

GLOBAL EDITION



Introduction to Biotechnology

FOURTH EDITION

William J. Thieman • Michael A. Palladino









INTRODUCTION TO BIOTECHNOLOGY

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To my wife, Billye, the love of my life,
and to the hundreds of biotechnology graduates
now doing good science at biotechnology companies
and loving every minute of it.

W. J. T.

To my "Auntie Ro," Rosanne Hansen, who fostered in
me a love and passion for biology at an early age.

M. A. P.

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About the Authors



Authors Bill Thieman and Michael Palladino

William J. Thieman taught biology at Ventura College for 40 years and biotechnology for 11 years before retiring from full-time teaching in 2005. He continues to serve as an advisor to the college biotechnology program. He received his B.A. in biology from California State University at Northridge in 1966 and his M.A. in Zoology in 1969 at UCLA. In 1995, he started the biotechnology program at Ventura College. In 1998, he added the laboratory skills course, and it was articulated as a state-approved vocational program. He identified technical skills needed for the program while serving three summer internships at Amgen, Biosource (now Invotrogen), and Biopool. The internships provided an opportunity to learn protocols, interact with lab directors, and query technicians, focusing on identifying the skills needed in these biotechnology companies. He routinely engaged his contacts at these biotechnology companies to lead lab protocols and describe their experiences to his classes.

Mr. Thieman has taught a broad range of undergraduate courses including general, human, and cancer biology. He received the Outstanding Teaching Award from the National Association of Biology Teachers in 1996 and the 1997 and 2000 Student Success Award from the California Community Colleges Chancellor's Office. The Economic Development Association presented its 1998 Program for Economic Development Award to the biotechnology training program at Ventura College for its work with local biotechnology companies. His success in acquiring grants to support the program was recognized at the 2007 Conference of the National Center for Resource Development.

Michael A. Palladino is Vice Provost for Graduate Studies, former Dean of the School of Science, and Professor of Biology at Monmouth University in West Long Branch, New Jersey. He received his B.S. in Biology from Trenton State College (now known as The College of New Jersey) in 1987 and his Ph.D. in Anatomy and Cell Biology from the University of Virginia in 1994.

Dr. Palladino has taught a wide range of courses including anatomy and physiology, biotechnology, cell and molecular biology, general biology, genetics, and endocrinology. He has received several awards for research and teaching, including the Distinguished Teacher Award from Monmouth University, the Caring Heart Award from the New Jersey Association for Biomedical Research, and the Young Andrologist Award from the American Society of Andrology. For more than 15 years he directed a laboratory of undergraduate student researchers supported by external funding from the National Institutes of Health, biopharma companies, and other agencies. He and his undergraduates studied molecular mechanisms involved in innate immunity and oxygen homeostasis of mammalian male reproductive organs.

Dr. Palladino started writing with Benjamin Cummings as a co-author of *BiologyLabs On-Line*, a series of Internet-based interactive laboratories for undergraduate students. He was Series Editor for the Benjamin Cummings *Special Topics in Biology* booklet series, and author of the first booklet in the series, *Understanding the Human Genome Project*. Dr. Palladino is a co-author on the writing team of W. S. Klug, M. R. Cummings, C. A. Spencer, and D. J. Killian for the textbooks *Concepts of Genetics* and *Essentials of Genetics*, both published by Pearson Education.

PREFACE

It is hard to imagine a more exciting time to be studying biotechnology. We began the preface with this statement in the first edition of *Introduction to Biotechnology* and this still holds true today. Advances are occurring at a dizzying pace, and biotechnology has made an impact on many aspects of our everyday lives. Now in its fourth edition, *Introduction to Biotechnology* remains the first biotechnology textbook written specifically for the diverse backgrounds of undergraduate students. *Introduction to Biotechnology* provides students with the tools for practical success in the biotechnology industry through its balanced coverage of a range of scientific disciplines, details on contemporary techniques and applications, the business of biotechnology, integration of ethical issues, coverage of important regulatory considerations, and career guidance.

Introduction to Biotechnology was designed with several major goals in mind. The text aims to provide:

- An engaging and easy-to-understand narrative that is appropriate for a diverse student audience with varying levels of scientific knowledge.
- Assistance to instructors teaching all major areas of biotechnology and help to students learning fundamental scientific concepts without overwhelming and excessive detail.
- An overview of historic applications while emphasizing modern, cutting-edge, and emerging areas of biotechnology.
- Insights on how biotechnology applications can provide some of the tools to solve important scientific and societal problems for the benefit of humankind and the environment.
- Inspiration for students to consider the many ethical issues associated with biotechnology.

Introduction to Biotechnology provides broad coverage of topics including cell and molecular biology, biochemistry, bioinformatics, genetics, genomics, proteomics, and others. We have striven to provide students with the tools and knowledge they need to understand varied and diverse areas of biotechnology.

In our effort to introduce students to the cutting-edge techniques and applications of biotechnology, we have dedicated specific chapters to constantly emerging areas such as microbial biotechnology (Chapter 5), agricultural biotechnology (Chapter 6), animal biotechnology (Chapter 7), forensic biotechnology (Chapter 8), bioremediation (Chapter 9), aquatic biotechnology (Chapter 10), and medical biotechnology (Chapter 11). Consideration of the many regulatory agencies and issues that affect the bio-technology industry are discussed in Chapter 12. In addition to the ethical issues included in each chapter as **You Decide** boxes, a separate chapter (Chapter 13) is dedicated to ethics and biotechnology.

New to the Fourth Edition

The fourth edition of *Introduction to Biotechnology* is thoroughly updated and includes several new features:

- **Case Studies—**New to this edition, each end-of-chapter question set, except for chapter 1, now concludes with a Case Study. We present an example of interesting, current research or a recent discovery related to the chapter content, provide a brief summary, and ask students to consider relevant questions. Two goals of the feature are (1) to engage students with contemporary research and (2) to ask higher order questions that require students to think critically.
- Expanded sets of end-of-chapter **Questions & Activities**, including more Internet-based exercises. Each chapter now has 20 Questions & Activities to provide a broader range of assessment options to help students learn.
- New **You Decide** entries have been added to stimulate student interest in, and critical thinking about, controversial areas of biotechnology related to legal, ethical, and social issues. We have expanded from 29 to 37 total You Decide boxes integrated throughout the chapters. Eighteen are new, and they cover topics such as the labeling

of genetically modified foods (Chapter 6), genetic screening to improve breast cancer prevention (Chapter 8), human consumption of transgenic salmon (Chapter 10), human embryo and germline editing (Chapter 11), regulating biotechnology beyond traditional settings (Chapter 12), and potential fast track approval of genetically modified wheat to help humans suffering from gluten intolerance (Chapter 13).

- **Nearly 70 new figures and 40 new photos** help simplify and explain complex topics in biotechnology.
- **Career Profiles**—New profiles have been developed for all chapters and contributed by professionals working in biotechnology. These profiles are designed to help students appreciate the wide range of careers available in the biotechnology industry, with tips and perspectives from experts doing the work. Career Profiles are available at the Companion Website where we can keep information up to date. Each profile includes a photo and background of the individual to help personalize his or her career stories.

In addition, each chapter has been thoroughly revised and updated to provide students with current information in all areas of biotechnology. Of special note are the following changes:

- **Chapter 1: The Biotechnology Century and Its Workforce**. Includes an updated overview of key topics to be discussed in the book, organized by chapter; the current state and trends of the biotechnology industry and its workforce; biotechnology and pharmaceutical company revenues; funding sources for starting a biotechnology company; trends in drug development; and a brief future example of precision medicine. We have added new coverage of Do-It-Yourself biotechnology, an introduction to industrial biotechnology, an introduction to genome editing by CRISPR; and several new figures.
- **Chapter 2: An Introduction to Genes and Genomes.** Includes streamlined content, a new section on noncoding RNAs, and a new section titled "Immune Response Mechanism in Prokaryotes Results in Extraordinary New Technology for Editing Genes *In Vitro* and *In Vivo*," which provides an introduction to genome editing by CRISPR-Cas and its roles in biotechnology.
- **Chapter 3: Recombinant DNA Technology and Genomics**. Includes condensed content on different types of vectors, as well as streamlined or eliminated coverage of libraries, mapping, Southern blotting, and microarrays reflecting a shift from these technologies and increased use of sequencing and other applications. Updated content on the Human Genome Project includes restructured content on "After the Human Genome Project," which focuses on ENCODE and personal genomics, whole exome sequencing, and single-cell sequencing. Major content updates have been made to DNA sequencing technologies, including a new section and figure on "third-generation sequencing." Additional new content includes RNA sequencing; analyzing gene function via protein expression, gene mutagenesis, and RNAi ; gene editing via transgenics, knockouts, and CRISPR; and a new section on systems biology and synthetic biology.
- **Chapter 4: Proteins as Products**. Explains why protein drugs produced by genetically engineered living organisms have largely supplanted pharmaceutical production methods; disease discoveries that have been made using new gene canceling technologies; instrumentation improvements for protein purification and identification; detection of significant protein-protein interactions; progress in identifying protein biomarkers that can detect disease at earlier stages; and the analysis of a contemporary study of protein interaction.
- **Chapter 5: Microbial Biotechnology**. Includes new content on whole genome sequencing; metagenomics and the Human Microbiome Project; vaccine development and major targets for new vaccines; synthetic genomes; and a new section on phage therapy, including a figure on CRISPR-Cas editing to treat antibiotic resistant microbes. In addition, there's a new You Decide box titled: "'Gain of Function' Experiments and Engineering Viral Pathogens."
- **Chapter 6: Plant Biotechnology**. Recognizes the impact of biotechnology on agricultural production in the world; briefly explains contemporary methods used to produce new plant products; discusses methods for using engineered gene vectors that can transfer genes for new products and insect resistance; provides a current list of genetically modified plants including their mechanism of action; discusses the expanding use of transgenic crops in developing countries; describes newly approved crops using gene silencing technology and the effect it has had on the USDA approval process; discuses the details of the new labeling of GM foods; and provides analysis of a contemporary study of an alternative method for insect resistance. There are two new You Decide boxes: "Labeling GM Foods" and "Is Roundup Toxic to the Environment?"

- **Chapter 7: Animal Biotechnology**. Includes a shift in direction from drugs to vaccines for humans of all ages and the rationale behind it; the significance of animal testing for drugs toward treatments for animal diseases; the benefits of cell-culture testing before animal testing for regulatory approval; the first approval of a drug produced in a transgenic goat to treat a type of stroke; new method for creating animals with gene knockouts and knock-ins; and the importance of a national project to determine the function of all the genes in a rat by using knockout technology. Two new You Decide boxes are included: "Can Gene Editing in Chickens Prevent Avian Flu Transfer to Humans?" and "Humans to Pets to Humans: Will the Public Accept This Type of Animal Testing?"
- **Chapter 8: DNA Fingerprinting and Forensic Analysis**. Includes the process for comparing DNA profiles with the new CODIS markers; the exclusion process for elimination of human suspects using profile examples; improvements in "touch DNA" analysis methods; the progress in utilizing personal DNA sequence markers as a precursor to diagnosis; new examples of DNA sequences to identify certified products; and the analysis of a contemporary example of human DNA contamination in a mouse DNA profile. Two new You Decide boxes are included: "Could Genetic Screening Improve Breast Cancer Prevention?" and "Will Rapid DNA Testing at a Crime Scene Help Law Enforcement?"
- **Chapter 9: Bioremediation**. Includes updated content on genomics and GM species for bioremediation, updates on the effects of bioremediation at the *Deepwater Horizon* oil spill in the Gulf of Mexico, new content and figure on endocrine disruptors, and a new section on ocean pollution by macro- and microplastics.
- **Chapter 10: Aquatic Biotechnology**. Includes new and revised content on aquaculture, coverage of the AquAdvantage salmon as the first GM animal approved by the U.S. FDA for human consumption, bioprospecting and recently approved novel medicines from aquatic species, a new Tools of the Trade on eDNA and environmental monitoring, and a new You Decide box: "Transgenic Salmon for Human Consumption: Safe or Not?"
- **Chapter 11: Medical Biotechnology**. Because of the rapid pace of change and progress in this field, Medical Biotechnology has undergone the most significant revision of all the chapters in the book. This includes reorganized and revised content on detecting and diagnosing human disease conditions, including new content on biomarkers and cell-free DNA; prognostic and diagnostic genetic tests; updates on approaches for genetic testing, including a new section on sequence analysis of individual genomes that explores the impact of whole genome sequencing including exon sequencing, sequencing and screening fetal genes from the maternal bloodstream, and pre-conception testing; updates on personal genomics to include RNA sequencing and single-cell sequencing; and a new section and figure on genome-wide association analysis. The chapter includes a renamed and revised section, Precision Medicine and Biotechnology; new content on the Precision Medicine Initiative and examples of cutting edge approaches including nanomedicine; and a new section on immunotherapies, including recently approved FDA immunotherapies using CAR-T cells that have been highly successful. It also includes revised and new content on gene therapy approaches, including CRISPR-Cas and recent trials with therapeutic RNA; and new and updated content on regenerative medicine, including new sections on 3D bioprinting of tissues, engineered organoids and organs, and updates on stem cell technologies and regulations. Nine new figures accompany these changes along with two new You Decide boxes: "Genetics Testing: Destiny Tests?" and "Human Embryo and Germline Editing."
- **Chapter 12: Biotechnology Regulations**. Includes an overview of international regulations; describes how FAO, the WHO, and the OIE contribute to the regulation of biotechnology products; explains how the Cartagena Protocol oversees the safe handling, transfer, and use of living-modified organisms; describes how the fair and equitable sharing of plant genetic resources for food and agriculture happens; and explains how patents on biotechnology products are protected under international regulations. Two new You Decide boxes ask: "How Should We Regulate Biotechnology Beyond Traditional Settings?" and "Should There be a Global Ban on Human Genome Editing?"
- **Chapter 13: Ethics and Biotechnology**. Includes reorganized content and an abbreviated chapter format beginning with Examples of Ethics and Biotechnology that includes new content on mitochondrial replacement therapy and so called "three-parent babies." There is new information on genome editing and germline modification,

new content on ethical issues related to gene patents and CRISPR-Cas, and six new You Decide boxes: "Should GM Wheat (for Gluten Sufferers) Be Approved Quickly?," "What Would Be the Effect of Banning GM Organisms?," "How Much Return on the Investment?," "Animal Organ Acceptance," "Regenerative Medicine: For the Rich Only?," and "Genome Hackers and 'Anonymous' Genomes Identify Individual DNA Donors."

Returning Features

Introduction to Biotechnology is specifically designed to provide several key elements that will help students enjoy learning about biotechnology and prepare them for a career in biotechnology.

Learning Objectives

Each chapter begins with a short list of learning objectives presenting key concepts that students should understand after studying the chapter.

Abundant Illustrations

Approximately 200 figures and photographs provide comprehensive coverage to support chapter content. Illustrations, instructional diagrams, tables, and flowcharts present step-by-step explanations that give students visual help to learn about the laboratory techniques and complex processes that are important in biotechnology. The new edition is enhanced by nearly 70 new figures and 40 new photos.

- **Forecasting the Future** at the beginning of each chapter briefly highlights exciting new areas of biotechnology that the authors predict will be worth watching in the future.
- **Making a Difference** at the end of each chapter spotlights particularly beneficial aspects of biotechnology applications that have had major impacts in improving the quality of life.



Career Profiles

A favorite feature of *Introduction to Biotechnology*, Career Profiles introduce students to different job options and career paths in the biotechnology industry and provide tips and information on job functions, salaries, guidance for preparing to enter the workforce, and other resources. New Career Profiles were written by different experts currently working in the biotechnology industry. For the fourth edition, all Career Profiles have moved from the book to the Companion Website where we can update profiles to provide current content and link to other relevant career resources. We strongly encourage students to refer to these profiles if they are interested in learning more about careers in the industry.



You Decide

From genetically modified foods to stem cell research, there are an endless number of topics in biotechnology that provoke strong ethical, legal, and social questions and dilemmas. **You Decide** boxes stimulate discussion in each chapter by presenting students with information that relates to the social and ethical implications of biotechnology, followed by a set of questions for them to consider. The goal of these boxes is to help them understand *how* to consider ethical issues and to formulate their own informed decisions. There are 37 You Decides integrated throughout the chapters, 18 of which are new to the fourth edition.



Tools of the Trade

Biotechnology is based on the application of various laboratory techniques or tools in molecular biology, biochemistry, bioinformatics, genetics, mathematics, engineering, computer science, chemistry, and other disciplines. **Tools of the Trade** boxes in selected chapters present modern or historically important techniques and technologies related to chapter content to help students learn about the techniques and laboratory methods that are the essence of biotechnology.

Questions & Activities

Questions are included at the conclusion of each chapter to reinforce student understanding of concepts. For the fourth edition, we expanded each chapter's question set to at least 20 questions and activities. We updated existing questions, added many new ones, and added Case Studies. Activities frequently include Internet assignments that ask students to explore a cutting-edge topic. Answers to these questions are provided in Appendix 1 at the end of the text.

Glossary

Like any technical discipline, biotechnology has a lexicon of terms and definitions that are routinely used in discussing processes, concepts, and applications. The

most important terms are shown in **boldface type** throughout the book and are defined as they appear in the text. Definitions of these key terms are included in a glossary at the end of the book.

Supplemental Learning Aids

Introduction to Biotechnology Companion Website

The Companion Website, available at **www.pearsonglobaleditions.com**, is designed to help students study for their exams and deepen their understanding of biotechnology. Each chapter contains learning objectives, quiz questions, flashcards, study tools, Internet and literature references, and biotech career information. For the fourth edition, **Career Profiles** have moved from the book to the website, providing engaging descriptions of various careers written by professionals working in the biotechnology industry.

Instructor Resource Center (IRC)

The Instructor Resource Center, **www.pearsonglobaleditions.com**, is designed to support instructors teaching biotechnology. The IRC is an online resource that supports and augments material in the textbook. Revised instructor supplements available for download include:

- **Computerized Test Bank:** 10 to 20 multiple-choice test questions per chapter
- **JPEG Art Files:** electronic files of all text tables, line drawings, and photos
- **PowerPoint Lecture Outlines:** a set of PowerPoint presentations consisting of lecture outlines for each chapter augmented by key text illustrations

Instructors using *Introduction to Biotechnology* can create a user account to access the Instructor Resource Center.

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A textbook is the collaborative result of hard work by many dedicated individuals, including students, colleagues, editors and editorial staff, graphics experts, and many others. First, we thank our family and friends for their support and encouragement while we spent countless hours on this project. Completing each new edition brings with it a great sense of accomplishment, and a certain degree of fatigue, overshadowed by the passion we have for helping faculty teach and students learn biotechnology. Without your understanding and patience, this book would not have been possible.

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As students ourselves, we too continue to learn about biotechnology every day. We wish you great success in your explorations of biotechnology!

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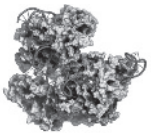
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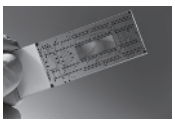
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CHAPTER ONE

The Biotechnology Century and Its Workforce



As you will learn in this chapter, the biotechnology industry presents a wide range of exciting career opportunities for students from research scientist positions in the lab or the field to positions in sales, marketing, communications and other disciplines.

After completing this chapter, you should be able to:

- Define biotechnology and describe the many scientific disciplines that contribute to biotechnology.
- Provide examples of historic and current applications of biotechnology.
- Appreciate the range of different topics that constitute modern biotechnology and how these influence everyday life.
- Discuss how medical diagnosis is changing as a result of biotechnology and provide examples of how genome data are being used to diagnose and treat human disease conditions.
- Give an example of a new plant biotechnology crop that reached the market recently and have a basic understanding of the controversies associated with genetically modified organisms.
- Understand the basics of how a biotechnology company is started, funded, and valued, and describe the organizational structure of a typical biotech company.
- Describe career opportunities and options in biotechnology and ways to explore them.

Can you imagine a world free of diseases, where food is abundant for everyone and the environment is free of pollution? These scenarios are exactly what many people in the biotechnology industry envision as they dedicate their lives to this exciting science. This chapter was designed to provide you with a basic introduction to the incredible range of biotechnology topics that you will read about in this book. As you will see, biotechnology is a multidisciplinary science with great potential for future discoveries and many powerful applications and products.

In this chapter, we present a brief introduction and overview of many topics that we discuss in greater detail throughout the book. We begin by defining biotechnology and outlining scientific disciplines that contribute to this incredible field. We highlight both historic and modern applications and describe the different types of biotechnology that you will study in this book. At the end of the chapter, we discuss aspects of the biotechnology workforce and skills required to work in the industry.

FORECASTING THE FUTURE

The discovery and creation of new medicines is expensive and difficult. In the past 40 years, thousands of small biotech companies have attempted to prove that they can do this better and less expensively than traditional pharmaceutical companies. Today, biotech companies generate global revenues of $163 billion. This makes up about 20 percent of the revenues generated by the pharmaceutical market. The current annual growth rate of biotech companies is more than 8 percent, which is double that of conventional pharmaceutical companies. It is likely that this rate of growth will continue in the foreseeable future. This means biotechnology companies, and the approaches they use to develop drugs, will continue to appeal to researchers and investors.

1.1 What Is Biotechnology and What Does It Mean to You?

Have you ever eaten a nonbruising apple or potato, been treated with a monoclonal antibody, received tissue grown from embryonic stem cells, or seen a "knockout" mouse? Have you ever eaten a corn chip, sour cream, yogurt, or cheese; had a flu shot; known a person with diabetes who requires injections of insulin; taken a home pregnancy test; used an antibiotic to treat a bacterial infection; sipped a glass of wine or milk; or made bread (**Figure 1.1**)? Although you may not have experienced any of the scenarios on the first list, at least one of the items on the second list must be familiar to you. If so, you have experienced the benefits of biotechnology firsthand.

Biotechnology is broadly defined as the science of using living organisms, or the products of living organisms, for human benefit (or to benefit human surroundings)—that is, to make a product or solve a problem. Remember this definition. As you learn more about biotechnology, we will expand and refine this definition with historical examples and modern applications from everyday life and look ahead to the future of biotechnology.

You would be correct in thinking that biotechnology is a relatively new discipline that is only recently getting more attention; however, it may surprise you to know that biotechnology involves several ancient practices. As we discuss in the next section, old and new approaches to biotechnology make this field one of the most rapidly changing and exciting areas of science. It affects our everyday lives and will become even more important during this century—what some have called the "century of biotechnology."

A Brief History of Biotechnology

If you asked your friends and family to define biotechnology, their answers might surprise you. They may have no idea what biotechnology is. Perhaps they might speculate that biotechnology involves serious-looking scientists in white lab coats secretively carrying out sophisticated "cloning" experiments in expensive laboratories. When pressed for details, however, your friends probably will not be able to tell you how these "experiments" are done, what information is gained from such work, and how this knowledge can or cannot be used. Although DNA cloning, the genetic manipulation of organisms, and even cloning entire organisms are exciting modern-day techniques, biotechnology is not a new science. In fact, many applications represent old practices with new methodologies. Humans have been using other biological organisms for their benefit in many processes for several thousand years. Historical accounts have shown that the Chinese, Greeks, Romans, Babylonians, and Egyptians, among many others, have been involved in biotechnology since about 2000 B.C.

Biotechnology does not mean hunting and gathering animals and plants for food; however, the domestication of animals such as sheep and cattle for use as livestock is a classic example of biotechnology. Our early ancestors also took advantage of **microorganisms** and used **fermentation** to make breads, cheeses, yogurts, and alcoholic beverages such as beer and

(a)







(b)



FIGURE 1.1 Examples of Biotechnology are in Your Home (a) Kitchen biotechnology includes breads, cheeses, yogurts, and many other foods and drinks. These are common basic examples of biotechnology. (b) A much more sophisticated and less common example includes smartphones that monitor vital signs and blood chemistry such as blood sugar levels. Shown here is a smartphone and glucose meter application that can detect blood glucose levels in a test strip.

wine. These practices continue today. During fermentation, some strains of yeast decompose sugars to derive energy, and in the process they produce ethanol (alcohol) as a waste product. When bread dough is being made, yeast such as *Saccharomyces cerevisiae* (commonly called baker's yeast) is added to make the dough rise. This occurs because during fermentation yeast release carbon dioxide, which causes the dough to rise and creates holes in the bread. Alcohol produced by the yeast evaporates when the bread is cooked. If you make bread or pizza dough at home, you have probably added store-bought *S. cerevisiae* from an envelope or jar to your dough mix.

For thousands of years, humans have used selective breeding as a biotechnology application to improve production of crops and livestock used for food purposes. In **selective breeding,** organisms with desirable features are purposely mated to produce offspring with the same desirable characteristics. For example, cross-breeding plants that produce the largest, sweetest, and most tender ears of corn is a good way for farmers to maximize their land to produce the most desirable crops (**Figure 1.2a**).

Similar breeding techniques are used with farm animals, including turkeys (to breed birds producing the largest and most tender breast meat), cows,

chickens, and pigs. Other examples include breeding wild species of plants, such as lettuces, strawberries, cabbage, and bananas, over many generations to produce modern plants that are cultivated for human consumption. Many of these approaches are really genetic applications of biotechnology. Without expensive labs, sophisticated equipment, PhD-trained scientists, and well-planned experiments, humans have been manipulating genetics for hundreds of years.

By selecting plants and animals with desirable characteristics, humans are choosing organisms with useful genes and taking advantage of their genetic potential for human benefit. As you will learn, zebrafish are important experimental **model organisms** (Figure 1.2b). Scientists at the Children's Hospital of Boston produced a transparent zebrafish named Casper. Casper was created by mating a zebrafish mutant that lacked reflective pigment with a zebrafish that lacked black pigment. Casper has also proven important for drug testing and *in vivo* (in the living organism) studies of stem cells and cancer. For example, to study how cancer cells spread, or **metastasize,** scientists injected fluorescent tumor cells into the fish's abdominal cavity and were able to track the migration of those cells to specific locations in the body.

One of the most commonly known applications of biotechnology is the use of **antibiotics,** substances produced by microorganisms that will inhibit the growth of other microorganisms. In the 1940s, penicillin became widely available for medicinal use to treat bacterial infections in humans. In the 1950s and 1960s, advances in biochemistry and cell biology made it possible to purify large amounts of antibiotics from many different strains of bacteria. **Batch (large-scale) processes**—in which scientists can grow bacteria and other cells in large amounts and harvest useful products in large batches—were developed to isolate commercially important molecules from microorganisms (explained further in Chapters 4 and 5).

Since the 1960s, rapid development of our understanding of genetics and molecular biology has led to exciting innovations and applications in biotechnology. As scientists unravelled the secrets of DNA structure and function, different laboratory technologies led to **gene cloning,** the ability to identify and reproduce a gene of interest, and **genetic engineering,** manipulating the DNA of an organism. Through genetic engineering, scientists are able to combine DNA from different sources. This process, called **recombinant DNA (rDNA) technology,** is used to produce hundreds of recombinant proteins of medical importance, including insulin, human growth hormone, and blood-clotting factors. From its inception, rDNA technology has dominated many areas of biotechnology and, as you will soon learn, many credit rDNA technology with starting modern

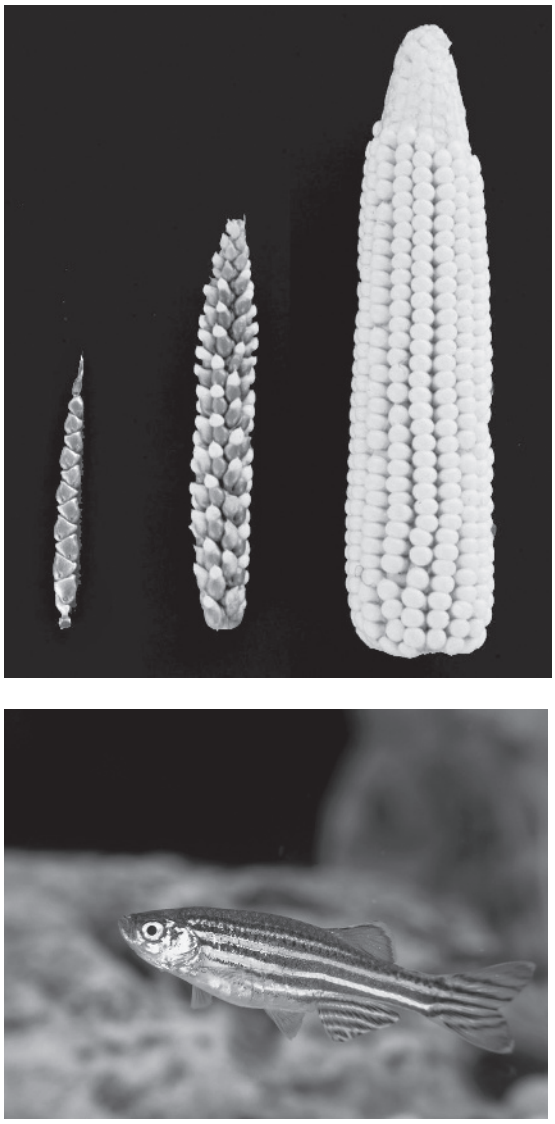


FIGURE 1.2 Selective Breeding Is an Old Example of Biotechnology That Is Still Common Today (a) Corn grown by selective breeding. From left to right is teosinte (*Zea canina*), selectively bred hybrids, and modern corn (*Zea mays*). (b) Zebrafish (*Danio rerio*).

biotechnology as an industry. You will learn that rDNA technology has led to hundreds of applications, including the development of disease-resistant plants, food crops that produce greater yields, crops engineered to be more nutritious, and genetically engineered bacteria capable of degrading environmental pollutants.

Gene cloning and rDNA technology have had a tremendous impact on human health through the identification of thousands of genes involved in human genetic diseases. Initiated in 1990 and completed in 2003, the **Human Genome Project** was the ultimate cloning project, and an international research effort with goals to identify and sequence all genes contained

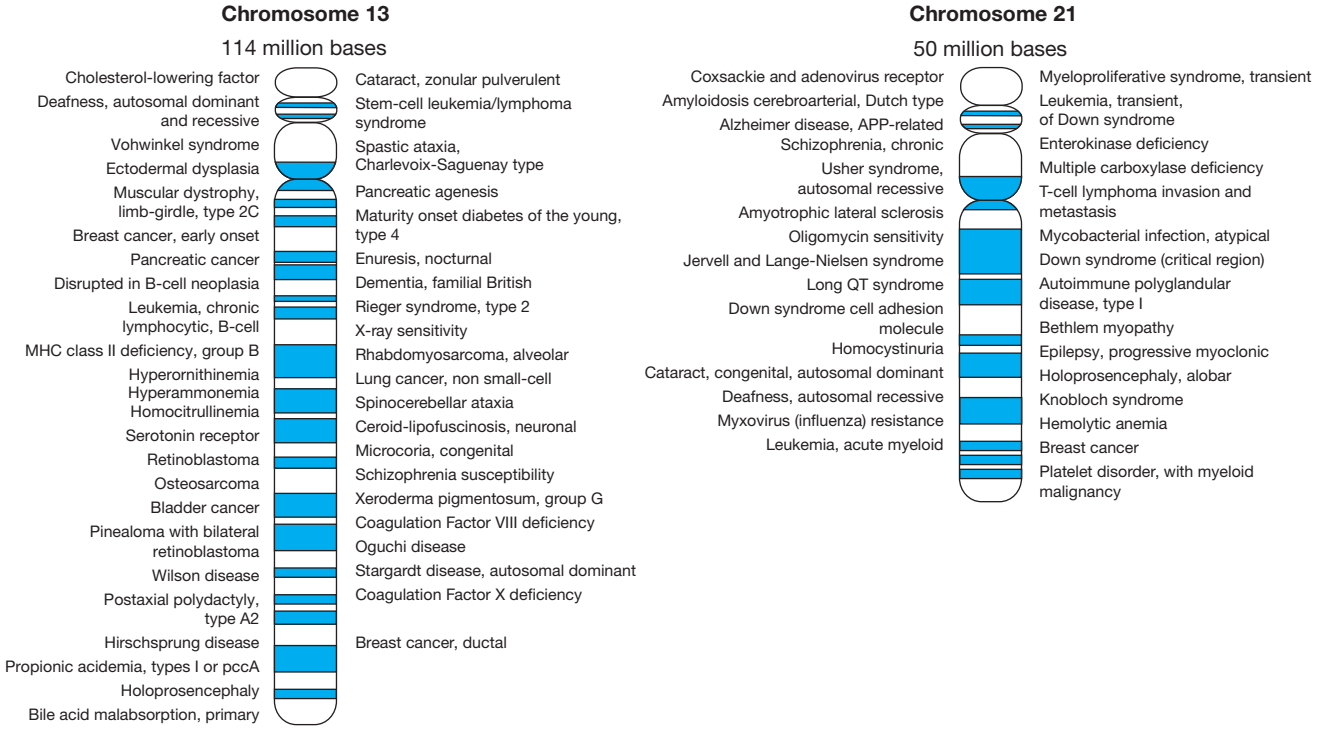


FIGURE 1.3 Gene Maps of Chromosomes 13 and 21 The Human Genome Project has led to the identification of nearly all human genes and has mapped their location on each chromosome. The maps of chromosomes 13 and 21 shown here display partial lists of genes known to be involved in human genetic diseases. Identifying such genes is an important first step toward developing treatments for many genetic diseases.

in the DNA of human cells (the **genome**) and to map gene locations to each of the 24 human chromosomes (chromosomes 1 to 22 and the X and Y chromosomes). The Human Genome Project has revealed the chromosomal location and sequence of every human gene, from genes that control normal cellular processes and determine characteristics such as hair color, eye color, height, and weight to the myriad of genes that cause human genetic diseases (**Figure 1.3**).

As you will learn, human genome data are now free and readily available in public databases. The Human Genome Project has significantly advanced the development of new diagnostic tools for detecting genetic diseases and molecular approaches for treating and curing human genetic diseases. As a result, new knowledge about human genetics is having, and will have, tremendous and wide-ranging effects on basic science and medicine now and in the near future.

The Human Genome Project ushered in an exciting new era of research in molecular biology and genetics known as **genomics** (the study of genomes), including the development of extraordinary new techniques for sequencing DNA. These techniques have made it possible to sequence the genomes of virtually any species and have resulted in the ability to sequence individual human genomes for a variety of reasons, from analyzing genetic ancestry to genetic testing and disease diagnosis. You will learn about creating **artificial** or **synthetic genomes** and the plans scientists have for these genomes. In addition, new approaches for **genome editing,** based on an exciting technology called **CRISPR-Cas,** is making it possible to correct genetic diseases and create novel genetic modification of genomes in many species—including humans. Throughout the book we extensively discuss these topics.

The Do-It-Yourself Biotechnology Movement

A movement under way called **do-it-yourself (DIY) biotechnology** moves biotechnology and related applications away from traditional research environments such as universities or established companies. The DIY movement encompasses individuals with many different backgrounds—from new doctoral students, to kitchen biologists with little formal training, to amateurs with an interest in tinkering, to entrepreneurs. Some have referred to DIY participants as "biohackers."

What DIY enthusiasts share in common is that more than 90 percent work in communal space, not garages

or basements; they are mostly under 45 years old; and about 20 percent have earned a doctorate. DIY folks are not necessarily rogue, inexperienced amateurs.

DIY biotech is working much the way Apple cofounders Steve Jobs and Steve Wozniak did when they built their first circuit boards at home in their bedrooms and garages. Inexpensive instruments for amplifying DNA and diagnostic devices for detecting malaria have resulted from DIY biotechnology. However, whether any DIY discoveries will result that will have anywhere close to the impact of Apple on technology remains to be seen.

Some of the methods they are using are fairly routine now. For example, in most parts of the world you could do basic gene-cloning experiments in your kitchen. Not exactly DIY, but about 4 years ago a group of undergraduate students in a genome course at Johns Hopkins University announced they had made a synthetic version of yeast chromosome 3 incorporating only essential elements of the genome. We mention this as an example of how students with relatively little training can do this work.

DIY participants often seek crowdsource funding via online fundraising campaigns, rent space, seek equipment donations, or share lab space with others. Concern has been raised about DIY scientists working in unregulated ways and what may result from their "research." For instance, German authorities discovered pathogenic antibiotic-resistant bacteria in a CRISPR kit sent from California. The kit contained common gut microbes. German regulators declared the environmental risk of modifying these drug-resistant bacteria as insignificant but banned all such imports from the company Odin except to certified high-safety laboratories that have some governmental oversight.

Right now because there is no government funding for DIY biotechnology, participants can largely do whatever they want in an unregulated environment as long as their work is not illegal.

Biotechnology: A Science of Many Disciplines

One of the many challenges you will encounter as you study biotechnology will be piecing together complex information from different scientific disciplines. It is impossible to talk about biotechnology without considering important contributions of biology, chemistry, mathematics, computer science, and engineering, in addition to fields such as philosophy, ethics, and economics. Biotechnology is an expansive, *interdisciplinary* field. Later in this chapter, we consider how biotechnology provides a wealth of employment opportunities for people who have been trained in diverse fields.

Figure 1.4 provides a diagrammatic view of the many disciplines that contribute to biotechnology. Notice that the "roots" are primarily formed by work in the **basic sciences**—research into fundamental processes of living organisms at the biochemical, molecular, and genetic levels. Basic science research, with the help of other disciplines, can lead to genetic engineering approaches that form the core or trunk of many, but not all, biotechnology applications. At the top of the tree, biotechnology applications create products or processes to help humans or our living environment. Many future processes have yet to be developed and await the intuitive participation of people working in biotechnology today.

A simplified example of the interdisciplinary nature of biotechnology can be summarized as follows. At the basic science level, scientists conducting research in microbiology at a college, university, government agency, or public or private company may discover a gene or gene product in bacteria that shows promise as an agent for treating a disease condition. Typically, biochemical, molecular, and genetic techniques would be used to determine the role of this gene. This process also involves using computer science in sophisticated ways to study the sequence of a gene and analyze the structure of the protein produced by the gene, an example of a field called **bioinformatics**.

Once basic research has arrived at a detailed understanding of this gene, the gene may then be used in a variety of ways, including drug development, agricultural biotechnology, and environmental and aquatic applications (Figure 1.4). The many applications of biotechnology will become much clearer as we cover each area. At this point keep in mind the important concept that biotechnology is a science that requires skills from many disciplines.

Products of Modern Biotechnology

Throughout the book, we consider many cutting-edge and innovative products and applications of biotechnology. We look not only at products for human use but also at biotechnology applications of microbiology, marine biology, and plant biology, among other disciplines. Sixty-five percent of biotechnology companies in the United States and 50 percent of the Australian and German biotech companies focus on producing medicines for the treatment of human health conditions. Many of these medicines are **recombinant proteins** named because they are produced by gene-cloning/recombinant DNA techniques. For example, the majority of these proteins are produced from human genes inserted into bacteria to make the recombinant proteins used to treat human disease conditions. In 1982, the California biotechnology company Genentech**,** widely regarded as the world's first biotech company, received approval for recombinant

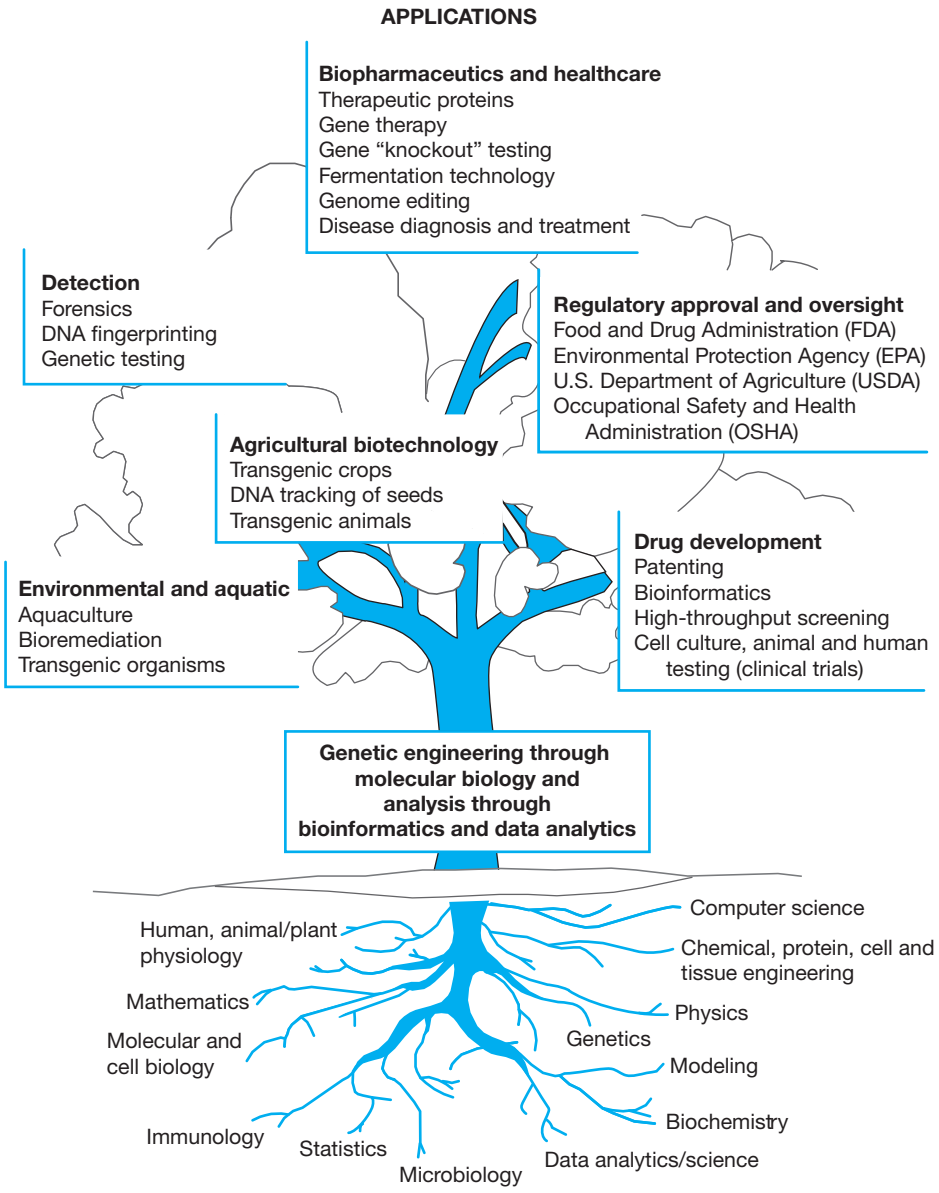


FIGURE 1.4 The Biotechnology Tree: Different Disciplines Contribute to Biotechnology The basic sciences are the foundation or "roots" of all aspects of biotechnology. The central focus or "trunk" for most biotechnological applications is genetic engineering. Branches of the tree represent different organisms, technologies, and applications that "stem" from genetic engineering and bioinformatics, central aspects of most biotechnological approaches. Regulation of biotechnology occurs through the FDA and EPA in the United States and the FAO, WHO, and OIE internationally (see Chapter 12).

insulin, used for the treatment of diabetes, as the first biotechnology product for human benefit (**Figure 1.5**). There are now several hundred drugs, vaccines, and diagnostics on the market, with more than 350 biotechnology medicines in development, targeting over 200 diseases.

Drug development by the biotechnology industry is focused on combating major diseases that affect humans, and over half of the new drugs in the development "pipeline" are designed to treat cancer. This focus of the industry is usually evident when reviewing types of new biotech drugs approved in the United States. For example, 2017 was a banner year for new biotech drug approvals with 46 novel drugs approved in the United States second only to 1996 when 53 biotech drugs were approved (**Figure 1.6**). As shown in Figure 1.6, cancer drugs received the most approvals. In comparison, 31 new drugs were approved in Germany in 2017. Approximately one-third of these were cancer drugs. Nine new cancer drugs received approval in Canada and 8 in Japan in the same year.

Table 1.1 provides a brief list of some of the top-selling biotechnology drugs and the companies that developed them. Diagnosis and/or treatment of a variety of human diseases and disorders—including acquired immunodeficiency syndrome (AIDS), stroke, diabetes, and cancer—make up the bulk of biotechnology products on the market. Many of the most widely used products of biotechnology are recombinant proteins (Table 1.2).

If Figure 1.6 and Tables 1.1 and 1.2 have not provided you with convincing examples of the importance of biotechnology for human health, consider that genes are being introduced increasingly into

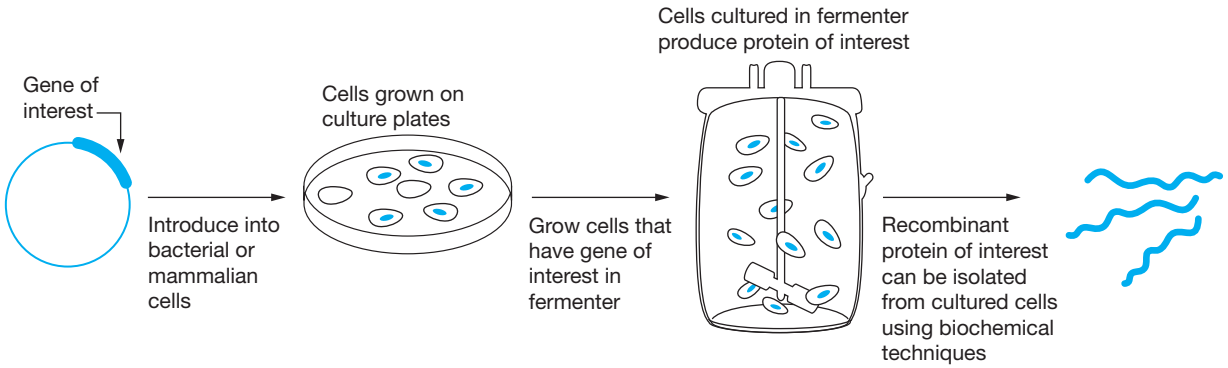




FIGURE 1.5 Using Genetically Modified Cultured Cells to Make a Protein of Interest Genes of interest can be introduced into bacterial or mammalian cells. Such cells can be grown using cell culture techniques. Recombinant proteins isolated from these cells are used in hundreds of different biotechnology applications. In this example, mammalian cells are shown, but this process is also commonly carried out using bacteria. The photograph shows a vial of human insulin produced by recombinant DNA technology.

human cells as **gene therapy** approaches are employed in attempts to treat and cure human disease conditions. Gene therapy involves delivering genes to treat or cure a genetic disorder. Genetics and tissue engineering may lead to the ability to grow organs for transplantation that would only rarely be rejected by their recipients. New biotechnology products from marine organisms are being used to treat cancers, strokes, and arthritis. There is no question that advances in modern medicine, driven by new knowledge from the Human Genome Project and biotechnology applications, will result in healthier lives and potentially increase the human life span.

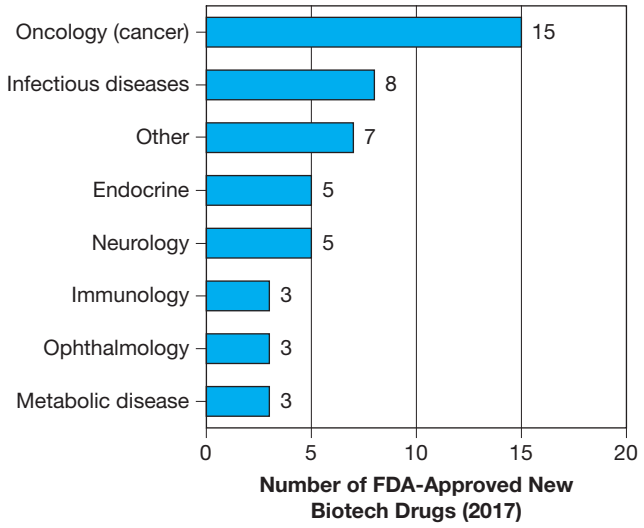


FIGURE 1.6 Investigational Biotechnology Drugs by Disease Category The production of drugs to combat cancer dominates the biotechnology industry's interest in treating human disease, with oncology-related research and treatment of infectious diseases such as the flu at the top of this list.

Ethics and Biotechnology

Just as in any other type of technology, the powerful applications and potential promise of biotechnology, including DIY experiments, raise many ethical concerns, and it should be no surprise to you that not everyone is a fan of biotechnology. A wide range of ethical, legal, and social implications of biotechnology inspire great debate and discussion among scientists, clergy, politicians, lawyers, and the general public (**Figure 1.7**). Throughout this book, we present ethical, legal, and social issues for you to consider.

Increasingly, you will be faced with ethical issues of biotechnology that may influence you directly. For instance, organism cloning in mammals such as sheep, cows, and monkeys has led some to suggest that human cloning should be permitted. How do you feel

TABLE 1.1 *2016—Top 10 Biotechnology Drugs (Each with Worldwide Sales over $5 Billion)

Drug Name	Developer	Drug Type	Function (Treatment of Human Disease Conditions)
Humira	AbbVie	Antibody (monoclonal)	Rheumatoid arthritis, Crohn's disease, Ulcerative colitis
Harvoni	Gilead Sciences	Small molecule	Hepatitis C
Rituxan	Roche	Antibody (monoclonal)	Non-Hodgkin's lymphoma
Revlimid	Celgene	Small molecule	Multiple myeloma
Avastin	Roche	Antibody (monoclonal)	Colorectal cancer; breast cancer; non–small cell lung cancer; ovarian, brain, and cervical cancer
Herceptin	Roche	Antibody (monoclonal)	Breast cancer, gastric cancer
Enbrel	Amgen	Recombinant protein	Rheumatoid arthritis, psoriasis
Prevnar 13	Pfizer	Vaccine	Pneumococcal (*Streptococcus Pneumoniae*) antibacterial vaccine
Lantus	Sanofi	Peptide	Diabetes mellitus types I and II
Neulasta	Amgen	Recombinant protein	Anemia (neutropenia/leukopenia)

*Data based on the most recent source available at the time of publication: Morrison C, Lähteenmäki R. Public biotech in 2016—the numbers. *Nat Biotechnol.* 2017;35:623–629.

about this? If, in the future, you and your spouse were unable to have children by any other means, would you want the opportunity to create a baby by cloning a replica of yourself? As another example, the potential for genome editing to create embryos with desired genetic characteristics has raised many ethically challenging questions.

If you choose to work in biotechnology, you will need to develop team working skills that allow for differences in opinion on many ethical issues, necessitating an understanding of the basis for the arguments supported by some of your colleagues. Look for the You Decide boxes in each chapter, in which we present scenarios or ethical dilemmas for you to consider. Realize that there are pros and cons and controversial issues associated with almost every application in biotechnology. Our goal is not to tell you *what* to think but to empower you with knowledge, and a framework for approaching ethical issues, that you can use to make your own decisions.

TABLE 1.2 Examples of Recombinant Proteins Manufactured from Cloned Genes

Product	Application
Blood Factor VIII (clotting factor)	Treat hemophilia
Epidermal growth factor	Stimulate antibody production in patients with immune system disorders
Growth hormone	Correct pituitary deficiencies and short stature in humans; other forms are used in cows to increase milk production
Insulin	Treat diabetes
Interferons	Treat cancer and viral infections
Interleukins	Treat cancer and stimulate antibody production
Monoclonal antibodies	Diagnose and treat a variety of diseases including arthritis and cancer
Tissue plasminogen activator	Treat heart attacks and stroke



FIGURE 1.7 Biotechnology Is a Controversial Science That Presents Many Ethical Dilemmas

1.2 Types of Biotechnology

Now that you have learned about the many areas of science that contribute to biotechnology, you should recognize that there are many different types of biotechnology. Chapters 2 and 3 provide important introductions to basic concepts you will need to be familiar with to understand biotechnology. In Chapter 2 you will learn about basic aspects of cell structure and function, DNA structure, genomes, and related topics. In Chapter 3 we discuss principles of gene cloning and recombinant technology that were the foundation for creating the biotechnology industry, as well as current trends in genomics that are key to understanding many modern applications of biotechnology.

Consider this section an introduction to what you will learn in greater detail in the chapters that follow.

Microbial Biotechnology

In Chapter 5, we explore the many ways in which microbial biotechnology affects society. As we discussed previously, the use of yeast for making beer and wine is one of the oldest applications of biotechnology. By manipulating microorganisms such as bacteria and yeast, microbial biotechnology has created better enzymes and organisms for making many foods, simplifying manufacturing and production processes, and making decontamination processes for the removal of industrial waste products more efficient. Microbes are used to create vaccines and to clone and produce batch amounts of important recombinant proteins used in human medicine.

Many studies are focusing on microbial genomics for a range of reasons, including to help us understand the roles microbes play in human health. Another recent controversial aspect we will explore is the creation of **synthetic genomes**—manmade DNA sequences that can be used to engineer microbes with desirable characteristics.

In microbial biotechnology you will also explore strategies used to detect microbes for diagnostic purposes in humans, food samples, and other sources; approaches to detect and combat microbes as possible bioweapons; and potential applications of microbes for producing biofuels.

Agricultural Biotechnology

Chapter 6 is dedicated to plant biotechnology and agricultural applications of biotechnology. In "ag biotech," we examine a range of topics from genetically engineered, pest-resistant plants that do not need to be sprayed with pesticides to foods with higher protein or vitamin content and drugs developed and grown as plant products. Agricultural biotechnology is already a big business that is rapidly expanding. The United Nations Food and Agriculture Organization has predicted that feeding a world population of 9.1 billion people in 2050 will require raising overall food production by some 70 percent (nearly 100 percent in developing countries). Agricultural biotechnology has provided solutions for today's farmers in the form of plants that are more environmentally friendly while yielding more per acre, resisting diseases and insect pests, and reducing farmers' production costs. Producing more for the expanding population will require new innovations from agricultural biotechnology.

A new generation of crops known as "gene-edited" rather than genetically modified is coming to the market. Created through CRISPR-Cas editing tools that snip and tweak DNA at precise locations, they largely fall outside current regulations, at least for now. In 2016, it was announced that a group at Pennsylvania State University used CRISPR-Cas to modify the common white button mushroom (*Agaricus bisporus*) to produce the first gene-edited crop approved for human consumption (**Figure 1.8**). By inactivating a gene that produces an enzyme responsible for the browning, the edited mushroom has a longer shelf life and resists browning when bruised or cut.

The current regulations were written for the earlier generation of genetically modified organisms, in which scientists used bacteria and viruses—typically from plant pests—to drop a payload of new genes into the nuclei of the plant cells, where they merge with the plant's DNA. That worked, but scientists could not control where the new genes would be inserted, and that led to worries of potentially dangerous genetic disruptions or cross-breeding with non-GM crops. For a long time, these regulations did not adequately distinguish between 'conventional GM' and the new gene-editing techniques, leaving confusion as to whether regulators



FIGURE 1.8 The Common White Button Mushroom Is the First Gene-Edited Crop Created by CRISPR to Be Approved for Human Consumption

will interpret products of gene editing as genetically engineered. Recently, Europe's high court has declared that gene-edited crops should be subject to the same regulations as conventional GM organisms.

Animal Biotechnology

In Chapter 7, we examine many areas of animal biotechnology, one of the most rapidly changing and exciting areas of biotechnology. Animals can be used as "bioreactors" to produce important products. For example, goats, cattle, sheep, and chickens are being used as sources of medically valuable proteins for human treatment, such as **antibodies**—protective proteins that recognize and help body cells to destroy foreign materials. Antibody treatments are being used to help improve immunity in patients with immune system disorders. Many other human therapeutic proteins produced from animals are in use, yet most of these proteins are needed in quantities that exceed hundreds of kilograms.

To achieve this large-scale production, scientists can create female transgenic animals that express therapeutic proteins in their milk. **Transgenic animals** contain genes from another source. For instance, human genes for clotting proteins can be introduced into goats for the production of these proteins in their milk. One of first examples was expressing silk genes from spiders in transgenic goats to produce silk in milk (so called "silk milk") from which silk could be purified and used to make clothes fabrics, including those for lighter and stronger military gear. More recently, Alexion Pharmaceuticals has produced a recombinant protein (sebelipase alfa), with the drug name Kanuma, that is expressed in eggs from transgenic chickens; it has been approved to treat a rare, fatal condition in humans (lysosomal acid lipase deficiency).

The Cayman Islands government intensified its efforts to protect inhabitants from mosquito-borne diseases by releasing genetically engineered (GE) mosquitoes. The GE mosquitoes, known as Friendly™ Aedes, are developed by Oxitec (United Kingdom). They contain a gene that kills the young insects at the larval stage to prevent the spread of dengue fever, Zika virus infection, chikungunya virus infection, and yellow fever. GE male mosquitoes mate with wild female mosquitoes and produce unviable larvae that die before adulthood. In two locations of mainland Malaysia, field trials involving the temporary small-scale releases of the same mosquitoes were approved by the Malaysian National Biosafety Board. The Malaysian government has made impressive efforts to establish a clear regulatory framework through the Biosafety Act 2007, which



YOU DECIDE

Genetically Modified Foods: To Eat or Not to Eat?

Many experts believe that genetically modified (GM) foods are safe and that they will provide significant benefits in the future. But public opinion on the use and safety of GM foods is mixed. A consumer survey on GM food conducted in all provinces in China indicates that only 11.9 percent of the Chinese consumers have a positive view towards GM food and an overwhelming 46.7 percent oppose it. Skeptics frequently comment that "GM foods are against nature," and some people worry about potential health effects such as food allergies.

The European Union (EU) has strict legislation and policy on GMOs to prevent any hostile effects on the environment and the health and safety of humans and animals. GMOs and food or feed obtained from GMOs are assessed for safety by the European Food and Safety Authority (EFSA) before they can be sold in or imported into the EU. The European Commission then grants authorizations for 10 years through a centralized procedure. Authorizations could also be given by national competent authorities that regulate the deliberate release of GMOs into the environment. A lot of consumers would look for another product rather than choose food labeled as genetically modified. To allow them to make informed choices, GMOs are assigned a unique identifier and are labeled as such.

The marketing and importing GMOs are regulated by the EU, but the cultivation of GMOs is left to the EU Members. Based on socioeconomic reasons and effects on local environments, they have the right to prohibit or restrict the sale or cultivation of approved GMOs that pose a health risk. EU members will be granted more flexibility to impose such measures after the approval of a pending Commission proposal, as amended by the European Parliament.



- What do you think about the use of GM foods?
- Would you be likely to buy GM foods if they were engineered to require fewer pesticide applications than "natural" foods?
- What if GM foods stayed fresher longer?
- What if they were more nutritious and less expensive?
- How much risk should consumers be willing to take to reap the benefits of GM foods?
- What are the potential negative impacts of GM crops affecting natural crops on nearby farms?

Consider these questions as you weigh the potential benefits or negative impacts of choosing between a GM or a non-GM food product. GM foods are here to stay, but you need to make an informed decision. You decide.

came into effect in December 2009, and is a party to the Cartagena Protocol on Biosafety. Approval processes for innovations like these is a requirement even for imminent problems like the spread of the Zika virus. These have been discussed in Chapter 12.

Gene editing is not being used only with plants. A Minnesota company, Recombinetics, is editing the genes of farm animals to create cattle without horns (**Figure 1.9**). On farms, cows are typically de-horned so that they don't hurt each other with their horns. A standard procedure is to burn off the horns when the cows are young, but researchers are looking for a way to avoid this. To genetically "remove" cow horns, a University of California–Davis team collaborated with Recombinetics to target the horn-growing gene using specific enzymes as a kind of "molecular scissors." This means that scientists sliced out the horn-growing gene naturally found in Holsteins and replaced it with a gene that stops horns from growing in the Angus breed.

How will this be regulated? According to the current regulatory regime, the FDA is in charge of regulating genetically engineered animals in the U.S.,



FIGURE 1.9 Hornless Cattle Without Pain The University of California–Davis team collaborated with a company called Recombinetics to target the horn-growing genes using "molecular scissors." This means that scientists sliced out the horn-growing genes naturally found in Holsteins and replaced them with genes that stop horns from growing in the Angus breed.

while the European Food safety authority is in charge of regulating this in Europe. This is based on the fact that the recombinant DNA construct is a new animal drug, and that affects the form or function of an animal. Some are not advocates of this change, because the hornless cows would be the first gene-edited animal to be entering the food supply, and the non-advocates think it would be wise to go through a mandatory pre-market approval process and test the milk to make sure it is substantially the same as milk from other Holstein cows that have their horns. Time will decide which ethical issue prevails: humane creation of hornless cattle or changes in regulations that require more testing than is currently required by regulatory agencies.

As yet another modern example of animal biotechnology, teams in the United Kingdom and the United States have been working on growing beef muscle cells in laboratory dishes to create cultured meat that can be used to make lab-grown burgers (**see Figure 1.10**). So far, the price tag for this meat is very high—about $18,000 a pound compared to lean ground beef, which sells for about $5.00 a pound. However, producing lab-grown meat could have tremendously positive impacts, considering the environmental harm caused by modern methods of running large animal farms and meat processing plants.

Forensic Biotechnology

In Chapter 8, we discuss forensic biotechnology. **DNA fingerprinting**—a collection of methods for detecting an organism's unique DNA pattern—is a primary tool used in forensic biotechnology. Forensic biotechnology is a powerful tool that allows law enforcement to examine DNA evidence that can lead to the inclusion or exclusion of a person with regard to suspicion. DNA fingerprinting can be accomplished using trace amounts of tissue, hair, blood, or body fluids left behind at a crime scene. It was first used in 1987 to convict a rapist in England but is now routinely used to provide evidence in court cases throughout the world to convict criminals as well as to free those wrongly accused of a crime.

DNA fingerprinting has many other applications, including use in paternity cases for pinpointing a child's father, identifying human remains, and even DNA testing of esteemed wines to certify their grapes of origin. Another application is the DNA fingerprinting of endangered species. This has already reduced poaching and led to convictions of criminals by analyzing the DNA fingerprints of their "catches." Scientists also use DNA fingerprinting to track and confirm organisms that spread disease, such as *Escherichia coli* in contaminated meat, and to track diseases such as AIDS, meningitis, tuberculosis, Lyme disease, and West Nile virus infection.

Liquid biopsies for cancer detection and monitoring are becoming a big area of forensic interest. (**Figure 1.11** illustrates new genetic markers that can be used to detect bladder cancer). Guardant Health, Exosome Diagnostics, and Illumina have invested millions in this blood-based analysis process. The San Diego–based Illumina announced in 2016 that its blood tests should reach the market by 2019, and cost around $1,000 or less. The new testing concept is referred to as "liquid biopsy." The technique uses "high-speed DNA sequencing machines to scour a person's blood for fragments of DNA released



FIGURE 1.10 Cultured Cells Produce Burger in a Dish Cultured beef muscle cells have been used to create meat with varied colors and incorporated fat cells to improve taste. Shown here is a burger made from cultured beef.

by cancer cells," *MIT Technology Review* reports. "If DNA with cancer-causing mutations is present, it often indicates a tumor is already forming, even if it's too small to cause symptoms or be seen on an imaging machine." The new tests will need to differentiate between mutated cells in the bloodstream caused by cancer, and mutations caused by polyps or moles, which can resemble tumors. The tests will require FDA approval but are expected to improve cancer diagnosis.

Bioremediation

In Chapter 9, we discuss **bioremediation,** the use of biotechnology to process and degrade a variety of natural and human-made substances, particularly those that contribute to environmental pollution. Bioremediation is being used to clean up many environmental hazards that have been caused by industrial progress. One of the most publicized examples of bioremediation in action occurred in 1989 following the *Exxon Valdez* oil spill in Prince William Sound, Alaska (**Figure 1.12**). By stimulating the growth of oil-degrading bacteria, which were already present in the Alaskan soil, many miles of shoreline were cleaned up nearly three times faster than they would have been had

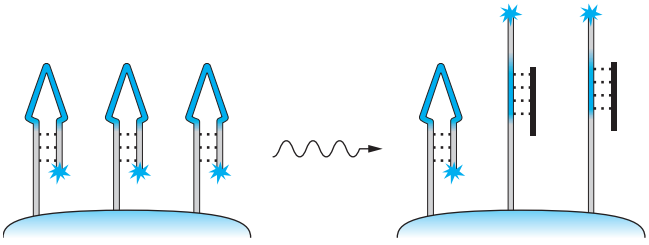


FIGURE 1.11 DNA Detection of Markers for Disease Has Expanded DNA Forensics Bladder cancer is the fourth most common form of cancer, and now microscopic detection has been aided by DNA marker detection. Three genes can be used as indicators of certain types of bladder cancer. Detection of these genes simultaneously in urine can be obtained with 90.9 percent accuracy. In the illustration, when tumor DNA is present, it combines with bound complementary DNA detectors to release a signal.



FIGURE 1.12 Bioremediation in Action Strains of the bacterium *Pseudomonas* were used to help clean Alaskan beaches following the *Exxon Valdez* oil spill. Scientists on this Alaskan beach are applying nutrients that will stimulate the growth of *Pseudomonas* to help speed up the bioremediation process.

chemical cleaning agents alone been used. As you will learn, the rapid degradation by microbes of the dispersed oil droplets from the *Deep Water Horizon* spill in 2010 enabled research into natural oil-degrading organisms and the enzymes that may be used to clean a future spill.

In this chapter we provide an overview of basic applications of bioremediation involving microbes, plants, and genetically engineered organisms to clean up different materials in various environmental conditions; discuss advantages and challenges of bioremediation approaches; and consider the possibility that energy can be derived from degrading waste, among other topics.

Aquatic Biotechnology

In Chapter 10, we explore the vast biotechnology possibilities offered by water—the medium that covers the majority of our planet. One of the oldest applications of aquatic biotechnology is **aquaculture,** raising finfish or shellfish in controlled conditions for use as food sources. Aquaculture is growing in popularity throughout the world, especially in countries such as Israel and Vietnam. Israel cultivates freshwater species like tilapia, carp, and mullet while Vietnam focuses on marine fish such as cobia, seabass, and grouper. It has been estimated that approximately 50 percent of all fish consumed by humans worldwide are produced by aquaculture.

In recent years, a wide range of fascinating new developments in aquatic biotechnology have emerged. These include the use of genetic engineering to produce disease-resistant strains of oysters and vaccines against viruses that infect salmon and other finfish. In 2015, the FDA approved AquAdvantage® salmon as the first GM animal for human consumption. As you will learn in Chapter 10, these transgenic salmon overproduce growth hormone, leading to extraordinary growth rates over short growing periods and thus decreasing the time and expense required to grow salmon for market sale (**Figure 1.13**). These salmon are among the most controversial biotechnology applications in recent years.

The uniqueness of many aquatic organisms is another attraction for biotechnologists. In our oceans, marine bacteria, algae, shellfish, finfish, and countless other organisms live under some of the harshest conditions in the world. Extreme cold, pressure from living at great depths, high salinity, and other environmental constraints are hardly a barrier because aquatic organisms have adapted to their difficult environments. As a result, such organisms are thought to be rich and valuable sources of new genes, proteins, and metabolic processes that may have important human applications and benefits. *Bioprospecting* efforts are ongoing around the world to identify aquatic organisms with novel properties that may be exploited for commercial purposes. For instance, certain species of marine plankton and snails have been found to be rich sources of antitumor and anticancer molecules. Intensive research efforts are under way to better understand the wealth of potential biotechnology applications that our aquatic environments may harbor.

Medical Biotechnology

In Section 1.1, we introduced the concept that many recombinant proteins are being manufactured for human medical applications; however, this is just one example of **medical biotechnology.** Chapter 11 covers a wide range of different applications. From detecting and diagnosing disease conditions through genetic testing and genome sequencing to innovative treatments of human disease conditions, including drug development, gene therapy, and immunotherapy, medical biotechnology has resulted in an amazing array of applications designed to improve human health. Over 325 million people worldwide have been helped by drugs and vaccines developed through biotechnology. Although many powerful applications have already been designed and are currently being applied, the biotechnology century will see some of the greatest advances in medical biotechnology in history.

It seems as though hardly a week goes by without news of a genetic breakthrough such as the discovery of a human gene involved in a disease process. Television, newspapers, journals, electronic news sources, social media, and popular websites all report important discoveries of new genes, genomic analysis, and other



FIGURE 1.13 Genetically Engineered Salmon Became the First GM Animals Approved for Human Consumption
Genetically modified salmon (top) bred to grow to adult size for market sale in half the time of a non-GM salmon (bottom).



FIGURE 1.14 Genes and Genomes Are Headline News Items Television, newspaper, magazine and electronic news and social media headlines frequently report the discovery of genes involved in human disease conditions and many other new developments involving DNA, including genome analysis.

headlines involving DNA (Figure 1.14). Every day, new information about human genetics is helping scientists identify defective genes and decipher the details of genetic diseases such as Alzheimer's and Parkinson's disease, sickle cell anemia, Tay-Sachs disease, cystic fibrosis, cancer, and infertility, to give just a few examples. The Human Genome Project has resulted in new techniques for genetic testing to identify defective genes and genetic disorders, including the ability to sequence and analyze entire genomes from individuals, and we explore many of these techniques in this book.

Gene therapy approaches, in which genetic disease conditions can be treated by inserting normal genes into a patient or replacing diseased genes with normal genes, are expanding at a rapid rate. In Chapter 11 and throughout the book you will learn about a powerful new approach called **CRISPR-Cas**, which is transforming the way scientists can modify a genome. CRISPR-Cas is a technique for genome editing that provides molecular scissors of sorts to cut out and replace a specific sequence of DNA. Promising studies with genome editing are under way, as are human trials using genome editing for gene therapy to treat diseases. As you will learn, applications of genome editing are also very controversial.

Stem cell technologies continue to be among the most promising aspects of medical biotechnology, but they are also among the most controversial topics in all of science. **Stem cells** are immature cells that have the potential to develop and specialize into nerve cells, blood cells, muscle cells, and virtually any other type of cell in the body. Stem cells can be grown in a laboratory and, when treated with different types of chemicals, can be coaxed to develop into different types of human tissue that might be used in transplantation to replace damaged tissue. There are many exciting potential applications for stem cells, but, as we discuss in the next section and in Chapters 11 and 13, many complex scientific, ethical, and legal issues surround their use.

We will also explore exciting new approaches that combine gene therapy and stem cell work to harness and direct the immune system for intriguing new applications called **immunotherapy** to treat disease such as certain types of cancer. Stay tuned—this is an extraordinarily exhilarating time to be studying biotechnology.

Biotechnology Regulations

An essential aspect of the biotechnology business involves the regulatory processes that govern the industry. In much the same way as pharmaceutical companies must evaluate their drugs based on specific guidelines designed to maximize the safety and effectiveness of a product, most biotechnology products must also be carefully examined before they are available for use.

Biotechnology regulations extend to the international level across a range of domains including food and feed safety, safe handling, transfer and use of biological materials, and protection of intellectual property rights. These regulations are primarily overseen by international organizations such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). In the United States, the Food and Drug Administration (FDA) sets the standards for biotechnology products, many times the U.S. Department of Agriculture (USDA) and the Environmental Protection Agency (EPA) are involved. In fact, it has been said that biotechnology is one of the most heavily regulated industries.

From procedures designed to ensure that biotechnology products meet strict standards for purity and performance to issues associated with granting patents and abiding by the regulatory processes required for clinical trials of biotechnology products in human patients, we consider these and other international biotechnology regulatory issues in Chapter 12.

The Biotechnology "Big Picture"

Although we have described different types of biotechnology as distinct disciplines, do not think about biotechnology as a field with separate and unrelated disciplines. It is important to remember that almost all areas of biotechnology are closely interrelated. For example, applications of bioremediation are heavily based on using microbes (microbial biotechnology) to clean up environmental conditions. Even medical biotechnology relies on the use of microbes to produce recombinant proteins, and all branches of biotechnology are regulated. A true appreciation of biotechnology involves understanding the biotechnology "big picture"—how biotechnology involves many different areas of science and how different types of biotechnology depend on each other. This interdependence of many areas of science will be put to the test in solving important problems in the twenty-first century.

A growing area of biotechnology that we do not address in a separate chapter but that exemplifies interdisciplinary science is **industrial biotechnology**—the application of biotechnology to industrial processes such as manufacturing. One key benefit is making products quickly, at reduced costs, in cleaner ways that are more environmentally friendly than traditional production processes. Many applications of industrial biotechnology involve the production of enzymes by microbes. These enzymes are used as *biocatalysts* to speed up chemical reactions.

One of the earliest, most well-publicized examples of industrial biotechnology was implemented in the 1970s to reduce water pollution problems caused by the use of phosphates in laundry detergent. As we discuss in Chapter 5, biotechnology companies created enzymes to remove stains from clothing instead of using phosphates for stain removal. This resulted in a product that cleans clothes at lower water temperatures and at reduced energy costs while dramatically decreasing phosphate-related algal blooms in water.

Some believe that the potential global impact of industrial biotechnology is greater than even that of medical biotechnology and agricultural biotechnology, and that it will be the next big area for innovation in biotechnology. Industrial biotechnology incorporates research from many different fields including genomics, proteomics, bioinformatics, and microbial biotechnology, and typically incorporates microorganisms such as bacteria, yeasts, and fungi. In some cases, natural enzymes are used; in other cases, new enzymes can be bioengineered with specific biocatalytic capabilities for certain industrial processes. We provide an introduction to the concept of industrial biotechnology here and encourage you to think about potential applications in this field as you learn about different topics throughout your studies of biotechnology.

1.3 What Will the New Biotechnology Century Look Like? An Example from Medical Biotechnology

Numerous problems and challenges have the potential to be solved by biotechnology. For many of the greatest challenges—such as curing life-threatening human diseases—the barriers to overcoming these challenges are not insurmountable. Answers lie in our ability to better understand biological processes and to design and adapt biotechnological solutions. Rather than speculating about all of the ways that biotechnology may affect society in this century (an

impossible task!), in this section we entice you with a few ideas on how medical biotechnology in particular is currently saving lives and will continue to do so in powerful ways in the years ahead.

As you read this example, do not focus on understanding the scientific details of the scenarios we describe. Instead, consider how each application can improve the quality of life for individuals so that you can develop a big picture view of the power of biotechnology.

A Scenario in the Future: How Might We Benefit from the Human Genome Project?

History will show that 2001 was a landmark in the biotechnology time line. In February 2001, some of the world's best-known molecular biologists gathered at a press conference to announce the publication of the rough draft of the human genome, a major accomplishment of the Human Genome Project. The DNA sequence—read as the letters A, G, C, and T—of human chromosomes was almost complete.

Identifying the chromosomal location and sequencing of all genes in the human genome has greatly increased our understanding of the complexity of human genetics. Basic research on the molecular biology and functions of human genes and controlling factors that regulate genes is providing immeasurable insight into how genes direct the activities of living cells, how normal genes function, and how defective genes are the molecular basis of many human disease conditions. Ultimately, an advanced understanding of human genetic disease conditions is intended to provide novel cures, and such information is already transforming medicine as it is currently practiced, and will provide even more powerful new therapies and cures in the future. A new era of medicine is on the horizon.

Understanding the human genome is not the "biological crystal ball" that will immediately solve all of our medical problems. A better understanding of human disease will require that we understand the structures and functions of the proteins that genes encode, the **proteome,** the collection of proteins responsible for human cells. But neither the genome nor the proteome is a software program that predetermines our health and our lives. Unlocking the mysteries of the human genome and human proteome alone makes the twenty-first century a most exciting time in which to be part of the scientific discovery process.

Imagine the following scene. A woman seeks advice at a local pharmacy. She recently switched from one major arthritis drug to another, and the current drug is not working any better than the first to alleviate her arthritis. She tells her pharmacist, "This drug is so expensive and doesn't work any better for me than the last one, but I don't want to waste it or throw it out." "Well, sometimes the drugs don't work for everyone," says the pharmacist. This exchange represents one difficulty inherent in most health care strategies.

Many people currently experience the same problem that the woman at the pharmacy encountered. The standard over-the-counter or routinely prescribed treatments available for arthritis and a host of other medical problems rarely work in the same way for everyone. Some drugs work for only some patients and in others have little to no effect. We use an arthritis example here but you could substitute almost any medical condition of choice. Does this sound familiar? Have you or someone you know had an experience like this with your doctor or a pharmacist?

How will the biotechnology century help this patient? How might this conversation be different in the future? Genome information has and will continue to result in the rapid, sensitive, and early detection and diagnosis of genetic disease conditions in humans of all ages, from unborn children to the elderly. In arthritis, we know there are different forms of this disease that have similar symptoms, and genetic analysis has revealed that these different forms of arthritis are caused by these genes.

Let us consider how identifying the genes causing arthritis in our imaginary patient might help her. From its inception, the Human Genome Project yielded immediate dividends in our ability to identify and diagnose disease conditions. The identification of disease genes has enabled scientists and physicians to screen for a wide range of genetic diseases through genetic testing. But, increasingly, sequencing entire individual human genomes, or **personal genomics,** accurately and at relatively low cost (although most insurance companies do not yet pay for genomic analysis) is being implemented. As one example of how genetic testing and personal genomics can aid in the diagnosis of genetic disease conditions, these applications can use **single-nucleotide polymorphisms (SNPs;** pronounced "snips"). SNPs are single-nucleotide changes, or **mutations,** in DNA sequences that vary from individual to individual (**Figure 1.15**). These subtle changes represent one of the most common examples of genetic variation in humans.

SNPs are the cause of some genetic diseases such as sickle cell anemia. SNPs associated with arthritis and many other conditions, such as stroke, cancer, heart disease, diabetes, and behavioral and emotional illnesses, have been discovered. Testing or sequencing one's DNA for different SNPs is one way to identify the specific disease genes that a person may be carrying.

The discovery of SNPs is partially responsible for the emergence of a field called **personalized** or

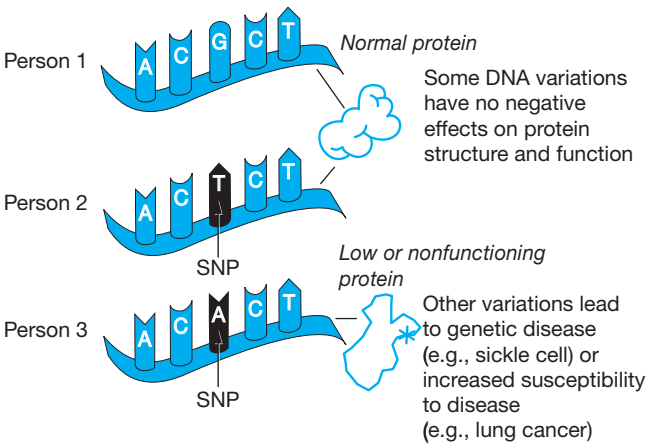


FIGURE 1.15 Single-Nucleotide Polymorphisms A small piece of a gene sequence for three different individuals is represented. For simplicity, only one strand of a DNA molecule is shown. Notice how person 2 has a SNP in this gene, which has no effect on protein structure and function. Person 3, however, has a different SNP in the same gene. This subtle genetic change may affect how this person responds to a drug, or it may influence the likelihood that person 3 will develop a genetic disease.

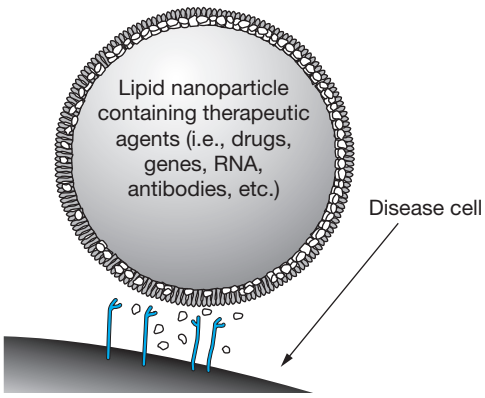


FIGURE 1.16 Nanotechnology in Action Nanoparticles containing drugs, which could also include genes, RNA, antibodies, and other therapeutic molecules, can be specifically directed to target disease cells by targeting specific proteins on the surface of those cells. In this way, drugs that cannot pass through the cell membrane can be released inside these target cancer cells.

precision medicine (this has also been referred to as **pharmacogenomics** in the past). Precision medicine is customized medicine. One aspect of precision medicine involves tailor-designing drug therapy and treatment strategies based on the specific genetic profile of a patient—that is, using his or her genetic information to determine the most effective and specific treatment approach (refer to Figure 11.8).

Can precision medicine help our arthritis patient? We know that arthritis is a disease that shows familial inheritance for some individuals and, as mentioned earlier, a number of different genes are involved in different forms of arthritis. In many other cases of arthritis, a clear mode of inheritance is not seen. There are likely additional genes or nongenetic factors, such as exercise and diet, that influence the severity of arthritis. A simple blood test from our patient could be used to prepare DNA for genetic analysis to determine which genes are involved in the form of arthritis that this woman has. Armed with this genetic information, a physician could design a drug-treatment strategy—based on the genes involved—that would be *specific* and *most effective* against this woman's type of arthritis. A second woman with a different genetic profile for her particular type of arthritis might undergo a different treatment than the first, and a man with arthritis must have a very different treatment strategy based on his genetic profile. This is the power of precision medicine in action.

Researchers can now study massive databases that combine genetic information, medical records, wearable devices, and patient surveys on diet, exercise, and side effects. These studies will result in the need for more diagnostic tools and precision-guided therapies that only biotechnology can provide. Much of this information will be available and provided directly to the patient through a smartphone, tablet, or other device, and increasingly in the future such devices will be used as diagnostic tools to monitor key vital signs (see Figure 1.1b).

Treating our arthritis patient might involve **nanotechnology,** or applications that incorporate extremely small devices (a "nano" scale). Nanotechnology is a relatively new field that has rapidly emerged as a major research area. One promising application of nanotechnology related to medical biotechnology has been the development of small particles that can be used to deliver drugs to cells (**Figure 1.16**). For our arthritis patient, these nanoparticles might deliver drugs, genes, RNA, antibodies, or even immune cells to help combat arthritis.

In addition to advances in drug treatment, gene therapy represents one of the ultimate strategies for combating genetic disease. **Gene therapy** technologies involve replacing or augmenting defective genes with normal copies of them. Scientists are working on a variety of ways to deliver healthy genes into humans and ways to replace defective genes, including through approaches we mentioned previously, such as genome editing. Think about the potential power of these approaches. Could gene therapy be used to treat or cure our arthritis patient? Many promising new gene therapy applications have recently emerged and you will learn about some of these in Chapter 11. However, many barriers must be overcome before gene therapy becomes a safe, practical, effective, and well-established approach to treating many different diseases.

Stem cell technologies are expected to provide powerful tools for treating and curing disease. Stem cells are immature cells that can grow and divide to produce different types of cells, such as skin, kidney, and blood cells. Stem cells can be coaxed to form almost any tissue of interest, depending on how they are treated. Cultured cells and tissues derived from stem cells are valuable for drug testing. "Organs on a chip" have been developed for most of the major organs in the body (see Chapter 7) and now make it possible to study drug interactions before the drugs are administered to patients (or test animals).

Imagine growing skin cells, blood cells, and even whole organs in the lab and using these to replace damaged tissue or failing organs such as the liver, pancreas, and retina (Figure 1.17). **Regenerative medicine** is the phrase used to describe this approach. In the future, scientists may be able to collect stem cells from patients with genetic disorders, genetically manipulate these cells by gene therapy, and reinsert them into the patient from whom they were collected to help treat genetic disease conditions. Stem cells are already being used to treat arthritis patients, but unfortunately most of these applications are highly unregulated and have not been rigorously tested by the scientific or medical communities. As you will learn in Chapter 11, recently, a young boy with a deadly skin condition caused by a genetic disorder underwent a remarkable treatment that combined gene therapy to correct a mutation and regenerative medicine to restore healthy skin to over 80 percent of his body.

Imagine the near future when whole-genome analysis and precision medicine, including specific drug and gene therapies, are routinely offered at your doctor's office. The future is not far away. We hope that the brief examples provided in this section demonstrated how the future is indeed bright for marvelous advances in medical biotechnology, and that at this early stage in your studies you look forward to learning much more about these and other applications.

Here we have discussed basic examples of medical applications in the biotechnology century, but in future chapters you will learn about exciting applications from other areas of biotechnology that will potentially change our lives for the better. Keep in mind that through biotechnology, many seemingly impossible problems may not be so insurmountable in the future. There is no better time to be part of biotechnology! We conclude our introduction to the world of biotechnology by discussing career opportunities in the industry.

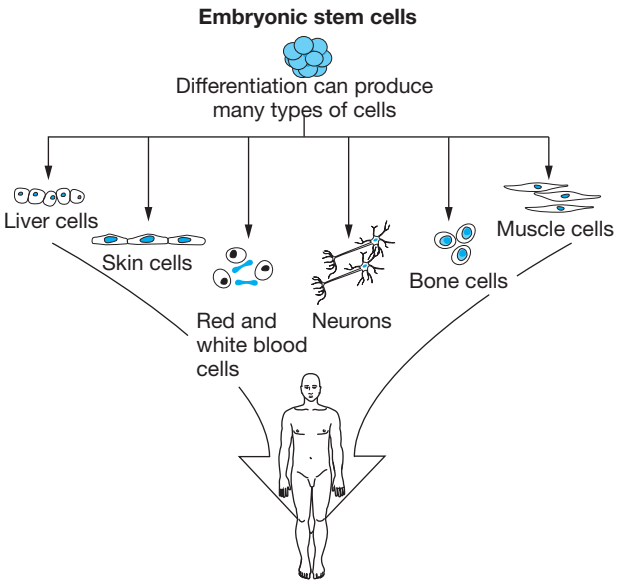


FIGURE 1.17 Embryonic Stem Cells Can Give Rise to Many Types of Differentiated Cells Embryonic stem cells (ESCs) are derived from embryos or early-stage fetuses; they are immature cells that can be stimulated to differentiate into a variety of cell types.

1.4 The Biotechnology Workforce

How will the world be preparing for the biotechnology century? Recent advances have created a range of new opportunities for biotechnology companies and individuals seeking employment in the biotechnology industry (Figure 1.18).

Ultimately, biotechnology companies are looking for people who are comfortable analyzing complex



FIGURE 1.18 The Biotechnology Industry Provides Exciting Opportunities for Many Types of Scientists From biologists and chemists to engineers, information technologists, and salespeople, the biotechnology industry offers a great range of high-tech employment opportunities. Shown here is a senior undergraduate student working on a biotechnology research project. Gaining research experience as an undergraduate is an excellent way to prepare for a career in biotechnology.

data and sharing their expertise with others in team-oriented, problem-solving work environments. The biotechnology workforce depends on important contributions from talented people in many different disciplines of science.

The Business of Biotechnology

In 1976, Genentech Inc., a small company near San Francisco, California, was founded. Genentech, part of the Roche Group since 2009, is generally recognized as the first biotechnology company, and its success ushered in the birth of this exciting industry with the release of recombinant human insulin—offering the first opportunity for diabetic persons to receive this protein. Today, biotechnology is a global industry with hundreds of products on the market.

Sales of therapeutic biological drugs (biotherapeutics) such as enzymes, antibodies, growth factors, vaccines, and hormones, including many recombinant proteins, in the United States are a significant part of this revenue. Annual sales of biotherapeutics are in excess of $225 billion worldwide and doubling about every 5 years! Many biotechnology companies are working on cures for cancer. It is the first or second leading cause of death before the age of 70 years in 91 of 172 countries, and it ranks third or fourth in an additional 22 countries, according to a study conducted by WHO in 2015. Over 350 biotechnology products are currently in development, targeting cancers, diabetes, heart disease, Alzheimer's and Parkinson's diseases, arthritis, AIDS, and many other diseases. Recombinant crops and seeds, and industrial products such as biofuels, are other large categories of revenue for biotechnology companies globally.

Top Regions for Biotechnology Jobs

North America, Europe, and Japan account for approximately 95 percent of the world's biotechnology companies, but biotechnology firms are found throughout the world in more than 54 countries. Countries without a traditional history in research and development (R/D) worldwide are turning to biotechnology for high-tech innovations. For example, biotechnology is a rapidly developing industry in India and China. Clustering of biotechnology companies happens close to public institutions of research where basic science ideas for biotechnological applications are generated.

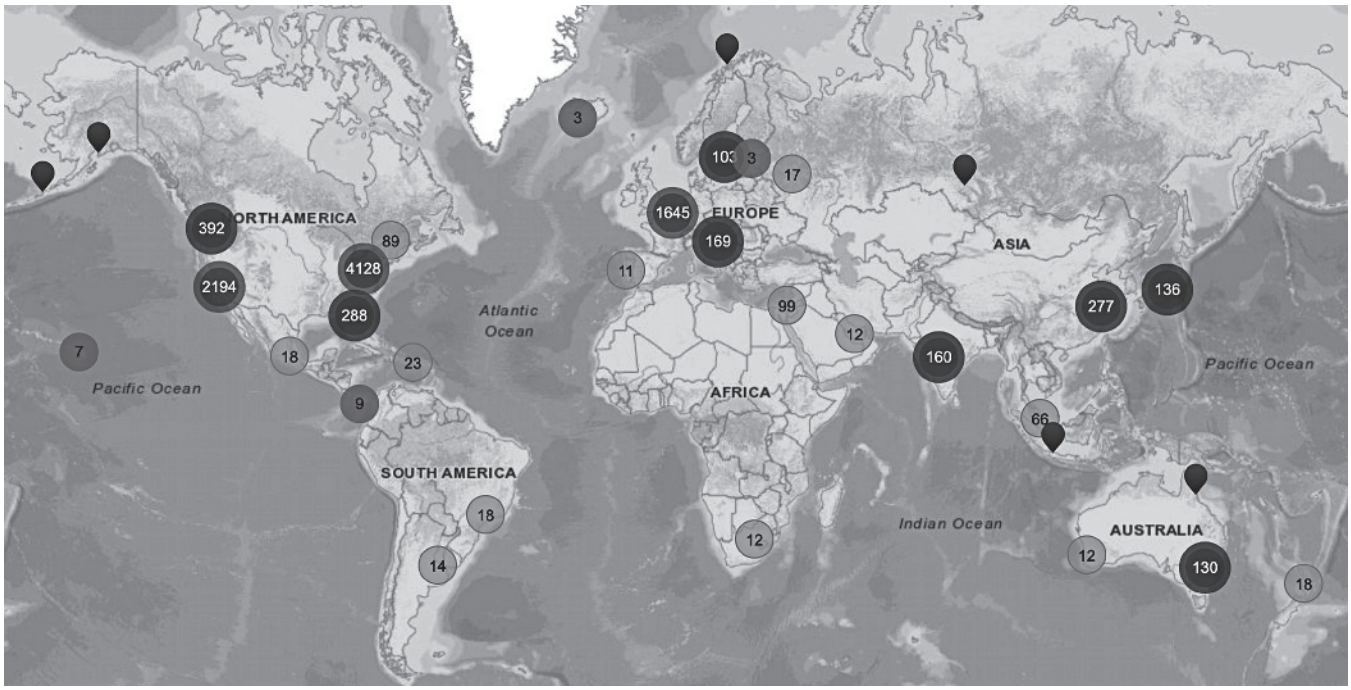


FIGURE 1.19 Biotech Companies around the World As of May 27, 2019, this map captures 10068 biotech employer locations worldwide. This interactive map is publicly available at https://www.biotech-careers.org/. Upon entering the website, you can click on the number within any circle to find out detailed information about the biotechnology companies in that region.

The United States continues to be a world leader in biotechnology. There are currently around 700 public biotechnology companies in the United States (and many other private companies). When the biotechnology industry first began, most companies were located in relatively small clusters or hubs in cities such as San Francisco and Boston. While individual rankings of states can vary from year to year, there are now clusters of biotechnology companies distributed across the United States.

The United Kingdom is the leading biotech cluster in Europe. The country offers over 12,0000 biopharma jobs and it also leads Europe in research funding. Germany has also become a top-tier cluster with one of the leading biotech companies, BioNTech, collaborating with Pfizer to develop mRNA-based flu vaccines in 2018. France has ranked third in biopharma employment in Europe. According to France Biotech, the country offers around 20,000 biotech jobs. The Netherlands continues to compete with the big players in the biotech industry. A lot of multinational companies like Johnson & Johnson and GlaxoSmithKline have chosen the Netherlands for their global operations. European countries like Spain, Switzerland, Belgium, Italy, and Denmark have also emerged as biotechnology hubs (see Figure 1.19).

We offer this perspective on the distribution of biotech clusters to emphasize that biotechnology employment opportunities are available across the world and are not restricted to just a few areas. So, if you want to work in the biotechnology industry, you can find job opportunities almost anywhere.

Visit the Biotech Careers website listed on the Companion Website to find biotechnology companies located around the world and to learn about their current products and career opportunities.

What Is a Biotechnology Company?

By now you may be wondering, "What is the difference between a biotechnology company and a pharmaceutical company?" Most people can name pharmaceutical companies such as Merck, Johnson & Johnson, or Pfizer because they or a family member may have used one or more of their products, but most people cannot name a biotechnology company (Table 1.3) or explain why a biotech company is different from a pharmaceutical company. The large pharmaceutical companies are commonly referred to as "big pharma." Generally speaking, **pharmaceutical companies** are involved in drug development by chemically synthesizing or purifying compounds used to make the drug—products such as aspirin, antacids, and cold medicines. Pharmaceutical companies typically do not use living organisms to grow or produce a product (such as a recombinant protein), as is the focus of biotechnology companies.

But for the past few decades the distinctions between the two industries are blurred, because many large pharmaceutical companies are often involved in biotechnology-related research and product development either directly or indirectly by partnering with a biotechnology company. Also remember that biotechnology involves much more than drug development. There are many different companies of varying sizes dedicated to working on specific areas of biotechnology.

TABLE 1.3 **Top Five Biotechnology Companies and Top Five Pharmaceutical Companies by Revenue in 2017**

Biotech Companies	Revenue (Billions)
Amgen	$129
Gilead Sciences	$103
Novo Nordisk	$ 96
Celgene	$ 77
Biogen	$ 66
Pharma Companies	**Revenue (Billions)**
Johnson & Johnson	$379
Novartis	$216
Pfizer	$208
Roche	$199
AbbVie	$153

Adapted from *Genetic Engineering & Biotechnology News*, https://www.genengnews.com/the-lists/top-25-biotech-companies-of-2017/77901002 and https://www.genengnews.com/the-lists/top-10-pharma-companies-of-2017/77901005. Revenue based on preliminary results reported by companies.

Biotechnology companies vary in size from small companies of less than 50 employees to large companies with over 300 employees. Historically, many biotechnology companies began as small **startup companies** formed by a small team of scientists who believe that they might have a promising product to make (such as a recombinant protein to treat disease). The team must typically then seek investors to fund their company so that they can buy or rent lab facilities, buy equipment and supplies, and continue the research and development necessary to make their product. But starting a biotechnology company is risky business; on average, a lot of startup companies close without providing any return to investors.

Biotechnology startup companies rely on financial investments or **capital** in the company, such as **venture capital (VC),** funds provided at an early stage to startup companies with a potential for success. Sources of VC funds can be individuals, endowments, foundations, financial institutions, and other companies, for example. Venture capital funds make money by owning equity in startup companies that have a promising

technology to develop. **Angel investors**—affluent individuals who provide capital for a startup in exchange for company ownership—are key to providing startup biotechnology companies with the funds needed to carry out the research and testing necessary to make a product.

VC investments are the essential pipeline of funds that supports biotech companies. During the economic downturn in 2008, as biotechnology VC investments experienced a 46 percent decrease between 2007 and 2008, many biotechnology companies had only enough cash flow for 12 months or less. But since this time, fundraising for biotechnology companies has rebounded fairly well, bolstered by many new innovative products such as biotherapeutics, new gene therapies, and immunotherapies that are showing strong potential for treating patients. Ultimately, most investors in a biotechnology company do so with an expectation that there will be some return on their investment and they will make a profit if the company is successful.

Eventually, if a startup biotechnology company is successful in bringing a product to the market (a process that takes around 10 years on average at a cost, in the United States, of over $2 billion for a medical drug!), many startups are often bought by larger, well-established companies. Bringing a promising product close to market ultimately creates value for a company, which may enable it to file for an **initial public offering (IPO),** which means that it is available for the public to purchase shares of company stock. For example, as biotech company funding rebounded after 2008, in 2013, 24 companies in the United States offered IPOs. Stocks in these companies rose by an average of 20 percent on the first day of trading, generating approximately $1.8 billion for the biotechnology industry.

There are similarities between how pharmaceutical companies and biotechnology companies are organized (Figure 1.20). In Figure 1.20, we provide a basic organizational structure for a typical medium-size

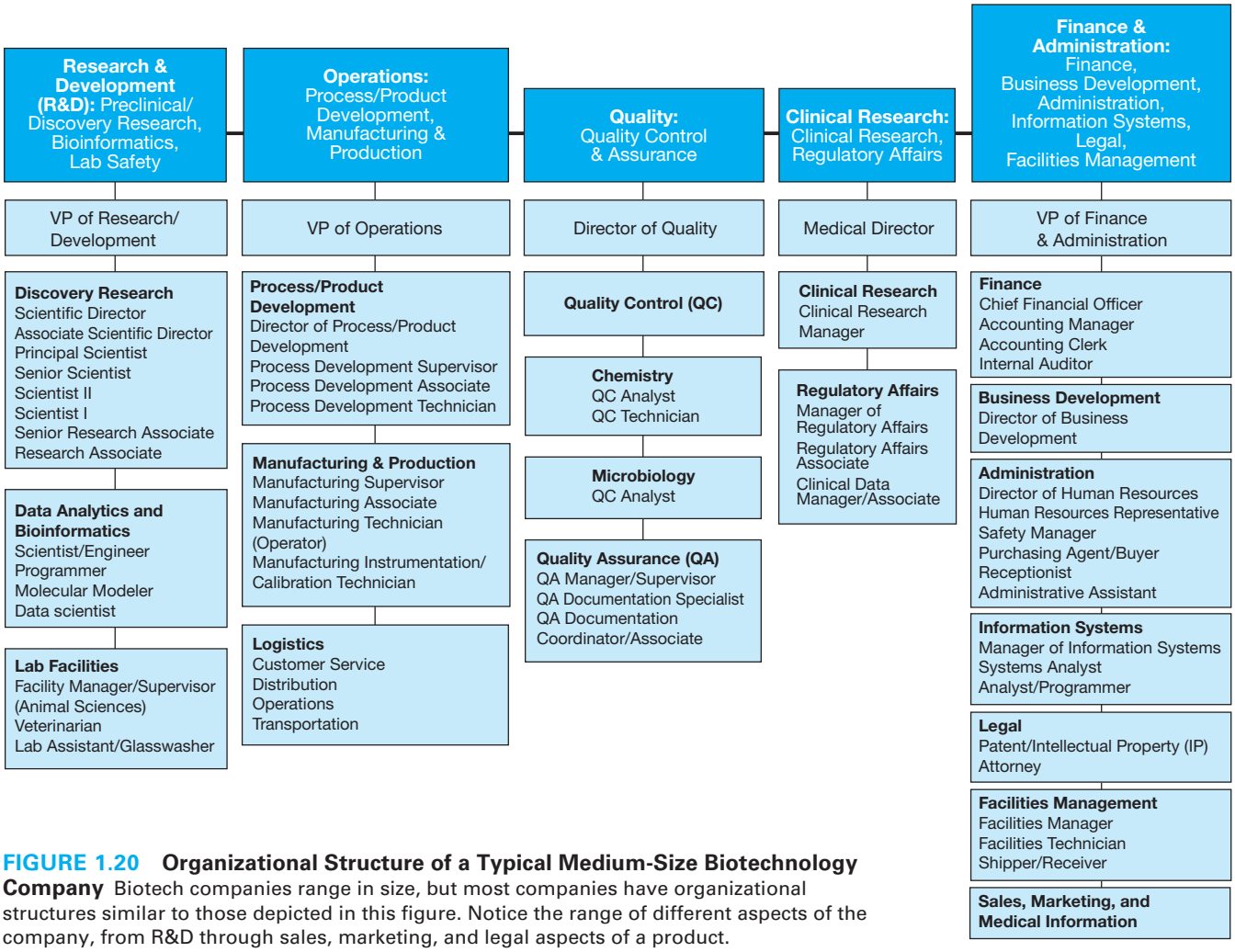


FIGURE 1.20 Organizational Structure of a Typical Medium-Size Biotechnology Company Biotech companies range in size, but most companies have organizational structures similar to those depicted in this figure. Notice the range of different aspects of the company, from R&D through sales, marketing, and legal aspects of a product.

biotechnology company for your reference, to help you appreciate the range of operations and the variety of employees involved in a successful company, beyond just research and development. We discuss many aspects of this in the next section, in which we describe different job opportunities in each area of a biotechnology company.

Jobs in Biotechnology

The biotechnology industry in the United States employs over 200,000 people. Biotechnology offers numerous employment choices, such as laboratory technicians involved in basic research and development, computer programmers, laboratory directors, and sales and marketing personnel. All are essential to the biotechnology industry. In this section, we consider some of the job categories available in biotechnology.

Research and development

Development of a new biotech product is a long and expensive process. Individuals in R&D are directly involved in the process of developing ideas and running experiments to determine if a promising idea (for example, using a recombinant protein from a cloned gene to treat a disease condition) can actually be developed into a product. It requires a great deal of trial and error. From the largest to the smallest biotechnology companies, all have some staff dedicated to R&D. On average, biotechnology companies invest at least four times more on R&D than any other high-tech industry. For some companies, the R&D budget is close to 50 percent of the operating budget. R&D is the lifeblood of most companies—without new discoveries, companies cannot make new products.

The majority of positions in R&D usually require a bachelor's or associate's degree in chemistry, biology, or biochemistry. **Laboratory technicians** are responsible for duties such as cleaning and maintaining equipment used by scientists and keeping labs stocked with supplies. Technician positions usually require a B.A. in science or a B.S. in biology or chemistry. **Research assistants** or **research associates** carry out experiments under the direct supervision of established and experienced scientists. These positions require a B.S. or M.S. degree in biology or chemistry. Research assistants and associates are considered "bench" scientists, carrying out research experiments under the direction of one or more principal or senior scientists. Assistants and associates perform research in collaboration with others. Involved in the design, execution, and interpretation of experiments and results, they may also be required to review scientific literature and prepare technical reports, lab protocols, and data summaries.

Principal or **senior scientists** usually have a Ph.D. and considerable practical experience in research and management skills for directing other scientists. These individuals are considered the scientific leaders of a company. Responsibilities include planning and executing research priorities of the company, acting as spokespeople on company research and development at conferences, participating in patent applications, writing progress reports, applying for grants, and serving as advisers to the top financial managers of the company. The job titles and descriptions we have given can vary from company to company; however, if you are interested in making new scientific discoveries, then R&D might be an exciting career option for you.

Bioinformatics, the use of computers to analyze and store DNA and protein data, requires an understanding of computer programming, statistics, and biology. Until recently, many experts in bioinformatics were computer scientists who had trained themselves in molecular biology or molecular biologists self-trained in computer science, database analysis, and mathematics. Today, people with computer science interests are being encouraged to take classes in biotechnology, and biotechnology students are being encouraged to take computer science classes. In addition, specific programs in bioinformatics have been developed at 4-year colleges and universities, technical colleges, and community colleges to train people to become **bioinformaticists.**

Massive amounts of data being generated by genome projects, drug studies, and other approaches contribute to what is referred to as "**big data.**" Storing, sharing, analyzing, and protecting (cybersecurity) large datasets is a significant challenge. Even having sufficient computing memory is a challenge when data are stored in the cloud. **Data scientists** or **analysts** are needed to keep biotechnology companies from drowning in the ever-increasing sea of data that has inundated modern science. Robust data-mining and data-warehousing systems are essential for everything from R&D to clinical trials and tracking patients' records (including electronic medical records that you may be familiar with from visiting a doctor's office). If you are interested in merging an understanding of biology with computer science skills, then bioinformatics and data analytics may be good career options for you to consider.

Operations, biomanufacturing, and production

Operations, biomanufacturing, and *production* are terms that describe the divisions of a biotechnology company that oversee specific details of product development, such as the equipment and laboratory processes involved in producing a product. This often includes **scale-up processes,** in which cultured cells making up a product must be grown on a large scale. This is not a trivial task. As a simple analogy, scaling-up is the difference between



YOU DECIDE **Generic Biotech Drugs?**

As the biotechnology industry has aged, many of the earlier biotech products that received patent approval have recently lost or will soon lose patent protection. Patents are designed to provide a monopoly right for the developer of an invention, and in the United States patents can last for up to 20 years. Controversy has arisen in the industry over whether many of these products will receive approval to become **generic drugs**. You may already know that generic drugs are copies of brand-name products that generally have the same effectiveness, safety, and quality as the original but are produced at a cheaper cost to the consumer than the brand-name drugs. One way that generics can cut costs is that they are often approved for use without having to undergo the same expensive safety and effects studies required for name drugs.

Many biotechnology companies are fighting the production of generic biotech drugs. Some doubt whether a generic, called a **biosimilar drug** when referring to therapeutic recombinant proteins and other biologically produced proteins such as antibodies, could be made at a greatly reduced price given that it is generally still more expensive to produce a biotech product than a pharmaceutical product and because biosimilars can be difficult to replicate exactly. Biosimilar drugs require the design of different (expensive) processes but must produce the same or better results, rather than simpler pharmaceutical changes in manufacturing. Globally, the biosimilar market value is estimated to exceed $55 billion by 2020. Similar questions about profit can be raised regarding drug costs in developing countries, where many of the people who need drugs cannot afford them, although many companies sell drugs in the developed world at a higher price and use some of these profits to provide drugs at low or no cost to developing nations. How do you think the cost of drugs should be regulated to be fair to consumers and provide an adequate profit for the innovators? You decide.

cooking a meal for yourself and preparing a full-course Thanksgiving dinner for 50 people. Biomanufacturing and production units maintain and monitor the large-scale and large-volume equipment used during production, and they ensure that the company is following proper procedures and maintaining appropriate records for the product. Biomanufacturing job details are specific to the particular product a company is manufacturing. Entry-level jobs include material handlers, manufacturing assistants, and manufacturing associates. Supervisory and management-level jobs usually require a bachelor's or master's degree in biology or chemistry and several years of experience in manufacturing the products or type of product being produced by that company. Manufacturing and production also involve many different types of engineers, including those trained in chemical, electrical, environmental, or industrial engineering. Engineering positions usually require a B.A. degree in engineering or a M.S. degree in biology or an area of engineering.

Quality assurance and quality control

Most products from biotechnology are highly regulated by organizations such as the U.S. FDA and the European Food Safety Authority. These federal agencies require that manufacturing follow exact methods approved by regulatory officials. As discussed previously, the overall purpose of quality assurance (QA) is to guarantee the final quality of all products. Quality control (QC) efforts are designed to ensure that products meet stringent regulations mandated by federal agencies. In addition to guaranteeing that components of the product manufacturing process meet the proper specifications, QA and QC workers are also responsible for monitoring equipment, facilities, and personnel, maintaining correct documentation, testing product samples, and addressing customer inquiries and complaints, along with other responsibilities. Entry-level jobs in QC and QA include validation technician, documentation clerk, and QC inspector. Jobs usually require at least a B.S. degree in biology, and managerial or supervisory positions require more education. **Customer relation specialists** or **product complaint specialists** often work in the QA divisions of a company. One function of such specialists is to investigate consumer complaints about a problem with a product and to follow up with the consumer to provide an appropriate response or solution to the problem encountered.

Clinical research and regulatory affairs

Developing a drug product is a long and expensive process of testing the new drug candidate in volunteer subjects to ultimately receive new drug approval from the FDA. The clinical trial process, along with many other clinical and nonclinical areas of biotechnology, is regulated by a number of different agencies. As a result, every biotechnology company has staff monitoring regulatory compliance to make sure

that proper regulatory procedures are in place and are being followed. Biotechnology companies involved in developing drugs for humans often have very large clinical research divisions with science and nonscience personnel who conduct and oversee clinical trials.

Marketing, sales, finance, and legal divisions

Marketing and selling a variety of biotechnology products, from medical instruments to drugs, is a critical area of biotechnology. Most people employed in biotechnology marketing and sales have a B.S. degree in the sciences and familiarity with scientific processes in biotechnology, perhaps combined with coursework in business or even a B.A. degree in marketing. **Sales representatives** work with medical doctors, hospitals, and medical institutions to promote a company's products. **Marketing specialists** devise advertising campaigns and promotional materials to target customer needs for the products a company sells. Representatives and specialists frequently attend trade shows and conferences. An understanding of science is important because the ability to answer end-user questions is an essential skill in marketing and sales.

Finance divisions of a biotechnology company are typically run by vice presidents or chief financial officers who oversee company finances and are also often involved in raising funds from partners or venture capitalists seeking investments in technology companies. **Legal specialists** in biotechnology companies typically work on legal issues associated with product development and marketing, such as copyrights, naming rights, and obtaining patents. These are essential issues for protecting the ideas and products that a biotechnology company works so hard to develop (see Chapter 12 for further discussion). Staff in this area will also address legal circumstances that may arise if there are problems with a product or litigation from a user of a product.

Salaries in Biotechnology

People working in the biotechnology industry are making groundbreaking discoveries that fight disease, improve food production, clean up the environment, and make manufacturing more efficient and profitable. Although the process of using living organisms to improve life is an ancient practice, the biotechnology industry has been around for only about 40 years, so it is still a relatively new industry. As an emerging industry, biotechnology offers competitive salaries and benefits, and employees at almost all levels report high job satisfaction.

Salaries for life scientists who work in the commercial sector are generally higher than those paid to scientists in academia (colleges and universities). Scientists working in the biotechnology industry are among the most highly paid of those in the professional sciences.

According to a survey of more than 400 biotechnology companies conducted by the Radford Division of AON Consulting, a person with a Ph.D. in biology, chemistry, and molecular biology with no work experience was starting at an average annual salary of $55,700, with senior scientists earning in excess of $120,000 a year. For individuals with an M.A. degree in the same fields, the average salary was $40,600 annually, with a range from $60,000 to $70,000 per year for research associates and $43,520 annually for those with a B.A. degree, with a range of $52,000 to $62,000 per year for research associates. Visit the Web Links provided for Chapter 1 at the Companion Website for references to current salary figures in the biotechnology industry.

Based on a national survey, 56 percent of the college students entering biotechnology training programs had little or no science background. If you have the proper background in biology and good lab skills, many good positions are available at many different levels, but increasingly educational training at the community college, technical college, 4-year college, or university level is becoming a requirement for employment in biotechnology.

Hiring Trends in the Biotechnology Industry

Career prospects in biotechnology are very good. The industry has more than tripled in size since 1992. Increased pricing pressures, patent expirations, increasing costs to develop new medicines, and costly instances in which drugs have failed in later stages of clinical trials have resulted in changes in drug development. As patents expire on drugs designed for large populations (blockbuster drugs), research and development investments have shifted to biological molecules that address serious diseases. Companies have shifted emphasis through mergers and acquisitions, emphasized their affiliations with universities and biotech hubs, and aligned their strengths through asset swaps between companies. Therapeutics for rare diseases have helped some companies owing to the FDA's Orphan Drug Act, which allowed quick approval and provided opportunities for expansion to a more mainstream population of patients with adaptations.

Another trend that has reached a stage of critical importance is the need for people with multiple skill areas. For instance, an individual with a degree in molecular biology or biochemistry, a minor in information technology, and coursework in mathematics can potentially have a great advantage in the job market, especially with companies seeking people with unique skill combinations.

In addition to good technical skills and an understanding of business and finance practices, companies also emphasize the importance of "soft skills," which they consider as important at technical skills. These include skills in writing and communication, presenting information to different audiences, and teamwork. Employment prospects in biotechnology are exciting indeed. Opportunities are excellent for individuals with solid scientific training and good verbal and written communication skills coupled with a strong ability to work as part of a team in a collaborative environment.

MAKING A DIFFERENCE

There are many ways to be part of biotechnology and to influence the lives of others, as we will explore at the end of each chapter in the Making a Difference feature. Here is a look at what's in store in the coming chapters. Early success in the use of cell-based beads that release the chemical levodopa into the brains of Parkinson's patients sets the stage for other cell-based therapies (Chapter 2). Recombinant therapeutic proteins such as insulin have improved the quality of life for many humans (Chapter 3). Diagnostic testing for biomarker proteins that reflect the early presence of a disease could boost the search for other treatments, bringing personalized medicine using purified proteins one step closer (Chapter 4). Antibiotics isolated from bacteria are one of the most successful examples of biotech products (Chapter 5). The plant biotech revolution has already occurred in the United States, and the majority of this expansion has occurred in other countries for the past 3 years. These methods have been used in the past to enhance the natural capacities of organisms with selected traits (Chapter 6). Studying the function of drugs in artificial organs (organ on a chip) is reducing the need for testing animals (Chapter 7). DNA forensics has expanded beyond crime scenes to food fraud, DNA diagnostics, and better methods for sensitive identification of smaller quantities of DNA (Chapter 8). Successful cleanup of groundwater pollution is one example of a bioremediation success story (Chapter 9). Novel drugs from aquatic organisms are improving patients' lives worldwide (Chapter 10). Bone marrow transplantations are a successful example of stem cell technologies (Chapter 11). Compliance with regulations that protect companies and employees is a necessary part of biotech and will continue to provide safe products and a safe working environment as well as protecting the intellectual property developed by biotech companies (Chapter 12). Finally, we'll explore many controversial and ethically challenging examples that will put together a range of topics you will learn about throughout the book (Chapter 13). The opportunity to be part of a science that truly makes a difference awaits you in the coming chapters!

QUESTIONS & ACTIVITIES

Answers can be found in Appendix 1.

1. Provide two examples of historical and current applications of biotechnology.
2. Pick an example of a biotechnology application and describe how it has affected your everyday life.
3. Which area of biotechnology involves using living organisms to clean up the environment?
4. Describe how precision medicine will influence the treatment of human diseases.
5. Do an Internet search for "DIY biotechnology" and be sure to visit the sites **biocurious.org** and DIYbio.org. What did you find? What are potential pros and cons of DIY? Consider ethical issues associated with DIY and what regulations might be needed for DIY biotechnology. For example, in 2017 an HIV-positive man injected himself (and live-streamed the event on Facebook) with an unregulated gene therapy intended to eliminate the HIV virus. Explain your answers.
6. Distinguish between QC and QA, and explain why both are important for biotechnology companies.
7. Access the library of the European Bioinformatics Institute (EMBL-EBI) (**https://www.ebi.ac.uk/**) and search for a source-example on adult-derived stem cell re-differentiation to learn more about stem cells. This free source will provide abstracts and titles for full-text articles that can be obtained at other libraries.
8. Visit ScienceDirect and search biotechnology news (**https://www.sciencedaily.com/news/plants_animals/biotechnology/**). Here you will find daily updates on exciting new developments in biotechnology described in student-friendly terms. Find a biotechnology topic that fascinates you and share your newly discovered knowledge with a friend or family member who is unfamiliar with biotechnology.
9. Interacting with others in a group setting is an essential skill in most areas of science. As you learned in this chapter, biotechnology involves groups of scientists, mathematicians, and computing experts with different backgrounds collaborating to solve a problem or achieve a common goal. Discussing biology with other people is fun and beneficial to everyone working on the same problem in a company, and working with other students is an excellent way to help you learn and enjoy your studies in biotechnology. Teaching

someone else is a great way to test your knowledge. Analyze your ability to work in a group by forming a study group for the next test. Assign a group leader who will be responsible for organizing meetings and keeping the study group focused on helping each person to learn. Make sure that everyone has a topic to present to the group and that all of you offer constructive criticism to his or her suggestions. If you cannot get together with your classmates in one room, share your thoughts via e-mail or ask your professor to set up an electronic bulletin board for discussion purposes.

10. The National Library of Medicine is a worldwide database of biology research publications in scientific journals, and it can be accessed for free at **http://www.ncbi.nlm.nih.gov**. Access PubMed and conduct a search on any topic of biotechnology that may be of interest to you to find recent papers on the latest new research developments in biotechnology.

11. As a preview to Chapter 7—Animal Biotechnology—we introduced the idea of culturing meat cells to produce lab-generated burgers. What do you think of this? Why are scientists interested in this application? Explain potential advantages and disadvantages of this approach. Do you consider this an ethically controversial example of biotechnology? If so, why? Explain your answers.

12. Organism cloning has led to concerns that humans may be cloned. In early 2018, it was reported that a research team in Shanghai, China, had cloned monkeys from cells grown in culture in the lab. At the time this book was published, no claims of human cloning had been proven to be valid, and most scientists are adamantly against human cloning. What do you think? Should humans be cloned?

13. Based on what you know so far, what is genome editing? Why do you think genome editing might be a highly controversial and ethically challenging concept in biotechnology?

14. Explain how biotechnology companies get started. Include in your answer a description of how a startup company is funded.

15. Describe differences and similarities between the pharmaceutical industry and the biotechnology industry.

16. Visit the FierceBiotech website (**http://www.fiercebiotech.com**) and sign up to receive free daily alerts on biotech industry news items. This is a great way to learn about exciting new discoveries in biotechnology, biotech company updates, career information, and more.

17. Consumers feel that they have the right to know their genetic health risks and that new diagnostics tests may provide them the therapy choices they desire. The FDA recently approved 10 of the genomics company 23andMe's screening tests, including one for Alzheimer's and one for a rare blood disorder. 23andMe utilizes the data it collects as well as those from the Michael J. Fox Foundation, the Parkinson's Disease Institute, and the biotechnology company Genentech. Together they have found new risk factors for Parkinson's and are exploring how specific genetic variations influence the side effects from some Parkinson's treatments. The FDA has developed Precision FDA, a site where researchers and companies pool data to develop standards for genetic sequencing tools and algorithms. Assess the FDA.gov site for Precision FDA and see if you agree that these screening tests are likely to predict Alzheimer's disease.

18. Drug pricing is a controversial topic in the biotechnology and pharmaceutical industries, with many ethical considerations. Do an Internet search for Martin Shkreli to learn about one of the most well-publicized and extreme recent examples of a drug-pricing controversy.

19. When studying biotechnology, one approach that might help you learn is to consider the problem or question to be addressed; the methods, techniques, or experimental approaches to solve this problem; and the biotechnology application (function, products, or purpose) that results. In your answer, consider potential ethical issues to address and regulations that might be necessary for this application. Apply this approach to something that you learned about in this chapter.

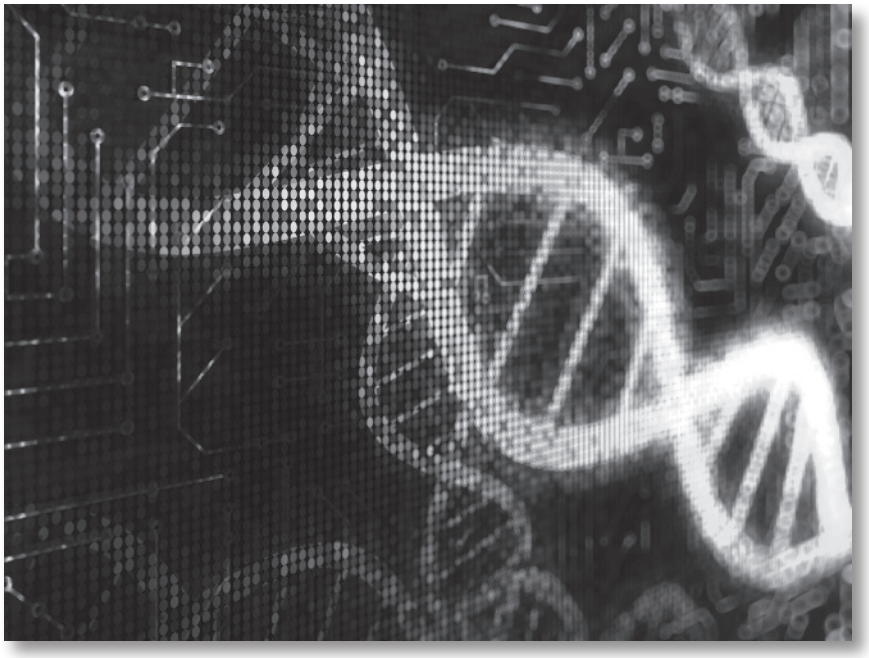
20. Biotechnology has a long and rich history. Future chapters are dedicated to exploring advances in biotechnology and looking ahead to what the future holds. As you study biotechnology, you will be introduced to what may seem to be an overwhelming number of terms and definitions. Be sure to use the index and glossary at the end of the book to help you find and define important terms.

Visit www.pearsonglobaleditions.com for the Companion Website

The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

CHAPTER TWO

An Introduction to Genes and Genomes



Encoded within DNA are genes that provide instructions controlling the activities of all cells. Genes enable the inheritance of traits from generation to generation. Genes influence our behavior; determine our physical appearances, such as skin, hair, and eye color; and can cause or be affected by genetic diseases.

After completing this chapter, you should be able to:

- Compare and contrast prokaryotic and eukaryotic cells.
- Describe the structure of a nucleotide and explain how nucleotides form double-helical DNA molecules.
- Explain the process of DNA replication and discuss the role of key proteins involved.
- Understand what genomes are and why biologists study them.
- Describe the process of transcription and understand how mRNA processing creates a functional mRNA molecule.
- Describe the process of translation, and understand the roles of mRNA, tRNA, and rRNA.
- Explain why noncoding RNAs are important to cells.
- Explain why gene expression regulation is important, and be familiar with different processes involved in regulating gene expression.
- Name different types of mutations and give examples of the consequences of mutations.
- Explain why the scientific community is excited about CRISPR-Cas applications in biotechnology.
- Appreciate why the epigenome is of interest to scientists in biotechnology.

Central to the study of biotechnology is an understanding of the structure of DNA as the molecule of life—the inherited genetic material. Later, we will consider how extraordinary techniques in molecular biology enable biologists to clone and engineer DNA—manipulations that are essential for many applications in biotechnology (Chapter 3). In this chapter, we review DNA structure and replication, discuss how genes code for proteins, provide an overview of genomics, and consider causes and consequences of mutations.

FORECASTING THE FUTURE: ARTIFICIAL CELLS

Although much of what we will discuss in this chapter describes basic information about cell structure and genes that scientists have known about for decades, there are many exciting potential future developments especially related to genomics. An active area of research is the creation of **programmable** or **artificial cells** in which synthetic genomes are assembled in a lab and introduced into cells to create cells with novel properties based on the inserted genome (for example, manufacturing drugs). Similarly, inserting small metal nanoparticles for controlling cell functions and delivering drugs, and artificial organelles such as ribosomes, are other approaches for manipulating cells that might be used to treat diseases in the future. These approaches have not yet advanced far enough to produce valuable applications. But signs indicate that synthetic genome strategies and nanotechnology approaches for making artificial cells with novel properties can work. Fundamental information about cells, gene expression, and genomes is playing a major role in the development of cell-based treatment strategies, such as using stem cells to create tissues and organs (which we will discuss in Chapter 11).

2.1 A Review of Cell Structure

Cells are the structural and functional units of life. Organisms such as bacteria consist of a single cell, whereas humans have approximately 75 trillion cells, including over 200 different types that vary in appearance and function. Cells vary greatly in size and complexity, from tiny bacterial cells to human neurons that may stretch for more than 3 feet from the spinal cord to muscles in the toes. Virtually all cells share a common component, genetic information in the form of **deoxyribonucleic acid (DNA). Genes** control numerous activities in cells by directing the synthesis of proteins. Genes influence our behavior; determine our physical appearance, such as skin, hair, and eye color; and affect our susceptibility to genetic disease conditions. Before we begin our study of genes and genomes, we will review basic aspects of cell structure and function and briefly compare different types of cells.

Prokaryotic Cells

Cells are complex entities with specialized structures that determine their functions. Generally, every cell has a **plasma (cell) membrane,** a double-layered structure of primarily lipids and proteins that surrounds its outer surface; **cytoplasm,** the inner contents of the cell between the nucleus and the plasma membrane; and **organelles** ("little organs"), structures in the cell that perform specific functions. Throughout this book, we not only consider how plant and animal cells play important roles in biotechnology but also cover many biotechnology applications involving bacteria, yeasts, and other microorganisms. Bacteria are referred to as **prokaryotic cells,** or simply prokaryotes, from the Greek words meaning "before nucleus," because they do not have a **nucleus,** an organelle that contains DNA in animal and plant cells. Prokaryotes include true bacteria (eubacteria) and cyanobacteria, a type of blue-green algae (Table 2.1), and members of the domain Archaea (ancient bacteria with some eukaryotic characteristics).

Bacteria have a relatively simple structure (**Figure 2.1**). Their outer boundary is defined by the plasma membrane, which is surrounded by a rigid cell wall that protects the cell. Except for ribosomes, which are used for protein synthesis, bacteria have few organelles. The cytoplasm contains DNA, usually in the form of a single circular molecule, which is attached to the plasma membrane and located in an area called the nucleoid region (Figure 2.1). Some bacteria also have a tail-like structure called a flagellum, which is used for locomotion.

Eukaryotic Cells

Plant and animal cells are considered **eukaryotic cells,** from the Greek words meaning "true nucleus," because they contain a membrane-enclosed nucleus and many organelles. Eukaryotes also include fungi and single-celled organisms called protists, which include most

TABLE 2.1 Prokaryotic and Eukaryotic Cells

	Prokaryotic Cells	Eukaryotic Cells
Cell types	True bacteria (eubacteria) Archaebacteria	Protists, fungi, plant, animal cells
Size	100 nm–10 μm	10–100 μm
Structure	No nucleus; DNA located in the cytoplasm. No organelles.	DNA enclosed in a membrane-bound cytoplasm. Nucleus. Many organelles.

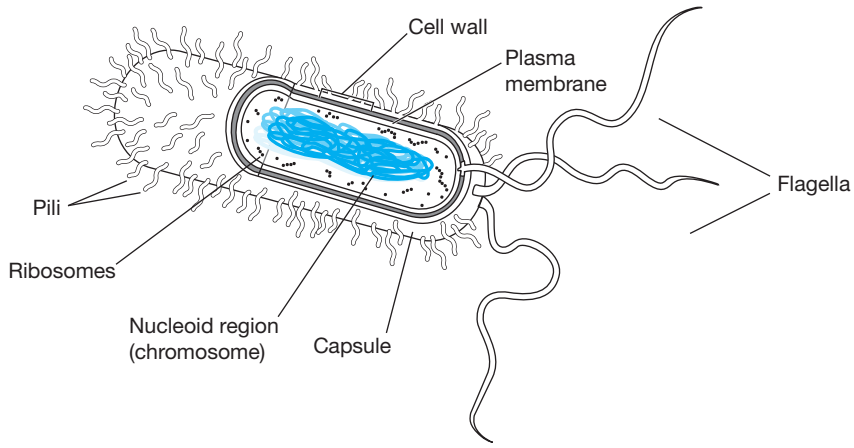


FIGURE 2.1 Prokaryotic Cell Structure Bacteria are prokaryotes. Shown here is a drawing of structures contained in a typical rod-shaped bacterium.

algae. Diagrams of plant and animal cells are shown in Figure 2.2. The plasma membrane is a fluid, highly dynamic, complex double-layered barrier composed of lipids, proteins, and carbohydrates. The membrane performs essential roles in cell adhesion, cell-to-cell communication, and cell shape, and it is essential for transporting molecules into and out of the cell. The membrane also serves important roles as a selectively permeable barrier, because it contains proteins involved in complex transport processes that control which molecules can enter and leave the cell. For example, hormones such as insulin are released from the cell in a process called secretion; other molecules, such as glucose, can be taken into the cell and, within **mitochondria,** can be converted into energy in the form of a molecule called **adenosine triphosphate (ATP).** Membranes also enclose or comprise many organelles.

The cytoplasm of eukaryotes consists of **cytosol,** a nutrient-rich, gel-like fluid, and many organelles. The cytoplasm of prokaryotes also contains cytosol, but few organelles. Think of organelles as the compartments in which chemical reactions and cellular processes occur. Organelles allow cells to carry out thousands of different complex reactions simultaneously. Each organelle is responsible for specific biochemical reactions. For instance, lysosomes break down foreign materials and old organelles; organelles such as the endoplasmic reticulum and Golgi apparatus synthesize proteins, lipids, and carbohydrates (sugars). By compartmentalizing reactions, cells can carry out a multitude of reactions in a highly coordinated fashion simultaneously without interference. Be sure to familiarize yourself with the functions of organelles presented in Figure 2.2 and Table 2.2.

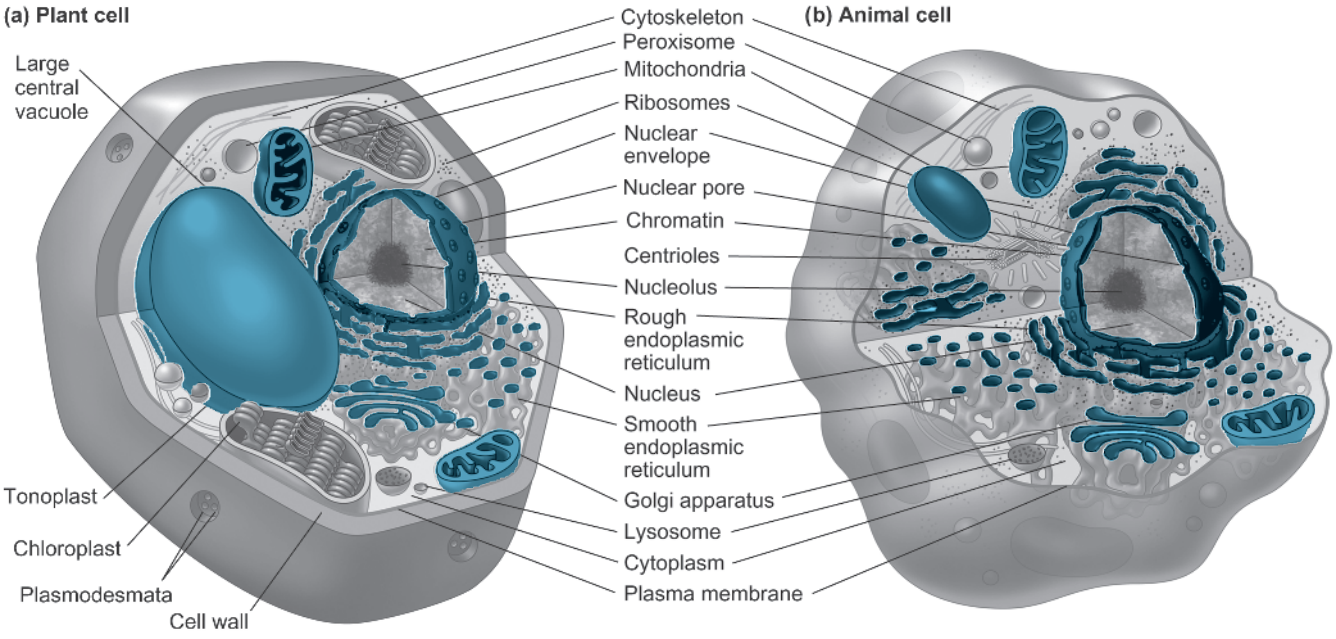


FIGURE 2.2 Eukaryotic Cell Structure Sketches of common structures present in plant (a) and animal cells (b).

TABLE 2.2 Structure and Functions of Eukaryotic Cells

Cell Part	Structure	Functions
Plasma membrane	Membrane made of a double layer of lipids (primarily phospholipids and cholesterol) within which proteins are embedded; proteins may extend entirely through the lipid bilayer or protrude on only one face; externally facing proteins and some lipids have attached sugar groups	Serves as an external cell barrier; acts in the transport of substances into or out of the cell; maintains an electrical potential essential for the functioning of excitable cells; externally facing proteins act as receptors (for hormones, neurotransmitters, and so on) and in cell-to-cell recognition
Cytoplasm	Cellular region between the nuclear and plasma membranes; consists of fluid cytosol (containing dissolved solutes), inclusions (stored nutrients, secretory products, pigment granules), and organelles (the metabolic machinery of the cytoplasm)	
Cytoplasmic organelles		
• Centrioles	Paired cylindrical bodies, each composed of nine triplets of microtubules	Organize a microtubule network during mitosis to form the spindle and asters; form the bases of cilia and flagella
• Cilia	Short, cell surface projections; each cilium is composed of nine pairs of microtubules surrounding a central pair	Move in unison, creating a unidirectional current that propels substances across cell surfaces
• Flagella	Like cilia but longer; the only example in humans is the sperm tail	Propels the cell
• Golgi apparatus	A stack of smooth membrane sacs and associated vesicles close to the nucleus	Packages, modifies, and segregates proteins for secretion from the cell, inclusion in lysosomes, and incorporation into the plasma membrane
• Intermediate filaments	Protein fibers; composition varies	Provide physical support and stability to the cytoskeleton of cells; resist mechanical forces acting on the cell; chromatin organization by anchoring DNA to the nuclear membrane
• Lysosomes	Membranous sacs containing hydrolases (digestive enzymes)	Sites of intracellular digestion
• Microfilaments	Fine filaments of the contractile protein actin	Involved in muscle contraction and other types of intracellular movement; help form the cell's cytoskeleton
• Microtubules	Cylindrical structures made of tubulin proteins	Support the cell and give it shape; involved in intracellular and cellular movements; form centrioles
• Mitochondria	Rod-like double-membrane structures; the inner membrane is folded into projections called cristae; contain circular chromosome which codes for rRNA, tRNA, and proteins involved in energy metabolism	Site of adenosine triphosphate (ATP) synthesis; powerhouse of the cell; also involved in cell death, signaling, and cellular differentiation
• Peroxisomes	Membranous sacs of oxidase enzymes	The enzymes detoxify a number of toxic substances; the most important enzyme, catalase, breaks down hydrogen peroxide
• Ribosomes	Dense particles consisting of two subunits, each composed of ribosomal RNA and protein; free or attached to rough endoplasmic reticulum	The sites of protein synthesis

TABLE 2.2 (*CONTINUED*)

Cell Part	Structure	Functions
• Rough endoplasmic reticulum	Membrane system enclosing a cavity (the cisterna) and coiling through the cytoplasm; externally studded with ribosomes	Sugar groups are attached to proteins within the cisternae; proteins are bound in vesicles for transport to the Golgi apparatus and other sites; external face synthesizes phospholipids and cholesterol
• Smooth endoplasmic reticulum	Membranous system of sacs and tubules; free of ribosomes	Site of lipid and steroid synthesis, lipid metabolism, and drug detoxification
• Vesicles	Small membrane-bound organelles including lysosomes, peroxisomes, transport vesicles, and others	Functions depend on the type of vesicle but include transport of molecules and various roles in metabolism
Nucleus	Largest organelle, surrounded by the nuclear envelope; contains fluid nucleoplasm, nucleoli, and chromatin	Control center of the cell; responsible for transmitting genetic information and providing the instructions for protein synthesis
• Chromatin	Granular, thread-like material composed of DNA and histone proteins	DNA contains genes
• Nuclear envelope	Double-membrane structure pierced by the pores; outer membrane continuous with the cytoplasmic endoplasmic reticulum	Separates the nucleoplasm from the cytoplasm and regulates the passage of large molecules into and out of the nucleus; inner membrane helps to anchor chromatin
• Nucleoli	Dense spherical (non-membrane-bound) bodies composed of ribosomal RNA and proteins	Site of ribosome subunit manufacture
Central vacuole (plant cells)	Large membrane-enclosed compartment	Used to store ions, waste products, pigments, protective compounds
Chloroplasts (plant cells)	Membrane-enclosed organelle containing stacked structures (grana) of chlorophyll-containing membrane sacs called thylakoids surrounded by an inner fluid (stroma)	Site of photosynthesis

The nucleus contains DNA. This organelle is a spherical structure enclosed by a double-layered membrane, the **nuclear envelope,** and is typically the largest structure in a eukaryotic cell. Nearly 6 feet of DNA is coiled into the nucleus of every human cell; if the DNA in all human cells were connected end to end, there would be enough to stretch to the sun and back about 500 times. Although the majority of DNA in a eukaryotic cell is contained within the nucleus, mitochondria and chloroplasts also contain small circular DNA molecules.

2.2 The Molecule of Life

Every high school or college biology course involves some discussion of DNA, and DNA is routinely manipulated by students in college biology laboratories and many high school classes. With the wealth of information available about many detailed aspects of DNA and genes, the study of biology in the twenty-first century might give you the impression that the structural details of DNA were always understood. However, the structure of DNA—and its function as genetic material—was not always well known. Many extraordinary researchers and incredible discoveries have contributed to our modern understanding of DNA structure and function. In this section we provide a brief overview of DNA structure.

DNA Structure

In 1869, Swiss biologist Friedrich Miescher identified a cellular substance from the nucleus that he called "nuclein." Miescher purified nuclein from white blood cells and found that it could not be broken down (degraded) by protein-digesting enzymes called

proteases. This discovery suggested that nuclein was not made only of proteins. Subsequent studies determined that this material had acidic properties, which led nuclein to be renamed "nucleic acids." DNA and **ribonucleic acid (RNA)** are the two major types of **nucleic acids.** While biochemists worked to identify the different components of nucleic acids, other scientists carried out experiments to demonstrate that DNA is the inherited genetic material of cells.

While evidence supporting DNA as hereditary material was building, a significant question still remained: what is the structure of DNA? Erwin Chargaff provided some insight into this question by isolating DNA from a variety of different species and revealing that the percentage of DNA bases called adenine was proportional to the percentage of bases called thymine, and that the percentage of cytosine bases in an organism's DNA was roughly proportional to the percentage of guanine. This valuable observation suggested that the bases adenine, thymine, cytosine, and guanine were somehow intricately related components of DNA structure—an important principle to remember because, as we explore next, these bases are essential components of DNA.

The building block of DNA is the **nucleotide** (Figure 2.3). Each nucleotide is composed of a (five-carbon) **pentose sugar** called deoxyribose, a phosphate molecule, and a **nitrogenous base.** The bases are interchangeable components of a nucleotide. Each nucleotide contains one base, either **adenine (A), thymine (T), guanine (G),** or **cytosine (C)**—the so-called A's, T's, G's, and C's of DNA.

Nucleotides are the building blocks of DNA, but how are these structures arranged to form a DNA molecule? Many scientists have contributed to the answer to this question, but the definitive structure of DNA was finally revealed by James Watson and Francis Crick, working at the Cavendish Laboratories in Cambridge, England. Chemists Rosalind Franklin and Maurice Wilkins, of University College, London, used x-ray crystallography to provide Watson and Crick with invaluable data on the structure of DNA. By firing an x-ray beam onto

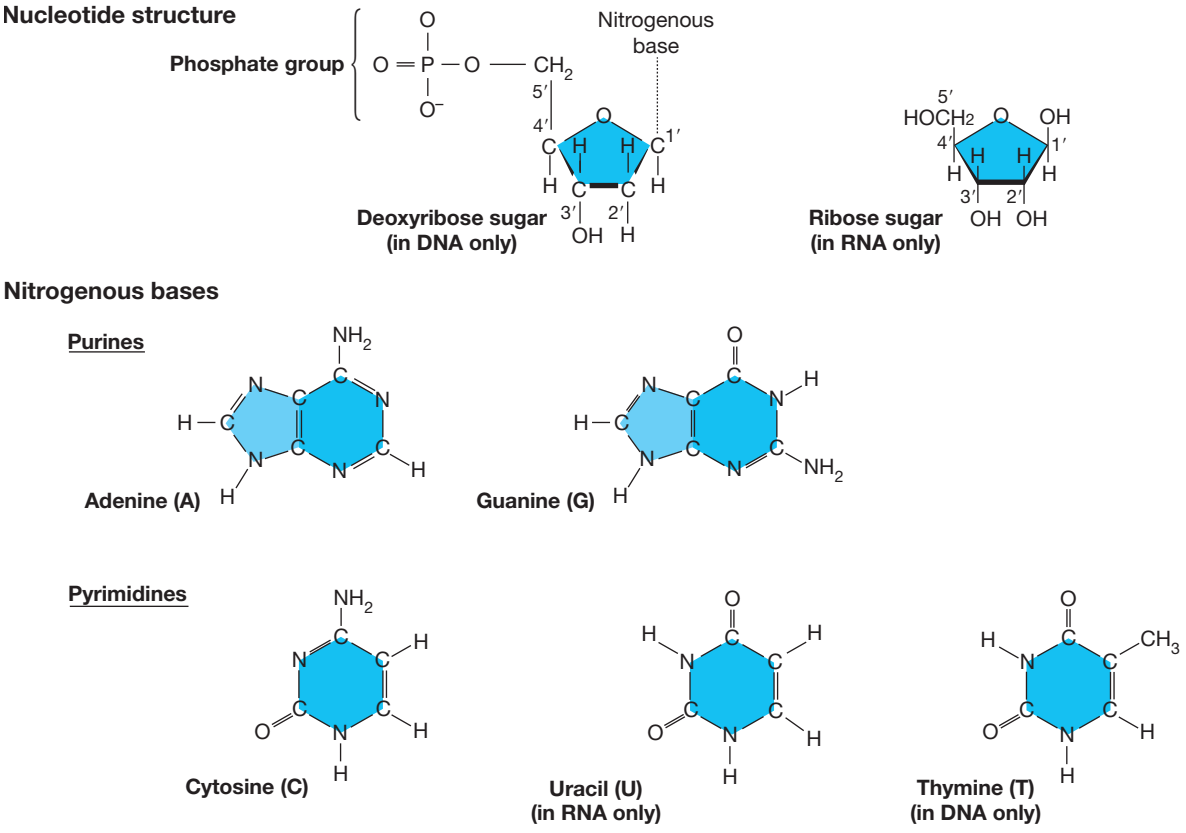


FIGURE 2.3 Nucleotide Structure All DNA nucleotides consist of a nitrogenous base, A, C, G, or T; a pentose sugar; and a phosphate group. The pentose sugar in DNA is called deoxyribose because it lacks an oxygen at carbon number 2 (2′) compared with the pentose sugar, called ribose, in RNA. A base is attached to carbon number 1 (1′) of the sugar; the phosphate group is attached to carbon number 5 (5′) of the sugar. Because of their structure, adenine and guanine belong to a group of bases called purines, whereas cytosine, thymine, and uracil belong to a group called pyrimidines.

crystals of DNA, Franklin and Wilkins revealed a model of DNA indicating that its structure could be helical. From these data, Chargaff's findings, and other studies, Watson and Crick assembled a wire model of DNA.

Watson and Crick published "The Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid" in the prestigious journal *Nature* on April 25, 1953. The first paragraph of this paper reads, "We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest." Given the importance of DNA and what we have learned about DNA structure over the past 65 years, this description might be one of the greatest understatements ever made in a published scientific paper. The significance of this discovery was appropriately recognized in 1962, when Watson, Crick, and Wilkins received the Nobel Prize in Medicine.

Watson and Crick determined that nucleotides form long strands of DNA and that each DNA molecule consists of two strands that join together and wrap around each other to form a double helix (Figure 2.4). A strand of DNA is a string of nucleotides held together by **phosphodiester bonds** that connect the sugar of one

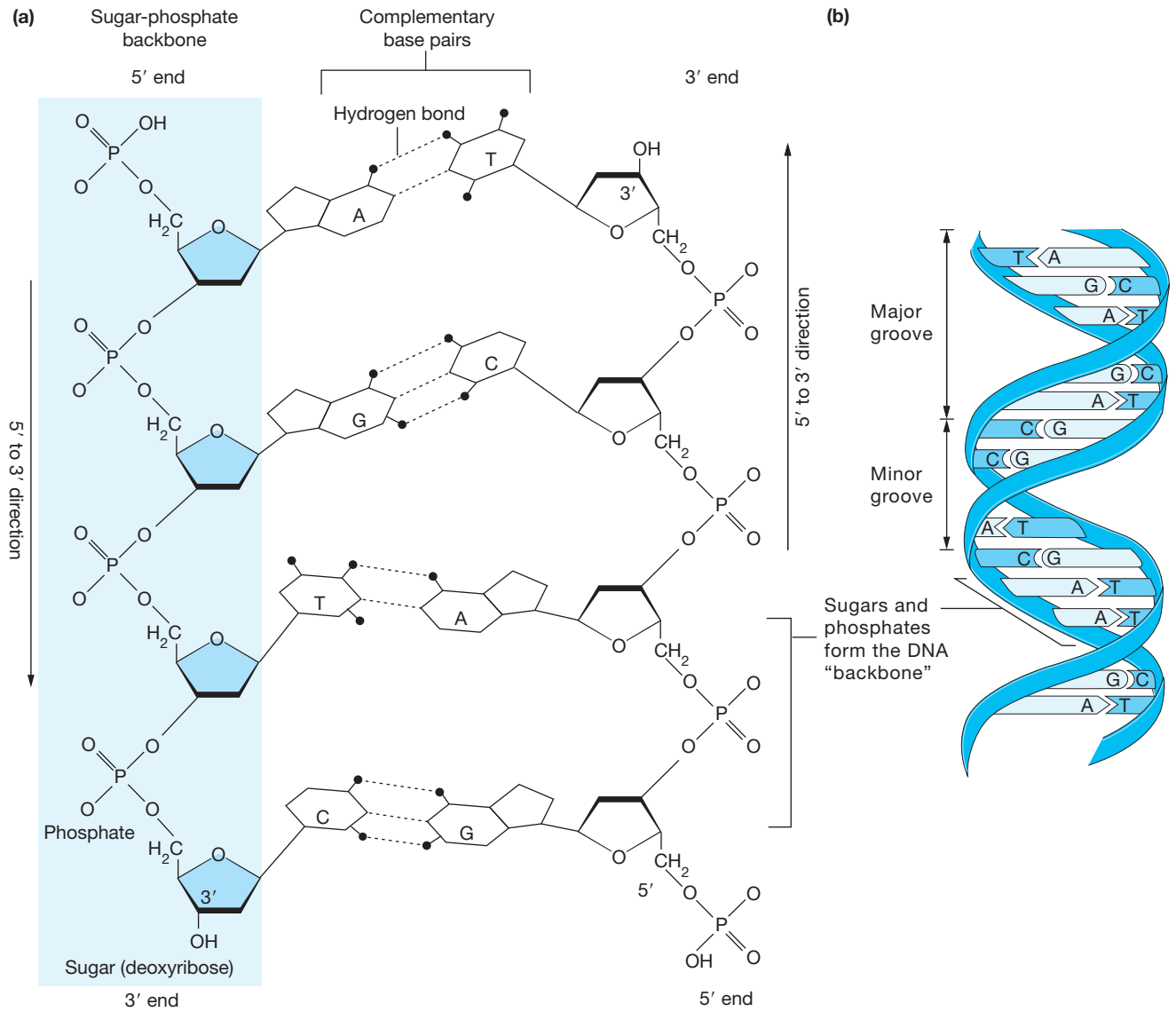


FIGURE 2.4 DNA Is a Double-Stranded Helix (a) Two strands of nucleotides are joined together by hydrogen bonds between complementary base pairs. Adenine bases (A) always base pair with thymine bases (T), and cytosine (C) base pairs with guanine (G). (b) The two strands wrap around each other so that the overall structure of DNA is a double-stranded helix with a sugar-phosphate "backbone," in which the bases are aligned in the center of the helix.

nucleotide to the phosphate group of an adjacent nucleotide (Figure 2.4). The sequence of bases in a strand can vary. For instance, a nucleotide containing a C can be connected by a phosphodiester bond to a nucleotide containing an A, T, G, or another nucleotide containing a C.

Each strand of nucleotides has a **polarity** to it; there is a *5′ end* and a *3′ end* to the strand (Figure 2.4). *Polarity* refers to the carbons of the deoxyribose sugar. At the 5′ end of a strand, the phosphate at carbon 5 is not bonded to another nucleotide, but carbon 3 is involved in a phosphodiester bond. At the 3′ end, the phosphate at carbon 5 is bonded to another nucleotide, but carbon 3 is not. Although this aspect of nucleotide structure may seem trivial, the polarity of DNA is important for replication and for the routine manipulation of DNA in the laboratory.

Watson and Crick determined that DNA molecules consist of two interconnecting strands that wrap around each other to form a right-handed double helix, perhaps the most famous molecular model in all of biology (Figure 2.4). The two strands are joined together by hydrogen bonds between **complementary base pairs** in opposite strands (Figure 2.4). Adenine base pairs with thymine, and guanine base pairs with cytosine. From this model, Chargaff's observations are easily understood. The proportions of A's and T's are equivalent in an organism's DNA, as are the proportions of G's and C's, because they pair with each other in a DNA molecule.

The two strands of nucleotides in a double helix are *antiparallel*—the polarity of each strand is reversed relative to the other (Figure 2.4). This orientation is necessary for the hydrogen bonds holding the complementary base pairs to align with one another. The double helix resembles a twisted ladder. The rungs of this ladder consist of the complementary base pairs, and the sides of the ladder consist of sugar and phosphate molecules, creating the "backbone" of DNA.

What Is a Gene?

Genes are units of inheritance, but what exactly is a gene? Until recently, a **gene** was often described as a sequence of nucleotides that provides cells with the instructions to synthesize a specific protein. But because we have learned that not all genes are used to make a protein, a more modern definition of a gene encompasses any DNA sequence that is used to produce RNA. For example, genes for transfer RNA (tRNA) are used to make tRNA molecules, and although tRNAs are required for protein synthesis, they are not translated to produce a protein. Most genes are approximately 1,000 to 4,000 nucleotides (nt) long, although many smaller and larger genes have been identified. Largely by controlling the proteins produced by a cell, genes influence how cells, tissues, and organs appear, both through the microscope and with the naked eye. These inherited appearances are called **traits.** Through the DNA contained in your cells, you have inherited traits from your parents, such as eye color and skin color. Genes influence not only cell metabolism and behavioral and cognitive abilities such as intelligence but also the inheritance of or susceptibility to certain types of genetic diseases.

Some traits are controlled by a single gene, but the majority of traits are determined by multiple genes, which produce many proteins that interact in complex ways. In Section 2.4, we explore how genes direct protein synthesis in cells. Throughout this book, we consider examples of genes, their functions, and their many applications in different areas of biotechnology.

2.3 Chromosome Structure, DNA Replication, and Genomes

Before we consider how genes function, it is important that you understand how and why DNA is organized into chromosomes and how DNA is replicated in cells.

Chromosome Structure

Suppose you were presented with a challenge. If you solved it, you would earn free tuition for the rest of your undergraduate courses. You are given a basket containing 46 packages of different-colored yarn all unraveled and intertwined. Your challenge is to sort the yarn into 46 even balls. How would you solve this challenge? If you started to cut the tangled pile of yarn randomly, you probably would not succeed. Of course, if you painstakingly unraveled the yarn and wound each color of yarn into a ball, you would eventually sort it into 46 even balls. This analogy provides a highly simplified view of the challenge presented to a human cell when it has to divide and sort its DNA into even packages.

The 3 billion base pairs (bp) of DNA in every human cell must be separated evenly when a cell divides; otherwise, the loss of DNA can have devastating consequences. Fortunately, such mistakes in DNA separation are rare, in part because cells effectively separate and package DNA into **chromosomes.**

Inside the nucleus, DNA exists in a relatively unraveled state. This does not mean that the DNA is uncoiled from its double-helical structure; rather, the DNA is somewhat loosely arranged and not fully compacted into tightly coiled chromosomes, although all of the DNA in a chromosome remains together within the nucleus. When a cell is not dividing, chromosomes in the nucleus exist as an intricate combination of DNA

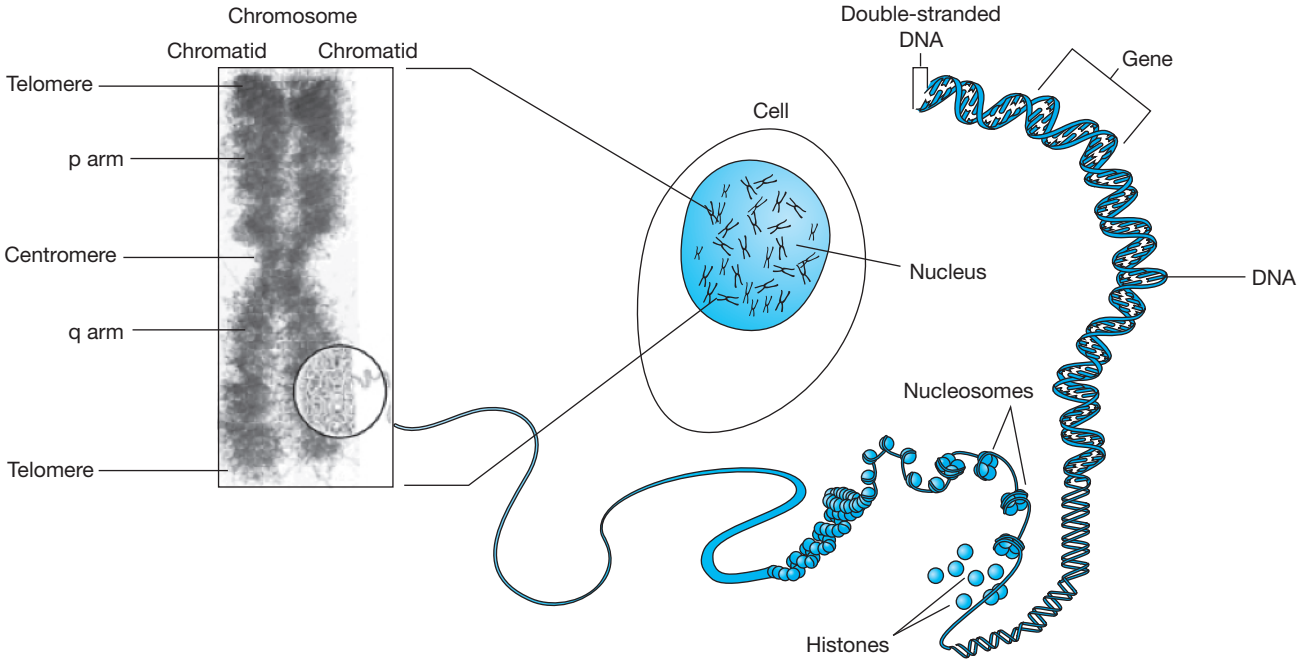


FIGURE 2.5 Chromosome Organization In a nondividing cell, chromosomal DNA exists in an unraveled state called *chromatin*. Histone proteins serve as particles around which DNA becomes tightly wound to give a "beads on a string" appearance when viewed with an electron microscope. When cells divide, chromatin is further compacted into tight fibers and supercoiled looped structures. Ultimately these supercoiled loops are tightly packed together with the assistance of other proteins to create an entire chromosome, a highly compact assembly of DNA. Each chromosome consists of two sister chromatids attached by a centromere. Chromosome arms are the portions of the chromatid on one side of the centromere, labeled as the *p* and *q arms*. The ends of a chromosome are called *telomeres*.

and DNA-binding proteins called **histones** to form strings called **chromatin.** During cell division, chromatin is coiled into tight fibers that eventually wrap around each other, so that chromosomes become highly coiled and tightly condensed packages of DNA and histones and other proteins (Figure 2.5). Compacting DNA into a chromosome is an amazing feat when you consider that DNA in most human cells is about two-meters long and must be compacted into a nucleus that is about one-thousandth of a millimeter in diameter!

The size and number of chromosomes vary from species to species. Most bacteria have a single circular chromosome, in the size range of several hundred thousand base pairs, which contains a few thousand genes. Eukaryotes typically contain one or more sets of chromosomes, which have a linear shape, and often these chromosomes are several million base pairs in size. Most human cells have two sets (pairs) of 23 chromosomes each, for a total of 46 chromosomes. Through the process of fertilization, you inherited 23 chromosomes from your mother **(maternal chromosomes)** and 23 chromosomes from your father **(paternal chromosomes).** These chromosome pairs are called **homologous pairs,** or **homologues.** Chromosomes 1 through 22 are known as the **autosomes;** the 23rd pair are called the **sex chromosomes—**consisting of X and Y chromosomes.

Human egg and sperm cells, known as the sex cells or **gametes,** contain a single set of 23 chromosomes, called the **haploid number (*n*)** of chromosomes. All other cells of the body—such as skin cells, muscle cells, and liver cells—are known as **somatic cells.** Somatic cells from many organisms have two sets of chromosomes, called the **diploid number (2*n*)** of chromosomes. Human somatic cells contain 46 chromosomes. Somatic cells of a normal human male have 22 pairs of autosomes and an X and Y chromosome; cells of a normal female have 22 pairs of autosomes and two X chromosomes.

Sex chromosomes were so named because they contain genes that influence sex traits and the development of reproductive organs, whereas the autosomes were originally thought primarily to contain genes that affect body features unrelated to sex, such as skin color and eye color. Although genes involved in sex organ determination are present on the Y chromosome, other genes involved in sex determination are present on autosomes, and the majority of genes

on the X chromosome are not required for development of the reproductive organs.

Several characteristics are common to most eukaryotic chromosomes. Each chromosome consists of two thin, rod-like structures of DNA called **sister chromatids** (Figure 2.5). The sister chromatids are exact replicas of each other, copied during DNA synthesis. During cell division, sister chromatids are separated so that newly forming cells receive the same amount of DNA as the original cell from which they arose. Each eukaryotic chromosome has a single **centromere,** a constricted region of the chromosome consisting of intertwined DNA and proteins that join the two sister chromatids to each other. This region of a chromosome also contains proteins that attach chromosomes to organelles called **microtubules,** which play essential roles in moving chromosomes and separating sister chromatids during cell division.

The centromere delineates each sister chromatid into two arms—the short arm, called the **p arm,** and the long arm, or **q arm.** Each arm of a chromosome ends with a segment known as a **telomere** (Figure 2.5). Telomeres are highly conserved repetitive sequences of nucleotides that are important for attaching chromosomes to the nuclear envelope. Telomeres are a subject of intense research. Changes in telomere length are associated with the cellular aging process (called **senescence**) and with the development of certain types of cancers.

Karyotype analysis for studying chromosomes

Analyzing chromosome structure and abnormalities is called **cytogenetics** or cytogenetic analysis. One common cytogenetic method for studying chromosome number and basic aspects of chromosome structure is to prepare a **karyotype.** In karyotype analysis, cells are spread on a microscope slide and then treated with chemicals to release and stain the chromosomes. For example, G-banding, in which chromosomes are treated with a DNA-binding dye called Giemsa stain, creates a series of alternating light and dark bands in stained chromosomes. Each stained chromosome shows a unique and reproducible banding pattern that can be used to identify different chromosomes. Chromosomes can be aligned and paired based on their staining pattern and size (Figure 2.6). In humans, chromosome 1 is the largest chromosome and chromosome 21 is the smallest.

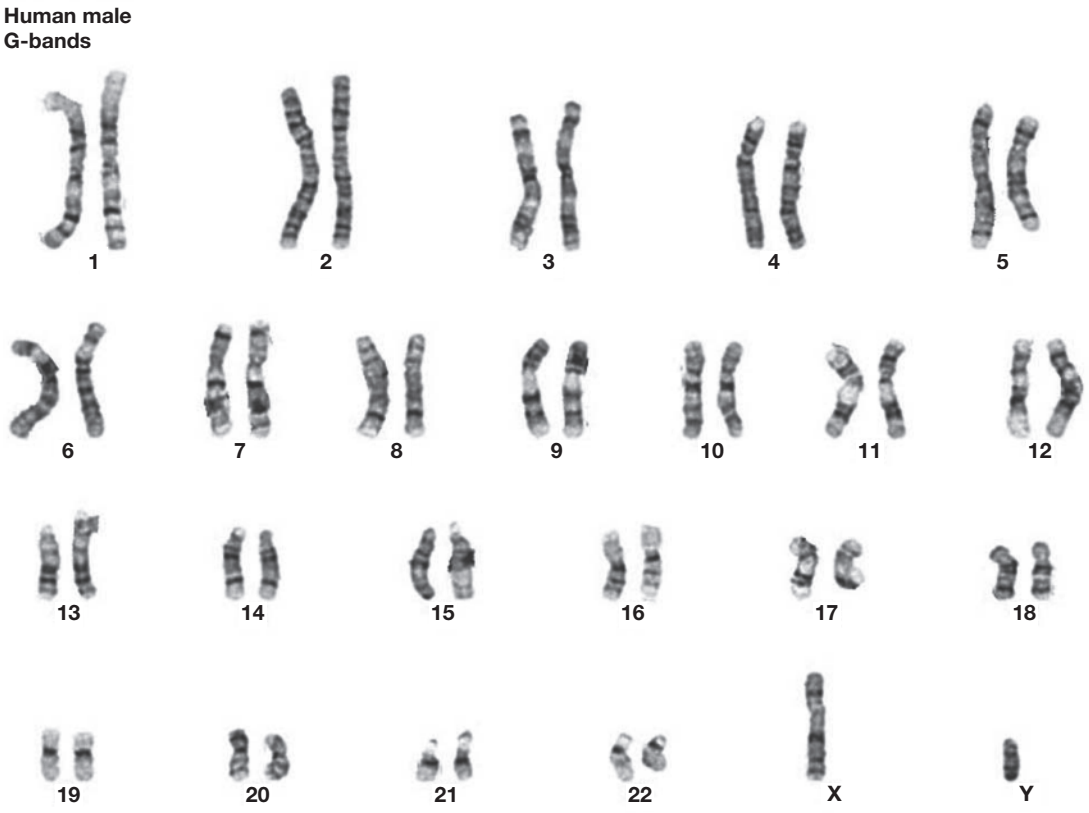


FIGURE 2.6 Karyotype Analysis In a karyotype, dividing cells are spread out onto a glass microscope slide to release their chromosomes. Chromosomes are stained and aligned based on their overall size, the position of the centromere, and their staining pattern to create a karyotype.

Modern methods for karyotype analysis, called **spectral karyotyping,** incorporate specific probes and techniques to colorize chromosomes. This provides a more detailed analysis of chromosome structure than traditional karyotypes, such as the one shown in Figure 2.6, which makes it easier to identify human genetic disease conditions associated with abnormalities in chromosomal structure and number. Similarly, a number of different approaches can be used to identify specific alterations in chromosome structure, including the presence or absence of a specific gene, by binding different DNA probes to chromosomes. One approach for doing this is called **fluorescence in situ hybridization,** or **FISH.** See Figure 3.13 for an example of FISH.

In Chapter 11, as well as other chapters, you will learn more about how karyotype analysis and other approaches for genetic testing enable scientists to diagnose genetic diseases.

DNA replication

When a cell divides, it is essential that the newly created cells contain equal copies of replicated DNA. Somatic cells divide by **mitosis,** wherein one cell divides to produce daughter cells, each of which contains an identical copy of the DNA from the original (parent) cell. For instance, a human skin cell divides to produce two daughter cells, each containing 23 pairs of chromosomes. Gametes are formed by **meiosis,** wherein a parent cell divides to create up to four daughter cells, which can be either sperm or egg cells. During meiosis, the chromosome number in daughter cells is cut in half to the haploid number. Sperm and egg cells each contain a single set of 23 chromosomes. Through sexual reproduction, a fertilized egg, called the **zygote,** is formed. The zygote, which divides by mitosis to form an embryo and eventually a complete human, contains 46 chromosomes: 23 paternal chromosomes and 23 maternal chromosomes.

Prior to cell division by either mitosis or meiosis, DNA must be replicated in the cell. Replication occurs by a process called **semiconservative replication.** Figure 2.7 shows an overview of this process. Before replication begins, the two complementary strands of the double helix must be pulled apart into single strands. Once separated, these strands serve as templates for copying two new strands of DNA. At the end of this process, two new double helices are formed. Each helix contains one original DNA (parental) strand and one newly synthesized strand—thus the term *semiconservative*.

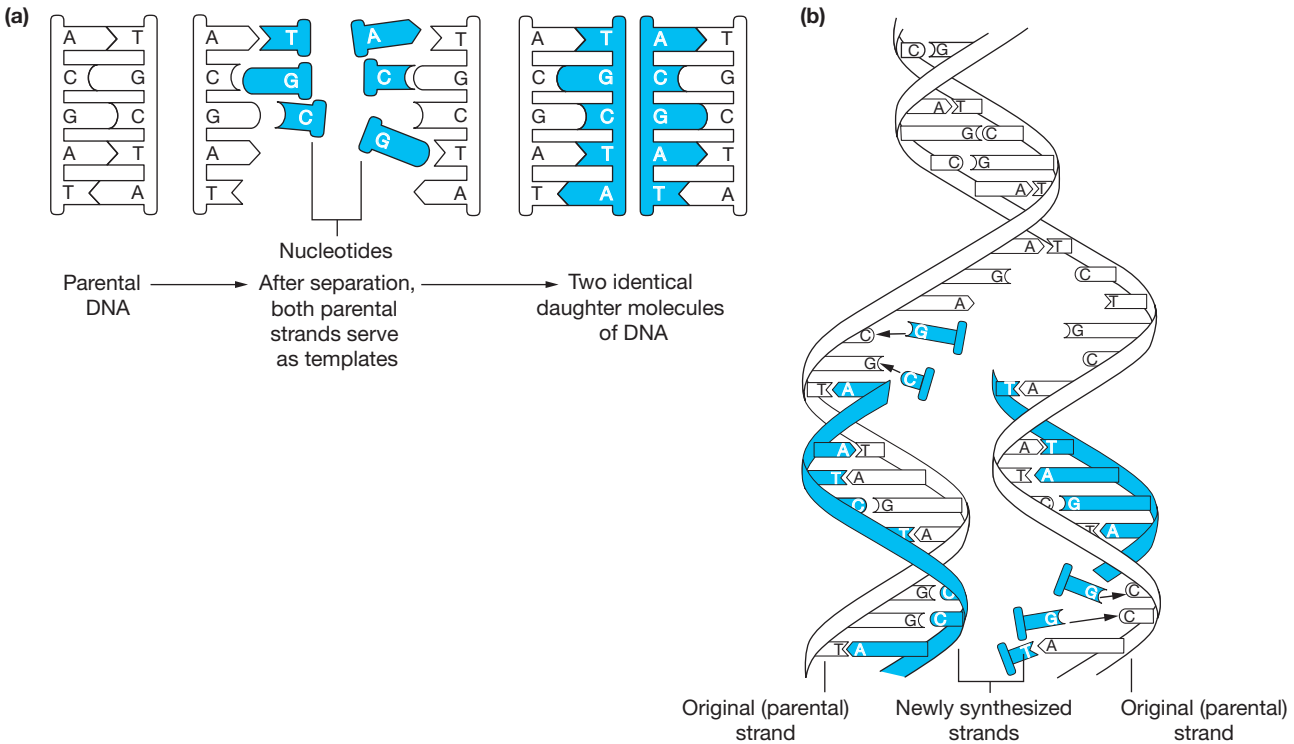


FIGURE 2.7 An Overview of DNA Replication Nucleotide strands in a DNA molecule must first be separated (a). Each strand serves as a template for the synthesis of new strands, producing two DNA molecules, each containing one original strand and one newly synthesized strand (b).

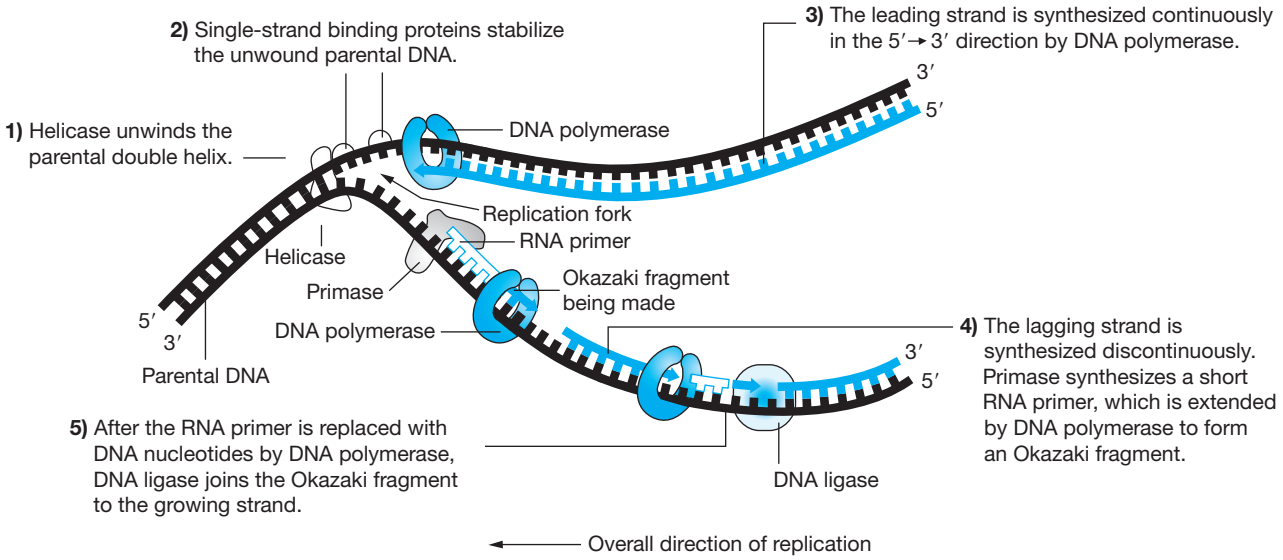


FIGURE 2.8 Semiconservative Replication of DNA

DNA replication occurs in a series of stages involving a number of different proteins. Because prokaryotes contain circular chromosomes, DNA replication in prokaryotes is slightly different from that in eukaryotes. Here we consider the main components and key concepts in DNA replication overall. But keep in mind that subtle differences distinguish the replication process in prokaryotes from that in eukaryotes. Replication is initiated by **DNA helicase,** an enzyme that separates the two strands of nucleotides, literally "unzipping" the DNA by breaking hydrogen bonds between complementary base pairs (Figure 2.8). The separated strands form a replication fork. As helicase unwinds the DNA, *single-strand binding proteins* attach to each strand to hold them apart and prevent them from base pairing and reforming a double helix during DNA replication. Strand separation occurs at sites called **origins of replication.** Bacterial chromosomes have a single origin. Because of their large size, eukaryotic chromosomes have multiple origins. Starting DNA replication at multiple origins allows eukaryotic chromosomes to be copied rapidly.

The next step in DNA replication involves the addition of short segments of RNA approximately 10 to 15 nucleotides long. These sequences, called **RNA primers,** are synthesized by an enzyme known as **primase** (in eukaryotes, a form of DNA polymerase called α [alpha] acts as the primase). Primers start the process of DNA replication because they serve as binding sites for **DNA polymerases,** the key enzymes that synthesize new strands of DNA. Several different forms of DNA polymerase are involved in copying DNA. In bacteria, the enzyme **DNA polymerase III** (called DNA polymerase δ [delta] in eukaryotes) binds to each single strand, moving along the strand and using it as a template to copy a new strand of DNA. During this process, DNA polymerase uses nucleotides present in the cell to synthesize complementary strands of DNA. DNA polymerase always works in one direction, synthesizing new strands in a 5′-to-3′ orientation and adding nucleotides to the 3′ end of a newly synthesized strand (Figure 2.8) by forming phosphodiester bonds between the phosphate of one nucleotide and the sugar in the previous nucleotide.

Because DNA polymerase proceeds only in a 5′-to-3′ direction, replication along one strand, the **leading strand,** occurs in a continuous fashion (Figure 2.8). Synthesis on the opposite strand, the **lagging strand,** occurs in a discontinuous fashion because DNA polymerase must wait for the replication fork to open. On the lagging strand, short pieces of DNA, called Okazaki fragments (named after Reiji and Tuneko Okazaki, the scientists who discovered these fragments), are synthesized as the DNA polymerase works its way out of the replication fork. Covalent bonds between Okazaki fragments in the lagging strand are formed by **DNA ligase** to ensure that there are no gaps in the phosphodiester backbone. Finally, the RNA primers are removed and these gaps are filled by DNA polymerase.

Remember the functions of enzymes involved in DNA synthesis. As we will discuss, DNA polymerase and DNA ligase are routinely used in the lab in working with cloned DNA (Chapter 3).

What is a genome?

DNA contains the instructions for life—genes. All of the DNA in an organism's cells is called the **genome.** Contained in the human genome are approximately 20,000 genes scattered among 3 billion base pairs of DNA. The study of genomes, a discipline called **genomics,** is currently one of the most active and rapidly advancing areas of biological science.

We probably discuss genomes more than any other topic in this book. In many chapters we discuss aspects of the **Human Genome Project,** a worldwide effort to identify all human genes on each chromosome. The Human Genome Project was an enormous undertaking in genomics that has provided exciting insight into human genes and gene functions, created new areas of research, and led to the development of novel ways to diagnose, treat, and—in some cases—cure human genetic diseases.

Also, as you will learn in Chapters 3 and 11, **DNA sequencing** (a method for determining the order of nucleotides in a DNA molecule) technology has progressed so rapidly that it is now possible to sequence *individual human genomes* fairly quickly, accurately, and at low cost! As a result, DNA sequencing is having major implications for diagnosing and treating human genetic disease conditions. In Chapter 11, we will consider many examples of genome sequencing as related to medical biotechnology.

2.4 RNA and Protein Synthesis

Genes govern the activities and functions within a cell often by directing the synthesis of proteins. Some of the myriad functions of proteins are as follows:

- Proteins are necessary for cell structure, for example, as important components of membranes, organelles, and the cytoplasm.
- Proteins carry out essential reactions in the cell as enzymes.
- Proteins perform critical roles as hormones and other "signaling" molecules that cells use to communicate with one another.
- Receptor proteins bind to other molecules, such as hormones, and transport proteins, enabling molecules to enter and leave cells.
- Proteins act as antibodies that recognize and destroy foreign materials in the body, creating immunity to foreign substance.

Quite simply, cells cannot function without proteins. How does DNA make proteins? Actually, DNA does not make proteins directly. To synthesize proteins, genes are first copied into molecules called **messenger RNA (mRNA)** (Figure 2.9). RNA synthesis is called **transcription,** because genes are literally transcribed (copied) from a DNA code into an RNA code. In turn, mRNA molecules, which are exact copies of genes, contain information that is deciphered into instructions for making a protein through a process known as **translation.**

RNA molecules are single-stranded, not double-stranded like DNA, but the chemical composition of RNA is very similar to that of DNA. The bases of RNA are also very similar to those of DNA. One key difference is that RNA contains a base called uracil (U) instead of thymine (T; see Figure 2.3). The other primary difference is that RNA contains a pentose sugar called ribose, which has a slightly different structure than the deoxyribose sugar contained in DNA.

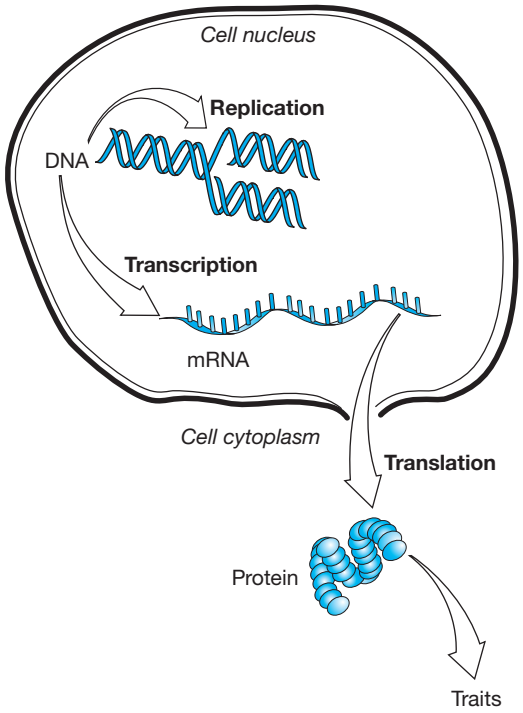


FIGURE 2.9 The Flow of Genetic Information in Cells DNA is copied into RNA during the process of transcription. RNA directs the synthesis of proteins during translation. Through proteins, genes control the metabolic and physical properties or traits of an organism.

An easy way to remember the difference between transcription and translation is to know that translation involves a change in code from RNA to protein, much like translating one language to another. Through the production of mRNA, other RNAs, and the synthesis of proteins, DNA controls the properties of a cell and its traits (Figure 2.9). This process of transcription and translation directs the flow of genetic information in cells, controlling a cell's activities and properties. Here we study basic principles of transcription and translation and aspects of how gene expression can be controlled by cells.

Copying the Code: Transcription

How is DNA used as a template to make RNA? **RNA polymerase** is a key enzyme for transcription. Inside the nucleus, RNA polymerase unwinds the DNA helix and then copies one strand of DNA into RNA (Figure 2.10). Unlike DNA replication, in which the entire DNA molecule is copied, transcription occurs only in segments



TOOLS OF THE TRADE

Enzymes Involved in DNA Replication Have Valuable and Essential Roles in Molecular Biology Research

This chapter introduces important principles of DNA structure, replication, transcription, and translation and provides an essential background on genes and genomes. These principles are important for understanding not only what genes are and how they function but also many of the components involved in processes such as DNA replication, which have become essential tools for research in molecular biology.

In the next chapter, you will learn how scientists can identify, clone, and analyze genes—a fascinating aspect of molecular biology research and biotechnology. The technology for cloning and studying genes became possible only as scientists learned more about the enzymes involved in processes such as DNA replication. For instance, DNA polymerase, the key enzyme that synthesizes DNA during semiconservative replication, is widely used in molecular biology labs to copy DNA with a technique called the polymerase chain reaction (PCR; see Figure 3.5). Similarly, RNA polymerase is also widely used in molecular research.

In addition, many DNA cloning procedures rely on DNA ligase to join together DNA fragments from different sources—a process called **recombinant DNA technology** (see Figure 3.2). As you will learn in Chapter 3, recombinant DNA technology created a new era in biotechnology (some say this created modern biotech!) because it made gene cloning possible, which soon led to recombinant products such as insulin and hundreds of other therapeutic proteins produced from cloned genes.

Because most of these enzymes are relatively inexpensive and readily available from supply companies, their use has become commonplace in molecular biology labs in industry and in academia, not only in colleges and universities but also in many advanced lab courses in high schools. Without our understanding of the enzymes that act on DNA and RNA and their functions, many applications of biotechnology and a majority of techniques routinely used in research labs around the world would be impossible.

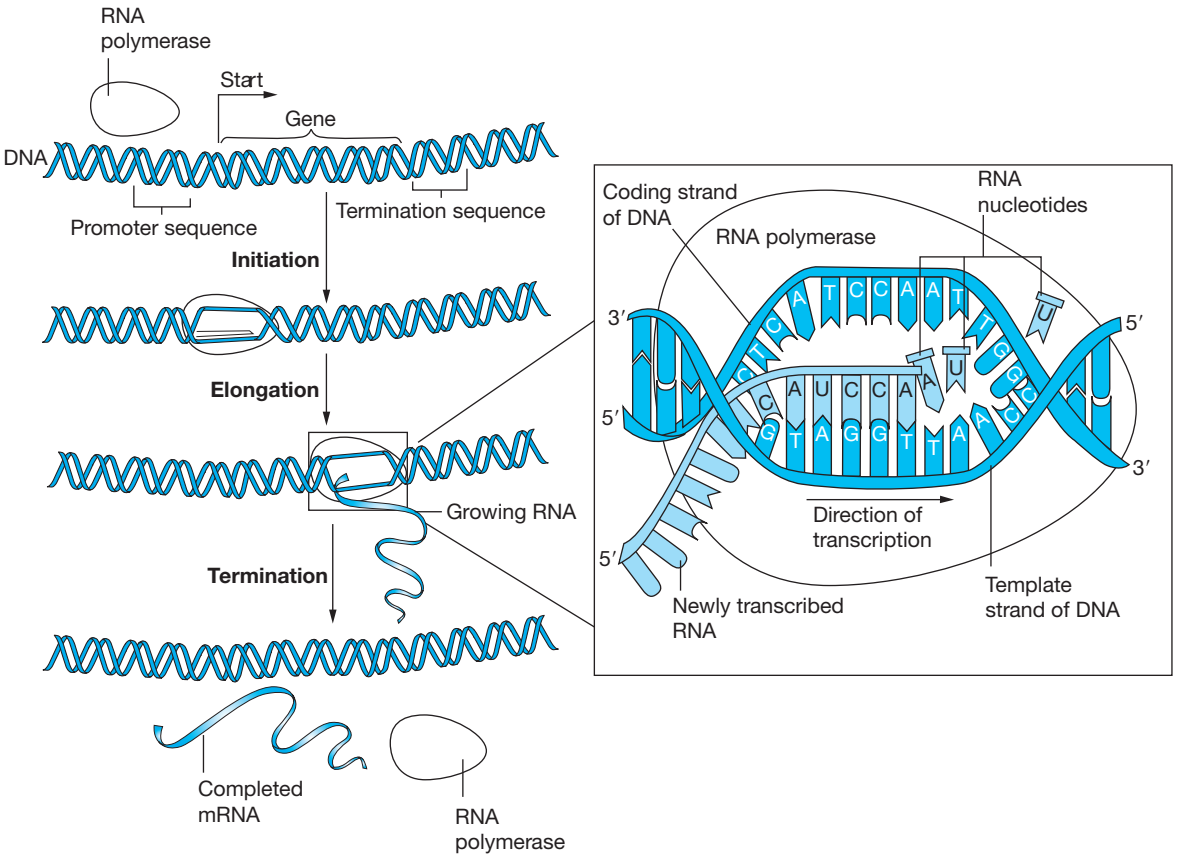


FIGURE 2.10 Transcription During transcription, RNA polymerase binds to DNA at a promoter region adjacent to a gene sequence and unwinds the DNA. RNA polymerase moves along the DNA template, copying one strand into a molecule of RNA. When RNA polymerase reaches a termination sequence, it releases from the DNA and transcription ends.

of a chromosome that contain genes. How does RNA polymerase know where to begin transcription? Adjacent to most genes is a **promoter,** specific sequences of nucleotides that allow RNA polymerase to bind at specific locations next to genes. Proteins called **transcription factors** help RNA polymerase find the promoter and bind to DNA; sequences called **enhancers** can also play important roles in transcription.

After RNA polymerase binds to a promoter, it unwinds a region of the DNA to separate the two strands. Only one of the strands, called the **template strand** (the opposite strand is called the *coding strand*), is copied by RNA polymerase. The presence of a promoter determines which strand of DNA will be transcribed. Once properly oriented, RNA polymerase proceeds along the DNA template strand to copy a complementary strand of RNA by forming phosphodiester bonds between ribonucleotides in a 5′-to-3′ direction (Figure 2.10).

When RNA polymerase reaches the end of a gene, it encounters a termination sequence. These sequences bind either specific proteins or base pairs to create loops at the end of the RNA. As a result, the RNA polymerase and newly formed RNA are released from the DNA molecule. Unlike DNA replication, in which the DNA is copied only once each time a cell divides, multiple copies of mRNA are transcribed from each gene during transcription. Sometimes a cell transcribes thousands of copies of mRNA from a gene. Later in this section, you will see that cells with a high requirement for a particular protein generally produce large amounts of mRNA to encode that protein.

Transcription produces different types of RNA

We have already seen that mRNA is produced when many genes are copied into RNA. Other types of RNA, **transfer RNA (tRNA)** and **ribosomal RNA (rRNA),** are also produced by transcription. Different RNA polymerases produce each type of RNA. As we will soon learn, only mRNA carries information that directly codes for the synthesis of a protein, but tRNA and rRNA are also essential for protein synthesis.

Only relatively recently have scientists discovered a new category of RNA molecules called **noncoding RNA (ncRNA),** so named because they do not code for proteins. These include tRNA and rRNA. But as you will soon learn, ncRNAs have diverse functions in cells. Later we'll briefly discuss different types of ncRNAs and their roles.

mRNA processing

In eukaryotic cells, the initial mRNA copied from a gene is called a **primary transcript (pre-mRNA).** This mRNA is immature and not fully functional. Primary transcripts undergo a series of modifications, collectively called mRNA processing, before they are ready for protein synthesis. One modification involves **RNA splicing** (Figure 2.11). When the details of transcription were first worked out, scientists were surprised to learn that genes are interrupted by stretches of DNA that do not contain protein-coding information, called **introns.** Introns are interspersed between **exons,** or protein-coding sequences of a gene. Introns and exons are copied during transcription of mRNA. Before mRNA can be used to make a protein, the exons must be spliced together. As a simple analogy, think of introns as randomly inserted letters in a sentence that must be removed before the sentence can make sense. In the splicing process, introns are cut out of the primary transcript and adjacent exons are spliced to form a fully functional mRNA molecule with no introns.

Splicing provides flexibility in the types of proteins that can ultimately be produced from a single gene. When genes were first discovered, scientists thought that a single gene could produce only one protein. But as the process of splicing was revealed, it became clear that when a gene contains several exons, splicing doesn't always occur in the same way. As a result, multiple proteins can be produced from a single gene. In a complex process called **alternative splicing,** splicing can sometimes join together certain exons and *cut out* other exons, essentially treating them as introns (Figure 2.11b). This process creates multiple mRNAs of different sizes from the same gene. Each mRNA can then be used to produce different proteins with varied, sometimes unique functions.

Thus, alternative splicing allows several different protein products to be produced from the same gene sequence. For instance, certain genes used to produce antibodies are alternatively spliced to produce some antibodies that attach to the surface of cells as well as other antibodies with different structures, causing them to be secreted into the bloodstream. Similar splicing occurs with neurotransmitter genes, among many others. In fact, scientists originally believed that the human genome contained approximately 100,000 genes based on a predicted number of proteins made by human cells. As you will learn, genome scientists were very surprised to find that the human genome contains only about 20,000 genes (Chapter 3). Much of the reason for this discrepancy is that many human genes can be spliced in different ways, and estimates suggest that approximately 95 percent of human genes use alternative splicing to create multiple different RNAs and proteins.

As we understand more about mRNA processing, increasingly scientists are identifying genetic diseases that result from splicing errors or alternative splicing. In 2016, one of the first gene therapies that targeted splicing for a disease was approved by the U.S. Food and Drug Administration (FDA) for the treatment of spinal muscular atrophy (SMA)—a debilitating condition and leading cause of death in infants. See Figure 11.19 in Chapter 11 and its accompanying text to learn more about SMA and the splicing therapy that has shown great promise so far.

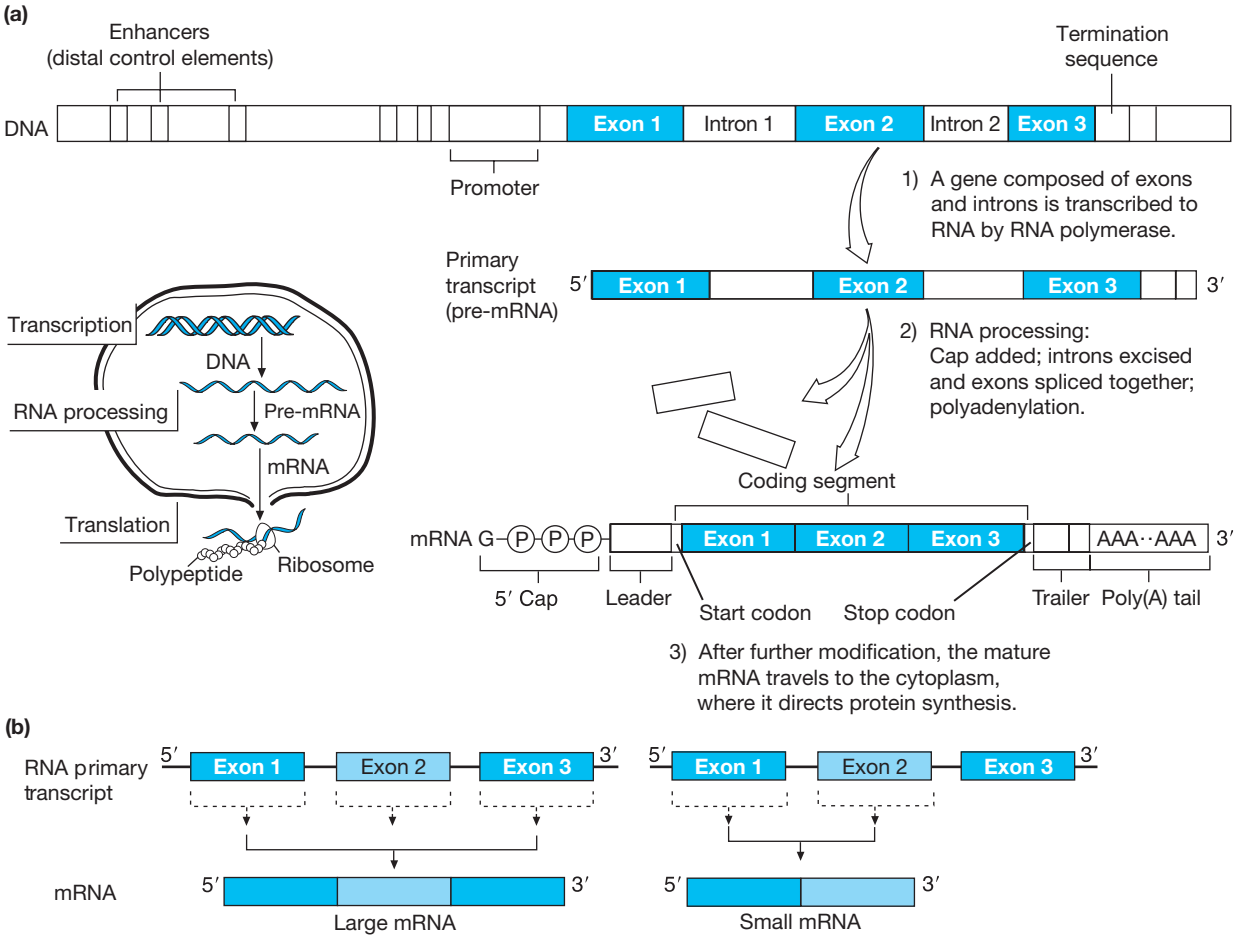


FIGURE 2.11 A Eukaryotic Gene and mRNA Processing (a) Transcription of a eukaryotic gene produces a primary transcript or pre-mRNA, which undergoes processing through RNA splicing, the addition of a 5′ cap, and polyadenylation. After processing, the final, mature mRNA is ready for export to the cytoplasm, where it will be translated into a protein. (b) Alternative splicing can produce different mRNAs and protein products from the same gene. Notice that the larger mRNA on the left contains three exons spliced together but that the shorter mRNA on the right contains only two exons spliced together.

Another type of processing occurs at the 5′ end of mRNA, where a guanine base containing a methyl group is added (Figure 2.11). Known as a 5′ cap, this structure plays a role in ribosome recognition of the 5′ end of the mRNA molecule during translation. Last, in a process called **polyadenylation,** a string of adenine nucleotides around 100 to 300 nucleotides in length is added to the 3′ end of the mRNA, creating a poly(A) "tail" (Figure 2.11). This tail protects mRNA from RNA-degrading enzymes in the cytoplasm, increasing its stability and availability for translation. Following processing, a mature, processed mRNA molecule leaves the nucleus and enters the cytoplasm, where it is now ready for translation (Figure 2.11).

Translating the Code: Protein Synthesis

The ultimate function of a protein-coding gene is to produce a protein. Here we look at a brief overview of translation, using information in mRNA to synthesize a protein from amino acids. Translation occurs in the cytoplasm of cells as a multistep process that involves several different types of RNA molecules. It will be much easier for you to understand the details of translation if you are familiar with important functions of each type of RNA. These are the major components of translation:

- Messenger RNA (mRNA)—a copy of a gene. Acts as a "messenger" by carrying the genetic code, encoded by DNA, from the nucleus to the cytoplasm, where this information can be read to produce a protein. Messenger RNAs vary in length from approximately 1,000 to several thousand nucleotides.
- Ribosomal RNA (rRNA)—short single-stranded molecules around 1,500 to 4,700 nucleotides long. Ribosomal RNAs are important components of **ribosomes,** organelles that are essential for protein

synthesis. Ribosomes recognize and bind to mRNA and "read" the mRNA during translation.

- Transfer RNA (tRNA)—molecules that transport amino acids to the ribosome during protein synthesis. Transfer RNA molecules are approximately 75 to 90 nucleotides in length.

We have already discussed the details of mRNA structure, but before we explore the details of translation, you need to be familiar with the genetic code of mRNA and the specific structures of ribosomes and tRNA.

The genetic code

What is the **"genetic code"** contained within mRNA? As you will learn shortly, ribosomes read the code and then produce proteins, which are formed by joining the building blocks called **amino acids** (see Appendix 2). A chain of amino acids linked together by covalent bonds is a **polypeptide.** Some proteins consist of a single polypeptide chain; others contain several polypeptide chains that must wrap and fold around each other to form complicated three-dimensional structures (see Figure 4.3). Proteins can contain combinations of up to 20 different amino acids, yet there are only four bases in mRNA molecules. So how does this code work? What information is the ribosome decoding to tell a cell what amino acids belong in a protein? If mRNA consists of combinations of only four nucleotides, how can mRNA provide information coding for 20 different amino acids?

The answers to these questions lie in the genetic code, a universal language of genetics used by virtually all living organisms. The code works in three-nucleotide units called **codons,** which are contained within mRNA molecules. Each codon codes for a single amino acid (Table 2.3). For instance, notice that the codon UAC codes for the amino acid tyrosine, and the codon UGC codes for the amino acid cysteine. Although each codon codes for one amino acid, the genetic code has flexibility. There are 64 different potential codons corresponding to all possible combinations of the four possible bases assembled into three nucleotide codons (4^3). But because there are only 20 amino acids, most amino acids may be coded for by more than one codon. For example, notice in Table 2.3 that the amino acid lysine may be coded for by AAA and AAG. Having a redundancy of codons increases the efficiency of translation. Some codons are present in mRNAs with greater frequency than others, just as some words in the English language are preferred over others with identical meanings.

Also contained in the genetic code are codons that tell ribosomes where to begin or end translation. The start codon, AUG, codes for the amino acid methionine and signals the starting point for mRNA translation. As a result, the first amino acid in many proteins is methionine, although this amino acid is removed shortly after translation in some proteins. Stop codons

TABLE 2.3 The Genetic Code

First Position (5′ End)	Second Position: U	Second Position: C	Second Position: A	Second Position: G	Third Position (3′ End)
U	UUU, UUC: Phenylalanine (Phe) UUA, UUG: Leucine (Leu)	UCU, UCC, UCA, UCG: Serine (Ser)	UAU, UAC: Tyrosine (Tyr) UAA: *Stop* UAG: *Stop*	UGU, UGC: Cysteine (Cys) UGA: *Stop* UGG: Tryptophan	U C A G
C	CUU, CUC, CUA, CUG: Leucine (Leu)	CCU, CCC, CCA, CCG: Proline (Pro)	CAU, CAC: Histidine (His) CAA, CAG: Glutamine (Gln)	CGU, CGC, CGA, CGG: Arginine (Arg)	U C A G
A	AUU, AUC, AUA: Isoleucine (Ile) AUG: Methionine (Met) START	ACU, ACC, ACA, ACG: Threonine (Thr)	AAU, AAC: Asparagine (Asn) AAA, AAG: Lysine (Lys)	AGU, AGC: Serine (Ser) AGA, AGG: Arginine (Arg)	U C A G
G	GUU, GUC, GUA, GUG: Valine (Val)	GCU, GCC, GCA, GCG: Alanine (Ala)	GAU, GAC: Aspartic Acid (Asp) GAA, GAG: Glutamic Acid (Glu)	GGU, GGC, GGA, GGG: Glycine (Gly)	U C A G

terminate translation. UGA is a commonly used stop codon, but UAA and UAG are other stop codons (Table 2.3). Stop codons do not code for amino acids; they simply signal the end of translation.

The genetic code is universal. It is used by cells in humans, bacteria, plants, earthworms, fruit flies, and all other species. There are some subtle differences to the code in certain species, but at the basic level it operates the same way throughout biology. Because of this, biologists can use techniques called **recombinant DNA technology** to clone a human gene such as the insulin gene and insert it into bacteria so that bacterial cells transcribe and translate insulin—a protein they normally do not produce. Another helpful aspect of the universal genetic code is that it enables scientists to clone a gene in one species, such as a mouse, and then use sequence information from the mouse gene to identify a similar gene in humans. Because different species share a common genetic code, this approach is a very common strategy for identifying human genes, including many involved in disease processes.

Ribosomes and tRNA molecules

Ribosomes are complex structures consisting of aggregates of rRNA and proteins that form structures called subunits. Each ribosome contains two subunits, the large and small subunits. These subunits associate to form two grooves, called the **A (aminoacyl) site** and the **P (peptidyl) site,** into which tRNA molecules can bind, and an **E site** through which tRNA molecules leave the ribosome (Figure 2.12). Incidentally, scientists are working on engineering ribosomes that may be manipulated in the lab to do things that naturally occurring ribosomes cannot do. For example, by connecting the two ribosomal subunits, it may be possible to increase the speed and efficiency of translation for rapidly synthesizing therapeutic and other commercially important proteins for the biotechnology industry.

Transfer RNAs are small molecules less than 100 nucleotides long. Transfer RNAs fold in intricate ways, and certain nucleotides in a tRNA base pair with each other. As a result, a tRNA assumes a structure called a cloverleaf, because as regions of the molecule base pair,

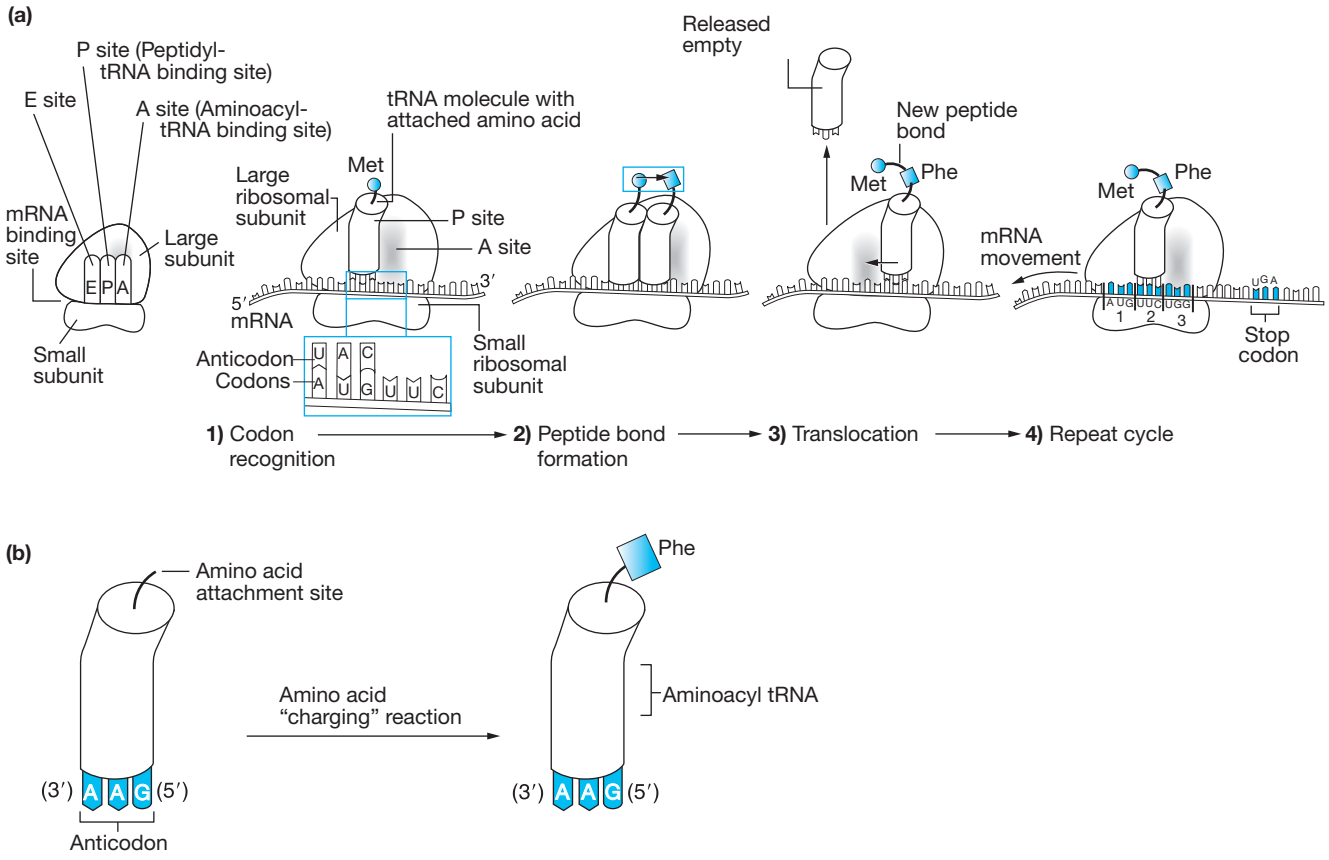


FIGURE 2.12 Stages of Protein Synthesis (a) Each ribosome contains a large and a small subunit. Shown here is a ribosome attached to mRNA; abbreviated steps of translation are shown in steps 1 to 4. (b) Diagrammatic example of the tRNA symbol used in this book. At one end of each tRNA is an amino acid binding site, and at the opposite end is a three-nucleotide anticodon sequence.

other unpaired segments create loops. At one end of each tRNA is an amino acid attachment site (Figure 2.12b). Enzymes in the cytoplasm called aminoacyl tRNA synthetases attach a single amino acid to each tRNA molecule, creating what is known as an **aminoacyl transfer RNA (tRNA)** or "charged" tRNA. Aminoacyl tRNA molecules carry their amino acids to the ribosome and bind within grooves of the ribosomes at the A site. At the opposite end of each tRNA molecule is a three-nucleotide sequence called an **anticodon.** Different amino acids have different anticodon sequences. Anticodons are designed to complementary base pair with codons in mRNA. Now that you know the "players" of translation—mRNA, ribosomes, and tRNA—we will examine how these components come together to produce a protein.

Stages of translation

There are some fundamental differences between translation in prokaryotes and that in eukaryotes. Here we provide an overview of basic aspects of the three major stages of translation in eukaryotes: initiation, elongation, and termination. The beginning of translation is called *initiation*. During initiation, the small ribosomal subunit binds to the 5′ end of the mRNA molecule by recognizing the 5′ cap of the mRNA. Other proteins, called initiation factors, are also involved in guiding the small subunit to the mRNA.

The small subunit moves along the mRNA until it encounters the start codon AUG. Pausing at the start codon, the small subunit waits for the correct tRNA, called the initiator tRNA, before translation can proceed (Figure 2.12). This tRNA has the amino acid methionine (met) attached to it (remember that most proteins begin with this amino acid) and contains the anticodon UAC. The UAC anticodon binds to the start codon by complementary base pairing (Figure 2.12); then the large ribosomal subunit binds to this complex containing the small subunit, initiation factors, mRNA, and initiator tRNA. Once these components are in place, the ribosome can start translating a protein.

The next step of translation is called *elongation*, because during this phase additional tRNAs enter the ribosome, one at a time, and a growing polypeptide chain is elongated. The ribosome, paused at the second codon, waits for the (second) tRNA to enter the A site. In Figure 2.12, notice that the second codon is UUC, which codes for the amino acid phenylalanine (phe). The phe-tRNA enters the A site of the ribosome and the anticodon (AAG) base pairs with the codon. After two tRNAs are attached to the ribosome, an enzyme in the ribosome called **peptidyl transferase** catalyzes the formation of a peptide bond between the amino acids (attached to their tRNAs). Peptide bonds join together amino acids to form a polypeptide chain.

After the amino acids are attached to each other, the initiator tRNA, without methionine attached, pauses briefly in the E site and then is released from the ribosome. Released "empty" tRNAs are recycled by the cell. A new amino acid is attached to the tRNA to create an aminoacyl tRNA, which can be used again for translation. The newly forming polypeptide remains attached to the tRNA in the A site. During a phase called *translocation*, the ribosome shifts so the tRNA and growing protein move into the P site of the ribosome. The tRNA with a growing polypeptide chain attached is called a peptidyl tRNA. The A site of the ribosome is now aligned with the third codon in sequence (UGG, which codes for tryptophan), and the ribosome waits for the proper aminoacyl tRNA to enter the A site. The cycle continues as described to attach the next amino acid (tryptophan) to the growing protein and repeats itself as the ribosome moves along the mRNA.



YOU DECIDE **Access to Biotechnology Products for Everyone?**

In Chapter 3 you will learn how genes can be identified, cloned, and studied in great detail. One benefit of gene cloning has been the identification of genes involved in human diseases. As a result, it is possible to make many gene products in the laboratory and use them for medical purposes. For instance, when the gene for insulin was cloned in bacteria, it became possible to produce large amounts of insulin for treating people with diabetes. Similarly, cloning of the gene for human growth hormone (hGH), which stimulates growth of bones and muscles during childhood, provided a readily available source of this hormone. Legally available by prescription only, hGH is used widely and effectively to treat children with certain forms of short stature or dwarfism. Illegal use of hGH by professional athletes has received much attention in recent years. Dwarfism is generally defined as a condition that results in an adult height of 4 feet, 10 inches or shorter. The availability of hGH and other products of biotechnology raises an ethical question. Should hGH be available to everyone who wants taller children or only those children with dwarfism? Suppose parents wanted their average-size son to be taller so that he would have a better chance of making his high school varsity basketball team. Should these parents be able to give their son hGH simply to enhance his height? You decide.

Elongation cycles continue to form a new protein until the ribosome encounters a stop codon (for instance, UGA). This signals the third stage of translation, called *termination*. Remember that stop codons do not code for an amino acid. Proteins called *releasing factors* interact with the stop codon to terminate translation. The ribosomal subunits come apart and release from the mRNA, and the newly synthesized protein is released into the cell. Ribosomes do recycle and subsequently can bind to any other mRNA molecule (not just the mRNA for one particular gene) and start the process of translation again.

2.5 Regulation of Gene Expression

The term **gene expression** refers to the production of mRNA (and sometimes protein) by a cell. Cells are exquisitely effective at controlling gene expression and translation to accommodate their needs. All genes are not transcribed and translated at the same rate in all cells. Gene expression is a dynamic process. Individual genes can be actively transcribed for a particular period of time, followed by periods of relatively little transcription, depending on the needs of a cell. How can genes be turned on and off in response to different signals? Biologists call this process **gene regulation.**

In addition, all cells of an organism contain the same genome, so how and why are skin cells different from brain cells or liver cells? Different cell types have different properties and carry out different functions because cells can *regulate* or control the genes they express. "Positive" and "negative" regulation of gene expression can occur. At any given time in a cell, only certain genes are "turned on" or expressed (positive regulation) to produce proteins, while many other genes are silenced or repressed (negative regulation). These genes may be expressed by cells only at certain times, in response to specific cues from inside or outside the cell, to make proteins as needed. These cues can be environmental signals such as temperature changes, nutrients in the external environment, hormones, or other complex chemical signals exchanged by cells.

Prokaryotic cells and eukaryotic cells regulate gene expression in a variety of complex ways (Figure 2.13). One common mechanism used by both types of cells, that has a significant impact on gene expression and the abundance of proteins produced, is called **transcriptional regulation**—controlling the amount of mRNA transcribed from a particular gene as a way to turn genes on or off. Estimates vary on the extent to which *post-transcriptional regulation* (such as mRNA degradation) translational regulation, and post-translational regulatory processes control protein abundance in cells. Here we provide an introduction to transcriptional regulation and consider basic examples of this process in eukaryotes and prokaryotes.

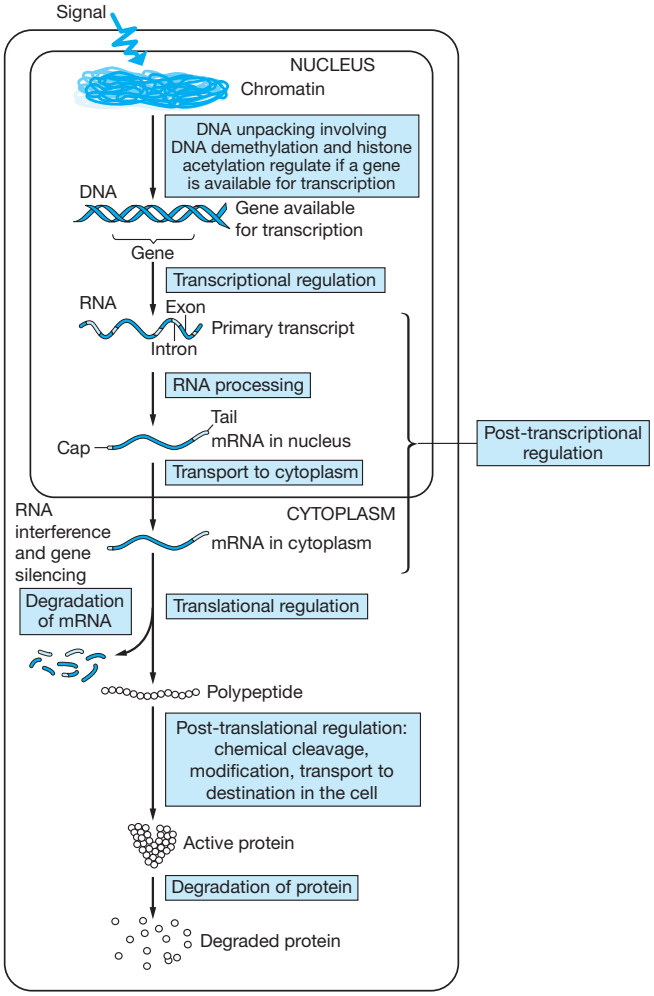


FIGURE 2.13 Levels of Gene Expression Regulation Prokaryotic and eukaryotic cells can regulate gene expression in a variety of complex ways. This figure summarizes the primary ways in which gene expression can be regulated in eukaryotic cells. Notice that gene expression regulation can occur at many different "levels" (highlighted in blue boxes here), beginning with how chromatin is folded or chemically modified to controlling degradation or turnover of a protein once it is made. Transcriptional regulation is a commonly used control mechanism in both prokaryotic and eukaryotic cells.

Transcriptional Regulation of Gene Expression

Because the amount of protein translated by a cell is often directly related to the amount of mRNA in the cell, cells can regulate the amount of mRNA produced for any given gene to control indirectly the amount of protein a cell produces. How do cells know which genes to turn on and which to shut off? To understand transcriptional regulation, we must look at the role of promoter sequences more closely.

Promoters are found "upstream" of gene sequences, meaning that they are found at the 5′ end of a gene.

Genes in prokaryotes and eukaryotes do not all use the same promoter sequences. In eukaryotes, common promoter sequences found upstream of many genes include a **TATA box** (TATAAAA), located about 30 nucleotides (–30) upstream of the start site of a gene, and a **CAAT box** (GGCCAATCT), located about 80 nucleotides (–80) upstream of a gene (Figure 2.14).

Earlier in this chapter we discussed how RNA polymerase initiates transcription by binding to promoter sequences adjacent to genes. For most eukaryotic genes, RNA polymerase cannot recognize and bind to a promoter unless transcription factors are present at the promoter. Transcription factors are DNA-binding proteins that can bind promoters and interact with RNA polymerase to stimulate transcription of a gene (Figure 2.14). In eukaryotes and prokaryotes, common transcription factors interact with promoters for many genes; however, both types of cells also use specific transcription factors that interact only with certain promoters. Transcription of some genes also depends on the binding of specific transcription factors to regulatory sequences adjacent to the promoter. In addition, many genes that are tightly regulated by cells also contain regulatory sequences called **enhancers.**

Enhancer sequences are usually located around 50 or more base pairs upstream of the promoter, but they can also be located downstream of a gene. Enhancer sequences bind regulatory proteins, generally referred to as *activators*. Activator molecules interact with transcription factors and RNA polymerase, forming a complex that stimulates (activates) transcription of a gene. Cells use a wide variety of different activator molecules. Each activator binds to a particular enhancer sequence. Some activator molecules can be hormones. For instance, the male sex steroid testosterone can be an activator to stimulate gene expression. You may know that testosterone stimulates cellular activities such as muscle and hair growth in adolescent boys, but how does testosterone work? This hormone binds to a receptor protein inside cells. The testosterone–receptor protein complex acts as an activator to bind to a specific enhancer element in DNA called an *androgen-response element* (5′-TGTTCT-3′). These elements are usually found close to a promoter. In turn, testosterone and its receptor stimulate gene expression. The female sex steroid estrogen works in a similar manner.

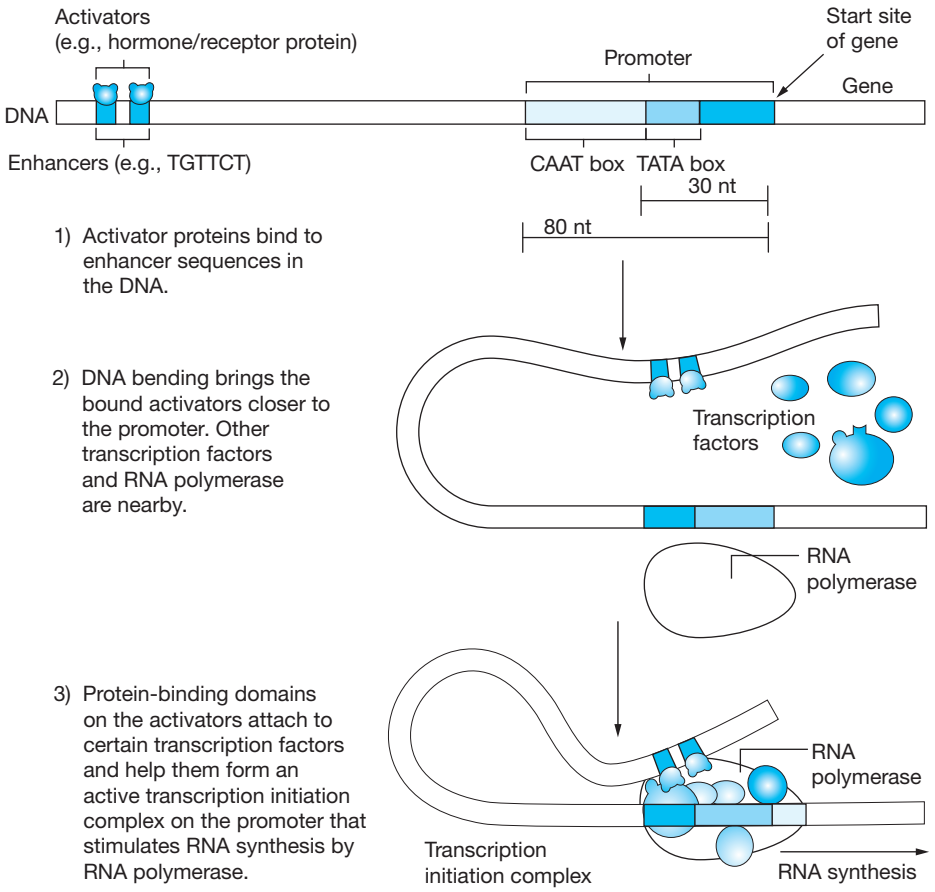


FIGURE 2.14 Promoters, Transcription Factors, and Enhancers

But testosterone and other activators don't stimulate expression of all genes in all cells. Activators act only on those genes that contain enhancer sequences to which they can bind. Testosterone stimulates expression of genes involved in muscle and hair growth because these genes contain androgen-response elements. Transcription of other genes without androgen-response elements are not directly affected by the hormone. Incidentally, steroid abuse by bodybuilders and athletes looking to increase muscle mass and tone can cause serious long-term health effects, in part because steroids abnormally stimulate gene expression for prolonged periods.

Through activators and enhancers, cells can use transcriptional control to regulate gene expression and control cellular activities. Some genes even contain repressor sequences that decrease transcription. Because different cells produce different transcription factors and activator molecules, genes can be turned on in some tissues and not others. Skin cells turn on different genes than muscle cells do, so each cell type produces different proteins, giving each cell type different functions. Consequently, tissue- and cell-specific gene expression is one way for cells to control the proteins they express even though all body cells contain the same genome. These important control mechanisms are part of why different cells have different functions.

In addition, identifying the promoters, enhancer sequences, and transcription factors that bind to regulatory regions of a gene is important for making biotechnology products. For instance, the identification of transcription factors that stimulate the expression of proteins needed for bone growth and development is helping scientists develop new drugs that can be used to stimulate bone growth in people suffering from forms of arthritis when their cells no longer produce the factors that stimulate bone growth.

Noncoding RNAs and Their Roles in Regulating Gene Expression

Earlier we mentioned the concept of noncoding RNAs (ncRNAs) and that rRNA and tRNA are examples of ncRNAs. These two ncRNAs are among the most abundant RNA molecules in a typical eukaryotic cell, comprising approximately 80 to 90 percent and 10 to 15 percent of the total RNA content of a cell, respectively. By comparison, protein-coding mRNA comprises approximately 3 to 7 percent of the RNA content of a cell.

Although ncRNAs are found in both eukaryotic and prokaryotic cells, depending on the cell type there are several other types of ncRNAs. Table 2.4 provides a summary of different ncRNAs, their approximate

TABLE 2.4 Noncoding RNAS (ncRNAs)

Type of ncRNA (abbreviation)	Average Size (nt)	Basic Functions
Ribosomal RNA (rRNA)	~7,000 nt	Translation
Transfer RNA (tRNA)	<100 nt	Translation
Small nuclear RNA (snRNA)	100–200 nt	mRNA splicing (bind specific proteins called snRNPs and form splicing complexes that catalyze pre-mRNA splicing)
Small nucleolar RNAs (snoRNA)	200 nt	Chemical modifications (such as methylation) of other RNAs including tRNA, rRNA, snRNA
MicroRNAs (miRNAs)	21–24 nt	RNA silencing and post-transcriptional regulation of gene expression
Long noncoding RNAs (lncRNA)	>200 nt (~1000–3,000 nt)	Regulation of transcription, post-transcriptional regulation, RNA splicing, RNA stability, inactivation of the X chromosome, among other roles
Small or short interfering RNAs (siRNAs)	20–25 nt, double-stranded	Similar to miRNAs involved in RNA interference (RNAi) pathway for gene expression regulation
Piwi interacting RNA (piRNA)	26–31 nt	One of the largest categories of ncRNAs; epigenetic and post-transcriptional gene expression regulation, particularly in developing spermatozoa
CRISPR-derived RNA (crRNA)	~20 nt	CRISPR-Cas–mediated immune responses in prokaryotes

Source: Adapted from Palazzo AF, Lee ES. Non-coding RNA: what is functional and what is junk? *Front Genet*. 2015;6. https://www.ncbi.nlm.nih.gov/pmc/articles/pmc4306305/table/t1/. Accessed June 24, 2017.

sizes, and examples of their functions. Individually, each of these ncRNAs is far less abundant than rRNA or tRNA, ranging anywhere from approximately 0.003 to 0.06 percent of the RNA content of a cell. With the exception of **long noncoding RNAs (lncRNAs),** the other ncRNAs in this table are often also collectively referred to as **small noncoding RNAs (sncRNAs).** Throughout this book you will learn more about different types of ncRNAs.

For example, in 1998, scientists studying the roundworm *Caenorhabditis elegans* discovered small (21 or 22 nt) double-stranded pieces of non-protein-coding RNA called **short interfering RNA (siRNA),** so named because they were shown to bind to mRNA and subsequently block or interfere with translation of bound mRNAs. For a short time it appeared that perhaps siRNAs were restricted to *C. elegans*, but while looking for siRNAs in other species, scientists discovered that **microRNAs (miRNAs)** are another category of small RNA molecules that do not encode proteins. Genes for miRNAs have been identified in all multicellular organisms studied to date, and miRNAs are part of a rapidly growing family of sncRNAs that play novel roles in regulating gene expression.

Like siRNAs, miRNAs regulate gene expression by "silencing" gene expression through blocking translation of mRNA or by causing degradation of mRNA. MicroRNA genes produce RNA transcripts that are processed by enzymes (Drosha and Dicer) into short single-stranded pieces 21 to 22 nucleotides in length (Figure 2.15). The processed miRNAs then bind to a complex of proteins (called RISCs), which allows them to bind to mRNAs that are complementary to the miRNA sequence. Binding of miRNA to an mRNA sequence can either block translation by ribosomes or trigger enzymatic degradation of an mRNA. Both mechanisms inhibit or silence expression by preventing the mRNA from being translated into a protein.

RNA-based mechanisms of gene silencing are generally referred to as **RNA interference (RNAi).** The identification of human genes that are silenced by miRNAs is currently a very active area of research. Several thousand human miRNAs have been identified and linked to normal cellular process and diseases in humans. Scientists are working on exploiting RNAi as a potentially promising way to use small RNA molecules to selectively turn off or silence genes involved in human disease conditions (discussed in Chapters 3 and 11). As evidence of how valuable RNAi is as a research technique, the 2006 Nobel Prize in Physiology or Medicine was awarded to Andrew Fire of Stanford University and Craig Mello of the University of the Massachusetts School of Medicine for their discovery of this method for switching genes off.

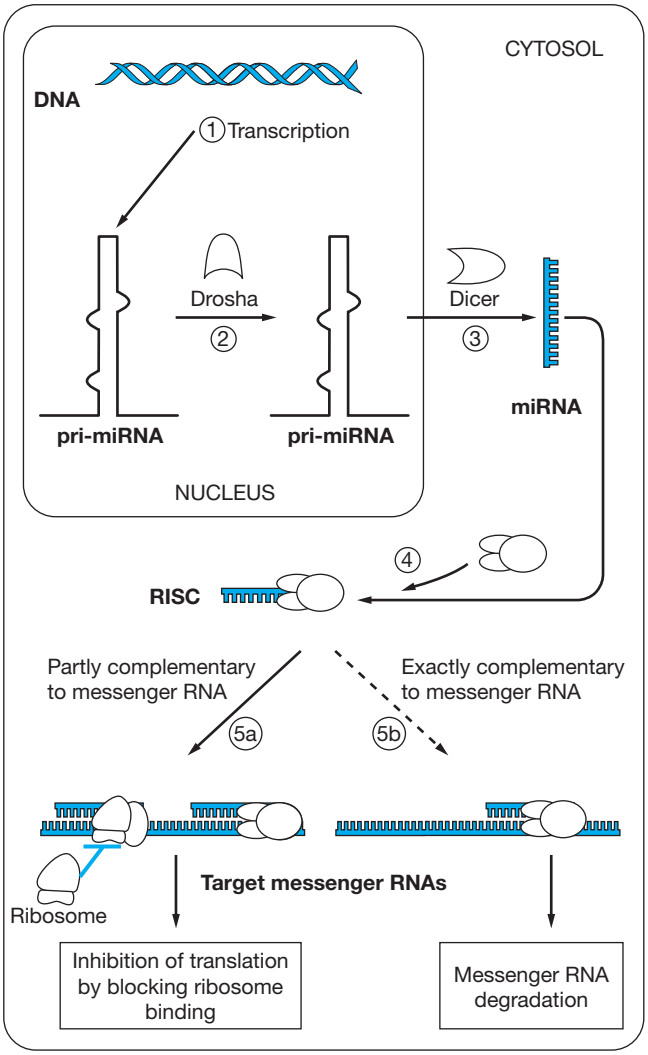


FIGURE 2.15 MicroRNAs Silence Gene Expression
Gene silencing by miRNAs is a multistep process. (1) MicroRNA genes transcribe a primary transcript called pri-RNA that (2) folds into a hairpin loop similar to the folding patterns of tRNA molecules. (3) The enzyme Dicer cleaves pri-mRNAs into single-stranded miRNAs that are 21 to 22 nucleotides long. (4) MicroRNAs bind to a cluster of proteins to form a ribonucleoprotein complex. (5) This miRNA complex can then bind to mRNA molecules by complementary base pairing with sequences that are an exact match or a close match to complementary sequences in the mRNA and miRNA. (5a) miRNA binding then silences gene expression by blocking translation or by targeting the bound mRNA for degradation by enzymes in the cell.

Bacteria Use Operons to Regulate Gene Expression

Bacteria are very important organisms for many applications of biotechnology, such as the production of human proteins for therapeutic purposes. Many initial studies on gene regulation were carried out in bacteria. Bacteria and other microorganisms can and

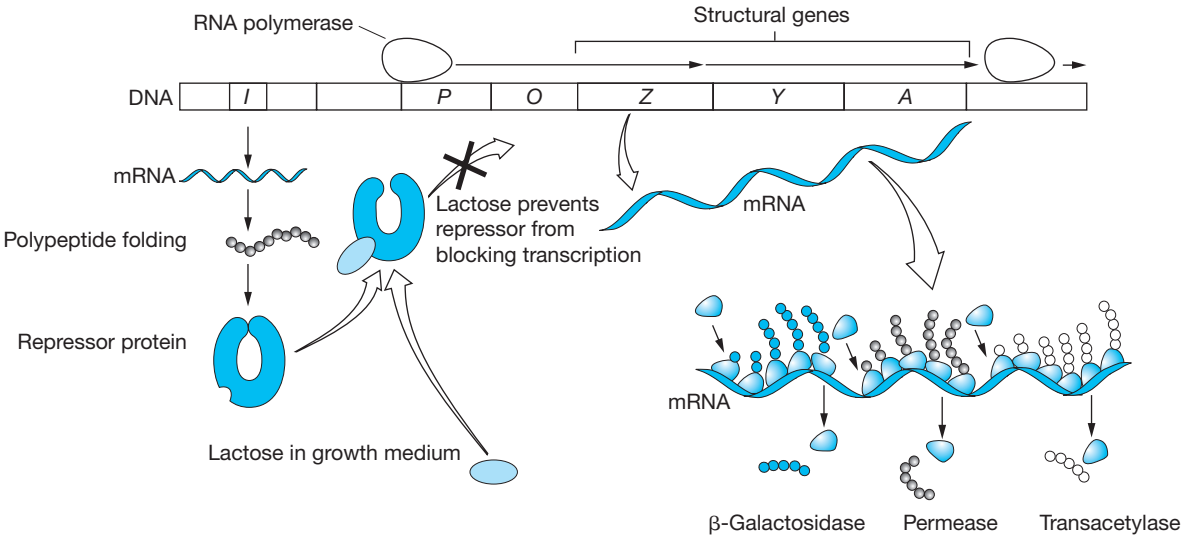


FIGURE 2.16 The *lac* Operon By controlling the *lac* operon, bacterial cells can regulate gene expression in response to availability of the sugar lactose. In the absence of lactose, the *lac* repressor binds to the operator, blocking its transcription. In the presence of lactose, lactose binds and inactivates the repressor, allowing transcription of the operon to occur.

must rapidly control gene expression in response to environmental stimuli such as growth nutrients and changes in temperature and light intensity. One interesting aspect of gene expression and regulation in bacteria is that many bacterial genes are organized in arrangements called **operons.** Operons are essentially clusters of several related genes located together and controlled by a single promoter.

The genes of an operon can be regulated in response to changes within the cell. Operons can be stimulated (induced) or inhibited (repressed) depending on the needs of a cell. Many genes controlling nutrient metabolism by bacteria are organized as operons. Bacteria can use operons to tightly regulate gene expression in response to their nutrient requirements. Here we present a well-studied classic example of gene regulation in bacteria by describing the ***lac* operon** (Figure 2.16).

The *lac* operon consists of the following three genes:

- *lacZ*, encoding the enzyme β-*galactosidase*
- *lacY*, encoding the enzyme *permease*
- *lacA*, encoding the enzyme *acetylase*

Together, these three enzymes are necessary for the transport and breakdown of lactose by bacterial cells. Lactose, a sugar present in milk, is an important energy source for many bacteria. For bacteria to metabolize lactose, the sugar must be transported into cells by permease and then degraded into glucose and galactose by β-galactosidase. The function of acetylase is not clear, although it may play a role in protecting cells from the toxic products of lactose degradation.

The *lac* operon is regulated by a protein called the ***lac* repressor,** which is encoded by a separate gene called the *lacI* gene. When bacteria are grown in the absence of lactose, the repressor protein binds a sequence within the *lac* operon promoter (p) called the **operator** (o). By binding to the operator, the repressor blocks RNA polymerase from binding to the promoter and blocks transcription of the *Z*, *Y*, and *A* genes in the operon (Figure 2.16). This is a nice way for bacteria to control their metabolism. Why expend energy transcribing genes and translating proteins if no lactose is available for producing energy?

Conversely, in the presence of lactose, the sugars act as inducer molecules that stimulate transcription of the *lac* operon. Lactose binds to the *lac* repressor, changing the shape of the repressor protein and preventing it from binding to the operator (Figure 2.16). With no repressor in its way, RNA polymerase can bind to the *lac* promoter and stimulate transcription of the operon. Transcribed mRNA from the operon is translated to produce the enzymes required by the cell to metabolize lactose.

2.6 Mutations: Causes and Consequences

We continue our overview of cell biology and genetics with a brief discussion of how genes can be affected by **mutations,** or changes in the nucleotide sequences of DNA. Mutations are a major cause of genetic diversity.

For instance, the underlying basis of the evolution of species to develop and acquire new characteristics is governed by mutations of genes over time. Mutations can also be detrimental. Mutation of a gene can result in the production of an altered protein that functions poorly or in some cases no longer encodes a functional protein. Such mutations can cause genetic diseases. In this section, we provide an overview of different types of mutations and their consequences.

Types of Mutations

Sometimes mutations occur through spontaneous events, such as errors during DNA replication. For instance, DNA polymerase can insert the wrong nucleotide into a newly synthesized strand of DNA, for example, inserting a T where a C belongs. Even though enzymes in cells work to detect and correct mistakes, errors occasionally occur during DNA replication. Mutations can also be induced by environmental causes. For example, chemicals called **mutagens,** many of which mimic the structure of nucleotides, can mistakenly be introduced into DNA and change DNA structure. Exposure to x-rays or ultraviolet light from the sun can also mutate DNA (that glowing tan you may enjoy during the summer is not as healthy as you think).

Regardless of how mutations arise, depending on the type of mutation, they may have no effect on protein production or they can dramatically change protein production and protein structure and function. Mutations can involve large changes in genetic information or single-nucleotide changes in a gene, such as changing an A to a C or a G to a T. The most common mutations in a genome are single-nucleotide changes (or a few nucleotide mutations) called **point mutations.** Such mutations are commonly referred to as **single-nucleotide polymorphisms (SNPs,** pronounced "snips"), and they represent one major type of genetic variation in the genomes of different humans. We will discuss SNPs in several chapters. Point mutations often involve base-pair substitutions, in which a base pair is replaced by a different base pair; insertions, in which a nucleotide is inserted into a gene sequence; or deletions, the removal of a base pair (**Figure 2.17**).

Mutations ultimately exert their effects on a cell by changing the properties of a protein, which in turn can affect traits.

Gene mutation can cause:
↓
Changes in protein structure and function; synthesis of a nonfunctional protein or no protein synthesized can lead to:
↓
Change or loss of a trait

Proteins are large, complicated molecules. To work correctly, most proteins must be folded into complex three-dimensional shapes. Changing one or two amino acids in a protein can alter its overall shape, dramatically disrupting its function or in some cases preventing it from functioning at all. A mutation may have no effect on a protein if it changes the codon sequence of a gene to another codon that codes for the same amino acid (Figure 2.17). This is a **silent mutation** because it has no effect on the structure and function of the protein. Similarly, a mutation can change a codon so that a different amino acid is coded for. Such **missense mutations** are also considered "silent" if the new amino acid coded for doesn't change the protein's structure and function. However, if the newly coded amino acid changes the structure of the protein, its function may be significantly altered. Later, we consider a dramatic example of how a SNP is responsible for the human genetic condition known as sickle cell disease.

Sometimes mutations called **nonsense mutations** change a codon for an amino acid into a stop codon, which causes an abnormally shortened protein to be translated, usually creating a nonfunctional protein. Insertions or deletions can also dramatically affect the protein produced from a gene by creating a **frameshift mutation.** As shown in Figure 2.17, the insertion of a nucleotide causes the reading frame of the codons to be shifted to the right of the insertion, changing the protein encoded by the mRNA. Frameshifts often create nonfunctional proteins.

Mutations can be inherited or acquired

It is important to realize that not all mutations have the same effect on body cells. The effects of a mutation depend not only on the type of mutation but also on the cell type in which it occurs. Gene mutations can be inherited or acquired. **Inherited mutations** are mutations passed to offspring through gametes—sperm or egg cells. As a result, the mutation is present in the genome of all the offspring's cells. Inherited mutations can cause birth defects or inherited genetic diseases.

Acquired mutations occur in the genome of somatic cells and are not passed along to offspring. Although not inherited, acquired mutations can cause abnormalities in cell growth, leading to the formation of cancerous tumors as well as metabolic disorders and other conditions. For instance, prolonged exposure to ultraviolet light can cause acquired mutations in skin cells, leading to skin cancer. The effort to understand the genetic basis of cancer and many other human diseases is a major area of biotechnology research.

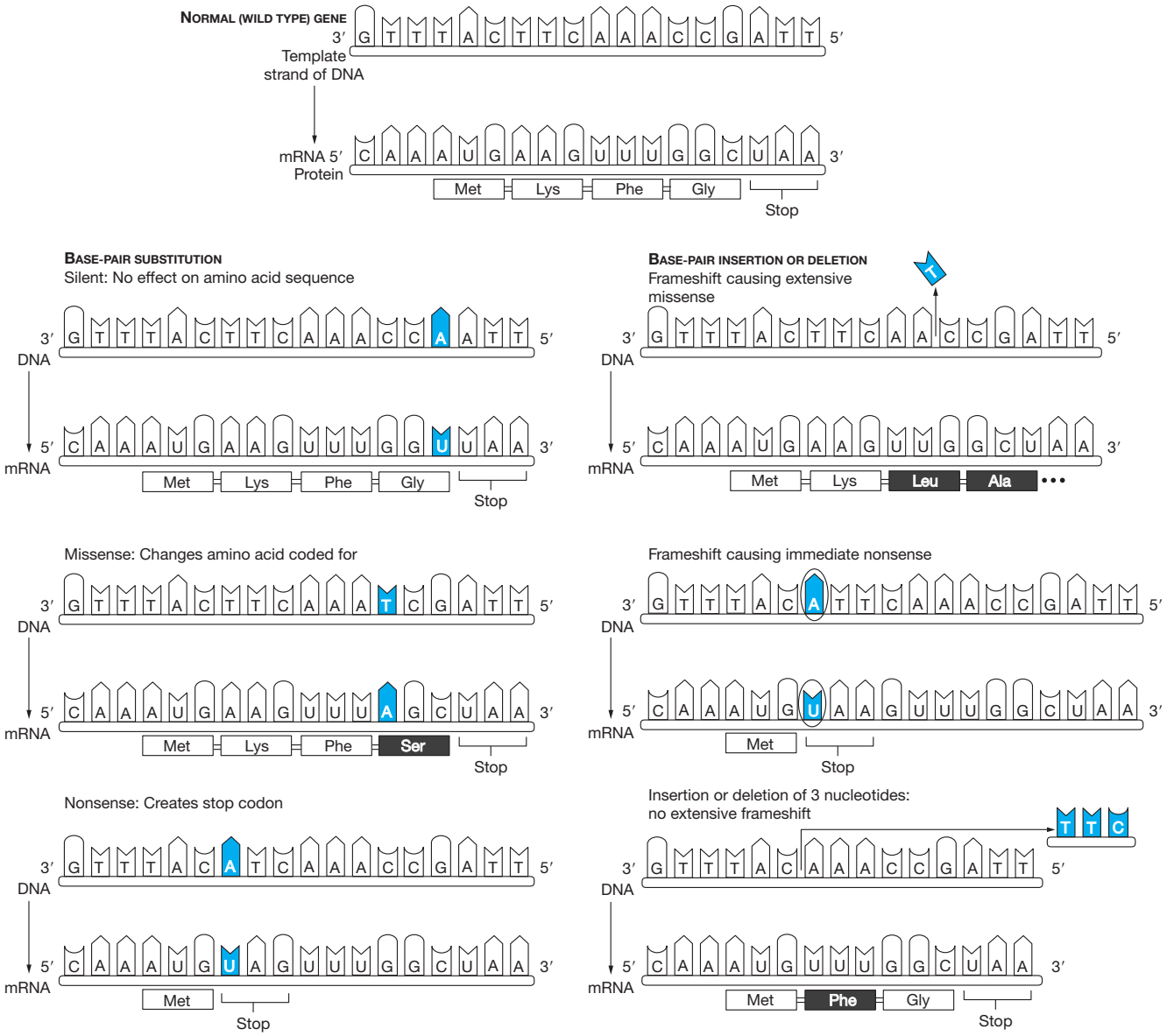


FIGURE 2.17 Types of Mutations Mutations can influence the genetic code of mRNA and resulting proteins translated from a gene. Shown here is a portion of mRNA copied from a gene, but mutations generally occur within DNA. Mutations in a gene can have different effects on the protein that is translated.

Mutations Are the Basis of Variation in Genomes and a Cause of Human Genetic Diseases

Mutations form the molecular basis of many human genetic diseases. Sickle cell disease was the first genetic disease discovered; its cause was pinpointed to a particular mutation (Figure 2.18). Sickle cell disease is created by a single-nucleotide change, a base-pair substitution, in the gene coding for one of the polypeptides in the protein **hemoglobin,** the oxygen-binding protein in red blood cells. Red blood cells contain millions of hemoglobin molecules, each of which consists of four polypeptide chains (Figure 2.18). A point mutation in the β-globin gene changes the genetic code so that the gene codes for a different amino acid at position 6 in one of the hemoglobin polypeptides. As a result, the amino acid valine, instead of glutamic acid, is inserted into sickle cell hemoglobin. Individuals with two defective copies of the hemoglobin gene suffer the effects of sickle cell disease. This subtle mutation alters the oxygen-transporting ability of hemoglobin and dramatically changes the shape of red blood cells to an abnormal sickled shape. Sickled cells block blood vessels, and patients suffer from poor oxygen delivery to tissues, causing joint pain and other symptoms. Sickle

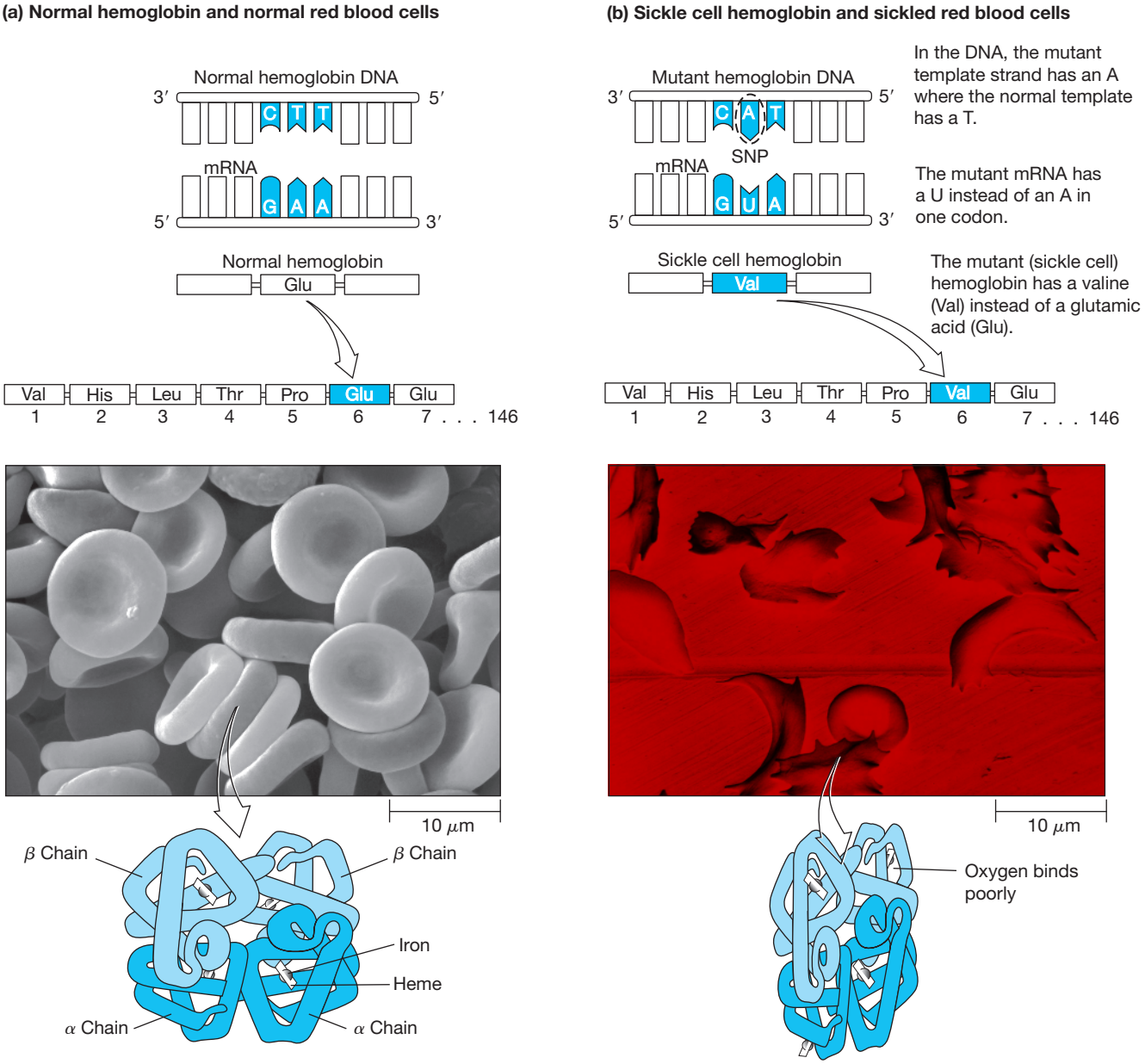


FIGURE 2.18 Molecular Basis of Sickle Cell Disease (a) Hemoglobin, the oxygen-binding protein of red blood cells, consists of four polypeptide chains. A portion of the normal hemoglobin gene is shown here along with its transcribed mRNA and the first seven amino acids for one of the hemoglobin polypeptides, which contains a total of 146 amino acids. (b) The defective gene that causes sickle cell disease contains a single nucleotide change in its sequence (an example of a SNP) that alters translation of the hemoglobin protein. This subtle change in protein structure alters the shape of red blood cells, causing them to sickle.

cell disease is one of the best-understood inherited genetic disorders.

As we have learned more about the human genome, we have discovered that DNA sequences from people of different backgrounds around the world are very similar. Regardless of ethnicity, human genomes are approximately 99.9 percent identical. In other words, your DNA sequence is about 99.9 percent the same as DNA sequences from French President Emmanuel Macron, Usain Bolt, Elton John, Julia Roberts, Helen Mirren, Bruce Lee—or virtually any other human!

But because there is about a 0.1 percent difference in DNA between one individual and another, or around one base out of every thousand, this means that there are roughly 3 million differences between different individuals. Many of these variations are created by mutation, and most of them have no obvious effects; however, other mutations strongly influence



"You're lucky nobody was hurt. Your base pairs are out of alignment and that has your reading frames all messed up."

FIGURE 2.19 Biotechnology Is Being Used to Detect and Correct Mutations

cell function, behavior, and susceptibility to genetic diseases. These variations are the basis of differences in all inherited traits among people, from height and eye color to personality, intelligence, and life span.

Most genetic variation between human genomes is created by SNPs. For instance, at a particular sequence, a certain base in a given region of DNA sequence from one person may read "A," another's sequence may read "T," and the same site in your sequence may read "G." Most SNPs are harmless because they occur in intron regions of DNA; however, when they occur in exons, they can affect the structure and function of a protein, which can influence cell function and result in disease. Sickle cell disease is caused by a SNP. Refer to Figure 1.11, which shows a comparison of a gene sequence from three different people. A SNP in this gene in person 2 may have no effect on protein structure and function if it is a silent mutation. Other SNPs (person 3) cause disease if they change protein structure and function.

In other chapters, we'll consider different genetic disease conditions, discuss how defective genes can be detected, and examine how scientists are working on **gene therapy** and other approaches to cure genetic diseases (Figure 2.19; see also Chapter 11 for more on genetic disease conditions, detection, and treatment).

2.7 Revealing the Epigenome

Our study of the genomes of humans and other species has provided great insight into the **epigenome** and its influences on gene expression. The term *epigenome* refers to modifications in chromatin structure, which *do not* involve mutations in DNA sequence. Some epigenetic modifications are inherited and can persist in several generations of offspring, whereas others vary from generation to generation. Some epigenetic modifications may be reversible, whereas others are long-lasting. Epigenetic changes can differ between cell types in the body and in normal and diseased tissues. Diet and environmental conditions can influence the epigenome. We also know that epigenetics plays a major role in clarifying how patterns of gene expression can vary during embryonic development.

The more we study the epigenome, the more we learn about how complex it is. We are only now beginning to truly appreciate the importance of the epigenome. So what kinds of chromatin modifications contribute to the epigenome? Epigenetic modifications affect both DNA and histones. For example, the addition of methyl groups (-CH3) to DNA, known as *methylation*, commonly occurs on cytosine bases. Methylation of histone proteins also occurs. Other examples of histone modifications include the addition of acetyl groups (CO CH_3), or *acetylation*, and the addition of phosphate groups (PO_4), or *phosphorylation* (Figure 2.20).

The extent to which methylation, acetylation, and phosphorylation affect a genome can vary greatly from one region of a chromosome to another. These chemical modifications work in complex ways to affect how DNA loops and folds in a chromosome. Such structural effects can ultimately influence how easily gene expression occurs in a particular region of a genome because epigenetic modifications affect how "accessible" a segment of DNA is to proteins involved in transcription. For instance, heavily acetylated regions of a genome cause the DNA to maintain a more open and less compact arrangement that allows for transcription. Removing acetyl groups from such a section of the genome typically inhibits transcription. Other epigenetic modifications that affect transcription include remodeling of histones and other DNA-binding proteins, which can mask or activate regions of a genome; variations in the types of histone proteins found in DNA; and the presence or absence of ncRNAs that can coat DNA and influence gene expression (Figure 2.20).

Because the epigenome regulates gene expression, almost every major pharmaceutical company and many biotech companies are working on epigenetics projects. We know that the epigenome is involved in many diseases, including cancer. Inhibiting or enhancing epigenetic modifications to target epigenetic changes that can affect gene expression for disease treatment is a very hot area of research. For example, a category of drugs called histone methyltransferases (HMTs), which affect gene expression by adding methyl groups to histone proteins, have shown promise in treating specific malignant tumors and leukemia. Keep in mind this brief introduction to the epigenome as you learn more about genomes and ways in which the biotechnology industry is working on treatments for genetic diseases.

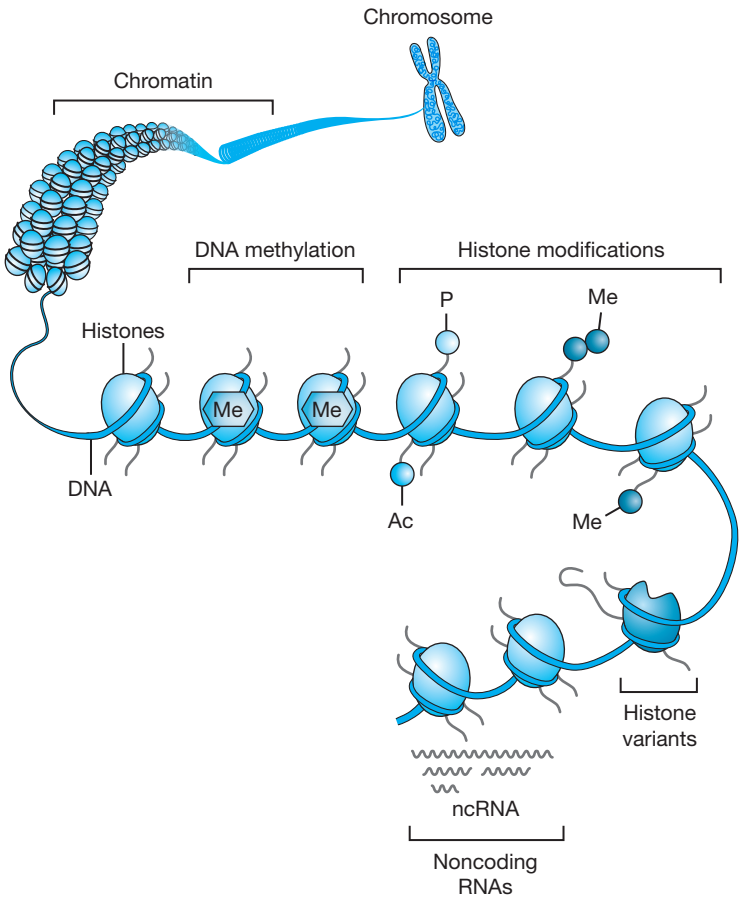


FIGURE 2.20 Chromatin Modifications Involved in Epigenetics The chromatin modifications shown here are responsible for many epigenetic influences on gene expression. These include DNA methylation (Me); histone modification by acetylation (Ac), phosphorylation (P), and methylation; remodeling of histones and other DNA-binding proteins; histone variations; and noncoding RNAs (ncRNAs).

2.8 Immune Response Mechanism in Prokaryotes Results in Extraordinary New Technology for Editing Genes *In Vitro* and *In Vivo*

We conclude this chapter with a brief discussion of a fundamental discovery about immune responses in prokaryotic cells that has led to unprecedented technical progress and excitement about how scientists can attempt to repair mutations *in vitro* and *in vivo*, including in humans receiving gene therapy.

The system called **CRISPR (Clustered Regularly Interspaced Palindromic Repeats)-Cas** was discovered by scientists trying to understand how bacteria fight viral infection. The original research on this system was not intended to create a technique for repairing mutations. CRISPR is a prokaryotic immune system that provides resistance to foreign genetic elements such as plasmids and phages **(Figure 2.21).** CRISPRs are segments of prokaryotic DNA consisting of short base-pair repeats. Each repeat is followed by a segment of spacer DNA, which comes from prior exposures to foreign plasmids or phages. CRISPR-associated proteins (Cas) function as nucleases. Cas9 was the first nuclease identified. Cas9 and other nucleases rely on specific ncRNAs called **CRISPR-derived RNAs (crRNAs)** transcribed from CRISPR sequences to target specific viral sequences for destruction. In prokaryotes, CRISPR spacer sequences can recognize viral DNA and use Cas nucleases to digest foreign DNA sequences, thus destroying them.

Scientists quickly realized that CRISPR-Cas could be adapted to target specific sequences in a genome that one wants to change or edit—thus, using CRISPR-Cas to repair mutations or to create specific changes in DNA sequence is referred to as **gene or genome editing.** As you will learn in Chapter 3, no approach to gene editing has garnered more attention, created more excitement, or generated as many early signs of success as CRISPR-Cas. In part, because of its simplicity as a laboratory technique, progress with CRISPR-Cas for gene editing of cultured cells or in whole organisms has been extraordinary. An introduction to CRISPR-Cas is a great conclusion to this chapter because it demonstrates how discovery of a fundamental process of cell biology can result in development of a powerful technology with the potential to save lives. This is what biotechnology is all about!

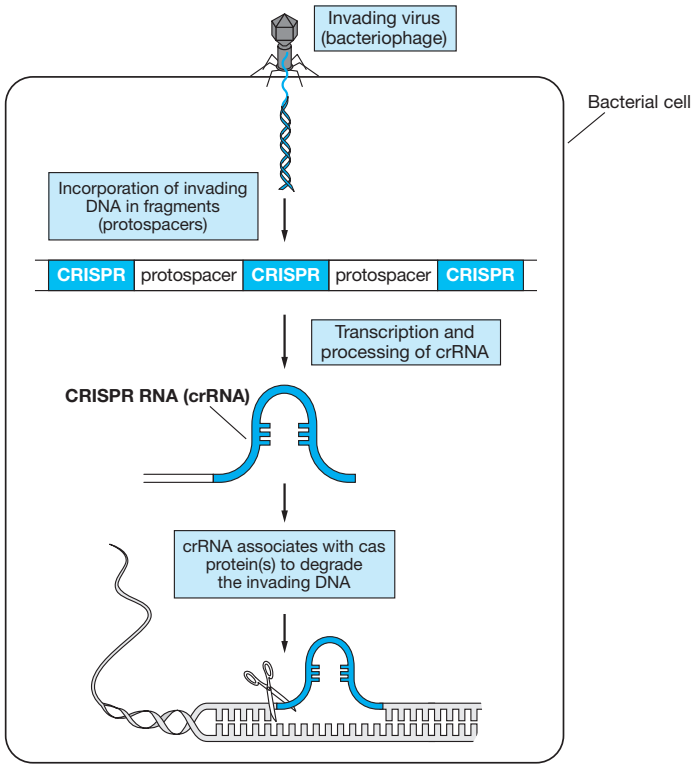


FIGURE 2.21 CRISPR-Cas Mediates Immune Responses in Prokaryotes

MAKING A DIFFERENCE

Scientists around the world are working on a variety of cell therapy approaches for the treatment of human disease. For example, researchers at Rush University Medical Center and Emory University are investigating applications of a novel cell therapy using retinal pigmented epithelial cells (RPE cells) attached to tiny gelatin beads. In early-stage studies, these beads have been implanted in the brains of patients with Parkinson disease (PD). Patients treated so far are showing improvements in symptoms associated with PD, such as tremors, rigidity, slowness of movements, and impaired balance and coordination. This cell therapy approach holds great promise, as some patients continue to show improvements 6 years or more after treatment. The RPE cells make levodopa, a precursor to the neurotransmitter dopamine. Loss of dopamine-producing neurons is a major cause of PD. In this approach, RPE cells are cultured in a lab and attached to gelatin beads, which are necessary for the cells to survive in the brain. Then the beads are implanted into patients. The implanted cells provide a source of levodopa, which can be used by local neurons to produce dopamine. Only time will tell if this approach will become a viable treatment option or cure for PD, but without question many cell-based strategies for treating disease are under way, strategies that will ultimately "make a difference" and alleviate pain and suffering.

QUESTIONS & ACTIVITIES

Answers can be found in Appendix 1.

1. Distinguish eukaryotic cells from prokaryotic cells by explaining at least three differences between these types of cells.
2. Compare and contrast genes and chromosomes, and describe their roles in the cell.
3. In a nucleotide of DNA, which carbon in the deoxyribose sugar is bonded to the nitrogenous base?
4. If the sequence of one strand of a DNA molecule is 5′ – ATTGCAGGAACCCTTA – 3′, what is the sequence of the complementary strand?
5. Suppose you identified a new strain of bacteria. If the DNA content of this organism's cells is 15 percent thymine, approximately what percent of this organism's genome consists of guanine? Explain your answer.
6. Provide at least three important differences between DNA and RNA.
7. Diagram the process of semiconservative replication of DNA by drawing a replication fork and indicating important enzymes, proteins, and other components involved in this process. Provide a *one-sentence* description of the function of each component.
8. Throughout *Introduction to Biotechnology* you will learn about genomes. Based on your studies so far, what is a genome and what information can be learned from studying genomes?
9. Consider the following sequence of mRNA: 5′ – GGCACCAUGCGUCGAAAAUCAAAGUGAAACACGGG – 3′. How many codons are included in this mRNA? How many amino acids are coded for by this sequence? Use Table 2.3 to determine the amino acid sequence encoded by this mRNA. Note: Remember that mRNA molecules are actually much larger than the very short sequence shown here.
10. Consider the following sequence of DNA:

    5′ – GGG ATG CCC TGG ACG CGG CGT TAG AT – 3′
    3′ – CCC TAC GGG ACC GGG GCC GCA ATC TA – 5′

    a. Transcribe each of these sequences into mRNA. Which DNA sequence (top or bottom strand) produces a functional mRNA containing a start codon? Which is the amino acid sequence of the polypeptide produced from this mRNA?

    b. For the DNA strand producing a functional mRNA, number each base from left to right with

the first based numbered "1′. Insert a "T" between bases 10 and 11, representing a base insertion mutation. Transcribe an mRNA from this new strand and translate it into a protein. Compare the amino acid sequence of this protein to the one translated in (a). What happened? Explain.

11. Name the three types of RNA involved in protein synthesis and describe the function of each.
12. What does the phrase "gene expression" mean?
13. Why are noncoding RNAs (ncRNAs) important for cells? In your answer, consider one or two examples of different ncRNAs and their functions.
14. What is gene expression regulation? Why is it important? Describe examples of ways in which gene expression can be regulated.
15. What is an operon? How do bacteria use operons to regulate gene expression?
16. Briefly describe transcriptional regulation of gene expression?
17. Can genes be "turned on" and "turned off"? Explain your answer.
18. Why do some mutations affect changes in protein structure and function that can result in disease, whereas other mutations have no significant effects on protein structure and function?
19. What is the epigenome, and why are biotechnology and pharmaceutical companies interested in the epigenome?
20. In this chapter we provided a brief introduction to CRISPR-Cas. Briefly describe why the discovery of CRISPR-Cas with its potential future applications is an excellent example of biotechnology that has generated tremendous excitement in the scientific community.

Visit www.pearsonglobaleditions.com for the Companion Website

The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

This case study has been included to give you an opportunity to become familiar with a research example applicable to this field of study. After you have analyzed the data and answered the questions, you can compare them to those provided to your instructor with the text materials.

CASE STUDY

Loss of Circular RNA Impacts miRNA Degradation and Brain Function

In this chapter we focused on many basic elements of cell structure and function that are important for understanding biotechnology. As much as we have known about RNA for many decades, recently scientists identified another category of noncoding RNAs known as **circular RNA (circRNAs)**. Most circRNAs are transcribed from the genome and exist as single-stranded, circular molecules (ends are covalently attached to form circles) in the cytoplasm.

In 2017, scientists at the Berlin Institute of Medical Systems Biology (BIMSB) in Germany provided one of the first reports correlating a specific circRNA, called CDR1as, with biological functions in humans. Review the news report link from *Science Daily* (**https://www.sciencedaily.com/releases/2017/08/170814092952.htm**), which provides access to a publication by Piwecka et al. (*Science*, 9/22/17), including photos of circRNAs.

From this news report and the *Science* paper, answer the following questions.

Questions

Answers can be found at www.pearsonglobaleditions.com

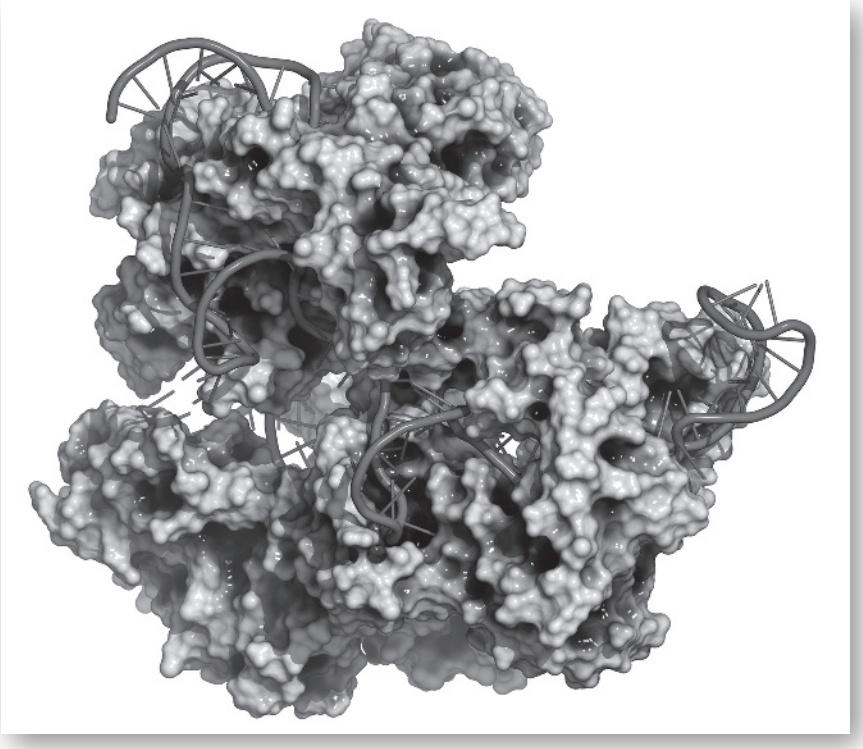
1. Based on this study, in what mouse and human tissues is CDR1as expressed?
2. What is the proposed function of CDR1as and how might it interact with other RNA molecules?
3. What results from experiments with mice led scientists to consider roles of CDR1as in humans?
4. In only one sentence, how would you summarize the potential importance of CDR1as and its role in the human brain?

CHAPTER THREE

Recombinant DNA Technology and Genomics

After completing this chapter, you should be able to:

- Define recombinant DNA technology and explain how it is used to clone genes and manipulate DNA.
- Compare and contrast different types of cloning vectors and describe their practical features and applications.
- Understand the importance of DNA libraries as collections of cloned DNA that were historically used to isolate specific genes.
- Describe how agarose gel electrophoresis, polymerase chain reaction (PCR), DNA sequencing, and other common laboratory techniques, including CRISPR-Cas for genome editing, are used to study gene structure, function, and expression for biotechnology applications.
- Describe how whole-genome sequencing and advances in DNA sequencing techniques enable scientists to rapidly analyze genomes.
- Understand the major goals and findings of the Human Genome Project.
- Explain why genomics-related "omics" disciplines are rapidly developing areas of biotechnology research.
- Provide examples of how bioinformatics can be used to analyze nucleic acid sequences and structures.
- Discuss basic concepts of systems biology and synthetic biology as emerging disciplines, and provide examples of potential applications of each field.



CRISPR-Cas protein complex bound to DNA. Since its discovery, no method has generated more potential and excitement as a genome editing tool for research and genetic therapies than CRISPR-Cas.

As you have learned, biotechnology is not a new science. However, the modern era of biotechnology began when DNA cloning techniques were developed. Since the 1970s and continuing today, amazing and rapidly developing methods in **recombinant DNA technology,** which enable the joining together of DNA from different sources, have led to **genetic engineering—**genetically modifying cells and organisms in various ways. Genetic engineering applications have changed molecular biology, basic science, and medical research forever.

In this chapter, we present an overview of recombinant DNA technology. We then take a look at an amazing range of techniques that scientists use to clone and manipulate genes and to study gene structure and function. The chapter concludes with an introduction to genomics and bioinformatics, which provide methods for studying genomes. From these introductions you will be well prepared to learn about modern applications of recombinant DNA and genomics in the biotechnology world.

FORECASTING THE FUTURE

There are many exciting potential future directions in recombinant DNA and genomics research. Without question, one area of ongoing and substantial progress is the completion of thousands of genome projects for different plants, animals, bacteria, viruses, and other organisms. Scientists continue to prospect genomes from organisms around the world to look for new genes with potential applications in biotechnology. Following on the success of the Human Genome Project, the use of genomic information for new applications—including novel methods for the detection of disease genes, targeted therapies based on genetics, and novel gene-based editing strategies for a range of diseases—holds tremendous promise for alleviating pain and suffering through biotechnology. As the cost of DNA sequencing decreases, the sequencing of individual genomes (personal genomics) will be more common.

3.1 Introduction to Recombinant DNA Technology and DNA Cloning

In their landmark paper detailing the double-helical structure of DNA, the scientists James Watson and Francis Crick hinted that DNA's very structure conveyed a means for its replication, thus underscoring the potential importance and impact of this discovery. However, not even these Nobel Prize winners could have imagined the astonishing pace at which molecular biology would advance over the next half century and beyond. In the late 1960s, many scientists were interested in copying DNA, or **gene cloning.** They speculated that it might be possible to clone DNA by cutting and joining together DNA from different sources (recombinant DNA technology). The terms *gene cloning*, *recombinant DNA technology*, and *genetic engineering* describe different, but closely interrelated, processes. Recombinant DNA technology is commonly used to make gene cloning possible, whereas genetic engineering often relies on recombinant DNA technology and gene cloning to modify an organism's genome. Often, the terms *recombinant DNA technology* and *genetic engineering* are used interchangeably.

The word *clone* is derived from a Greek word that describes a cutting (of a twig) that is used to propagate or copy a plant. A modern biological definition of a clone is a molecule, cell, or organism produced from another single entity. The laboratory methods required for gene cloning as described in this chapter are different from the techniques used to clone whole organisms, which we discuss in Chapter 7.

Restriction Enzymes and Plasmid DNA Vectors

Many nearly simultaneous discoveries and collaborative efforts among several researchers led to the discovery of two essential components that made recombinant DNA techniques and gene cloning possible—**restriction enzymes** and **plasmids (plasmid DNA).** Restriction enzymes are DNA-cutting enzymes, and plasmid DNA is a small, circular form of DNA naturally present in many bacteria, which scientists have learned to manipulate in order to carry and clone other pieces of DNA.

Much of what we know about DNA replication and DNA-synthesizing enzymes that made cloning possible was learned from studying DNA structure and replication in bacteria and in **bacteriophages.** Bacteriophages, often simply called *phages*, are viruses that infect bacterial cells.

Microbiologists in the 1960s discovered that some bacteria are protected from destruction by bacteriophages because they can *restrict* phage replication. Swiss scientist Werner Arber proposed that restricted growth of phages occurred because these bacteria contained enzymes that could cut viral DNA into small pieces, thus preventing viral replication. Because of this ability, these enzymes were called *restriction enzymes.* In 1970, working with the bacterium *Haemophilus influenzae*, Johns Hopkins University researcher Hamilton Smith isolated *Hin*dIII, the first restriction enzyme to be well characterized and used for DNA cloning. Restriction enzymes are also called restriction endonucleases (*endo*, "within"; *nuclease*, "nucleic acid–cutting enzyme") because they cut *within* DNA molecules at specific sequences as opposed to enzymes (exonucleases) that cut beginning at the free ends of DNA molecules, and

not at specific sequences. Smith demonstrated that *Hin*dIII could be used to cut or digest DNA into small fragments. In 1978, Smith shared a Nobel Prize with Werner Arber and Daniel Nathans for their discoveries of restriction enzymes and their applications.

Restriction enzymes are primarily found in bacteria, and they are given abbreviated names based on the genus and species names of the bacteria from which they are isolated. For example, one of the first restriction enzymes to be isolated, *Eco*RI, is so named because it was discovered in the *Escherichia coli (E. coli)* strain called RY13. Restriction enzymes cut double-stranded DNA by cleaving the phosphodiester bond (in the sugar-phosphate backbone) that joins adjacent nucleotides in a DNA strand. However, restriction enzymes do not just randomly cut DNA, nor do all restriction enzymes cut DNA at the same locations.

Like other enzymes, restriction enzymes show specificity for certain **substrates.** For these enzymes, the substrate is a specific sequence within double-stranded DNA. As shown in **Figure 3.1a**, restriction enzymes bind to, recognize, and cut (digest) DNA within specific sequences of bases called **restriction sites.** Why don't restriction enzymes digest DNA in bacterial cells? Bacteria protect their DNA from restriction enzyme digestion because some of the nucleotides in their DNA contain methyl groups that block restriction enzymes from digestion (Figure 3.1b).

Restriction enzymes are commonly referred to as four- or six-base-pair cutters because they typically recognize restriction sites with a sequence of four or six nucleotides. Eight-base-pair cutters have also been identified. Each restriction site is a *palindrome*—the arrangement of nucleotides reads the same forward and backward on opposite strands of the DNA molecule. (Remember the word *madam* or the phrase *a Toyota* as examples of palindromes.) Some restriction enzymes, such as *Eco*RI, cut DNA to create DNA fragments with overhanging single-stranded ends called "sticky" or **cohesive ends** (see Figure 3.1a); other enzymes generate fragments with double-stranded ends called **blunt ends.**

Table 3.1 shows some common restriction enzymes, their source microorganisms, and their restriction sites. Notice that the first three enzymes in the table are six-base-pair cutters that produce DNA molecules with cohesive ends. The fourth enzyme (*Taq*I) is a four-base-pair cutter that produces cohesive ends, and the lower three enzymes produce blunt-ended DNA fragments. Enzymes that produce cohesive ends are often favored over blunt-end cutters for many cloning experiments because DNA fragments with cohesive ends can easily be joined together. DNA from *any* source—such as bacteria, humans, dogs, cats, frogs, dinosaurs, or ancient human remains—can be digested by a particular restriction enzyme as long as the DNA has a restriction site for

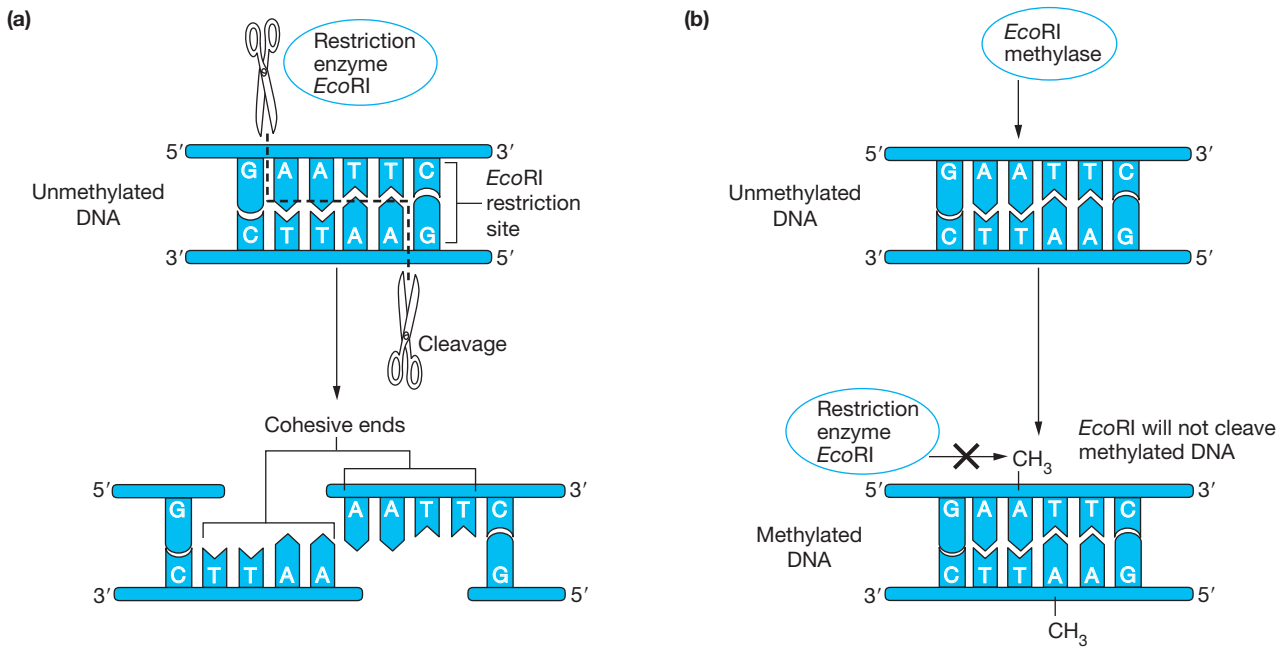
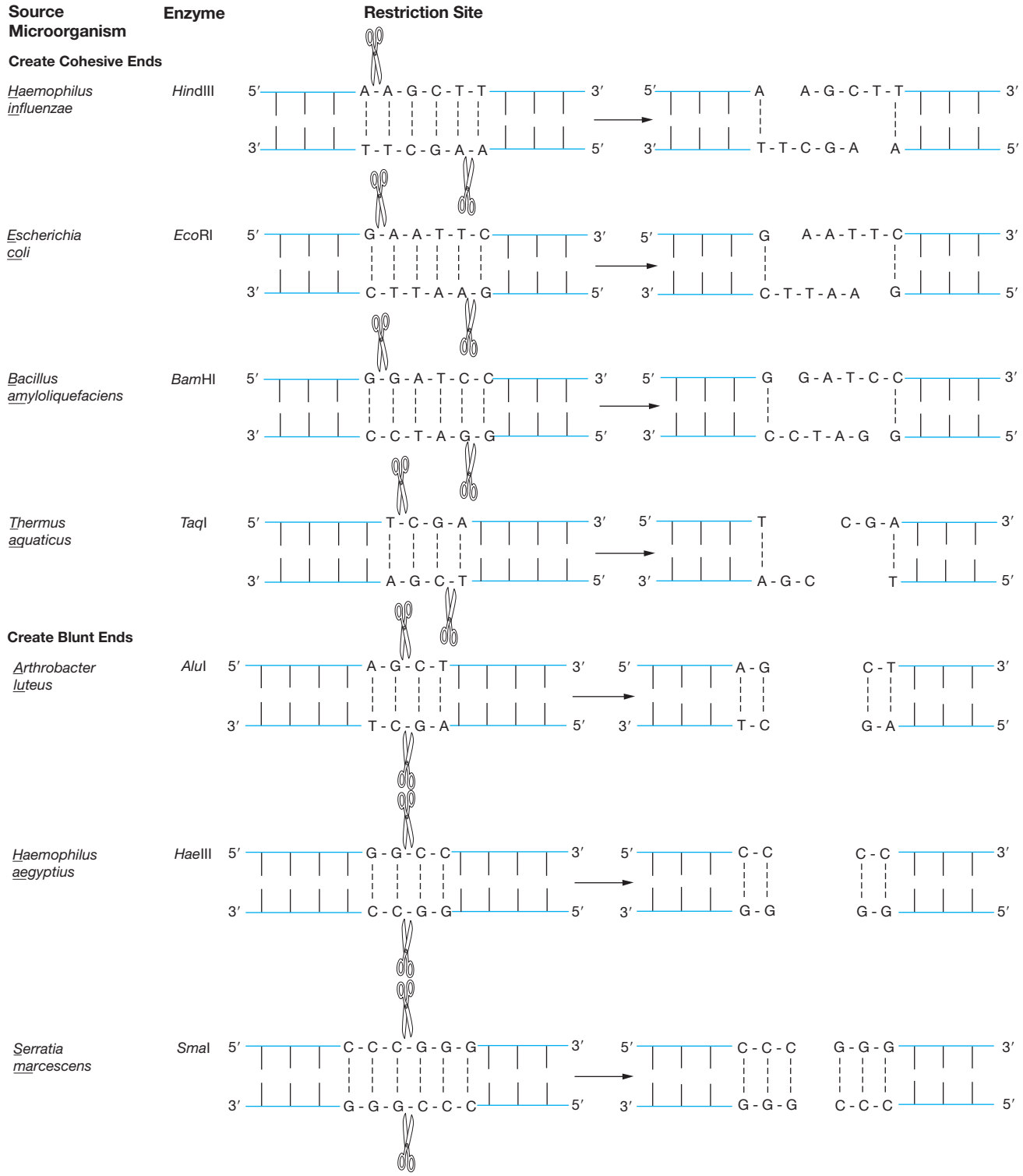


FIGURE 3.1 Restriction Sites and Restriction Enzyme Action (a) Digestion of DNA by *Eco*RI produces DNA fragments with cohesive ends. (b) Methylation of the *Eco*RI restriction site by the enzyme *Eco*RI methylase blocks DNA cleavage by *Eco*RI. Note: Methylation of the *Eco*RI restriction site occurs on an adenine nucleotide, but methylation more frequently occurs on cytosine nucleotides.

TABLE 3.1 Common Restriction Enzymes





TOOLS OF THE TRADE Restriction Enzymes

Restriction enzymes are sophisticated "scissors" that molecular biologists use to manipulate DNA. Working with restriction enzymes has become much easier since Hamilton Smith and others pioneered their use. Now over 600 restriction enzymes are commercially available rather inexpensively. These enzymes are readily available because they have been cloned using recombinant DNA technology, so they are made and isolated in large quantities. Commercially prepared enzymes come in conveniently sized prepackages with buffer solutions that provide all the components necessary for optimal enzyme activity. If researchers must work with an enzyme with which they are unfamiliar, they can use restriction enzyme databases such as REBASE (**http://rebase.neb.com**), an outstanding tool for locating technical specifications and suppliers of enzymes.

In addition, a variety of software packages and websites are available to assist scientists who work with restriction enzymes and DNA sequences. For example, imagine that you are a molecular biologist who just cloned and sequenced a 7,200–base-pair (bp) piece of DNA and you want to see if there is an enzyme that will cut your gene to create a 250-bp piece of DNA to use as a probe for detecting similar genes in other species. Not too long ago, if you had a lot of enzymes in your freezer, you could digest this DNA and run gels to see if you could get a 250-bp piece, but this imprecise approach took a lot of time and resources. If you had sequenced your gene, you could scan the sequence with your eyes, looking for a restriction site of interest—a very time-consuming and eye-straining effort! The Internet makes this task much easier, because many websites function as online tools for analyzing restriction enzyme cutting sites. For example, in Webcutter, DNA sequences can be entered and searched to determine restriction enzyme cutting patterns (see Problem 9 in "Questions & Activities" at the end of the chapter).

The widespread application of recombinant DNA techniques in many areas of biological and medical research has led to hundreds of technique books, websites, and journals. In *Bio Techniques* (a popular monthly journal), biologists publish and share information on molecular cloning techniques. Several sites that are commonly used for designing PCR primers, as well as other applications, are provided in Keeping Current: Web Links, on the Companion Website.

that enzyme. In the simplest sense, the discovery of restriction enzymes provided molecular biologists with the "scissors" needed to carry out gene cloning.

In the early 1970s, Paul Berg, Herbert Boyer, Stanley Cohen, and colleagues at Stanford University used gene cloning to change molecular biology forever. Berg founded recombinant DNA technology when he created a piece of recombinant DNA by joining together, or *splicing*, DNA from the *E. coli* chromosome and DNA from a primate virus called simian virus 40 (SV40). Berg first isolated chromosomal DNA from *E. coli* and DNA from SV40. Then he cut both DNA samples with *Eco*RI, added *E. coli* and viral DNA fragments to a reaction tube with the enzyme DNA ligase, and succeeded in creating a hybrid molecule of SV40 and *E. coli* DNA. The importance of this discovery was fully recognized when Paul Berg won the 1980 Nobel Prize in Chemistry for this experiment, which demonstrated that DNA could be cut from different sources with the same enzyme and that the restriction fragments could be joined to create a recombinant DNA molecule. Berg shared this prize with Walter Gilbert and Frederick Sanger, who independently developed methods for sequencing DNA—a process we discuss later in this chapter.

While Berg worked with chromosomal DNA, Cohen was interested in the molecular biology of small circular pieces of DNA known as **plasmids.** Plasmid DNA is primarily found in bacteria. Plasmids are considered *extrachromosomal* DNA because they are present in the bacterial cytoplasm in addition to the bacterial chromosome. Plasmids are small; most average approximately 1,000 to 4,000 bp in size and they are replicated independently of the chromosome. Cohen studied plasmid replication, the transfer of plasmids between bacteria, and the contribution of plasmid transfer to the spread of antibiotic resistance.

Cohen postulated that plasmids could be used as **vectors—**pieces of DNA that can accept, carry, and replicate (clone) other pieces of DNA. Cohen and Boyer worked with two bacterial plasmids to clone DNA successfully. They published results describing these experiments in 1973. Many consider this work the informal birth of recombinant DNA technology. Using *Eco*RI, a restriction enzyme previously isolated by Herbert Boyer, they cut both plasmids and then joined fragments from each plasmid together, using DNA ligase to create new hybrid (recombinant) plasmids. Recall from Chapter 2 that DNA ligase catalyzes the formation of

phosphodiester bonds between nucleotides. Ligase can join together DNA with cohesive ends as well as blunt-ended fragments. As a result of these and other experiments, Cohen and Boyer produced the first plasmid vector for cloning purposes, called pSC101 and named "SC" for Stanley Cohen. pSC101 contained a gene for tetracycline resistance and restriction sites for several enzymes including *Eco*RI and *Hin*dIII. In subsequent work they used similar experiments to clone DNA from the South African claw-toed frog *Xenopus laevis* (another important model organism in genetics and developmental biology) into the *Eco*RI site of pSC101. **Figure 3.2**

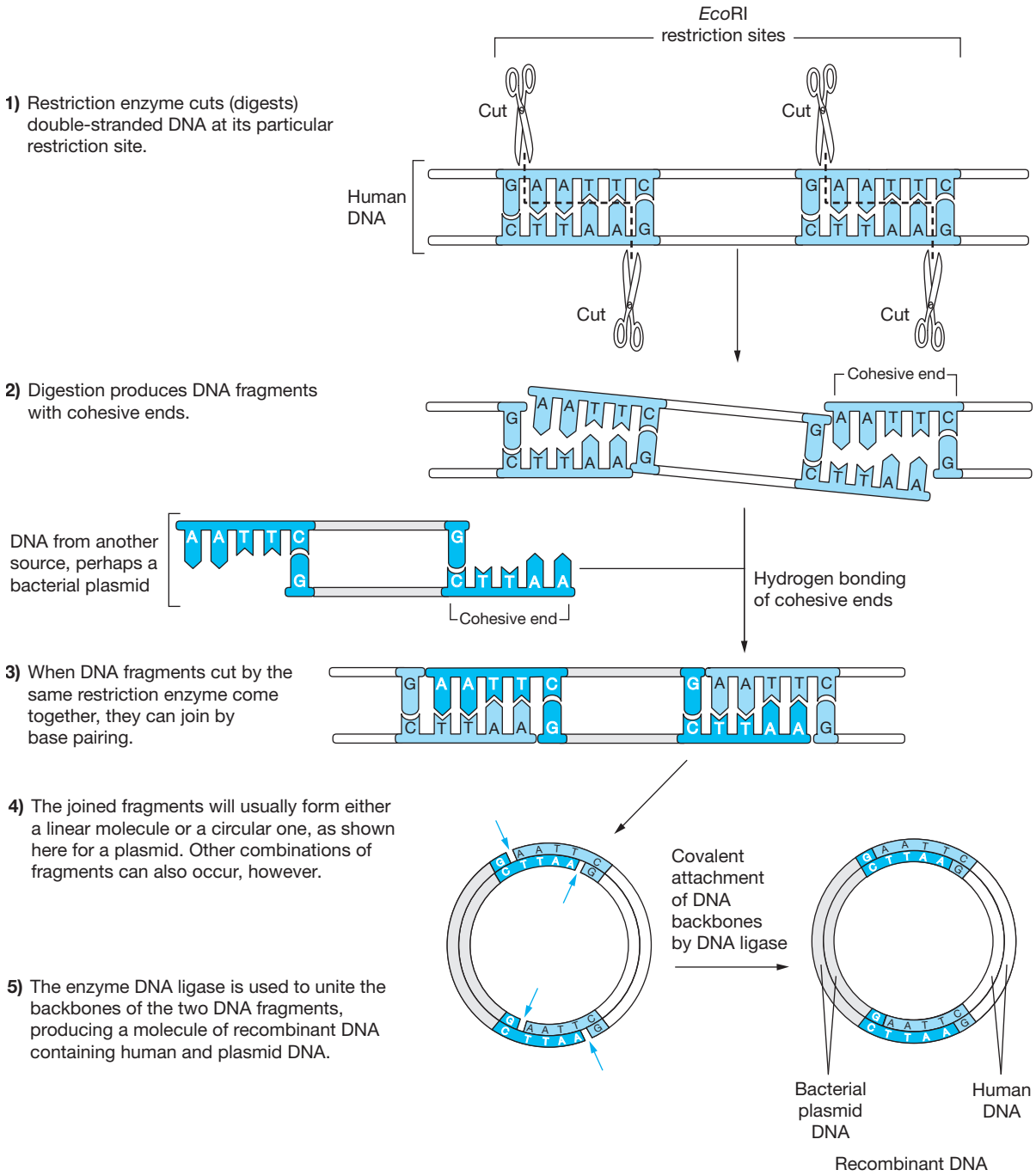


FIGURE 3.2 Creating Recombinant DNA *Eco*RI binds to a specific restriction site (5′-GAATTC-3′) and then cleaves the DNA backbone, producing DNA fragments. The single-stranded ends of the DNA fragments can form hydrogen bonds with each other because they have complementary base pairs. DNA ligase can then catalyze the formation of covalent bonds in the DNA backbones (→) of the fragments to create a piece of recombinant DNA.

illustrates how recombinant DNA can be formed in a process similar to that used in the Cohen and Boyer experiments.

Cohen and Boyer had created the first DNA cloning vector—a vehicle for the insertion and replication of DNA—and in 1980 were awarded patents for pSC101 and for the gene splicing and cloning techniques they had developed. These experiments ushered in the birth of modern biotechnology, because many of the current techniques used for gene cloning and gene manipulations are based on these fundamental methods of recombinant DNA technology.

In 1974, as a direct result of the Berg, Cohen, and Boyer experiments, gene cloning pioneers and critics voiced concerns about the safety of genetically modified organisms. Scientists were concerned about what might happen if recombinant bacteria were to leave the lab or if such bacteria could transfer their genes to other cells or survive in other organisms, including humans. In 1975, an invited group of well-known molecular biologists, virologists, microbiologists, lawyers, and journalists gathered at the Asilomar Conference Center in Pacific Grove, California, to discuss the benefits and potential hazards of recombinant DNA technology. As a result of the historic Asilomar meeting, the **National Institutes of Health (NIH)** formed the **Recombinant DNA Advisory Committee (RAC),** which was charged with evaluating the risks of recombinant DNA technology and establishing guidelines for recombinant DNA research. In 1976, the RAC published a set of guidelines for working with recombinant organisms. The RAC continues to oversee gene cloning research, and compliance with RAC guidelines is mandatory for scientists working with recombinant organisms.

Transformation of Bacterial Cells and Antibiotic Selection of Recombinant Bacteria

Cohen also made another important contribution to gene cloning, which made the pSC101 cloning experiments possible. His laboratory demonstrated how **transformation,** a process for inserting foreign DNA into bacteria, could be used to reliably introduce DNA into bacteria. Cohen discovered that if he treated bacterial cells with calcium chloride solutions, added plasmids to cells chilled on ice, and then briefly heated the cell and DNA mixture, plasmids entered bacterial cells. Once inside bacteria, plasmids are replicated by the cell and their genes are expressed. Transformation techniques are explained in greater detail later (Chapter 5). A more modern transformation method, called **electroporation,** involves applying a brief (millisecond) pulse of high-voltage electricity to create tiny holes in the bacterial cell wall that allow DNA to enter. Electroporation can also be used to introduce DNA into mammalian cells and to transform plant cells.

Ligation of DNA fragments and transformation by any method are somewhat inefficient. During ligation, some of the digested plasmid ligates back to itself to create recircularized plasmid that lacks foreign DNA. During transformation, a majority of cells do not take up DNA. Now that we have seen how DNA can be inserted into a vector and introduced into bacterial cells, we consider how recombinant bacteria—those transformed with a recombinant plasmid—can be distinguished from a large number of nontransformed bacteria and bacterial cells that contain plasmid DNA without foreign DNA. This screening process is called **selection** because it is designed to facilitate the identification of (selecting *for*) recombinant bacteria while preventing the growth of (selecting *against*) nontransformed bacteria and bacteria that contain plasmid without foreign DNA.

Cohen and Boyer used **antibiotic selection,** a technique in which transformed bacterial cells are plated on agar plates with different antibiotics, as a way to identify recombinant bacteria and nontransformed cells. For many years antibiotic selection was a widely used approach. Modern cloning techniques often incorporate other more popular selection strategies, such as **"blue-white" selection** (the reason for this name will soon be obvious). In blue-white selection, DNA is cloned into a restriction site in the *lacZ* gene, as illustrated in **Figure 3.3**. Recall that the *lacZ* gene encodes β-galactosidase (β-gal), an enzyme that degrades the disaccharide lactose into the monosaccharides glucose and galactose (Chapter 2). When the *lacZ* gene is interrupted by an inserted gene, no functional β-gal enzyme is produced.

Transformed bacteria are plated on agar plates that contain an antibiotic—ampicillin, in this example. Nontransformed bacteria cannot grow in the presence of ampicillin because they lack plasmids containing an ampicillin resistance gene (*ampR*). But antibiotic selection alone does not distinguish transformed bacteria containing nonrecombinant plasmids (plasmids that have recircularized and do not contain insert DNA) from transformed bacteria containing recombinant plasmids (plasmids that do have insert DNA). To identify recombinant bacteria, the agar must also contain a chromogenic (color-producing) substrate for β-gal called X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). X-gal is similar to lactose in structure and turns blue when cleaved by β-gal. As a result, nonrecombinant bacteria—those that contain plasmid that ligated back to itself without insert DNA—contain a functional *lacZ* gene,

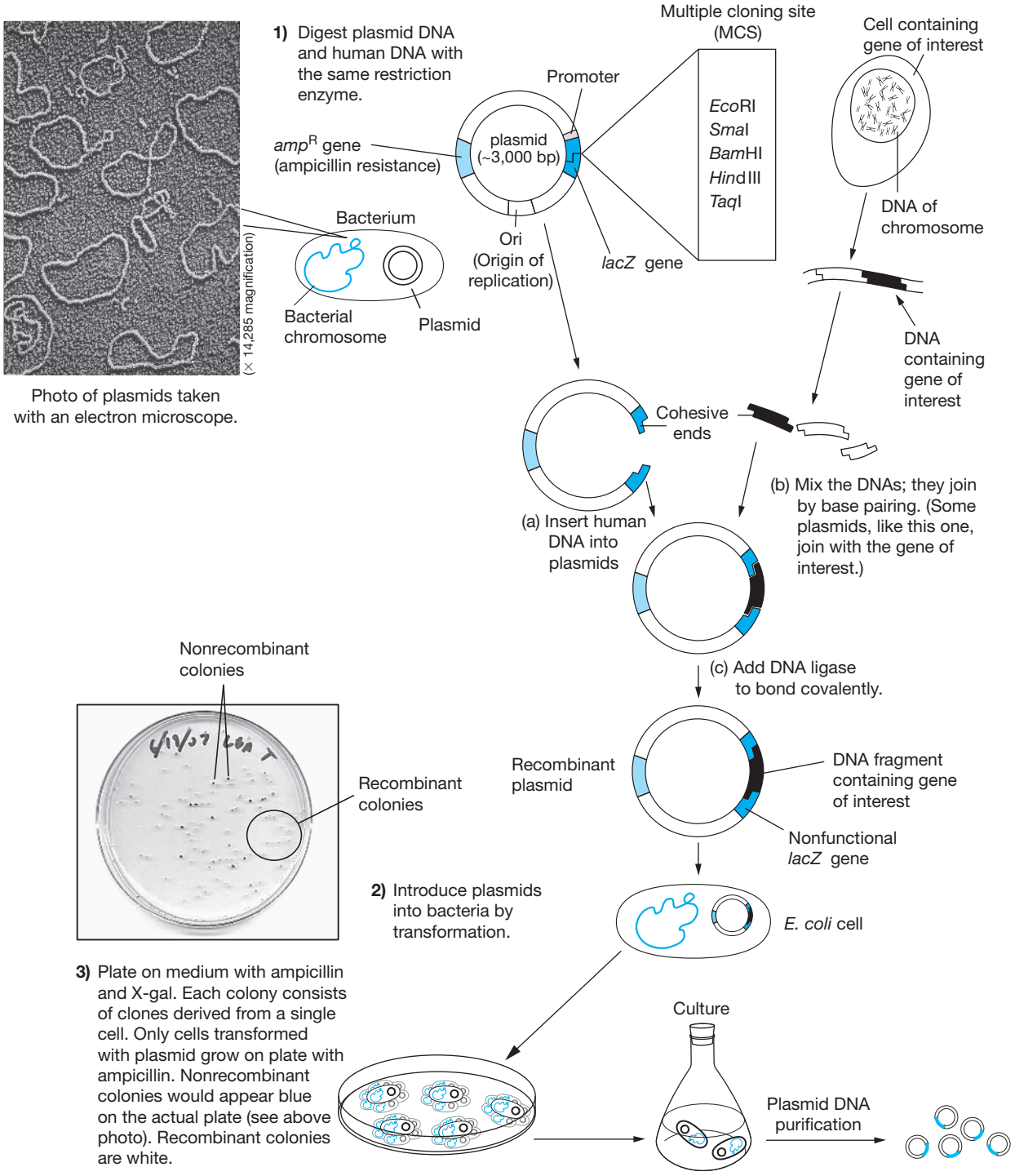


FIGURE 3.3 Cloning a Gene in a Plasmid and Blue-White Selection

produce β-gal, and turn blue. Conversely, recombinant bacteria are identified as white colonies. Because these cells contain plasmid with foreign DNA inserted into the *lacZ* gene, β-gal is not produced, and these cells cannot metabolize X-gal. Therefore, through blue-white selection, nontransformed and nonrecombinant bacteria are *selected against* and white colonies are identified or *selected for* as the desired colonies containing recombinant plasmids. Colonies containing recombinant plasmids are **clones**—genetically identical bacterial cells, each containing copies of the recombinant plasmids.

Introduction to Human Gene Cloning and Expressing Proteins for Biotechnology Applications

Restriction enzymes, DNA ligase, and plasmids are major tools of molecular biologists for cloning and manipulating genes from virtually any source, although you will soon learn about many other methods that are also important for cloning. With transformation, scientists had a way to introduce recombinant DNA into bacterial cells. Recombinant DNA technology made it possible to cut and join together DNA fragments, insert DNA into a plasmid (DNA cloned into a plasmid is commonly called *insert* DNA), and produce large amounts of the insert DNA by allowing bacteria to be the workhorses for replicating recombinant DNA.

If the cloned DNA fragment is a gene that encodes a protein product, bacterial cells could be used to synthesize the protein product of the cloned gene. We call this *expressing* a protein. Molecular biologists recognized that if human genes could be cloned and expressed, recombinant DNA technology would become an invaluable tool with powerful and exciting applications in research and medicine. Because bacteria can be grown in large-scale preparations, scientists can produce large amounts of the cloned DNA and isolate quantities of protein that would normally be very difficult or expensive to purify without cloning (these processes are described in more detail in Chapters 4 and 5). Because of recombinant DNA technology, a wide range of valuable proteins that are otherwise difficult to obtain can be produced from cloned genes.

The first commercially available human gene product of recombinant DNA technology was human **insulin,** a peptide hormone produced by cells in the pancreas called beta cells. When blood glucose rises—for example, after eating a sugar-rich meal—insulin lowers blood glucose by stimulating glucose storage in liver and muscle cells as long chains of glucose called glycogen. Individuals with **type 1,** or **insulin-dependent,** diabetes mellitus do not produce insulin on their own. As a result of this insulin deficiency, people with diabetes experience excessively high blood sugar levels (hyperglycemia), which, over time, can lead to serious damage to many bodily organs.

In 1977, the insulin gene was cloned into plasmids, expressed in bacterial cells, and isolated by scientists at **Genentech** (named for *gen*etic *en*gineering *tech*nology), the San Francisco biotechnology company cofounded in 1976 by Herbert Boyer and Robert Swanson. Details of the techniques used for cloning insulin are examined in Chapter 5. Genentech is generally regarded as the first biotechnology company to produce a product using recombinant DNA technologies.

In 1982, the recombinant form of human insulin, called **Humulin,** became the first recombinant DNA product to be approved for human applications by the **U.S. Food and Drug Administration (FDA).** Shortly after insulin became available, growth hormone—used to treat children who suffer from a form of dwarfism—was cloned. Because of recombinant DNA technology, a wide variety of medically important proteins that were once difficult to obtain in adequate amounts became readily available.

Prior to recombinant DNA technology, important hormones like insulin and growth hormone had to be isolated from tissues. Growth hormone was isolated from the pituitary glands of human cadavers. Not only was this process expensive and inefficient, but these isolations also carried with them the risk of unknowingly co-purifying viruses and other pathogens as contaminants that could be passed to people receiving the hormone. There are now several hundred commercially available products manufactured using recombinant DNA technology and having widespread applications in basic research, medicine, and agriculture.

With a basic understanding of the techniques involved in manipulating a piece of DNA, in the next section we go on to examine some important aspects of DNA vectors and how different vectors are chosen and used depending on what is to be accomplished.

What Makes a Good Vector?

Plasmids form the foundation for many techniques used daily in a molecular biology laboratory, and are still commonly used cloning vectors because they allow for the routine cloning and manipulation of small pieces of DNA. In addition, it is fairly simple to transform bacterial cells with plasmids and relatively easy to isolate plasmids from bacterial cells. One of the first widely used plasmid vectors, called pBR322, was designed to include genes for ampicillin and tetracycline resistance and several useful restriction sites. However, plasmid cloning vectors have been engineered to incorporate a number of other important features that have made early plasmids such as pBR322 obsolete.

Practical features of DNA cloning vectors

Plasmid cloning vectors usually include most of the following desirable and practical features:

- *Origin of replication* (ori)—The ori is a site for DNA replication that allows plasmids to replicate separately from the host cell's chromosome. The number of plasmids in a cell is called *copy number.* The copy number of naturally occurring plasmids in most bacterial cells is small (usually less than 12 plasmids per cell); however, many of the most desirable cloning plasmids are known as high-copy-number

plasmids because they replicate to create hundreds or thousands of plasmid copies per cell.

- *Multiple cloning site* (MCS)—The MCS is a segment of plasmid DNA with recognition sites for different common restriction enzymes (see Figure 3.3). These sites are engineered into the plasmid so that digestion of the plasmid with restriction enzymes does not result in the loss of a fragment of DNA. Rather, the circular plasmid simply becomes linearized when it is digested with a restriction enzyme. An MCS provides for great flexibility in the range of DNA fragments that can be cloned into a plasmid because it is possible to insert DNA fragments generated by cutting with many different enzymes.
- *Selectable marker genes*—These genes allow for the selection and identification of bacteria that have been transformed with a recombinant plasmid. Some of the most common selectable markers are genes for ampicillin resistance (*ampR*), tetracycline resistance (*tetR*), and the *lacZ* gene used for blue-white selection.
- *RNA polymerase promoter sequences*—These sequences are used for the transcription of RNA *in vivo* and *in vitro* by RNA polymerase. Recall that RNA polymerase copies DNA into RNA during transcription (Chapter 2). *In vivo*, these sequences allow bacterial cells to make RNA from cloned genes, which in turn leads to protein synthesis. *In vitro*, transcribed RNA can be used to synthesize RNA "probes," which are useful in studying gene expression (Section 3.4).
- *DNA sequencing primer sequences*—These sequences permit nucleotide sequencing of cloned DNA fragments that have been inserted into the plasmid (Section 3.4).

Types of vectors

Just as one screwdriver cannot be used for all sizes and types of screws, bacterial plasmid vectors cannot be used for all cloning applications. One primary limitation is the size of the DNA fragment that can be inserted into a plasmid. Insert size usually cannot exceed approximately 6 to 7 kilobases (1 kb = 1,000 bp). In addition, because of differences in translation between bacterial cells and eukaryotic cells, sometimes bacteria express proteins from eukaryotic genes poorly. Because of these and other limitations, molecular biologists have developed many other types of DNA vectors, each of which has particular benefits depending on the cloning application. Table 3.2 compares the important features, sources, and applications of different types of cloning vectors.

For example, DNA from phage lambda (λ) was one of the first bacteriophage vectors used for cloning. The λ chromosome is a linear structure approximately

TABLE 3.2 A Comparison of DNA Vectors and Their Applications

Vector Type	Maximum Insert Size (kb)	Applications	Limitations
Bacterial plasmid vectors (circular)	~6-12	DNA cloning, protein expression, subcloning, direct sequencing of insert DNA	Restricted insert size; limited expression of proteins; copy number problems; replication restricted to bacteria
Bacteriophage vectors (linear)	~35	cDNA, genomic and expression libraries	Packaging limits DNA insert size; host replication problems
Cosmid (circular)	~45	cDNA and genomic libraries, cloning large DNA fragments	Phage packaging restrictions; not ideal for protein expression; cannot be replicated in mammalian cells
Bacterial artificial chromosome (BAC, circular)	~300	Genomic libraries, cloning large DNA fragments	Replication restricted to bacteria; cannot be used for protein expression
Yeast artificial chromosome (YAC, circular)	200–2,000	Genomic libraries, cloning large DNA fragments	Must be grown in yeast; cannot be used in bacteria
Ti vector (circular)	Varies depending on type of Ti vector used	Gene transfer in plants	Limited to use in plant cells only; number of restriction sites randomly distributed; large size of vector not easily manipulated

49 kb in size. Cloned DNA is inserted into restriction sites in the center of the λ chromosome. Recombinant chromosomes are then packaged into viral particles *in vitro*, and these phages are used to infect *E. coli* growing as a lawn (a continuous layer covering the plate). When λ infects *E. coli* as a host, sequences in the λ chromosome allow it to circularize and then replicate. An advantage of these vectors is that they allowed for the cloning of larger DNA fragments (up to approximately 25 kb) than plasmids. Phage vectors are no longer very widely used.

Bacterial artificial chromosomes (BACs) are large low-copy-number plasmids present as one to two copies in bacterial cells. BACs can accept DNA inserts in the 100- to 300-kb range. **Yeast artificial chromosomes (YACs)** are small plasmids grown in *E. coli* and introduced into yeast cells such as *Saccharomyces cerevisiae*. A YAC is a miniature version of a eukaryotic chromosome containing an ori, selectable markers, two telomeres, and a centromere that allows for replication of the YAC. Foreign DNA fragments are cloned into a restriction site in the center of the YAC alongside the chromosomes naturally found within the yeast cells. YACs are useful for cloning large fragments of DNA from 200 kb to approximately 2 megabases (Mb = 1 million bases) in size. Like BACs, YACs played an important role in the cloning efforts of the Human Genome Project. But increasingly, modern advances in DNA sequencing make applications of BACs and YACs less important.

Expression vectors

Protein **expression vectors** allow for the high-level synthesis (expression) of eukaryotic proteins within bacterial cells because they contain a prokaryotic promoter sequence adjacent to the site where DNA is inserted into the plasmid. Bacterial RNA polymerase can bind to the promoter and synthesize large amounts of RNA (for the insert), which is then translated into protein. Expressed proteins may then be isolated using biochemical techniques (described in Chapter 4).

However, it is not always possible to express a functional protein in bacteria. For example, bacterial ribosomes sometimes cannot translate eukaryotic messenger RNA (mRNA) sequences due to suble differences in codons that some bacteria use for translation. If a protein is produced, it may not fold and be processed correctly, as occurs in eukaryotic cells that use organelles to fold and modify proteins. Also, making some recombinant products in bacteria can be a problem because *E. coli* often does not secrete proteins; therefore, expression vectors are often used in *Bacillus subtilis*, a strain more suitable for protein secretion. In some cases, the host bacteria can recognize recombinant proteins as foreign and degrade the protein, whereas in others the expressed protein is lethal to the host bacterial cells.

Certain viruses, such as SV40, can be used to deliver expression vectors into mammalian cells. Typically, SV40-derived vectors contain a strong (viral) promoter sequence for high-level transcription and a poly(A) addition signal for adding a poly(A) tail to the 3′ end of synthesized mRNAs. Variations of such vectors have been used for human gene therapy, as we will discuss in Chapter 11.

Ti vectors

Ti vectors are naturally occurring plasmids, around 200 kb in size, isolated from the bacterium *Rhizobium radiobacter* (formerly called *Agrobacterium tumefaciens* and renamed on the basis of genome data), a soil-borne plant pathogen that causes a condition in plants called crown gall disease. When *R. radiobacter* enters host plants, a piece of DNA (T-DNA) from the Ti plasmid (Ti stands for tumor-inducing) inserts into the host chromosome. T-DNA encodes for the synthesis of a hormone called auxin, which weakens the host's cell wall. Infected plant cells divide and enlarge to form a tumor (gall). Plant geneticists recognized that if they could remove auxin and other detrimental genes from the Ti plasmid, the resulting vector could be used to deliver genes into plant cells. Ti vectors are widely used to transfer genes into plants, as you will learn in Chapter 6 (see Figure 6.3).

Now that we have considered different types of vectors and their applications, in the next section we will turn our attention to how scientists can use recombinant DNA technology to identify and clone genes of interest.

3.2 How Do You Identify and Clone a Gene of Interest?

Cutting and pasting different pieces of DNA to produce a recombinant DNA molecule soon became a routine technique in molecular biology. But the types of cloning experiments we have described so far allow for the *random* cloning of DNA fragments based on restriction enzyme cutting sites, not precise cloning of a single gene or a particular piece of DNA of interest. For example, if you were interested in cloning the insulin gene and you simply took DNA from the pancreas, cut it with enzymes, and then ligated digested DNA into plasmids, you would create hundreds of thousands of recombinant plasmids and not just recombinant plasmids that contain only the insulin gene.

Molecular biologists call this approach "shotgun" cloning, because many fragments are randomly cloned at once and no individual gene is specifically targeted for cloning. How would you know which recombinant

plasmid contained the insulin gene? If the insulin gene contains a restriction site for the enzyme you used, it may have been cut into fragments so that you cloned only part of the gene. Moreover, if sequences adjacent to the insulin gene do not have restriction sites for the restriction enzyme you used, you might not have any recombinant plasmids containing the insulin gene. Even if you did create plasmids with the insulin gene, how would you separate these from the other recombinant plasmids? So how do you find a particular gene of interest and clone only the DNA sequence that you want to study? In the early years of DNA cloning, these questions could often be answered by a cloning approach involving DNA libraries.

As you will learn in Section 3.4, an approach called **whole-genome sequencing** and new sequencing methodologies are replacing DNA libraries because they effectively allow one to sequence an entire genomic DNA sample without the need for inserting DNA fragments into vectors and cloning them in host cells. But understanding the concept of a DNA library is still of fundamental importance for a number of modern applications. In Section 3.4 we will also consider how DNA sequence analysis using **bioinformatics** allows one to identify protein-coding and non-coding sequences in cloned DNA.

Here we briefly discuss DNA libraries and then introduce DNA amplification by the **polymerase chain reaction** for cloning genes.

DNA Libraries: Collections of Cloned Genes

During the first several decades of DNA cloning, many cloning strategies began by preparing a **DNA library—** a collection of cloned DNA fragments from a particular organism contained within bacteria or viruses as the hosts. Libraries can be saved for relatively long periods and "screened" to pick out different genes of interest. Two types of libraries were typically used for cloning, **genomic DNA libraries** and **complementary DNA libraries (cDNA libraries).** Figure 3.4 on the next page shows how genomic libraries and cDNA libraries are constructed.

In a genomic library, chromosomal DNA from the tissue of interest is isolated and then digested with a restriction enzyme (see Figure 3.4a). This process produces fragments of DNA that include the organism's entire genome. A plasmid, BAC, YAC, or bacteriophage vector is digested with the same enzyme, and DNA ligase is used to ligate genomic DNA pieces and vector DNA randomly. In theory, all DNA fragments in the genome will be cloned into a vector. Recombinant vectors are then used to transform bacteria, and each bacterial cell clone will contain recombinant vector with a plasmid containing a genomic DNA fragment. Consider each clone a "book" in this "library" of DNA fragments. One disadvantage of creating this type of library for eukaryotic genes is that non-protein-coding pieces of DNA, called introns, are cloned in addition to protein-coding sequences (exons). Because a majority of DNA in any eukaryotic organism consists of introns, many of the clones in a genomic library will contain non-protein-coding pieces of DNA. Another limitation of genomic libraries is that many organisms, including humans, have such large genomes that searching for a gene of interest would be like searching for a needle in a haystack.

By contrast, in a cDNA library, mRNA from the tissue of interest is isolated and used for making the library. However, mRNA cannot be digested with restriction enzymes, so it must be converted to a double-stranded DNA molecule. An enzyme called **reverse transcriptase (RT)** is used to catalyze the synthesis of single-stranded DNA from the mRNA (see Figure 3.4b). This enzyme is made by viruses called **retroviruses—**so named because they are exceptions to the usual flow of genetic information. Instead of having a DNA genome that can be used to make RNA, retroviruses have an RNA genome. After infecting host cells, retroviruses use RT to convert their RNA-encoded viral genome into DNA, so that they can replicate. Human immunodeficiency virus (HIV) is a retrovirus. As we will discuss, retroviruses also have important applications in biotechnology as gene therapy vectors (Chapter 11). Because RT synthesizes DNA that is an exact copy of mRNA, it is called **complementary DNA (cDNA).** The mRNA is degraded by treatment with an alkaline solution or enzymatically digested; then DNA polymerase is used to synthesize a second strand to create double-stranded cDNA.

Because cDNA sequences do not necessarily have a convenient restriction site at each end, short, double-stranded DNA sequences called *linker sequences* are often enzymatically added to the ends of the cDNA. Linkers contain restriction sites. Different linkers for different restriction sites are commercially available. By adding linkers, cDNA can now be ligated into a convenient restriction site in a vector of choice, often a plasmid. The recombinant plasmid is then used to transform bacteria.

One primary advantage of cDNA libraries over genomic libraries is that they are a collection of actively *expressed* genes in the cells or tissue from which the mRNA was isolated. Also, introns are not cloned in a cDNA library. This is one reason why cDNA libraries are typically preferred over genomic libraries when attempting to clone and express a gene of interest. Another advantage of cDNA libraries is that they can be created and screened to isolate genes that are primarily expressed only under certain conditions in a

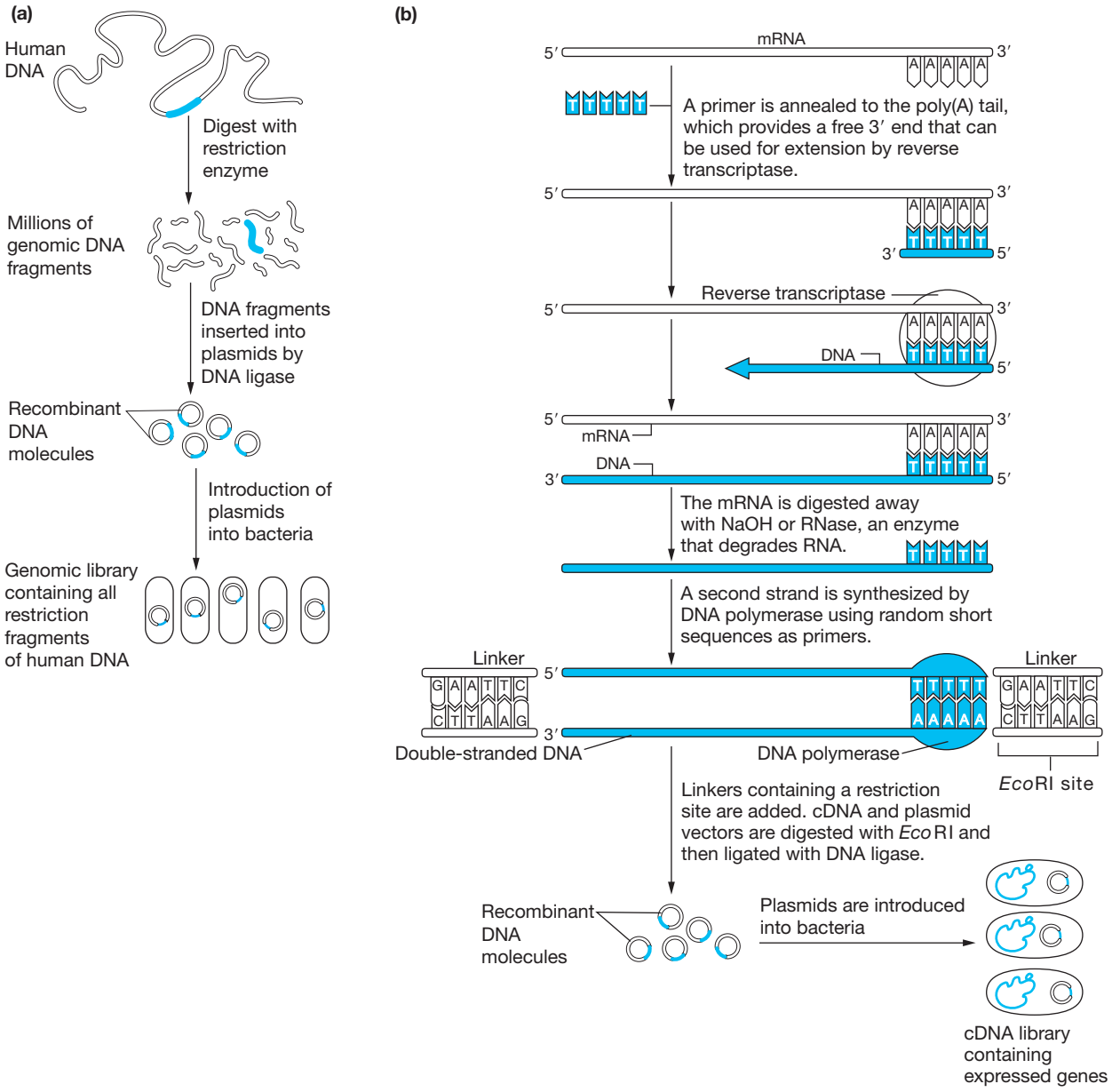


FIGURE 3.4 Comparison of a Human Genomic DNA Library and a cDNA Library (a) Human DNA is digested with a restriction enzyme to create a series of smaller fragments that are cloned into plasmids or other vectors. A (human) genomic library consists of a collection of bacteria, each containing a different fragment of human DNA. In theory, all DNA fragments from the genome will be represented in the library. (b) In a cDNA library, mRNA is converted into cDNA by the enzyme reverse transcriptase. Linkers containing a restriction site are added to the cDNA to create cohesive ends. The cDNA can now be cloned into a plasmid for subsequent replication in bacteria.

tissue—for example, during development, cell death, cancer, and other biological processes. One can use these libraries to compare expressed genes from normal tissues and diseased tissues. This approach has been widely used to identify genes involved in cancer formation, such as those genes that contribute to progression from a normal cell to a cancer cell and genes involved in cancer cell metastasis (spreading).

Libraries have become such a routine aspect of molecular biology that many companies sell libraries prepared from a range of tissues from different species. One disadvantage is that cDNA libraries can be difficult to create and screen if a source tissue with an abundant amount of mRNA for the gene is not available. But as you will learn, a technique called the *polymerase chain reaction (PCR)* can frequently solve this problem.

Specific genes can be isolated from a library by screening

Genomic and cDNA libraries often consist of several hundred thousand different DNA clones, much as a library may have many books but only a few of interest to your studies in genetics. So how can a specific gene of interest be located within a library? To do this, we need to identify and isolate only the clone or clones containing that gene. We must also determine whether a given clone contains all or only part of the gene we are studying. For several decades an approach called *library screening* was routinely used to sort through a library and isolate specific genes of interest. Many of the first genes to be cloned and sequenced were identified this way.

Library screening usually involves use of a **probe,** any DNA or RNA sequence that is complementary to some part of the target gene or sequence to be identified in a library. Probes are derived from a variety of sources—often related genes isolated from another species can be used if enough of the DNA sequence is conserved. For example, genes from rats, mice, or even *Drosophila* that have conserved sequence similarity to human genes can be used as probes to identify human genes during library screening. A probe must be labeled or tagged in different ways so that it can be identified. When used in a hybridization reaction, the probe binds to any complementary DNA sequences present in one or more clones. Initially, probes were labeled with radioactive isotopes, but modern applications use probes labeled with nonradioactive compounds that undergo colorimetric, fluorometric, or enzymatic reactions to indicate the location of a specific clone in a library.

Probes can be used in a variety of ways to screen cells in a library for a gene of interest, and to then subsequently isolate that gene. Refer to the Companion Website for a figure demonstrating one approach for screening. Libraries still have their place for certain applications in biotechnology. However, as you will learn later in this chapter, the basic methods of recombinant DNA technology, including DNA libraries, were the foundation for the development of powerful techniques for whole-genome cloning and sequencing, which led to the **genomics** era of modern genetics and molecular biology. Genomic techniques, in which entire genomes are being sequenced without creating libraries, have largely replaced libraries, at least for cloning and isolating one or a few genes at a time.

Polymerase chain reaction

Although libraries were effective for cloning and identifying a gene of interest, the **polymerase chain reaction (PCR)** is a much more rapid approach to cloning. Developed in the mid-1980s by Kary Mullis, PCR turned out to be a revolutionary technique that has had an impact on many areas of molecular biology. In 1993, Mullis won the Nobel Prize in Chemistry for his invention. PCR is a technique for making copies or amplifying a *specific sequence* of DNA in a short period of time, and it quickly became the technique of choice when cloning genes. However, it also has many other applications. An excellent tutorial on PCR can be viewed at the Cold Spring Harbor DNA Learning Center website listed at the Companion Website.

The concept behind a PCR reaction is remarkably simple. Target DNA to be amplified is added to a thin-walled tube and mixed with deoxyribonucleotides (dATP, dCTP, dGTP, dTTP), buffer, and DNA polymerase. A paired set of **primers** is added to the mixture. Primers are short single-stranded DNA oligonucleotides usually around 20 to 30 nucleotides long. These primers are complementary to nucleotides flanking opposite ends of the target DNA to be amplified (**Figure 3.5**).

The reaction tube is then placed in a *thermal cycler*. In the simplest sense, a thermal cycler is a sophisticated heating block that is capable of rapidly changing temperature over very short time intervals. The thermal cycler takes the sample through a series of reactions called a PCR cycle (Figure 3.5). Each cycle consists of three stages. In the first stage, *denaturation*, the reaction tube is heated to approximately 94°C to 96°C, causing separation of the target DNA into single strands. In the second stage, *hybridization* (or *annealing*), the tube is then cooled slightly to between 55°C and 65°C, which allows the primers to hydrogen bond, or *hybridize,* to complementary bases at opposite ends of the target sequence. During *extension* (or *elongation*), the last stage of a PCR cycle, the temperature is usually raised slightly (to about 70°C to 75°C), and DNA polymerase copies the target DNA by binding to the 3′ ends of each primer and using the primers as templates. DNA polymerase adds nucleotides to the 3′ end of each primer to synthesize a complementary strand.

At the end of one complete cycle, the amount of target DNA has been doubled. The thermal cycler repeats these three stages again according to the total number of cycles determined by the researcher, usually 20 or 30 cycles. Amplification of the target DNA continues exponentially based on the number of amplification cycles. Thus the greatest advantage of PCR is its ability to amplify millions of copies of target DNA from a very small amount of starting material in a short time. Because the target DNA is doubled after every round of PCR, after 20 cycles of PCR, approximately 1 million copies (2^{20}) of target DNA are produced from a reaction starting with one molecule of target DNA.

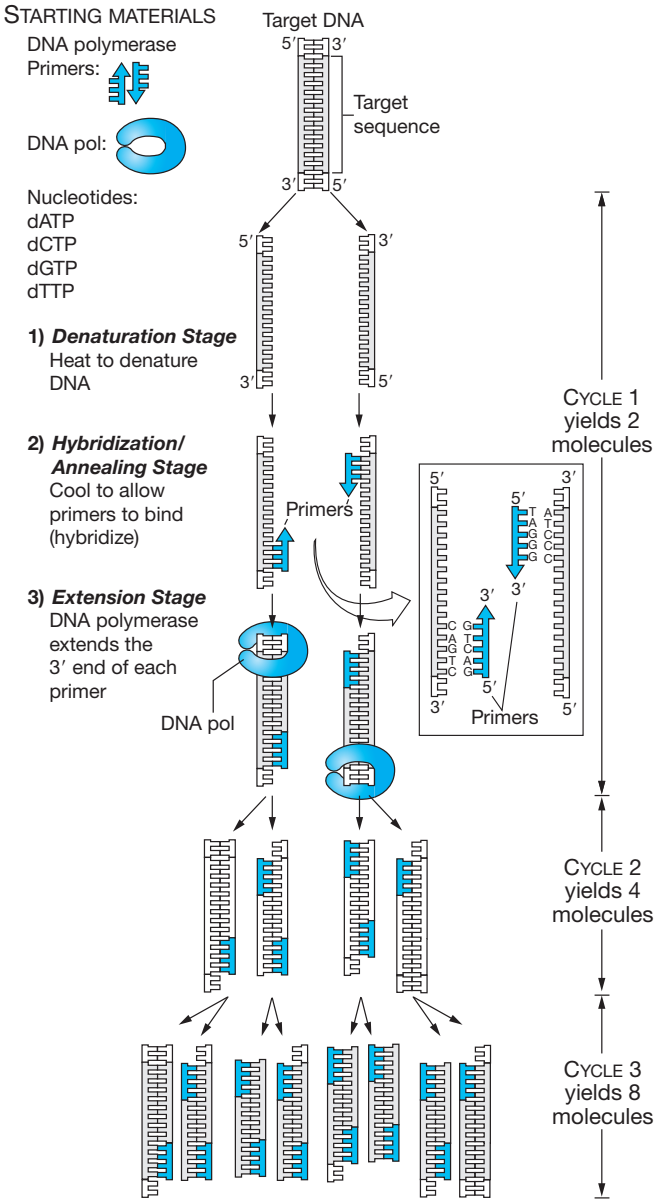


FIGURE 3.5 The Polymerase Chain Reaction

One key to PCR is the type of DNA polymerase used in the reaction. The repeated heating and cooling required for PCR would denature and destroy most DNA polymerases after just a few cycles. Several sources of PCR-suitable DNA polymerases are available. One of the first and most popular enzymes for PCR is known as ***Taq* DNA polymerase.** ***Taq*** is isolated from the domain Archaea called *Thermus aquaticus*, a species that thrives in hot springs. Because *T. aquaticus* is adapted to live in hot water (it was first discovered in the hot springs of Yellowstone National Park), it has evolved a DNA polymerase that can withstand high temperatures. Because *Taq* is stable at high temperatures, it can withstand the temperature changes necessary for PCR without being denatured. Thermostable polymerases such as *Taq* are essential for PCR. Incidentally, now most commercially available thermostable polymerases are produced by DNA cloning but the initial identification and purification of *Taq* is itself a great example of biotechnology.

There are many variations and different applications of PCR technology. For example, **real-time or quantitative PCR (qPCR)** uses specialized thermal cyclers that enable researchers to quantify amplification reactions as they occur. We will discuss qPCR later in this chapter (see Figure 3.16).

PCR has widespread applications in research and medicine, such as studying gene expression, amplifying minute amounts of DNA to detect viral pathogens and bacterial infections, amplifying DNA to diagnose genetic conditions, detecting trace amounts of DNA from tissues at a crime scene, and even amplifying ancient DNA from fossilized dinosaur tissue (**Figure 3.6**). Many PCR applications are described throughout the book.

Cloning PCR products

PCR is often used for cloning a gene because it is rapid and effective (**Figure 3.7**). A disadvantage of PCR cloning is that, to design primers, you need to know something about the DNA sequences that flank your gene of interest. Cloning by PCR is easiest if the gene has already been cloned in another species—for instance, using primers for a gene cloned previously from mice to clone the equivalent gene from humans, or if the DNA sequence of the gene you want to clone has already been determined.

There are many ways to clone a gene using PCR. One approach, called *TA cloning*, takes advantage of an interesting quirk of thermostable polymerases. As DNA is copied, *Taq* and other polymerases used for PCR normally add a single adenine nucleotide to the 3′ end of all PCR products (Figure 3.7). After amplifying a target gene, cloned PCR products can be ligated into plasmids called T vectors. T vectors contain a single-stranded thymine nucleotide at each end that can complementarily base pair with overhanging adenine nucleotides in PCR products. Once ligated into a T vector, the recombinant plasmid containing the cloned PCR product can be introduced into bacteria and its nucleotide sequence can be determined.

PCR is also frequently used to *subclone* genes—that is, to clone smaller pieces of a gene, rather than the entire gene—to make a probe that will express parts of a gene in a cell for the purpose of studying gene and protein function. Now that we have explored some of the most common strategies used to clone genes, in the next section we will consider a wide range of different approaches that scientists use to study cloned genes.

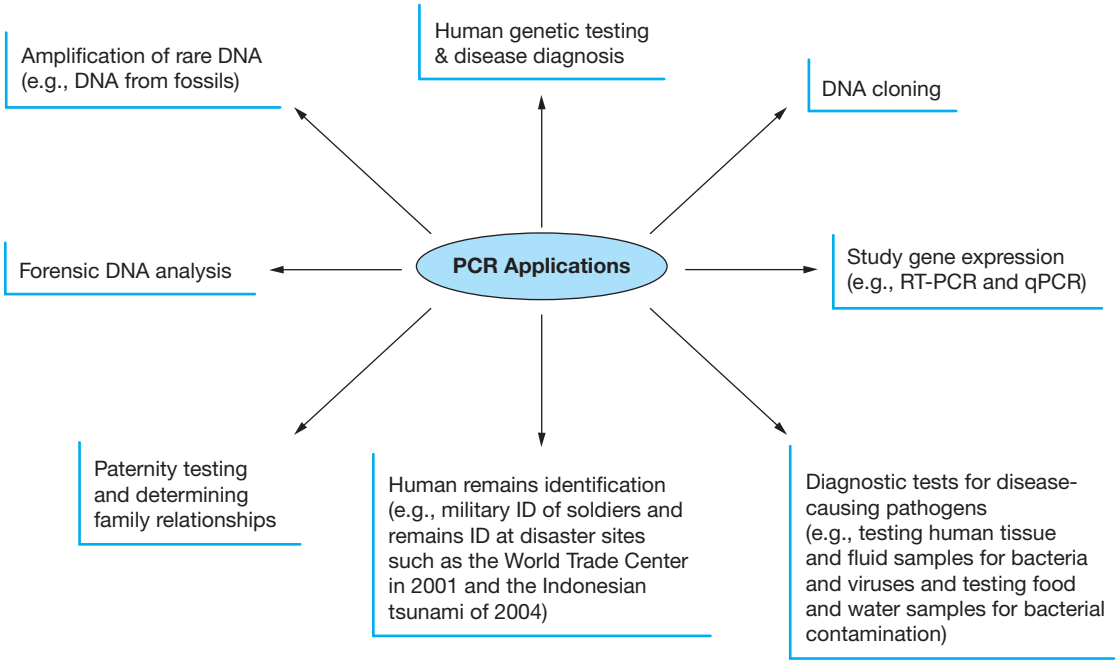


FIGURE 3.6 PCR Applications The amplification of DNA by PCR has become an essential technique in molecular biology with a wide range of different applications. Examples of some of the more common biotechnology-related applications are represented in this figure.

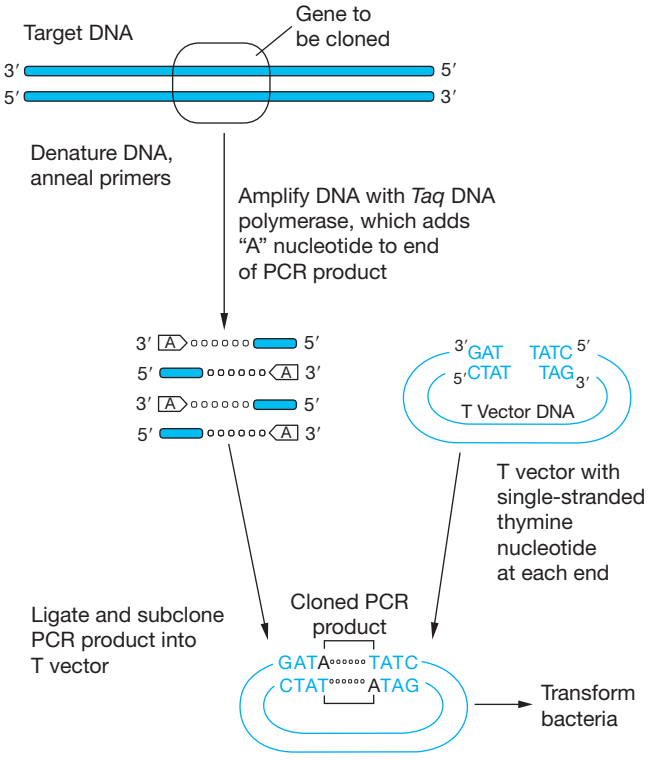


FIGURE 3.7 Cloning a Gene by PCR

3.3 Laboratory Techniques and Applications of Recombinant DNA Technology

What can you do with a cloned gene? The applications of gene cloning and recombinant DNA technology are numerous. **Figure 3.8** summarizes common applications, many of which are discussed further in other chapters. In this section, we present some important routine molecular biology laboratory techniques and basic applications of gene cloning.

Agarose Gel Electrophoresis

Agarose gel electrophoresis is one of the most common laboratory techniques used when working with DNA because it allows one to separate and visualize DNA fragments based on size (**Figure 3.9**). Agarose is a material that is purified from seaweed, melted in a buffer solution, and poured into a plastic tray. As the agarose cools, it solidifies to form a horizontal semisolid gel containing small holes or pores through which DNA fragments will travel. The percentage of agarose used to create the gel determines its ability to resolve DNA fragments of different sizes. Most applications generally involve gels that contain 0.5 to 2 percent agarose. A gel with a high percentage of agarose (for example, 2 percent) is better

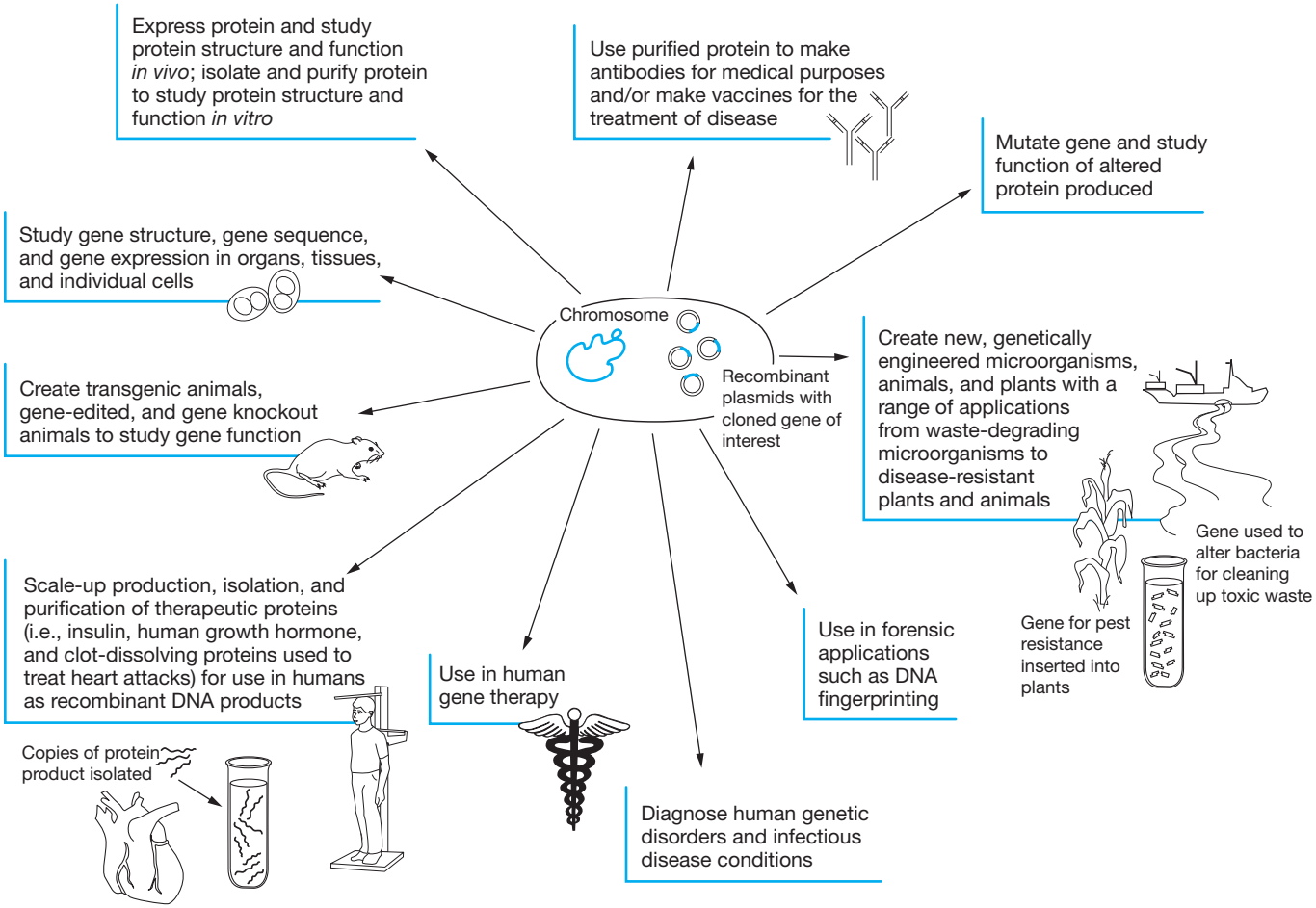


FIGURE 3.8 Applications of Recombinant DNA Technology

suited for separating small DNA fragments because they will snake their way through the pores more easily than large fragments, which do not separate through the dense gel very well. A lower percentage of agarose is better suited for resolving large DNA fragments.

To run a gel, it is submerged in a buffer solution that will conduct electricity. DNA samples are loaded into small depressions called wells, and an electrical current is applied through electrodes at opposite ends of the gel. Separating DNA by electrophoresis is based on the principle that DNA migrates through a gel according to its charge and size (in base pairs). The sugar-phosphate backbone renders DNA negatively charged at physiological pH; therefore, when DNA is placed in an electrical field, it migrates toward the anode (positive pole) and is repelled by the cathode (negative pole). Because all DNA is negatively charged regardless of the length or source, the rate of DNA migration and separation through an agarose gel depends on the *size* of a DNA molecule. Migration distance is inversely proportional to the size of a DNA fragment, so large DNA fragments migrate short distances through a gel relatively slowly and small fragments migrate farther.

Tracking dyes are added to monitor DNA migration during electrophoresis. After the desired time of electrophoresis, DNA in the gel is stained using dyes such as **ethidium bromide,** which intercalate (penetrate) in between the base pairs of DNA. These dyes fluoresce when they are exposed to ultraviolet light. A permanent record of the gel is obtained by taking a digital photograph of the gel while it is exposed to ultraviolet light (Figure 3.9b). Notice how the *Hin*dIII-digested *E. coli* DNA produces a smear of bands unlike the set of discrete fragments visualized with *Hin*dIII-digested λ DNA. This smearing is due to the large size of the *E. coli* chromosome and the large number of cutting sites for *Hin*dIII; so many fragments are created that it is not possible to visualize discrete bands. You will encounter techniques involving agarose gel electrophoresis throughout this book and, as a student, you are very likely to learn to run agarose gels during your biotechnology training (or perhaps you had experience with gels as a high school student).

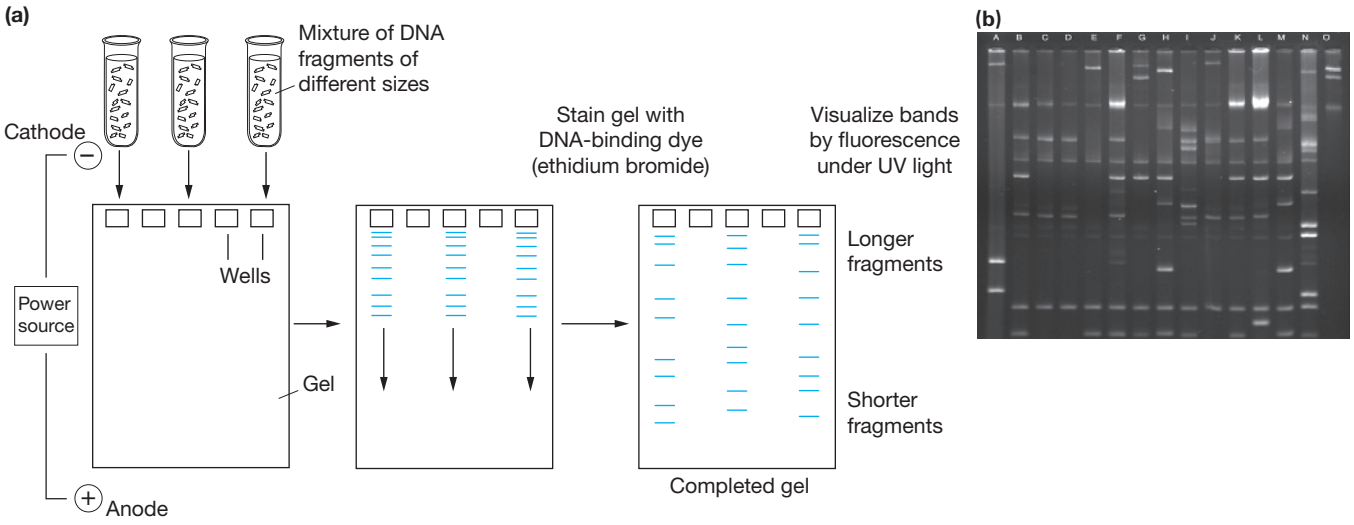


FIGURE 3.9 Agarose Gel Electrophoresis (a) DNA fragments can be separated and visualized by agarose gel electrophoresis. (b) Photograph of an agarose gel stained with ethidium bromide. Lanes loaded with different DNA samples are labeled as A-O.

Restriction Mapping

In the early days of gene cloning, soon after a gene was cloned, typically a type of physical map of the gene would be created to determine which restriction enzymes cut the cloned gene and to pinpoint the location of these cutting sites. Knowing the **restriction map** of a gene was very useful for subcloning and manipulating many relatively small pieces of DNA (for example, 100 to 1,000 bp) to sequence DNA and to prepare DNA probes for studying gene expression. Before DNA sequencing and bioinformatics became popular, restriction maps were created by digesting DNA with different restriction enzymes and separating DNA fragments by gel electrophoresis.

Once the DNA samples were digested and visualized by gel electrophoresis, creating the actual restriction map was like assembling a puzzle; the digestion pattern of fragments could then be interpreted to determine the location of restriction sites for different enzymes. At the beginning of the Human Genome Project, restriction maps of the human genome were important for digesting the genome into pieces that could be sequenced.

Because DNA sequencing of large and small pieces of DNA is now routine, restriction mapping is typically done using bioinformatics software (such as Webcutter, described in Problem 9 in "Questions & Activities") to identify restriction cutting sites in a DNA sequence without having to actually digest DNA and create a map experimentally. However, because of the historical importance of restriction mapping and its occasional use today, an awareness of this technique is still of value.

DNA Sequencing

Determining the nucleotide sequence of a segment of DNA—its exact order of A's, G's, T's, and C's—is a routine technique in molecular biology and biotechnology laboratories. Knowing the DNA sequence of a gene can be helpful (1) to understand chromosome structure; (2) to deduce the amino acid sequence of a protein encoded by a cloned gene; (3) to identify regulatory elements such as promoter sequences; (4) to identify differences in genes created by gene splicing; and (5) to identify genetic mutations, including those used for human genetic testing, among many other reasons. DNA sequencing is equally valuable for studying and understanding non-coding regions of the genome.

Many different methods of **DNA sequencing** are available. Over the past several decades, sequencing methods progressed from manual approaches to techniques for PCR "cycle" sequencing and computer-automated DNA sequencing. Sequencing technologies continue to rapidly improve each year. Initially, the most widely used sequencing approach was chain-termination sequencing, a manual method developed in 1977 by Frederick Sanger and often referred to as Sanger sequencing. In this technique, a DNA primer was hybridized to denatured template DNA, such as a recombinant plasmid to be sequenced, in a reaction tube containing deoxyribonucleotides and DNA polymerase. Because many modern plasmids are designed with sequencing primer binding sites adjacent to the multiple cloning site, DNA

polymerase could be used to extend a complementary strand from the 3′ end of primers hybridized to the plasmid. The original approach utilized primer sequences labeled with radioactive phosphorus or sulfur.

A small amount of a modified nucleotide called a **dideoxyribonucleotide (ddNTP)** was mixed in with the vector, primer, polymerase, and deoxyribonucleotides. A ddNTP differs from a normal deoxyribonucleotide (dNTP) because it has a hydrogen group attached to the 3′ carbon of the deoxyribose sugar instead of a hydroxyl group, OH (see **Figure 3.10a** on the next page). When a ddNTP is incorporated into a chain of DNA, the chain cannot be extended because the absence of a 3′-OH prevents the formation of a phosphodiester bond with a new nucleotide; hence, the chain is "terminated."

In the original Sanger approach, four separate reaction tubes were set up. Each tube contained vector, primer, and all four dNTPs, but each tube also contained a small amount of one ddNTP. As synthesis of a new DNA strand from the primer begins, DNA polymerase randomly inserts a ddNTP into the sequence instead of a normal dNTP, preventing further synthesis of a complementary strand. Over time, a ddNTP will be incorporated at all positions in the newly synthesized strands, creating a series of fragments of varying lengths that are terminated at dideoxy residues.

The DNA strands were separated on a thin polyacrylamide gel, which could separate sequences that differ in length by a single nucleotide. Autoradiography was used to detect the radioactive sequencing fragments. The sequence determined from the autoradiogram was "read" from the bottom to the top as individual nucleotides. Sanger sequencing has largely been replaced by computer-automated approaches, as we discuss in the next section.

Computer-automated high-throughput sequencing replaces the original Sanger sequencing technique

Because of limitations in running a sequencing gel, the original Sanger method could be used only to sequence approximately 200 to 400 nucleotides in a single reaction; therefore, when sequencing a longer piece of DNA—for example, 1,000 base pairs—it was necessary to run multiple reactions to create overlapping sequences that could be pieced together to determine the entire, continuous sequence of 1,000 base pairs. Because of this limitation, the Sanger approach was a cumbersome method for large-scale sequencing efforts such as the Human Genome Project. As you will soon learn, ultimately the project was completed ahead of schedule in part because of the development of **computer-automated high-throughput DNA sequencing methods** capable of sequencing longer stretches of DNA.

For about two decades since the early 1990s, high-throughput sequencing relied on the basic chemistry that was developed by Sanger sequencing. Initially, computer-automated sequencing reactions used either ddNTPs, each labeled with a different colored nonradioactive fluorescent dye, or a sequencing primer labeled at its 5′ end with a visibly colored dye (Figure 3.10). A single reaction tube was used, and the manual-style approach of using polyacrylamide gels was replaced by separating sequencing reactions through an ultrathin diameter tube gel called a *capillary gel*. As DNA fragments move through the gel, they are scanned with a laser beam. The laser stimulates the fluorescent dye on each DNA fragment, which emits different wavelengths of light for each ddNTP. The emitted light is collected by a detector that amplifies and then feeds this information to a computer to process and convert the light patterns and reveal the DNA sequence (Figure 3.10).

Automated DNA sequencers such as these can contain multiple capillary gels several feet long, each generating approximately 1,000 bp of DNA sequence per reaction. These systems were a big improvement over manual Sanger systems because they could generate relatively large amounts of DNA sequence in a relatively short time, with about 99.999 percent accuracy for around $0.50 per kilobase. Automated DNA sequencers of this time period often contain multiple capillary gels (as many as 96) and could process several thousand bases of sequences so that many of these instruments make it possible to generate over 2 million bp of sequences in a day. As a result, these sequencers became known as high-throughput sequencers because of their ability to process and generate large amounts of sequence data in relatively short periods.

These sequencers became essential for the rapidly accelerating progress of the Human Genome Project. They are still often used in both academic research laboratories and biotechnology because they are relatively inexpensive compared to new instruments, particularly for quick applications and sequencing relatively small pieces of DNA. But by around 2005 and in the years since, sequencing technologies were to improve dramatically. For laboratories engaged in large-scale sequencing efforts that need to generate even larger amounts of sequence data faster and more efficiently, next-generation and third-generation sequencing are the leading technologies.

Next-Generation Sequencing (NGS)

Genomics along with the desire to sequence and analyze entire genomes (including sequencing and analyzing human genomes for detecting and treating genetic diseases) has spurred a demand for sequencers that are faster and capable of generating millions of bases of DNA sequence in a relatively short time, leading to the development of **next-generation sequencing (NGS)** technologies designed to produce highly accurate and long

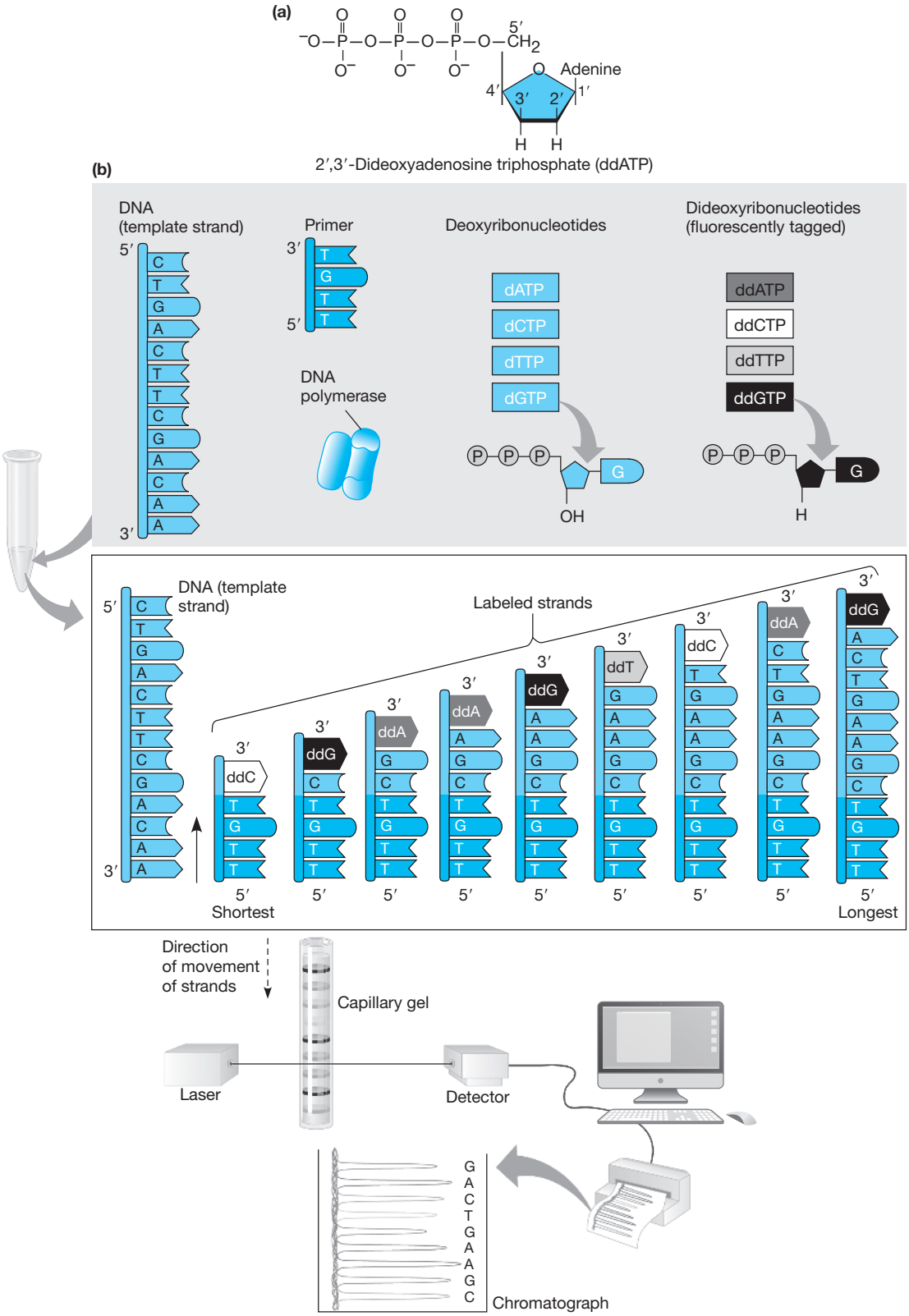


FIGURE 3.10 Computer-Automated DNA Sequencing (a) The structure of a dideoxynucleotide (ddNTP). Note that the 3′ group attached to the carbon is a hydrogen rather than a hydroxyl group (OH). Because another nucleotide cannot be attached at the 3′ end of a ddNTP, these nucleotides are the key to DNA sequencing by the Sanger method, as shown (b). Computer-automated approaches separate DNA fragments using a capillary gel. A laser and detector are used to detect fluorescence of each dideoxynucleotide.

stretches of DNA sequence, greater than 1 gigabase (1 billion bases) of DNA per reaction, at a low cost. NGS approaches dispense with Sanger chemistry and capillary electrophoresis methods in favor of sophisticated, parallel formats (simultaneous-reaction formats) that use state-of-the art fluorescence imaging techniques and software for comparing and averaging sequence data for many strands sequenced simultaneously. NGS technologies provided an unprecedented capacity for generating massive amounts of DNA sequence data rapidly and at dramatically reduced costs per base. NGS has become so routine that often when scientists talk about "sequencing" in general terms they are referring to NGS.

The desire for next-generation sequencing among the research community and challenges such as the $1,000 genome (discussed later in this chapter) led to an intense technology race among many companies eager to produce NGS methods. As a result, several NGS technologies emerged by 2005. Some of the first instruments were capable of producing as much data as 50 capillary electrophoresis systems and were up to 200 times faster and cheaper than conventional Sanger approaches. NGS instruments generally produce short read lengths of approximately 50 to 400 bp, and then these snippets of sequence are stitched together using software to produce a coherent, complete genome.

A biotechnology company called 454 Life Sciences, subsequently acquired by Roche, was the first company to commercialize a NGS technology. This approach sequenced genomes using a so-called solid-phase method in which beads were attached to fragmented genomic DNA, which was then PCR-amplified in separate water droplets in oil for each bead, loaded into multiwell plates, and mixed with DNA polymerase. Multiwell plates could contain more than a million wells, each serving as a reaction tube for sequencing. A reaction called *pyrosequencing* was then used to sequence DNA on the beads in each well. With this method, 454 sequencing could generate on the order of 400 million bases (Mb) of data per 10-hour run. Incidentally, NGS sequencing technologies are also creating major data-management challenges for saving and storing such large image data files. But by 2013, sequencing technology was advancing so rapidly that Roche disbanded 454 Life Sciences because even this NGS approach was not competitive with newer systems.

The Illumina HiSeq, and variations of this instrument, are among the most common NGS platforms currently used in cutting-edge sequencing laboratories. This system uses a *sequencing by synthesis* approach. This method involves using DNA fragments as templates for synthesizing new strands, incorporating nucleotides labeled with different dyes, washing away unincorporated nucleotides, imaging incorporated nucleotides, and repeating this cycle. The method developed by Illumina attaches DNA fragments to a solid support and then, using reactions similar to those of the Sanger method, fluorescently tagged terminator nucleotides are added and detected. Then the fluorescent tags and terminator portions of the nucleotide are removed to allow another cycle of extension. This approach can now generate about 600 gigabases (Gb = 1 billion bp) of data in 10 days—enough to sequence four complete human genomes, with each base sequenced an average of 30 times for accuracy. The instrumentation needed to run these platforms is expensive. For example, the Illumina HiSeq instrument costs about $700,000. But given the massive amounts of sequence data the NGS methods can generate, the average cost per base is much lower than that in Sanger sequencing.

Third-Generation Sequencing Technology

Shortly after NGS methods were commercialized, companies were announcing progress on **third-generation sequencing (TGS).** TGS methods are based on strategies that sequence a *single molecule* of single-stranded DNA, and at least four different approaches are being explored. Pacific Biosciences' PacBio technology is one of the leaders in TGS and involves an approach known as **single-molecule sequencing in real time (SMRT)**. The PacBio instrument works by attaching single-stranded molecules of the DNA to be sequenced to a single molecule of DNA polymerase anchored to a substrate and then visualizing, in real time, the polymerase as it synthesizes a strand of DNA (see **Figure 3.11**). The DNA polymerase is confined within a hole, a nanopore of about 10 nm in diameter located within a thin layer of metal on a glass substrate. This configuration allows for the illumination necessary to detect individual nucleotides as they are added to single strands synthesized in the nanopore.

The polymerase adds fluorescent dye–labeled nucleotides to a newly synthesized growing strand of DNA. Because the dye is attached to a terminal phosphate chain of the nucleotide, it is cleaved when incorporated into the newly synthesized strand. The fluorescent dye flashes as the base is incorporated into the DNA strand. Each base flashes with a characteristic color that is detected and recorded. Other nanopore sequencing technologies apply an electrical current across the pore and measure disruptions in current unique to each nucleotide to determine the sequence as a newly synthesized chain is created.

The PacBio was one of the first "long-read" instruments to reach the market. It generates read lengths of over 1,500 bp. Recently, the PacBio was used to sequence the genomes of five strains of *Vibrio cholerae* involved in a cholera outbreak in Haiti in less than an hour. This genetic determination of the *Vibrio* strains resulted in rapid action to successfully treat the outbreak with antibiotics to which these bacteria were not resistant. Most TGS technologies still have somewhat high error rates for sequencing accuracy—about 15 percent errors in

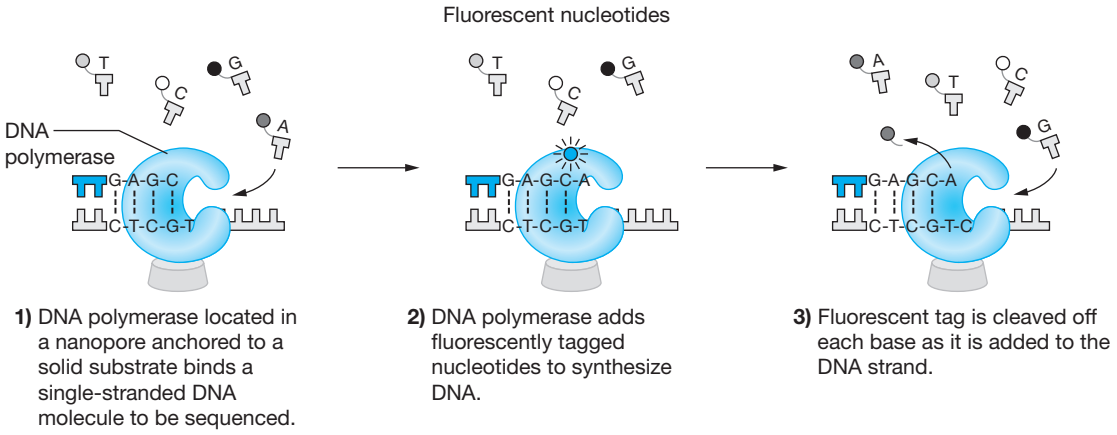


FIGURE 3.11 Third-Generation Sequencing A simplified version of one approach to TGS is shown here. In this example, a DNA polymerase molecule anchored within a nanopore binds to a single strand of DNA. As the polymerase incorporates fluorescently labeled nucleotides into a newly synthesized strand (shown in blue), each base emits a characteristic color that can be detected.

sequence generated. At around $750,000, these technologies also remain very expensive. A few years ago, Oxford Nanopore Technologies developed a handheld portable, single-molecule sequencer called the MinION that is the size of a USB memory stick! Although accuracy of this sequencer limits its applications, there is no reason to think that the technology for highly accurate pocket-sized sequencers will not advance in the near future.

We briefly present TGS to provide a context for how far sequencing technologies have developed since Sanger sequencing and even NGS. The detailed methodologies of TGS are less important than the conceptual significance of these approaches—a shift toward sequencing individual molecules faster, cheaper, and more accurately than previous methods. Without doubt, the development of TGS technologies represents another significant sequencing milestone for genomic studies.

Moore's law, stated by Intel cofounder Gordon Moore, is that computing power tends to double (and its price effectively halves) every 2 years. In the computing world this has generally been true for about 50 years. Scientists have often applied Moore's law to evaluate the costs of DNA sequencing. Through 2006, new sequencing technologies were cutting sequencing costs in half about every 2 years and keeping pace with Moore's law (**Figure 3.12**). But since 2007 the price of

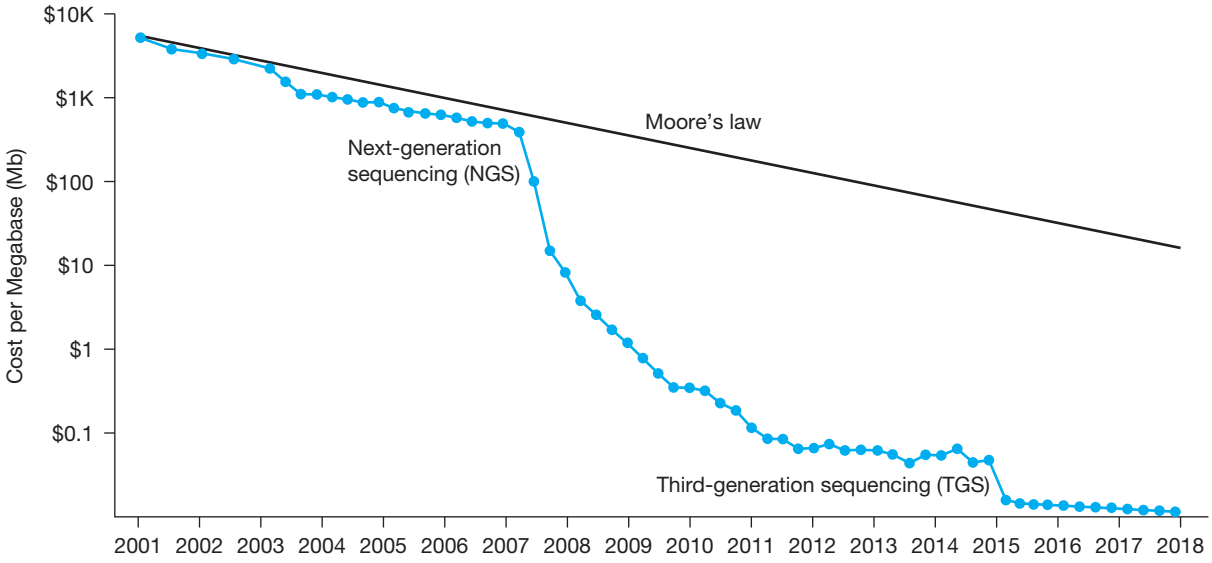


FIGURE 3.12 Advances in sequencing technology have led to rapidly decreasing sequence costs for genome sequencing. Notice how the development of NGS led to a substantial decrease in cost per megabase. Another major decrease occurred with the advent of TGS.

sequencing a human genome has plummeted dramatically, far outpacing Moore's law, in large part because of the impact of NGS entering the market.

Fluorescence In Situ Hybridization

A technique called **fluorescence in situ hybridization (FISH;** *in situ* means "in place") can be used to pinpoint a gene of interest on a particular chromosome. FISH can also be used to study RNA expression *in vivo*. In FISH, chromosomes are isolated from cells such as white blood cells and spread out on a glass microscope slide. A DNA or RNA probe for the gene of interest is labeled with fluorescent nucleotides and then incubated in solution with the slide. The probe hybridizes with complementary sequences on chromosomes on the slide. The slide is washed and then exposed to fluorescent light. Wherever the probe has bound to a chromosome, the fluorescently labeled probe is illuminated to indicate the presence of probe binding (**Figure 3.13**).

To determine which of the 23 human chromosomes show fluorescence, they are aligned according to the length and staining patterns of their chromatids to create a karyotype. Fluorescence on more than one chromosome indicates either multiple copies of the gene or related sequences that may be part of a gene family. FISH is also used to analyze genetic disorders. For example, FISH analysis can be performed on chromosomes contained in fetal cells harvested from the fetal portion of the placenta (through **chorionic villus sampling**) or from the amniotic fluid (**amniocentesis**; both of these techniques are discussed in Chapter 11), to screen for abnormal genes or incorrect numbers of chromosomes in the developing fetus of a pregnant woman.

When a gene is expressed in an organ with many different cell types—for example, kidney or brain tissue—FISH can also be used to determine the cell type that is expressing a particular mRNA. In this approach, the tissue of interest is preserved in a fixative solution and then embedded in a wax-like material or medium that can be frozen. This allows researchers to slice the tissue into thin sections about 1 to 5 mm thick and to attach them to a microscope slide. The slide is incubated with a fluorescent dye–tagged RNA or DNA probe for the gene of interest. The probe hybridizes to mRNA within cells in their native place, and probe binding is determined by detecting fluorescence. For some studies, PCR can even be performed directly on tissue sections in situ as a way of determining cell-type expression for a given gene.

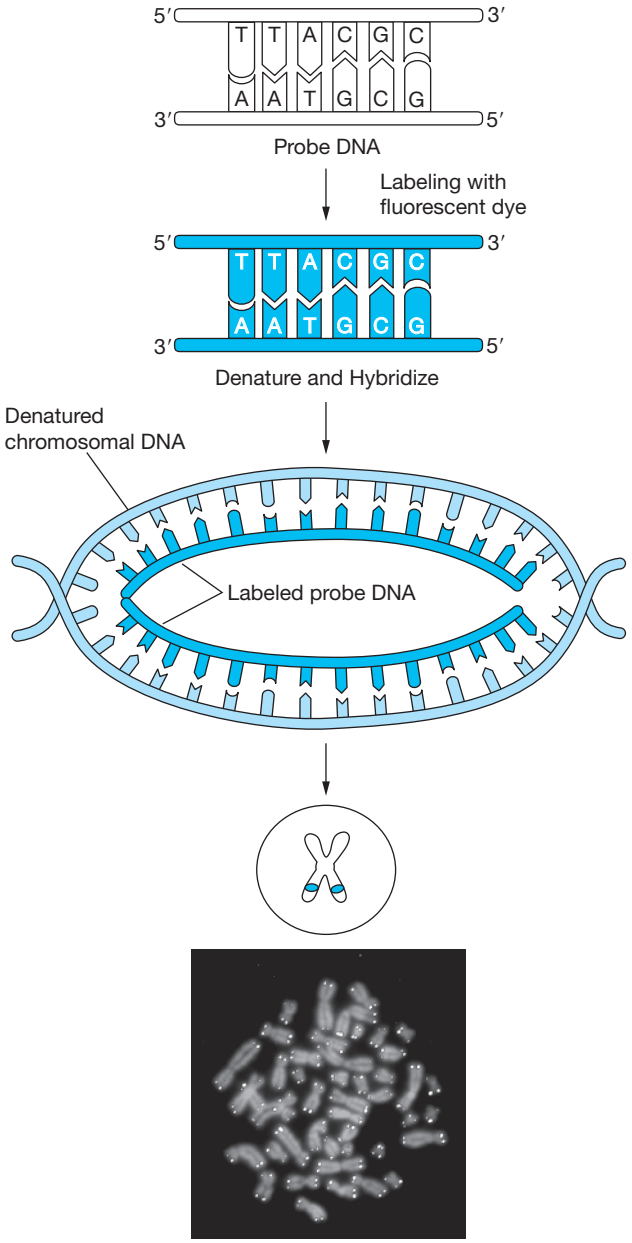
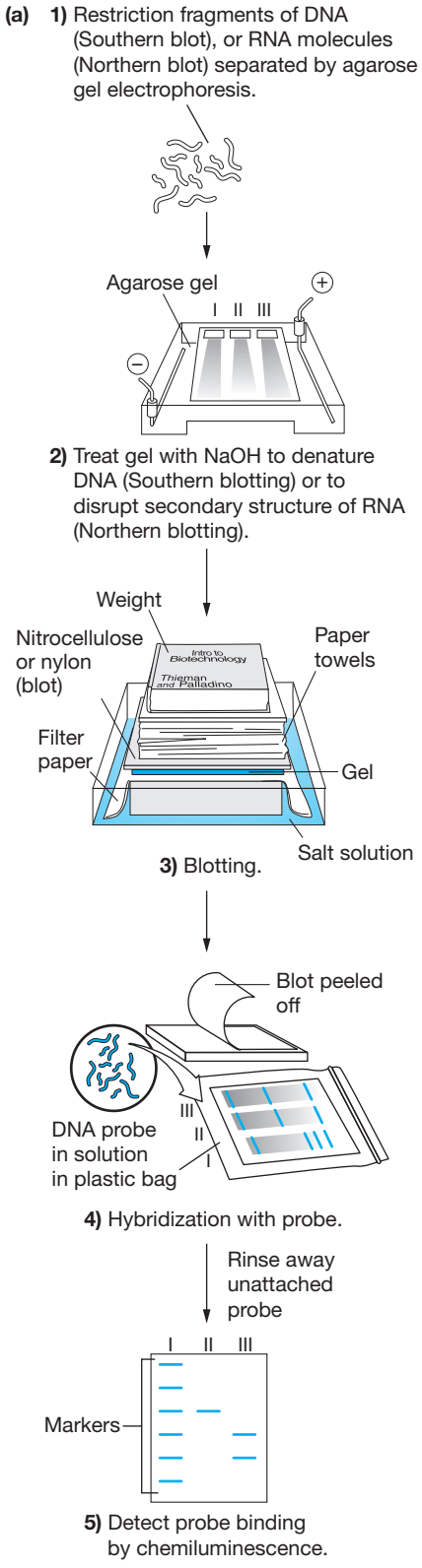


FIGURE 3.13 Fluorescence In Situ Hybridization White spots at the tips of each chromosome indicate fluorescence from a probe binding to telomeres.

Southern Blotting

Prior to widespread use of DNA sequencing, a technique called **Southern blot analysis** (Southern blotting or hybridization) was frequently used to determine gene copy number (the number of copies of a particular gene in a genome) among a range of other applications. Many traditional applications of Southern blotting have been rendered antiquated by DNA sequencing. But the development of Southern blotting was an important technique because it demonstrated how DNA hybridization could be carried out—which became essential for library screening, PCR, and DNA sequencing. And Southern blotting established the basic techniques for **Northern blotting** (the separation and blotting of RNA molecules, as discussed in the



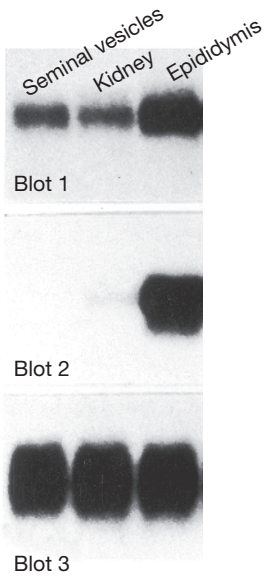


FIGURE 3.14 Analyzing Gene Expression by Northern Blot Analysis Northern blot analysis and RT-PCR are two common techniques for analyzing the amount of mRNA produced by a tissue (gene expression). (a) Shown here is an overview of the basic steps involved in Southern blotting and Northern blotting. (b) Blot 1 shows results from a Northern blotting experiment in which RNA from three rat tissues—seminal vesicles (a male reproductive organ), kidney, and the epididymis (another male reproductive organ)—was blotted onto nylon and then hybridized to probe for a gene involved in protecting tissues from damage by harmful free radicals. Notice how the amount of mRNA detected in the epididymis is much greater (as indicated by the size and darkness of each band) than the amount of mRNA in the seminal vesicles or kidney. Blot 2 shows results when the same blot as shown in blot 1 was stripped of bound probe and reprobed with a probe for a different gene. Notice how this gene is expressed primarily in the epididymis but is not readily detected in the seminal vesicles or kidney. Blot 3, the same blot shown in the other two panels, was stripped of bound probe and probed for a gene called cyclophilin that is expressed at nearly the same levels in virtually all tissues.

next section; see Figure 3.14) and **Western blotting** (the separation and blotting of proteins).

Developed by Ed Southern in 1975, Southern blotting involved digesting DNA, such as chromosomal DNA, into small fragments with restriction enzymes and separating those fragments by agarose gel electrophoresis. However, the number of restriction fragments generated by digesting chromosomal DNA is often so great that simply running a gel and staining the DNA does not resolve discrete fragments. Rather, digested DNA appears as a continuous smear of fragments in the gel (refer to Figure 3.9b). Southern blotting allowed one to visualize only *specific* fragments of interest. Following electrophoresis, the gel is treated with an alkaline solution to denature the DNA; then the fragments are transferred onto a nylon or nitrocellulose membrane using a technique called *blotting*.

Blotting can be achieved by setting up a gel sandwich in which the gel is placed under the nylon membrane, filter paper, paper towels, and a weight to allow for wicking of a salt solution through the gel, which will transfer DNA onto the nylon by capillary action. The single strands of DNA stick to the blot, positioned in bands exactly as on the gel. The nylon blot is then baked or briefly exposed to ultraviolet (UV) light to attach the DNA permanently. Next the blot is incubated with a labeled probe. The probe hybridizes (binds to complementary base pairs) to molecules of DNA on the blot to which the probe has similar sequence. Unbound probe molecules are then washed away. For many decades radioactive probes were used, and blots were visualized by exposing x-ray film to the radioactivity of the probe in a process called *autoradiography*. But autoradiography has largely been replaced by nonradioactive **chemiluminescence** approaches.

In chemiluminescence, probes are labeled with enzymes or other molecules that can bind to and cleave light-releasing substrates. The released photons are captured by a digital imaging system or a light-detecting device called a scanning luminometer.

Northern and Western blotting techniques were not named after scientists named "Northern" and "Western"; rather, they were named as tongue-in-cheek references to Ed Southern, the founder of Southern blots. In the research lab and in biotechnology, Southern blotting is still used for applications including gene mapping, detecting genetic mutations, PCR product confirmation, and DNA fingerprinting (a topic we discuss in Chapter 8).

Studying Gene Expression

Molecular biologists around the world are involved in research studying gene expression and the regulation of gene expression. As you will learn throughout the book, a range of applications in biotechnology, including human genetic disease diagnosis, rely on measuring and quantifying RNA expression. Numerous different molecular techniques are available for studying gene expression. Most methods involve analyzing mRNA produced by a tissue. This is often a good measure of gene expression because the amount of mRNA produced by a tissue is frequently equivalent to the amount of protein the tissue makes.

Northern blot analysis

The basic methodology of a Northern blot is similar to that of Southern blot analysis. PCR experiments have replaced this method in popularity, but because you will still encounter applications of Northern blotting, an introduction to this approach has value. In Northern blotting, RNA is isolated from a tissue of interest and separated by gel electrophoresis (the RNA is not digested with enzymes). RNA is blotted onto a nylon membrane and then hybridized to a probe, as described for Southern blots. Exposed bands on the autoradiogram show the presence of mRNA for the gene of interest and the size of the mRNA (**Figure 3.14**). In addition, the amounts of mRNA produced by different tissues can be compared and quantified.

Reverse transcription PCR

PCR allows for detecting minute amounts of mRNA from even very small amounts of starting tissue. Because the amount of RNA produced by a tissue can be below the level of detection by Northern blot analysis, once PCR was discovered it became a very powerful method for studying gene expression. For instance, PCR has been a great tool for studying gene expression in embryos and developing tissues in which the amount of tissue available for analysis is very small. Because RNA cannot be directly amplified by PCR, a technique called **reverse transcription or reverse transcriptase PCR (RT-PCR)** is carried out. In RT-PCR, isolated mRNA is converted into double-stranded cDNA by the enzyme reverse transcriptase in a process similar to how cDNA for a library is synthesized. The cDNA is then amplified with a set of primers specific for the gene of interest. Amplified DNA fragments are electrophoresed on an agarose gel and evaluated to determine expression patterns in a tissue (see **Figure 3.15**). The amount of cDNA produced in a RT-PCR reaction for a particular gene of interest reflects the amount of mRNA, and thus the level of gene expression, for that particular gene in a given tissue.

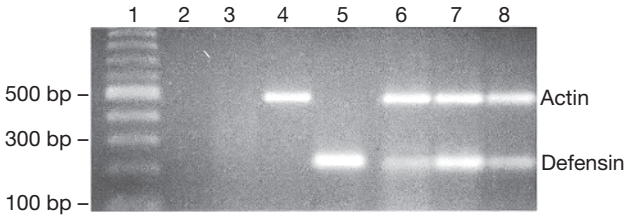


FIGURE 3.15 RT-PCR Analysis of RNA Expression Agarose gel showing results from experiments in which RNA from rat tissues was reverse-transcribed and amplified with primers for β-actin (an important component of the cytoplasm of cells) and/or β-defensin-1 (a gene that encodes a peptide that protects tissue against bacterial infections). Lane 1 contains DNA size standards of known size (often called a ladder) increasing in 100-bp increments. Lane 2 is a negative control sample in which primers were added to a PCR experiment without cDNA. Lane 3 is a negative control sample in which cDNA was added to a PCR experiment without primers. Notice that lanes 2 and 3 do not show any amplified PCR product because amplification will not occur without cDNA as target DNA (lane 2) or without primers (lane 3). Lane 4 shows kidney cDNA amplified with actin primers. Lane 5 shows kidney cDNA amplified with defensin primers. Lanes 6, 7, and 8 show PCR products from cDNA of three different rat reproductive tissues that were amplified with both actin and defensin primers. Notice how lanes 6 and 8 show relatively even amounts of actin and defensin PCR products, whereas higher amounts of defensin PCR product are shown in lane 7. These differences reflect the varying amounts of defensin mRNA expressed by each tissue.

Real-time PCR

Real-time or quantitative PCR (qPCR) enables researchers to quantify amplification reactions as they occur in "real time" (**Figure 3.16**). There are several ways to run real-time PCR reactions, but the basic procedure involves the use of specialized thermal cyclers that use a laser to scan a beam of light through the top or bottom of each PCR tube. Each reaction tube contains either a dye-containing probe or a DNA-binding dye that emits fluorescent light when illuminated by

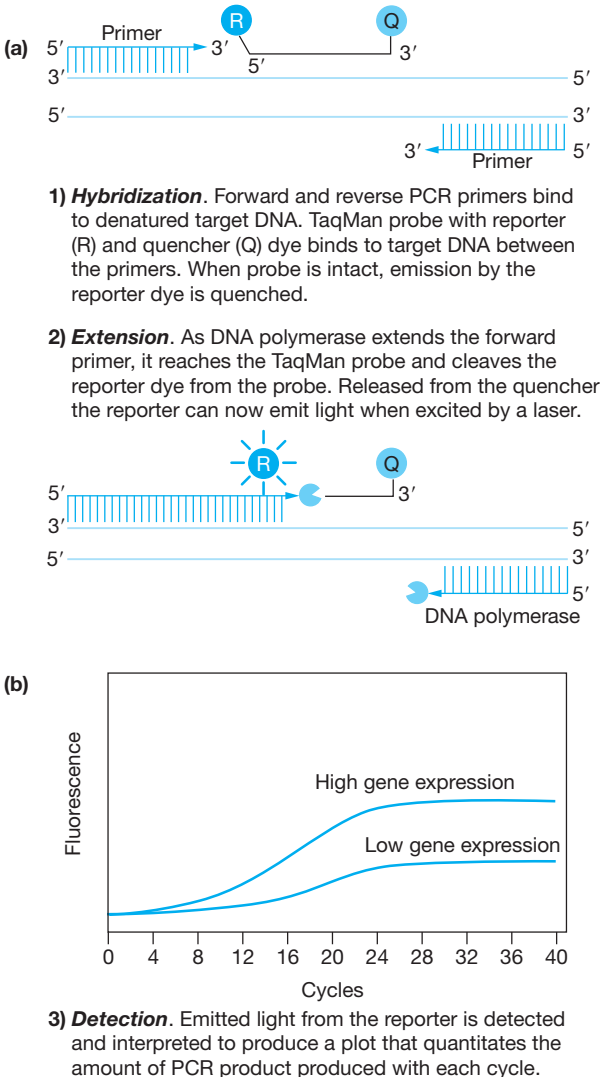


FIGURE 3.16 Real-Time PCR (a) The TaqMan method of real-time PCR involves a pair of PCR primers along with a probe sequence complementary to the target gene. The probe contains a reporter dye (R) at one end and a quencher dye (Q) at the other. When the quencher dye is close to the reporter dye, it interferes with fluorescence released by the reporter dye. When *Taq* DNA polymerase extends a primer to synthesize a strand of DNA, it cleaves the reporter dye off the probe, allowing the reporter to give off energy. (b) Each subsequent PCR cycle removes more reporter dyes, so that increased light emitted from the dye can be captured by a computer to produce a readout of fluorescence intensity with each cycle.

the laser. The light emitted by these dyes correlates with the amount of PCR product amplified. Light from each tube is captured by a detector, which relays information to a computer to provide a readout on the amount of fluorescence after each cycle; this readout can be plotted and analyzed to quantitate the number of PCR products produced after each cycle.

Two commonly used approaches for real-time PCR involve the use of TaqMan probes and a dye called SYBR Green. SYBR Green binds double-stranded DNA. As more double-stranded DNA is copied with each round of real-time PCR, there are more DNA copies to bind SYBR Green, which increases the amount of fluorescent light emitted. TaqMan probes are complementary to specific regions of the target DNA between where the forward and reverse primers for PCR bind (Figure 3.16). Because real-time PCR does not involve running gels, it is a powerful and rapid technique for measuring and quantitating changes in gene expression, particularly in multiplex analyses, in which multiple samples and different genes are being analyzed simultaneously.

Gene microarrays

DNA microarray analysis is another technique for studying gene expression that rapidly gained popularity over the past two decades because it enables researchers to quickly analyze all the genes expressed in a tissue—often called the **transcriptome** (Figure 3.17 on page 106). A microarray, also known as a **gene chip,** is created with the use of a small glass microscope slide. Single-stranded DNA molecules are attached or "spotted" onto the slide using a computer-controlled high-speed robotic arm called an *arrayer*, which is fitted with a number of tiny pins. Each pin is immersed in a small amount of solution containing millions of copies of different DNA molecules, such as cDNAs for different genes. The arrayer fixes this DNA onto the slide at specific locations (points or spots) recorded by a computer. A single microarray can have over 10,000 spots of DNA, each containing unique sequences of DNA for a different gene.

There are several different kinds of microarrays. Figure 3.17 shows a representative example of how a microarray can be used to study gene expression. Scientists begin by extracting all the mRNA molecules from a tissue of interest. Then cDNA synthesized from this mRNA is labeled with a fluorescent dye. Labeled cDNA is incubated overnight with the microarray, where it hybridizes to spots on the microarray that contain complementary DNA sequences. The microarray is washed and scanned by a laser that causes the cDNA hybridized to the microarray to fluoresce. Fluorescent spots reveal which genes are expressed in the tissue of interest, and the intensity of fluorescence indicates the relative amount of expression, producing a so-called heat map. The brighter the spot, the more mRNA is expressed in that tissue. Microarrays can also be run by labeling cDNAs from two or more tissues with different-colored fluorescent dyes. Gene expression patterns from the different tissues are compared based on the color of spots that appear following hybridization and detection.

For many species, including humans, entire genomes are available on microarrays. Researchers are

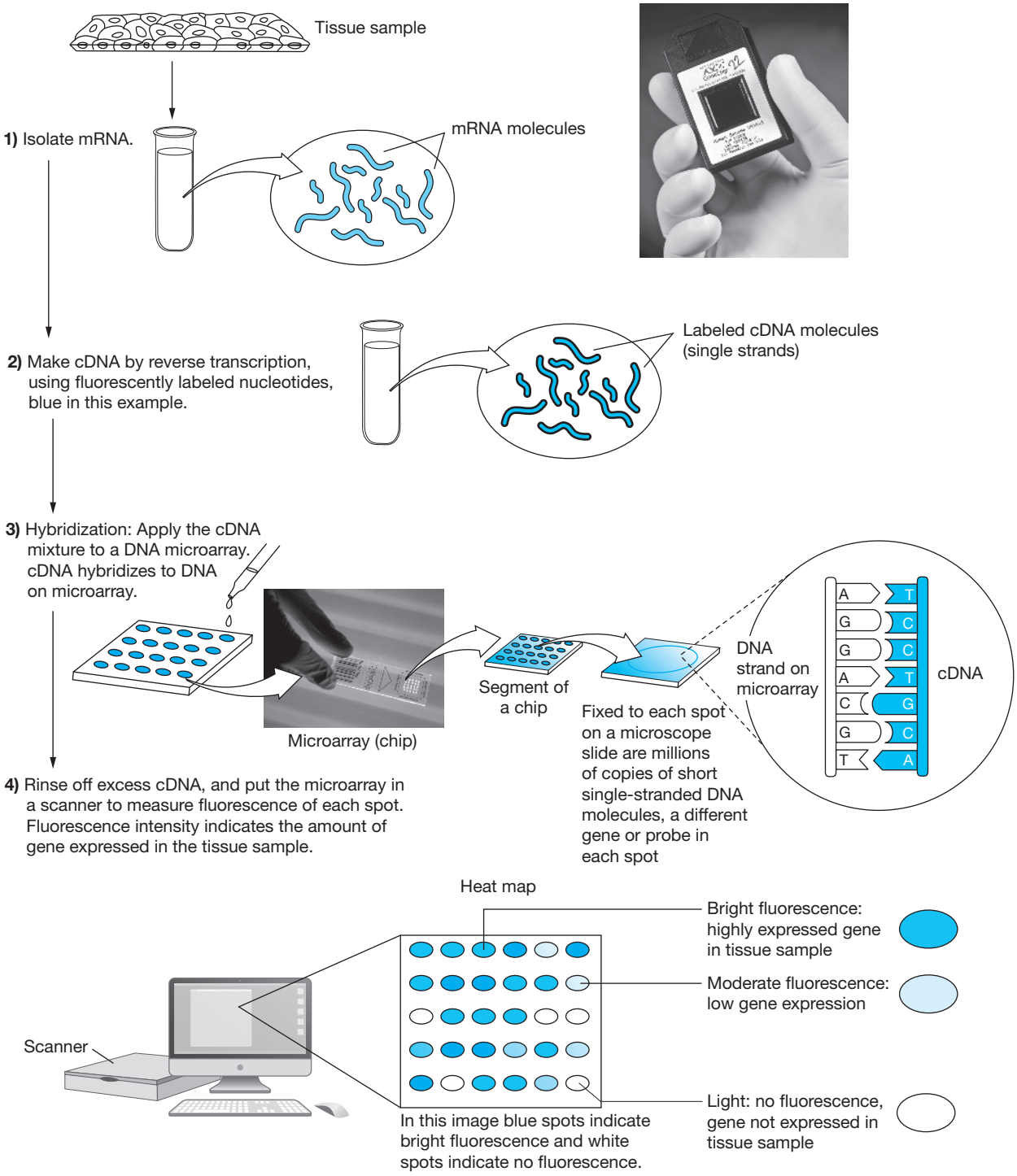


FIGURE 3.17 Gene Microarray Analysis

using microarrays to compare patterns of expressed genes in tissues under different disease conditions. For example, cancer cells can be compared with normal cells to look for genes that may be involved in cancer formation. Results of such microarray studies can be used to develop new drug therapy strategies to combat cancer and other diseases (Chapter 11). Certain applications of microarrays are being replaced by DNA sequencing, which may eventually render microarrays obsolete. But microarrays are still widely used and you will learn about different microarray applications throughout this book.

RNA sequencing allows for in situ analysis of gene expression

In recent years, significant progress has been made on direct **RNA sequencing (RNA-seq),** also called whole-transcriptome shotgun sequencing. One limitation of microarrays is that the investigator is restricted to studying expression of only those genes with probes on the chip. Conversely, RNA-seq not only allows for quantitative analysis of all RNAs (mRNA as well as non-coding RNAs such as microRNAs, long non-coding RNAs, etc.) expressed in a particular tissue but also provides actual sequence data. And RNA-seq can also be carried out inside the cell (in situ). For this application of RNA-seq, the cell itself can serve as a "gene chip."

Generally, most RNA-seq methods incorporate reverse transcribing RNA in situ, sequencing cDNA, mapping sequences to a reference genome (to determine which sequences were transcribed from specific genes), and quantifying gene expression. Methods incorporating fluorescence in situ RNA-seq are enabling scientists to visualize where specific RNAs are being transcribed in intact cells and tissues.

As you will learn in Section 3.4, it is now also becoming possible to carry out DNA sequencing and RNA-seq on *individual* cells. Several groups are taking an integrated approach to sequence genomes and transcriptomes in the same cell. This strategy enables researchers to correlate genetic variability and mRNA expression variability simultaneously in single cells—thus analyzing both the genome and the transcriptome. We will consider applications of RNA-seq in Chapter 11, but clearly this approach has already demonstrated great value for disease diagnosis, including understanding how variations in a person's genome affect transcriptome expression.

Analyzing Gene Function

Although many of the methods we have described in this section can contribute to an understanding of gene function, a number of specific techniques can be used to understand gene function *in vitro* and *in vivo,* and some of these result in biotechnology applications that you will learn about throughout the book. Because of this, here we provide a brief overview of a few key approaches that you will study in biotechnology and learn about in greater detail in other chapters of this book.

Protein expression and purification

Bacteria that have been transformed with recombinant plasmids can often be used to produce the protein product of the isolated gene. Protein expression itself is one way to learn about gene function, because the protein product of a particular gene can be studied. As you will learn in Chapter 4, for the production of recombinant proteins for therapeutic applications, large quantities of bacteria can be grown in a fermenter, and the protein can be isolated.

Gene mutagenesis studies

Mutated versions of a gene can be expressed to produce proteins missing certain sections as one way to dissect apart functional domains of a protein. One such approach is called **site-directed mutagenesis.** In this technique, mutations can be created in specific nucleotides of a cloned gene contained in a vector. The gene can then be expressed in cells, which results in the translation of a mutated protein. This allows researchers to study the effects of particular mutations on protein structure and serves as a way of determining which nucleotides are important for specific functions of the protein. Site-directed mutagenesis can be a very valuable way to help scientists identify critical sequences in genes that produce proteins involved in human diseases.

RNA interference

In 1998, researchers Craig C. Mello of the University of Massachusetts Medical School and Andrew Z. Fire of Stanford University published groundbreaking work in which they used double-stranded pieces of RNA (dsRNA) to inhibit or silence expression of genes in the nematode roundworm *Caenorhabditis elegans*. This naturally occurring mechanism for inhibiting gene expression is known as **RNA interference (RNAi).** As Mello, Fire, and other researchers discovered, dsRNA can be bound by an RNA-digesting enzyme called dicer, which cuts dsRNA into 21- to 25-nucleotide-long snippets of RNA molecules referred to as **small interfering RNAs (siRNAs).**

These siRNAs are bound by a protein-RNA complex called the RNA-induced silencing complex (RISC). RISC unwinds the double-stranded siRNAs, releasing single-stranded siRNAs that bind to complementary sequences in mRNA molecules. Binding of siRNAs to mRNA results in degradation of the mRNA (by the enzyme slicer) or blocks translation by interfering with ribosome binding (**Figure 3.18**). RNAi is similar in mechanism to the gene-silencing actions of siRNAs, but a primary difference is that siRNAs are generated from dsRNA (see Chapter 2).

Initially it appeared that the discovery of siRNAs and miRNAs was perhaps a relatively esoteric finding. Predictions now indicate that mammalian cells express thousands of siRNAs or miRNAs, and these may in turn regulate hundreds of genes. Biotechnology and

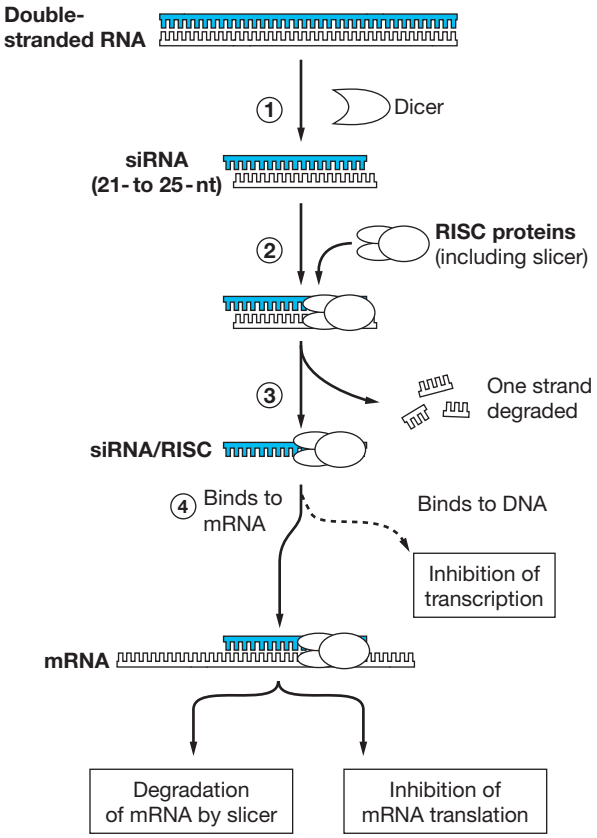


FIGURE 3.18 RNA Interference (1) Double-stranded RNA is cleaved by the enzyme dicer into siRNAs. (2) RISC proteins bind siRNAs and degrade one of the two strands (3) to produce single-stranded siRNAs. (4) Single-stranded siRNAs bind to complementary sequences on mRNA molecules in the cytoplasm and interfere with (silence) gene expression through triggering mRNA degradation by slicer or by inhibiting translation of the mRNA by ribosomes.

pharmaceutical companies are looking for ways in which siRNAs and miRNAs may be exploited for therapeutic purposes. Techniques incorporating RNAi have developed rapidly as methods for regulating gene expression and as a potential way to target and inactivate specific genes with high efficiency. We'll consider examples of how companies are working on RNAi techniques for silencing genes involved in human diseases in Chapter 11. RNAi has become such a rapidly developing technology that recent estimates indicate the use of RNAi reagents will grow from about $400 million in 2005 to over $1.5 billion by 2019. The 2006 Nobel Prize in Physiology or Medicine was awarded to Andrew Fire and Craig Mello for their discovery of this natural method for switching genes off. Nobel Prizes are typically awarded decades after the honored work has been completed, so this unusually quick recognition is a clear indication of the value of RNAi as a powerful and promising research tool.

Genome editing approaches to studying gene function

Genome editing methods allow researchers to create changes in a specific sequence to remove, correct, or replace a defective gene or parts of a gene. Genome editing is based on using different nucleases to create breaks in the genome in a sequence-specific manner. In Chapter 7 we discuss how gene-targeting methods with *zinc finger nucleases (ZFNs)* can be used for the *in vivo* genetic engineering of mice, rats, and other species. In Chapter 7 we will also discuss how **transgenic animals,** animals genetically engineered with one or more genes from different sources, can be created (sometimes also called "knock-in" animals), and gene editing can be applied to produce **knockout animals** in which a specific gene or genes can be disrupted or removed. As you will learn, both transgenic and knockout approaches are often used to understand gene function.

No approach to genome editing has garnered more attention, created more excitement, or generated as many early signs of success as the **CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas system.** In brief, CRISPR-Cas was discovered by scientists trying to understand how bacteria fight viral infection. The original research on this system was not intended to create a gene editing technique. CRISPR is a prokaryotic immune system that provides resistance to foreign genetic elements such as plasmids and phages.

CRISPRs are segments of prokaryotic DNA consisting of short base-pair repeats. Each repeat is followed by a segment of spacer DNA, which comes from prior exposures to foreign plasmids or phage. CRISPR-associated proteins (Cas) function as nucleases. Cas9 was the first nuclease identified. In prokaryotes, CRISPR spacer sequences can recognize viral DNA and use Cas nucleases to digest foreign DNA sequences. By delivering the CRISPR-Cas9 nuclease complexed with a synthetic guide RNA (gRNA) into eukaryotic cells, the genome can be targeted and cut a specific location—thus allowing genes to be removed or new genes inserted in cultured cells or in whole organisms (see **Figure 3.19**). Scientists are also working on CRISPR as a tool for editing RNA, for altering single bases in the genome, and for ways to turn CRISPR on and off to carefully regulate genome editing as needed, among other applications.

Because of its simplicity and relatively low cost, many scientists are turning to CRISPR applications for genome editing *in vitro* and *in vivo* instead of RNAi. Since its first demonstration in 2012, in recent years dozens of scientific papers describing CRISPR gene editing applications are being published each week. In Chapter 11 we discuss recent examples of promising gene therapy applications involving CRISPR-Cas.

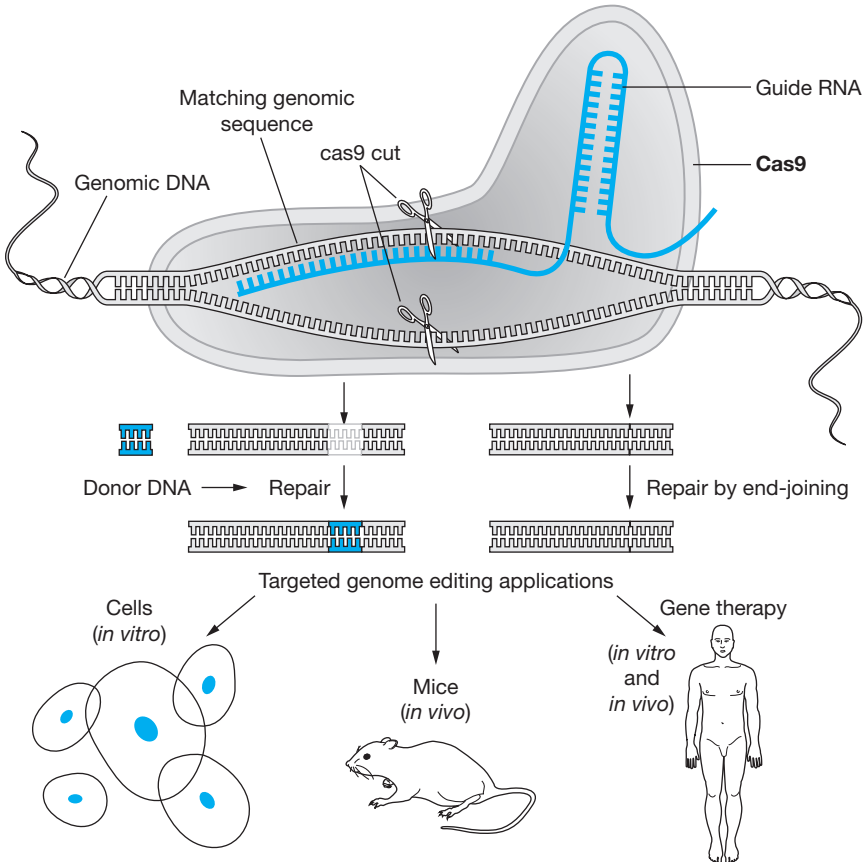


FIGURE 3.19 CRISPR-Cas Applications CRISPR-Cas allows for targeted editing of specific genes. The donor DNA sequence can be almost any sequence that scientists want to use to replace an existing gene, thus enabling gene editing applications for cells *in vitro* and whole organisms *(in vivo)* including humans.

3.4 Genomics and Bioinformatics: The Hottest Disciplines in the History of Biotechnology

The recombinant DNA approaches that we described at the start of this chapter provided the necessary foundation for cloning and analyzing genes. However, during the past two decades, a number of advances in cloning and sequencing technologies have increasingly led to the widespread use of new strategies for cloning, sequencing, and analyzing *entire genomes*—the field of **genomics.** No discipline has had a greater impact on biotechnology than genomics. You will learn about many applications of genomics as you study biotechnology. Here we provide an introduction to selected applications of genomics and bioinformatics, which is intended to form the basis for discussions we will have throughout subsequent chapters.

Whole-Genome Sequencing

As powerful as traditional recombinant DNA techniques are, it became increasingly apparent that if scientists wanted to study complex biological processes that involve many genes—such as most cancers—cloning one or even a few genes at a time was too slow and thus inefficient. As a result, scientists started working on strategies for cloning and sequencing entire genomes—a strategy commonly called **whole-genome sequencing (WGS).** Initially WGS was often referred to as whole-genome "shotgun" cloning. The analogy is that cloning individual genes using libraries is equivalent to using a rifle to hit a specific spot on a target (e.g., cloning a specific gene), whereas the pellets from a shotgun would randomly hit many spots on a target with little precision. In shotgun sequencing, the entire genome, introns and exons, is sequenced. The complete genome sequence is assembled with the help of software programs, and then individual genes are identified through **bioinformatics—**an interdisciplinary field, now a little over 20 years old, that applies computer science and information technology to analyze and help us understand biological data, such as sequence data.

One common WGS approach involves using restriction enzymes to digest pieces of entire chromosomes (**Figure 3.20**). This process can produce thousands of overlapping fragments called **contigs (contiguous sequences).** Each contig is sequenced,

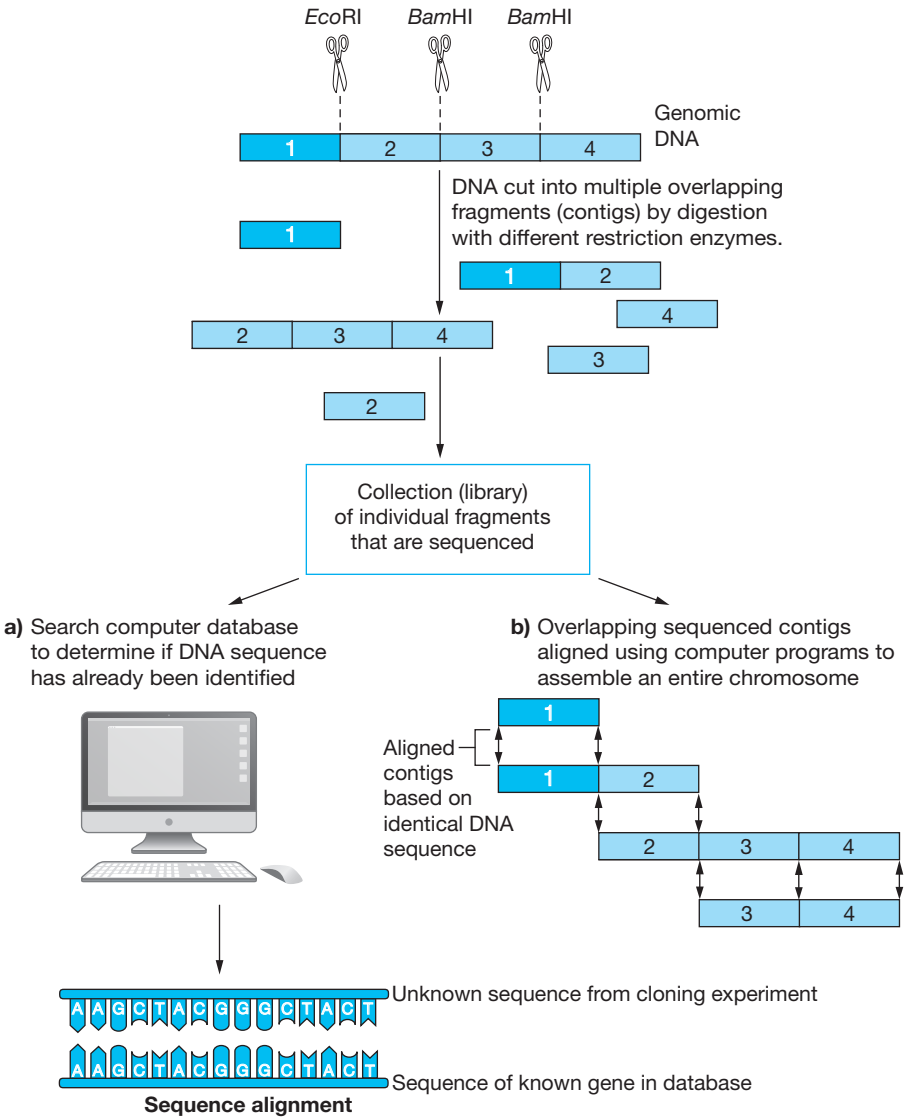


FIGURE 3.20 Whole-Genome Shotgun Sequencing and Two Examples of Bioinformatics Applications Shown is a simplified example of a piece of genomic DNA cut into smaller pieces (labeled 1, 2, 3, and 4) by *Eco*RI and *Bam*HI. Regardless of how a piece of DNA is cloned, DNA sequence analysis is an essential part of learning more about the cloned DNA. (a) Such analysis can begin by searching the unknown sequence against a database of known sequences. In this example, sequence alignment reveals that the unknown sequence is an exact match with a sequence of a "known" gene already in the database. Note: For simplicity, only one strand of sequenced DNA is being compared with the database. (b) Another application of bioinformatics involves the use of computer programs to align DNA fragments (contigs) based on nucleotide sequence overlaps. The scheme shown here is similar to one approach used in the Human Genome Project to assemble completed sequences of entire chromosomes. However, when contigs are compared, typically only relatively short sequences at the end of each contig are aligned to assemble an entire chromosome.

and then computer programs are used to align the fragments based on overlapping sequence pieces (Figure 3.20b). This approach enables scientists to reconstruct the entire sequence of a whole chromosome and, as you can tell from the figure, software to organize and compare the DNA sequences of these fragments is essential. This is one example of bioinformatics in action.

In 1995, scientists at The Institute for Genomic Research were the first to successfully use WGS to sequence an entire genome for any organism when they sequenced the 1.8-million-bp genome of the bacterium *Haemophilus influenzae*. Many doubted that WGS strategies could be used effectively to sequence larger genomes, but development of this technique and novel sequencing strategies rapidly accelerated the progress of the Human Genome Project.

Bioinformatics: Merging Molecular Biology with Computing Technology

When scientists sequence a newly identified gene or DNA sequence, they report their findings in scientific publications and submit the sequence data to databases so that other scientists who may be interested in this sequence information can have access to it. Database manipulations of DNA sequence data were among the first applications of bioinformatics, which involves the use of computer hardware and software to study, organize, share, and analyze data related to gene structure, gene sequence and expression, and protein structure and function.

As genome data have rapidly accumulated, resulting in an enormous amount of information being stored in public and private databases, bioinformatics

has become an essential tool that allows scientists to share and compare data, especially DNA sequence data. We discuss basic applications of bioinformatics throughout this book; however, we introduce the field here with a few very common applications.

Examples of Bioinformatics in Action

Even before WGS projects began, scientists were accumulating sequence information from a variety of different organisms, necessitating the development of sophisticated databases that could be used by researchers around the world to store, share, and obtain the maximum amount of information from protein and DNA sequences. Databases are essential tools for archiving and sharing data with researchers and the public. Because computer-automated DNA sequencing techniques were developed nearly simultaneously with expansion of the Internet as an information tool, many DNA sequence databases became available through the Internet.

When scientists who have cloned a gene enter their sequence data into a database, these databases search the new sequence against all other sequences in the database and create an *alignment* of similar nucleotide sequences if a match is found (Figure 3.20a). This type of search is often one of the first steps taken after a gene is cloned using recombinant DNA techniques because it is important to determine if the sequence has already been cloned and studied or if the gene sequence is a novel one. Among other applications, these databases can also be used to predict the sequence of amino acids encoded by a nucleotide sequence and to provide information on the function of the cloned gene.

The largest publicly available DNA sequence databases worldwide is called GenBank. It is widely used and contains the National Institutes of Health (NIH) collection of DNA sequences. GenBank shares and acquires data from databases in Japan (DNA Data Bank of Japan) and Europe (the European Nucleotide Archive). Maintained by the National Center for Biotechnology Information (NCBI), GenBank contains more than 225 billion bases of sequence data from over 100,000 species, and it doubles in size roughly every 18 months. The NCBI has designed user-friendly ways to access and analyze data on protein sequences, molecular structures, genome data, and even scientific literature. Recently, GenBank along with DDBJ and the ENA launched the International Nucleotide Sequence Database Collaboration initiative to better capture, preserve, and provide nucleotide sequence information.

An NCBI feature called **Basic Local Alignment Search Tool (BLAST; www.ncbi.nlm.nih.gov/BLAST)** can be used to search GenBank for sequence matches between cloned genes and to create DNA sequence alignments. See Questions & Activities Problem 11 to carry out a simple BLAST search. **Figure 3.21** shows a BLAST search alignment comparing the human and mouse gene sequences for an obesity gene (*Lep*) that produces a hormone called *leptin*. Leptin plays a role in fat metabolism, and mutations in the leptin gene can contribute to obesity (see also Figure 11.1). Notice that the nucleotide sequence for these two genes is very similar, as indicated by the vertical lines between identical nucleotides.

The Human Genome Nomenclature Committee, supported by the NIH, establishes rules for assigning names and symbols to newly cloned human genes. You should recognize the convention of naming genes. A variety of different guidelines affect the number of characters, use of numbers, and so forth, when naming genes. Gene names are italicized and given symbols or short abbreviated names. For human genes, the symbol is italicized and all letters are in uppercase. For rats and mice, the symbol is italicized, but only the first letter is in uppercase and the other letters are in lowercase. For instance, in Figure 3.21, notice that the symbol for the human leptin gene is *Lep*, but the symbol for the corresponding mouse gene is *Lep*. To avoid

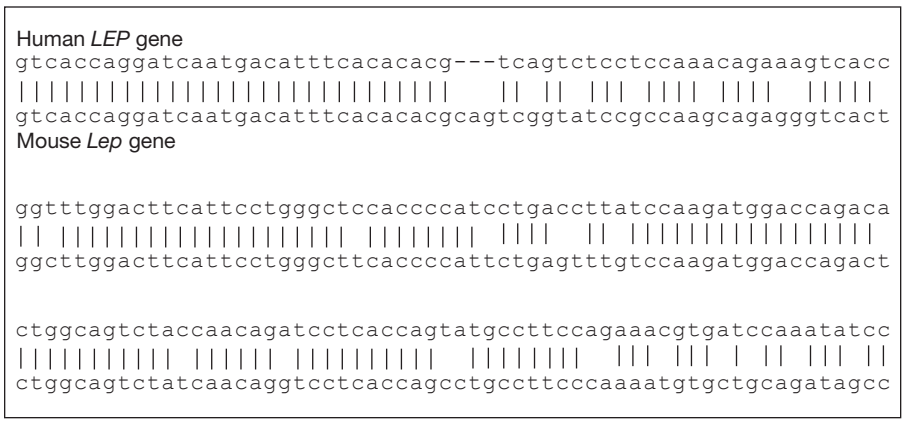


FIGURE 3.21 Comparison of the Human *LEP* and Mouse *Lep* Genes
Partial sequences for these two genes are shown, with the human gene sequence on top and the mouse gene sequence below it.

confusing gene symbols and protein symbols, proteins are typically noted in capital letters without italics. Thus the leptin protein is abbreviated as LEP.

Each entry into GenBank is provided with an **accession number** that scientists can use to refer back to that cloned sequence. For example, go to the GenBank website listed at the Companion Website and then type in the accession number U14680 and click "GO." What gene is identified by this accession number? Click on the accession number link. Notice that GenBank provides the original journal reference that reported this sequence, the single-letter amino acid code of the protein encoded by this gene, and the nucleotide sequence of this gene. Alternatively, you can type in the name of a gene or a potential gene that you are interested in to see if it has already been cloned and submitted to GenBank.*

GenBank is only one example of an invaluable database that is essential for bioinformatics. Many other specialized databases exist with information such as single-nucleotide polymorphism data, as well as protein databases that catalog amino acid sequences and three-dimensional protein structures. Rapid advances in computer science have allowed bioinformatics to develop quickly. Similarly, as sequencing technologies improve, the number of genomes being sequenced is also increasing at extraordinary rates, with some predictions estimating that the number of genomes sequenced may double every 12 months. This explosion of genome data, one example of an increase in data for science and medicine that is often referred to as **"big data,"** is challenging scientists to keep up with an abundance of DNA sequence data that can require up to several terabytes of storage space. Simply having enough hard drive and server storage space for sequence data can be a significant expense for some labs.

A Genome Cloning Effort of Epic Proportion: The Human Genome Project

It is a very exciting time to be studying genomics. The completion of an unprecedented project that involved many of the techniques described in this chapter, the **Human Genome Project (HGP),** led to the creation of new disciplines having extraordinary impacts on the progress of biotechnology. Initiated in 1990 by the U.S. Department of Energy (DOE), the HGP was an international collaborative effort with a 15-year plan to identify all human genes, originally estimated at 80,000 to 100,000 in number, and to sequence the approximately 3 billion base pairs thought to make up the 24 different human chromosomes (chromosomes 1 to 22, X, Y). The HGP was also designed to accomplish the following:

- Analyze genetic variations among humans.
- Map and sequence the genomes of **model organisms,** including bacteria, yeast, roundworms, fruit flies, mice, and others.
- Develop new laboratory technologies such as high-powered automated sequencers and computing technologies, as well as widely available databases of genome information, which can be used to advance our analysis and understanding of gene structure and function.
- Disseminate genome information among scientists and the general public.
- Consider the ethical, legal, and social issues that accompany the HGP and genetic research.

In the United States, public research on the HGP was coordinated by the National Center of Human Genome Research, a division of the NIH and the DOE. The project funded seven major sequencing centers in the United States. For example, the DOE Joint Genome Institute (JGI) in California was created to integrate genome data and research expertise from several different DOE genome centers. Specifically, JGI generated complete sequences of chromosomes 5, 16, and 19. Over time, the project grew to become an international effort with contributions from scientists in 18 countries. The work was primarily carried out by the International Human Genome Sequence Consortium, which involved nearly 3,000 scientists working at 20 centers in six countries: China, France, Germany, Great Britain, Japan, and the United States.

The estimated budget for completing the genome was $3 billion, a cost of $1 per nucleotide. Driven in part by competition from private companies and the development of computer-automated DNA sequences (such as the example in Figure 3.10) and bioinformatics, the HGP turned out to be a rare government project that completed all of its initial goals and several additional goals more than 2 years ahead of schedule—and under budget.

The most aggressive competitor on the project was a private company called Celera Genomics (aptly named from a Latin word meaning "swiftness") directed by Dr. J. Craig Venter, who was the scientist directing The Institute for Genomic Research when it used whole-genome "shotgun" cloning to sequence the genome for *H. influenzae*, as discussed earlier in this section. Celera announced its intention to use its novel shotgun sequencing approach, as well as newly

developed, high-throughput computer-automated DNA sequencers, to sequence the entire human genome in 3 years. Fearful of how private corporations might control the release of genome information, U.S. government agencies involved in the HGP were effectively forced to keep pace with private groups to stay competitive in the race to complete the genome.

In 1998, as a result of accelerated progress, a revised target date of 2003 was set for completion of the project. On June 26, 2000, leaders of the HGP and Celera Genomics participated in a press conference with President Bill Clinton to announce that a rough "working draft" of approximately 95 percent of the human genome had been assembled (nearly 4 years ahead of the initially projected timetable). At a joint press conference on February 12, 2001, Dr. Francis Collins, director of the NIH National Human Genome Research Institute and of the HGP, and Dr. J. Craig Venter of Celera announced that a series of papers describing the initial analysis of the genome working draft sequence were published by their research groups in the prestigious journals *Nature* and *Science*, respectively. Scientists spent the next 2 years working to fill in thousands of gaps in the genome by completing the sequencing of pieces not yet finished, correcting misaligned pieces, and comparing sequences to ensure the accuracy of the genome. On April 14, 2003, the International Human Genome Sequencing Consortium announced that its work was done. A *reference sequence* of the human genome was essentially complete, with virtually all bases identified and placed in their proper order and potential genes assigned to a chromosome (see **Figure 3.22**). This reference sequence represents a baseline of major elements of the human genome, but our knowledge of the human genome and its organization, functions, and reasons for individual variations continues to expand each year since the genome was completed.

What Have We Learned from the Human Genome?

One of the most surprising findings of the project was that the human genome consists of approximately 20,000 protein-coding genes, not 100,000 genes, as predicted. The prediction was based primarily on estimates that human cells make approximately 100,000 to 150,000 proteins. One reason why the actual number of genes is so much lower than the predicted number is the discovery of large numbers of gene families with related functions. In addition, many genes that code for multiple proteins through alternative splicing have been found. It has been estimated approximately 95 percent of human genes produce multiple proteins through alternative splicing. Incidentally, the total number of proteins present in human cells is now thought to be anywhere from approximately 200,000 to 1 million.

Analysis of human genes by functional categories has provided genome scientists with a snapshot of the numbers of genes involved in different molecular functions. Not surprisingly, many genes encode enzymes, whereas other large categories of genes encode proteins involved in signaling and communication within and between cells and DNA- and RNA-binding proteins. But even today, approximately 40 percent of human genes have no clearly known function. Keep this in mind if you are interested in a career in genetics research, because efforts to discover what these genes do will provide exciting career opportunities for many years into the future. Another excellent resource on the Internet is the PANTHER (Protein

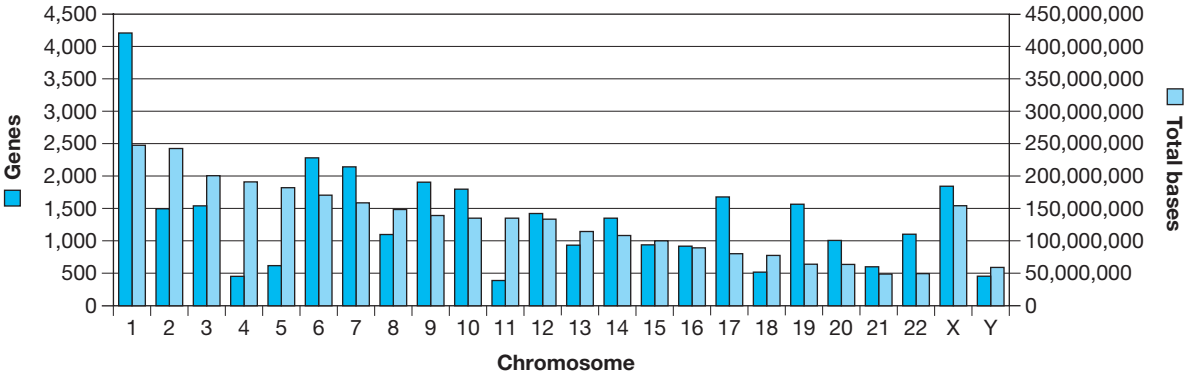


FIGURE 3.22 Approximate Number of Genes on Each Chromosome and Approximate Size in Base Pairs (bp) of Each Human Chromosome Figure-compiling statistics for human chromosomes are based on the Sanger Institute's human genome information in the Vertebrate Genome Annotation (VEGA) database. Number of genes is an estimate, as it is in part based on gene predictions, which is why this value is higher than the estimated 20,000 genes generally accepted as the number of human genes.

ANalysis THrough Evolutionary Relationships) database. This provides functional classifications of genes and encoded proteins. Refer to the Case Study for an exercise that will take you to a pie chart view of current functional categories for human genes.

Here is a summary of some of the basic concepts scientists have learned from the HGP:

- The human genome consists of approximately 3.1 billion base pairs.
- The genome is approximately 99.9 percent the same between individuals of all nationalities. Single-nucleotide polymorphisms (SNPs) and **copy number variations (CNVs)—**such as long deletions, insertions, and duplications in the genome—account for much of the genome diversity identified between humans.
- Approximately 2 percent of the genome codes for proteins. The genome contains about 20,000 protein-coding genes. The vast majority of our DNA is non-protein-coding, and repetitive DNA sequences account for at least 50 percent of the non-coding DNA. Many human genes are capable of making more than one protein, allowing human cells to make at least 100,000 proteins from only about 20,000 genes.
- The functions of at least 40 percent of human genes are still unknown.
- Chromosome 1 contains the highest number of genes. The Y chromosome contains the fewest genes.
- Many of the genes in the human genome show a high degree of sequence similarity to genes in other organisms.
- Thousands of human disease genes have been identified and mapped to their chromosomal locations.

How have we and how will we continue to benefit from the HGP? The complete sequence of the human genome has been described as the genetic "blueprint" of humanity, containing the scientific keys to understanding our biology and behaviors. Identifying all human genes was not as important as understanding *what* these genes do and *how* they function. We will not fully understand the function of all human genes for many years, if ever. One immediate impact of the HGP on biotechnology has been the identification of genes associated with human genetic diseases, and the subsequent development of new treatment strategies and cures. We will discuss some of the most effective ways the HGP has changed diagnosis and treatment of genetic diseases in Chapter 11. For updated information on the HGP and for an outstanding overview of goals, sequencing and mapping technologies, and the ethical, legal, and social issues of the HGP, visit the human genome sites listed at the Companion Website.

Accessing Human Genome Information via the Internet

It is relatively easy to access databases and other sites on the Internet that display maps for all human chromosomes. **Figure 3.23** displays a partial gene map for chromosome 12 that was taken from an NCBI database called Map Viewer. The first image shows an ideogram, or cytogenetic map, of chromosome 12. To the right of the ideogram is a column showing the contigs (arranged lying vertically) that were aligned to sequence this chromosome. The UniGene clusters column displays a histogram representation of gene density on chromosome 12. Notice that relatively few genes are located near the centromere. Finally, gene symbols, loci, and gene names (by description) are provided for selected genes; in this figure only 20 genes are shown. When accessing these maps on the Internet, one can magnify, or zoom in on, each region of the chromosome, revealing all genes mapped to a particular area.

You can see that most of the genes listed here have been assigned descriptions based on the functions of their products, some of which are transmembrane proteins; some enzymes such as kinases; some receptors, including several involved in olfaction; and so on. When viewing maps such as these, sometimes genes are described in terms of hypothetical products; they are presumed to be genes based on their sequence, but their function remains unknown. Increasingly, as we learn more about the functions of all human genes, far fewer hypothetical genes are noted on these maps than when the HGP was first completed.

The Human Genome Project Started an "Omics" Revolution

The HGP and genomics are largely responsible for ushering in a new area of biological research—the "omics." It seems that every year, new or existing areas of biological research are being described as having an omics connection. For example:

- Glycomics—studying the carbohydrates of a cell
- Metabolomics—studying proteins and enzymatic pathways involved in cell metabolism
- Metagenomics—the analysis of genomes of organisms collected from the environment
- Pharmacogenomics—customized medicine based on a person's genetic profile for a particular condition

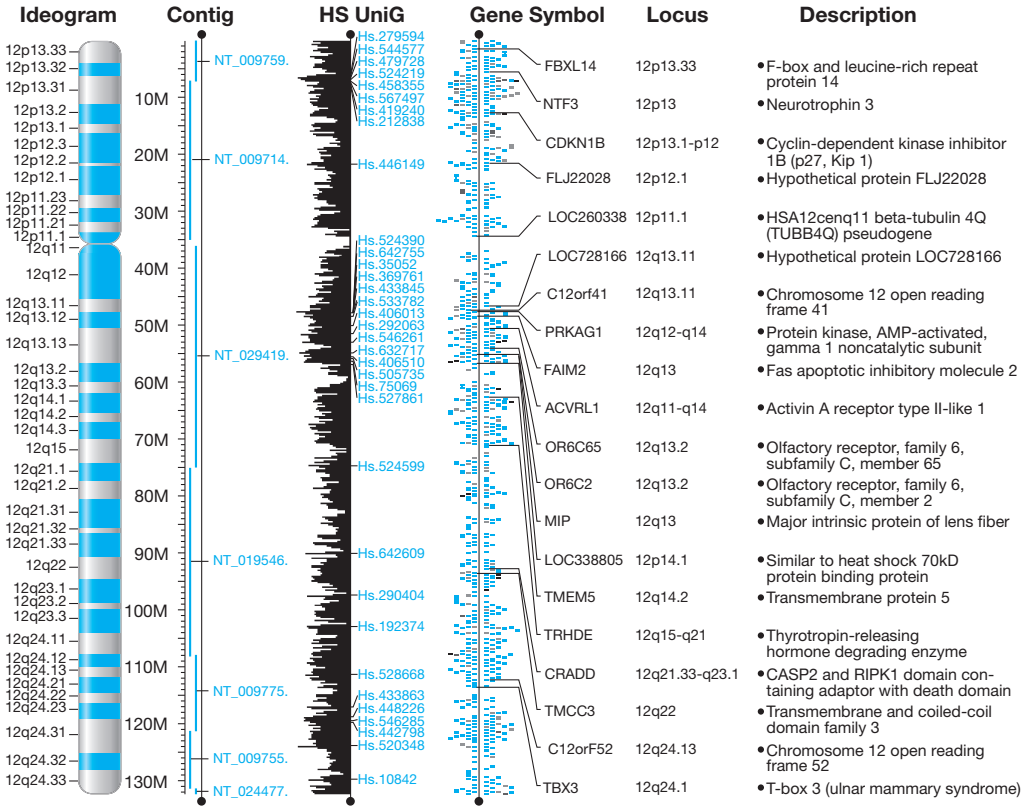


FIGURE 3.23 A Gene Map for Human Chromosome 12 from the NCBI Database Map Viewer

- Proteomics—studying all of the proteins in a cell
- Toxicogenomics—the analysis of the effects of toxic chemicals on genes, including mutations created by toxins and changes in gene expression caused by toxins
- Transcriptomics—studying all genes expressed (transcription) in a cell
- Vaccinomics-creating specific vaccines based on an individual's genetic profile

If any of the "omics" areas defined here sound of interest to you, do some Internet research on these topics and you will find vast amounts of information about each area. We will touch on many of these omics areas throughout the book. As further evidence of the impact of genomics, a field of nutritional science, called **nutrigenomics,** has emerged. Nutrigenomics is focused on understanding interactions between diet and genes. Several companies offer services employing microarrays or other genetic tests to analyze your genotype for genes thought to be associated with different medical conditions or aspects of nutrient metabolism. These companies then provide a customized report on nutrition with claims that they can suggest dietary changes you should make to improve your health and prevent illness based on your genes. Whether nutrigenomics is actually a valid scientific approach is a subject of debate among scientists because currently there are very few genes clearly correlated to nutrition for which treatment is known to have a scientifically or medically proven benefit.

Comparative genomics

It might surprise you that in addition to studying the human genome, the HGP involved mapping and sequencing genomes from a number of model organisms, including *E. coli*, a model plant called *Arabidopsis thaliana*, the yeast *Saccharomyces cerevisiae*, the fruit fly *Drosophila melanogaster*, the nematode roundworm *Caenorhabditis elegans*, and the mouse *Mus musculus*, among other species. Complete genome sequences of these model organisms have been incredibly useful for **comparative genomics** studies, which allow researchers to study gene structure and function in these organisms in ways designed to understand gene structure and function in other species, including humans. Because we share many of the same genes as flies, roundworms, and mice, such studies will also lead to a greater understanding of human evolution. The number of genes we share with other species is very high, ranging from about 30 percent of the genes in yeast to about 80 percent of the genes in mice and about 95 percent of the genes in chimpanzees (Table 3.3).

Comparative genomic analysis of the genome for "man's best friend" has revealed that we share about 75



YOU DECIDE To Patent or Not?

Craig Venter and colleagues were the first scientists to completely sequence the genome of a living organism, the bacterium *Haemophilus influenzae*. This group applied for patents on the nucleotide sequence for *H. influenzae* and on the bioinformatics technology used to analyze this genome.

Previously, Venter and his colleagues described a set of experiments in which they randomly cloned short pieces of cDNAs from human brain cells. These short sequences—called **expressed sequence tags (ESTs)**—could in theory be used as probes to identify full-length cDNAs. Some of Venter's ESTs were found to be identical to genes that had already been cloned or to a portion of a gene; others appeared to be novel gene sequences or junk DNA. Hoping to gain proprietary rights to full-length genes that could be identified from Venter's ESTs, the Institute for Genomic Research applied for a patent. At the time this request generated a great deal of controversy. It is estimated that the U.S. Patent and Trademark Office has granted patents on more than 35,000 genes or gene sequences, and an estimated 20 percent of human genes. Incidentally, the patenting of human genes has led some to use the term *patentome*. Examples of questions raised by patenting DNA include:

- Should scientists be allowed to patent DNA sequences from naturally living organisms?
- What if a patent is awarded for only small *pieces of* a gene—even if no one knows what a DNA sequence does—just because a company or some individuals want a patent to stake their claim to having cloned a piece of DNA first?
- What if there are no clear uses for the DNA sequences cloned?
- Can or should investigators who sequence a complete genome be allowed to patent the entire genome of any organism?
- When they are granted a patent, scientists essentially have protection on use of patented information for two decades from the patent filing date. Could or should a group stake a claim to a gene, thereby preventing others from working on it or developing a product from it? Many believe that the hoarding of genome information is against the tradition of sharing information to advance science.
- Should a genetically engineered living organism be patented? Engineered bacteria (for example, those used to clean up environmental pollution) and transgenic animals have been patented.
- Can a group claim rights to anticipated future uses of the gene even if there are no data to substantiate such claims? What about individuals who figure out *what to do* with the gene? What rights do they have to patent or protect their discovery?
- What if a gene sequence is involved in a disease for which a genetic therapy may be developed?

In recent years, the Supreme Court of the United States has ruled on cases related to patenting of human genes and any sequences, functions, or correlations to naturally occurring products from a gene. Genes in their natural state as products of nature cannot be patented but applications (such as genetic testing) and potential applications of genes and DNA sequences can be patented. The High Court of Australia also ruled that naturally occurring genes are not patentable. However, human DNA sequences have still remained patentable in European countries under the European Patent Convention. Even patenting of genetic tests is also under increased scrutiny in part because of concerns that a patented test can create monopolies in which patients cannot get a second opinion if only one company holds the rights to conduct a particular genetic test. This issue has been raised with the most common genetic test for breast cancer.

Recent analysis has estimated that as many as 64 percent of patented tests for disease genes make it very difficult or impossible for other groups to propose a different way to test for the same disease. From a commercial standpoint, one advantage of patenting is that it provides private companies with a financial incentive to get a medicine or technology to the marketplace and enables them to make a profit after many millions (or billions in some cases) of dollars are spent on research and development (R&D) to make a product. To patent or not? You decide.

percent of our genes with dogs. Human DNA even contains around 100 genes that are also present in many bacteria. When researchers completed the 814-million-bp genome of the sea urchin (*Strongylocentrus purpuratus*), an invertebrate that has served as an important model organism particularly for developmental biologists, we learned that of the 23,500 genes in the urchin genome, many are genes with important functions in humans.

The genomes of many model organisms have been completely sequenced, and hundreds of other

TABLE 3.3 Comparison of Selected Genomes

Organism (scientific name)	Approximate Size of Genome (date completed)	Number of Genes	Approximate Percentage of Genes Shared with Humans	Web Access to Genome Databases
Bacterium (*Escherichia coli*)	4.1 million bp (1997)	4,403	Not determined	www.genome.wisc.edu/
Chicken (*Gallus gallus*)	1 billion bp (2004)	~20,000–23,000	60%	https://useast.ensembl.org/Gallus_gallus/Info/Index
Dog (*Canis familiaris*)	2.5 billion bp (2003)	~18,400	75%	https://www.ncbi.nlm.nih.gov/genome?term=canis%20lupus%20familiaris
Chimpanzee (*Pan troglodytes*)	~3 billion bp (initial draft, 2005)	~20,000–24,000	96%	http://www.nature.com/nature/focus/chimpgenome/index.html
Fruit fly (*Drosophila melanogaster*)	165 million bp (2000)	~13,600	50%	www.fruitfly.org
Humans (*Homo sapiens*)	~3.1 billion bp (2004)	~20,000–25,000	100%	www.doegenomes.org
Mouse (*Mus musculus*)	~2.5 billion bp (2002)	~30,000	~80%	www.informatics.jax.org
Plants Thale cress (*Arabidopsis thaliana* is a model organism widely used by plant geneticists)	119 million bp (2000)	~26,000	Not determined	www.arabidopsis.org
Rice (*Oryza sativa*)	389 million bp (2005)	~41,000	Not determined	http://www.plantgdb.org/OsGDB/
Rat (*Rattus norvegicus*)	~2.75 billion bp (2004)	~22,000	80%	www.hgsc.bcm.tmc.edu/projects/rat
Roundworm (*Caenorhabditis elegans*)	97 million bp (1998)	19,099	40%	http://www.wormbase.org
Yeast (*Saccharomyces cerevisiae*)	12 million bp (1996)	~5,700	30%	https://www.yeastgenome.org

genomics projects have been completed with hundreds of others under way worldwide. Other recent headline-grabbing genomes that have been completed include:

- The apple (more than 57,000 genes) and the tomato (31,760 genes) have many more genes than humans!
- The potato, a plant that shares 92 percent of its DNA with tomatoes, turns out to be a fruit.
- The chickpea is one of the earliest cultivated legumes and the second most widely grown legume after the soybean.
- The red-spotted newt has a genome almost 10 times the size of a human's.
- The first genome for a tree, the black cottonwood (a type of poplar), was sequenced several years ago. At the time, the poplar's 45,555 genes were the highest number found in a genome. Scientists anticipate using data from this project to help the forestry industry make better products, including biofuels or even genetically engineered poplars, to capture high levels of carbon dioxide from the atmosphere.
- The honeybee genome has been completed, and information from this project will be used to

understand bee genetics and behavior to help the honey-producing industry as well as advance an understanding of how bee toxins produce allergic responses.

Genome scientists around the world are working on a project to sequence 10,000 vertebrate genomes, the **Genome 10K plan.** This ambitious plan proposes to assemble 10,000 genomes in 5 years—about one genome a day. A similar project is under way to sequence about 10,000 invertebrate genomes. Scientists in China and the U.S. have proposed the Earth BioGenome Project (EBP), an effort to sequence move than 1.5 million eukaryotic genomes. In addition to its own genome sequencing research, the EBP would incorporate data from the Genome 10K plan and many others.

Throughout other chapters of this book we will discuss genomics projects and you will learn about many applications of genomics. For example, in Chapter 5 we discuss microbial genome sequencing projects that are sequencing genomes for literally hundreds of newly identified microbes, including microbes that live in and on humans (the **Human Microbiome Project**) and microbes present in water, soil, and air samples from around the world (**metagenomics**). In Section 3.5 and in Chapter 5, we will also discuss **synthetic genome** approaches designed to create novel life forms from artificially constructed genomes.

Stone age genomics

As yet another intriguing example of genomics, a number of labs around the world are involved in analyzing "ancient" DNA. These studies are generating fascinating data from minuscule amounts of ancient DNA from bone and other tissues and fossil samples that are tens of thousands of years old. Analysis of DNA from more than 90 Egyptian mummies, mammoths, platypuses, Pleistocene-age cave bears, ancestral horse species (one sequence from more than 700,000 years ago is the oldest completed sequence to date), and Neanderthals are some of the most prominent examples of **stone age genomics,** also called **paleogenomics.**

About a decade ago researchers published partial sequence data (about 13 million bp) from a 27,000-year-old woolly mammoth found frozen and nearly intact in Siberia. This study showed that there is approximately 98.5 percent sequence identity between mammoths and African elephants. Subsequent work by other scientists has used whole-genome sequencing of mitochondrial and nuclear DNA from Siberian mammoths to provide data on the mammoth genome. These studies suggest that the mammoth genome differs from that of the African elephant by as little as 0.6 percent. These studies are a great demonstration of how stable DNA can be under the right conditions, particularly when frozen. Also intriguing are similarities that have been revealed between the mammoth and human genomes. When human chromosomes were aligned with the mammoth genome, approximately 50 percent of mammoth genes show sequence alignment with human genes on autosomes.

Svante Pääbo at the Max Planck Institute for Evolutionary Anthropology in Germany and colleagues have been leaders in creating drafts of the Neanderthal (*Homo neanderthalensis*) genome encompassing more than 3 billion bp of Neanderthal DNA and about two-thirds of the genome. In 2006, Pääbo's group, along with a number of scientists in the United States, reported the first sequence of about 65,000 bp of nuclear DNA isolated from bone of a 38,000-year-old Neanderthal sample from Croatia. A sequence recently recovered from calcified remains of a Neanderthal that lived over 200,000 years ago is thought to be the oldest Neanderthal DNA ever analyzed. Because Neanderthals are close relatives of humans, the sequencing of the Neanderthal genome provides a tremendous opportunity to use comparative genomics to advance our understanding of evolutionary relationships between humans.

The human and Neanderthal genomes are 99 percent identical. Some of these sequences are involved in cognitive development and sperm motility. Of the many genes shared by these species, *FOXP2* is a gene that has been linked to speech and language ability. Many genes influence speech, so this finding does not mean that Neanderthals spoke as we do. But because Neanderthal had the same modern human *FOXP2* gene, scientists have speculated that Neanderthals possessed linguistic abilities. The realization that modern humans and Neanderthals lived in overlapping ranges as recently as 30,000 years ago has led to speculation about the interactions between modern humans and Neanderthals. Genome studies suggest that there was interbreeding between Neanderthals and modern humans an estimated 45,000 to 80,000 years ago in the eastern Mediterranean. These exciting studies, previously thought to be impossible, are having ramifications in many areas of human evolution, and it will be interesting indeed to follow the progress of this work.

After the HGP: What Is Next?

In the 15 years since completion of a draft sequence of the human genome, studies on the human genome continue at a very rapid pace. As a result of the HGP, many other major theme areas for human genome research have emerged, including analysis of the epigenome (the **Human Epigenome Project,** which is creating hundreds of maps of epigenetic changes in different cell and tissue types and evaluating potential

roles of epigenetics in complex diseases), as well as characterization of SNPs (the **International HapMap Project**) and CNVs for their role in genome variation, disease, and pharmacogenomics applications.

Here we discuss several major human genome-related research project areas: the ENCODE Project, personal genomics, and cancer genome projects.

Encyclopedia of DNA Elements (ENCODE) Project

As the HGP was being completed, several hundred researchers in about three dozen labs around the world began the **Encyclopedia of DNA Elements (ENCODE) Project.** The main goal of ENCODE is to use both experimental approaches and bioinformatics to identify and analyze functional elements (such as transcriptional start sites, promoters, and enhancers) that regulate expression of human genes. ENCODE projects have also been initiated for mouse, worm, and fly genomes.

Because less than 2 percent of the human genome codes for proteins, ENCODE focuses not on protein-coding genes but on all the rest of the sequences, commonly referred to as "junk" DNA in the past. So what are all these other bases in the genome doing? The term *junk DNA* has always been a misnomer. We know that such sequences are important for chromosome structure, the regulation of gene expression, and other roles. Just because these sequences themselves do not code for protein does not mean that they are unimportant. ENCODE studied gene expression in 147 different cell types because genome activity differs from cell to cell. After about a decade of research and a cost of $288 million, in 2012 a group of 30 research papers were published that revealed the major initial findings of the ENCODE project. Selected highlights revealed in those initial papers and recent highlights include the following:

- The *majority*, approximately 80 percent, of the human genome is considered functional. This is partly because large segments of the genome are transcribed into RNA. Most of these RNAs do not encode proteins. These various RNAs include tRNA, rRNAs, and miRNAs, and long non-coding RNAs (lncRNAs)—defined as non-protein-coding transcripts longer than 200 nucleotides. A conservative estimate is that there may be over 17,000 genes for lncRNAs. It may turn out that the number of non-coding RNA sequences will outnumber protein-coding genes.
- The functional sequences also include gene-regulatory regions: approximately 70,000 promoter regions and nearly 400,000 enhancer regions.
- There are 20,687 protein-coding genes in the human genome.
- SNPs associated with disease are enriched within non-coding regions of the genome, often residing near protein-coding genes.

The ENCODE findings have broadly defined the functional roles of the genome to include encoding proteins or non-coding RNAs and displaying biochemical properties such as binding regulatory proteins that influence transcription or chromatin structure. It is worth noting, however, that a relatively large body of geneticists and other scientists do not agree with ENCODE's definition of functional sequences. But research teams are using information from ENCODE to identify risk factors for certain diseases, with the hope of developing appropriate cures and treatments.

Personal genomics

Because of rapid advances in sequencing technologies, many companies proposed to sequence whole genomes for individual people, or **personal genomics.** There were several competitions in which significant prize money was offered to groups that could develop technologies capable of sequencing individual genomes for less than $10,000 per genome. Then quickly the target became a personal genome for a mere $1,000! Several companies have now developed third-generation sequencing technologies to sequence a complete human genome for less than $1,000. Some companies have set their sights on sequencing an entire genome for less than $100. Whether the $1,000 mark represents the costs of reagents to sequence a genome or actual costs when sequence preparation, labor, and analysis of the genome are taken into account can be debated.

Regardless of how the actual cost of sequencing a genome is calculated, the modern cost is substantially lower than the $3 billion required for the HGP. Personal genomics may eventually be affordable enough for individuals to acquire a readout of their own genetic blueprint as part of routine medical care.

A number of larger-scale personal genomics projects are in progress around the world. For example, a project is under way to sequence whole genomes for centenarians (people aged 100 or over). The thought here is that DNA from older humans may reveal important clues about longevity that could one day help more people live longer, disease-free. As another example, in 2012, the United Kingdom launched the **100,000 Genomes Project.** This initiative aims to sequence whole genomes from individuals with certain rare disorders and different infectious diseases (50,000 people) and common cancers (25,000 people who will have their DNA sequenced twice, once from normal cells and once from tumor cells). The goal is that genome data with medical data can be used to improve understanding of the causes of these disease conditions, eventually resulting in the development of more effective treatments or cures.



YOU DECIDE **To Sequence or Not to Sequence?**

As of early 2017 (the last date for which data was available before *Introduction to Biotechnology* published), more than 500,000 individual human genomes had been sequenced (compared to only about a dozen in early 2010). Would you want your genome sequenced? What would you pay for your genome sequence? What would you do with this sequence information? Who would have access to your sequence information? Who should have access to your sequence information? You decide.

Because such a small portion of the genome consists of protein-coding genes, personal genomic analysis is shifted toward **whole-exome sequencing (WES),** that is, sequencing only the approximately 180,000 exons (protein-coding sequences) in a person's genome. WES reveals mutations involved in disease because there are more disease-related genetic variations in the exome than in other regions of the genome. WES can be done at a cost of much less than $1,000. Of course, a limitation of this approach is its failure to identify mutations in gene-regulatory regions that influence gene expression. As the cost of WGS continues to drop, scientists and clinicians trying to detect disease-causing mutations are debating whether it makes sense to simply sequence the entire genome or to just sequence exomes first to find mutations.

Finally, sequencing technology has now advanced to where we have the ability to sequence the genome from a *single cell*! **Single-cell sequencing (SCS)** typically involves isolating genomic DNA from a single cell that is then subjected to *whole-genome amplification (WGA)* by PCR to produce sufficient DNA to be sequenced. Amplification of the genome to produce enough DNA for sequencing without introducing errors remains a major challenge that researchers are working on so that SCS can become a more reliable and accurate technique for genetic testing. Genomic sequencing from single cells is valuable for analyzing both *somatic cell mutations*—for example, mutations that arise in somatic cells, such as in a skin cancer, which are not heritable—and *germline mutations*, which are heritable mutations transmitted to offspring via gametes.

So, as you can see, genomics has advanced quite a bit since the HGP. We return to our discussion of personal genomics and related topics in Chapter 11 when we consider applications of individual genome data for genetic testing and controversies associated with these applications.

Cancer genome projects

Cancer as a disease has many genetic components. In 2006, the NIH launched a cancer genome project called the **Cancer Genome Atlas Project (TCGA)** to map important genes and genetic changes involved in cancer. The TCGA project sequenced genomes from nearly three dozen types of cancers to date and concluded in 2017. TCGA is just one example of many major projects worldwide designed to identify genetic changes in cancer cells. Ultimately, the identification of key genes involved in tumor formation and metastasis (spreading) will lead to improved diagnostic techniques for the detection of cancer and more effective treatments for curing cancer. This is already happening for many cancers, as we will discuss in Chapter 11.

3.5 Systems Biology and Synthetic Biology

We conclude this chapter by briefly introducing **systems biology** and **synthetic biology,** two rapidly emerging disciplines that incorporate data from genomics, transcriptomics, proteomics, and other areas of biology, as well as engineering applications and other problem-solving approaches.

Systems biology involves interpreting genomic information in the context of the structure, function, and regulation of biological pathways. Biological systems are very complex. By studying relationships between all components in an organism, biologists are trying to build a "systems"-level understanding of how organisms function. Systems biologists typically combine recently acquired genomics and proteomics data with traditional studies of gene and protein structure and function, along with modeling data. Much of this information is retrieved from databases such as PubMed, GenBank, and other newly emerging genomics, transcriptomics, and proteomics resources. Systems models are used to diagram interactions within a cell or an entire organism, such as protein-protein interactions, protein–nucleic acid interactions, and protein-metabolite interactions (e.g., enzyme-substrate binding).

These models help biologists understand the components of interacting pathways and the interrelationships of molecules in an interacting pathway. In recent years,

the term **interactome** has arisen to describe the interacting components of a cell. Systems biologists use several different types of models to diagram protein interaction pathways. One of the most common model types is a **network map**—a sketch showing interacting proteins, genes, and other molecules. These diagrams are essentially the equivalent of an electrical wiring diagram. One disadvantage of network maps is that they are static diagrams that typically lack information about when and where each interaction occurs. Even so, they are a useful foundation for generating computational models that allow the running of simulations to determine how signaling events occur. For example, major groups of kinases, enzymes that phosphorylate other proteins to affect their activity, have been network mapped to show their interactions with each other. Because kinases play such important roles in the regulation of most critical cellular processes, such information about the "kinome" has been very valuable for companies developing drug treatments targeted to certain metabolic pathways.

Network maps are helping scientists model intricate potential interactions of molecules involved in normal and disease processes. Figure 3.24 shows an example of a network map, which depicts a human disease network model illustrating the complexity of interactions between genes involved in 22 different

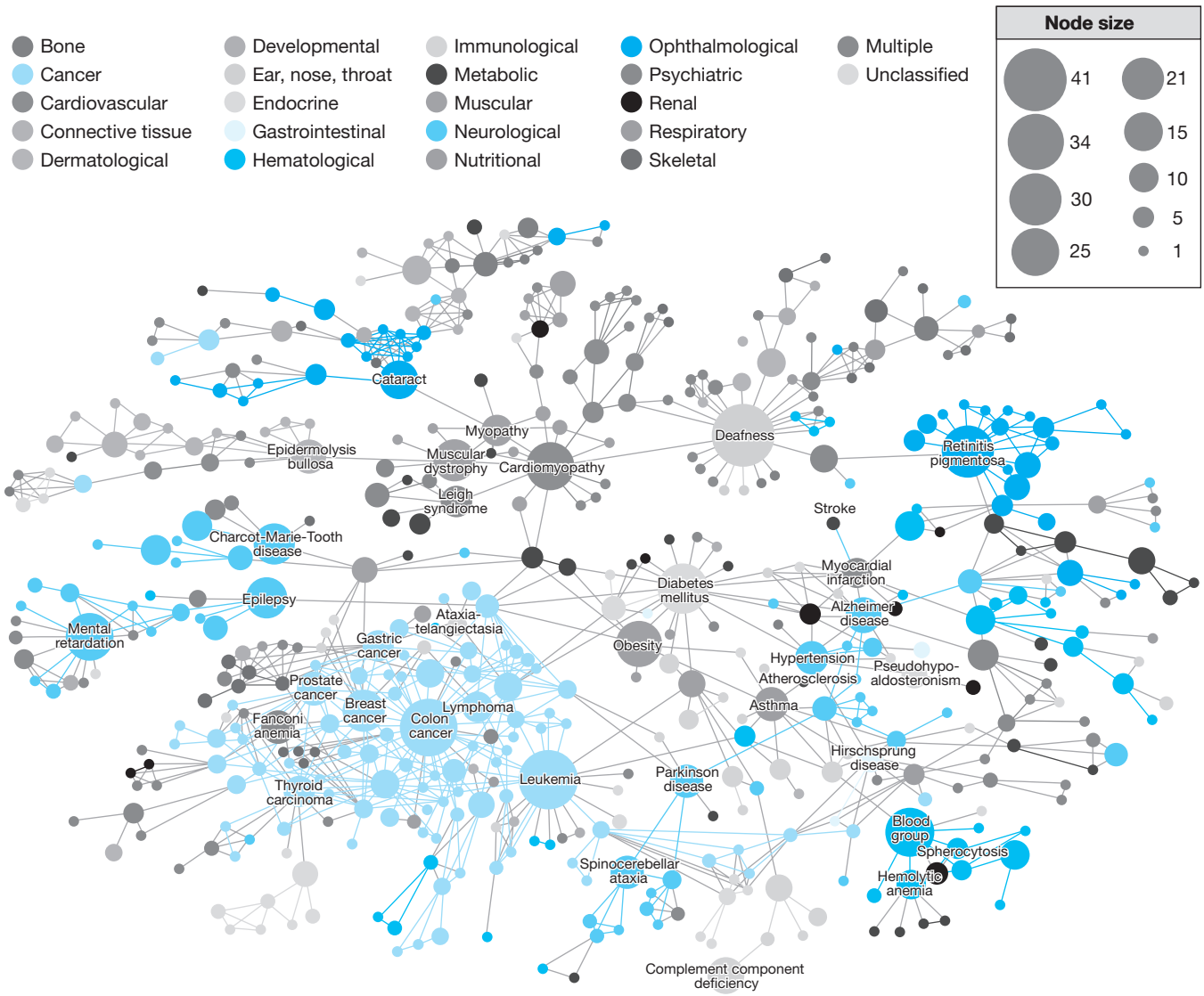


FIGURE 3.24 A Systems Biology Model of Human Disease Gene Interactions
The model shows nodes corresponding to 22 specific disorders colored by class. Node size is proportional to the number of genes contributing to the disorder. Although the shading for categories of different disease can be difficult to distinguish, this figure illustrates the complex genetics interactions and overlaps between different diseases.

human diseases. One aspect of the map that should be immediately obvious is that a number of cancers share interacting genes even though the cancers affect different organs. Knowing the genes involved and the protein interaction networks for different cancers is a major breakthrough for informing scientists about target genes and proteins to consider for therapeutic purposes. Systems biology is becoming increasingly important in the drug discovery and development process, where its approaches can help scientists and physicians develop a conceptual framework of gene and protein interactions in human disease that can then serve as the rationale for effective drug design.

In Chapter 5 you will learn about novel research from a team led by HGP pioneer Craig Venter; this team created a functional *synthetic genome*, an artificially synthesized genome, for a microbe. This work was hailed as a defining moment in the emerging field of **synthetic biology.** Synthetic biology applies engineering principles and designs to biological systems, with the idea of engineering living cells (prokaryotic and eukaryotic) for specific purposes such as manufacturing food, drugs, and different materials. Many fundamental questions about synthetic genomes, however, need to be answered. But early studies have provided key "proof of concept" that synthetic genomes can be produced, assembled, and successfully transplanted into cells. This accomplishment brings scientists closer to producing novel synthetic genomes that incorporate genes for specific traits of interest. An international effort is now under way to produce synthetic chromosomes comprising an entire yeast genome, about 12.5 million bases. In 2014, a synthetic version of yeast (*S. cerevisiae*) chromosome III was created, the first such eukaryotic chromosome.

What are potential applications of synthetic genomes and synthetic biology? One is to create microorganisms that can be used to synthesize biofuels. Other possibilities exist, such as the creation of synthetic microbes engineered to degrade pollutants (bioremediation); the synthesis of new biopharmaceutical products including vaccines; the production of chemicals and fuels from sunlight and carbon dioxide; and the development of genetically programmed bacteria to help us heal, of cells as biosensors to detect pollutants, and of "semisynthetic" crops that contain synthetic chromosomes encoding genes for beneficial traits such as drought resistance or improved photosynthetic efficiency.

In addition, scientists have been interested in using synthetic genomes to create simple switches with specific functions. You are familiar with many everyday examples of switches—a place where an input leads to an output. A light switch provides a good example. Flip the light switch (input) and a light turns on (output). Or you push a key on the keyboard (input) and a letter is typed (output). "Switches" exist throughout cells, too. Cells receive various inputs, and turn something on (or off), such as expressing a gene or producing a protein, in response (output). Synthetic biologists have created databases of genome parts (promoters, genes, etc., that can be combined using recombinant DNA technology). Using synthetic biology, scientists have combined parts of a gene, such as promoter sequences and other regulatory elements, with genes encoding specific proteins, including proteins that fluoresce, to create synthetic *DNA circuits* that switch on and off in response to different stimuli. When incorporated into cells, such circuits cause cells to fluoresce when exposed to pollutants, for instance.

Could synthetic biology be used to create microbes or other cells engineered to seek out and detect diseased cells throughout the body, such as cancer cells, and then release molecules that could be detected in blood, urine, or stool to signal the presence of these cells? Might these same cells be engineered to release a therapeutic drug or antibody when they encounter a diseased cell? These and many other questions are being addressed by synthetic biologists, and you will learn more about recent applications in Chapter 5. Another project in process, called *Human Genome Project-Write (HGP-Write)*, proposes to synthesize a complete human genome.

In 2017 alone, synthetic biology companies raised over $1.7 billion in investments for the development of innovative technologies in various areas. Many challenges lie ahead, along with many decades of research—but both systems biology and synthetic biology are rapidly evolving, and it is likely that the most incredible applications of these disciplines have not been realized yet.

MAKING A DIFFERENCE

One of the most successful examples of how recombinant DNA technology is saving lives is also one of its first applications for treating human disease. Earlier in the chapter we discussed how the recombinant form of insulin, called Humulin, became the first product of recombinant DNA technology to be approved by the FDA for use in humans. Prior to recombinant insulin, insulin was isolated from animals. In many persons with diabetes, insulin from animal sources was ineffective and caused a range of complications. Producing recombinant insulin was not easy (see Figure 5.8). Insulin is composed of two different polypeptide chains. Both polypeptides had to be expressed in recombinant form and assembled to form the active hormone. Needless to say, it was quite a feat to successfully engineer insulin, such a challenging recombinant protein, as the first product of this technology. This demonstration that a

recombinant approach for producing a complex protein was possible made scientists highly optimistic about the possibilities of recombinant DNA approaches to making other proteins of therapeutic value. Humulin was originally developed by Genentech and then widely distributed by Eli Lilly and Company, which continues to produce Humulin today. Since it first became available in 1982, Humulin has been used successfully and safely to treat millions of patients. The vast majority of insulin given to insulin-dependent diabetes mellitus patients worldwide is recombinant insulin. Many variations of Humulin are now available, most of which are different mixtures that determine whether the insulin is fast- or slow-acting once injected. Recently, researchers have been working on techniques to produce recombinant insulin in plants, which is expected to dramatically reduce biomanufacturing costs. The insulin story is an outstanding example of how recombinant DNA techniques are being used to make a difference in alleviating human pain and suffering.

QUESTIONS & ACTIVITIES

Answers can be found in Appendix 1.

1. Distinguish among gene cloning, recombinant DNA technology, and genetic engineering by describing each process and discussing how they are interrelated. Provide examples of each approach as described in this chapter.
2. Restriction enzymes are an essential component in gene cloning and recombinant DNA techniques. Does the sequence below contain a palindromic recognition sequence for a restriction enzyme listed in Table 3.1? If so, what is the double-stranded sequence of the palindrome, and what enzyme would cut at this sequence?

   ATCCGTCATACCGAATTCGTAAGG

3. A paired set of primers is essential to a PCR reaction. What are these primers and what is their function in a PCR reaction?
4. What features of plasmid cloning vectors make them useful for cloning DNA? Provide examples of different types of cloning vectors and discuss their applications in biotechnology.
5. Your lab just determined the sequence of a rat gene thought to be involved in controlling the fertilizing ability of rat sperm. You believe a similar gene may control fertility in human males. Briefly describe how you could use what you know about this rat gene combined with PCR to clone the complementary human gene. Be sure to explain your experimental approach and the necessary lab materials. Also explain in detail any procedures necessary to confirm that you have a human gene that corresponds to your rat gene.
6. If you performed a PCR experiment starting with only one copy of double-stranded DNA, approximately how many molecules would be produced after 15 cycles of amplification?
7. Describe the major difference between reverse transcription PCR (RT-PCR) and real-time or quantitative PCR (qPCR). Consider what can be done by combining these two technologies.
8. Visit the Online Mendelian Inheritance in Man Site (OMIM) at the Companion Website and then click on "Search the OMIM database." Type *diabetes* in the search box and then click "Submit Search." What did you find? Try typing *114480* in the search box. What happened this time? Alternatively, search for a gene you might be interested in and see what you can find in OMIM. If the results of your OMIM search are too technical, visit the "Genes & Disease" section of the NCBI site from the Companion Website, then search for *diabetes*.
9. Software analysis of DNA sequences makes it much easier for molecular biologists to study gene structure. This activity is designed to help you experience applications of DNA analysis software. Imagine that the following very short sequence of nucleotides, CAGAATTCCC-GGGAAGCTTGG, represents one strand of an important gene that was just mailed to you for your research project. Before you can continue your research, you must find out which restriction enzymes, if any, cut this piece of DNA.

   Go to the Webcutter site from the Companion Website. Scroll down the page until you see a text box with the title, "Paste the DNA sequence into the box below." Type the sequence of your DNA piece into this box. Scroll down the page, leaving all parameters at their default settings until you see "Please indicate which enzymes to include in the analysis." Click on "Only the following enzymes:" and then use the drop-down menu and select *Hind*III. Scroll to the bottom of the page and click "Analyze sequence" button. What did you find? Is your sequence cut by *Hind*III? Analyze this sequence for other cutting sites to answer the following questions. Is this sequence cut by *Eco*RI? How about *Bam*HI? What happens if you do a search and scan for cutting sites with all enzymes in the database?

10. Go to the molecular biology section of the Biology Project website from the University of Arizona (you can find the address on the Companion Website). Link to "Recombinant DNA Technology" and test your knowledge of recombinant DNA technology by working on the questions at this site.
11. The NCBI program called Basic Local Alignment Search Tool (BLAST; **www.ncbi.nlm.nih.gov/BLAST**) can be used to search GenBank for sequence matches between cloned genes and to create DNA sequence alignments. Go to the BLAST website and click "standard nucleotide-nucleotide BLAST [blastn]." In the search box, type in the following sequence: AGCCCTCCAG GACAGGCTGC ATCAGAAGAG. Imagine that this sequence is from a piece of a gene that you just cloned and sequenced and you want to know if anyone cloned this gene before you. Click the "Blast!" button. Your results will be available in a minute or two. Click the "Format!" button to see the results of your search. A page will appear with the results of your search (you may need to scroll down the page to find the sequence alignment). What did you find?
12. Diagram a basic outline of the process of whole-genome sequencing to demonstrate that you understand key steps involved in sequencing a large genome.
13. Explain why next-generation and third-generation sequencing approaches have rapidly advanced the analysis of genomes.
14. Describe several key findings of the Human Genome Project.
15. Search the Web for the company MyHeritage. What services do they provide?
16. Biology is experiencing an "omics" revolution. What does this mean? In your answer, describe two "omics" disciplines and provide examples of biotechnology applications involving these fields.
17. How can personal genomics be used to diagnose and treat genetic diseases?
18. Will you or your friends be interested in giving your genomes to pharmaceutical or similar companies for research? What are the things to consider before you proceed with this?
19. Why is systems biology of interest to biotechnology companies and scientists studying complex diseases?
20. What is synthetic biology? Describe possible biotechnology applications of synthetic biology in the future.

Visit www.pearsonglobaleditions.com for the Companion Website

The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

This case study has been included to give you an opportunity to become familiar with a research example applicable to this field of study. After you have analyzed the data and answered the questions, you can compare them to those provided to your instructor with the text materials.

CASE STUDY

The PANTHER Database

Recall that when the HGP was completed, more than 40 percent of the genes identified had unknown functions. The PANTHER database provides access to comprehensive and current functional assignments for human genes (and genes from other species). See **http://www.pantherdb.org/data/**.

In the frame on the left side of the screen, locate the "Quick Links" and use the "Whole genome functions" link to view a pie chart of current functional classes for human genes. Mouse over the pie chart to answer the following questions.

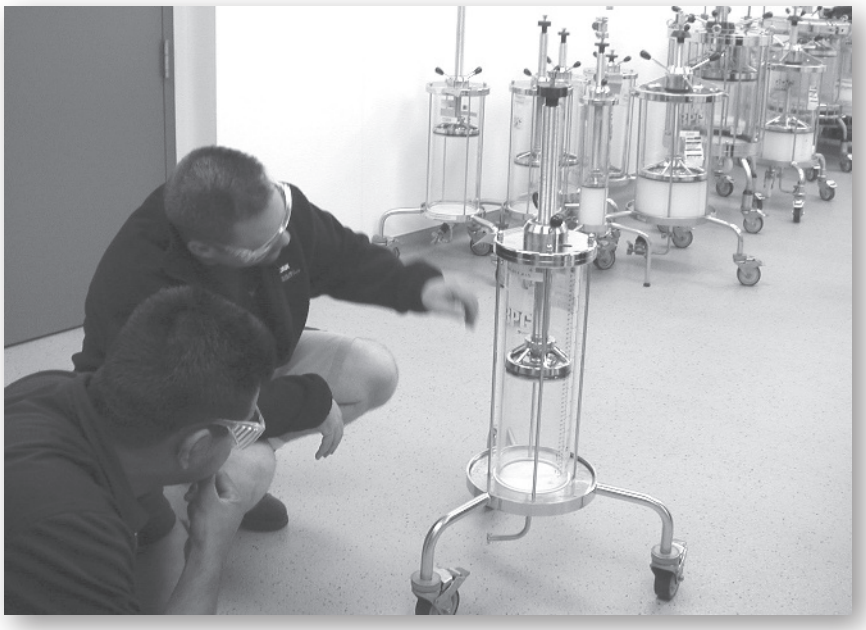
Questions

Answers can be found at www.pearsonglobaleditions.com

1. What percentage of human genes encode transcription factors?
2. What percentage of human genes encode cytoskeletal proteins?
3. What percentage of human genes encode transmembrane receptor proteins?

CHAPTER FOUR

Proteins as Products



Lab supervisor describing a purification column to separate a commercial biological product.

After completing this chapter, you should be able to:

- Explain some advantages for producing proteins biotechnologically.
- Explain why some common household products may include manufactured proteins as ingredients.
- Provide three examples of proteins produced for medical applications.
- Discuss the advantages and disadvantages of bacterial, fungal, plant, and animal sources of expressed proteins.
- Explain why *Escherichia coli* frequently is used for protein production, and describe *E. coli*'s limitations.
- Explain why the need for protein post-translational modification may determine the choice of a protein expression system.
- Describe a general scheme for protein purification of a protein such as hemoglobin, based on its known chemical characteristics.
- Explain how a target protein can be separated from other cell proteins in a specific purification sequence.
- Discuss the benefits of being able to predict a protein's structure from the DNA sequence (i.e., proteomics).
- Describe the value that understanding protein biology/chemistry has for achieving the delivery of small molecules across membranes, such as occurs in drug delivery.

FORECASTING THE FUTURE

According to the European Federation of Pharmaceutical Industries and Associations (EFPIA), the biotech and pharmaceutical industry together make up one of Europe's top performing high technology sectors. The discovery and creation of new medicines are expensive and difficult, with the cost of researching and developing a new chemical or biological drug estimated to be almost €2 billion. With advances in science and technology, the biotech and pharmaceutical industries play a key role in advancing modern medicine by developing innovative treatments, many involving the use of proteins as therapeutics. These companies harness living cells to express these high molecular weight proteins (biologic drugs), whereas small molecule drugs produced by pharmaceutical companies rely on chemical synthesis for production. Biologic medicines are some of the most expensive drugs and much effort is being made to produce biosimilars for biologic drugs that are scheduled to go off patent, particularly in Europe. European Medicines Agency (EMA) has authorized 21 biosimilar medicines since 2006.

Tropical rainforests, the deepest reaches of the ocean, boiling geysers in Iceland, and whale skeletons—these are on the frontier of the scientific quest for proteins. **Proteins** are large molecules required for the structure, function, and regulation of living cells. Each protein molecule has a unique function in the biochemical reactions that sustain life. As researchers explore the proteins that occur in nature, they unlock secrets that govern growth, speed of chemical decomposition, and protection from disease.

The applications of proteins are as numerous as proteins themselves. Consider whale skeletons: during the natural decomposition process, bones are often colonized by bacteria, some of which have evolved especially to digest the fatty residue remaining on them. The proteins that the bacteria produce to break down the fats are adapted to the frigid waters of the deep sea. Researchers recognized that a substance with the ability to dissolve fats at cold temperatures would make a great additive for commercial laundry detergents.

A study carried out in 2012 also showed that six artificial nucleotides, called XNAs, could store genetic information and evolve through natural selection (like DNA). Next, researchers identified four different types of synthetic enzyme proteins formed from these synthetic DNAs. Like naturally occurring enzymes, these are capable of powering simple biochemical reactions. **XNAzymes** are much more stable than naturally occurring enzymes, and could be useful in developing new therapies for a range of diseases, including cancers and viral infections, which exploit the body's natural processes. Artificial enzymes are based on an understanding of naturally occurring enzymes, and represent a new discovery approach for biotechnology.

In 2000, the National Institutes of Health launched the Protein Structure Initiative (PSI), a 10-year, $600 million effort to identify the structure of human proteins. The PSI is a federal university and industry effort aimed at reducing costs and lessening the time it takes to determine three-dimensional (3D) protein structures. The initial goal of the PSI was to make the structures of most proteins easily obtainable from knowledge of their corresponding DNA sequences. In the current phase, which began in 2010, investigators are using high-throughput structure determination, which was successfully developed during the earlier phases of the PSI, to study a broad range of important biological and biomedical problems.

The public database that is part of the initiative currently holds more than 33,000 protein sequences. The goal of the initiative is to be able to model unknown protein structures based on structural comparisons to those stored in the database. An international team of researchers has created an initial catalog of the **human proteome**, "or all of the proteins in the human body." Using 30 different human tissues, the team identified proteins encoded by 17,294 genes, which is about 84 percent of all the genes in the human genome predicted to encode proteins. The project, led by researchers at The Johns Hopkins University and the Institute of Bioinformatics in Bangalore, India, also reported the identification of 193 novel proteins that came from regions of the genome not predicted to code for proteins.

We will begin with a quick survey of the many applications of proteins in a variety of industries. Then we look at the nature of protein structures, paying special attention to the process of protein folding. With that as a foundation, we delve into some details of protein processing, beginning with the methods of purifying individual proteins. We then learn how expressed proteins are purified and examine the processes used to analyze and verify the final product. Although there is no one best method for purifying individual proteins, several common techniques are available. In this chapter, we look at those techniques, keeping in mind that the specifics of protein processing vary from protein to protein.

4.1 Proteins as Biotechnology Products

The use of proteins in manufacturing processes is a time-tested technology. For example, two of the oldest food-processing endeavors depend on proteins: beer brewing and winemaking. Fermentation depends not only on yeast-produced enzymes but also on others

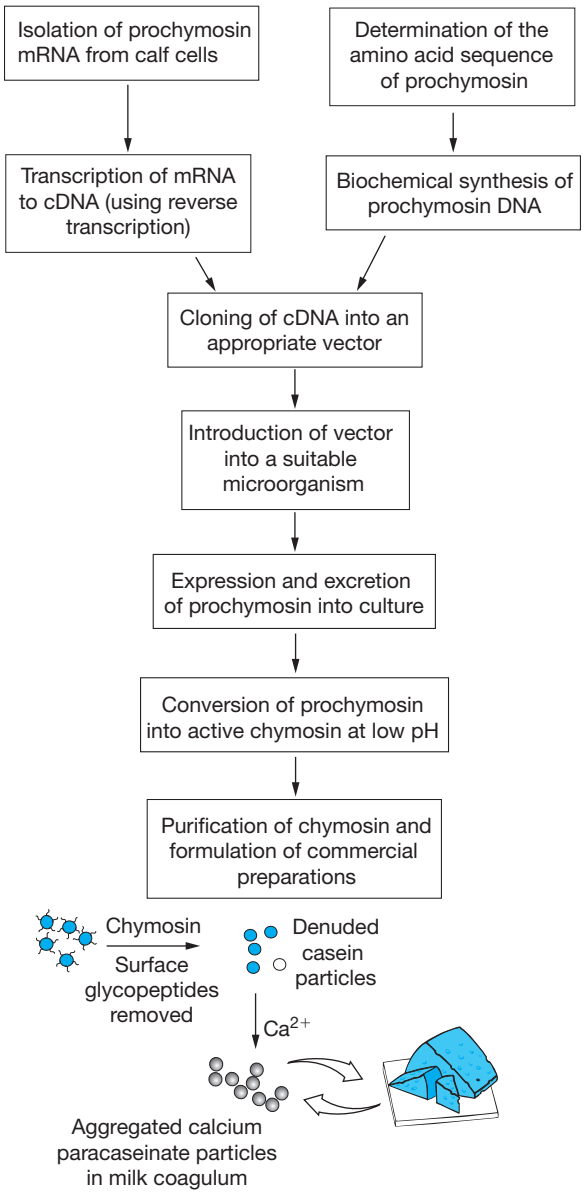


FIGURE 4.1 Cheese Production Casein, the primary ingredient in cheese, is the result of a chemical conversion that depends on chymosin. This enzyme has been obtained from the stomach walls of unweaned calves for centuries. Today 80 percent of the chymosin manufactured is produced (and purified) from genetically modified cells.

added directly to the batch. Cheese making is another food-processing industry that has always used proteins and, thanks to bioengineering, the protein source now used is from engineered bacteria rather than the calves' stomachs that originally provided the cheese-making enzyme (Figure 4.1).

Even though the value of proteins in manufacturing had long been evident, we were not able to further this knowledge until the 1970s, when recombinant DNA technology was first developed and it became possible to produce specific proteins on demand. Since that time, the production of proteins has been the driving force behind the development of new products in a wide variety of industries.

TABLE 4.1 Some Enzymes and Their Industrial Applications

Enzyme	Application
Amylases	Digest starch in fermentation and processing
Proteases	Digest proteins in detergents, meat/leather, cheese, brewing/baking, animal/human digestive aids
Lipases	Digest lipids (fats) in dairy and vegetable oil products
Pectinases	Digest enzymes in fruit juice/pulp
Lactases	Digest milk sugar
Glucose isomerase	Produce high-fructose syrups
Cellulases/ hemicellulases	Produce animal feeds, fruit juices, brewing converters
Penicillin acylase	Produces penicillin
Alpha-galactosidase	Treatment for a rare type of lysosomal storage disease
Alpha-L-iduronidase	Treatment for a rare type of lysosomal storage disease

Other industries are also benefiting from the ready availability of bioengineered proteins. Many of these applications depend on the power of a group of proteins called **enzymes** to speed up chemical reactions. Enzymes serve myriad purposes, such as making detergents work better, increasing the flow of oil in drilling operations, and cleaning contact lenses. Enzymes are used in manufacturing to break down large molecules—a process called **depolymerization.** These enzymes include glycosidases (carbohydrases) like **amylase,** which breaks down starch; **proteases,** which break down other proteins; and **lipases,** which break down fats (Table 4.1). Such enzymes (like chymosin, for cheese) are used in food and beverage production and for bulk processing in industries. You may wish to visit "How Enzymes Work" on the Companion Website for a review of this process.

Biotech Drugs and Other Medical Applications

Biotechnology proteins have revolutionized the health care and pharmaceutical industries in the past several decades. Many illnesses, from common conditions like

TABLE 4.2 Some Protein-Based Pharmaceutical Products (Most Produced as Recombinant Proteins)

Protein	Application
Erythropoietins	Treatment of anemia
Interleukins 1, 2, 3, 4	Treatment of cancer, AIDS; radiation- or drug-induced bone marrow suppression
Monoclonal antibodies	Treatment of cancer, rheumatoid arthritis; used for diagnostic purposes
Interferons (α, β, κ, including consensus)	Treatment of cancer, allergies, asthma, arthritis, and infectious disease
Colony-stimulating factors	Treatment of cancer, low blood cell count; adjuvant chemotherapy; AIDS therapy
Blood clotting factors	Treatment of hemophilia and related clotting disorders
Human growth factor	Treatment of growth deficiency in children
Epidermal growth factor	Treatment of wounds, skin ulcers, cancer
Insulin	Treatment of types 1 and 2 diabetes mellitus
Insulin-like growth factor	Treatment of type 1 diabetes mellitus
Tissue plasminogen factor	Treatment after heart attack, stroke
Tumor necrosis factor	Cancer treatment
Vaccines	Vaccination against hepatitis B, malaria, herpes

diabetes to rare diseases like Gaucher's disease, can be treated by replacing missing proteins. In the case of diabetes, the missing protein is the hormone insulin. Insulin once had to be harvested from pigs and cows. This was less than ideal because human immune systems often rejected this foreign protein. Researchers overcame the problem by turning to an unlikely source: the bacterium *E. coli*. By inserting human genes into *E. coli*, they created microscopic insulin factories. The FDA approved this new insulin in 1982, making it the first recombinant DNA drug. The ability to produce an abundant supply of human insulin has improved the health and lives of millions of people. (Table 4.2 lists some other protein-based pharmaceutical products.)

Therapeutic proteins—such as monoclonal antibodies, blood proteins, and enzymes produced by living organisms to fight disease—can also be thought of as biotech drugs. Unlike other medicines, these drugs are not synthetically produced (i.e., chemically synthesized by adding one compound at a time) but are usually produced through microbial fermentation or by mammalian cell culture. Today, more than 400 biotechnology medicines are in the pipeline, and the majority are proteins. If even a small percentage of these drugs succeed, it will significantly add to the approximately 50 biotech drugs currently in use.

Each person has a unique response to a medication that will determine whether or not that particular drug will work for him or her. If certain biological markers (**biomarkers**) are known and measured, they could be used to predict the effectiveness of any particular drug for a particular patient. For example, patients with pancreatic cancer were found to have increased levels of each of three proteins when their urine samples were compared to those from healthy patients. These biomarkers can detect patients with stages I to II pancreatic cancer with more than 90 percent accuracy.

Producing biotech drugs is a complicated and time-consuming process. Researchers can spend many years just identifying the relevant therapeutic protein, determining its gene sequence, and working out a process to make sufficient quantities of the protein molecules using biotechnology. Once this method is determined, technicians can produce large batches of the protein products in bioreactors, under carefully controlled conditions, by growing host cells that have been transformed to contain the therapeutic gene (a bioreactor is a sterile production container designed to produce biological products). The cells are stimulated to produce the target proteins through precise culture conditions that include a balance of temperature, oxygen, acidity, and other variables. At the appropriate time, the proteins are isolated from the cultures, stringently tested at every step of purification (which we discuss later in this chapter), and formulated into pharmaceutically active products. Manufacturing technicians monitoring bioreactors must strictly comply with industry regulations at all stages of the procedure.



YOU DECIDE **Testing for the Best Product: Who Should Pay?**

It takes over $2.6 billion to bring a drug to market. Included in this cost is the price of researching drugs that are not approved owing to an adverse reaction or ineffectiveness. Usually, the purification process has already been developed and the product is in human trials when this happens. Many people do not realize that high drug prices reflect, in part, the cost of research on products that were not approved. If we also add the ability to determine which drug is best for each patient (using **pharmacogenetics**) to the cost of bringing a drug to market, the cost climbs higher (see Chapter 1). Because biotechnology companies are supposed to make a profit for their stockholders, it is more profitable for the company to have everyone buy their drug, even if the drug is not entirely suited for each individual. What do you think is the best solution: higher prices and "cutting edge" drugs, or lower prices and drugs that do not always work and may even have negative side effects? You decide.

Using novel computational and biochemical approaches, scientists have accurately designed and built from scratch 10 large protein icosahedra, similar to viral capsids that usually carry viral DNA. The designed structures are made of two different engineered proteins, present in 60 copies each, which self-assemble into icosahedra. Researchers at the Institute for Protein Design used Rosetta, a protein-design computer program developed over many years, to optimize amino acid residues that form the interface between proteins to make them fit with each other. Because the work is so computationally intensive, the researchers relied on a crowd-sourcing platform called Rosetta@home, which allows members of the public to donate cycles of their idle computers to run jobs for computing protein structures. The researchers used the cryo-electron microscope (see cryo-EM in Section 4.4), and they were able to confirm that the designs came out very close to predictions from the computational models.

Smart bandages that produce antibiotic proteins

Imagine "smart bandages" that would detect an infection and then begin producing the appropriate antimicrobial protein to treat the infection. Massachusetts Institute of Technology (MIT) researchers developed tiny pellets containing enzymes, ribosomes, RNA, and DNA that can be stored at room temperature, taken where needed, and unleashed simply by adding water. The freeze-dried pellets were devised by MIT researchers. The cellular components are simply freeze-dried into pellets, which remain stable for at least a year. To activate protein production, the researchers add water to rehydrate the pellets. Basically, the cell-free extracts can enable on-the-spot biomanufacturing for health care workers, military personnel, and educators—as well as travelers and adventurers who would appreciate unusually sophisticated first-aid kits. In the MIT study, the researchers produced small proteins that could be used as a diphtheria vaccine, as well as antimicrobial peptides.

Bioremediation: Treating Pollution with Proteins

In addition to reducing the quantity of pollutants produced during industrial processes, proteins can be used to clean up harmful wastes. Organic wastes from feedlots, homes, and businesses are a growing threat to the environment, especially aquatic ecosystems. Enzymes can be used to digest such organic wastes before they cause trouble.

Another promising new application for proteins is in neutralizing heavy metal pollutants such as mercury and cadmium. These dangerous elements can persist in the environment, causing harm to organisms throughout the food chain. These metals resist enzymatic breakdown, but this does not mean that proteins cannot be used to neutralize them (see Chapter 9). Some microorganisms have a sticky coat of **metallothioneins,** proteins that actually capture heavy metals. In this case, the pollutant is not dismantled or digested but simply made less dangerous. When toxic metals are bound to bacteria, they are less likely to be absorbed by plants and animals. Researchers are currently using the power of genetic engineering to create new, better biological tools to attack and destroy toxic substances.

4.2 Protein Structures

To understand the processes of expressing and harvesting proteins, we must look at the molecular structure of proteins more closely. Proteins are complex molecules built of chains of amino acids. Like all molecules, proteins have specific molecular weights. They also have an electrical charge that causes them to

interact with other molecules. This ability to interact is the key to the biological activity of proteins. Consider, for example, the way the chemical structure and electrical charge of an amino acid can influence its interactions with water: The molecules will be either **hydrophilic** (water loving, attracted to water molecules) or **hydrophobic** (water hating, as if the water molecules and amino acids repelled one another).

Structural Arrangement

Proteins are capable of four levels of structural arrangement. These are the primary, secondary, tertiary, and quaternary structures of proteins (see **Figure 4.2**). The exact arrangement that a protein takes depends on the specific chemical sequence of its amino acids and the types of side groups that are present. (See Appendix 2 to view the side groups of amino acids and the alphabet used for their sequences.)

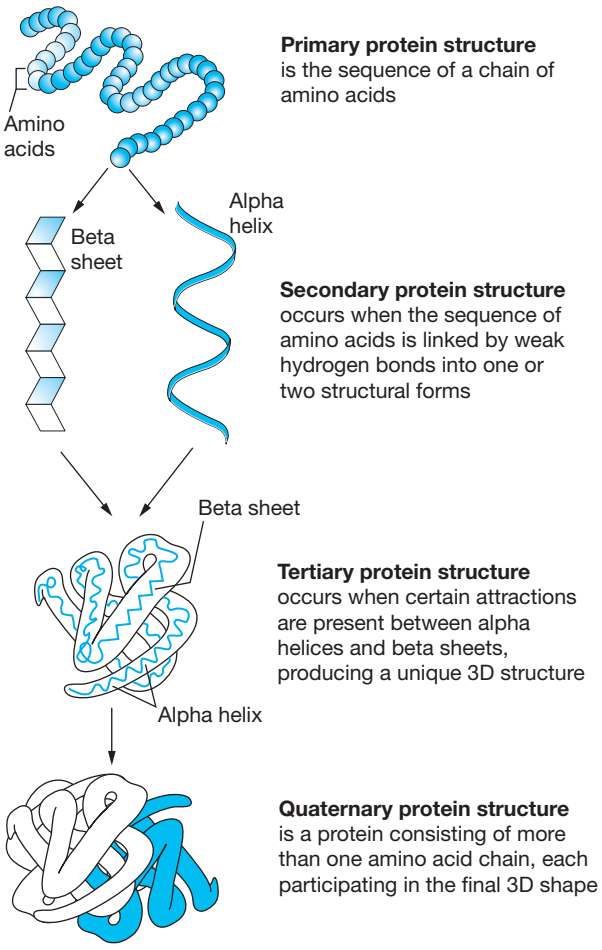


FIGURE 4.2 The Four Levels of Protein Structure
The proper folding of proteins is necessary for full functional capability. Purification methods must guarantee that proper folding is retained.

Primary structures

The 20 commonly occurring amino acids are the building blocks that make up proteins. Ten to 10,000 amino acids can be linked together in a head-to-tail fashion to form the sequence of a protein. The sequence in which amino acids are linked together at the ribosome is known as the *primary structure*. Altering a single amino acid in the sequence can mean that the protein loses all function. Genetic diseases are often the result of these protein mutations. You may wish to visit the Companion Website to view "Overview of Protein Synthesis" as a refresher on this process.

Secondary structures

Secondary protein structures occur when chains of amino acids fold or twist at specific points, forming new shapes due to the formation of hydrogen bonds between hydrophobic amino acids. The most common shapes, alpha helices and beta sheets, are shown in the section on protein folding. In the alpha-helix arrangement, the amino acids form a right-handed spiral. The hydrogen bonds stabilize the structure, linking an amino acid's nitrogen atom to the oxygen atom of another amino acid. Because the links occur at regular intervals, a spiraling chain is formed. In the beta-sheet structure, the hydrogen bonds also link the nitrogen and oxygen atoms; however, because the atoms belong to amino acid chains that run side by side, an essentially flat sheet is formed. The sheets can be either "parallel" (if the chains all run in the same direction) or "antiparallel" (in which case the chains alternate in direction). One of the fundamental elements of protein structure, the beta turn, occurs when a single chain loops back on itself to form an antiparallel beta sheet. Both the alpha-helix and beta-sheet structures exist because they are the most stable structures the protein can assume.

Tertiary structures

Tertiary protein structures are 3D polypeptides (amino acid chains) that are formed when secondary structures are cross-linked. The bonds that hold together tertiary structures occur between amino acids capable of forming secondary covalent bonds (like cysteine, which can form disulfide bonds with another adjacent cysteine, cross-linking the protein into a unique shape). (A quick look at Appendix 2 will show the SH side group of cysteines that can cross-link to form disulfide bridges.) You may wish to visit the Companion Website to view "Hydrogen Bonds" as a way to reinforce your understanding of this bonding process. The tertiary structure of a protein determines its function, such as binding a cellular receptor or catalyzing a chemical reaction.

No matter what secondary and tertiary structure a protein takes, it is important to remember that structures are fragile. Hydrogen bonds can be broken easily,

changing the protein structure. Anyone who has heated an egg has realized that the fluid egg proteins (albumin) quickly change shape (egg white) as the hydrogen and cross-linking tertiary bonds are broken by heat. Most proteins used in the lab are kept on ice to maintain their fragile structure and prevent them from being destroyed by the heat in the lab.

Quaternary structures

Quaternary protein structures are unique, globular, 3D complexes built of several polypeptides. Hemoglobin, which carries oxygen in the blood, is an example of a protein with quaternary structure; it is composed of two tertiary proteins linked together. You may benefit from the animation "Protein Structure" on the Companion Website, with its visual examples.

Protein-Protein Interaction Map Created

A newly developed protein interaction map is already helping spot individual proteins that could be at the root of complex human disorders. An international research team studied cells from numerous organisms, from amebae to worms to mice to humans, to show how proteins fit together to build different tissues and bodies. They uncovered tens of thousands of new protein interactions, accounting for about a quarter of all estimated protein contacts in a cell. Many proteins come together to form so-called molecular machines that play key roles, such as building new proteins or recycling those no longer needed, by literally grinding them into reusable parts. The yeast two-hybrid system described in Chapter 3 is an early test for protein interaction. A newly discovered molecular machine, called **Commander**, consists of about a dozen individual proteins. Genes that encode some of Commander's components had previously been found to be mutated in people with intellectual disabilities, but it was not clear how these proteins worked. Because Commander is present in all animal cells, the scientists went on to disrupt its components in tadpoles, revealing abnormalities in the way brain cells are positioned during embryo development and providing a possible origin for a complex human condition. It is fully expected that this open source map will lead to human discoveries through protein linkages found in organisms.

Protein Folding

The first breakthrough in understanding the fundamental forms of proteins came in 1951, when Pauling and Corey described the alpha helix and beta sheet, which are the most common components of protein structure. Both structures result from hydrogen bonding that ties together the chain of amino acids.

Everything that is important about a protein—its structure, its function—depends on folding. Folding describes how different strands of amino acids take their shape; for example, sickle cell anemia results from a misfolding due to a single amino acid replacement at a strategic location in the primary structure. If a protein folds incorrectly, not only will the desired function of the protein be lost but also the resulting misfolded protein can be detrimental. For example, the plaque that forms in Alzheimer's disease accumulates because a misfolded protein cannot be broken down by the enzymes present in brain cells.

Post-Translational Protein Modifications

More than 100 **post-translational modifications** (like glycosylation) occur within a eukaryotic cell. This change can have a significant effect on a protein's activity by increasing solubility and orienting proteins into membranes to extend the active life of a molecule in an organism. Post-translational modification (PTM) refers to the covalent and generally enzymatic modification of proteins during or after protein biosynthesis. Proteins are synthesized by ribosomes translating mRNA into polypeptide chains, which may then undergo PTM to form the mature protein product. Post-translational modifications can occur on the amino acid side chains or at the protein's C- or N-terminal end. Modifications such as peptide cleavage, lipidation, glycosylation, phosphorylation, and disulfide bond formation can be achieved.

Phosphorylation is a very common mechanism for regulating the activity of enzymes and is the most common post-translational modification. Many eukaryotic proteins also have carbohydrate molecules attached to them in a process called glycosylation (see **Figure 4.3**), which can promote protein folding and improve stability as well as serve regulatory functions. Attachment of lipid molecules often targets a protein or part of a protein attached to the cell membrane.

Glycoproteins can be used in the treatment of disease, as scientists with the Scripps Research Institute have discovered. This discovery represents a new way to target and destroy a type of cancerous cell. A glycoprotein can be combined with a nanoparticle (a small synthetic molecule) loaded with a chemotherapy drug, resulting in a new method to target and destroy cancer cells. By targeting B lymphoma cancers with chemotherapeutic-loaded nanoparticles, the effective dose of the drug is increased while simultaneously protecting normal tissues. It's clear that the findings of the Scripps scientists could lead to the development of other novel drug therapies based on glycosylation to treat leukemia, lymphomas, and related cancers.

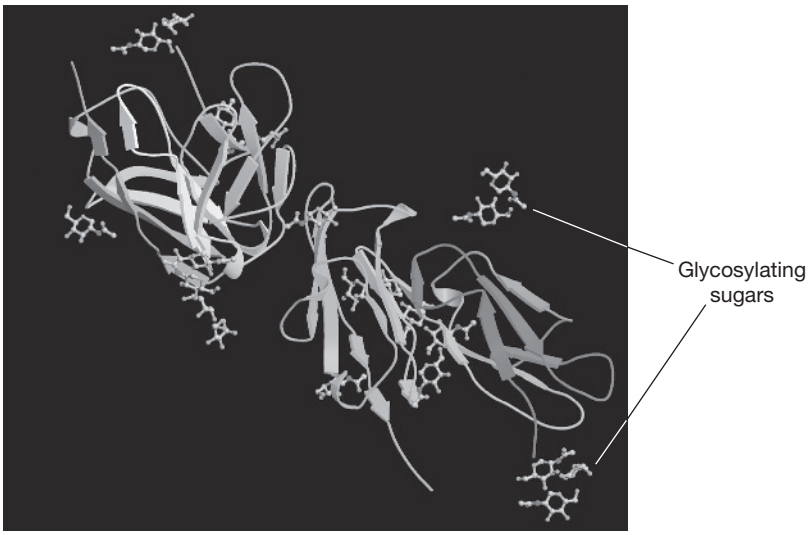


FIGURE 4.3 3D Protein Structure with Glycosylated Side Chains Glycosylation occurs within eukaryotic cells and probably extends the life of the protein by protecting it from natural cellular mechanisms that destroy proteins.

Protein Engineering by Directed Molecular Evolution

Biotechnology often relies on protein engineering. At times it is necessary to introduce specific, predefined alterations in the amino acid sequence. This can be done with directed molecular evolution technology. Enzymes have been modified over millions of years of evolution to catalyze specific chemical reactions in cells. Within the last decade, scientists have used directed evolution and rational design (designing a protein to fit a surface) with varying degrees of success to improve activity, stability, and selectivity of native enzymes. Much progress has been made in David Baker's lab at the University of Washington using a rational design program called Rosetta. The team designed an enzyme not found in nature by modeling the active site part of the enzyme and then finding a protein scaffold structure to attach to the part. After testing 84 constructs for activity, they selected 50 and inserted their DNA sequence into *E. coli* for expression. The final chosen enzyme functioned, but not as well as the native enzymes. This required them to mutate the active site many times to achieve better activity. This research shows that the day when proteins can be designed rationally is yet to come, but progress is being made.

At Harvard University, in Massachusetts, researchers have been focused on developing methods to evolve proteins and other biomolecules termed **PACE** (for **phage-assisted continuous evolution**), adapted to the life cycle of the M13 filamentous bacteriophage. Using the phage as a scaffold, researchers have achieved selection by linking a protein of interest to the phage coat protein. Their work showed that PACE is 100 times faster than conventional directed evolution approaches when it comes to evolving biomolecules because the phage life cycle is only 10 to 15 minutes, with the capability of evolving proteins at a rapid rate. Directing modification of proteins to produce a functional structure is illustrated in the "Spy" protein (see **Figure 4.4**).

Protein misfolding is an abnormal event but does occur in some disease conditions such as Alzheimer's, Parkinson's, Huntington's, cystic fibrosis, Gaucher's disease, emphysema, chronic liver disease, and some others (see **Figure 4.5**). When misfolding is not corrected, accumulation of the misfolded proteins usually leads to adverse effects. Prions serve as an example of misfolded proteins. A prion is an infectious agent, specifically a protein in a misfolded form. The protein itself, whether in its misfolded or its correctly folded form, can be referred to as the prion protein (PrP). A protein as an infectious agent stands in contrast to all other known infectious agents, like viruses, fungi, or parasites—all of which must contain nucleic acids (either DNA or RNA). Prions are responsible for mammalian transmissible spongiform encephalopathies, including BSE, also known as mad cow disease and scrapie in sheep. All known prion diseases in mammals affect the structure of the brain or other neural tissue, and all are currently untreatable and universally fatal.

In 2013, a study revealed that 1 in 2,000 people in the United Kingdom might harbor the infectious prion protein that causes the human form of the disease (Creutzfeldt-Jakob disease, or CJD). All known prions induce the formation of an amyloid fold, in which the protein polymerizes into an aggregate consisting of tight beta sheets. This altered structure is extremely stable and accumulates in infected tissue, causing tissue damage and cell death. This structural stability means that prions are resistant to natural denaturation. The prion protein (PrP) is found throughout the body, even in healthy people and animals. However, PrP found in

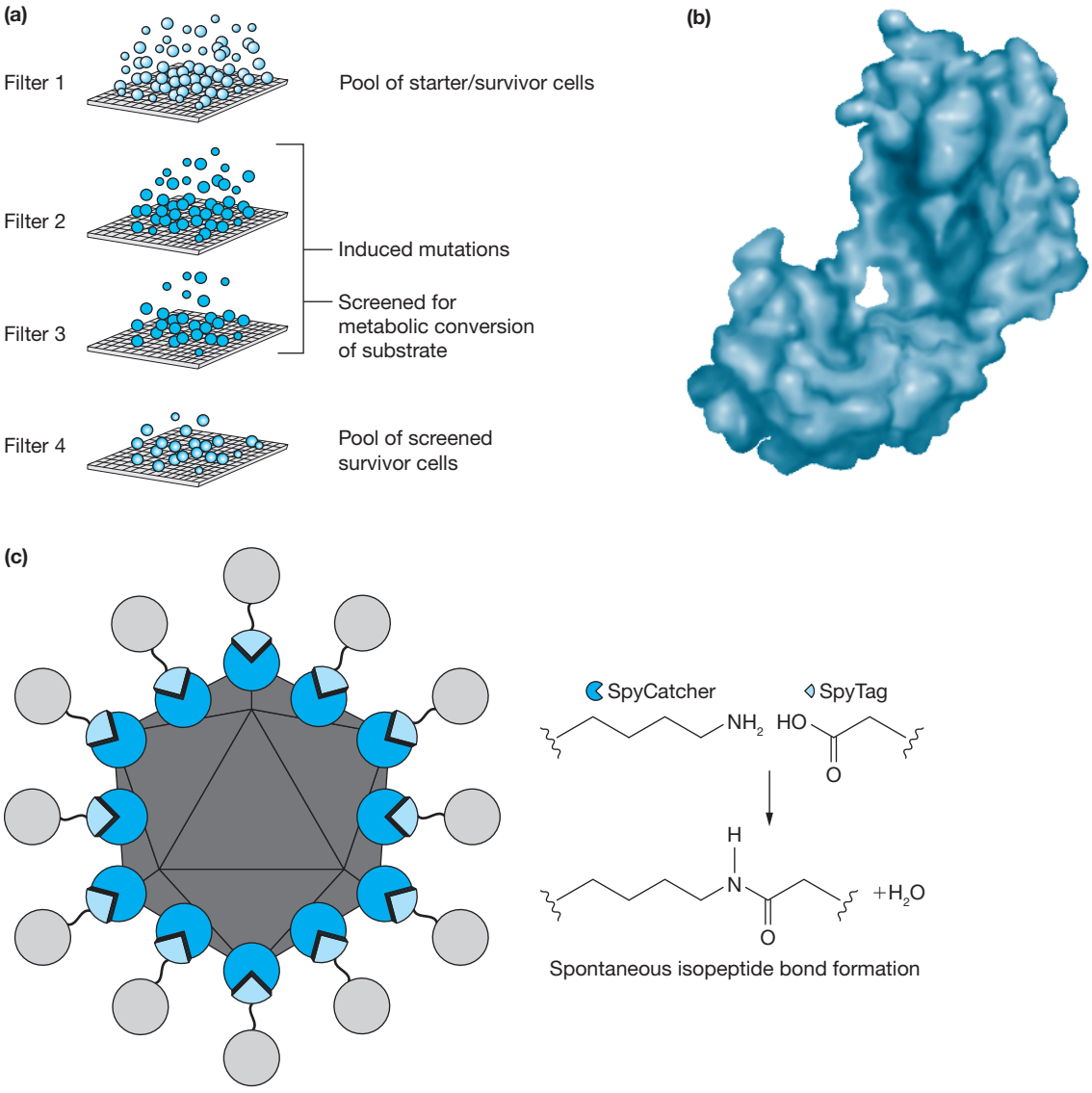


FIGURE 4.4 Directed Molecular Evolution Technology (a) Genes that express proteins can be subjected to mutational events, generating a diverse group of novel gene sequences. Organisms with mutated genes are screened for unique properties. After an improvement in protein function, the process can be repeated until the maximum function is obtained. Unlike natural selection, this process focuses on the properties of the protein, not the organism, and can achieve changes that may never occur in nature. It may not have an evolutionary advantage, but the proteins it produces will have more commercial value. (b) Scientists have discovered a protein used by bacteria to improve protein folding. The "Spy" protein reduces the misfolding that commonly occurs when bacteria produce recombinant proteins for drug or industrial use. (c) It has been found that *Streptococcus pyogenes*, like many other Gram-positive bacteria, contains extracellular proteins stabilized by spontaneous intramolecular peptide bonds. The fibronectin binding protein FbaB is found in invasive strains of *S. pyogenes*, and is essential for phagocytosis-like uptake of the bacteria by endothelial cells (Spy protein). By splitting FbaB into peptide and protein fragments, followed by rational modification of the parts, researchers at the University of California, San Francisco, developed a peptide tag of 13 amino acids that rapidly formed a covalent bond with its protein partner, called SpyTag. The irreversible linkage of SpyTag should provide a target in cells and a design opportunity for new protein architectures. For example, many vaccines are built as virus-like particles (VLPs), which resemble viruses but carry drugs or other substances. SpyTag should improve design of VLPs for drug delivery within cells.

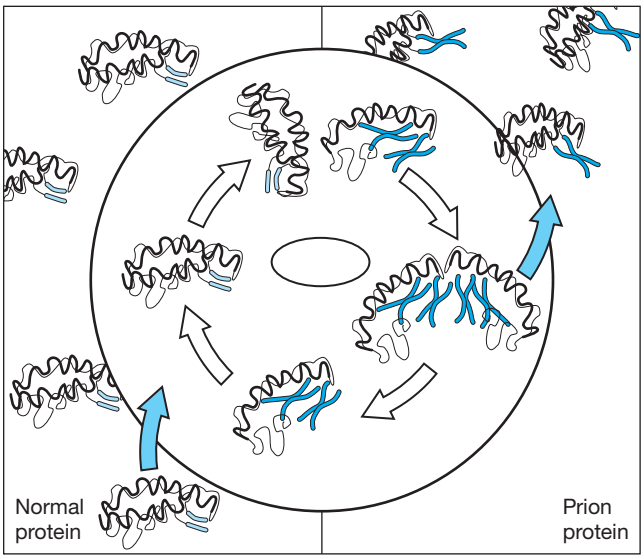


FIGURE 4.5 Prions Are Misfolded Proteins A misfolding of proteins that occurs in prion disorders can be duplicated in the lab to produce large quantities of prion proteins, which can then be studied to create diagnostic kits. Follow the arrows from the left as a prion protein attracts a normal protein and changes it into a prion, resulting in an accumulation of prions within the cell due to the inability of cellular enzymes to degrade the changed structure.

infectious material has a different structure and is resistant to proteases. A model of prion replication must explain both how prions propagate and why their spontaneous appearance is so rare. Manfred Eigen showed the heterodimer model (called PrPSc) to be an extraordinarily effective catalyst, increasing the rate of the conversion reaction of proteins by a factor of about 10^{15}. A gene for the normal protein has been identified: the *PRNP* gene. In all inherited cases of prion disease, there is a mutation in the *PRNP* gene. Many different PRNP mutations have been identified, and these proteins are more likely to fold into abnormal prions.

4.3 Protein Production

By now, two things should be evident: (1) Proteins are valuable, and (2) they are complex, fragile products. With these points in mind, we now examine the work of biotechnology in the production of proteins.

Producing a protein in the lab is a long, painstaking process, and at every stage there are many methods of production from which to choose. We refer to the two major phases used in producing proteins as **upstream processing** and **downstream processing.** Upstream processing includes the actual expression of the protein in the cell. During downstream processing, the protein is first separated from other parts of the cell and isolated from other proteins. Purity and functional abilities are then verified. Finally, a stable means of preserving the protein is developed. The choices made during upstream processing can simplify downstream processing.

Protein Expression: Upstream Processing

We begin a detailed discussion of protein processing by looking at the first decision made in upstream processing: selecting the cell to be used as a protein source. Microorganisms, fungi, plant cells, and animal cells all have unique qualities that make them good choices in the right circumstances.

Bacteria

Bacteria are an attractive protein source for several reasons. First, the fermentation processes of bacteria are well understood. Also, they can be cultured in large quantities in a short time. In industrial applications, this ability to generate the product on a large scale is often essential. Bacteria are also relatively easy to alter genetically.

Several methods of recombinant DNA technology can be used to increase the level of production of a bacterial protein. One is the introduction of additional copies of the relevant gene to the host cell. In most cases the relevant gene introduced into the organism is under the control of expression by a more powerful transcriptional promoter (see Chapter 3). You may wish to watch the animation "The *lac* Operon in *E. coli*" on the Companion Website for a refresher.

Another microbial protein factory is the Gram-positive bacterium *Bacillus subtilis*, an inhabitant of the upper layers of the soil that has the capacity to secrete proteins in the gram per liter range. The engineering of *B. subtilis* into a next-generation super-secreting cell factory has occurred. The analysis of protein secretion in *Bacillus* was initiated in the 1980s with classical molecular genetics approaches, providing valuable insights into cellular components involved in this process. The many applications of *B. subtilis* are related to the high-level secretion of proteins that has focused major research interests on the **secretome**, which includes both the protein secretion machinery and the secreted proteins. A very important outcome of the secretome analyses was the definition of secretion signals, the so-called signal peptides, for all secreted proteins of *B. subtilis*. The efficient production of secreted enzymes by microorganisms like *B. subtilis* is one of the key drivers of today's successes in the protein industry.

In some instances, the target gene (in the form of **cDNA)** for the desired protein product is attached directly to a complete or partial *E. coli* gene. In these

TABLE 4.3 **Advantages and Disadvantages of Recombinant Protein Production in *E. coli***

Advantages	Disadvantages
E. coli genetics are well understood	Foreign proteins produced as inclusion bodies must be refolded
Almost unlimited quantities of proteins can be generated	Proteins cannot be folded in ways needed for many proteins active in mammalian systems
Fermentation technology is well understood	Some proteins are inactive in humans

cases, the genetically engineered *E. coli* produce the desired protein, but it is in the form of a **fusion protein.** In fusion proteins**,** a target protein is fused to a bacterial protein; therefore, an additional step is required to break the two apart. The fused bacterial protein is usually an enzyme that will bind to its substrate and can be attached to a purification column (see description of affinity columns, in the text that follows). The majority of proteins synthesized naturally by *E. coli* are intracellular (within the cell). In most cases, the resultant foreign protein accumulates in the cell's cytoplasm in the form of insoluble clumps called **inclusion bodies,** which must be purified (and usually refolded) from the other cell proteins before they can be used. You may wish to visit "Use of GFP Fusions for Protein Localization" on the Companion Website for an example.

There are some limitations to the use of microorganisms in protein production. All bacteria, including *E. coli,* are prokaryotic. Prokaryotes are unable to carry out certain processes, such as glycosylation. For this reason, some proteins can be produced only by eukaryotic cells, as seen in Table 4.3.

Although it is possible to conduct the entire protein production process in a small flask in the laboratory, genetically engineered microorganisms can also be grown in large-scale fermenters (anaerobic) or bioreactors (aerobic) (Figure 4.6).

Computers monitor the environment in bioreactors, keeping oxygen levels and temperature ideal for cell growth. Cell growth is monitored carefully, because when the phase of growth is highest, the promoter must be activated to stimulate foreign gene expression. Activating a gene in a recombinant organism requires correct timing. It must be done after the organism has completed synthesizing important natural proteins needed for its metabolism.

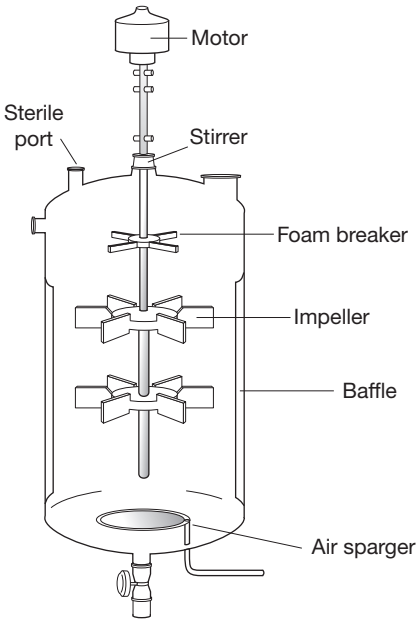


FIGURE 4.6 **Bioreactor** Large-volume cell culture is commonly performed in a self-contained, closed (sterile) system until the products are harvested. Through sterile ports, workers can adjust pH, gas concentrations, and other variables based on input from internal sensors. Sterile bags as large as 2,000 liters can be used on rocking tables as alternatives to stainless steel bioreactors.

Fungi

Fungi are the source of a wide range of proteins used in products as diverse as animal feed and beer. In addition to naturally occurring proteins, many species of fungi are good hosts for engineered proteins. Unlike bacteria, fungi are eukaryotic and capable of some post-translational modification (like folding human proteins correctly and other modifications) and are used for that synthesis, as illustrated in Table 4.4.

Plants

Plant cells can also be used for protein expression. In fact, plants are an abundant source of naturally occurring, biologically active molecules, and 85

TABLE 4.4 **Some Recombinant Proteins from Fungi**

Protein	Fungi
Human interferon	*A. niger*, *A. nidulans*
Human lactoferrin	*A. oryzae*, *A. niger*
Bovine chymosin	*A. niger*, *A. nidulans*
Aspartic proteinase	*A. oryzae*
Triglyceride lipase	*A. oryzae*

percent of all current drugs originated in plants. One example of a plant-derived protein produced on an industrial scale is the **proteolytic** (protein-degrading) enzyme **papain.** Papain, or vegetable pepsin, is a protease used as a meat-tenderizing agent. It digests the collagen present in connective tissue and blood vessels that makes meat tough.

Plants can be genetically modified to produce specific proteins that do not occur naturally. This process encourages rapid growth and reproductive rates in plants, which can be a distinct advantage. For example, tobacco, the first plant to be genetically engineered, can produce a million seeds from a single plant. As a non-food plant, it makes a good choice for biotech protein production. Once the genetic material is integrated, a million new "plant protein factories" can fill the fields.

There are also disadvantages to using plants as protein producers. Not all proteins can be expressed in plants, and, because they have tough cell walls, the process of extracting proteins from them can be time-consuming and difficult. Finally, although plant cells can often properly glycosylate proteins, the process is slightly different from that of animal cells. This may rule out using plants as biofactories for the expression of some proteins. We will discuss transgenic plants in greater detail later in Chapter 6.

Mammalian cell culture systems

It is possible to culture animal cells, growing them in a medium until it is time to harvest the proteins. This process is challenging because the nutritional requirements of mammalian animal cells are complex. Mammalian cells also grow relatively slowly, and the opportunity for mammalian cell cultures to become contaminated is greater than that of other culture systems. Despite these issues, mammalian cells are still the best choice for proteins destined to be used in humans.

Animal bioreactor production systems

Cells in culture are not the only option in using animal cells; sometimes living animals are protein producers. Consider, for example, the technique used to harvest monoclonal antibodies. Monoclonal antibodies react against only one target, making them valuable in diagnostic and therapeutic applications (see Chapter 7). Antibodies are proteins produced in reaction to **antigens** (usually an invading virus or bacteria). Antibodies can combine with and neutralize an antigen, protecting the organism. The production of antibodies is part of the immune response that helps living things resist infectious disease. When the production of a monoclonal antibody is the goal, mice are injected with an antigen. Then, the mouse's antibody-producing tissue is fused with cancer cells (to make them immortal). When fluid from the tumor that results is collected, the monoclonal antibodies can be purified from it.

Another method of animal bioreactor protein production uses the milk or eggs from transgenic animals (animals that contain genes from other organisms). These animal products contain the proteins from the recombinant gene that was introduced and can be purified from the milk or egg proteins. In 2009, the FDA approved the first human drug produced from a goat: ATryn, which treats a rare bleeding disorder in humans (see Chapter 7).

Insect systems

Insect systems are another avenue of protein production from animal cells. **Baculoviruses** (viruses that infect insects) are used as vehicles to insert DNA, causing the desired proteins to be produced by the insect cells. However, there are instances in which the post-translational modification of proteins is slightly different in insects than it is in other eukaryotes. Some covalent modifications, such as glycosylation and disulfide-bond formation, are frequently present in eukaryotic proteins and are often essential for correct protein folding and activity. Insect expression systems are commonly used when small quantities of proteins are needed in research.

Protein Purification Methods: Downstream Processing

Once a protein is produced, downstream processing begins (**Figure 4.7**). First, the protein must be harvested. If the protein is intracellular, the entire cell is harvested; if it is extracellular, the protein is excreted into the culture medium that is collected. Harvesting, though, is just the beginning of downstream processing. Next, the real work begins: The protein must be purified. This is the process of separating the target protein from the complex mixture of biological molecules in which it was produced.

Purity in this context is a relative term. Generally, the FDA requires that a sample be composed of 99.99 percent of the target protein. Separating proteins from all other cellular contents is not easy, and isolating the target protein from the other proteins in the sample can be even more difficult. To understand the process of purifying proteins, we look at some steps commonly followed in purification.

Step one: Preparing an extract for purification

If the protein is intracellular, the first task is **cell lysis,** disrupting the cell wall to release the protein. There are many methods for doing this: freezing and thawing (which disrupts cell membranes and releases cell contents), detergents (used to dissolve cell walls),

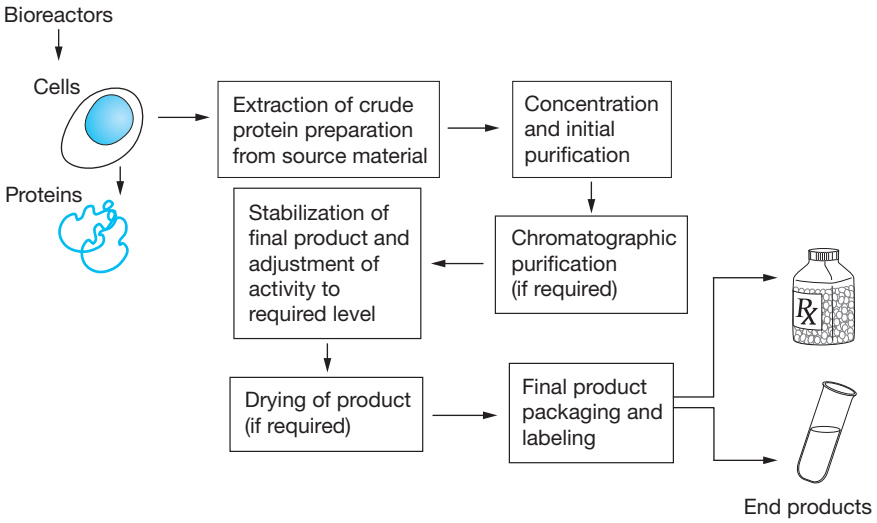


FIGURE 4.7 Basic Steps in Bioprocessing Purifications can be accomplished from raw materials or from bioreactors. The steps in the process must be devised and are often unique (and patentable).

and mechanical methods (ultrasonics or grinding with tiny glass beads). Given the fragility of proteins, freeing them from the cell without degrading them entirely is challenging. The disruption process releases the protein of interest as well as the entire intracellular content of the cell.

After the cells have been ruptured, organic alcohols or salts may be added to the mixture. Both of these take advantage of the hydrophobic orientation of the proteins by attracting water from the proteins, causing them to coalesce. These agents increase the interactions between the protein molecules to separate them from the mixture.

Step two: Stabilizing proteins in solution

Next, the proteins must be stabilized. Recall that it is important to maintain the bioactivity of the protein and that proteins are relatively fragile molecules. As a consequence, precautions must be taken to protect the protein during the purification process.

Maintaining a low temperature is crucial to protecting proteins, so most purifications must occur at low temperatures. Heat as moderate as room temperature limits the activity of proteins. Maintaining the proper pH for the activity of a protein is also important, and most active proteins are suspended in buffering agents to preserve maximal function.

Natural proteases that can digest the target proteins in a preparation are another threat. Protease inhibitors and antimicrobials can be added to prevent the protein molecules from being dismantled but must be removed later, as must any additive used in the purification process. Still another potential problem is mechanical destruction by foaming or shearing of the proteins into useless fragments. Once again, additives can help prevent foaming and shearing from destroying the protein, but the additives must be removed later.

As we've seen, some purification methods are powerful enough to damage the target protein. It takes a balancing act to extract and purify proteins successfully. Although it is essential that the protein be purified, it is equally important that the protein maintain its biological activity.

Step three: Separating the components in the extract

The last step in the purification process can be the most important. Similarities between proteins permit us to separate them from material such as lipids (fats), carbohydrates, and nucleic acids, which are also released when a cell is disrupted. Differences between individual proteins are then used to separate the target proteins from others. Following are several methods used for protein separation.

Protein precipitation

Proteins often have hydrophilic amino acids on their surfaces that attract and interact with water molecules. That characteristic is used as the basis for separating proteins from other substances in the extract. Salts, most commonly ammonium sulfate, can be added to the protein mixture to **precipitate** the proteins (to cause them to settle out of solution). Ammonium sulfate precipitation is frequently a first step in protein purification, resulting in a protein precipitate that is relatively stable. Problems associated with ammonium sulfate precipitation make it a poor choice in some industrial situations, however. Ammonium sulfate is highly reactive when it contacts stainless steel, for example, and many industrial purification facilities are made of stainless steel. Other solvents frequently used

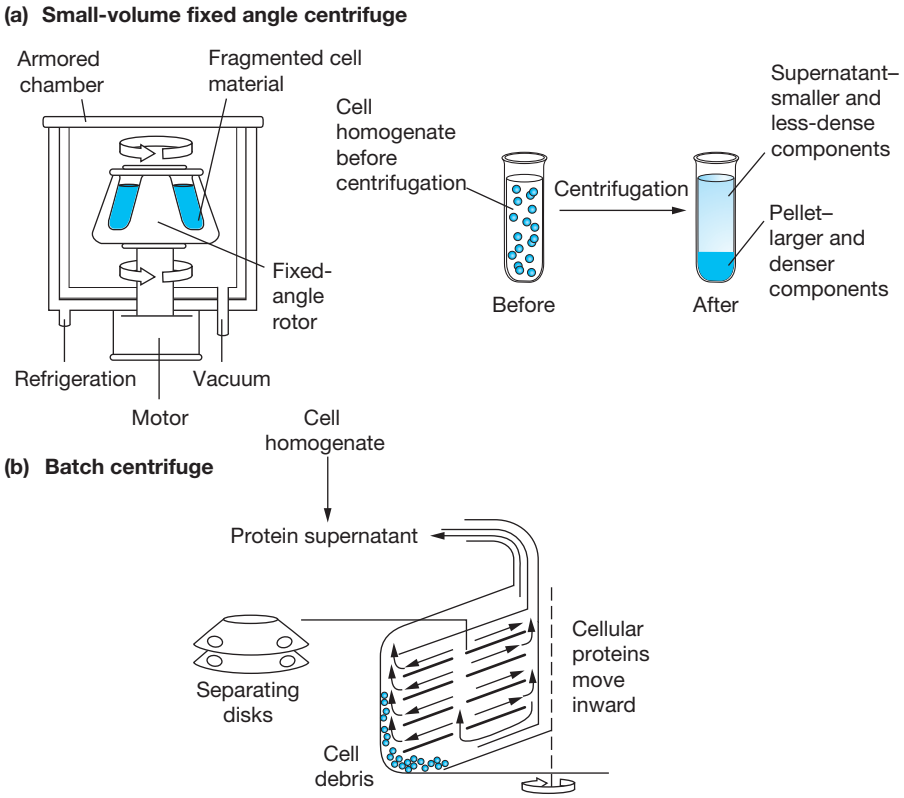


FIGURE 4.8 Fixed-Angle and Batch Centrifugation Fixed-angle centrifuges (a) can develop extremely high gravitational forces (*g* forces) but are limited to smaller quantities and must be run separately for each batch. Batch centrifuges (b) were developed to allow continuous flow of materials and separation of cell debris from cell proteins. The continuous pressure of the inflow and the centrifugal force can be adjusted to maximize the separation and outflow of the protein supernatant.

to promote protein precipitation include ethanol, isopropanol, acetone, and diethyl ether. Just like ammonium sulfate, these solvents cause protein precipitation by removing water from between the protein molecules.

Filtration (size-based) separation methods

There are a variety of ways to separate molecules based on size and density. **Centrifugation** separates samples by spinning them at high speed. With this process, proteins can often be isolated in a single layer or separated from heavier cell components. Small-volume centrifuges are capable of processing only a few liters at each run. Large reactors can process hundreds or thousands of liters (see **Figure 4.8**). Industrial-scale centrifugation is normally achieved using continuous-flow centrifuges that allow continuous processing of the contents of a bioreactor.

Filters of various sizes and types can also be used to separate protein from other molecules in the mixture. In this process, known as **membrane filtration,** thin membranes of nylon or other engineered substances with varying pore sizes are used to filter out all of the cellular debris from a solution. First, **microfiltration** removes the precipitates and bacteria. **Ultrafiltration** then uses filters that can catch molecules such as proteins and nucleic acids. Some ultrafiltration processes can even separate large proteins from smaller ones. (Refer to www.amicon.com to view some of these devices.) One of the main shortcomings of membrane filtration systems is their tendency to clog easily. On the plus side, the use of these filtration systems takes less time than centrifugation.

Diafiltration and **dialysis** are filtration methods that rely on the chemical concept of equilibrium, the migration of dissolved substances from areas of higher concentration to areas of lower concentration. As shown in **Figure 4.9**, dialysis depends on the ability of some molecules to pass through semipermeable membranes (osmosis) while others are halted or slowed because of their size. Dialysis is often required to remove the smaller salts, solvents, and other additives used earlier in the purification. The salts are then replaced with buffering agents that help stabilize the proteins during the remainder of the process. Diafiltration adds a filtering component to dialysis.

Chromatography

The initial steps in any purification process liberate a protein from the cell, remove undesired contaminants and particulates, and concentrate the proteins. **Chromatography** methods allow the sorting of proteins by size or by how they cling to, or separate from, other substances. In chromatography, long glass tubes are filled with microscopic resin beads and a buffered solution. The protein extract is then added and flows through the resin beads in a glass column. Depending on the resin used, the protein either sticks to the beads or passes through the column while the beads act as a filtration system.

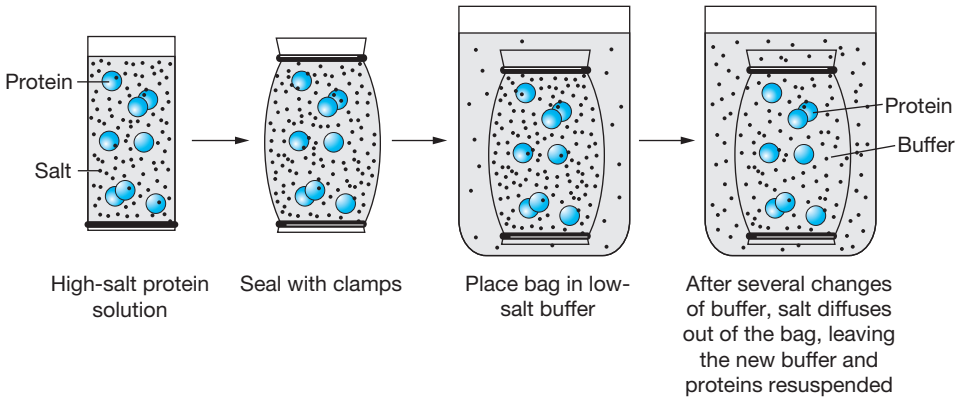


FIGURE 4.9 Dialysis This procedure can be used to remove salt by the process of diffusion and replace it with a buffer that is better suited for protein stability.

Size-exclusion chromatography (SEC) uses gel beads as a filtering system. Larger protein molecules quickly work their way around the gel beads while smaller molecules pass through more slowly because they are able to slip through tiny holes in the beads, as shown in Figure 4.10. The gels are available in a variety of pore sizes, and the necessary gel for proper separation depends on the molecular weight of the contaminants or proteins being separated. This method can make only preliminary separations, however, and can pose problems in industrial settings because it requires very large columns.

Ion-exchange (IonX) chromatography relies on an electrostatic charge (like static cling) to bind proteins to resin beads in a column. While the charged proteins cling to the resin, other contaminants pass through and out of the column, as shown in Figure 4.11. The proteins can then be eluted (released from the resin) by changing the electrostatic charge; this is done by rinsing the column with salt solutions of increasing concentrations. The bound protein is then released from its attachment (detected by viewing under UV 280) and collected.

Affinity chromatography relies on the ability of most proteins to bind specifically and reversibly to uniquely shaped compounds called **ligands.** Ligands are small molecules that bind to a particular large molecule in a protein. Think of ligands fitting with a unique protein molecule the way a key fits a lock (Figure 4.12). After the proteins have bound to the

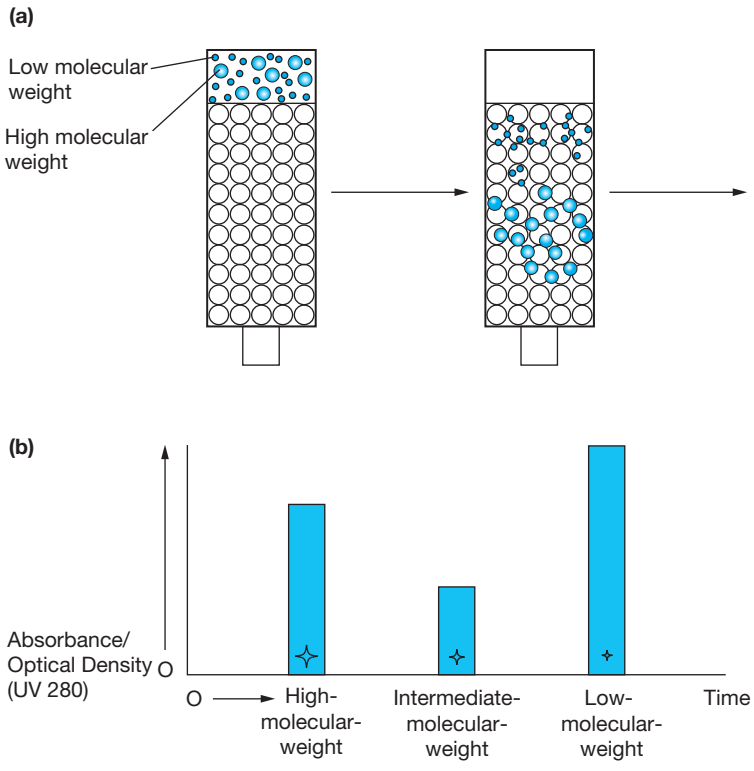


FIGURE 4.10 Protein Purification by Size-Exclusion Chromatography (a) Low- and high-molecular-weight proteins travel through a size-exclusion chromatography column. (b) The diagram shows how the low and high molecular weight proteins appear as they come off the column when monitored for proteins (ultraviolet [UV] 280 nm absorption by aromatic amino acids used for detection). Notice that the high-molecular-weight proteins move quickly through the buffer, whereas the low-molecular-weight proteins are slowed by the matrix of the column resin. Resins may be purchased with many different pore sizes.

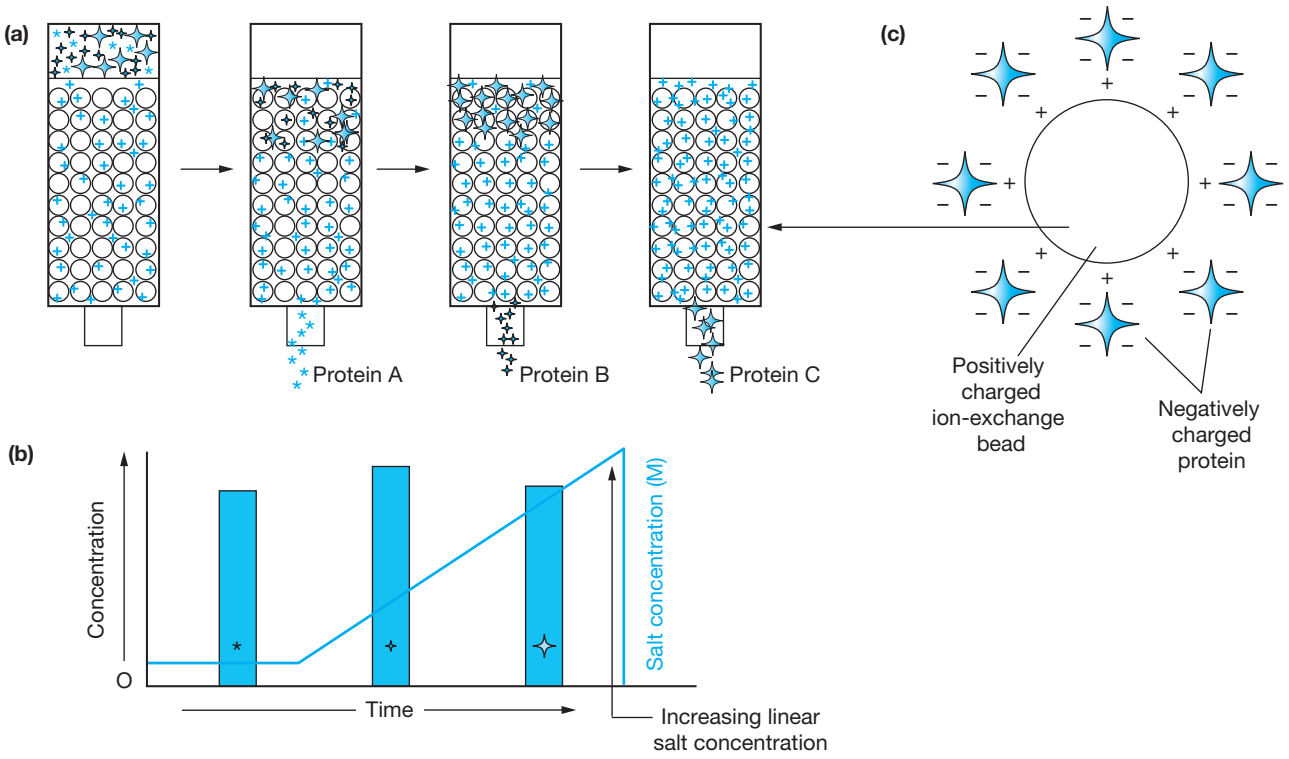


FIGURE 4.11 Ion-Exchange Chromatography (a) Charged amino acids bind to ionic resin beads. Increasing the ionic strength of the buffer displaces proteins (based on their binding strength) after they have bound to the column resin. (b) Protein A is released at the lowest concentration of the displacing salt gradient; protein B has a higher binding strength and is released second; protein C has the highest binding strength and requires a high salt concentration to displace it from the ion-exchange beads of the column. (c) Anion-exchange resin is positive; cation-exchange resins are negatively charged.

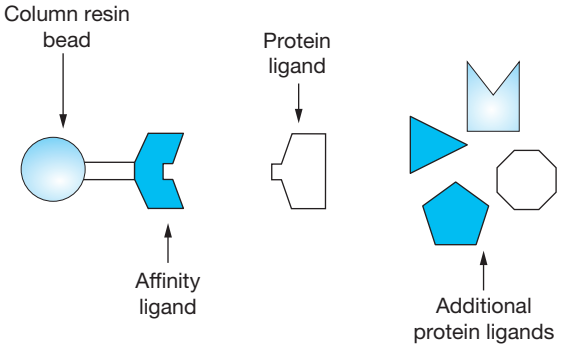


FIGURE 4.12 Affinity Chromatography Affinity ligands are designed to bind specifically to unique 3D chemical components of the protein being purified. The nonbound proteins wash through the column. Increasing the ionic strength of the buffer can displace the bound protein (after preferential binding) and regenerate the affinity column. The displaced (pure) protein can be collected and concentrated. For example, enzyme proteins will bind to column resin with their attached substrate.

resin beads, a buffer solution is used to wash out the unbound molecules. Finally, special buffer solutions are used to cause desorption (to break the ligand bonds) of the retained proteins. Fusion proteins, as mentioned earlier, can be used in affinity chromatography because the substrate (ligand) of the bacterial protein can be part of the affinity column, attracting the fused protein (bacterial enzyme protein) to the column. Affinity chromatography may shorten the purification process by reducing the number of steps.

As we have seen, amino acids are either attracted to or repelled by water molecules. In **hydrophobic interaction chromatography (HIC),** proteins are sorted on the basis of their repulsion of water. The column beads in HIC are coated with hydrophobic molecules, and the hydrophobic amino acids in a protein are attracted to the similar chemicals in the beads, shown in Figure 4.13 on page 141.

Isoelectric focusing is often used in quality control during purification to identify two similar proteins that are difficult to separate by other means. Each protein has a specific number of charged amino acids on its surface in specific places. Because of this unique combination of charged groups, each protein has a unique electronic signature known as its **isoelectric point (IEP),** where the charges on the protein match

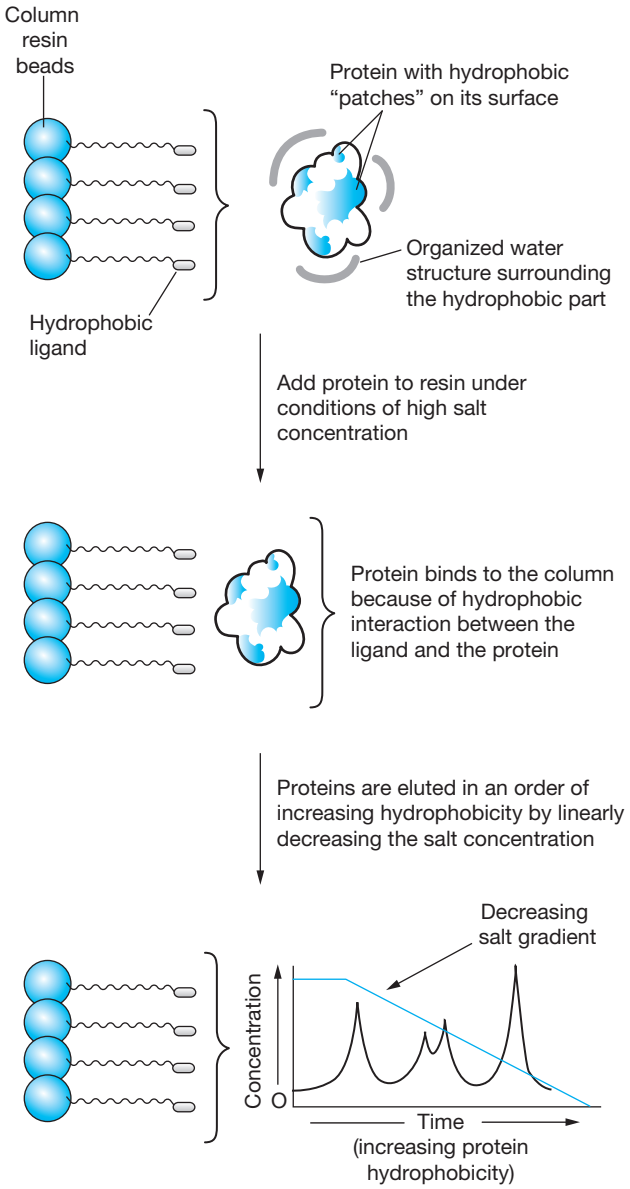


FIGURE 4.13 Hydrophobic Interaction Chromatography Increasing the nonpolar concentration of the buffer can cause the hydrophobic portions of proteins to combine with a hydrophobic ion-exchange-column resin. Further reducing the ionic concentration displaces the protein from the column resin and replaces its attachment with a nonpolar solvent. Fractions can be collected and protein concentration determined based on detection by spectrophotometric analysis at UV 280 nm.

the pH of the solution. The IEP can be used to separate similar proteins from one another. Isoelectric focusing is the first dimension of **two-dimensional electrophoresis.**

Two-dimensional electrophoresis separates proteins based on their electrical charge and size. It is essentially the combination of two methods, IEP and gel electrophoresis. In this technique, researchers introduce a solution of cell proteins onto a specially prepared strip of polymer. When the strip is exposed to an electrical current, each protein in the mixture settles into a layer according to its charge. Next, the strip is aligned with a gel and again exposed to electrical current. As the proteins migrate through the gel, they separate according to their molecular weight.

Analytic methods

High-performance liquid chromatography (HPLC) adds a twist to the previously described chromatography methods, which depend on gravity flow or very low pressure pumps to move the extract through the columns. Such low-flow methods can take several hours to process a single sample. In contrast, HPLC systems use greater pressure to force the extract through the column in a shorter time. HPLC systems have limitations, however. Less protein is separated, so the technique is more useful in analytic situations than in mass production.

Mass spectrometry (mass spec) is a highly sensitive method used to identify small differences between proteins. In fact, it is used frequently on the outflow of HPLC systems. All mass spectrometers do three things: suspend the sample molecules into a charged gas phase, separate the molecules based on their mass-to-charge ratios by acceleration down a narrow tube, and finally detect the separated ions. A sample as small as one picogram (one-billionth of a gram) can be analyzed by this process, which is illustrated in Figure 4.14. A definitive readout is produced, which indicates the identity and size of most of the proteins or fragments analyzed.

An important application of this process is in protein sequencing. A larger protein can be digested into fragments (peptides) and analyzed to determine the amino acid sequence. Mass spectrometry is now the preferred method and, in biotech companies, has largely replaced the slower Edmond end-group analysis method used for amino acid sequencing. Mass spectrometry can detect the difference between isomers of the same protein, and its capabilities are improving faster than most lab instruments in terms of critical analysis.

Fast protein liquid chromatography (FPLC) is different from mass spec, and is often used to analyze or purify mixtures of proteins. As in other forms of chromatography, separation is possible because the different components of a mixture have different affinities for two materials, a moving fluid (the "mobile phase") and a porous solid (the stationary phase). In FPLC the mobile phase is an aqueous solution, or "buffer." The buffer flow rate is controlled by a positive-displacement pump and is normally kept constant, while the composition of the buffer can be varied by drawing fluids in different proportions from two or more external reservoirs. The stationary phase is a resin composed of beads,

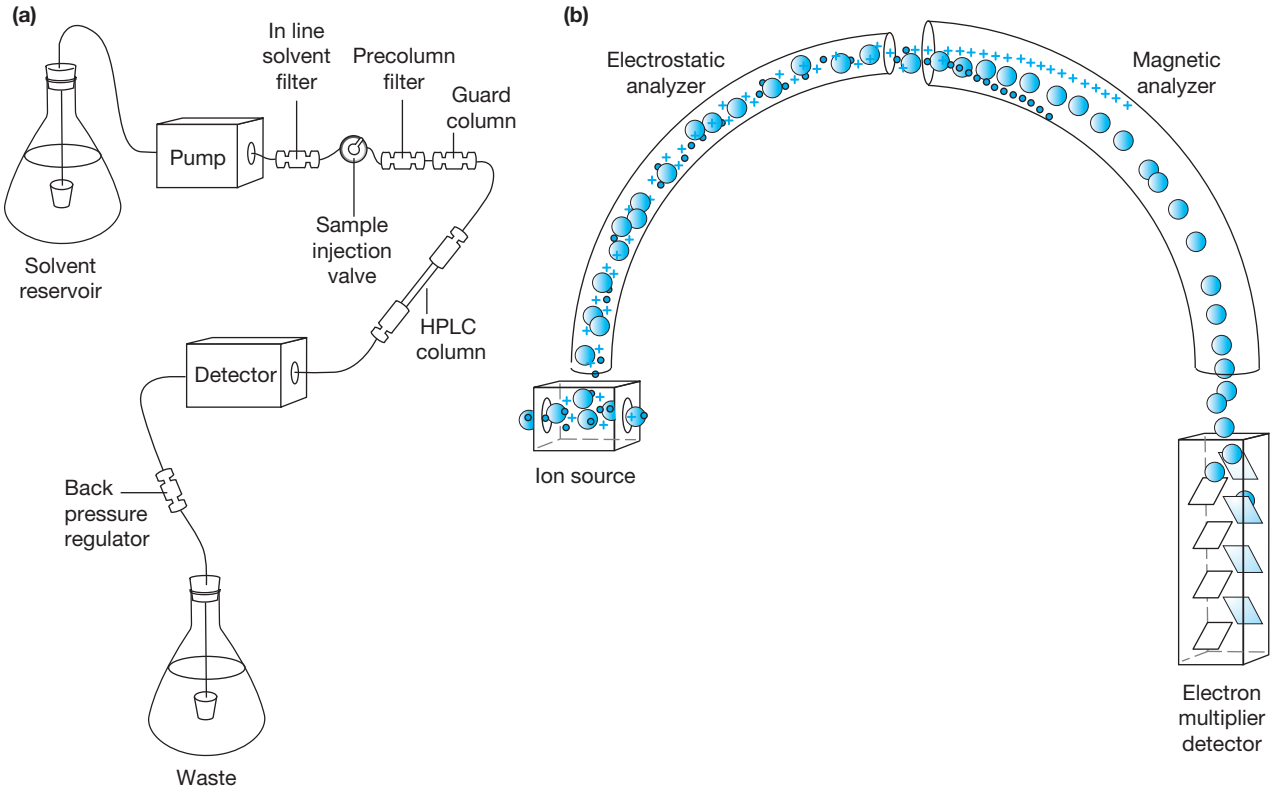


FIGURE 4.14 High-Performance Liquid Chromatography and Mass Spectrometry
High-performance liquid chromatography is often coupled with mass spectrometry to analyze proteins. (a) HPLC uses noncompressible resin beads under very high pressures to separate proteins that are often similar in size. (b) Mass spectrometry follows this initial separation to detect subtle differences in ionized and accelerated proteins that will be analyzed (by distance or time) as they travel down a vacuum-filled tube.

usually of cross-linked agarose, packed into a cylindrical glass or plastic column. FPLC resins are available in a wide range of bead sizes and surface ligands, depending on the application. The correct balance of buffer and ligand will bind the protein of interest.

Verification

At each step of the experimental purification process, it is important to verify that the target protein has not been lost and that concentration efforts have been successful. **SDS-PAGE** (polyacrylamide gel electrophoresis) is often used for verification. In this method, a detergent called sodium dodecyl sulfate (SDS) is added to a sample of the protein mixture and the mixture is heated. The sulfate charges are distributed evenly along the denatured (heated) protein, making separation dependent on the size of the protein. After this treatment, the protein sample is loaded onto a special gel matrix (PAGE), where it forms a single band at a specific location, depending on its molecular size and mass, as seen in **Figure 4.15**.

By adding a dye that combines with proteins, such as **Coomassie stain,** a colored band results, and a known size marker can be compared with the stained sample. When the sample and the known marker are equivalent, we have evidence that the protein of interest is indeed being concentrated. Since this test is run at each step in the purification process, the colored band should become increasingly intense, proving that the protein has not been lost.

A specific detection method for proteins separated by SDS-PAGE is Western blotting (similar to Southern blotting for DNA in Chapter 3). In Western blotting, protein is transferred from a gel to a nitrocellulose membrane and detected with a specific antibody that recognizes that protein by its unique structure. The nitrocellulose membrane binds all proteins. Once your proteins are transferred, you must "block" the rest of the membrane with non-reactive proteins. This allows you to apply the antibody (also a protein) so that it will not bind non-specifically to the membrane wherever it is not covered by a transferred protein. An enzyme

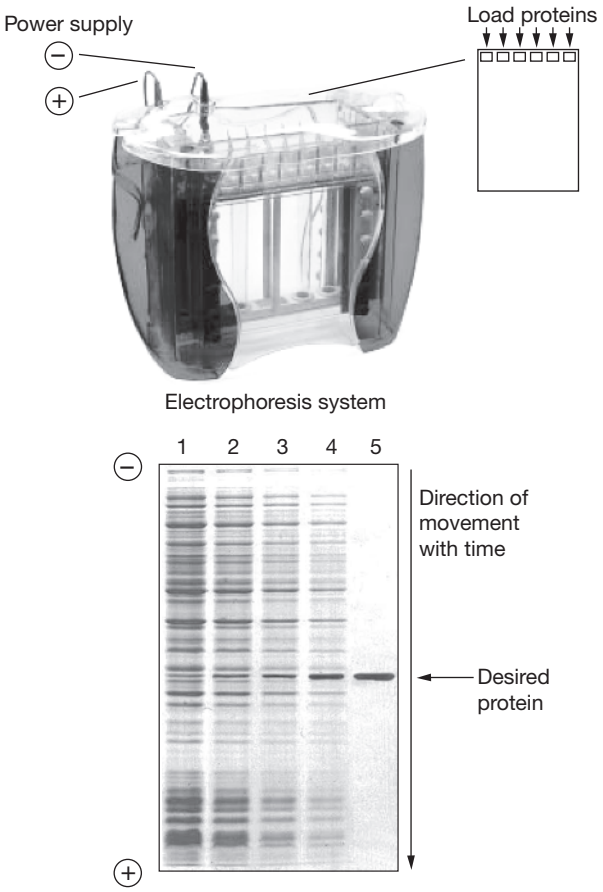


FIGURE 4.15 SDS-PAGE Polyacrylamide gel electrophoresis of proteins that have uniform charges (produced by heating in sodium dodecyl sulfate) can be separated by size, based on their migration in a vertical electrical field. This procedure commonly follows each major step in purification to verify that the band of desired protein is becoming more concentrated (and has not been lost in the separation). Notice that the 65-kilodalton (kD) protein has the highest concentration in the final step (lane 2) of this procedure (lane 5) when proteins of known size are used for comparison. The smallest proteins will reach the + pole first.

attached to the antibody can be used to convert a fluorescent substrate so as to produce a permanent record of the detection. In this procedure, an electric current is applied to the gel. The separated proteins then migrate through the gel and onto the membrane in the same pattern as they separate on the SDS-PAGE. All sites on the membrane can then be blocked in such a way that antibody (serum) will not bind to them nonspecifically to detect the antigen blotted on the membrane; a primary antibody (serum) is then added at an appropriate dilution and incubated with the membrane. If there are any antibodies present that are directed against one or more of the blotted antigens, those antibodies will bind to the protein(s) while other proteins will be washed away at the end of the incubation. To detect the antibodies that have bound, anti-antibodies (or secondary antibodies) coupled to a reporter group, such as the enzyme alkaline phosphatase, are added. Finally, after excess secondary antibody is washed free of the blot, a substrate is added that precipitates upon reaction with the conjugate, resulting in a visible band where the primary antibody bound to the protein (see Figure 4.15).

Antibody detection of a specific protein can also be carried out using an enzyme-linked immunosorbent assay (**ELISA**). The most commonly used ELISA requires two antibodies: one to capture the unique protein and one attached to an enzyme to produce a color reaction (see Figure 4.12). ELISA occurs on a multiwell plate using affinity chromatography. The first antibody to the unique protein is plated onto a multiwell ELISA plate, the protein is added, and—after a series of wash and blocking steps—the second antibody (enzyme attached) is added. The addition of a substrate permits a color reaction to occur if the antibody has bound. ELISA plates are designed to capture many important proteins currently under research.

Preserving Proteins

After the target protein has been isolated, collected, and purified, it must be saved in a manner that will preserve its activity until it can be used. One means of preserving the protein is **lyophilization,** or freeze-drying. In this process, a purified liquid protein solution is frozen. A vacuum is then used to hasten the evaporation of water from the fluid. In lyophilization, ice crystals are converted directly to water vapor without melting into liquid water first. The containers of freeze-dried material are sealed after the water is removed, leaving the dried proteins behind. Lyophilization has been demonstrated to maintain protein structure, making it a commonly chosen method for the preservation of biotechnologically derived proteins. Many freeze-dried proteins may be stored at room temperature for prolonged periods.

Scaling Up Protein Purification

Protein purification protocols are usually designed in the laboratory on a small scale. These techniques at this level of productivity are feasible if the product is needed in only small amounts. Orders of monoclonal antibodies, for example, are in the range of grams per year—a relatively small demand. A single laboratory-scale bioreactor can usually produce adequate quantities. However, other proteins are required in much larger quantities, which means that the production methods must be scaled up.

Scaling up is not always easy to accomplish. Laboratory methods that may work on a small scale may not always be adaptable to large-scale production. Furthermore, changes in the purification process can invalidate earlier laboratory-scale clinical studies. For example, when the FDA approves a bioengineered protein, it also approves the process for producing it. To change the process, it may be necessary to seek FDA approval once again. For this reason, bioprocess engineers are involved in the early stages of purification and work to ensure that it will be possible to scale up the process later on.

Postpurification Analysis Methods

During research, it is often helpful to look closely at a purified protein. The goal may be to better understand the molecular structure of a specific protein or to alter its structure to change the protein's function. The following two methods are used in the postpurification analysis of proteins.

Protein sequencing

Recall from our discussion of protein structures that each protein has a specific sequence of amino acids, known as the primary sequence. To understand a protein completely, it is important to determine its primary sequence. Automated protein sequencers make this task possible (see Figure 4.14). In the mass spectrometry method, peptide masses are identified by their unique signatures (retention times). By converting peptides into ions and subjecting them to acceleration in a vacuum, it is possible to identify many unique proteins in a matter of minutes.

X-ray crystallography

X-ray crystallography is used to determine the complex tertiary and quaternary structures of proteins. The method requires pure crystals of proteins that have been carefully dehydrated from solutions. Bombarded with x-rays, a pure protein creates specific shadow patterns based on its configuration. Computer analysis of these patterns allows the generation of "ribbon" diagrams, which not only describe the structure of the proteins but also make it possible to plan potential modifications of the protein molecule to improve function. Protein crystallography is usually a requirement for approval by the FDA because it verifies that the process produces the product being approved.

Cryo-EM creates images of proteins

For decades x-ray crystallography has been used to obtain images of macromolecular structures, especially 3D images of proteins. Recent technological improvements in instrumentation are now allowing researchers to use **cryo-electronic microscopy** (cryo-EM) to achieve near atomic resolution of complex protein structures. After applying a purified protein to a scaffold lattice supported by a metal frame, the 3D proteins fill the holes in different orientations. Next the grid is flash frozen in a vacuum for the scanning EM. With software, numerous two-dimensional (2D) images in different orientation can be used to create a 3D structure and provide more detailed structural information (and win the three researchers who developed it the Nobel Prize in Chemistry in 2017). In the words of one of the researchers, Richard Henderson, "now we can see the intricate details of the biomolecules in every corner of our cells."

4.4 Proteomics

Many diseases are the result of flaws in protein expression, but not all of those diseases can be understood simply in terms of genetic mutation. Because proteins undergo post-translational modification, the puzzle can be far more complicated. A new scientific discipline, **proteomics,** is dedicated to understanding the complex relationship of disease and protein expression.

In proteomics, **proteomes** (the PROTEin complement to a genOME) are compared between healthy and diseased states. The variations of protein expression are then correlated to the onset or progression of a specific disease. The goal of proteomic research is the discovery of protein markers that can be used in new diagnostic methods and the development of targeted drugs for treating disease. For example, scientists at the University of California, Davis, purified the protein produced by *BRCA2*, an oncogene linked to breast cancer. The researchers were then able to synthesize the protein to study *BRCA2's* role in breast cancer. *BRCA2* is susceptible to the drug trastuzumab (Herceptin; Genentech, San Francisco), which has increased the breast cancer survival rate to nearly 70 percent. Herceptin use is dependent on the presence of an overexpressed *HER2* receptor, which is not always the case in a *BRCA2* mutation (*BRCA1, BRCA2,* and *HER2* negatives are insensitive to trastuzumab). Herceptin targets an outer membrane growth signal receptor, and not the DNA repair complex. In this way, only patients with specific *BRCA2* biomarkers receive trastuzumab, saving discomfort and unnecessary treatment of all breast cancer patients.

Although the *BRCA2* gene was discovered in 1994, purifying the protein made by the gene proved

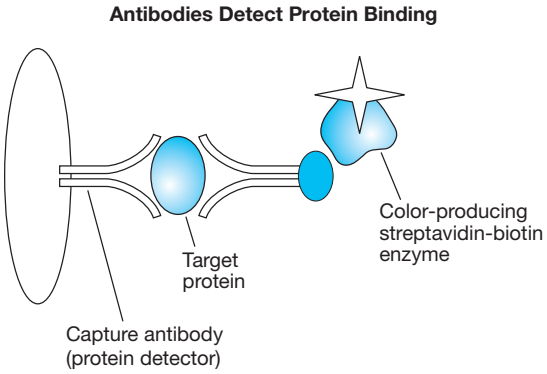


FIGURE 4.16 Protein Microarrays Protein microarrays that uniquely bind to single proteins are being perfected. When a protein binds, it releases a signal as the enzyme bound to the interacting protein degrades a substrate. Enzyme-linked immune sorbent assays (ELISA) bind protein targets in this way for detection.

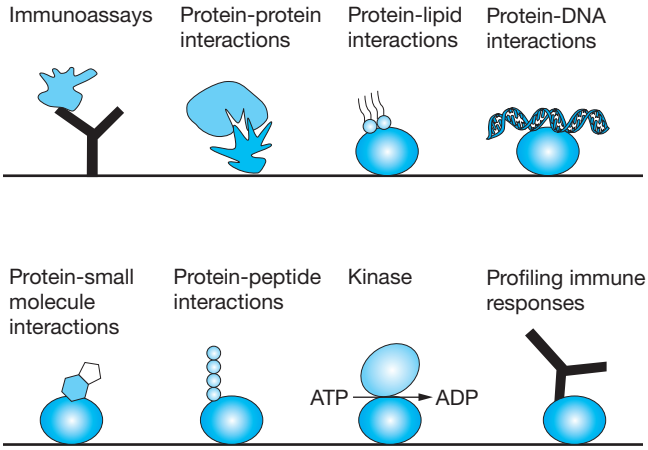


FIGURE 4.17 Protein Microarrays Can Detect More Than Just Proteins The capacity of protein microarrays has increased. Functional protein microarrays have recently been applied to the discovery of protein interactions (i.e., protein-protein, protein-lipid, protein-DNA, and protein-drug interactions), expanding knowledge of the significance of protein interactions to normal cell function. Binding antibodies or other proteins to the matrix allows detection when the binding allows a colored product to be produced.

difficult, since it is very large, does not express well, and degrades easily. The U.C. Davis researchers tested many different cell lines and succeeded in introducing a *BRCA2* gene into a human cell line and expressing it as a whole protein. Other researchers used genetic engineering techniques to manufacture the human protein in yeast. They then tested the purified protein for its function in repairing damaged DNA. Experiments with the *BRCA2* protein confirmed that it plays a role in repairing damaged DNA, and when it is damaged in breast cancer, the DNA fails to function. Research continues on the function of BRCA2 and other proteins involved in breast cancer, but this progress would not have been possible without proteomics.

Proteomics applies several techniques described earlier: two-dimensional electrophoresis is used to separate proteins, mass spectrometry verifies the protein's identity, and the protein is characterized using amino acid sequencing. Some of this labor-intensive work may be assisted by automation in the future. A **protein microarray** is a set of proteins immobilized on a surface—usually a glass slide—which has been coated with a reagent that will indicate binding by color change (**Figure 4.16**). The capacity of these arrays has increased but does not rival that of DNA arrays because of the difficulties of production. A single DNA array can monitor the expression of an entire genome. Functional protein microarrays have recently been applied to the discovery of protein interactions: protein-protein, protein-lipid, protein-DNA, and protein-drug interactions (**Figure 4.17**).

MAKING A DIFFERENCE

Protein (e.g., drug) delivery has improved with nanoparticles. Nanoparticles interacting with long polypeptide chains for drug delivery have been the direction for some time, but have had the drawback of needing to be implanted surgically. To combat this problem, hydrogels have been designed to deliver drugs. Owing to their tunable properties, hydrogels can be molded into specific shapes and designed to release their payload over a specified time period. They are made up of two components, one is a type of nanoparticle formed of polyethylene glycol (PEG)-peptide polymers mixed with another polymer, cellulose. Using two components to form the gel provides the opportunity to deliver two different drugs at the same time. These nanoparticles have an inner core that is ideally suited to carry hydrophobic small-molecule drugs, which include many chemotherapy drugs. Meanwhile, the polymers, which exist in a watery solution, can carry hydrophilic molecules such as proteins, including antibodies and growth factors. One drawback of hydrogels is the high concentration of polymeric constituents that is required in the formulation. This can create poor resilience, resulting in a strong 'burst' release effect of drugs. In order to circumvent this limitation, Cambridge University researchers created a self-assembled hydrogel with very high water content (up to 99.75 percent) that has improved biocompatibility for sustained drug release.



TOOLS OF THE TRADE Diagnostic Proteomics

Proteomes, the collection of proteins associated with a specific life function, have become more important since the discovery of the human genome. As mentioned earlier, the cost of bringing a drug to market is about $2.6 billion and takes between 5 and 11 years. Because most of the drugs produced by the biotechnology industry are proteins (such as growth factors, antibodies, and synthetic hormones) that replace missing or nonfunctional proteins in humans, companies are particularly interested in developing less expensive and faster methods of detecting how proteins function in disease detection and treatment—specifically, new protein microarrays. Like DNA microarrays, these miniature devices detect proteins associated with disease and those present in abnormal concentrations. As new protein structures are identified, new microarrays are constructed.

Protein microarrays have many uses. Most current biotech drugs function at the protein level, interacting with receptors, triggering events, and targeting other proteins in cells of the body. The detection of biomarker proteins using microarrays has improved the monitoring of drug action in many diseases. For example, we have all experienced the benefit of vaccines: proteins that stimulate our immune systems to recognize disease organisms without our having to contract the disease. Antibody production in response to vaccination is an example of the effect of a protein biomarker and can indicate positive immunity.

Until recently, acceptance of proteomics as a discovery method for diagnostics has been slow. Recent advancements in technology, however, allowed the measurement of enough proteins to make diagnostic proteomics a viable option. As with whole genome sequencing, developing proteomic tests requires computing vast amounts of data to find the signal in an ocean of proteins that will allow development of bioinformatics tools to aid in the search. The results of proteomics can now be interpreted on a large scale and used to draw conclusions about a disease in subgroups of patients. One of the early fields to benefit is oncology, with both diagnostics and targeted medicines improving the patient's quality of life. The ability to measure a multitude of different proteins from a single source lagged behind the ability to measure genetic changes. What was needed was the ability to measure thousands to hundreds of thousands of proteins simultaneously. Scientists overcame some of these challenges using highly sensitive mass spectrometry and software that provides reliable analytic data.

Coupling mass spec with the advanced analytic approaches developed for highly complex data enables scientists to discover clinically relevant disease classifiers much faster than was previously possible. Identifying patient subgroups with serum-based mass spec protein signatures resulted in predictive outcomes for cancer patients. As the knowledge of a patient's immune response to a specific cancer tumor is measured, clinicians are beginning to look at how it can be boosted to fight the disease—another essential key to precision medicine. This technology can also screen out nonresponders before they are subjected to treatments that won't modify their disease. It improves patients' risk-benefit profile and allows clinicians to focus on therapies that will improve their quality of life. The potential of proteomics to benefit patient care is being recognized by insurance companies as well as National health insurance programs such as Medicare in the United States and the National Health Service (NHS) in the United Kingdom.

They observed a highly sustained release of protein for 160 days. This proved the use of this material as a potential candidate for sustained therapeutic use.

QUESTIONS & ACTIVITIES

Answers can be found in Appendix 1.

1. While working with your favorite organism you discover that it has an enzyme capable of digesting a unique substrate. How would you use the public protein database to begin to determine whether your enzyme is unique?
2. You are tasked with producing an enzyme to be used in a new "steam washing machine" that will withstand 100°C temperatures. You want to start

with a bacterium from The Great Geysir in Iceland that has a similar enzyme, but you need to increase its substrate activity and temperature tolerance. Describe how you would do this using directed molecular evolution.

3. The metalloprotein hemocyanin has shown that it can be replaced by scaffolded histidines in the active site of an artificial enzyme. How does this artificial enzyme discovery add to the knowledge of the 3D enzyme structure?
4. Patents are not allowed on proteins produced from "natural DNA." How could you use the amino acid sequence of a protein to produce a DNA sequence that was patentable?
5. How is the study of prion protein structure likely to lead to discoveries that will benefit treatment or prevention of diseases like Alzheimer's disease?
6. Under what circumstances would the use of animal bioreactors be preferable to industrial bioreactors for unique protein production?
7. How would you set up an affinity column that preferentially bound an enzyme that you wanted to purify?
8. List the protein separation methods primarily used in analytic protein identification rather than in protein purification.
9. How would your knowledge of a protein's isoelectric point (pI) help you to select the type of column chromatography to use for purification?
10. If a PhARMA company discovers a chemical (structure) that can be used to treat a disease, how could a biotech company provide an economical protein that could serve the structural need more economically?
11. Why do you think that artificial enzymes (XNAzymes) are not degraded as quickly as natural enzymes in biological systems?
12. Access the human proteome site: **www.humanproteomemap.org**. Select "query" (deselect "Adult tissues" and "Hematopoietic cells" and only select "Fetal heart") and watch it provide the results. Select EGFR. What do the letter sequences mean? What do the colors ranging from green to red on the top bar signify?
13. Explain how the cryo-EM can verify that a protein structure predicted from the amino acid sequence has a high degree of structural similarity to a naturally occurring protein?
14. What types of DNA or RNA sequences would you expect to find in a smart bandage designed to treat an infection? How would it work?
15. The yeast two-hybrid system (Chapter 3) is one of the oldest protein interaction measuring techniques. Explain how it can be used to determine if two proteins interact for a cellular event.
16. Disulfide bond formation is an example of post-translational modification that commonly occurs to produce the 3D structure of proteins. Which amino acids are most likely to participate in PTM?
17. How would you use your knowledge that a PRNP mutation caused abnormal protein folding (prions) in a mice model study? How would you detect the misfolding in the brain at an early stage?
18. Knowing that the bacterium *B. subtilis* secretes proteins and avoids the problems of refolding proteins produced in bacterial bioreactors, how would you determine if it was a good candidate for a protein that you wished to produce in quantity?
19. If you had used a protein precipitating agent like ammonium sulfate to precipitate all the proteins in a crude extract, how would you reestablish the structure of your target protein?
20. Some ELISA systems use multiple attachment sites on the primary antibody for attachment of the secondary antibody. How does this improve detection?

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The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

CASE STUDY

Tau-Tau Protein Interaction in Alzheimer's Disease Study

It has been established that disease forms of tau protein (hyperphosphorylated, aggregated, and truncated) are a major cause of dementia (Alzheimer's disease, or AD). There is a link between two AD pathologies, tau and amyloid plaques (with tau pathology being downstream to amyloid pathology), yet tau pathology can develop and occur independently of amyloid plaques. There is direct evidence for tau pathology developing independently of amyloid, and being sufficient to cause dementia and neurodegeneration. These findings supported the concept that amyloid toxicity is tau-dependent and that blocking or reducing the pathological effects of tau may be protective against the harmful effects of amyloid pathology. Immunotherapy in animals through vaccine development has recently taken the lead in research.

In the human brain, the tau proteins constitute a family of six isoforms with the range from 352 to 441 amino acids. Tau is a phosphoprotein with 79 potential serine (Ser) and threonine (Thr) phosphorylation sites on the longest tau isoform. Phosphorylation has been reported on approximately 30 of these sites in normal tau proteins. Hyperphosphorylation of the tau protein (tau inclusions, pTau) can result in the self-assembly of tangles of paired helical filaments and straight filaments, which are involved in the pathogenesis of Alzheimer's disease. Results of one study of tau peptide in rats are shown in the following figure.

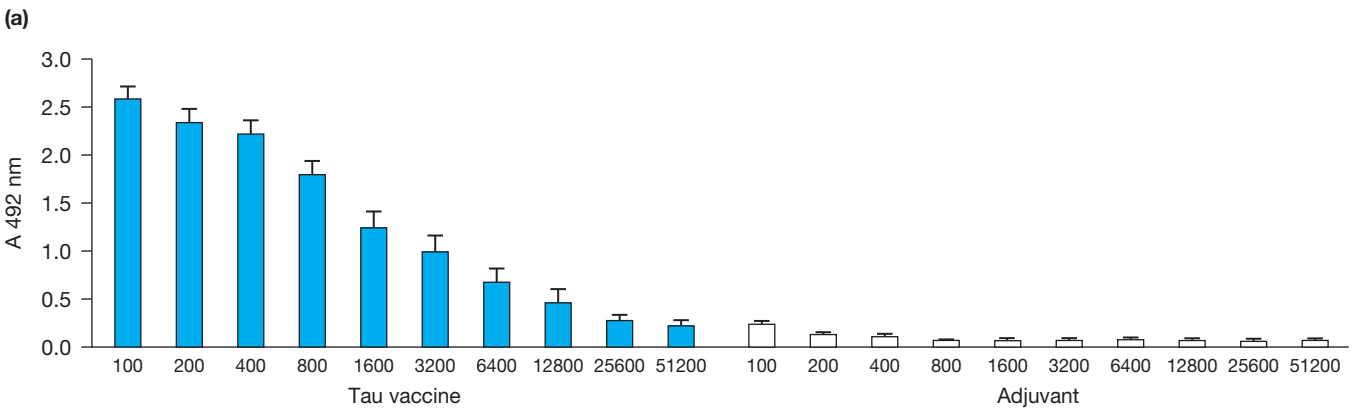
Serial dilutions (1:100 to 1:51,200) of each serum were tested against the synthetic peptide ^{294}KDNIKHVPGGGS^{305}, against mis-disordered tau (151-391/4R), and against physiological tau 2N4R using indirect ELISA.

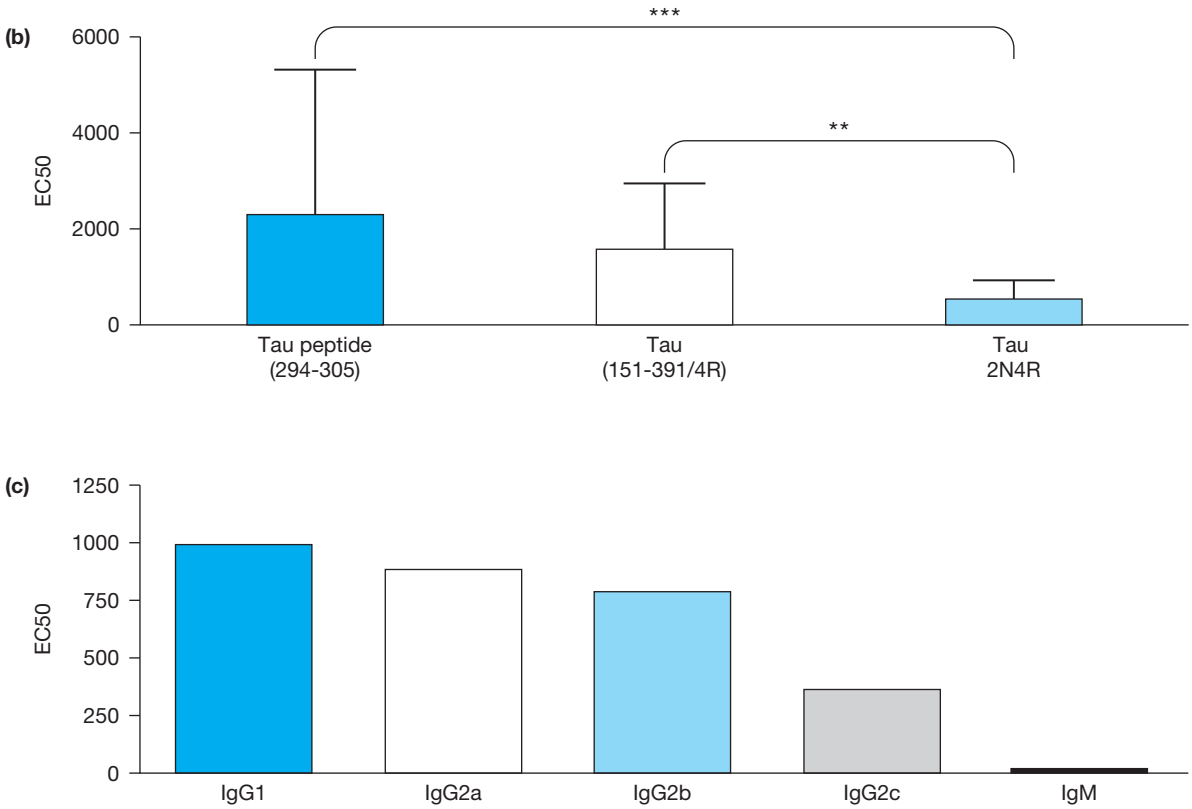
Questions

Answers can be found at www.pearsonglobaleditions.com

1. What do the letters ^{294}KDNIKHVPGGGS^{305} refer to? And why were they tested?
2. Why were the results compared between immune response to tau isoforms and the response to adjuvant?
3. Why were serial dilutions of the tau antigens tested?
4. How was ELISA used to test the antibody response?

Source: Rosenmann H, Blum D, Kayed R, Ittner, LM. Tau protein: function and pathology. *Int J Alzheimers Dis.* 2012;2012:707482. Published online August 15, 2012. doi:10.1155/2012/707482.





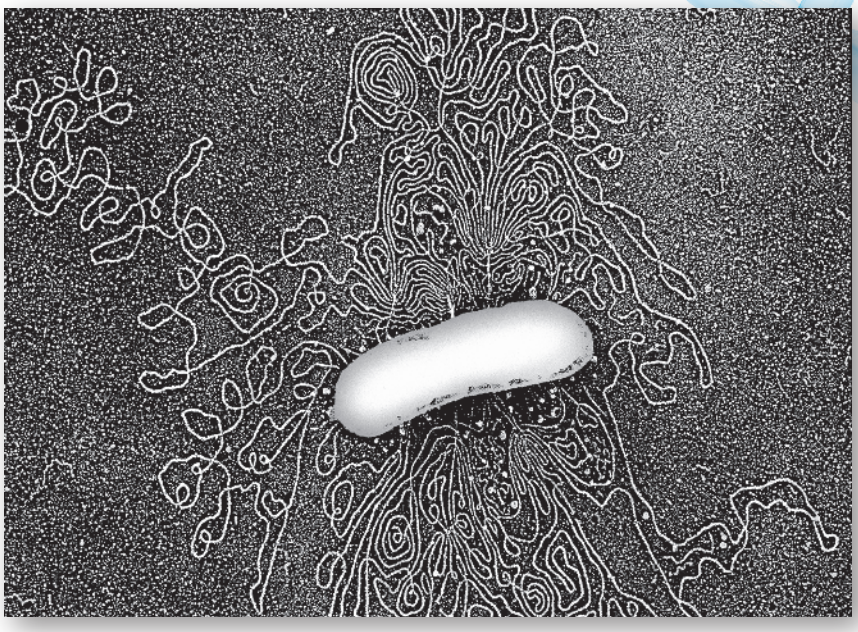
Antibody Response in Transgenic Rats Immunized with Tau Peptide Vaccine Determined by Enzyme-Linked Immunosorbent Assay Tau peptide vaccine induces high antibody levels specific to the tau peptide ^{294}KDNIKHVPGGGS^{305}. No antibody response was observed in the mice immunized with adjuvant only. Data shown are serial twofold dilutions of animal sera (a). Antibodies elicited by vaccination exhibited statistically significantly higher binding activity to tau peptide and to mis-disordered tau (151-391/4R) than to full-length tau 2N4R (***$P = 0.0003$ and **$P = 0.0028$, respectively) (b). EC_{50}, Half-maximal effective concentration. Vaccine-induced antibodies specific to mis-disordered tau are prevalently of the immunoglobulin G (IgG) isotype (c). The data shown are means with error bars representing SD.

CHAPTER FIVE

Microbial Biotechnology

After completing this chapter you should be able to:

- Describe features of bacteria that make them useful for biotechnology.
- Appreciate the importance of microbial biotechnology in creating products that we use on a daily basis.
- Explain how alcohol and lactic acid fermentation is used to produce common foods and beverages.
- Provide examples of valuable proteins that are produced in bacteria using recombinant DNA technology.
- Describe the role microorganisms play in the development and production of antibiotics and vaccines; provide examples of different types of vaccines, and appreciate important infectious disease targets for new vaccine development.
- Discuss why studying microbial genomes is valuable.
- Understand why scientists are interested in synthetic genomes and synthetic biology for biotechnology applications.
- Explain metagenomics; describe major goals and findings of the Human Microbiome Project.
- Describe applications of microbial diagnostics for detecting infectious disease outbreaks.
- Understand roles that biotechnology can play in combating bioterrorism.
- Consider how microbes may be used to produce biofuels in the future.



Microbes, such as the *Escherichia coli* (*E. coli*) cell shown in the center of this photo, have a long history of applications in biotechnology. The cell wall and plasma membrane of this cell have been burst open to reveal the bacterial chromosome.

Just when Italian art restorers were at a loss to save one of the world's largest collections of fourteenth- and fifteenth-century medieval frescoes (paintings done on plaster) decorating a cemetery in Pisa near the Leaning Tower, soil bacteria called *Pseudomonas stutzeri* came to the rescue. Restorers were trying to clean the frescoes of paint-clouding grime contained within glue that had been used to repair them after damage in World War II. Traditional chemical reagents only further damaged the paintings. Knowing that *P. stutzeri* could degrade nitrogens, plastic resins, and pollutants, scientists cultured a strain of these microbes in the lab and applied them to the frescoes. Within 6 to 12 hours, the bacteria had cleaned approximately 80 percent of the glue residue and the remaining residue was removed with a light washing.

Microorganisms, or **microbes,** are tiny organisms too small to be seen individually by the naked eye; they must be viewed with the help of a microscope. Although the most abundant microorganisms are bacteria, microbes also include viruses; fungi, such as yeast and mold; algae; and single-celled organisms called protozoa. Bacteria have existed on the earth for over 3.5 billion years, and they greatly outnumber humans. It has been estimated that microbes constitute over 50 percent of the earth's living matter. Yet less than 1 percent of all bacteria have been identified, cultured, and studied in the laboratory. We are literally surrounded by bacteria. They live on our skin, in our mouths, and in our intestines; they are in the air and on virtually every surface we touch. Bacteria have also adapted to live in some of the harshest environments on the planet: polar ice caps, deserts, boiling hot springs, and under extraordinarily high pressure in deep-sea vents miles under the ocean's surface.

The rich abundance of bacteria and other microbes provides a wealth of potential biotechnology applications. Well before the development of gene-cloning techniques, humans used microbes in biotechnology. In this chapter, we primarily discuss the important roles bacteria have played in both old and new practices of biotechnology.

FORECASTING THE FUTURE

Speculating on the future directions of microbial biotechnology is not particularly easy because there are so many active areas of research with great potential. Two areas of great intrigue are synthetic genomes and biofuels. Research involving engineering microbes with synthetic genomes is progressing rapidly. What will be some of the future applications of this technology and of the field of synthetic biology? For biofuels, there is a great need to reduce our global dependence on fossil fuels and generate alternative energy sources. Can microbes be used in metabolic processes to create fuels or to break down materials to release components that can become fuels in a way that is effective and cost-efficient for widespread use? Microbial biotechnology is a dynamic field that is producing many new applications. Ongoing drug development to combat infectious diseases (which can potentially be advanced in new directions by synthetic biology) and using microbes as "bots" to deliver drugs are topics to keep an eye on. We could also forecast several other topics as hot areas of microbial biotechnology in the future, but we think it will be particularly interesting to keep an eye on developments in synthetic biology and biofuels.

5.1 The Structure of Microbes

Before you can consider the many applications of microbial biotechnology, it is important that you can distinguish microorganisms from plant and animal cells. Recall that cells can be broadly classified as eukaryotes or prokaryotes based on the presence or absence, respectively, of the nucleus that contains a cell's DNA (Chapter 2). *Eukaryotic cells* include plant and animal cells; fungi (such as yeast); algae; and single-celled organisms called protozoans (such as amoebas). Unlike eukaryotic cells, *prokaryotic cells* lack most membrane-bound organelles, including a nucleus. Prokaryotes include the **domains** (taxonomic categories above the kingdom level) **Bacteria** and **Archaea**—single-celled prokaryotic microorganisms, but not bacteria, with properties unique from those of eukaryotes and bacteria. The cellular structure of microorganisms is important in determining both where they will thrive and how they can be used in biotechnology. For example, archaea that live in extreme environments such as very salty conditions are called halophiles or hot environments are called thermophiles; as a result, they have very unique metabolic properties.

Moreover, many structural features of bacteria make them excellent microorganisms to use for biotechnology research and applications. Bacterial cells are much smaller (1 to 5 micrometers, or μm; 1 μm = 0.001 millimeter) than eukaryotic cells (10 to 100 μm) and have a much simpler structure (refer to Figure 2.1 for a diagram of prokaryotic cell structure). Bacterial cells also exhibit the following structural features:

- DNA is not contained within a nucleus and typically consists of a single circular chromosome that lacks histone proteins.

- Bacteria may contain plasmid DNA.
- Bacteria lack membrane-bound organelles.
- The cell wall that surrounds the plasma membrane is structurally different from the plant cell wall. Composed of a complex polysaccharide and protein substance called **peptidoglycan,** the cell wall forms a rigid outer barrier that protects the cells and determines their shape. In archaea, this structure does not contain peptidoglycans.
- Some bacteria contain an outer layer of carbohydrates, which form a structure called the *capsule.*

Most bacteria are classified by the **Gram stain,** a technique in which dyes are used to stain the cell wall of bacteria. Gram-positive bacteria stain purple and have simple cell wall structures rich in peptidoglycans, whereas Gram-negative bacteria stain pink and have complex cell wall structures with less peptidoglycan. Bacteria do not form multicellular tissues like animal and plant cells, although some bacteria can associate with each other to form chains, filaments, or layers (biofilms, discussed later in the chapter) of many connected cells.

Bacteria vary in their size and shape. The most common shapes include spherical cells called **cocci** (singular, coccus), rod-shaped cells called **bacilli** (singular, bacillus), and corkscrew-shaped or **spiral** bacteria (**Figure 5.1**). As you study microbes, you will learn that the names of bacteria frequently give you a tip about the shapes of those cells. For instance, *staphylococci* are spherical bacteria that live on the surface of our skin. These grow in bunches likes grapes, which reflects their naming from a Greek term referring to bunches of grapes.

The single circular chromosome that constitutes the genome of most bacteria is relatively small. Bacterial chromosomes average in the range of 2 million to 4 million base pairs in size, compared with 200 million base pairs for a typical human chromosome. As you learned in Chapter 3, some bacteria also contain plasmids in addition to their chromosomal DNA. Plasmid DNA often contains genes that are not essential for life—for example, genes for antibiotic resistance and genes encoding proteins that form connecting tubes called *pili,* which allow bacteria to exchange DNA between cells (see Figure 2.1). As you know, commercially available plasmid DNA is an essential tool for molecular biologists because it can be used in various ways for recombinant DNA experiments.

Bacteria grow and divide rapidly. Under ideal growth conditions, many bacterial cells divide every 20 minutes or so, whereas eukaryotic cells often grow for 24 hours or much longer before they divide. Therefore, under favorable growth conditions in the laboratory, a small population of bacteria can divide rapidly to produce millions of identical cells. Because bacteria are so small, millions of cells can easily be grown on small dishes of agar or in liquid culture media. When grown on culture plates, each bacterial cell typically divides to form circular colonies that contain thousands or millions of cells (**Figure 5.2a**). For many applications in biotechnology, bacteria are often grown in fermenters that can hold several hundred or thousand liters of liquid culture medium (**Figure 5.2b**).

It is also relatively easy to make mutant strains of bacteria that can be used for molecular and genetic studies. Mutants can be created by exposing bacteria to x-rays, ultraviolet light, and a variety of chemicals that mutate DNA (mutagens), as well as by gene editing using CRISPR (Clustered Regularly Interspaced Palindromic Repeats)–Cas. Thousands of mutant strains are available. For these reasons and many more, bacteria are not only the favorite organisms of many microbiologists but also ideal model organisms for studies in molecular biology, genetics, biochemistry, and biotechnology.

Yeasts Are Important Microbes, Too

Although this chapter primarily focuses on applications of bacteria in biotechnology, **yeasts** have served many important roles in biotechnology. Yeasts are

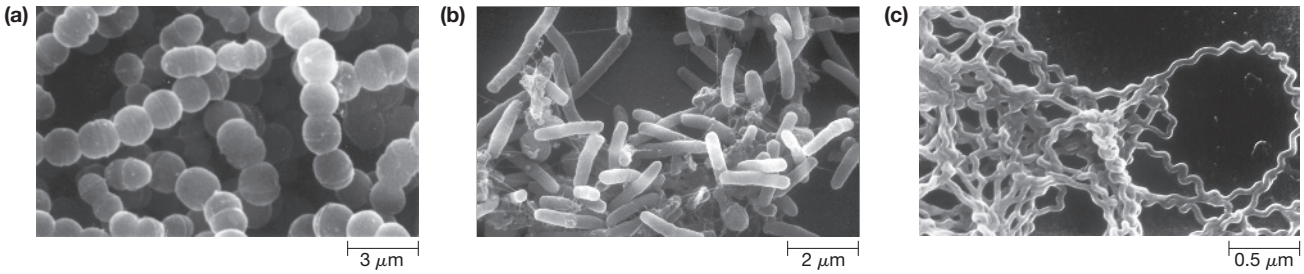


FIGURE 5.1 Shapes of Bacteria The most common shapes of bacteria are (a) spherical, called cocci; (b) rod-shaped, called bacilli; and (c) corkscrew-shaped, referred to as helical or spiral.

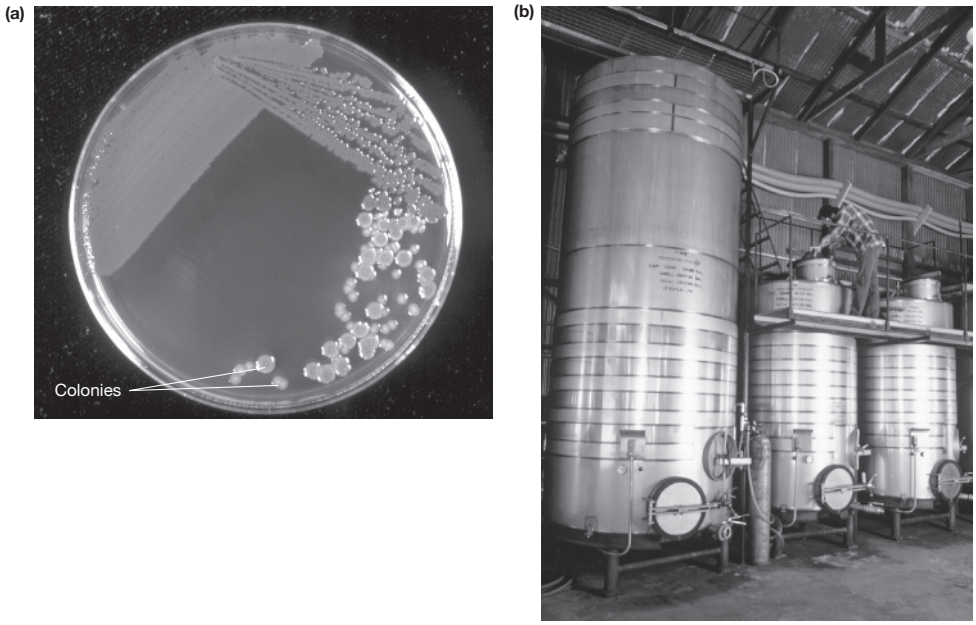


FIGURE 5.2 Bacteria in Culture (a) On solid media, many bacteria grow in circular clusters called colonies, which typically begin with a single cell that divides rapidly to produce a spot visible to the naked eye. A single colony may contain millions of individual bacterial cells. (b) Bacteria can also be grown in large quantities. The fermenters shown here contain several hundred liters of bacteria growing in liquid culture broth. These fermenters function as bioreactors that serve many purposes, such as growing bacteria for isolating recombinant proteins and culturing yeast to produce wine.

single-celled eukaryotic microbes that belong to the **Kingdom Fungi.** Archeologists have uncovered recipes on ancient Babylonian tablets from 4300 B.C. for brewing beer using yeast, which is one of the oldest documented applications of biotechnology. Much more recently, yeast-producing recombinant human antibodies have been generated.

There are well over 1.5 million species of fungi, yet only around 10 percent of these have been identified and classified, so there is significant potential for identifying more valuable products from fungi. For instance, fungi are important sources of antibiotics and drugs that lower blood cholesterol. In addition to having many structures similar to those of other eukaryotic cells, yeasts contain a number of membrane-enclosed organelles in the cytoplasm, a cytoskeleton, and chromosome structures similar to human chromosomes. Yeast cells also have larger genomes than most bacteria. These features make yeasts very valuable model organisms for studying chromosome structure, gene regulation, cell division, and cell-cycle control.

Different types of yeast vary greatly in size, but a majority are larger than bacteria and spherical, elliptical, or cylindrical in shape. Many can grow in the presence of oxygen **(aerobic conditions)** or in the absence of oxygen **(anaerobic conditions)** and under a variety of nutritional growth conditions. A wide number of different types of yeast mutants are also available. *Saccharomyces cerevisiae,* a commonly studied strain of yeast, was the first eukaryotic organism to have its complete genome sequenced. It has 16 linear chromosomes that contain over 12 million base pairs of DNA and ~6,300 genes. Mechanisms of gene expression in yeast resemble those in human cells. Human disease genes have also been discovered in yeast and, by studying them, scientists can learn a great deal about how these genes function in humans and our evolutionary relatedness to yeast.

A strain of yeast called *Pichia pastoris* has proven to be a particularly useful organism. *Pichia* grows to a higher density (biomass) in liquid culture than most laboratory strains of yeast, has a number of strong promoters that can be used for high production of proteins, and can be used in **batch processes** to produce large numbers of cells.

In the next section we consider how microorganisms can be used by scientists as valuable tools for biotechnology research.

5.2 Microorganisms as Tools

Microorganisms in either their natural state or genetically modified forms have served as useful tools in a variety of fascinating ways.

Microbial Enzymes

Microbial enzymes have been used in applications from food production to molecular biology research. Some of the first commercially available enzymes isolated for use in molecular biology were DNA

polymerases and restriction enzymes from bacteria. Initially isolated primarily from *Escherichia coli,* DNA polymerases became available for a range of recombinant DNA techniques such as labeling DNA sequences to make probes and the polymerase chain reaction (PCR) to amplify DNA.

Taq is a heat-stable enzyme essential for PCR that was isolated from the hot-spring archaean *Thermus aquaticus*. In Chapter 3, you were introduced to *Taq* DNA polymerase as a **thermostable enzyme**. Because of their ability to grow and thrive under extreme heat, these microbes are called **thermophiles** (from the Greek words meaning "heat-loving"). Many similar thermostable enzymes have been identified in other thermophiles. *Pfu* DNA polymerase, a popular thermostable enzyme widely used for PCR, is derived from the thermophile *Pyrococcus furiosus,* a species of Archaea originally present in geothermally heated marine sediments. Several companies have permission from the U.S. government to prospect geysers in Yellowstone National Park to identify other potentially valuable microorganisms that might contain novel and valuable enzymes. These **bioprospecting** projects are occurring all around the world. *Methanopyrus kandleri* is a species of Archaea isolated from hydrothermal vents on the floor of the northeast Pacific Ocean. This species has been shown to survive at 121°C, which may be the record discovered so far for the upper temperature limit at which life can exist.

The enzyme **cellulase** from *E. coli* degrades cellulose, a polysaccharide that forms the plant cell wall. Cellulase is widely used to make animal food more easily digestible. Have you ever owned a pair of stonewashed denim jeans? These soft and faded jeans are not produced by a literal washing with stones. Instead, the denim is treated with a mixture of cellulases from fungi such as *Trichoderma reesei* and *Aspergillus niger*. These cellulases mildly digest cellulose fibers in the cotton used to make the pants, resulting in a softer fabric.

The protease **subtilisin,** derived from *Bacillus subtilis,* is a valuable component of many laundry detergents, in which it functions to degrade and remove protein stains from clothing. Several bacterial enzymes are also used to manufacture foods, such as carbohydrate-digesting enzymes called amylases that are used to degrade starches for making corn syrup. The enzyme examples we mentioned here are all examples of **industrial biotechnology,** a rapidly growing field in which synthetic and manufacturing processes can incorporate biotechnology to improve yield, reduce costs, and produce environmentally cleaner products.

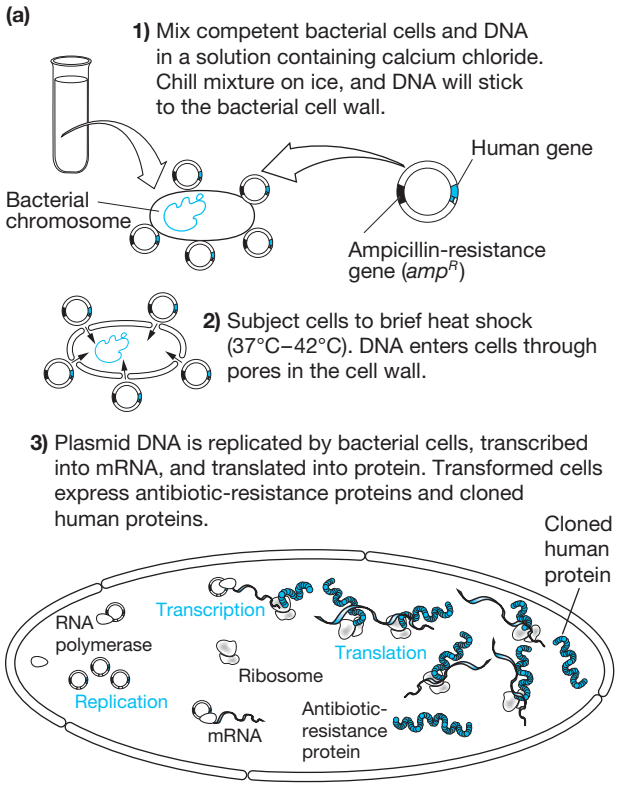
Bacterial Transformation

Recall that **transformation—**the ability of bacteria to take in DNA from their surrounding environment—is an essential step in the recombinant DNA cloning process (Chapter 3). In DNA cloning, recombinant plasmids can be introduced into bacterial cells through transformation so that the bacteria can replicate the recombinant plasmids. Most bacteria do not take up DNA easily unless they are treated to make them more receptive, so-called *competent cells*. One technique for preparing competent cells involves treating cells with an ice-cold solution of calcium chloride. Positively charged atoms (cations) in the calcium chloride disrupt the bacterial cell wall and membrane to create small holes through which DNA can enter. These cells can then be frozen at ultralow temperatures (–80°C to –60°C) to maintain their competent state and then be used in the laboratory as needed.

Once competent cells are prepared, they can be transformed with DNA relatively easily, as shown in **Figure 5.3a**. Typically, the DNA to be introduced into bacteria is inserted in a plasmid containing one or more antibiotic-resistance genes. The recombinant plasmid vector is mixed with competent cells and the mixture placed on ice for a few minutes. The exact mechanism of transformation is not fully understood, but we do know that the cells must be kept cold, during which time DNA sticks to the outer surface of the bacteria. Cold conditions probably also serve to create gaps in the lipid structure of the cell membrane that allow for the entry of DNA. Cations in the calcium chloride are thought to play a significant role in neutralizing the negative charges of phosphates in the cell membrane and the DNA that would otherwise cause them to repel each other. The cells are then heated briefly, for about a minute, at temperatures between 37°C and 42°C. During this brief heat "shock," DNA enters the bacterial cells.

After these cells have been allowed to grow in culture broth, they can be plated onto an agar medium containing antibiotics. Only those cells that were transformed with plasmid DNA containing the appropriate antibiotic-resistant genes will grow to produce colonies. This technique is called *antibiotic selection* (see Chapter 3). Plasmid DNA is replicated (cloned) by transformed bacteria, and genes in the plasmid are transcribed and translated into protein. Thus, the transformed bacterial cells now express recombinant proteins.

This process is called transformation because one can "transform" the properties of bacterial cells by introducing foreign genes. Transformed cells have been genetically altered with new properties encoded by the DNA, enabling them to produce substances they would not normally produce. For example, *E. coli* can be transformed with a gene called green fluorescent protein (GFP), which comes from jellyfish (**Figure 5.3b**; we look more closely at useful applications of GFP in Chapter 10). As we will discuss in Section 5.3, bacterial cells have



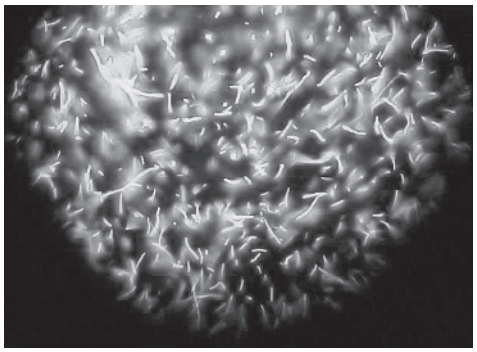


FIGURE 5.3 Transformation of Bacterial Cells by Calcium Chloride Treatment (a) In a laboratory environment, most bacterial cells can be induced to take up foreign DNA by calcium chloride treatment. (b) *E. coli* transformed with a plasmid containing the jellyfish gene for green fluorescent protein (GFP). These bacterial cells glow bright green—a dramatic example of transformation—indicating that they have taken up plasmids containing the *Gfp* gene and expressed *Gfp* mRNA and protein.

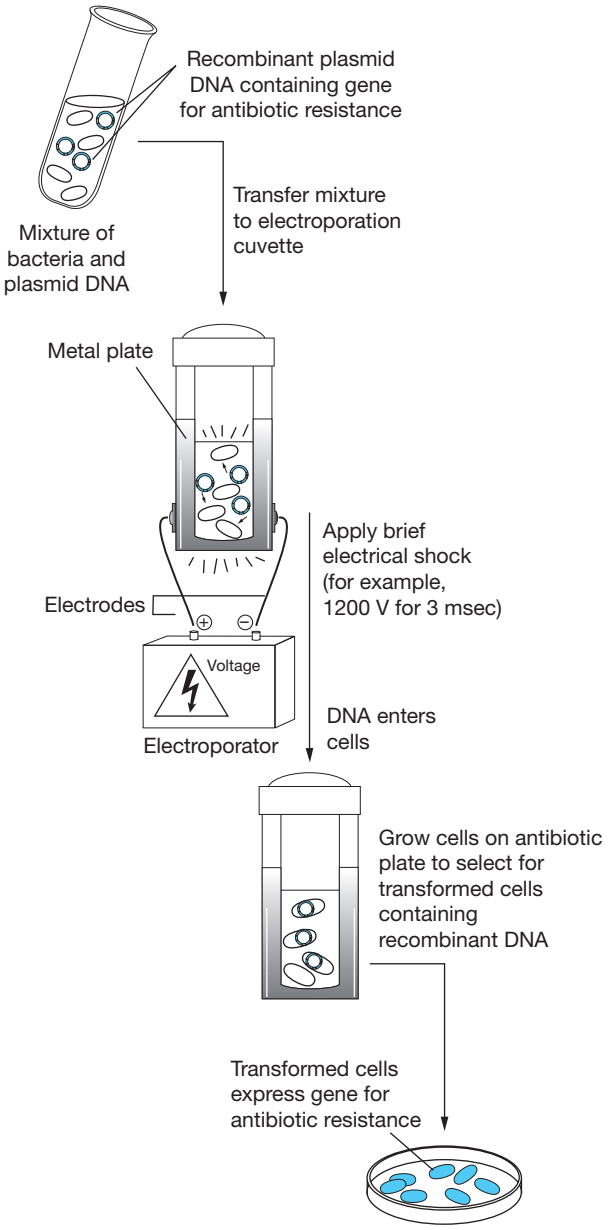


FIGURE 5.4 Electroporation Is a Rapid and Effective Technique for Transforming Bacteria In electroporation, a mixture of bacteria and plasmid DNA is placed into a cuvette. Upon the application of a brief electrical shock to the cuvette, DNA quickly enters cells. Cells containing recombinant DNA can then be selected for by growth on agar containing an antibiotic or another selection component.

been transformed to express a wide range of valuable genes, including human genes, for various applications in biotechnology.

Electroporation

Another common technique for transforming cells is called **electroporation** (Figure 5.4). In this approach, an instrument called an electroporator produces a brief electrical shock that introduces DNA into bacterial cells without killing most of them. Electroporation offers several advantages over the calcium chloride treatment, although competent cells are still required for electroporation. Electroporation is rapid, requires fewer cells, and can also be used to introduce DNA into many other cell types including yeast, fungi, animal, and plant cells. In addition,

electroporation is a much more efficient process than calcium chloride transformation. A greater majority of cells will receive foreign DNA through electroporation than by calcium chloride treatment. Because of this, much less DNA can be used to transform cells (picogram amounts of DNA are sufficient). One drawback to this technique is that it is more costly than calcium chloride transformation because of the cost of an electroporator and competent cells that can tolerate electrical shock. Regardless of how bacterial cells are transformed, once the DNA of interest is introduced into bacteria, a variety of useful techniques can be carried out.

Cloning and Expression Techniques

In addition to replicating recombinant DNA, transformed bacteria are valuable because they can frequently be used to mass-produce proteins for a variety of purposes.

Creating bacterial fusion proteins to synthesize and isolate recombinant proteins

One popular technique for using bacteria for the synthesis and isolation of recombinant proteins involves making a **fusion protein.** Fusion protein techniques are used to create purified proteins to study protein structure and function and to isolate medically important recombinant proteins (see Figure 5.8 later in the chapter). There are a variety of ways to produce a fusion protein, but the basic concept is to use recombinant DNA methods to insert the gene for a protein of interest into a plasmid containing a gene for a well-known protein or amino acid sequence that serves as a "tag" (Figure 5.5). The tag then allows for the isolation and purification of the recombinant protein as a fusion protein. Plasmid vectors for making fusion proteins are often called **expression vectors** because they enable bacterial cells to produce or express large amounts of protein. Commonly used expression vector tags include

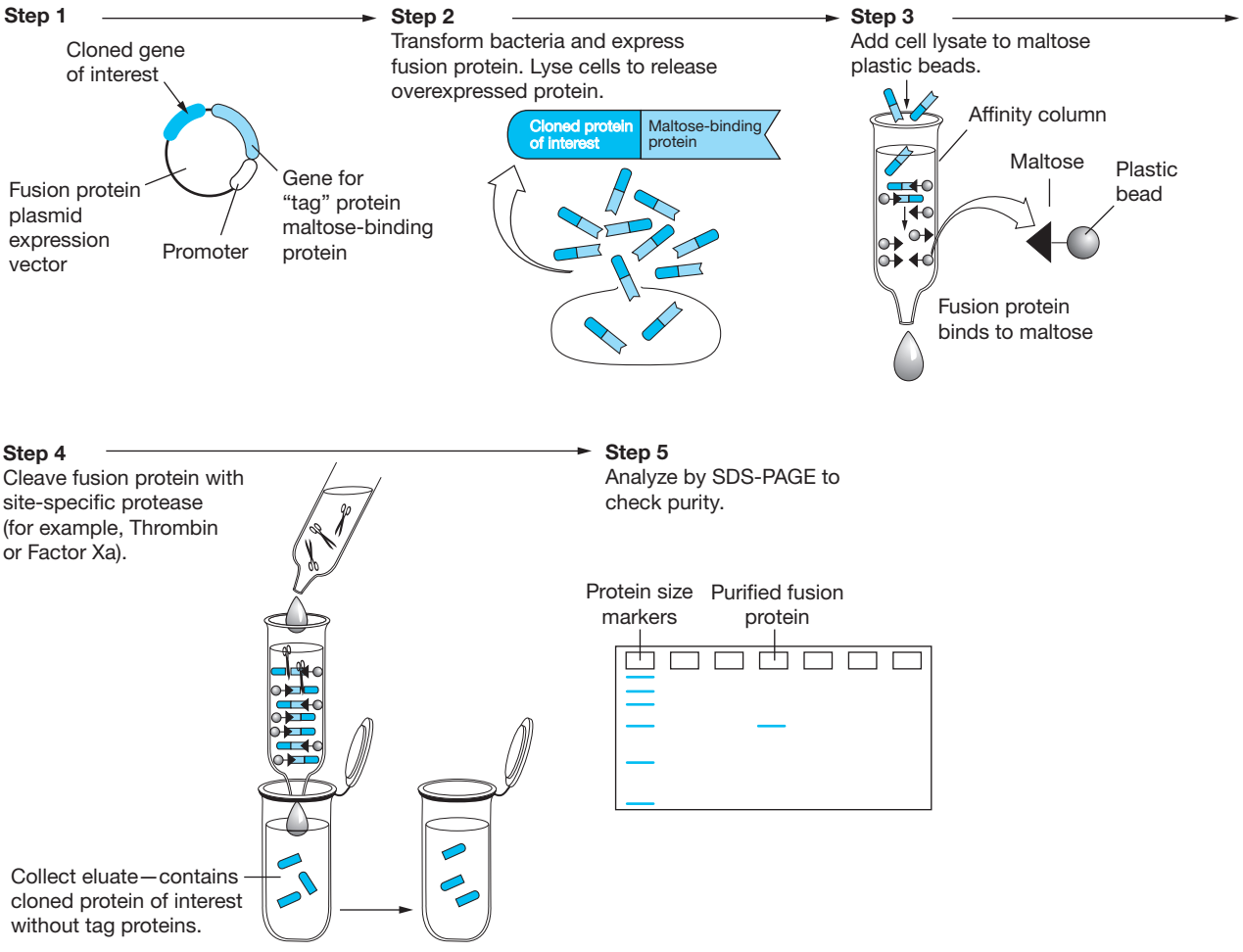


FIGURE 5.5 Fusion Proteins To make a fusion protein, a gene of interest is ligated into an expression vector. Bacterial cells are transformed with recombinant DNA. Transformed cells express the fusion protein, which is isolated by binding it to an affinity column.

maltose-binding protein (shown in Figure 5.5), glutathione S-transferase, histidine, luciferase, green fluorescent protein, and β-galactosidase.

Expression vectors incorporate prokaryotic promoter sequences so that, once the recombinant expression vector containing the gene of interest is introduced into bacteria by transformation, bacteria synthesize messenger RNA (mRNA) and protein from this plasmid. The mRNA strands transcribed are hybrid molecules that contain sequence coding for the gene of interest and the tag protein. As a result, a fusion protein is translated from this mRNA consisting of the protein of interest joined (fused) to the tag protein (Figure 5.5).

To isolate the fusion protein and separate it from other proteins normally made by the bacteria, cells are broken open (lysed) and homogenized to create a bacterial milkshake of sorts known as an *extract.* The extract is then passed through a tube called a *column.* One common approach is to fill a column with plastic beads coated with molecules that will bind to the tag protein portion of the fusion protein. This technique is called **affinity chromatography** because the beads in the column have an attraction, or "affinity," for binding to the tag protein. For example, in Figure 5.5, plastic beads are attached to the sugar maltose, which will be bound by maltose-binding protein. Next, an enzyme treatment that uses protein-cutting enzymes called *proteases* cuts off and releases the protein of interest from the tag protein.

Some techniques for making fusion proteins incorporate short peptide tags of just a few amino acids. For example, poly-His tags are a short string of the amino acid histidine. One benefit of this approach is that, unlike large protein tags, the small tags typically don't affect the structure and function of the protein they are fused to, so they usually do not need to be removed. *E. coli* and the Gram-positive rod-shaped bacterium *Bacillus subtilis* are commonly used microbes for producing fusion proteins. In particular, *B. subtilis* is a favored microbe for many applications when producing fusion proteins for human proteins because it will secrete them into growth media where they can easily be harvested and purified. And unlike some bacteria, *B. subtilis* often processes proteins in such a way as to maintain their three-dimensional folding and function.

Microbial proteins as reporters

According to recent estimates, close to three-fourths of all marine organisms can release light through **bioluminescence.** For marine fish, bioluminescence in lines of cells along the side of a fish and in its fins can be used to attract mates in dark ocean environments. Bioluminescence is often created by bacteria such as *Vibrio fischeri* that use the marine organism as a host (**Figure 5.6**). *Vibrio* have been used as biosensors to detect cancer-causing chemicals, called carcinogens; environmental pollutants; and chemical and bacterial contaminants in foods. *Vibrio fischeri* and another marine bioluminescent strain

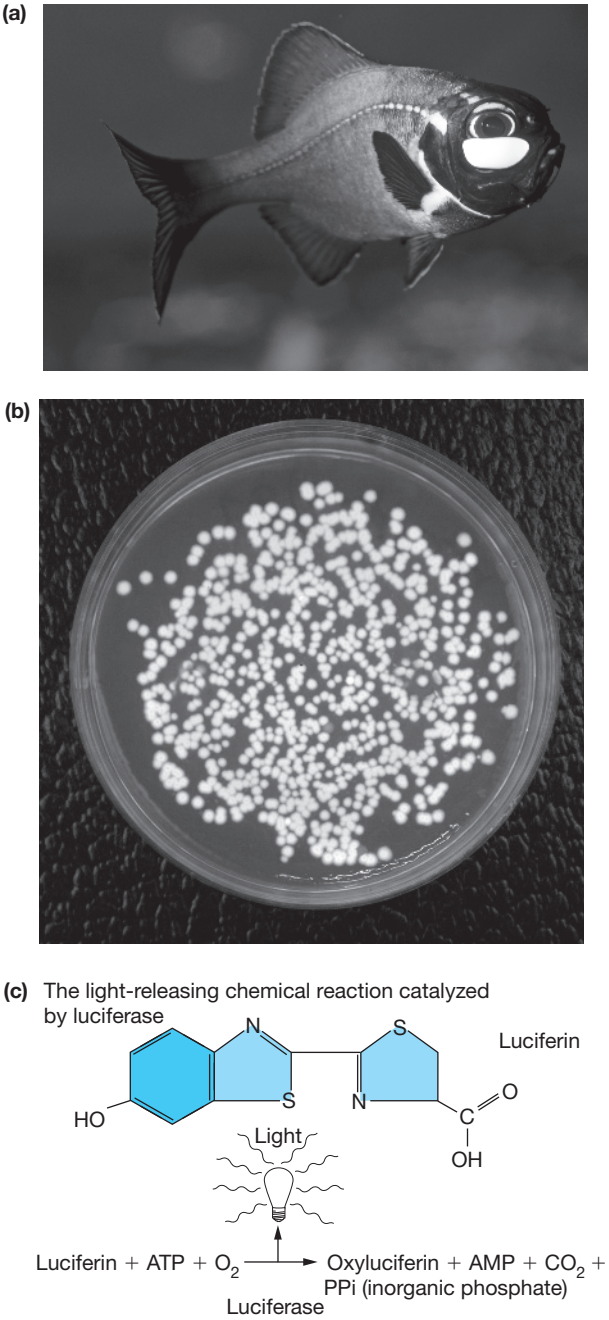


FIGURE 5.6 Bioluminescent Marine Bacteria Bioluminescent marine bacteria, such as *Vibrio fischeri* shown (a) glowing in the light-releasing organs of a deep-sea fish and (b) growing in the laboratory, generate light. (c) *Lux* genes encode the enzyme luciferase that uses oxygen and stored energy (ATP) to convert luciferin into oxyluciferin. This reaction releases light. *Lux* genes serve as reporter genes, allowing biologists to study gene expression. Expression is indicated by glowing cells.

called *Vibrio harveyi* create light through *lux* genes. Several *lux* genes encode protein subunits that form an enzyme called **luciferase** (derived from the Latin *lux ferre,* meaning "light bearer").

Luciferase is the same enzyme that allows fireflies to produce light. The *lux* genes have been cloned and used to study gene expression in a number of unique ways. For instance, by cloning *lux* genes into a plasmid, the *lux* plasmid can be used to produce fusion proteins. Also, *lux* genes can serve as valuable **reporter genes.** If inserted into animal or plant cells, the luciferase encoded by the *lux* plasmid causes these cells to fluoresce (Figure 5.6). In this manner, the *lux* plasmid is acting as a "reporter" to provide a visual indicator of gene expression.

Lux genes have been used to develop a fluorescent bioassay to test for tuberculosis (TB). TB is caused by the bacterium *Mycobacterium tuberculosis,* which grows slowly and can exist in a human for several years before TB develops (TB is discussed in Section 5.4). For the TB bioassay, scientists introduced *lux* genes into a virus that infects *M. tuberculosis.* Saliva from a patient who may be infected with *M. tuberculosis* is mixed together with the *lux*-containing virus. If *M. tuberculosis* is in the saliva sample, the virus infects these bacterial cells, which can be detected by their glowing. Similarly, engineered strains of *E. coli* have been used to detect arsenic contamination in water. Reporter genes have many valuable roles in research, bioremediation (Chapter 9), and medicine (Chapter 11). In the next section we explore a range of everyday applications that involve microbes.

5.3 Using Microbes for a Variety of Everyday Applications

Harnessing the great potential of microbes for making foods and for developing and producing new drugs are among the most common everyday applications of microbial biotechnology.

Food Products

Microbes are used to make many foods, including breads, yogurt, cheeses, and sauerkraut, as well as beverages such as beer, wine, champagne, and liquors. As a child you probably learned the classic nursery rhyme "Little Miss Muffet." The tale of Miss Muffet, sitting on her tuffet, eating "curds and whey" might seem like an improbable way to discuss biotechnology, but the treat in Miss Muffet's bowl was the result of biotechnology! Curds and whey are formed from coagulated milk, and milk coagulation is an important step during cheese production. To make cheese, milk from cows, goats, or sheep is treated to help it coagulate (*curd*). The watery liquid that remains after curd forms is called *whey.*

One way to make cheese from coagulated milk is to treat the milk with an acidic solution, but the best-tasting cheeses are typically made using the enzyme **rennin.** In the early days of cheese production, rennin was traditionally obtained by extracting it from the stomachs of calves and other milk-producing species such as goats, sheep, horses, and even zebras and camels. Rennin coagulates milk to produce curd by digesting a family of proteins called *casein,* which is a major component of milk. Digested casein forms an insoluble mixture of proteins that clumps (coagulates) in a process similar to what happens when milk spoils. When compressed together, adjusted for moisture content, and flavored, this coagulate forms cheese.

In the 1980s, using recombinant DNA techniques, scientists cloned rennin and expressed it in bacterial cells and fungi such as *Aspergillus niger.* Recombinant rennin, called **chymosin,** from microbes is widely used by cheese makers as an inexpensive substitute for extracting rennin from calves. In 1990, rennin was the first recombinant DNA food ingredient approved by the Food and Drug Administration (FDA). For some types of cheese, certain strains of bacteria called lactic acid bacteria (*Lactococcus lactis*, *Lactobacillus acidophilus*) are used for coagulation. These bacteria degrade casein and use an enzyme called *lactase* to break down sugars in the milk that are eventually used by bacteria for **fermentation.**

Fermenting microbes

Fermentation is an important microbial process that produces many food products and beverages including a variety of breads, beers, wines, champagnes, yogurts, and cheeses. One of the earliest applications of microorganisms—the brewing of beer and wine—involves fermentation by yeast (**Figure 5.7a**). To appreciate how making beer, wine, and bread requires microbes, you need to know more about the process of fermentation.

Animal and plant cells and many microbes obtain energy from carbohydrates such as glucose by using electrons from these sugars to create **adenosine triphosphate (ATP),** a molecule cells use for energy. ATP production occurs as a series of reactions. The first major reaction, **glycolysis,** converts glucose into two molecules of *pyruvate.* During this conversion, electrons are transferred from glucose to electron carrier molecules called NAD^+ (nicotinamide adenine dinucleotide), which capture electrons to produce NADH (**Figure 5.7b**). NADH transports electrons to the

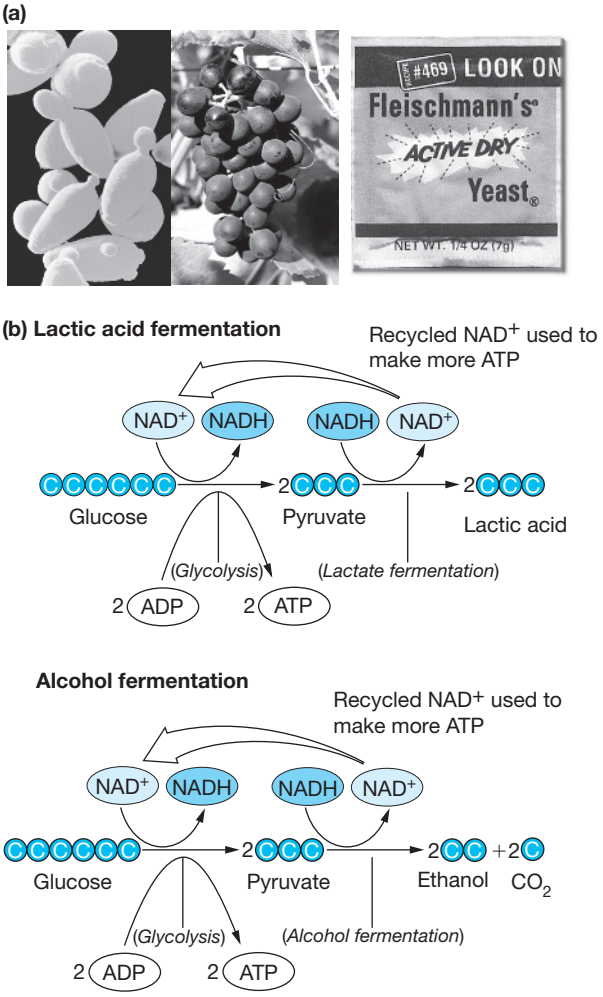


FIGURE 5.7 Fermentation Certain yeasts and bacteria are capable of producing energy from sugars (glucose) through fermentation. (a) Yeast such as *S. cerevisiae* (left), shown here, causes bread dough to rise; other strains of *Saccharomyces* grow on grapevines and are important for making wine (center photo). (b) Anaerobic bacteria that undergo lactic acid fermentation make lactic acid (lactate) as a waste product; alcohol-fermenting bacteria create ethanol and carbon dioxide (CO_2) as waste products.

subsequent reactions of **cellular respiration,** which results in large amounts of ATP being produced in the presence of oxygen. Microbes that use oxygen for ATP production are called **aerobes** because they undergo oxygen-dependent (aerobic) cellular respiration.

Many microbes live in areas where oxygen is rare or absent, such as the intestines of animals, deep water, or soil. Because these organisms must survive without oxygen, they have evolved the ability to derive energy from sugars in the absence of oxygen (anaerobic conditions). This is fermentation, and microbes that use fermentation are called **anaerobes.** Fermentation utilizes glycolysis in that NAD^+ is used to capture electrons to make NADH and pyruvate; however, neither the NADH nor the pyruvate has anywhere to go. In aerobic metabolism, oxygen is required to use electrons from NADH to make ATP, but under anaerobic conditions there is little or no oxygen, so the NADH cannot be used to make ATP. All organisms must recycle NADH into NAD^+. Fermentation enables anaerobes to do this in the absence of oxygen, and some anaerobes are capable of either fermentation or aerobic respiration, depending on the presence or absence of oxygen. In the absence of oxygen, anaerobes have evolved to acquire fermentation reactions as a way to solve the problem of recycling NADH into NAD^+. Fermenting microbes use pyruvate as an electron acceptor molecule to take electrons from NADH and thus to regenerate NAD^+ (Figure 5.7b). Two of the most common types of fermentation are **lactic acid fermentation** and **alcohol (ethanol) fermentation.**

In lactic acid fermentation, electrons from NADH are used to convert pyruvate into lactic acid, also called lactate; during alcohol fermentation, electrons from NADH convert pyruvate into ethanol. NAD^+ is regenerated when electrons are removed from NADH and transferred to pyruvate to make lactate or ethanol in the final step of fermentation. During alcohol fermentation, carbon dioxide gas is also produced as a waste product.

There are many strains of fermenting bacteria and yeast. Other types of fermentation create sauerkraut from cabbage and produce such useful products as acetic acid in vinegar; citric acid in fruit juices; and acetone and methanol, two chemicals often used in laboratories for cleaning glassware. Furthermore, these microbial products have the advantage of being produced more efficiently and cheaply than by other means. Lactic acid fermentation also occurs in human muscle cells during strenuous exercise. The burning you feel in your legs when you run because you are late to class is created by fermentation in muscle cells, creating lactic acid.

So how are fermenting microbes used to make foods and beverages? To make beer and wine, many processes rely on wild strains of yeast that live on grapevines and on such domestic strains of yeast as *Saccharomyces cerevisiae,* which are very good at alcohol fermentation. Large barrels, or fermenters, containing crushed grapes and yeast are mixed together under carefully controlled conditions. Fermenting yeast converts sugars from the grapes into alcohol. Fermentation rates are monitored and carefully controlled by changing both the amount of oxygen in the fermenter and the temperature.

By manipulating fermentation rates, wine makers can control the alcohol content of the brewing wine until the desired alcohol content and flavor are

achieved. Bottles of champagne and other sparkling wines are capped while the yeast is still in the liquid and actively fermenting, thereby trapping carbon dioxide gas in the bottle and releasing it when the bottle is opened, producing the characteristic champagne bottle "pop." Production of some wines relies also on lactic acid bacteria such as *Oenococcus oeni,* which will convert bitter-tasting malic acid that is formed during fermentation into softer-tasting lactic acid and thus give the wine a milder flavor and aroma.

Lactic acid–fermenting bacteria are used to produce cheeses, sour cream, and yogurts; the sharp or sour flavors of these products are primarily due to lactic acid. Yogurt was first created in Bulgaria and has been around for centuries. Yogurt production typically involves a blend of bacteria that often includes strains of anaerobic lactic acid–fermenting microbes such as *Streptococcus thermophilus,* a strain called *Lactobacillus* (*Lactobacillus delbrueckii* and *Lactobacillus bulgaricus*), and another strain called *Lactococcus* (*Lactococcus lactis*). Active cultures of these lactic acid–fermenting microbes are added to mixtures of milk and sugar in a fermenter, which is maintained at carefully controlled temperatures. Microbes in the mixture use sugars to produce lactic acid. Fruit and other flavorings may then be added to the yogurt before it is cooled to refrigeration temperature (4°C–5°C) to prevent changes in its composition. When you enjoy a spoonful of yogurt, you are also eating large numbers of fermenting microbes that are still in the yogurt. Lactic acid and other products of fermentation contribute to the sweet and sour taste of yogurt and assist in the coagulation of the yogurt.

Another lactic acid bacterium, *Lactobacillus sakei,* is found naturally on fresh meat and fish. *L. sakei* serves as a natural biopreservative in meat products such as sausage, where it wards off growth of other undesirable microbes that will spoil the food or cause illness. In addition to lactic acid, this microbe produces molecules called *bacteriocins,* which act as naturally occurring antimicrobial agents to kill other microbes.

Therapeutic Proteins

The development of recombinant DNA technology quickly led to using bacteria to produce such important medical products as therapeutic proteins. Insulin was the first recombinant molecule expressed in bacteria for use in humans. Here we use insulin production as an example of how microbes can be used to make therapeutic proteins.

Producing recombinant insulin in bacteria

Insulin is a hormone produced by cells in the pancreas, called *beta cells.* When insulin is secreted into the bloodstream by the pancreas, it plays an essential role in carbohydrate metabolism. One of its primary functions is to stimulate the uptake of glucose into body cells such as muscle cells, where the glucose can be broken down to produce ATP as an energy source. **Type 1,** or **insulin-dependent, diabetes mellitus** occurs when beta cells do not produce insulin. The lack of insulin results in an elevated blood glucose concentration, which can cause a number of health problems such as high blood pressure, poor circulation, cataracts, and nerve damage. People with type 1 diabetes require regular injections of insulin to control their blood sugar levels.

Prior to recombinant DNA technology, insulin used to treat diabetes was purified from the pancreases of pigs and cows before being injected into diabetic individuals. The purification process was cumbersome and expensive, and often produced impure batches of insulin. Also, purified insulin was ineffective in some individuals, and many others developed allergies to insulin from cows. In 1978, insulin was cloned into a plasmid, expressed in bacterial cells, and isolated by scientists at Genentech, a biotechnology company near San Francisco, California. In 1982, this recombinant human insulin, called Humulin, was the first biotechnology product to be approved for human applications by the FDA.

Bacteria do not normally make insulin, and producing human insulin in recombinant bacteria was a significant advance in biotechnology. It remains an outstanding example of microbial biotechnology in action. Human insulin consists of two polypeptides called the A (21 amino acids) and B (30 amino acids) chains or subunits; they bind to each other by disulfide bonds to create the active hormone (**Figure 5.8**). In the pancreas, beta cells synthesize both insulin chains as one polypeptide that is secreted and then enzymatically cut (cleaved) and folded to join the two subunits. When the human genes for insulin were cloned and expressed in bacteria, genes for each of the subunits were cloned into separate expression vector plasmids containing the *lacZ* gene encoding the enzyme β-galactosidase (β-gal) and then used to transform bacteria (Figure 5.8).

Because the insulin genes are connected to the *lacZ* gene, when bacteria synthesize proteins from these plasmids, they produce a protein that contains β-gal attached to the human insulin protein to create a β-gal–insulin fusion protein. As we saw in Section 5.2, making a fusion protein enables scientists to isolate and purify a protein of interest such as insulin. Bacterial extracts are passed over an affinity column to isolate the β-gal–insulin fusion proteins (Figure 5.8). The fusion protein is chemically treated to cleave off the β-gal, releasing the insulin protein; then, purified A and B chains of insulin are mixed together under conditions that allow the two subunits to bind and form the active hormone. After further purification to conform

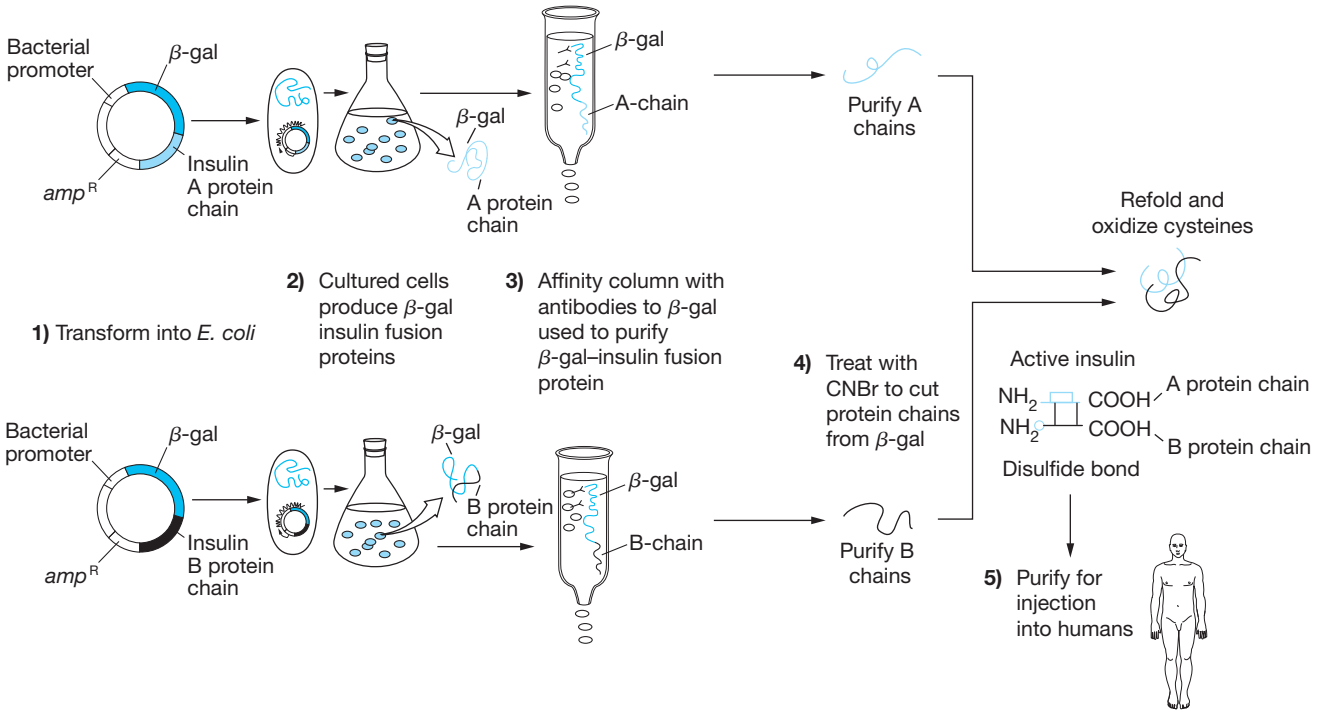


FIGURE 5.8 Using Bacteria for the Production of Human Insulin Insulin was the first protein expressed in recombinant bacteria to be approved for use in humans. Insulin consists of two protein chains (A and B) produced from separate genes. To make recombinant insulin, scientists cloned the insulin genes into plasmids containing the *lacZ* gene, which encodes the enzyme β-galactosidase. Recombinant plasmids were used to transform bacteria, enabling them to produce β-gal–insulin fusion proteins. Affinity chromatography was used to isolate fusion proteins, which were then chemically treated to separate the cloned insulin from β-gal proteins. Purified forms of the A and B protein chains of insulin could then combine to form active insulin, which is given to people with diabetes to control blood sugar levels.

to FDA guidelines, the recombinant hormone is ready for use in patients as an injectable drug.

Shortly after insulin became available, growth hormone—used to treat children who suffer from a form of dwarfism—was cloned in bacteria and became available for human use. A short time later, a wide variety of other medically important proteins that were once difficult to obtain became readily available as a result of recombinant DNA technology and expressing proteins in bacteria. As shown in Table 5.1, many other therapeutic proteins with valuable applications for treating medical illness in humans have been expressed in and isolated from bacteria. A major category of medical products from recombinant bacteria are vaccines. We cover vaccines in Section 5.4.

Using Microbes against Other Microbes

Antibiotics are substances produced by microbes that inhibit the growth of other microbes. Antibiotics are a type of **antimicrobial drug,** a general category defined as any drug (whether produced by microbes or not) that inhibits microorganisms. Penicillin was the first antibiotic to be used widely in humans, and its discovery is an excellent example of how some microbes protect themselves from others by making antimicrobial substances. Alexander Fleming was the microbiologist who, in 1928, discovered that colonies of the mold *Penicillium notatum* inhibited growth of the bacterium *Staphylococcus aureus.* When cultured together on a petri dish, *S. aureus* would not grow in a small zone of agar surrounding mold colonies. A dozen years later, scientists used *P. notatum* to isolate the drug they called penicillin, which was subsequently mass-produced and used to treat bacterial infections in humans.

A majority of antibiotics are isolated from bacteria, and most of these substances inhibit the growth of other bacteria. Since penicillin was discovered, thousands of other antibiotic-producing microbes have been discovered, and hundreds of different antibiotics have been isolated. Table 5.2 shows examples of common antibiotics and their source microbes.

How do antibiotics and other antimicrobial drugs affect bacterial cells? Most of these substances act in a few key ways (called mechanisms of action). Typically, they either prevent bacteria from replicating or kill

TABLE 5.1 Examples of Therapeutic Proteins from Recombinant Bacteria

Protein	Function	Medical Application(s)
DNase	DNA-digesting enzyme	Treatment of patients with cystic fibrosis.
Erythropoietin	Stimulates production of red blood cells	Used to treat patients with anemia (low number of red blood cells).
Factor VIII	Blood clotting factor	Used to treat certain types of hemophilia (bleeding diseases due to deficiencies in blood clotting factors).
Granulocyte colony-stimulating factor	Stimulates growth of white blood cells	Used to increase production of certain types of white blood cells; stimulate blood cell production following bone marrow transplants.
Growth hormone (human, bovine, porcine)	Hormone stimulates bone and muscle tissue growth	In humans, used to treat individuals with dwarfism. Improves weight gain in pigs and cows; stimulates milk production in cows.
Insulin	Hormone required for glucose uptake by body cells	Used to control blood sugar levels in patients with diabetes.
Interferons and interleukins	Growth factors that stimulate blood cell growth and production	Used to treat blood cell cancers such as leukemia; improve platelet counts; some used to treat different cancers.
Superoxide dismutase	An antioxidant that binds and destroys harmful free radicals	Minimizes tissue damage during and after a heart attack.
Tissue plasminogen activator (tPA)	Dissolves blood clots	Used to treat patients after heart attack and stroke.
Vaccines (e.g., hepatitis B vaccine)	Stimulate the immune system to prevent bacterial and viral infections	Used to immunize humans and animals against a variety of pathogens; also used in some cancer tumor treatments.

TABLE 5.2 Common Antibiotics

Antibiotic	Source Microbe	Common Uses of Antibiotic
Bacitracin	*Bacillus subtilis* (bacterium)	First-aid ointment and skin creams
Erythromycin	*Streptomyces erythraeus* (bacterium)	Broad uses to treat bacterial infections, especially in children
Neomycin	*Streptomyces fradiae* (bacterium)	Skin ointments and other topical creams
Penicillin	*Penicillium notatum* (fungus)	Injected or oral antibiotic used in humans and farm animals (cattle and poultry)
Streptomycin	*Streptomyces griseus* (bacterium)	Oral antibiotic used to treat many bacterial infections in children
Tetracycline	*Streptomyces aureofaciens* (bacterium)	Used to treat infections of the urinary tract in humans; commonly used in animal feed to reduce infections and stimulate weight gain
Vancomycin	*Amycolatopsis orientalis* (soil bacterium)	In use for > 50 years against a wide range of bacteria; important for treatment of methicillin-resistant *Staphyloccoccus aureus* (MRSA), a major cause of infections acquired in hospitals

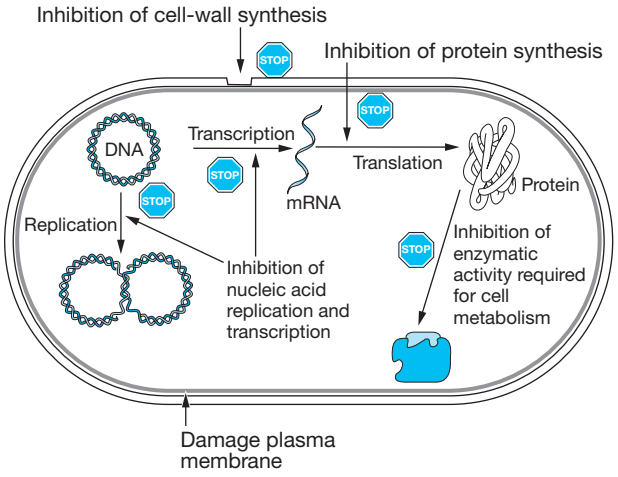


FIGURE 5.9 Antibiotics and Other Antimicrobial Drugs Work against Microbes in a Variety of Ways

microbes directly, which of course also prevents affected cells from replicating. Antibiotics can damage the cell wall or prevent its synthesis (which is how penicillin acts), block protein synthesis, inhibit DNA replication, or inhibit the synthesis or activity of an important enzyme required for bacterial cell metabolism (Figure 5.9).

You sneeze, your body aches, your nose is running, your throat is sore, and you can't sleep, but you still have an exam to take tomorrow afternoon. How will you do it? By going to your doctor and asking for antibiotics of course! But are antibiotics what you really need? Because antibiotics are effective only against bacteria, they do not work against viruses such as those that cause flu. Also, bacterial resistance to antibiotics has become a major problem. Improper use and overuse of antibiotics in humans and farm animals has led to dramatic increases in antibiotic-resistant bacteria, including some strains that do not respond to many antibiotics that were effective in the past. In 2006, the European Union banned feeding antibiotics to farm animals solely for enhancing their growth to minimize the spread of antibiotic-resistant microbes. The routine use of antibiotics in farming will also be banned from 2022.

Antibiotic-resistant strains of *S. aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *M. tuberculosis*, and many other deadly strains of human **pathogens** (disease-causing microorganisms) are major problems for hospitals and farms. These and other newly emerging strains of drug-resistant microbes are often referred to as "superbugs," and in recent years they are responsible for killing around 33,000 people in Europe alone and 700,000 people annually around the world. You may have heard of **"MRSA" (methicillin-resistant *Staphylococcus aureus*),** a microbe that can cause deadly skin, lung, and blood infections. MRSA is a significant problem in hospitals because this superbug has become resistant to several major categories of antibiotics including methicillin, the most commonly used antibiotic to combat *S. aureus*. New categories of antibiotics called synthetic retinoid antibiotics are showing promise against MRSA in mice but more research is needed to determine if these drugs will be safe and effective in humans.

Because most antibiotics attack bacterial cells in a limited number of ways (Figure 5.9), resistance to one antibiotic often leads to resistance to others—known as *multidrug resistance*. Thus, the spread of antibiotic-resistant pathogens is a major problem. In the past 50 years, only one antibiotic with a new mechanism of action has been discovered and put into use! Consequently, there is an urgent need for the discovery and development of new antimicrobial drugs with different mechanisms of action for use in humans as well as for treating pets and farm animals such as cows, pigs, and chickens. Changing the chemical properties of existing antibiotics to create new mechanisms of action is one approach researchers are using. But clearly new antibiotics need to be developed. From treetops to polar ice caps, deserts and soil samples from many different environments, and the ocean's depths, scientists are bioprospecting many microorganisms as potential sources of new antimicrobial substances.

Another way to create new antimicrobial drugs is to study bacterial pathogens and identify toxins and properties that cause illness. By understanding these factors, scientists can develop new strategies to kill or inhibit bacteria. Researchers are working on antibiotics that interfere with chemical signals that microbes use to communicate in populations in synchronized ways (what scientists call quorum sensing) that can lead to the formation of **bacterial biofilms**—layers of microbes that can accumulate and coat surfaces including medical devices and your teeth. For example, biofilms of *P. aeruginosa*, the primary pathogen in cystic fibrosis infections, in the lungs are very difficult to treat. We discuss biofilms further in Chapter 10.

In Section 5.5 we will discuss the Human Microbiome Project and how "good" microbes in our body produce molecules that protect us from pathogens. Studying genes from these microbes is also actively under way to identify new categories of small molecules that could be developed into antimicrobial drugs.

A company called Oragenics in Florida, United States is involved in clinical trials with a recombinant form of *Streptococcus mutans* to evaluate its safety and efficacy for reducing tooth decay. Unlike naturally occurring strains of *S. mutans* found in the oral cavity, the recombinant strain cannot metabolize sugars to produce lactic acid. Because lactic acid dissolves enamel and dentin in teeth, it leads to cavities and tooth decay. Scientists are attempting to use recombinant *S. mutans* to colonize the oral cavity and replace natural strains of *S. mutans* and then determine if tooth decay is reduced.

In another creative approach, scientists at the University of California–Los Angeles (UCLA) created a sugar-free lollipop containing an ingredient in licorice that kills *S. mutans,* and these bacteria-killing lollipops are now available for purchase.

Finally, engineering microbes to target and destroy other microbes is another potential opportunity. A recent example involved coating nonpathogenic *E. coli* with nanoparticles made from nickel and tin coated in gold. When these bacteria attach to diseased tissue or pathogenic *E. coli*, the beads can be heated with infrared light to destroy surrounding cells. In other research, scientists are actively exploring using bacteria for transporting nanoparticles as microbial "bots" to deliver drugs to diseased cells.

Phage therapy and CRISPR-Cas applications

The antibiotic resistance problem has also led to renewed interest in applications of **phage therapy**—using bacteriophages to treat infections. Recall from Chapter 3 that phages are viruses that infect and replicate in bacteria, and some can kill bacteria. Phage therapy has been used in countries such as Russia and Poland but has not been widely accepted in the West. But in early 2017, phage therapy made headlines when a patient in San Diego, California, with a severe and widespread bacterial infection resistant to antibiotics was treated intravenously with a mixture of phage as a last resort. Within a few days of treatment, the patient awoke from an infection-induced coma with seemingly no ill effects from the phage therapy. Most phage therapy in the United States as well as in some European Union countries, including France, Belgium, and Switzerland, is still in preclinical stages; as of 2017 there were several clinical trials in progress for conditions such as *P. aeruginosa* lung infections in cystic fibrosis, *S. aureus* infections, and *E. coli* and *P. aeruginosa* burn and skin infections. Most approaches involve using phages that were originally found in the environment and then genetically modified to have particular features. This can also include using synthetic biology approaches to modify phage.

There are potential advantages of phage therapy for combating antibiotic-resistant microbes. For example, phages work through completely different mechanisms of action than antibiotics, so they can be effective against multidrug-resistant bacteria, including, potentially, "superbugs." Also, phages are species-specific, so in theory they can be engineered to target specific pathogens so that they also avoid destroying naturally occurring bacteria we rely on for proper health.

CRISPR-Cas is being explored to target antibiotic-resistance genes in pathogenic, but not native, strains of bacteria (**Figure 5.10**). This is a very exciting area of research. A group at MIT has developed an approach in which a phage injects bacteria with a guide RNA that targets antibiotic-resistance genes and Cas9 cuts up this sequence, killing the bacteria. Early trials demonstrated that these phages could kill more than 99 percent of antibiotic-resistant bacteria but not interfere with nonresistant cells. This early "proof of concept" is promising and tests are under way to treat antibiotic resistance in mice, which, if successful, may lead to trials in humans. Approaches have also been used to deliver CRISPR-Cas to protect bacteria from phage infection. Guide RNA can be targeted to recognize phage DNA and when phage attempt to infect cells, CRISPR-Cas disables the phage DNA.

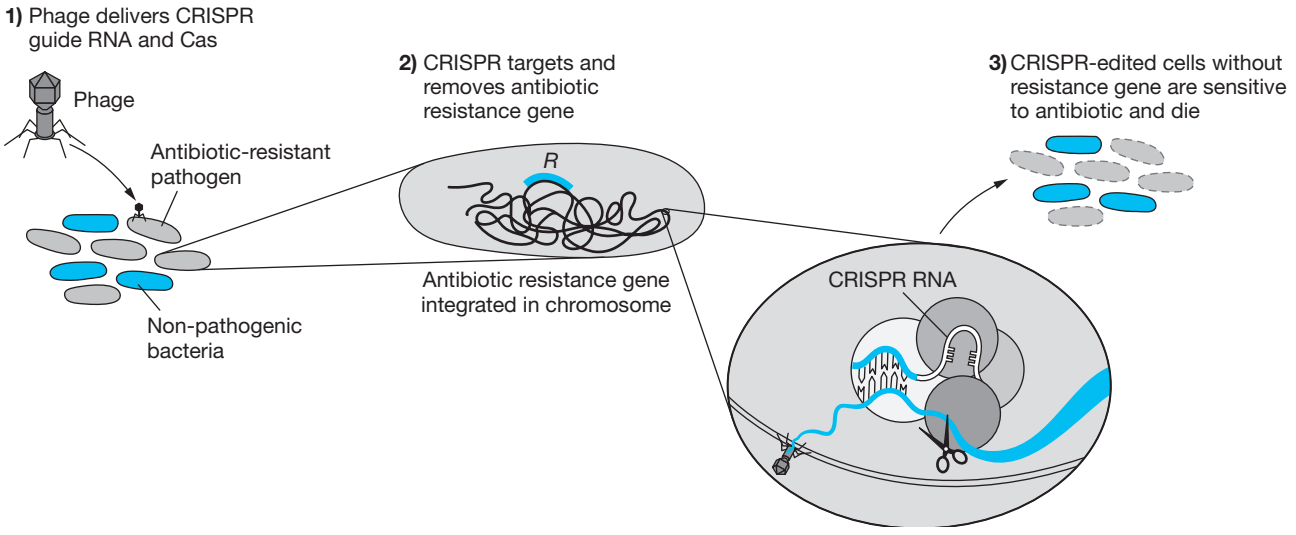


FIGURE 5.10 Phage Therapy Incorporating CRISPR-Cas



YOU DECIDE **Microbes on the Loose**

One of the many controversies surrounding microbial biotechnology is the possibility that recombinant microbes can enter the environment, through accidental introduction or intentional release. If recombinant microbes are loose in the environment, how can we know what will ultimately happen to these organisms? The first field application of genetically modified (GM) bacteria was developed at the University of California by plant pathologist Steven Lindow and colleagues. They identified a common strain of the bacterium *Pseudomonas syringae*, which makes proteins that stimulate ice crystal formation. Lindow's group created ice-minus bacteria by removing the ice protein–producing genes from *P. syringae.* They proposed that releasing ice-minus bacteria onto plants would cause the ice-minus bacteria to crowd out normal, ice-forming *P. syringae* and provide frost-sensitive crop plants with protection from the cold, thus extending the growing season and increasing crop yields.

Surrounded by a great deal of controversy, Lindow received approval in 1987 to test *P. syringae* on a crop of potatoes. Around the same time, other scientists received permission to test ice-minus bacteria on strawberries in a small town in California. This was the first time that GM microbes were ever intentionally released into the environment in the United States. In both experiments, a majority of plants were damaged by activists concerned about the release of GM microbes. Ice-minus *P. syringae* have shown some promise for frost protection, but they have not been as effective at crowding out the growth of normal ice-forming *P. syringae* as Lindow and others had hoped. Incidentally, in recent years many ski resort operators use commercial snow makers that contain naturally occurring strains of *P. syringae* to accelerate crystal formation to create snow.

Because gene transfer between bacteria is a natural process that occurs in the wild, scientists are concerned about **horizontal gene transfer,** the spread of genes to related microbes. As a result of genetic recombination, strains of microbes may be produced with different characteristics based on the genes they inherit.

Regarding the matter of "microbes on the loose," certain questions and considerations arise, such as the following:

- What would happen if GM microbes transfer genetically altered genes into other microorganisms for which they were not originally intended (for example, naturally occurring strains of *P. syringae*?
- For instance, what would happen if ice-minus bacterial genes were transferred to strains of bacteria that are accustomed to living under cold conditions?
- Once recombinant microbes escape or are released into the environment, we cannot simply call them back into the lab if we do not like what they are doing in the field.
- Can we prevent the escape of GM microbes in field experiments? It is a difficult, if not impossible, task when wind, rain, and other weather elements are involved.

Should GM microbes be released even in "controlled" experiments that might result in beneficial applications of biotechnology? You decide.

5.4 Vaccines

Antibiotics and vaccines have proven to be very effective for treating pathogen-induced infectious disease conditions in humans and animals. However, pathogens with resistance to widely used antibiotics and vaccines have emerged and challenge their effectiveness. Infectious diseases affect everyone, and worldwide over 60 percent of deaths among children before age 4 are due to infectious diseases. Without question, our ability to prevent and treat infectious diseases is an important aspect of microbial biotechnology, and vaccines play key roles in this process.

The first vaccine was developed in 1796, when Edward Jenner demonstrated that a live cowpox virus could be used to vaccinate humans against smallpox. Smallpox and cowpox are closely related viruses. Smallpox epidemics ravaged areas of Europe, and an estimated 80 percent or more of Native Americans on the East Coast of the United States died from smallpox infections carried by European settlers. Cowpox produces blisters and lesions on the udders of cows and similar skin ulcers in humans. Based on a milkmaid's claim that cowpox infections protected her from smallpox, Jenner prepared his vaccine. He took fluid from cowpox blisters on the milkmaid and used needles containing this fluid to scratch the skin of healthy volunteers. His first "patient" was an

8-year-old boy. A majority of Jenner's volunteers did not develop cowpox or smallpox even when subsequently exposed to persons infected with smallpox. Exposure to cowpox fluid had stimulated the immune system of Jenner's volunteers to develop protection against smallpox.

These experiments demonstrated the potential of **vaccination** (named from the Latin word *vacca,* meaning "cow")—using infectious agents to provide immune protection against illness. Although the United States stopped routine vaccinations for smallpox in 1972, by 1980, subsequent widespread applications of the vaccine had eradicated this disease. In the United States many vaccines are routinely given to newborns, children, and adults. Although you may not remember your first vaccination (which usually occurs sometime from 2 to 15 months of age), you were probably vaccinated with the **DPT vaccine,** which provides several years of protection against three bacterial toxins called *d*iphtheria toxin, *p*ertussis toxin, and *t*etanus toxin. Diphtheria can cause breathing problems, paralysis, and heart failure. Pertussis causes whooping cough, which involves episodes of coughing paralysis so severe that it becomes hard for infants to eat, drink, or breathe. Tetanus can cause lockjaw, preventing opening of the mouth.

Another childhood vaccine is the **MMR** (*m*easles-*m*umps-*r*ubella) vaccine. As you may know, measles results in a rash, fever, and cough; it can also cause a variety of other complications, such as ear infections, seizures, and even death. Mumps causes fever, swollen glands, and headaches, but it can also lead to deafness. Rubella, or German measles, causes a fever, rash, and arthritis.

You were probably also vaccinated with **OPV** (*o*ral *p*olio *v*accine) for the poliovirus, a strain that infects neurons in the spinal cord, causing a devastating paralysis called poliomyelitis (polio). The OPV is the vaccine of choice in developed countries and has dramatically decreased the incidence of polio. Polio has virtually been eliminated in North America, South America, and most of Europe; however, it still exists in some areas of the world. Once a much more common disease, polio ravaged millions of children worldwide prior to 1954, when Jonas Salk developed the first polio vaccine. Salk's original vaccine required injection; Albert Sabin developed the current version, which can be taken by mouth, in 1961. To understand how vaccines work, you must be familiar with the basic aspects of the human immune system.

A Primer on Antibodies

The immune system in humans and other animals is extremely complex. Numerous cells throughout the body work together in intricate ways to recognize foreign materials that have entered our body and mount an attack to neutralize or destroy those materials. Foreign substances that stimulate an immune response are called **antigens.** They may be whole bacteria, fungi, and viruses or individual molecules such as proteins or lipids found on pollen. For instance, people with food allergies have immune responses to proteins, carbohydrates, and lipids in certain foods.

The immune system responds to antigens in part by producing antibodies. This response is called **antibody-mediated immunity** (Figure 5.11). When exposed to antigens, **B lymphocytes** (simply called **B cells**), which are a type of white blood cell, or **leukocyte,** recognize

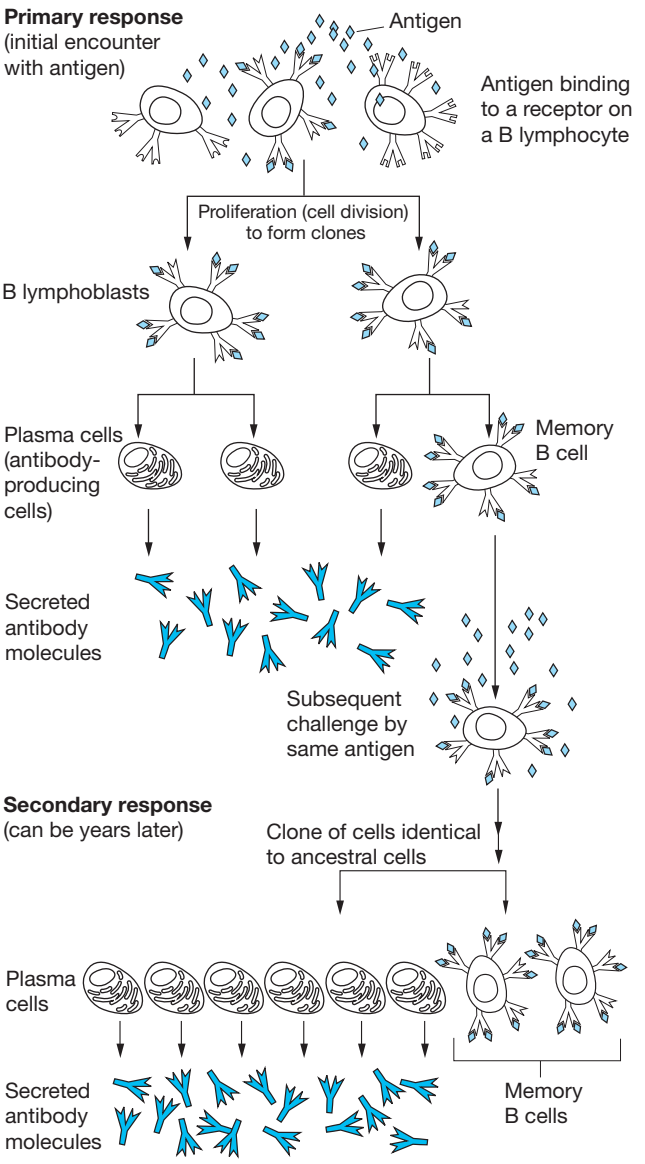


FIGURE 5.11 Antigens Stimulate Antibody Production by the Immune System In response to the initial exposure to antigens, which constitute whole cells or individual molecules, B cells divide repeatedly to form many other B cells (clones). B cells differentiate into plasma cells, which produce antibodies specific to the antigen. During this process, memory B cells are formed. If a person is exposed to the same antigen again in the future, even many years later, memory B cells can recognize and produce a stronger and more rapid response to the antigen.

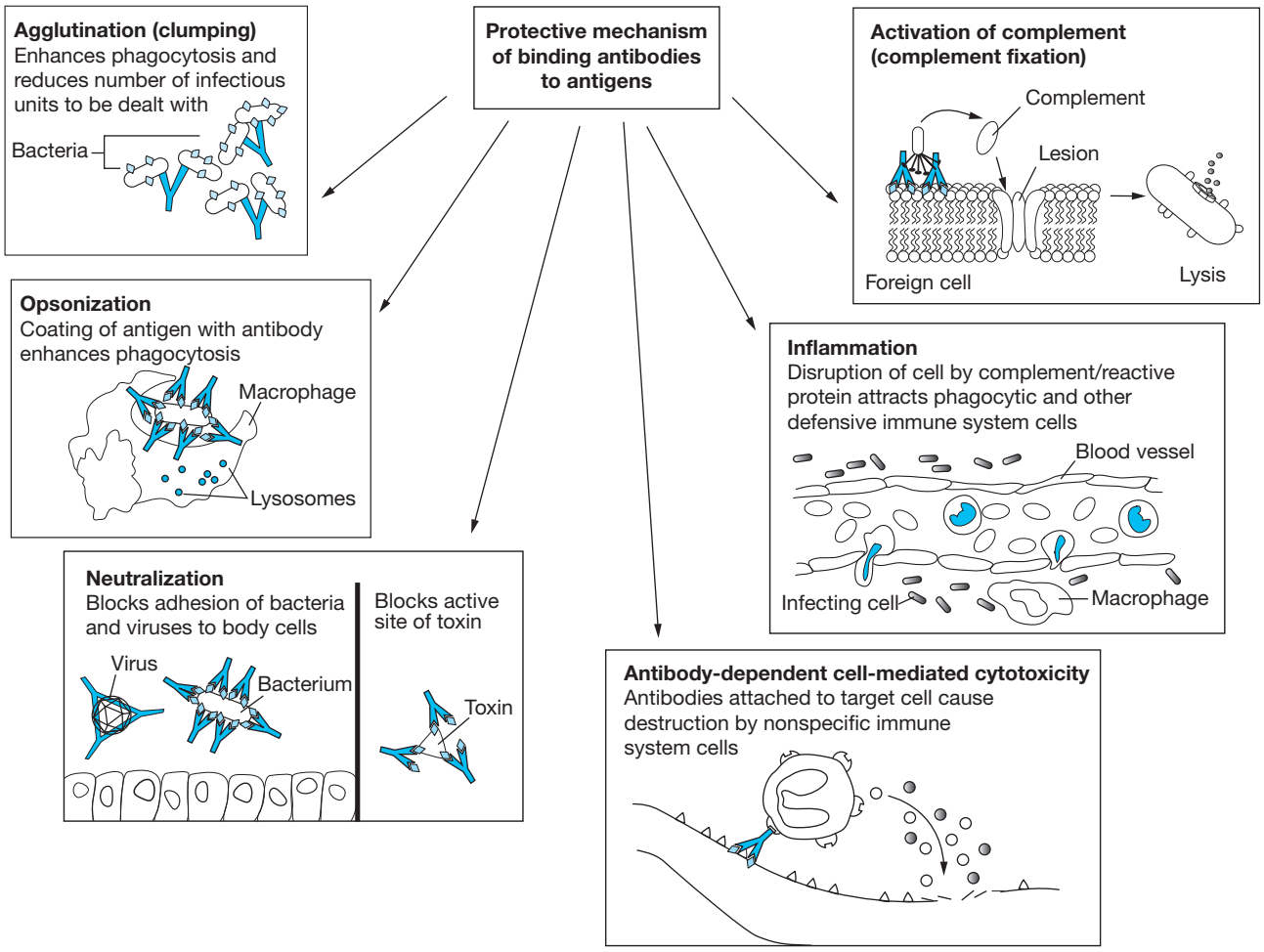


FIGURE 5.12 Mechanisms of Antibody Action Antibodies can inactivate and destroy antigens in a number of ways.

and bind to antigen. **T lymphocytes (T cells)** play essential roles in helping B cells recognize and respond to antigen. After antigen exposure, B cells develop to form **plasma cells,** which produce and secrete antibodies. Most antibodies are released into the bloodstream, but there are also antibodies in saliva, tears, and the fluids lining the digestive system, among others.

Another purpose of antibody production is to provide lasting protection against antigens. During the process of B cell development, some B cells become "memory" cells, which have the ability to recognize foreign materials years later and in response grow and produce more plasma cells and antibodies, which provide the body with long-term protection against antigens (Figure 5.11).

Antibodies are very specific for the antigen for which they were made, but how do these proteins protect the body against foreign materials? Many antibodies bind to and coat the antigen for which they were made (**Figure 5.12**). After antigens are covered with antibodies, a type of leukocyte called a **macrophage** can often recognize them. Macrophages are cells that are very effective at phagocytosis (which literally means "cell eating," derived from the Greek terms *phago,* "eating," and *cyto,* "cell"). In phagocytosis, macrophages engulf antigen covered with antibody; then, organelles in the macrophage called *lysosomes* unleash digestive enzymes that degrade the antigen (Figure 5.12). When the antigen is a foreign cell such as a bacterium, some antibodies are involved in mechanisms that rupture the cell through a process called *cell lysis.*

We are constantly being exposed to antigens, against which our immune system develops antibodies. But sometimes our natural production of antibodies is not sufficient to protect us from pathogens such as smallpox, viruses that cause hepatitis, and **human immunodeficiency virus (HIV),** the cause of **acquired immunodeficiency syndrome (AIDS).** Biotechnology can help our immune systems by boosting our immunity through the use of vaccines.

Types of Vaccines: How Are Vaccines Made?

Vaccines are parts of a pathogen or whole organisms that can be given to humans or animals by mouth or by injection to stimulate the immune system against infection by those pathogens. When people or animals are vaccinated, their immune systems recognize the vaccine as an antigen and respond by making antibodies and B memory cells. By stimulating the immune system, the vaccine has pressured it into stockpiling antibodies and immune memory cells that can go to work on exposure to the real pathogen in the future, should such exposure occur. Therefore, many but not all vaccines are designed to be *preventative* or *prophylactic* (by providing protection against a pathogen should you be exposed) and not *therapeutic*—that is, a cure once you have become infected or developed a particular condition. Remember also that vaccination is used in pets, farm animals, zoo animals, and even wild animals.

So, how are vaccines made? Currently, several new experimental approaches are active areas of vaccine development, but generally four major strategies have traditionally been used to create immune responses with vaccines.

1. **Subunit vaccines** are made by injecting portions of viral or bacterial structures, usually proteins or lipids from the microbe, to which the immune system responds. A fairly effective vaccine against hepatitis B virus was one of the first examples of a recombinant expressed subunit vaccine (discussed in the text that follows), and vaccines for tetanus, anthrax, and meningococcal disease (which you may have been vaccinated for, because this vaccine is required by many colleges and universities) are also subunit vaccines.
2. **Attenuated vaccines** involve using live bacteria or viruses that have been weakened through aging or by altering their growth conditions to prevent their replication after they are introduced into the recipient. The Sabin vaccine for polio (OPV) is an attenuated vaccine. So are the MMR, tuberculosis, cholera, and chickenpox (varicella) vaccines as well as many others. One downside of inactivated vaccines is that the live virus can mutate and regain disease-causing properties. For example, OPV-derived outbreaks of polio occur almost every year in different areas of the world.
3. **Inactivated (killed) vaccines** are prepared by killing the pathogen and using the dead or inactive microorganism for the vaccine. A mixture of inactivated poliovirus is used in the Salk vaccine against polio. The rabies vaccines administered by injection to dogs, cats, and humans, the DPT vaccine, and the influenza (flu) vaccine, which has become common in recent years, are also examples of inactivated vaccines. Inactivated flu vaccine is also delivered as a nasal spray.
4. **DNA-based vaccines** have been attempted, but so far they have not proven to be widely effective. In 2005, the U.S. Department of Agriculture (USDA) approved the world's first licensed DNA vaccine, a vaccine designed to protect horses against the mosquito-borne West Nile virus (WNV). Equine infections of WNV are on the rise, and about one-third of horses infected with WNV will die or will have to be euthanized. In 2016, the European Medicine Agency approved the first DNA vaccine in the European Union against salmon alphavirus that causes the salmon pancreas disease. This vaccine consists of a plasmid containing a gene for a human enzyme (tyrosinase). Clinical trials for a DNA-based vaccine against Zika virus (discussed in the next section) are in progress. Similarly, several trials are under way using plasmid DNA encoding protein antigens from HIV. However, a major limitation of DNA-based vectors, evident in the HIV research, has been that they typically result in very low production of protein encoded by delivered genes, and thus the immune response in vaccinated persons is insufficient to provide the desired protection. Work on DNA-based vaccines, and recently RNA vaccines, continues to be an active area of exploration, but whether they will ever have significant roles in the vaccine market remains to be seen.

Immunity from vaccinations can fade with time, particularly for inactivated vaccines, which often do not produce a strong immune response. As a result, many vaccines require immunization *booster* shots every few years to re-stimulate the immune system so that it will continue to provide protective levels of antibodies and immune memory cells. For instance, the DPT vaccine is effective for about 10 years, as is the tetanus vaccine, and the flu vaccine that you may have received requires annual injections because new strains of influenza virus are developing each year.

Attenuated and inactivated vaccines were among the first vaccines developed. Some subunit vaccines against bacteria were made prior to recombinant DNA technology by growing bacterial pathogens in liquid culture. Many bacteria release proteins into the surrounding media, and these proteins can be purified and mixed with compounds that will help stimulate an immune response when injected into humans. But as scientists have learned more about the molecular structure of many pathogens, attempts at making

recombinant subunit vaccines have become more popular.

For instance, **hepatitis B** is a blood-borne virus transmitted by exposure to body fluids, sexual intercourse, and contaminated blood transfusions. Hepatitis B causes deadly liver diseases. When vaccines for hepatitis B were first prepared, scientists isolated the virus from the blood of infected patients and then used biochemical techniques to purify viral proteins. These proteins were then injected into humans as a vaccine. Hepatitis B virus vaccination has been added to the routine vaccination programs of 179 countries around the world. The hepatitis B vaccine is also recommended for international travelers, particularly people visiting Africa and Asia, and health care workers and others who may come in contact with hepatitis-infected persons or their body fluids.

Currently, a majority of subunit vaccines, including the vaccine for hepatitis B, are made using recombinant DNA approaches in which the vaccine is produced in microbes. To produce the recombinant subunit vaccine for hepatitis B, scientists cloned genes for proteins on the outer surface of the virus into plasmids. Yeasts transformed with these plasmids are used to express large amounts of viral protein as fusion proteins, which are then purified and used to vaccinate people against hepatitis B infections. This approach is a common strategy for producing subunit vaccines, although sometimes fusion proteins are expressed in bacteria or in cultured mammalian cells.

In 2006, the pharmaceutical company Merck received FDA approval for Gardasil, a recombinant subunit vaccine against cervical cancer and the first cancer vaccine to be approved by the FDA. Gardasil is a quadrivalent vaccine because it targets four specific strains of **human papillomavirus (HPV).** Among women, cervical cancer ranks as the fourth most frequently diagnosed cancer and the leading cause of cancer death with an estimated 570,000 new cases and 311,000 deaths worldwide in 2018. Unfortunately, approximately 90 percent of deaths caused by cervical cancer occur in low and middle-income countries. HPV can also cause genital warts in men, penile cancer, and anal, head, and neck cancers in men and women.

The HPV vaccines are prophylactic type, which means they need to be taken to provide immune protection prior to exposure to HPV. Therefore, in certain countries, the vaccine is given to girls aged between 9 and 14 years in a series of two to three doses with intervals stipulated by the manufacturers. However, different health care systems and infrastructure have resulted in different implementation strategies in high, middle, and low-income countries. Since 2014, three prophylactic HPV vaccines—bivalent, quadrivalent, and nonavalent—targeting 9 HPV types that are responsible for approximately 90 percent of cervical cancers are available in over 100 countries for primary prevention of cervical cancer and other HPV-associated diseases.

Major Targets for Vaccine Development

Pathogens are changing all the time, giving rise to both drug- and vaccine-resistant strains of disease-causing bacteria and viruses. More infectious microbes exist than there are vaccines. As a result, there are many research priorities for improving existing vaccines and producing new ones, and biotechnology companies have in excess of 50 targets for vaccine development. Several of the major vaccine targets include dengue fever; Ebola virus; hepatitis C and E; sexually transmitted diseases such as herpes, gonorrhea, and chlamydial infection; MRSA; pneumococcal disease; rotavirus; West Nile Virus; and Zika virus. In addition, nonpathogenic human diseases such as Alzheimer's disease, multiple sclerosis, allergies, drug addiction, diabetes, and hypertension are targets for *therapeutic* vaccines. Even vaccine development for treating snakebites is an active area of research. More than 5 million people are bitten by snakes worldwide each year, resulting in over 100,000 deaths. Here, we consider selected infectious disease targets for new vaccine development, including the "big three" targets: HIV/AIDS, tuberculosis, and malaria.

The flu is caused by a large number of viruses that belong to the **influenza** family of viruses. Although most people experience flu symptoms that last for a few days and can be readily treated by over-the-counter medications, influenza kills some 500,000 to 1 million people worldwide each year. Despite many efforts to create a universal vaccine against most flu viruses, because flu viruses mutate so rapidly, no single vaccine protects against all strains. New flu vaccines are generated each year based on the three main flu virus strains expected to be prevalent during the upcoming flu season.

The **World Health Organization (WHO)**—an international group that monitors infectious diseases and epidemics—has established centers in over 80 countries so that it can collect and screen samples of influenza for analysis and then develop vaccine treatment strategies. Infectious disease scientists work through a "global lab" to monitor strains of influenza viruses around the world, replicate these viruses (which are grown in eggs), and then use recombinant DNA techniques to produce subunit vaccines in response to new viral strains detected. This strategy is a surveillance and response approach to keeping up with new pathogens and producing vaccines as needed (see Chapter 12).

Influenza A represents one of the greatest potential threats to human health through a *pandemic,* a global outbreak. Pandemic strains of influenza A have arisen in the past. In 1918, influenza virus killed at least 20 million people. Other pandemics occurred in 1957 and 1968, and epidemiologists predict that a future pandemic could be much worse than previous episodes. A strain of **avian flu (H5N1)** received a lot of media attention because of its presence in chickens. Variations in influenza A are due to two glycoproteins called hemagglutinin (HA) and neuraminidase (NA), which project from the surface of the virus. Avian flu is an influenza A subtype called H5N1 because of the variation of these two proteins contained in this strain (abbreviated as H and N when used in strain names).

In 2003, strain H5N1 caused a pandemic in chickens in Asia that resulted in the killing of over 200 million birds in an effort to halt the spread. Scientists were concerned that this strain, like other viruses, may mutate and make the jump into humans. Although a few isolated cases of human infection were seen, widespread infection did not occur; however, bird-to-pig transmission of the virus has occurred, and mutation of the virus in pigs could produce a strain that would infect humans. Because of the devastation such a virus could cause, the WHO declared development of a vaccine to protect against H5N1 a high priority and vaccines were subsequently produced. In 2009, **swine flu (H1N1)** led to significant public health concerns, but vaccination against this virus has proven effective at controlling its spread in humans.

In 2014, an outbreak of **Ebola virus** in West Africa killed over 1,500 people, with many more cases likely unreported. Ebola causes hemorrhagic fever and produces fatality rates of approximately 90 percent. There is currently no effective treatment for curing or preventing Ebola virus infection. But antibodies against Ebola expressed in tobacco leaves are showing promise in ongoing clinical trials. Mice were used to create monoclonal antibodies against the virus. The antibody genes were then introduced into tobacco plants. The transgenic tobacco plants express high quantities of the antibody proteins, which can then be isolated and purified for use in humans. Transgenic tobacco plants are commonly used for expressing recombinant proteins because of the large size of their leaves and relatively high yield of recombinant proteins compared to other plants. Also, a live attenuated whole-virus vaccine approach has shown promise in nonhuman primates (macaques). Other vaccines are under development at an unprecedented pace of moving from the lab bench to clinical trials, and Ebola vaccine scientists are optimistic that a successful vaccine may be developed soon.

In 2015, an outbreak of the mosquito-borne **Zika virus (ZIKV)** in the Caribbean and the Americas was identified as the cause of congenital defects, such as microcephaly, in babies from women infected with ZIKV. Thus, a priority was to develop a vaccine to establish ZIKV immunity in women of childbearing age and pregnant women at risk for ZIKV infection that could harm a developing fetus. Early-stage trials with DNA expressing ZIKV proteins have been shown to create levels of antibodies in mice and Rhesus macaques capable of neutralizing the virus and protecting against ZIKV infection. Clinical trials of the ZIKV DNA vaccine have already begun, so it will be very interesting to see if this approach is effective in humans.

HIV is responsible for more deaths than any other infectious disease; approximately 6,000 people are newly infected each day, and more than 33 million people are infected worldwide. Various antiviral drugs enable many to live with AIDS as a manageable disease, whereas before these drugs HIV infection for most people led to their death. But in the more than three decades since HIV was discovered as the cause of AIDS, there is still no effective vaccine against HIV. Thus, the need for a vaccine to treat and stop the spread of HIV is critical if we are to curb this devastating disease and eventually eliminate the AIDS epidemic. Several vaccines for the prevention of HIV infections or the treatment of HIV-infected individuals have been tried in humans; most of these vaccines target viral surface proteins, but to date none of these have worked. In 2007, a promising vaccine from Merck failed in phase II clinical trials when it was determined that the vaccine increased the risk of HIV infection in some men. Two subsequent trials related to this vaccine were ineffective.

One obstacle facing HIV vaccine scientists is the high mutation rate of HIV. Thus, trying to prevent infection by creating antibodies against genetically diverse and rapidly mutating strains of virus is a major challenge for vaccine developers. Another obstacle is that relatively few surface proteins on HIV can be targeted by neutralizing antibodies. Consequently, the multi-subunit vaccines called *cocktails*—those that contain mixtures of many viral proteins, together with antiviral drugs that block viral replication—may be a more effective strategy to combat HIV than using either vaccines or antiviral drugs alone. Similar strategies are being developed for treating other viruses, such as hepatitis B and C.

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis,* is responsible for between 2 and 3 million deaths each year, second only to HIV. Inhaled particles of *M. tuberculosis* can infect lung tissue, creating lumpy lesions called *tubercles.* The spread of TB has

been effectively controlled in many areas of the world by the use of antibiotics and vaccines. The most widely used vaccine, called BCG, is an attenuated vaccine but it largely protects only infants. There has been a resurgence of TB because *M. tuberculosis* has proved to be very adept at evolving new strains that are resistant to treatment. Concern over such new strains is so great that the WHO has declared TB a global health emergency. The Bill and Melinda Gates Foundation and other organizations have provided over $30 million in support of efforts to develop new vaccines against TB. The genome for *M. tuberculosis* has been sequenced and, as a result, new proteins have been discovered, leading to the development of new TB vaccines, many of which are currently in clinical trials.

Malaria is caused by the protozoan parasite *Plasmodium falciparum* and transmitted by insects. Worldwide, *Plasmodium* strains are developing resistance to the most commonly used antimalarial drugs. Although these drugs have been effective in parts of the world, the death rate from malaria is still unacceptable. Each year approximately half a billion cases of *Plasmodium* infections occur in children and cause over 1 million deaths in Africa alone. Genomic analysis of *Plasmodium* is helping scientists identify new gene and protein targets for inhibiting this parasite. A subunit vaccine called RTS,S—produced by GlaxoSmithKline and tested in children—is the most effective vaccine developed so far. But RTS,S is still only effective at protecting approximately 50 percent of children treated because it targets a particular stage of parasite development while in the bloodstream but is not effective against the parasite once it enters the liver. Several other experimental vaccines are in clinical trials currently, but to date none have emerged as fully effective at protecting against malaria.

In the next section, we will consider the many reasons for sequencing microbial genomes and the tools used to carry out this work.

5.5 Microbial Genomics

In 1994, as an extension of the Human Genome Project, the U.S. Department of Energy initiated the **Microbial Genome Program (MGP).** A goal of the MGP was to sequence whole genomes of microorganisms with potential applications in environmental biology, research, industry, and health, such as bacteria that cause tuberculosis, gonorrhea, and cholera, as well as genomes of protozoan pathogens such as *Plasmodium*, which causes malaria. In 1995, the Institute for Genomic Research, which played a major role in the Human Genome Project, reported the first completed sequence of a microbial genome when it published the sequence for *Haemophilus influenzae.* Since then, over 2,000 microbial genomes have been sequenced, and work is being carried out on the genomes for hundreds, if not thousands, of other microbes.

Why Sequence Microbial Genomes?

A team of researchers at the University of California–Los Angeles analyzed DNA sequences from 101 college students, 49 of whom had acne and 52 of whom did not. Over 1,000 strains of *Propionibacterium acnes* were isolated. Using whole-genome sequencing and bioinformatics, researchers clustered these strains into 10 related strain types. Six of these types were more common among acne-prone students, and one type appeared repeatedly in skin samples from students without acne. Sequence analysis of types associated with acne indicated groups of genes that may contribute to the skin disease, and this information is helping dermatologists develop new drugs targeted at killing acne-causing strains of *P. acnes.*

This is just one example of the potential benefits of microbial genomics. By sequencing microbial genomes, scientists will be able to identify many secrets of bacteria, from genes involved in bacterial cell metabolism and cell division to genes that cause human and animal illnesses. In addition, researchers will find bacterial genes that may enable scientists to develop new strains of microbes that can be used in bioremediation and to reduce atmospheric carbon dioxide and other greenhouse gases, to find disease-causing organisms in food and water, to detect biological weapons, to synthesize plastics, to make better food products, and to produce genetically altered bacteria as biosensors for detecting harmful substances, among many other examples.

Bacteria perform a wealth of biochemical activities, which is reflected in their genomes. Of the microbial genomes sequenced to date, a large percentage of genes identified produce proteins of unknown function, and often many genes discovered by sequencing produce proteins that are unique to the bacterial genome sequenced. Therefore, the potential for identifying new genes and proteins with unique properties that may have important applications in biotechnology is very high.

Of the millions of different bacteria that have been identified, which ones are of greatest interest to microbial genome researchers? As shown in Table 5.3, many of the bacterial genomes that have received the most attention are from microbes responsible for serious illnesses and diseases in humans. For example, *P. aeruginosa* is a major human pathogen causing urinary tract infections, skin infections, and persistent lung infections that are a

TABLE 5.3 Selected Microbial Genomes

Bacterium	Human Disease Condition	Approximate Genome Size (megabases, mB)	Approximate Number of Genes
Bacillus anthracis	Anthrax	5.23	5,000
Borrelia burgdorferi	Lyme disease	1.44	853
Chlamydia trachomatis	Eye infections, genitourinary tract infections (e.g., pelvic inflammatory disease)	1.04	896
Escherichia coli O157:H7	Severe food-borne illness (diarrhea)	4.10	5,283
Haemophilus influenzae	Serious infections in children (eye, throat, and ear infections, meningitis)	1.83	1,746
Helicobacter pylori	Stomach (gastric) ulcers	1.66	1,590
Listeria monocytogenes	Listeriosis (serious food-borne illness)	2.94	2,853
Mycobacterium tuberculosis	Tuberculosis	4.41	3,974
Neisseria meningitidis (*MC58*)	Meningitis and blood infections	2.27	2,158
Pseudomonas aeruginosa	Pneumonia, chronic lung infections	6.30	5,570
Rickettsia conorii	Mediterranean spotted fever	1.30	1,374
Rickettsia prowazekii	Typhus	1.11	834
Streptococcus pneumoniae	Acute (short-term) respiratory infection	2.16	2,236
Yersinia pestis	Plague	4.65	4,012
Vibrio cholerae	Cholera (diarrheal disease)	4.00	3,885

significant cause of death in cystic fibrosis patients, as mentioned earlier in the chapter. *P. aeruginosa* is particularly problematic because it is resistant to many antibiotics and disinfectants commonly used to treat other microbes. From sequencing its genome and learning more about the genes involved in the metabolism, replication, and the breakdown of antibiotics, scientists are developing new approaches for treating *P. aeruginosa* infections.

Streptococcus pneumoniae—a bacterium that can cause bacterial meningitis, as well as ear and lung infections, including pneumonia—kills approximately 3 million children worldwide annually. Infections of *S. pneumoniae* have been effectively treated since 1946. But many of the vaccines are ineffective in young children, who are particularly susceptible to infection. The *S. pneumoniae* genome has been sequenced, revealing many genes encoding previously undiscovered proteins on the surface of these bacteria. Researchers are optimistic that this new understanding of the *S. pneumoniae* genome will lead to new treatments for pneumonia.

One of the first microbes targeted for genomic analysis was *Vibrio cholerae,* typically found in polluted waters in areas of the world with poor sanitary practices. This bacterium causes **cholera,** characterized by severe diarrhea and vomiting, leading to massive fluid loss, which can cause shock and even death. Antibiotic-resistant *V. cholerae* are causing recurring problems in Asia, India, Latin America, and even areas of the Gulf Coast in the United States. Understanding the genome of *V. cholerae* is helping scientists identify toxin genes, genes for antibiotic resistance, and other genes that will augment current methods for combating this microbe.

Scientists have been studying the genetics of lactic acid bacteria for about 35 years to understand how these bacteria contribute to the flavor and texture of cheeses, milk, and other products we discussed earlier. Genome projects have been completed for several dozen dairy-related lactic acid bacteria. For example, scientists have sequenced the genome for *Lactococcus lactis,* a strain that is important for making cheese. Such projects are helping



YOU DECIDE

Should Pathogen Genome Sequences Remain in Public Databases?

Science as a process relies on scientists sharing data and information through presentations at conferences, in publications, and via Web resources such as databases. Now that genomes have been completed for many human pathogens—such as those that cause cholera, anthrax, meningitis, and smallpox—considerable debate has arisen about whether genome sequences for potential bioweapons should be publicly available in DNA databases. There is concern that terrorists could use sequences to develop recombinant proteins for pathogen toxins as a way to produce bioweapons. Others have suggested that terrorists could use genome data to make more effective "superweapon" pathogens resistant to existing vaccines and drugs. However, many genome scientists have spoken in favor of keeping pathogen sequences in public databases, citing the importance of open access to information and claiming that there is so much that we don't understand about the genomes of these pathogens that no human health threat is posed by making their sequences available. What do you think? Should pathogen genome sequences remain in public databases?

You decide.

food scientists better utilize different strains to make specific cheeses with enhanced flavor characteristics and to refine culture conditions to maximize the growth abilities of different microbes.

Genome biologists are also focusing on microorganisms that may be used as biological weapons. In Section 5.7, we discuss how biotechnology can be used to detect and combat bioweapons.

Metagenomic Studies Sequence Genomes from Microbial Communities

Metagenomics involves sequencing genomes for entire communities of microbes. Metagenomics projects are sequencing microbial genomes from environmental samples of water, air, and soils as well as from oceans throughout the world, glaciers, mines—virtually every corner of the globe. Metagenomics projects have been launched all around the world.

Human genome pioneer J. Craig Venter, whom we discussed in Chapter 3, left Celera in 2003 to form the J. Craig Venter Institute (JCVI), and his group played a central role in establishing metagenomics as an emerging area of genomics research. One of the institute's major initiatives was a global expedition to sample marine and terrestrial microorganisms from around the world and to sequence their genomes. Called the *Sorcerer II* Global Ocean Sampling (GOS) Expedition, between 2004 and 2006 Venter and his researchers traveled the globe by yacht, in a sailing voyage covering 32,000 nautical miles, described as a modern-day version of Charles Darwin's famous voyage on the *H.M.S. Beagle*. One of the earliest expeditions by this group sequenced bacterial genomes from the Sargasso Sea off Bermuda. This project yielded over 1.2 million novel DNA sequences from 1,800 microbial species, including 148 previously unknown bacterial species, and identified hundreds of photoreceptor genes. Scientists are interested in learning more about photoreceptors to help develop ways in which photosynthesis may be used to produce hydrogen as a fuel source. Medical researchers are also very interested in photoreceptors because, in humans and many other species, photoreceptors in the eye are responsible for vision.

A key benefit of metagenomics is its potential for teaching us more about millions of yet uncharacterized species of bacteria. Many new viruses, particularly bacteriophages, have also been identified through metagenomic studies of water and soil samples. Metagenomics is providing important new information about genetic diversity in microbes that is key to understanding complex interactions between microbial communities and their environment, as well as allowing phylogenetic classification of newly identified microbes. Metagenomics also has great potential for identifying genes with novel functions, some of which may have valuable applications in medicine and biotechnology.

The general method used in metagenomics often involves isolating DNA directly from an environmental sample without requiring cultures of the microbes or viruses, then sequencing the DNA. Such an approach is necessary because often it is difficult to replicate the complex array of growth conditions the microbes need to survive in culture. Estimates suggest that over 99 percent of currently known microbial diversity exists in organisms that cannot be cultured; thus, one advance of metagenomics is that sequences are generated from environmental samples without the need to grow microbes in culture.

An example of how metagenomics can provide novel insight into the microbial world around us is reflected by a study of the microbiota found in New

York City subways. This project revealed that most microbes present are non-disease-causing bacteria normally prevalent on human skin and in the gastrointestinal tract, although, occasionally, pathogens such as *Bacillus anthracis* were identified. But almost half of the DNA sequenced did not match any organism in eukaryotic, prokaryotic, archaeal, and viral genome databases!

The Human Microbiome Project

In 2007 the National Institutes of Health initiated the **Human Microbiome Project (HMP),** a $170 million project to complete the genomes of an estimated 600 to 1,000 bacteria, viruses, yeasts, and other microorganisms that live on and inside humans. Many microbes, such as *E. coli* in the digestive tract, have important roles in human health, and, of course, other microbes make us ill. At the start of the project it was thought that microorganisms make up some 1 to 2 percent of the human body, outnumbering human cells by about 10 to 1, although this number has been questioned. In addition, an estimated 1,200 different bacteriophages are found in the gut, and we know virtually nothing about more than half of them.

The HMP has several major goals, including:

- Determining if individuals share a core human microbiome.
- Understanding how we acquire and maintain microbial communities.
- Understanding how changes in the microbiome can be correlated with changes in human health and the conditions (such as stress and diet) that affect the microbiome.
- Developing new methods, including bioinformatics tools, to support analysis of the microbiome.
- Addressing ethical, legal, and social implications raised by human microbiome research. Does this sound familiar? Recall that addressing ethical, legal, and social issues was a goal of the Human Genome Project (HGP).

The HMP involved about 200 scientists at 80 institutions. In 2012 a series of papers were published summarizing findings from the HMP. The HMP analyzed 15 body sites from males and 18 sites from females from 242 healthy individuals in the United States and applied whole-genome sequencing of microbes and viruses present at these sites. Each person was sampled up to three times over nearly 2 years. In addition, sequencing of 16S ribosomal (rRNA) genes, which are unique for almost every bacterial species, was also used. More than 3,000 reference sequences for microbes isolated from the human body were developed. The HMP amassed more than 1,000 times the sequencing data generated by the HGP.

What are some of the key concepts that we have learned about the human microbiome?

- Sequence data from the HMP have identified an estimated 81 to 99 percent of the microbes and viruses distributed among body areas in human males and females.
- As many as 1,000 bacterial strains may be present in each person.
- The microbiome starts at birth. Babies acquire bacteria from their mothers' microbiome.
- A surprise to HMP scientists, the microbiome can be substantially different from person to person. Based on variation between individuals, an estimated 10,000 bacterial species may be part of the total human microbiome.
- In the human gut, although the microbiome differs from person to person, it remains relatively stable over time in individuals.

Based on these findings, there is no single reference human microbiome to which people can be compared. Microbial diversity varies greatly from individual to individual, and a personalization of the microbiome occurs in individuals. For instance, comparing sequences of the microbiomes from two healthy people of equivalent age reveals microbiomes that can be quite different. There are, however, similarities in certain parts of the body, with signature bacteria and characteristic genes associated that are specific to certain locations. Some scientists think that microbiomes may even be used for DNA fingerprinting to identify individuals at crime scenes in the future!

Knowledge about the personalized nature of the microbiome is already proving valuable for improving human health and medicine, which in the future will include microbiome-specific therapies. Increased promotion and use of *probiotics* (live microorganisms that provide health benefits) in recent years is a direct result of what we have learned about critical roles the microbiome plays in so many aspects of our health. Criteria are being considered to define a healthy microbiome, which is expected to help determine the role of bacteria in maintaining normal health. This may provide insight into factors that disturb a person's microbiome, and why certain individuals are susceptible to certain diseases, especially chronic conditions such as psoriasis, irritable bowel syndrome, and even obesity, based on their microbiome. Similar projects are under way to study the microbiomes of dogs and other animals. Keep an eye on these and other metagenomics

TABLE 5.4 Examples of Medically Important Viral Genomes That Have Been Sequenced

Virus	Human Disease or Illness
Ebola virus	Ebola hemorrhagic fever
Hepatitis A virus	Hepatitis A
Hepatitis B virus	Hepatitis B
Hepatitis C virus	Hepatitis C
Herpes simplex virus, type I	Cold sores
Human immunodeficiency virus (HIV-1)	Acquired immunodeficiency syndrome (AIDS)
Human papillomavirus	Cervical cancer
Human poliovirus	Poliomyelitis
Human rhinovirus	Common cold
Influenza A virus	
• Subtype H5N1 (avian flu)	Severe flu
• Subtype H5N1 (swine flu)	Severe flu
Severe acute respiratory coronavirus (SARS-CoV)	Severe acute respiratory syndrome (SARS)
Variola virus	Smallpox

projects, because they are providing very interesting data about the microbes among us.

Viral Genomics

The study of viral genomes is another hot area of research (Table 5.4). This is true in part because many deadly viruses mutate quickly in response to vaccine and antiviral treatments. Antiviral drugs are designed to work in several ways. Some antiviral drugs block viruses from binding to the surface of cells and infecting cells, whereas others block viral replication after the virus has infected body cells. Research on viral genomes helps scientists learn how viruses cause disease and leads to the development of new and effective antiviral drugs.

Creating Synthetic Genomes

In 2010 JCVI scientists published the first report of a functional **synthetic genome.** In this project they designed and had chemically synthesized more than a thousand 1,080-bp segments covering the entire 1.08-Mb genome of the bacterium *Mycoplasma mycoides* (Figure 5.13). To assemble these segments correctly, the segments had 80-bp sequences at each end, which overlapped with their neighbor sequences. These



YOU DECIDE Should We Create Life Synthetically?

If applications of synthetic biology eventually become routine, will we have simplified or demystified life, making it less sacred? Should humans be such "creators" of life? Of course, we have been creating life in the lab for a long time; ***in vitro* fertilization** is one such example. But synthetic biology is a very different approach. Not surprisingly, one fear raised about synthetic biology is that bioterrorists could use synthetic genome strategies to recreate deadly bacteria or viruses (read more about bioweapons in Section 5.7) from benign bacteria and viruses. To what extent should we use this technology to remake naturally occurring cells with features we deem better or more desirable? In the future, could synthetic genomes be used to create life from inanimate components? Should this be done if it is technically possible? You decide.

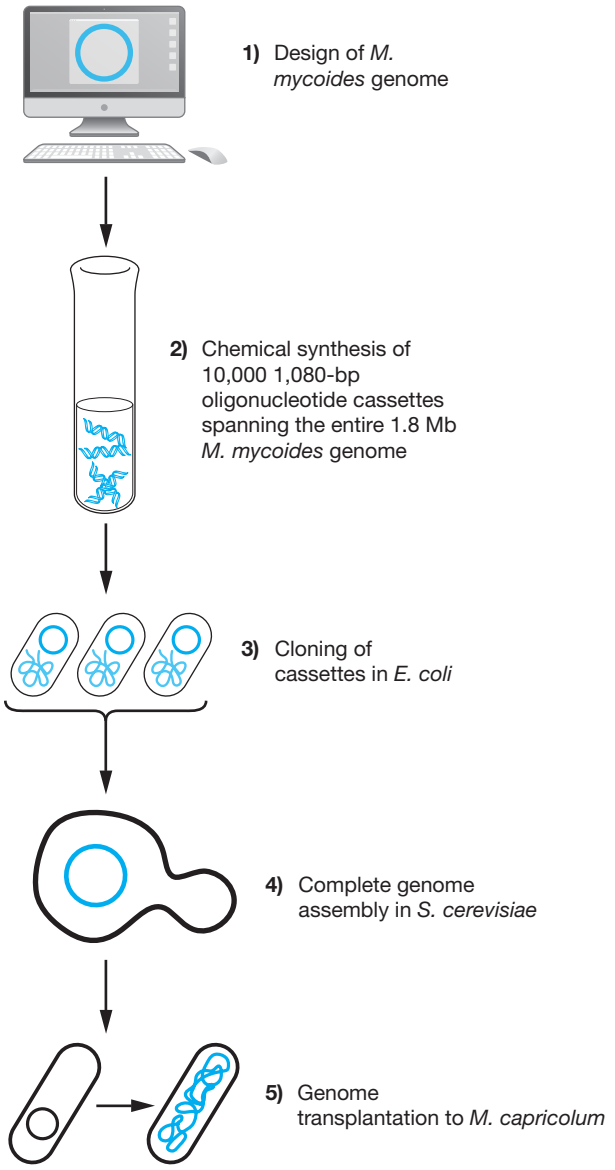


FIGURE 5.13 Building a synthetic version of the 1.08-Mb *Mycoplasma mycoides* genome JCVI-syn1.0. Shown here is an overview of the approach used to produce *M. mycoides* JCVI-syn1.0.

sequences were cloned in *E. coli*. Then, using the yeast *Saccharomyces cerevisiae,* they assembled the sequences into 11 separate 10-kb assemblies, which were eventually combined to completely span the entire *M. mycoides* genome. The assembled genome, called JCVI-syn1.0, was then transplanted into a close relative, *M. capricolum,* as recipient cells, resulting in new cells with the JCVI-syn1.0 genotype and the phenotype of a new strain of *M. mycoides*. JCVI determined that the recipient cells were taken over to become JCVI-syn.10 *M. mycoides* because they were shown to express the *lacZ* gene, which was incorporated into the synthetic genome. Selection for tetracycline resistance and a determination that recipient cells made proteins characteristic of *M. mycoides* and not *M. capricolum* were also used to verify strain conversion.

One particularly impressive accomplishment of these experiments was that the synthetic DNA was "naked" because it did not contain any proteins from *M. mycoides;* therefore, it was capable of transcribing the appropriate genes and translating all of the protein products necessary for life as *M. mycoides*. This is not a trivial accomplishment. The synthetic genome effectively rebooted the *M. capricolum* recipient cells to change them from one form to another. This research did not create life from an inanimate object, since it was based on converting one living strain into another. J. Craig Venter claimed, "This is equivalent to changing a Macintosh computer into a PC by inserting a new piece of PC software." This was tedious work, spanning over 15 years. Ninety-nine percent of the experiments involved failed!

Venter and others have used recombinant DNA technology to construct synthetic copies of viral genomes. Researchers at Stony Brook University in New York made headlines when they assembled approximately 7,500 bp of synthetically produced DNA sequences to synthesize proteins and lipids; these were assembled into a recreated polio virus: the first synthetically made virus. The genome for the 1918 influenza strain responsible for the pandemic was also assembled in this way.

Venter's work with *M. mycoides* JCVI-syn1.0 was hailed as a defining moment in **synthetic biology.** There are many fundamental questions about synthetic genomes and genome transplantation that need to be answered. But clearly these studies provided key "proof of concept" that synthetic genomes could be produced, assembled, and successfully transplanted to create a microbial strain encoded by a synthetic genome and bring scientists closer to producing novel synthetic genomes incorporating genes for specific traits of interest.

In recent years, JCVI has continued refining the synthetic genome of *M. mycoides* to determine the minimal number of genes necessary for life (which appears to be 473 genes).What are potential microbial biotechnology applications of synthetic genomes and synthetic biology? JCVI claims that its ultimate goal is to create microorganisms that can be used to synthesize biofuels. Many other possibilities exist, such as creating synthetic microbes with genomes engineered for bioremediation, producing alternative fuels, synthesizing new biopharmaceutical products, developing genetically programmed bacteria to help us heal, and making "prosthetic genomes." Yeast engineered by synthetic biology have already been used to

produce compounds that provide the aroma of grapefruits and oranges in perfumes and cosmetics. There are even teams of scientists around the world using synthetic biology to engineer yeast designed to produce certain by-products of fermentation when brewing beer to produce desired tastes and aromas!

Recently, scientists used a synthetic biology approach to engineer yeast to produce opiate precursors from sugar that can be turned into morphine and hydrocodone—powerful but addicting painkillers that have been harvested from poppy plants for thousands of years. Yeast were engineered with genes from three different poppies. Through this approach it may be possible to engineer effective but less addictive painkillers. Policy experts also worry that morphine-producing yeast strains could allow illegal drugmakers to produce opiates as easily as homebrewers can create craft beers. Yeast-derived opiates are not yet for sale. But with an opiate market estimated to be worth more than $8 billion, there is reason to expect that a company will enter this market with GM yeast-producing opiates. Keep an eye on applications of synthetic biology in the future!

5.6 Microbial Diagnostics

Microorganisms cause a number of diseases in humans, pets, and agriculturally important crops. Scientists can use a variety of molecular techniques to detect and track microbes—approaches called **microbial diagnostics.**

Detecting and Tracking Disease-Causing Microorganisms

Before the development of molecular biology techniques, microbiologists relied on biochemical tests and culturing bacteria on different growth media to identify strains of disease-causing bacteria. For example, when doctors take a throat culture, they use a swab of bacteria from your throat to check for the presence of *Streptococcus pyogenes,* a bacterium that causes strep throat. Even though these and other similar techniques still have an important place in microbial diagnosis, molecular biology allows for the rapid detection of bacteria and viruses with great sensitivity.

Initially, molecular techniques such as restriction fragment length polymorphism **(RFLP)** analysis were used in bacterial identification for diagnostic purposes. Currently, PCR and DNA sequencing are the predominant techniques widely used (**Figure 5.14**). **Whole-genome sequencing (WGS)** in particular is rapidly taking over as the tool for microbial diagnostics because entire microbial genomes can be sequenced in less than a day.

Many databases of PCR patterns and bacterial DNA sequences are available for comparison of samples. If a doctor suspects a bacterial or viral infection, samples including blood, saliva, feces, and cerebrospinal fluid from the patient can be used to isolate bacterial and viral pathogens. DNA from the suspected pathogen is then isolated and subjected to PCR or sequencing. PCR is an important tool in clinical microbiology laboratories and widely used to diagnose infection caused by microbes such as the hepatitis viruses (A, B, and C), *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (both of which cause sexually transmitted diseases), HIV-1, and many other bacteria and viruses.

Increasingly, next-generation sequencing (NGS) and third-generation sequencing (TGS) methods are being used for WGS and pathogen identification. WGS can be a quick way to identify pathogenic bacteria, viruses, or other microorganisms, and it is also very valuable for tracking genetic variations in microorganisms. This allows public health officials to track patterns and the evolution of disease-causing microbes to help develop proactive approaches to combat outbreaks of new, deadly strains. Another advantage of WGS is that, combined with bioinformatics, it is also possible to quickly identify many different species in a particular sample.

As an example, NGS provided a large body of data on evolving strains of Ebola virus during the 2014–2015 outbreak in West Africa, information essential for advancing vaccine development. From the first confirmed hospitalized case of a woman with Ebola in Sierra Leone in late May 2014, blood samples from patients testing positive for Ebola were shipped to a lab in Cambridge, Massachusetts, for sequencing. By mid-June 149 blood samples from 78 patients had been processed for deep sequencing. Alignment of these sequences yielded 78 confirmed cases of Ebola and provided 99 full-length genomes. New mutations were mapped to a reference genome. From genomic analysis, it was estimated that strains from the current outbreak diverged from a Central African version of the Ebola virus less than 10 years ago. Avian influenza, Middle East respiratory syndrome (MERS), and Zika virus are among other pathogens that have been identified and characterized by WGS.

Tracking disease-causing microbes is very important for the food services industry. Bacterial contamination of food is a significant problem worldwide. In 2010, 582 million cases of food-borne disease due to microbes occurred worldwide, resulting in 351,000 deaths. *Norovirus* was the leading cause of food-borne illness contributing to 125 million cases, followed by *Campylobacter* spp., which caused 96 million cases.

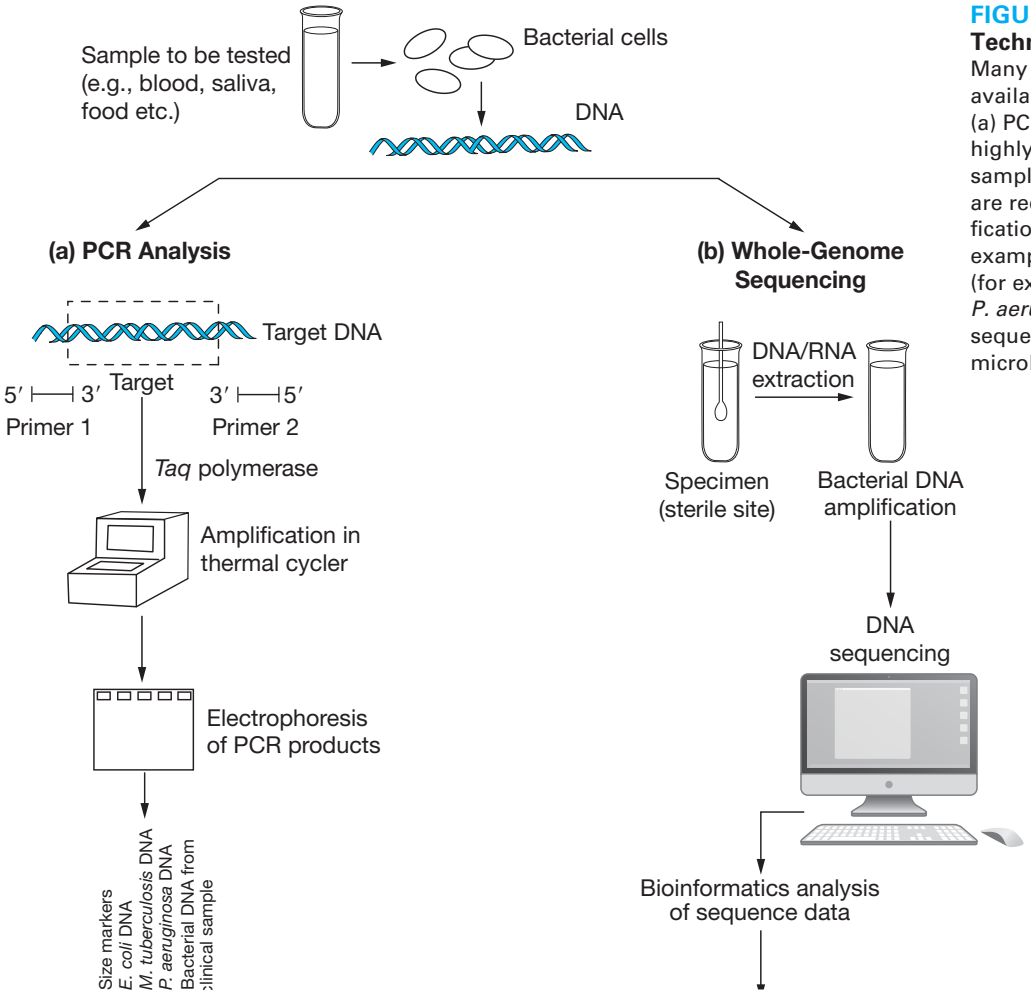


FIGURE 5.14 Using Molecular Techniques to Identify Bacteria Many molecular techniques are available for identifying bacteria. (a) PCR has the advantage of being highly sensitive; therefore, small samples and small amounts of DNA are required, allowing for fast identification of a microbe. In this example, DNA from a clinical sample (for example, blood) matches *P. aeruginosa*. (b) Routinely, DNA-sequencing strategies are used for microbial identification.

As you know, microbes play important roles in the production of dairy products such as yogurts and cheeses. But dairy products are susceptible to contamination with pathogenic microorganisms. Information about the microbes in milk can be used to determine the quality of milk and milk spoilage. You have probably also heard of the bacterium *Salmonella*, which can contaminate meats, poultry, and eggs. *Salmonella* can infect the human intestinal tract, causing serious diarrhea and vomiting, symptoms commonly called food poisoning.

A little over 20 years ago after successfully responding to an outbreak of meat contaminated with *E. coli*, which affected more than 700 people and resulted in four deaths, the **Centers for Disease Control and Prevention (CDC)** and the USDA created a national network of laboratories for determining causes of food-borne illnesses, called **PulseNet.** PulseNet enables scientists to rapidly identify microbes involved in a public health condition using variations of the techniques shown in Figure 5.14. Initially, PulseNet relied heavily on DNA fingerprinting but is gradually migrating more toward PCR-based methods and DNA sequencing. Results can be compared with a database to identify outbreaks of microbes in contaminated foods and to decide how to respond so that a minimal number of people are affected. PulseNet monitors *E. coli* O157, *Salmonella*, *Shigella*, and *Listeria*, and many non-food-borne diseases such as tuberculosis. Incidentally, recall that earlier in the chapter we discussed phage therapy. Several companies have developed phage therapy for controlling food-borne pathogens such as O157:H7 and *L. monocytogenes* and *Salmonella* at salad bars!

Microarrays have been used for detecting and identifying pathogens and for examining host responses to infectious diseases. One of the first applications was the SARS-CoV GeneChip, developed by microarray pioneer Affymetrics Inc., which contained about 30,000 probes representing the entire viral genome for the coronavirus that causes **severe acute**

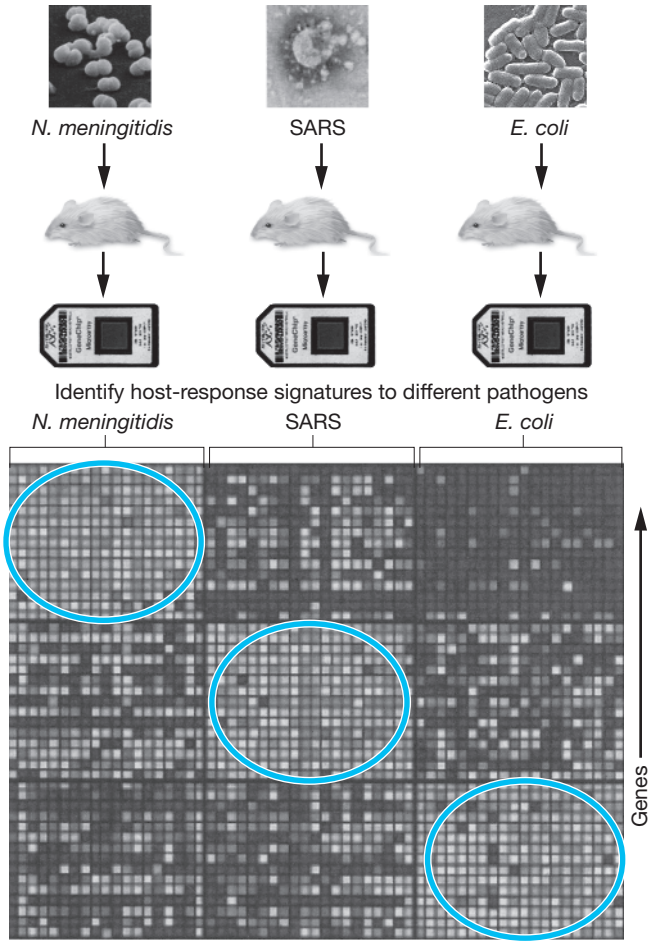


FIGURE 5.15 Host-Response Gene Expression Signatures for Pathogen Identification In this animal model, mice were infected with *Neisseria meningitidis* (the bacterium that causes meningitis), SARS, or *E. coli,* and microarray analysis was carried out to examine changes in gene expression following infection by each pathogen. In this example, actively expressed genes are shown as light spots. Notice how each pathogen activates a specific subset of genes (circled), creating a gene expression "signature" that researchers can use to identify the infecting pathogen.

respiratory syndrome (SARS). SARS is a highly contagious respiratory virus that has infected approximately 10,000 people and killed over 1,000 since first detected in 2002. Similar chips have been used to detect flu strains H1N1 and H5N1. Microarrays are also being used to study gene expression changes that occur when an organism is infected with a pathogen (Figure 5.15), providing a "signature" for infection by a particular organism. With these chips, patterns of genes that are stimulated or inhibited by a pathogen can be analyzed as a hallmark signature unique to that particular pathogen. Notice how the three pathogens used in Figure 5.15 stimulate different sets of genes in mice. But here, too, WGS techniques and even RNA sequencing are beginning to replace microarrays for pathogen detection. Related, a low-cost, paper-based ELISA-style test detecting viral RNA sequences has been developed to detect low levels of Zika virus in blood samples.

In the next section, we provide a brief glimpse of biological agents that can pose a threat as weapons and discuss how biotechnology can be used to detect and combat bioterrorism.

5.7 Combating Bioterrorism

Bioterrorism is broadly defined as the use of biological materials as weapons to harm humans or the animals and plants we depend on for food. Biotechnology by its very definition is designed to improve the quality of life for humans and other organisms. Unfortunately, bioterrorism represents the most extreme abuse of living organisms. Cases of hostile uses of biotechnology have been witnessed over the last few decades. The most well-known case is the 2001 anthrax attacks in the United States when letters contaminated with dried powder spores of the bacterium *Bacillus anthracis* were mailed to two senators, other legislators, and members of the press (Figure 5.16a). Five people were killed and 17 were infected. The Japanese Aum Shinrikyo cult also attempted several attacks in the early 1990s but was unsuccessful, with probable failures in both producing and weaponizing the bacteria they were working on (*Clostridium botulinum* and *Bacillus anthracis*).

Bioterrorism has been a legitimate concern for centuries. In the fourteenth century, bodies of bubonic plague victims were used to spread the bacterium *Yersinia pestis,* which caused bubonic plague during wars in Russia and other countries (Figure 5.16b). This subsequently played a part in causing the Black Death pandemic that ravaged Europe. Early European settlers of the New World spread measles, smallpox, and influenza to Native Americans. Although the introduction of these diseases may not have been intentional, it led to the deaths of hundreds of thousands of Native Americans because they had virtually no immunity to these pathogens. Between 1990 and 1995, aerosols of toxins from the bacterium *Clostridium botulinum* were released at crowded sites in downtown Tokyo, Japan, although no infections resulted from these attempts. In the past few decades, many other lesser-known and, fortunately, unsuccessful incidents have occurred around the world.

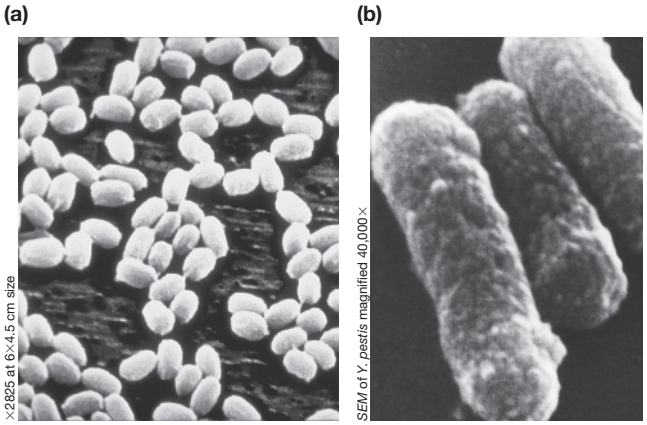


FIGURE 5.16 Deadly Microbes as Bioweapons
Potential bioweapons might include the organisms that cause anthrax [*Bacillus anthracis* (a)], and plaque [*Yersinia pestis* (b)].

Microbes as Bioweapons

New strains of infectious and potentially deadly pathogens are evolving every day all around the world. The threat posed by these disease-causing microorganisms, which could be used as **bioweapons,** may conjure images of science fiction novels; however, the potential for a bioterrorism attack is real and of significant concern.

Even though thousands of different organisms that infect humans are potential choices as bioweapons, most experts believe that only a dozen or so organisms could feasibly be cultured, refined, and used in bioterrorism (Table 5.5). These agents include *Bacillus anthracis,* the Gram-positive bacillus that causes anthrax, and deadly viruses such as smallpox and Ebola. The possibility that unknown organisms could be used as bioweapons is disquieting because they would probably be very difficult to detect and neutralize. However, a little-known microbe would probably be difficult to deliver as a bioweapon.

As a bioweapon, smallpox, which is a disease caused by the variola virus, is of concern for several reasons. Virtually all humans are susceptible to smallpox infection because widespread vaccination stopped over 30 years ago. The last confirmed cases of smallpox were reported in 1977 and in 1980, after which the WHO declared the disease to be eradicated throughout the world, in large part as a result of vaccines. Recent outbreaks of a monkey smallpox strain, however, may also revive smallpox as a concern. After 2001, the CDC worked with biotechnology companies to mass-produce and stockpile supplies of smallpox vaccines. Mandatory vaccination of certain military personnel and a voluntary campaign to vaccinate health care workers and those first responders most

TABLE 5.5 Potential Biological Weapons

Agent	Disease Threat and Common Symptoms
Bacillus anthracis (bacterium)	Anthrax. Skin form (cutaneous) produces skin-surface lesions that are generally treatable. Inhalation anthrax initially produces flu-like symptoms leading to pulmonary pneumonia, which is usually fatal.
Brucella (bacteria)	Different strains of *Brucella* infect livestock such as cattle and goats. They can cause brucellosis in animals and humans. Prolonged fever and lethargy are common symptoms. The disease can be mild or life-threatening.
Clostridium botulinum (bacterium)	Botulism. Caused by ingestion of food contaminated with *C. botulinum* or its toxins. Varying degrees of paralysis of the muscular system created by botulinum toxins are typical. Respiratory paralysis and cardiac arrest often cause death.
Ebola virus or Marburg virus	Both are highly virulent viruses that cause hemorrhagic fever. Symptoms include severe fever, muscle/joint pain, and bleeding disorders.
Francisella tularensis (bacterium)	Tularemia. Lung inflammation can cause respiratory failure, shock, and death.
Influenza viruses (a large, highly contagious group)	Influenza (flu). Severity and outcome depend largely on the strain of the virus.
Rickettsia (several bacteria strains)	Different strains cause diseases such as Rocky Mountain spotted fever and typhus.
Variola virus	Smallpox. Chills, high fever, backache, headache, and skin lesions.
Yersinia pestis (bacterium)	Bubonic plague. High fever, headache, painful swelling of lymph nodes, shock, circulatory collapse, organ failure, and death within days after infection in a majority of cases.



YOU DECIDE

"Gain of Function" Experiments and Engineering Viral Pathogens

Various researchers around the world have carried out so-called *gain-of-function* experiments designed to help understand how certain pathogens can become more deadly and potentially transmissible to humans. For example, flu researchers at the University of Wisconsin reported a highly controversial finding that they had genetically engineered H5N1 flu resulting in air-borne transmission between ferrets. Recall that H5N1 is normally only transmitted between birds. The same group engineered H1N1 using genes related to those from the 1918 pandemic strain. The engineered virus was transmissible in mammals and more dangerous than the natural strain of H1N1. Thus, this team concluded that a pandemic flu could potentially originate from wild avian flu viruses.

In response to concerns about such research, there had been a ban in the United States on federal funding for gain-of-function research that increases the virulence of influenza viruses or the viruses that cause SARS or Middle East respiratory syndrome (MERS). In early 2018, the ban was lifted, based in part on studies concluding that few gain-of-function studies pose a significant public health risk. New policies will be put in place to assess and approve research on pathogens with pandemic potential. Researchers argued that viruses are always mutating and studies such as these can help us predict genetic changes to look for to help us combat infectious disease outbreaks. Do these researchers have a point? Should restrictions be placed on what viral pathogens can or cannot be altered? For example, should engineering an air-borne form of HIV or Ebola be permitted? You decide.

likely to come in contact with the variola virus were also instituted.

Targets of Bioterrorism

Antibioterrorism experts expect that bioterrorists will target cities or events where large numbers of humans gather at the same time. Experts, however, have had a poor track record of predicting where and how such acts might occur. At best, we can speculate that there may be many different potential ways to deliver a bioweapon to injure or kill humans. Bioterrorists are most likely to use a limited number of approaches to achieve quick and effective results. Widespread application of most agents might occur via some type of aerosol release in which small particles of the bioweapon are released into the air and inhaled. The aerosol could be created by grinding the bioweapon into a fine powder and producing a "silent bomb" that would release a cloud of the bioweapon into the air. This aerosol cloud would be gas-like, colorless, odorless, and tasteless. Such a silent attack could go undetected for several days. If exposed to a biological agent, in the days following an attack, a few people might develop early symptoms, which physicians might misdiagnose, or their symptoms might closely resemble common illnesses. Meanwhile, if the biological agent could be spread from human to human, larger numbers of people in other states and even other countries would become infected as infected individuals traveled from place to place and spread their illness. Experts also suspect that biological agents might be delivered by crop-duster planes or disseminated into water supplies.

Experts are also concerned with preventing bioweapon attacks that could cause severe damage to crops, food animals, and other food supplies (see Table 5.6). Not only could such an attack affect human health, but this approach could cripple the agricultural economy of a country if food animals such as cows were infected. If there were general concerns about the safety of beef and milk, many consumers would likely shy away from buying these products for fear of contamination.

Using Biotechnology against Bioweapons

Most countries are poorly prepared for major disease outbreaks, whether of natural origin or caused by a deliberate misuse of biology. Several initiatives have been taken to build preparedness against bioterrorism attacks. For instance, the Global Health Security Agenda brings together over 50 nations, international organizations, and non-governmental stakeholders to achieve specific and measurable targets around biological threats. The World Bank's Pandemic Emergency Financing Facility provides surge funding to prevent high-severity disease outbreaks from becoming pandemics. A 2015 report by the InterAcademy Partnership Biosecurity Working Group, which is also relevant to the Biological and Toxins Weapons Convention, highlighted several areas in which biotechnology has advanced since 2011: understanding of disease; detection of disease; diagnosis and surveillance; preventing, mitigating and treating disease using vaccines and drugs; industrialization of biotechnology; and delivery of drugs. Even with increased budgets for antibioterrorism work, new

TABLE 5.6 Potential Biological Pathogens for a Bioweapons Attack on Food Sources

Disease	Target/Vector	Agent
Animal diseases		
Avian influenza	Birds, pigs	Influenza virus (orthomyxovirus)
Foot-and-mouth disease	Livestock	Foot-and-mouth virus
Goat pox	Sheep, goats	Goat pox and sheep pox virus (Capripoxvirus)
Hog cholera	Pigs	Hog cholera virus
Newcastle disease	Chickens, turkeys	Paramyxovirus
Plague	Animals, fowl, fish	*Yersinia pestis* (bacterium)
Rabies	Livestock	Rabies virus (Lyssavirus)
Plant diseases		
Citrus canker	Citrus	*Xanthomonas axonopodis* pv. *citri* (bacterium)
Potato blight	Potato	*Phytophthora infestans* (fungus)
Rice blast	Rice	*Pyricularia grisea* (fungus)
Southern corn leaf blight	Corn	*Bipolaris maydis* (fungus)
Stem rust for cereals	Oat, barley, wheat	*Puccinia* spp. (fungus)
Zoonoses (diseases that can be transmitted from animals to humans)		
Brucellosis	Livestock	*Brucella melitensis* (bacterium)
Cutaneous anthrax	Livestock	*Bacillus anthracis* (bacterium)
Japanese encephalitis	Mosquitoes	Japanese encephalitis virus

Source: Gilmore R. U.S. food safety under siege? *Nat Biotechnol.* 2004;22:1503–1504.

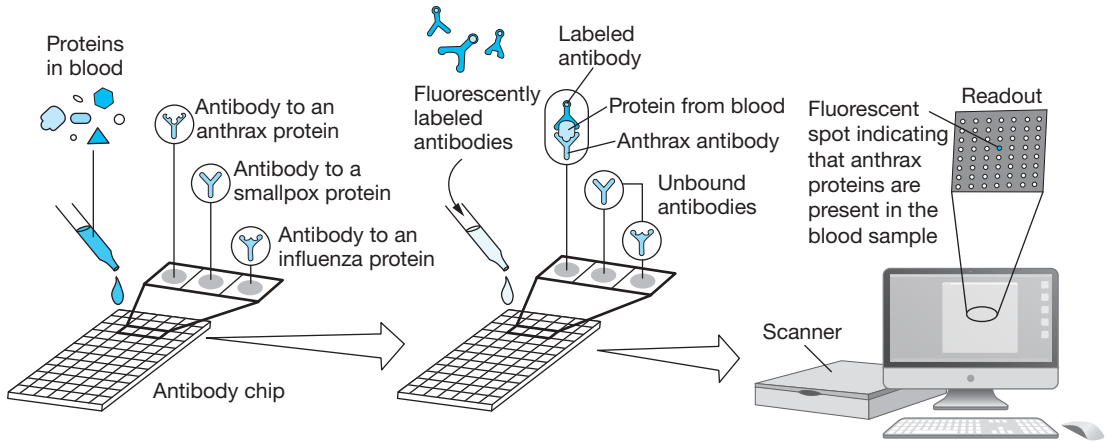
laws, and international treaties, no measures can guarantee that bioterrorism will never occur or that we can adequately detect and protect people against illness from such an attack if it were to occur. Worldwide efforts to prevent bioterrorism are essential, but how can biotechnology help to detect bioweapons and respond to an attack if it did occur?

Field tests are an essential step in the detection of an attack. Some field tests involve antibody-based tests such as enzyme-linked immunosorbent assays (ELISAs) to determine whether pathogens, or specific molecules from a pathogen, are present in an air or water sample. Such units were put in place around the Pentagon in 2001, the Gulf War, and the wars in Afghanistan and Iraq. These units use antibodies to detect pathogens in the air, but this technique is flawed because many of these instruments are not very sensitive and cannot detect small quantities of pathogens. In fact, these sensors have been known to detect harmless, naturally occurring microbes. Thus, more sensitive and highly-specific biosensors must be developed.

Variations of protein-based assays include rapid handheld immunoassays and protein microarrays used by the U.S. military to detect air-borne pathogens such as anthrax spores and the smallpox virus (**Figure 5.17**). Designed for use in lab settings or even quick diagnostic tests in field settings, these arrays detect whole pathogens or pathogen components such as proteins or spores.

Emergency response teams and cleanup crews will also be called to action to evaluate the extent of contamination and determine appropriate cleanup procedures based on the bioweapon released (**Figure 5.18**). Should an attack occur, drugs such as antibiotics will be needed. For instance, the antibiotic ciprofloxacin (Cipro) was in high demand during the anthrax threats of 2001.

Countries must build a stockpile of drugs and vaccines that can be widely distributed to large numbers of people if necessary. The basic problem with vaccines is that they must be administered *before* exposure to bioweapons in order to be effective—giving them to infected individuals after an attack is useless. However, even drugs and vaccines may be ineffective if an attack involves organisms that have been engineered against most conventional treatments or if an unknown organism is used as the bioweapon.



1) Apply blood from a patient to a chip, or array, consisting of antibodies assigned to specific squares on a grid. Each square includes multiple copies of an antibody able to bind to a specific protein from one organism and so represents a distinct disease-causing agent.

2) Apply fluorescently labeled antibodies able to attach to a second site on the proteins recognizable by the antibodies on the chip. If a protein from the blood has bound to the chip, one of these fluorescent antibodies will bind to that protein, enclosing it in an antibody "sandwich."

3) Feed the chip into a scanner to determine which organism is present in the patient's body. In this case, the culprit is shown to be *Bacillus anthracis*, the bacterium that causes anthrax.

FIGURE 5.17 Protein Microarrays for Detecting Bioweapon Pathogens

An unfortunate reality is that somewhere in this world, someone may be working to plan an attack with biological weapons. Will we be prepared?

5.8 Microbes for Making Biofuels

The world consumed over 4.6 million tons of oil equivalent per year in 2017, and only about 84 metric tons of oil equivalent biofuel, including ethanol and biodiesel, was produced in the same year. The production of biofuels has the potential to provide alternative energy sources and reduce global warming resulting from the burning of fossil fuels.

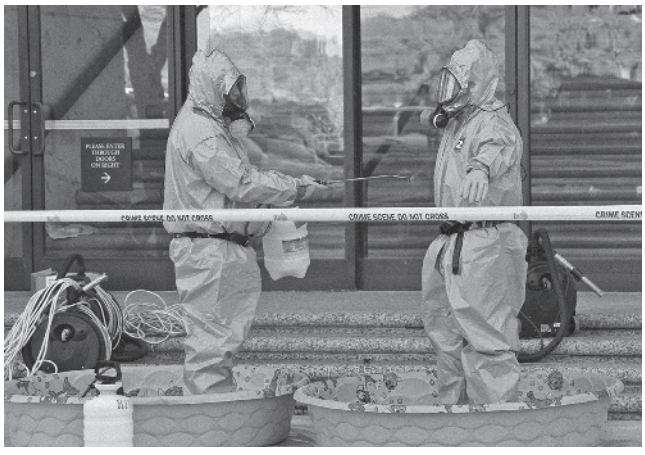


FIGURE 5.18 Battling Bioterrorism Hazardous material workers from the U.S. Coast Guard decontaminate a coworker after working inside the Hart Senate Office Building to clean the building of anthrax spores during the anthrax attacks in 2001.

To produce ethanol, alcohol-fermenting microbes convert glucose and other sugars in grain to ethanol, but this process is not cost-effective or efficient. It takes a lot of corn kernels to produce relatively small amounts of ethanol. Although, as you will learn in Chapter 6, cellulosic biomass such as corn stalks is a readily available and abundant source of sugars for making ethanol, breaking down glucose from cellulose is no simple matter. The trick is to break the cellulose down into individual glucose molecules, which can then be used to create ethanol by fermentation processes. Cellulose in the plant cell wall is somewhat resistant to breakdown naturally in the environment. You also know that you and I cannot digest cellulose; therefore, it contributes fiber to our diet. Some techniques use chemical treatments to help loosen the cellulose structure, but these chemicals inhibit many microbes that could be used to make ethanol.

Many companies working on alternative energy sources consider cellulosic sugars for ethanol production as a sustainable source of biofuels for reducing the world's reliance on fossil fuels. One concept is to create biorefineries in which leftover biomass from conventional crops—such as corn husks and stalks, wood waste such as chips, saw dusts, and yard

clippings—would incorporate microbial enzymes to process sugars with great efficiency from biomass into fuels. Researchers are improving strains of bacteria, such as *Zymomonas mobilis,* to help with this process. Key genes encoding enzymes that will convert sugars into ethanol at higher rates than yeasts can have been engineered into these bacteria, but their effectiveness in scale-up processes for making ethanol is unproven so far.

Recombinant DNA technology is being used to produce *E. coli* with an increased ability to produce ethanol, as well as bacteria with an increased ability to ferment sugars into ethanol by alcohol fermentation. Others have genetically engineered *E. coli* to secrete cellulose-degrading enzymes, and synthetic biology approaches are under way to engineer microbes with a number of genes that can drive production of enzymes involved in biofuel production. This includes microbes that may produce sugars by photosynthesis that can then be degraded into fuels by the same synthetically engineered microbes or by other microbes.

In 2017, a team from the University of California–Berkeley reported that by adding a small amount of cadmium to a naturally occurring non-photosynthetic strain of bacteria called *Moorella thermoacetica*, the bacteria can produce cadmium sulfide crystals on their cell surface. Serving as tiny photosynthetic solar panels, these crystals allow the cells to produce acetic acid (the major component of vinegar) from carbon dioxide, water, and light. These bacteria can be mixed with genetically engineered *E. coli* that can use acetic acid as a food source to create butanol—a component of lighter fluid—and polymers that can be turned into plastics. In the lab, these bacteria were much more effective at harvesting sunlight than plant cells. It will be interesting to follow how this research progresses in the future to see if it leads to the development of applications for generating biofuels.

Finally, major bioprospecting efforts are ongoing worldwide to identify bacteria that produce enzymes that could help to process biomass into fuel. Cyanobacteria, often confused for algae because of how they grow in water, produce and release sugars into water. When grown under the right conditions, cyanobacteria can produce significant quantities of sugar per liter of biomass. A bacterium called *Prochlorococcus* is the smallest but most abundant photosynthetically active cell in the ocean that has been discovered to date. Some estimate that this tiny microbe accounts for approximately 5 percent of global photosynthesis! Different strains of *Prochlorococcus* have an estimated 80,000 genes. What can we learn from *Prochlorococcus* that might be important for reducing carbon pollution in the environment and bolstering oxygen production? In Chapter 9, we will briefly discuss how bioremediation approaches are investigating the use of microbes to degrade components in sediments as a way of generating energy.

It is unclear whether microbial biotechnology can produce biofuels as a major solution to our energy problems and to help reduce our reliance on fossil fuels in the future. But ongoing research over the next decade is expected to result in significant improvements in biofuel technology.

MAKING A DIFFERENCE

For over 80 years, antibiotics have had a significant impact on successfully treating infections in humans, pets, livestock, and other animals and limiting the spread of infectious microbes around the world. Without question, antibiotics as a biotechnology product have made a major difference in improving our quality of life when it comes to combating disease. There are also many examples in which overuse of antibiotics is causing health concerns in individuals on long-term antibiotic treatments, and such use can lead to new strains of antibiotic-resistant microbes. Thus, bioprospecting continues in our war against infection as biotechnology companies continue to seek novel antibiotics and other drugs to combat microbial pathogens.

QUESTIONS & ACTIVITIES

Answers can be found in Appendix 1.

1. Explain how prokaryotic cell structure differs from eukaryotic cell structure by describing at least three structural differences. Provide specific examples of how prokaryotic cells have served important roles in biotechnology.
2. Describe how yeasts differ from bacteria and describe the role(s) of yeast in at least two important biotechnology applications.
3. Several research teams have demonstrated that microbes such as *E. coli* can be genetically modified to produce morphine and other pain killers. If GM yeast or bacteria producing opioids were commercially available, what restrictions should be in place for their use? Explain your answers.
4. During what step of the calcium chloride transformation procedure does DNA bind to cells? What step in this procedure causes DNA to enter cells? Describe several advantages of

electroporation over the calcium chloride procedure for transformation.

5. What is a fusion protein and how can it be used to isolate a recombinant protein of interest?
6. Why are anaerobic microbes important for making many foods?
7. If you were interested in producing a sweet-tasting wine with low alcohol content, explain how you might attempt to control the oxygen concentration in your bioreactor to achieve the desired results.
8. In this chapter we mentioned the urgent need to develop new antibiotics with different mechanisms of action. So far, a few new classes of antibiotics have been discovered, and some of them have been used in clinical trials. Do an Internet search to learn about "lantibiotics" and "odilorhabdins" and what makes them different from each other.
9. Discuss the four major ways in which vaccines can be made to fight a virus or bacterium, and describe how each vaccine works.
10. Search the ECDC website (**https://ecdc.europa.eu/en/home**) and the WHO website (**http://www.who.int/**) to determine (1) which influenza strains are recommended for vaccine development for the year, (2) how many types of seasonal influenza vaccines are available in the EU/EEA member states, and (3) which vaccine formulations will be used in northern and southern hemispheres.
11. We have become accustomed to being vaccinated against pathogens such as those that cause measles and polio. The hepatitis B vaccine is a prophylactic vaccine to prevent an infection caused by the hepatitis B virus (HPV). Therefore, the WHO recommends that newborn infants receive a shot of the vaccine, preferably within 24 hours of birth. This should be followed by two or three doses to complete the primary series of the vaccination. Do you think such birth-dose routine of hepatitis B vaccination is necessary? Although hepatitis B vaccine is not expensive, do you think the vaccination process is cost-effective in low and middle-income countries?
12. Most vaccines are designed to be preventative, or prophylactic. What does this mean?
13. What are the "big 3" infectious disease targets for vaccine development?
14. How can information gained from studying microbial genomes be used in microbial biotechnology? Provide three examples. What is metagenomics? In your answer provide an example of why metagenomics projects can be valuable.
15. Why are the JCVI synthetic genome experiments considered a breakthrough for synthetic biology?
16. Describe potential future applications of microbial biotechnology that may arise from synthetic biology.
17. Launched in 2011, the Global Microbial Identifier (**www.globalmicrobialidentifier.org/**) has emerged as an important resource. Visit the GMI website to learn about what this resource is used for.
18. Visit the PulseNet International website (**http://www.pulsenetinternational.org/**) and click on "Molecular Typing" to learn about the diagnostic techniques (e.g., PFGE, MLVA, and WGS) used for food-borne disease surveillance.
19. If you were in charge of protecting citizens from a bioterrorism attack, what biotechnology strategies would you consider for monitoring infectious agents? Do you think that vaccination against a bioweapon such as the *Variola vaccinia* virus, which causes smallpox, should be mandatory for all citizens even if the vaccine causes life-threatening side effects in some people?
20. In this chapter, we covered a wide range of topics related to microbial biotechnology. Many of these topics are controversial and evoke ethical questions about their applications in everyday life. From your perspective, describe what you think are the top three ethically challenging topics in microbial biotechnology and why these topics are controversial, and consider each of these from deontological and utilitarian perspectives.

Visit www.pearsonglobaleditions.com for the Companion Website

The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

This case study has been included to give you an opportunity to become familiar with a research example applicable to this field of study. After you have analyzed the data and answered the questions, you can compare them to those provided to your instructor with the text materials.

CASE STUDY

Designer Microbes Rely on Synthetic Amino Acids

In 2015 (**Go to doi.org and search for 10.1038/nature14095**), Farren Isaacs and colleagues at Yale University reported the production of GM bacteria whose growth is restricted by expressing different essential amino acids that are derived from synthetic, not naturally occurring, amino acids. Similarly, George Church and colleagues at Harvard (**Go to doi.org and search for 10.1038/nature14121**) described a GM version of *E. coli*, also created by synthetic genomics, with a reprogrammed genetic code that makes these microbes dependent on a synthetic amino acid. In 2017, Church's lab also described the design of a synthetic genome for *E. coli* in which seven codons were replaced with synonymous alternative codons (see Ostrov N, et al. *Science*. 2017;353:819–822). These experiments suggest approaches for using synthetic biology methods to create GM microbes dependent on artificial nutrients. Review these papers and consider the following.

Questions

Answers can be found at www.pearsonglobaleditions.com

1. Explain how these approaches might be used to prevent the escape and replication of GM microbes in the environment.
2. Based on what you know about the ability of microbes to mutate and adapt, are synthetic biology approaches such as these a guarantee that GM microbes will retain their engineered characteristics? Explain your answers.
3. If this approach works for bacteria, are there potential applications for animal and plant cells?
4. Can you think of potential concerns about this approach to creating GM microbes? Explain your answers.

CHAPTER SIX

Plant Biotechnology



Plants being cloned for evaluation of traits

After completing this chapter, you should be able to:

- Describe the impact of biotechnology on agricultural crop production.
- Outline how plant biotechnology could reduce hunger and malnutrition around the world.
- Explain some advantages of using genetic engineering to improve plants.
- List and describe several methods used in the genetic engineering of plants.
- Describe the use of the Ti plasmid from *Agrobacterium* as a gene vector.
- Explain the value of using gene stacks in developing new plant varieties.
- Explain how gene deletion has accelerated approval of new plant varieties.
- Describe some of the controversies in labeling genetically modified foods.
- List some opportunities for using plant biotechnology to produce protein drugs.
- List the environmental impacts, both positive and negative, of biotechnologically enhanced plants.

Over the past 40 years, the world population has nearly doubled, while the amount of land available for agriculture has increased by a scant 10 percent. Yet we still live in a world of comparative abundance. In fact, world food production per person has increased 25 percent over the past 40 years. How has it been possible to feed so many people with only a marginal increase in available land?

Part of the answer lies in fertilizers, pesticides, irrigation, and other elements of modern agriculture—technologies that bring great benefits but that also pose risks to the environment. Agricultural productivity contributed approximately 24 percent of global greenhouse gas emissions according to the latest figures. Agriculture uses 70 percent of global freshwater, and fertilizer and pesticide runoff from farms contributes to the contamination of water supplies and coastal areas.

The other half of the story is, simply, that today's crop plants are better than their forebears—more productive, more resistant to disease, and able to grow under a wider range of conditions. These advances were achieved in part through conventional selective breeding, but increasingly this process is abetted and accelerated by biotechnology. **Figure 6.1** shows the steady growth in total area planted in biotech crops. As the world's population continues to increase, we are likely to rely increasingly on techniques of bioengineering. Used well, these techniques can produce plants that are more nutritious and that can be grown with less use of pesticides and fertilizers and a lower demand for water and fossil fuels. Yet the power and novelty of these techniques raise concerns that they could be misused or have unintended consequences.

In this chapter, we'll survey some of the key biotechnologies used in agriculture and see how they are applied. We will then look at the concerns they have raised and at the regulatory structures that are in place to prevent unwanted consequences.

FORECASTING THE FUTURE

Since the early 1990s, some biotech companies have proposed using crop plants as miniature factories for producing pharmaceutical proteins, including vaccines. Traditional vaccines must be created in high-tech pharmaceutical facilities, and many vaccines require continuous refrigeration and must be administered by injection. By contrast, a plant-based vaccine could be grown inexpensively in a field or greenhouse near where it is needed. Administering the vaccine could be as simple as giving the recipient a capsule containing dried and ground leaves, or even a fruit or vegetable to eat. The need for inexpensive vaccines that do not require refrigeration was first voiced by the World Health Organization in the early 1990s and has resulted in studies of vaccines cultivated in bananas, potatoes, tomatoes, rice, wheat, soybeans, corn, and tobacco.

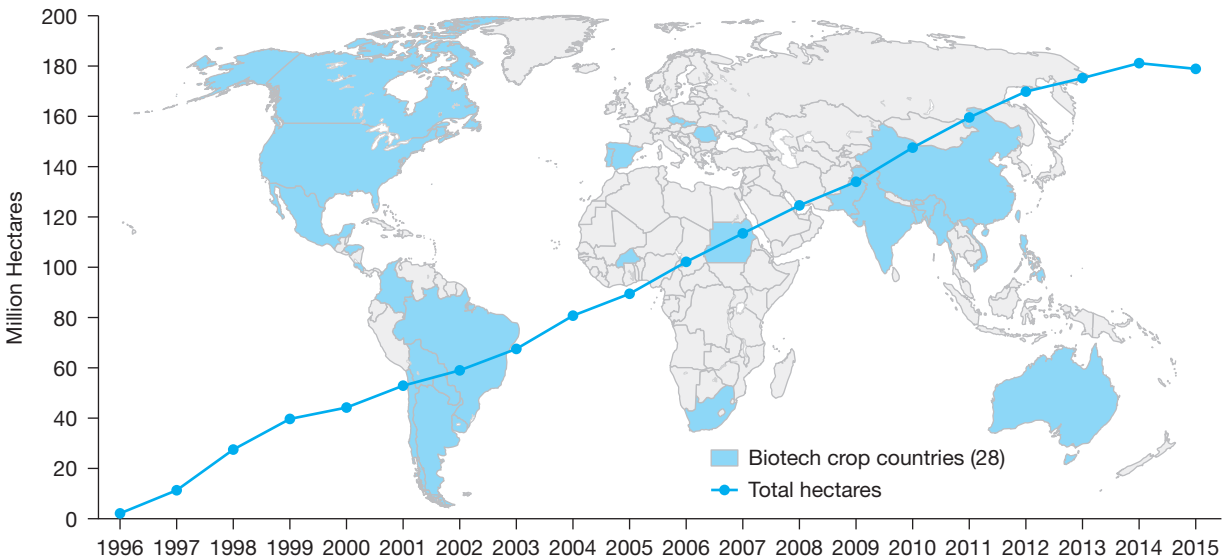


FIGURE 6.1 Global Area of Biotech Crops in Million Hectares (1996-2015) Since 1996, 2 billon hectares of cultivatable land—an area more than twice the size of China or the United States—has been planted with biotech crops, according to the Crop Biotech Update Special Edition 13, April 2016, www.isaaa.org/kc/cropbiotechupdate.

At the time of this writing, a vaccine against influenza (flu) that is produced by genetically engineered tobacco plants is in phase III clinical trials. It is quite possible that your next flu shot will come from this source.

It is necessary to ensure that plants engineered to produce drugs or vaccines do not enter the food supply or pass their genes to food crops, so these plants typically must be grown in greenhouses or in fields subject to isolation protocols. Tobacco has the advantage of being a non-food plant and therefore is unlikely to contaminate the food supply.

6.1 Uses of Biotechnology to Enhance Selective Breeding

Humans have engaged in selective breeding ever since we first domesticated plants. At first, this consisted simply in planting seeds from plants with desirable characteristics. Later, armed with an increasingly sophisticated understanding of inheritance and genetics, we learned to cross different lines to create plants with the best characteristics of both. However, this process of conventional selective breeding remained slow, unpredictable, and labor-intensive. The famous plant breeder Luther Burbank created a pure white blackberry—but at the cost of 65,000 unsuccessful crosses. The problem is particularly acute for tree crops: The offspring from a single cross may take years (and acres) to mature and bear fruit, but a fast-moving new disease can cause rapid devastation.

Within the past few decades, techniques from biotechnology have revolutionized selective breeding, making it much faster and more efficient. The following sections examine some of these techniques.

Marker-Assisted Selection

In conventional plant breeding, the only way to tell whether a cross had worked was to wait for the offspring—or, in the case of a recessive trait, *their* offspring—to mature enough to exhibit the trait in question. A technique known as **marker-assisted selection,** or MAS, can shortcut this process.

The first step is to identify genetic variants that are associated with desired traits, such as productivity or disease resistance. Next, genetic markers located near these alleles are identified. Unlike genes, these markers, which are short DNA sequences, can be identified quickly and inexpensively.

Suppose that a cross between two fruit trees—one with good fruit and the other with a disease-resistance trait—has yielded a large number of seedlings. Instead of planting all the seedlings and waiting while they grow, a small tissue sample can be taken from each of them and tested for the presence of markers inherited with the alleles for fruit quality and disease resistance. A seedling that has these markers almost certainly has inherited the desired alleles as well. These seedlings can then be selected and grown, while the rest are discarded. This technique of marker-assisted selection can also be used to select for traits that are difficult to measure or that exhibit low heritability.

Mutation Breeding

Conventional selective breeding can work only with traits that are available in the species of interest or in close relatives with which it can interbreed. For instance, Luther Burbank could not breed for a white blackberry until he obtained a mutant wild strain that had pale berries (along with the small, tart fruit typical of a wild plant). Such spontaneous mutations are rare, however.

To create many mutants and thus increase the pool of available traits, breeders can perform **mutation breeding,** the process of exposing seeds to mutation-causing chemicals or radiation. Most of the resulting mutants will not be interesting, but a few may have useful traits, such as a new fruit color or larger seeds. From 1930 to 2014, more than 3,200 plants were released that had mutagenesis in their background. Crop plants account for 75 percent of mutation-derived species with the remaining 25 percent as ornamentals or decorative plants. For example, almost all red grapefruit that we buy in the store today were produced by this process.

Protoplast Fusion

Occasionally, in nature, two plants breed in such a way that the offspring inherits the full, diploid genome of each parent. This process can allow plants that would not ordinarily interbreed to produce fertile offspring, and the offspring are often larger and more vigorous than the parents. As an example, modern wheat derives from two such spontaneous crosses, which successively combined the genomes of three wild grass species.

This rare spontaneous process can be duplicated in the lab through a technique called **protoplast fusion,** shown in Figure 6.2. This technique begins with the creation of a **callus**—a mass of cells that grows over the site of a wound in plants. These cells have the ability to form a complete new plant if cultured appropriately. (If you have ever rooted a cutting, you have taken advantage of this ability.) Like all plant cells, callus cells are surrounded by a thick cell wall made of cellulose. Fortunately, the cell wall can be dissolved with the enzyme cellulase, leaving a denuded cell called a **protoplast.** It

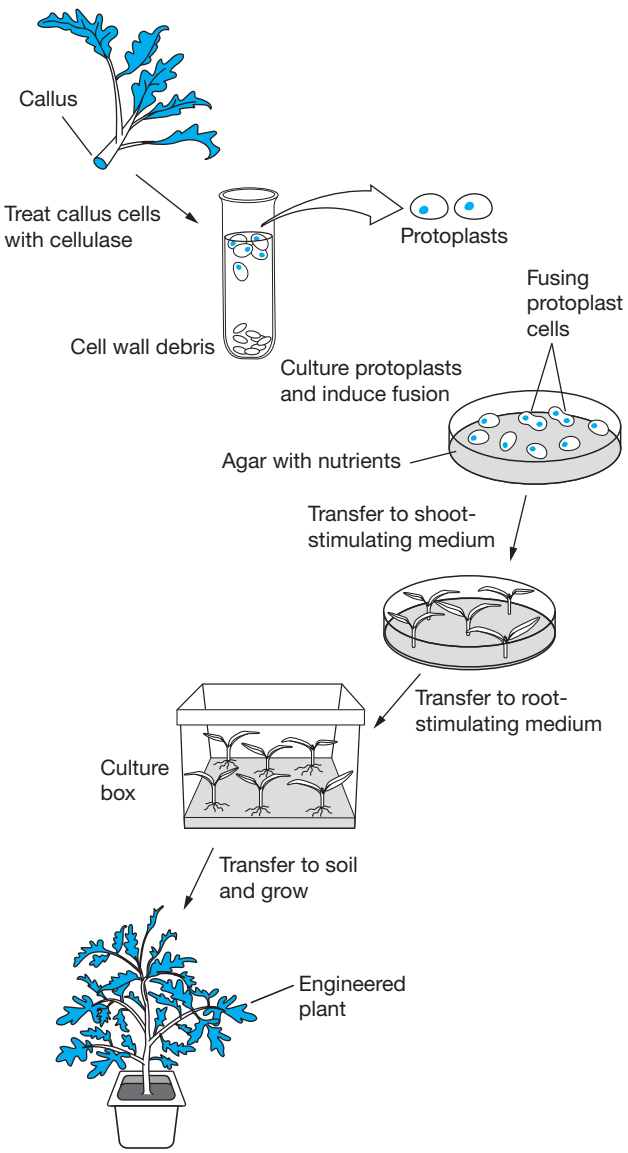


FIGURE 6.2 Protoplast Fusion and Regeneration of a Hybrid Plant Protoplasts are created by digesting the cell wall with the enzyme cellulase. To create a hybrid plant, protoplasts from different plants are fused by culturing them in a sterile medium with plant hormones to stimulate growth and development.

is also possible to start from a plant cell culture that has been denuded and sterilized. In cell culture, the protoplast can then be fused with another protoplast from a different plant, creating a fused cell that can grow into a hybrid plant. Protoplast fusion has been used to create broccoflower, a fusion of broccoli and cauliflower. It has also been used to combine different species of wheat, and to combat potato leaf roll disease.

6.2 Genetic Engineering of Plants

Now we will turn to techniques that directly manipulate the plant genome. Most of these techniques insert new genes into the target plant, creating a transgenic plant. This process is called **plant transformation**. Plant transformation can achieve results that are impossible with conventional breeding methods, such as the introduction of bacterial genes for pest resistance, or engineering a crop plant to be resistant to herbicides. The 83 percent of the world production of soybean, 75 percent of cotton, and 29 percent of maize are genetically modified. Among the countries growing GM crops, the USA (70.9 Mha), Brazil (44.2 Mha), Argentina (24.5 Mha), India (11.6 Mha), and Canada (11 Mha) are the largest users. Table 6.1 lists current genetically modified crops.

Using *Agrobacterium* to Insert Genes

One of the main ways in which genes are inserted into plants is through the use of a soil bacterium called *Agrobacterium tumefaciens* (recently reclassified as *Rhizobium radiobacter* by genome analysis, but the name *Agrobacterium* is still in common use). In nature, this bacterium makes its living by entering a plant through a wound and inserting DNA into cells of the plant. This DNA integrates into the cell's chromosomes and then directs the cell to proliferate into a tumor that both houses and feeds the bacteria. The tumors are known as **crown galls** (see Figure 6.3). Specifically, *A. tumefaciens* infects the plant cell by means of a large, circular double-stranded DNA plasmid, which is known as a Ti plasmid (from "tumor-inducing"). The portion of the Ti plasmid that integrates into the plant's chromosomal DNA is called T-DNA.

To use *Agrobacterium* for genetic engineering, the T-DNA is modified by removing the genes that cause tumor formation and replacing them with the genes to be inserted into the plant.

The Leaf Fragment Technique

To put transferred DNA (T-DNA) to use, researchers may employ the **leaf fragment technique.** In this method, small discs cut from a leaf are cultured briefly in a medium containing genetically modified *Agrobacterium,* as shown in Figure 6.4. The Ti plasmid has been disarmed of tumor genes, and cloned genes have been inserted. During this exposure, the DNA from the Ti plasmid can integrate into the DNA of the host cell. The leaf discs are then treated with plant hormones to stimulate shoot and root development before the new plants are placed in soil.

TABLE 6.1 Current Genetically Modified Crops

Crop	Traits	Genetic Modification	Date Introduced; Percent of Total Crop Grown
Alfalfa	Resistance to glyphosate or glufosinate (herbicide resistance)	Genes added	Planted in US 2005–2007; deregulated 2011
Canola/rapeseed	Herbicide resistance	Genes added	2005; 21–87%
Corn	Herbicide resistance; Bt (insect resistance); amylase added for ethanol production (biofuel)	Genes added	2013 herbicides; 2013 stacked genes; 26–85%
Cotton (cottonseed oil)	Herbicide and insect resistance	Genes added	2013 herbicide; 2013 Bt; 2013; stacked genes 49–80%
Papaya (Hawaiian)	Papaya ringspot virus resistance	Genes added	1998; 80%
Potato (food)	Innate potato: less acrylamide and bruises	RNA interference	2015; 0%
Potato (starch)	Better starch production	Gene modification	2018; 0%
Soybeans	Less saturated fats; insect resistance	Gene knockout; Bt	2013; 77–90%
Zucchini, cucumber	Resistance to yellow mosaic viruses	Virus coat protein genes added	2005; 13%
Sugar beet	Herbicide resistance	Genes added	2010–11 regulated; 2012 deregulated; 59%
Sugarcane	Insect resistance; sugar increase	Genes added	Not reported
Sweet peppers	Resistance to cucumber mosaic virus	Virus coat protein genes added	Small quantity grown in China
Tomatoes	PG gene suppression	Antisense gene	Small quantity grown in China
Wheat	Herbicide resistance	Genes added	Unknown
Arctic apples	Non-browning	Genes deleted	2017; unmeasured %
Eggplant	Reduced flavonoids	Genes deleted	2017; unmeasured %
White mushroom	Non-browning	Genes deleted	2017; unmeasured %

Source: "Executive Summary: Global Status of Commercialized Biotech/GM Crops: 2014—ISAAA Brief 49-s2014 | ISAAA.org" and https://pubs.acs.org/doi/abs/10.1021/acs.jafc.7b05617, March 2018.

Gene Guns

Another way to introduce DNA into a plant cell is with a gene gun. As shown in **Figure 6.5**, a gene gun shoots tiny DNA-coated pellets of gold or tungsten directly into plant cells. Once in the cell, the DNA separates from the metal and may integrate into the cell's DNA. This method is particularly useful for monocots (flowering plants whose seeds typically contain only one embryonic leaf, or cotyledon), such as wheat or maize, for which transformation using *Agrobacterium* has been less successful. DuPont Pioneer has recently found that it can use a gene gun to transform almost any variety of corn, a plant that had been a challenge to transform by other methods.

Chloroplast Engineering

Plant cells contain chloroplasts, the organelles responsible for photosynthesis. Chloroplasts contain their own DNA, which codes for some of their proteins. The chloroplast DNA can be an inviting target for bioengineers (see **Figure 6.6**). This DNA can accept several

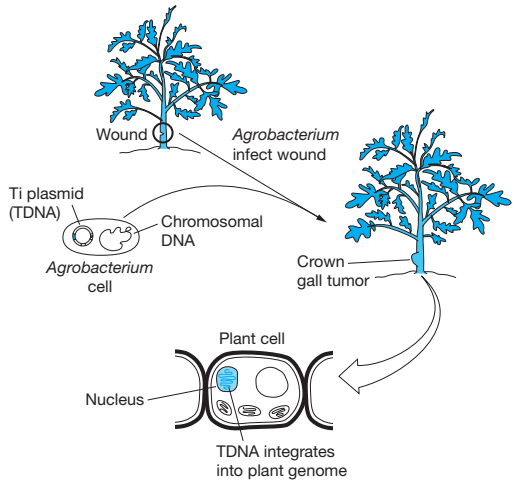


FIGURE 6.3 Process of Crown Gall Formation The Ti plasmid of *Agrobacterium* causes "crown galls" in susceptible plants. Through genetic engineering, it is possible to silence the tumor-inducing genes and insert desirable genes into the plasmid, making it a vector for gene transfer.

new genes at once. Also, a high percentage of genes inserted into the chloroplast will remain active when the plant matures, which is not always the case for genes inserted into the nuclear DNA. Another advantage is that pollen lacks chloroplasts, so chloroplast DNA cannot be transferred via pollen to other crop plants. In addition, both nuclear genomes and chloroplast genomes can be modified with several genes at once. A number of challenges remain, including gene regulation and unwanted off-target effects.

Chloroplast transformation is expected to offer unique advantages in a number of applications, including the production of industrial enzymes, biofuels, and biomaterials, as well as the production of antibiotics, vaccines, and other drugs. Chloroplast transformation has been achieved in tobacco, lettuce, *Arabidopsis* (a mustard), tomato, carrot, rapeseed potato, cabbage, cotton, petunia, soybean, sugarcane, sugar beet, rice, eggplant, cauliflower and poplar.

Gene Inactivation Using CRISPR-Cas Technology

The CRISPR-Cas technology can be used to delete a gene from a cell with great accuracy. In some cases, this is a desired effect. For instance, inactivating genes that code for polyphenol oxidase (PPO), an enzyme that causes browning, can yield button mushrooms that do not go brown, improving shelf life and allowing automated mechanical harvesting.

Moreover, many countries, starting with the European Union (EU) in 2015, have decided that a plant in

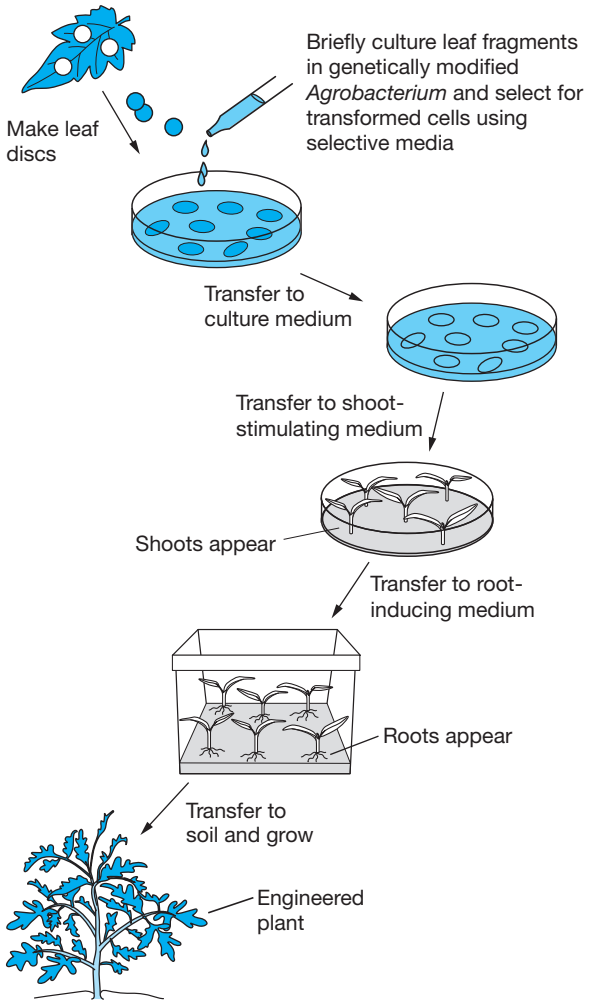


FIGURE 6.4 Transfer of Genetically Modified Ti Plasmid to Susceptible Plants through Tissue Culture Ti plasmids from *Agrobacterium*, genetically engineered to contain new genes, can be cultured with leaf fragments in sterile medium and stimulated to grow into plant embryos that can regenerate into engineered plants.

which an existing gene has been deleted through genetic engineering does not fall under the definition of a genetically modified organism (GMO). This means that these plants can be cultivated without restriction, and has led to the speedy development of new varietals in the EU. In the U.S., Canada, Brazil, and several other countries, CRISPR-Cas9–edited plants, such as waxy corn and white button mushrooms, are not treated legally as GM plants because those plants have been transformed by inactivating or altering genes, not by introducing foreign genes from other organisms. In contrast, Europe's highest court ruled in July 2018 that gene-edited crops should be subject to the same strict regulations as conventional genetically modified ones. This law makes it difficult for developing GM crops for food.

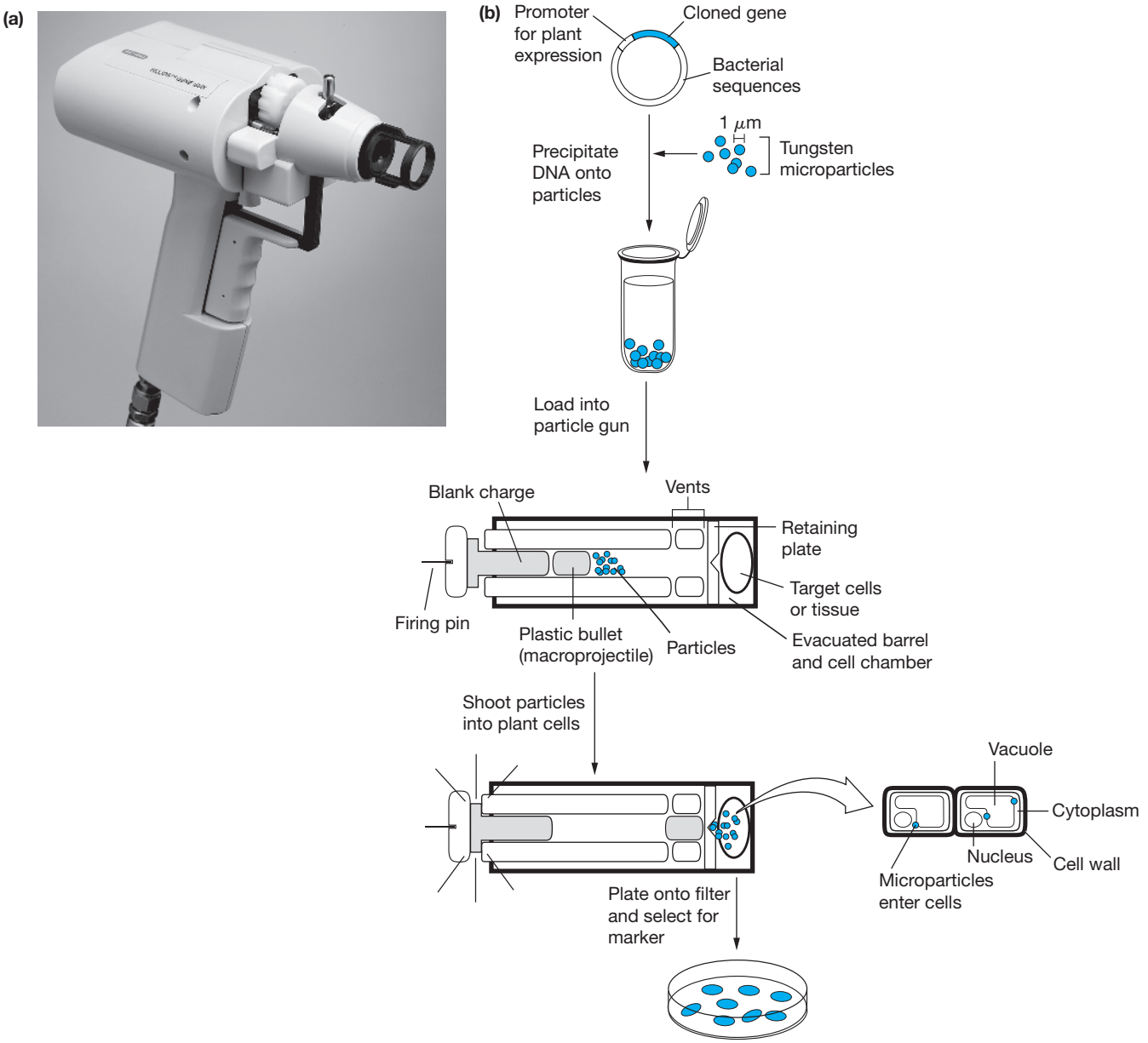


FIGURE 6.5 Gene Guns (a) A gene gun. (b) The "bullets" of the gun are gold or tungsten beads 1 micrometer in diameter, which are coated with DNA and fired with a velocity of about 430 meters per second. The targets can include suspensions of embryonic cells, intact leaves, and soft seeds. Transformed cell from this process are surrounded by masses of untransformed cells. Thus, selectable marker genes (usually antibiotic or herbicide resistance genes) are included in transformation vectors. This allows selection to be performed after transformation. After plant selection, a clump of transformed cells (callus) survives, and then must be regenerated with shoot induction and root induction media.

A team of scientists from Purdue University and the Chinese Academy of Sciences has developed a genetically engineered variety of rice that would not have been possible to create through traditional breeding methods. The variety produced 25 percent more grain in a field test in Shanghai and 31 percent more in a field test conducted in China's Hainan Island. The FDA, in 2015, has also approved new varieties of CRISPR-engineered apples (Arctic Apples) and potatoes (that are resistant to browning and contain less asparagine).

Antisense Technology

Fresh tomatoes are red, juicy, and tasty—and extremely perishable. When picked ripe, most tomatoes will turn to mush within days. But the Flavr Savr tomato, introduced in 1994 after years of experimentation and briefly available in stores, stayed ripe and fresh for weeks. It was created with **antisense technology,** in which a

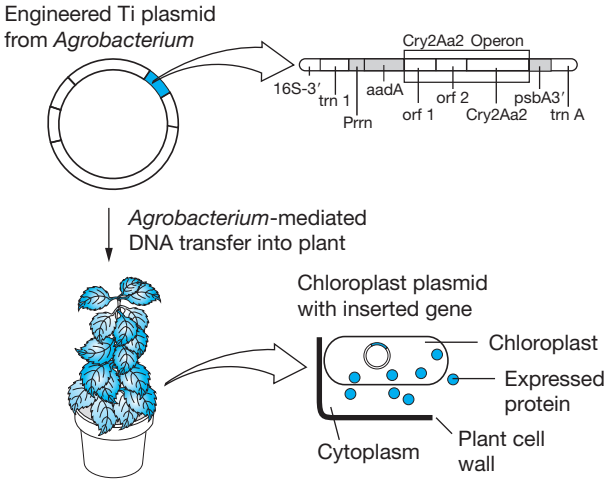


FIGURE 6.6 Chloroplast Genetic Engineering
Transgenes can be inserted into the DNA of the chloroplast, as opposed to the nuclear DNA. Doing so offers several advantages.

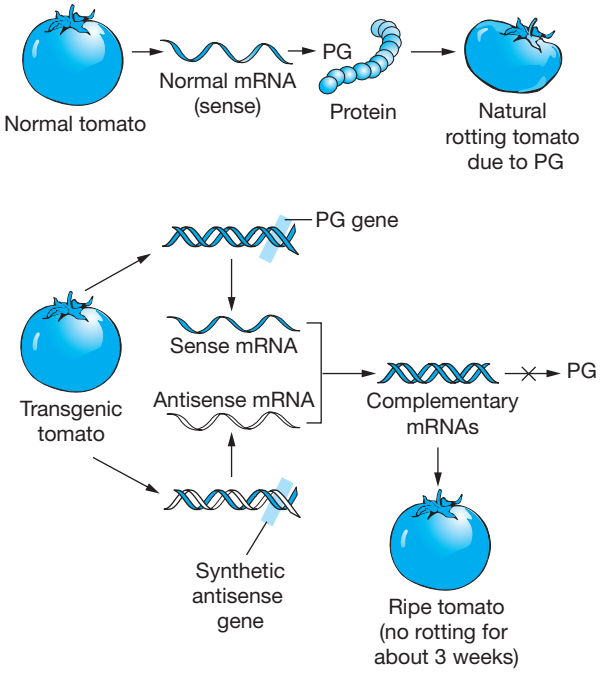


FIGURE 6.7 Gene Silencing Using Antisense RNA to Produce Flavr-Savr Tomatoes Flavr Savr tomatoes with reduced softening were produced by first isolating the gene that encodes polygalacturonase (PG), creating a complementary version (double stranded DNA) of it, and inserting this version into the plant. Plants with both genes produce both normal (sense) and complementary (antisense) mRNA. These molecules align and bind to each other, canceling the production of the PG enzyme. This slows the rotting process and produces a tomato that will last for about 3 weeks after ripening.

complementary copy of an existing gene is inserted into the plant. Both the normal ("sense") and the complementary ("antisense") versions of the gene are transcribed into messenger RNA (mRNA), but the two molecules then join together, preventing the gene from being translated into protein.

In the Flavr Savr tomato, antisense technology was used to prevent the ripe fruit from producing the enzyme **polygalacturonase,** which digests pectin in the fruit cell walls and thus causes the fruit to soften rapidly (see **Figure 6.7**).

The Flavr Savr tomato was the first genetically modified food approved by the FDA, and although it was not an economic success, other varieties of genetically modified foods created by antisense technology are commonly found on the market today.

Gene Stacking

For both conventional breeders and genetic engineers, the goal is often to move more than one desired gene into a plant—for instance, two disease resistance genes from wild relatives, or genes for pest resistance and herbicide resistance from different engineered lines (**Figure 6.8**). Such combinations of two or more inserted genes are called **gene stacks.** A common way to stack genes is to cross parental lines that each have one of the genes, then select offspring that inherit both. This process is called hybrid stacking. If the parent plants containing the genes have already been approved for commercial use by regulatory agencies, the offspring of hybrid stacking will inherit that approval. Consequently, most of the commercially available biotech stacks (including triple and quadruple stacks) are products of serial hybrid stacking.

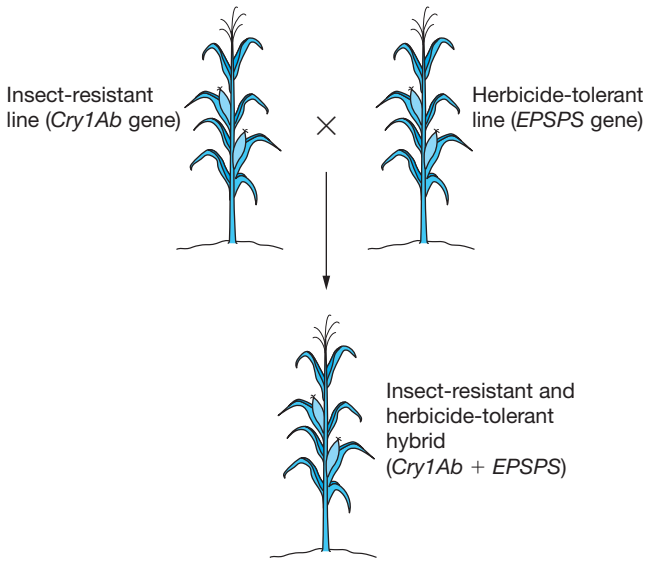


FIGURE 6.8 Creating a Gene Stack In this case, the gene stack is created via conventional selective breeding from two lines in which the genes were inserted through genetic engineering.

Another way to stack genes, called molecular stacking, is to introduce them via genetic engineering, either simultaneously or sequentially. Approximately 51 million hectares of stacked GM crops were planted in 2014 in 13 different countries. This represents 28 percent of the 181 million hectares of GM crops planted worldwide.

6.3 Practical Applications

Protecting plants from viruses, insects, and weeds while improving their nutritional properties is the goal of commercial growers, and biotechnology has offered some powerful tools to help growers.

Protecting Plants from Viruses

Crop plants are vulnerable to a wide range of plant viruses. Infections can lead to reduced growth rates, poor crop yields, and low crop quality. Fortunately, farmers can protect their crops by using **virus-like particles** to stimulate a plant's natural defenses against disease. These "plant vaccines" consist of inactivated or partial versions of the plant virus. They turn on the plant's version of an immune system, making the plant resistant to the real virus, somewhat similarly to how vaccines work for humans. For example, researchers have inserted a gene from the tobacco mosaic virus (TMV) into tobacco plants. The gene produces a protein found on the surface of the virus, which turns on the plant's immune system. This viral coat protection arises from RNA silencing of viral mRNAs. Tobacco plants with this gene are immune to tobacco mosaic virus infection (see Figure 6.9).

Plant vaccines have proven themselves in a wide variety of crops. As another example, the papaya industry in Hawaii was ravaged by papaya ringspot virus. Starting in 1984, researchers from Cornell University and the University of Hawaii worked to develop a variety resistant to this virus, called the Rainbow papaya. Within 4 years of its commercial introduction in 1998, this strain not only stopped the decline of the Hawaiian papaya industry but brought production back to nearly its pre-ringspot levels.

Genetic Pesticides

For the past 50 years, farmers have relied on a natural bacterial pesticide to prevent insect damage to crops. The bacterium *Bacillus thuringiensis* **(Bt)** produces a family of crystallized proteins, called Cry, that kills harmful insects and their larvae. The molecular structure of at least three Cry proteins has been studied. When a susceptible insect larva consumes a crystal of Cry protein while eating a plant, the crystal dissolves in the insect's midgut, and the protein molecules attack and kill the gut cells, causing the insect to die (Figure 6.10). These proteins are harmless to mammals

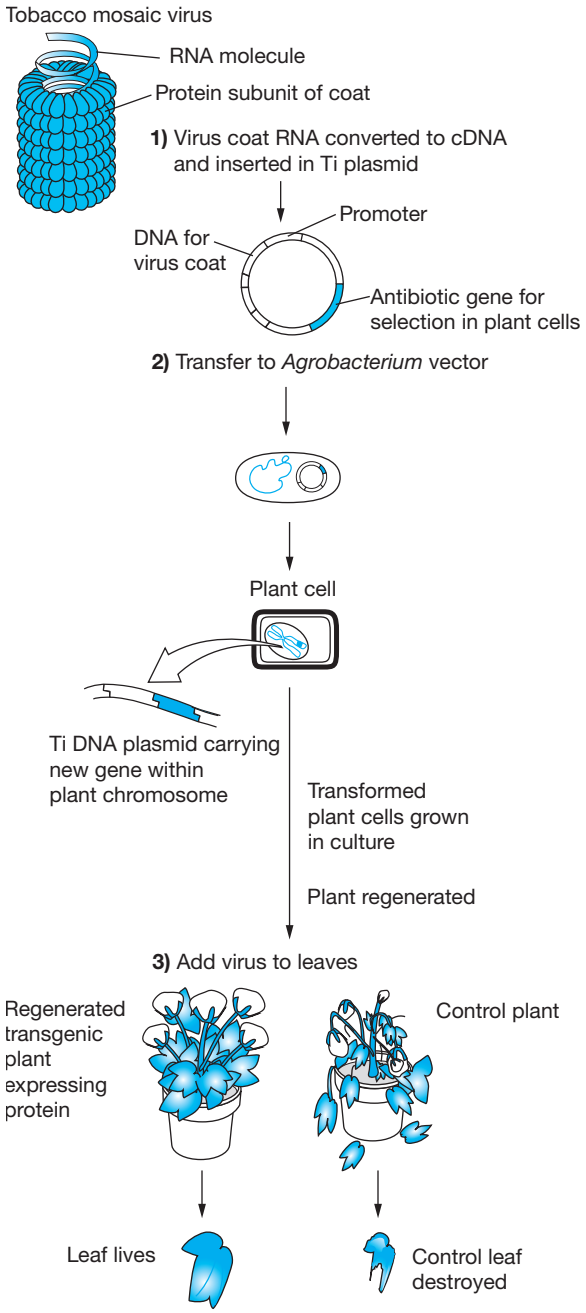


FIGURE 6.9 Virus Protection for Plants Researchers have inserted a gene from the tobacco mosaic virus (TMV) into tobacco plants. The gene produces a protein that is found on the surface of the virus and that activates the plant's immune system. Tobacco plants with this gene are immune to tobacco mosaic virus infection.

(including humans) and to birds; in fact, they can function only in the alkaline environment of the insect gut—the acidic environment of our gut destroys them.

In the past, the only way to use Bt toxins as a pesticide was to spray them on crops, where they risked being washed off by rain or inactivated by sunlight. More recently, genetic engineering has been used to insert *Cry* genes directly into plants, so that the plants make their

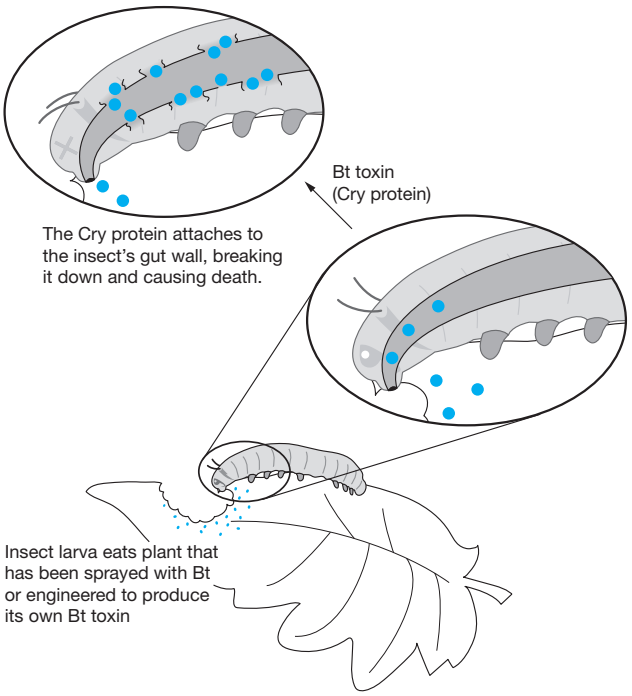


FIGURE 6.10 How Bt Toxin Works When the insect ingests the Cry protein (Bt toxin), the insect's digestive enzymes break the protein into two portions, one of which attacks and destroys the insect's gut wall, causing death.

own pesticides. Tobacco, tomatoes, corn, and cotton have all been engineered in this way. In fact, most cotton seeds planted today contain genes for Bt toxin, which effectively kills most cotton-infesting insects.

Herbicide Resistance

Traditional weed killers have a fundamental flaw: They often kill desirable plants along with weeds. However, genetic engineering has been used to make crop plants resistant to specific herbicides, making herbicides an effective way to manage weeds. In addition, the advent of safer herbicides has reduced environmental damage and increased crop production by reducing weeding. Glyphosate, glufosinate, 2-4D, and dicamba are the most commonly used herbicides.

Glyphosate

Since 1999, the most common transgenic trait in plants has been resistance to the herbicide glyphosate, which is the active ingredient in Roundup and other herbicide products. Glyphosate works by blocking the enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), which functions in a biochemical pathway responsible for the synthesis of aromatic amino acids and other compounds vital to plant growth and survival. Through bioengineering, scientists have created transgenic crops that produce an alternative enzyme that is not affected by glyphosphate, meaning that weeds are susceptible while desirable plants are not (see Figure 6.11). The enzyme attacked by glyphosate is not present in animals.

Glyphosate is degraded readily by soil microorganisms. Both glyphosate and AMPA (aminomethylphosphonic acid), its breakdown product, adsorb strongly onto soil particles, meaning that they are not likely to move into groundwater. In the soil of a typical field, glyphosate has a half-life of about 47 days. One problem that has occurred is drift of the herbicide spray into adjacent areas; work to address this problem is ongoing.

Glufosinate

Glufosinate is an herbicide that works by interfering with the synthesis of the amino acid glutamine and with ammonia detoxification. Plants have been engineered to resist this herbicide by using a gene first isolated from *Streptomyces* bacteria. Crops that are resistant to the herbicide glufosinate have been engineered for resistance to multiple herbicides, permitting growers to use a mixed group of two to four different chemicals that combat herbicide resistance.

2-4D and dicamba

Dow AgroSciences has requested approval of seeds that are resistant to 2-4D quizalofop (a grass herbicide) and glufosinate. Additionally, Monsanto has requested approval for a stacked strain that is tolerant to both glyphosate and dicamba. Both 2-4D and dicamba have been used individually on millions of acres for decades without widespread damage. Dicamba controls annual and perennial rose weeds in grain crops and highlands, and it is used to control brush and bracken in pastures. Dicamba functions by increasing plant growth rate. The soil bacterium *Pseudomonas maltophilia* (strain DI-6) converts dicamba to dichlorosalicylic acid, which is adsorbed to soil much more strongly than is dicamba. The rate of decomposition for this herbicide is similar to that for glyphosate (by soil microorganisms, in acidic soils).

Enhanced Nutrition

One goal of biotechnology is to make crops that have enhanced nutrition, with the potential to save millions of people from the crippling effects of malnutrition. One potential weapon against malnutrition is **Golden Rice**—rice that has been genetically modified to produce large amounts of beta carotene, a provitamin that the body converts to vitamin A. According to recent estimates, 500,000 children in many parts of the world

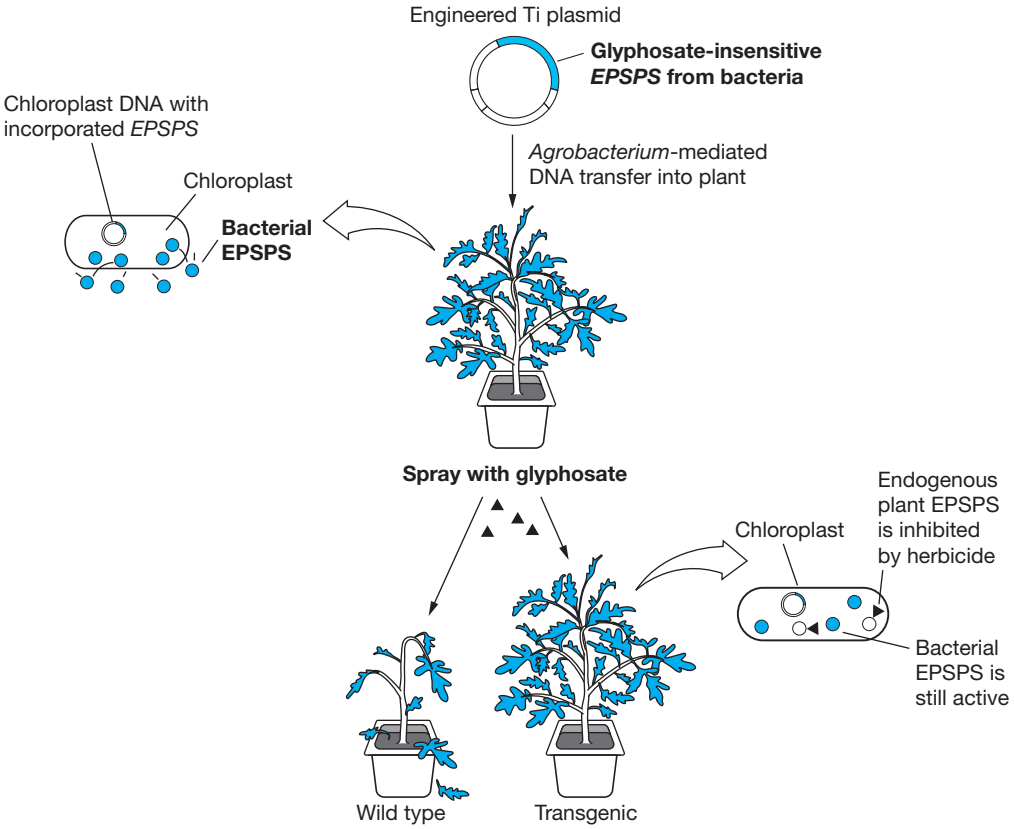


FIGURE 6.11 Engineering Herbicide-Resistant Plants Glyphosate acts by inhibiting the native EPSPS protein of plants. Plants can be engineered to include a bacterial *EPSPS* gene, which produces a protein that is not affected by glyphosate. Transgenic plants with this bacterial gene can safely be sprayed with glyphosate, while susceptible weeds are killed by it.

will eventually become blind because of vitamin A deficiency. Currently, health workers carry doses of vitamin A from village to village in an effort to prevent blindness. Simply adding this nutrient to a common food like rice would be more efficient and, in theory, more effective. One limitation of Golden Rice is that the provitamin it supplies must dissolve in fat before it can be used by the body. Hence, Golden Rice might offer limited benefits to children who get very little fat in their diet.

The use of genetically modified crops to address malnutrition is controversial. As of 2017, Golden Rice has not yet been planted by farmers, largely because of concerns voiced by environmental organizations. As an alternative to engineered crops, some groups endorse programs such as Harvest Plus, which is a collection of 12 crops that aim to boost levels of vitamin A, iron, and zinc, and relies on conventional breeding. Other groups have supported development of transgenic crops. The Bill and Melinda Gates Foundation, for example, is spending $36 million to support Golden Rice, as well as GM cassava, sorghum, and bananas.

"Biopharming"

Since the early 1990s, some biotech companies have proposed using engineered crops as miniature factories for producing pharmaceutical proteins and industrial chemicals (called "biopharming"). A single field of a transgenic crop can produce a large amount of commercially valuable and extractable proteins, including protein drugs and vaccines. At the start of this chapter, we described the creation of tobacco plants that produce vaccines against influenza in their leaves. Studies have also been conducted on producing vaccines in bananas, potatoes, tomatoes, rice, wheat, soybeans, and corn. Researchers at Cornell University have recently created tomatoes and bananas that produce a human vaccine against the virus hepatitis B.

Other protein pharmaceuticals that could be produced in plants include antibodies, blood products, cytokines, growth factors, hormones, and recombinant enzymes. The diseases these could treat include cystic fibrosis, non-Hodgkin's lymphoma, hepatitis, Norwalk virus, rabies, and a range of gastrointestinal illnesses.



FIGURE 6.12 Tomatoes Whose Form Has Been Modified by Using CRISPR Technology to Edit Promoter Sequences. CRISPR technology was used to modify the promoter sequences that control the number of locules (seed compartments) in these tomatoes, as well as fruit size. These tomatoes are not transgenic, as no foreign DNA was inserted.

Engineered Deletion of Gene Promoters Rather Than Genes

So far, we've talked about adding and deleting genes. It is also possible to use CRISPR gene editing techniques to modify the promoter sequences that control when, where, and how much an existing gene is expressed. Researchers at Cold Spring Harbor Laboratory used these techniques to fine-tune tomato genes that control yield, including genes that regulate the number of floral organs and locules (the gelatinous seed cavities within the tomato), which can determine just how big the fruit will grow (see **Figure 6.12**). The study could provide a catalog of beneficial tomato variants that growers could use to easily choose the best growth traits and adjust them during future growing seasons. Because this approach does not add foreign genes, it is likely to receive fast-track, deregulated U.S. Department of Agriculture (USDA) approval and may elicit less resistance.

6.4 Health and Environmental Concerns

Ever since the inception of transgenic plants, people have worried about potentially harmful effects to humans and the environment. In an age when "natural" is often equated with "safe," these decidedly unnatural plants carry an air of danger. Activists have staged protests against companies producing genetically modified plants.

Concerns about Human Health

One concern is that the proteins coded for by foreign genes could cause allergic reactions or other problems when eaten. This has, in fact, happened. In 1996, the *New England Journal of Medicine* reported that an attempt to breed a nutritious protein from Brazil nuts into soybeans intended for animal feed produced plants that cause a strong allergic reaction in people sensitive to Brazil nuts. The problem was uncovered during the crop's development, and the breeding effort was terminated. On the one hand, this incident can be seen as demonstrating the dangers of genetic engineering. On the other, it can be seen as a success story: the system detected the threat before it reached the public.

Another concern centers on antibiotic resistance genes. These genes are often inserted into a plant along with a gene of interest, so that antibiotics can be used to select for cells or seedlings that have acquired the gene. The worry is that these antibiotic genes could then move into bacteria, conferring resistance to antibiotics. However, bacteria do not normally acquire genes from plants. According to a recent report in the journal *Science*, there is only a "minuscule" chance that an antibiotic-resistance gene could ever pass from a plant to a bacterium. In contrast, bacteria do readily acquire antibiotic resistance genes from each other, and resistant bacteria can be selected for when a patient or an animal is treated with antibiotics. Antibiotic resistance is a very serious concern, but transgenic plants are unlikely to play any role in it.

Concerns about the Environment

Scientific studies have put to rest fears that bioengineered corn could kill large numbers of monarch butterflies (In 2001 multiple follow-up studies concluded that "the most common types of Bt maize pollen are not toxic to monarch butterflies in concentrations the insects would encounter in the fields"). However, worries about the environmental damage due to genetically modified plants are not baseless. For one thing,



YOU DECIDE Labeling GM Foods

In July 2016, the U.S. Senate voted 63-30 to require labels on food produced with genetic engineering techniques, but with a few options. The Agriculture Secretary would develop a program that requires producers to add a symbol or notice about GMOs on packages, or a QR code that consumers can scan with a smartphone. Producers can also opt to distribute the information via a website or toll-free phone number (see the figure). The food industry supports the bill, in part because it allows companies flexibility on how to convey the information. A single federal law also reduces the need for the 50 states to create their own unique labeling systems. Proponents claim that this labeling would answer the argument that the true content of foods is being hidden from consumers, and that consumers will benefit from accurate labels that allow them to make informed decisions about the food they eat.





This QR code (when scanned by your cell phone) provides detailed information on nutrition, ingredients, allergens, brand, and other details.

The bill excludes meat from animals that consumed genetically engineered feed, and more dispensations may follow. In addition, the GMO distinction is arguably arbitrary, since nearly everything humans eat has been genetically modified through breeding techniques or biotechnology. It can be argued that this legislation won't add to the sum of consumer knowledge, because genetic modification is not an ingredient, but an innovation—one that has made farming more sustainable and food more affordable. A Purdue University study presented the (hypothetically significant) crop yield loss and other economic effects of banning GM crops in the United States. The model showed that if all genetically modified organisms in the United States were eliminated, greenhouse gas emissions would increase significantly as more land would be needed for agricultural production. In addition, corn prices would increase as much as 28 percent and soybeans as much as 22 percent, due to lower crop yields. In contrast, GM crop planting practices have helped world farmers produce more for less.

Another unanswered question is how to deal with foods that are created by editing existing genes, rather than by inserting new genes or deleting existing ones. CRISPR-Cas, the system mentioned earlier as a tool to delete genes, is also increasingly able to edit and alter them. As mentioned earlier, foods created with these techniques are deregulated because they do not contain transgenes. The Arctic Apples approved in 2015 and commercially released in 2017 have a scannable QR code on the bag that directs consumers to a website with information on their production. Approximately 34,000 products already have SmartLabel™ pages using QR codes providing detailed information on their nutrition, ingredients, allergens, and brand. Pre-marketing research indicates that about 60 percent of customers say they will buy these products for their improved characteristics. An additional 10 to 15 percent are staunchly opposed, but the research indicates that some of them will change their mind after they have experienced the product. Do you think that food plants produced by gene deletion, like these examples, will still be received by the public as "genetic modification" or as an improved product without the GMO distinction? You decide.

genetic enhancement of crops has led to superweeds—that is, to weeds that have adapted traits such as herbicide resistance from crop plants. Further studies are needed to gauge the full extent of this threat and develop ways to minimize the risk. The risks posed by biotechnologically enhanced crops must be weighed against the benefits they offer. First and foremost, biotechnology can dramatically reduce the use of

less-selective chemical pesticides. In countries where biotech crops have been planted, pesticide use on four biotech crops—soybeans, corn, cotton, and canola—has fallen by 791 million pounds per year (8.8 percent). This has resulted in a 17.2 percent reduction in the associated environmental impact. The 2016 National Academies of Sciences report: "Genetically Engineered Crops, Experiences and Prospects" presents the positive and adverse effects of GE crops and anticipates what emerging genetic-engineering technologies hold for the future. This report indicates where there are uncertainties about the economic, agronomic, health, safety, or other impacts of GE crops and food, and makes recommendations to fill gaps in safety assessments, increase regulatory clarity, and improve innovations in and access to GE technology. The National Academy of sciences report is available on line.

Regulations

Biotechnology is not a lawless frontier. Several different agencies regulate the production and marketing of genetically modified foods. In the United States, the FDA (United States Food and Drug Administration) regulates foods on the market, the USDA (United States Department of Agriculture) oversees growing practices, and the Environmental Protection Agency (EPA) controls the use of plants that function as pesticides. European Food Safety Authority (EFSA) evaluates the safety of GMO products before they can be authorized for use as food or for cultivation in the EU member states. In countries like Japan, GMOs in food are regulated under the Cartagena Protocol on Biosafety. The approach of these agencies has changed over the years, but they are all actively involved in approving plant crops.

As early as 1992, at the beginning of the biotechnology revolution, the FDA announced that genetically altered food products would be regulated according to the same tough standards applied to regular foods. Even though they were not bound by law, food companies voluntarily consulted with the FDA before marketing any product. In 2001, the agency adopted a stricter, more formal approach. Rules require companies to notify the FDA at least 120 days before a genetically altered food reaches the market. The manufacturer must also provide evidence that the new product is no more dangerous than the food it replaces. The Center for Food Safety and the Grocery Manufacturers Association and the Food Products Association have informed the USDA of their "strong opposition to the use of food crops to produce plant-manufactured pharmaceuticals in the absence of controls and procedures that assure safety of essentially 100% of the food supply." There are many non-food plants and contained-growth plant systems that will satisfy this concern, and no manufacturer has violated these rules to date.

MAKING A DIFFERENCE

Large biological molecules, or biologics, have been manufactured inside genetically engineered mammalian, avian, and bacterial cells for more than two decades. Insulin, for example, has been produced using genetically modified *Escherichia coli* bacteria since 1982.



YOU DECIDE — Is Roundup Toxic to the Environment?

Glyphosate, the active ingredient in the herbicide Roundup, is designed to be toxic to certain pest plants, but scientists have observed some harmful effects on animals in the lab. Some studies suggest that glyphosate (at high concentrations,) can reduce mitochondrial function as well as sperm motility in zebrafish and can alter neurotransmitter activity in the brains of rats. This spurred the researcher to compare the effects of glyphosate alone to the effects of Roundup (containing the same glyphosate concentration) in zebrafish. Remarkably, she found that the effect of Roundup was opposite to that of glyphosate by itself: The fish moved more, and their basal respiration was higher.

Researchers at Monsanto are not surprised by such results. The surfactants used in Roundup are similar to those used in regular household products, they explain, which cause membrane degradation and subsequent mitochondrial breakdown in high doses. They claim that you would see the same thing with dish detergent or hand soap. An analysis in 2017, drawing on data from the Agricultural Health Study, which included some 90,000 farmworkers and their spouses in Iowa and North Carolina over nearly two decades, showed no significant association between glyphosate and non-Hodgkin's lymphoma, nor with overall cancer risk (although it did show a weak association with acute myeloid leukemia).

Since herbicide makers often change their formulations, there should be a responsibility of scientists to continue to screen for any overlooked side effects, because these chemicals are sold and used in vast amounts. Do you think that regular product formula testing should be a requirement of a company's approval of a product like Roundup?

The biologics market is estimated at around $100 billion per year. One example of a needed biologic is the protein that could be used to treat Gaucher's disease.

Type 1 Gaucher's disease is a rare genetic liposomal storage disorder that afflicts about 10,000 people around the world. It occurs when insufficient quantities of the enzyme glucocerebrosidase are produced, causing lipids to collect in the spleen, liver, kidneys, and other organs. This in turn produces liver or spleen damage, anemia, low blood platelet counts, and bone problems. Until last year the leading treatment for Type 1 Gaucher's disease was Cerezyme (imiglucerase), made by Genzyme (Cambridge, Massachusetts) in mammalian cell culture. Genzyme's production of Cerezyme was halted in June 2009 after a virus contaminated its Allston, Massachusetts, facility, resulting in a years-long, worldwide shortage of the drug.

Another company, Protalix (Israel), has devised a solution to this problem of bioreactor contamination. Protalix's approach is to introduce a normal version of the human gene that is affected in Gaucher's disease into modified carrot cells and extract the enzyme the cells produce. This enzyme is then processed into an injectable solution for long-term enzyme replacement therapy. The resulting drug, called Elelyso, is the first biological drug produced inside modified plant cells to win FDA approval for a human disease. Production of biologics in plant cells may continue for other drugs that can satisfy a similar production need.

QUESTIONS & ACTIVITIES

Answers can be found in Appendix 1.

1. Why are the genes that induce a plant tumor usually removed from Ti plasmid during construction of plasmid T-DNA that will serve as a vector for plant transformation?
2. List some of the benefits and drawbacks of using plants to produce pharmaceutical proteins, such as vaccines and the protein that is missing in persons with Gaucher's disease.
3. Why is it likely that weeds will eventually develop resistance to any single herbicide?
4. Is it likely that CRISPR-Cas technology will become globally dominant as a gene technology for plant transformation in the near future?
5. How are hybrid gene stacking and molecular gene stacking different from each other?
6. Why do you think that agricultural biotechnology advancements are occurring faster in less-developed countries?
7. Why was the genetically modified Arctic Apple awarded deregulated USDA approval?
8. Do you think that plants genetically modified to be drought resistant will be more favorably received by the public than other genetically modified plants? Why?
9. How is *Agrobacterium tumefaciens* employed to introduce transgenes into plants?
10. How can marker-assisted selection speed the creation of a genetically modified plant?
11. Explain how the Cry proteins of *Bacillus thuringiensis* kill target insects.
12. Explain how inserting a transgene into the chloroplast genome can pose less environmental risk than inserting it into the nuclear genome.
13. Why does the USDA consider plants from which genes have been deleted to be "as safe and nutritious as their conventional counterparts," allowing faster approval of plant varieties by this method?
14. Why wouldn't the introduction of viral DNA via a plasmid to transform a plant also induce a viral infection?
15. How would a new genetically engineered food crop be proven to be "no more dangerous to the environment than the crop that it replaces"?
16. Search *Genetically_modified_food_controversies* on the Internet and summarize the primary objections to the introduction of new GMOs.
17. What types of pharmaceutical proteins would you expect to have the greatest potential application for production in plants (biopharming)?
18. Explain how antisense technology is able to block the PG enzyme in tomatoes.
19. Why are most of the commercially available biotech stacks products of serial hybrid stacking?
20. Describe the benefit of modifying gene promoters in plants.

Visit www.pearsonglobaleditions.com for the Companion Website

The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

This case study has been included to give you an opportunity to become familiar with a research example applicable to this field of study. After you have analyzed the data and answered the questions, you can compare them to those provided to your instructor with the text materials.

CASE STUDY

Can RNA Interference Silence Genes in a Citrus Pest?

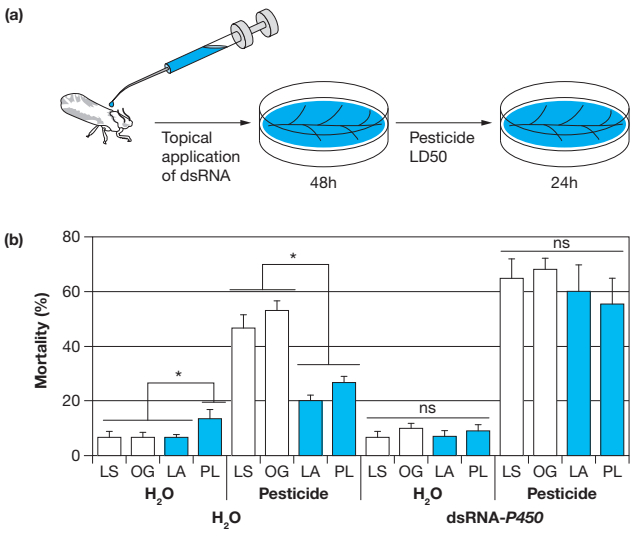
Silencing of insecticide resistance genes through RNA interference (RNAi) in insects has gained momentum during the past few years. RNA interference has been used to cause insect mortality, inhibit insect growth, increase insecticide susceptibility, and prevent the development of insecticide resistance. The cytochrome P_{450} monooxygenases are an important group of enzymes that are involved in the metabolism of xenobiotic compounds (such as insecticides) in insects and are associated with insecticide resistance. Intense insecticide use has led to the development of varying levels of insecticide resistance in populations of *Diaphorina citri* (the Asian citrus psyllid insect) in Florida.

Florida investigators measured the efficacy of topically applied dsRNA to induce RNAi for five cytochrome P_{450} genes, family 4 (*CYP4*), in *D. citri*. It was previously reported that these *CYP4* genes are associated with the development of insecticide resistance in adult *D. citri*. The investigators targeted five *CYP4* genes that share a consensus (common) sequence with one dsRNA construct (please see Chapter 3 for the dsRNA mechanism producing RNAi). Mortality was significantly higher in adults treated with this dsRNA than in adults treated with water. The researchers' results indicate that topically applied dsRNA can penetrate the cuticle of *D. citri* and induce RNAi against *CYP4* (resistance) genes. These results broaden the scope of RNAi as a mechanism to manage pests by targeting a broad range of genes. The results also support the development of RNAi as a viable tool to overcome insecticide resistance in *D. citri* populations.

Constructing dsRNA

A consensus sequence, derived from five previously published *CYP4* sequences, was used to design *CYP4*-specific primers for the genetic transcript. The *CYP4*-specific primers were tailed with a T7 promoter sequence to generate sense and antisense transcripts separately. They also used dsRNA-*gfp* as an irrelevant dsRNA control of green fluorescent protein and evidence of gene insertion. A new approach to plant protection may involve vaccinating plants against specific pathogens with double-stranded RNA molecules that can be sprayed directly on the leaves.

Refer to the diagram to answer the following questons.



(a) Illustration of the protocol used to test the effect of dsRNA on insect resistance. (b) Percent mortality of *D. citri* after exposure to imidacloprid (a neonicotinoid insecticide that acts on the central nervous system of insects, but with much lower toxicity to mammals) for 24 hours. Insects were maintained on non-treated plant leaf discs for 48 hours after treatment with the dsRNA to P_{450} (the consensus *CYP4* gene sequence), prior to exposure to imidacloprid. LS, Laboratory susceptible population; OG, Susceptible population collected from an organic grove; PL, Resistant population collected from commercially managed Polk County, Florida. LA, Resistant population collected from commercially managed Lake County, Florida. Asterisks indicate significant differences, while ns indicates no significant differences ($P < 0.05$).

Questions

Answers can be found at www.pearsonglobaleditions.com

1. What happened to the mortality of the insects after application of the dsRNA and then the insecticide?
2. What caused the mortality to change after application of the dsRNA?
3. If dsRNA is applied topically to plants, is it considered genetic modification of the plant? Why or why not?

CHAPTER SEVEN

Animal Biotechnology



Ninety nine percent of animals used in research in the U.S. are mice, rats, fish and birds.

After completing this chapter, you should be able to:

- Explain how an animal is chosen as a model for drug studies.
- List some of the medical advances made using animal research models.
- Describe the benefits and limitations of "organ on a chip" as an alternative to animal models.
- Discuss some of the regulations protecting animals in animal research.
- Outline the process used to clone an animal after gene transfer.
- Describe the process used to create monoclonal antibodies from hybridomas.
- Discuss the benefits of nuclear transfer to mitochondrial disease studies.
- List some of the benefits of using transgenic animals as bioreactors.
- Explain why CRISPR-Cas has become the predominate gene editing method used in animal research.
- Discuss the current progress of xenotransplantation research for organ donation.

Animal research is a necessary and expanding field for many reasons. Research and Markets released its 2018 report *Animal Biotechnology—Technologies, Markets, and Companies*. According to the report, biotechnology has potential applications in the management of several animal diseases, such as foot-and-mouth disease, classical swine fever, avian flu, and bovine spongiform encephalopathy. The most relevant biotech products are vaccines, particularly genetically engineered DNA vaccines. Scientists are also starting to use gene therapy for diseases of pet animals. It is a fast-developing field of study because many of the technologies used in clinical trials of humans were developed in animals and many of the diseases of cats and dogs are similar to those in humans.

Animals can also be used for purposes that may seem inhumane and unethical, but government regulations have largely prevented the widespread introduction of such controversial practices.

Animals offer biotechnological opportunities, but they also present many tough scientific and ethical challenges. Researchers who successfully meet those challenges have the potential to make important contributions to science and society. For example, the global animal health market size was valued at US $44.74 billion in 2018. With a continuous growth rate of 5.7 percent, the value is estimated to be US $69.44 by 2026.

This chapter reviews many aspects of animal biotechnology. We begin by looking at the use of animals in research and then visit some of the issues at the cutting edge of biotechnology: cloning, transgenics, and the use of animals as bioreactors.

FORECASTING THE FUTURE

One of the most lethal genetic diseases, Duchenne muscular dystrophy (DMD), affects 1 in 3,500 male births worldwide. It is characterized by rapid muscle degeneration caused by the replacement of strong muscle fibers with fatty tissue, a progression that reduces the average life expectancy of DMD patients to 25 years. Biologists at the University of Texas Southwestern Medical Center have successfully used CRISPR-Cas9 genome editing to correct the genetic mutation responsible for DMD in mice. They saw that the muscle gets progressively rescued even for animals with a relatively low percentage of corrected genes. Gene surgery is a future cure for genetic diseases in animals as a first test for human cures, and this new (CRISPR-Cas) technology is speeding the process.

7.1 Animals in Research

The white mouse is an icon of scientific research, as it represents the first step of discovery. Although animals have been central to research methods for decades, their use is controversial. Next, we explore the role of animals in research and the resulting issues.

Why Use Animals in Research?

Research using animals has been key to most medical breakthroughs in the past century. Without question, such research has prevented much human suffering. Here are a few examples:

- Without the polio vaccine, which was developed using animals, thousands of children and adults would die or suffer debilitating side effects from the disease each year.
- Without dialysis, which was tested on animals, tens of thousands of patients suffering from end-stage kidney disease would die.
- Without cataract surgery techniques, which were perfected on animals, more than a million people would lose sight in at least one eye this year.

Animal research has also directly benefited animal health. Genomics, transgenics, and cloning technologies provide new approaches for advancing the quality and efficiency of the production of meat, milk, and eggs and reducing the environmental impact of agriculture. These technologies are also being used to help preserve endangered species.

You might wonder why testing on cells *in vitro* (cells cultured outside the body) would not be sufficient. The answer is that new drugs and medical procedures have effects beyond single cells in tissues and organs and must be tested in animals to determine the impact of the treatment on an entire organism. For example, a drug may be a powerful agent able to kill cancer cells, but it may also be destructive to unrelated organs.

One drug with unexpected side effects is finasteride (Propecia), which is used to encourage hair growth. It carries a clear warning that pregnant women should not handle broken or crushed pills.

TABLE 7.1 Animal Testing in the United States

Animal	Purpose	Problems	Benefit
Fruit flies	Genetic understanding	Immune system differs from that of humans	Large numbers
Roundworms	Transparent body	Immune system differs from that of humans	Cellular embryonic fate tracking
Mice	Early studies of human drugs (majority of animals used)	Some differences from humans	99% genetic similarity to humans
Rats	Early studies of human drugs	Harder to manipulate than mice	High genetic similarity to humans
Cats	Neurological studies	Highly regulated use	Neurological similarity
Dogs	Similarity to some human disease processes	Highly regulated use	Cardiology, endocrinology, and bone and joint studies
Macaques, marmosets, spider monkeys, squirrel monkeys, baboons, and chimpanzees	Polio, AIDS, and deep brain stimulation studies	Highly regulated use, abnormal containment behaviors	Behavior and cognition, reproduction, genetics, and xenotransplantation
Pigs, goats, sheep	Gene transfer, knockout	Limited understanding of long-term effects	Biopharming, xenotransplantation studies

That warning and a special protective coating on the pills themselves are the direct result of information collected during testing on animal subjects. Those tests revealed serious birth defects (malformations of the reproductive organs) in male offspring born to animal mothers given large oral doses of the drug. Even though merely handling the pills is unlikely to result in similar defects in human infants, the warnings were put in place before the drug was marketed. In this case, experiments using animal subjects helped to prevent unexpected disasters. Thanks to animal testing, pregnant women are never given the drug.

Types of Animals Used in Research

The differences between mice and humans are obvious, but there are many genetic and physiological similarities between the two. For this reason, research conducted on mice—or other animals, for that matter—can tell us much about ourselves (Table 7.1). The animals used most often in research are purebred mice and rats (for which the genetics are known and controlled), but other species are also used. Researchers have developed model animal systems using both vertebrates (zebrafish) and invertebrates (fruit flies) and worms. The tiny zebrafish (*Danio rerio*), an extremely valuable research animal, is a popular and hardy aquarium fish (**Figure 7.1**).

Zebrafish are small (adult length is 3 cm, about the length of a paper clip), making it possible to house large numbers of animals in small spaces. Spawning is virtually continuous by this active warm-water fish, with only about 3 months between generations. The average number of progeny per female per week can exceed



FIGURE 7.1 Zebrafish Are Commonly Used in Animal Research Zebrafish are fast-growing alternatives to other animals for gene studies and toxicity analyses. After transplanting genes into their embryos it becomes possible to study developmental changes (like blood vessel growth) in the embryos and the adults. The transparent embryos, fast growth rate, and ease of study of zebrafish make them common animal models for testing.

200. Zebrafish eggs complete embryogenesis (early organ development) in about 120 hours; the gut, liver, and kidneys are developed in the first 48 to 72 hours. Because the fish grow so rapidly, scientists can test for toxicity or adverse effects in about 5 days. If a drug is toxic to a zebrafish, it is probably toxic to humans.

There are times when mice, fish, or rats are not the best test animals. For example, the lung and cardiovascular systems of dogs are similar to those of humans, making dogs a better choice for the study of heart disease and lung disorders. HIV/AIDS research is conducted on monkeys and chimpanzees because they are the only known animals that share humans' vulnerability to the virus. Although cats, dogs, and primates like monkeys and chimps are used in specific instances when their particular biology is important to research, they make up less than 1 percent of the total number of research animals (the majority are mice). In addition, the number of those animals used in experiments has been declining for the past 20 years because of the increased use and availability of alternative methods of testing, which we will discuss shortly.

Monoclonal antibodies are a good example of using animals in the research and development of drugs (see **Figure 7.2**). Monoclonal antibodies are typically made by cell culture that involves fusing myeloma cells with mouse spleen cells immunized with the desired antigen. A selective medium in which only fused cells can grow is used to isolate monoclonals. A more complete description of the process for producing monoclonal antibodies will be described later in this chapter.

Regulations in Animal Research

According to regulations enforced by the U.S. Food and Drug Administration (FDA), new drugs, medical procedures, and even cosmetic products must pass safety tests before they can be marketed. Safety testing involves a rigorous scientific methodology known as **phase testing,** as illustrated in Table 7.2. Other drug regulatory agencies like the European Medicine Agency (EMA) and Medicines and Healthcare products Regulatory Agency (MHRA) in the UK follow similar testing procedures.

As part of phase testing, the FDA requires manufacturers to conduct a statistically significant number of trials on cell cultures as well as trials in live animals and human research participants before such products can be made available to the general public.

If initial tests on cell cultures indicate dangerous levels of toxicity, the product is never tested on live animals. The choice of animal to test is usually determined by its genetic similarity to humans or potential to mimic conditions in humans. Two or more species are used in the tests because different effects may be revealed in different animals. Toxicity and unexpected nontarget effects can often be detected by using more than one species with genes homologous to those of humans. The rate of absorption, specific chemical metabolism, and time required to excrete the substance can all be examined in animal models. If significant problems are detected in the animal tests, human research participants are never recruited to participate in trials, and are informed of this potential as a requirement of informed consent.

Alternatives to the Use of Animals

Whenever possible, researchers use cell cultures and computer-generated models in initial testing. Both of these tools are significantly less expensive than research animals and can save time as well.

As mentioned earlier, cell culture studies are often used as a preliminary screen to check on the toxicity of substances. In addition, fundamental questions about biology are often best answered by collecting evidence at the cellular level. Clues about which compounds might make the best drugs are often the result of *in vitro* research, like those using "organs on a chip."

Organs on a chip

Organs on a chip are microengineered biological mimic systems that represent key functional models of human organs like the liver, heart, kidney, intestine,

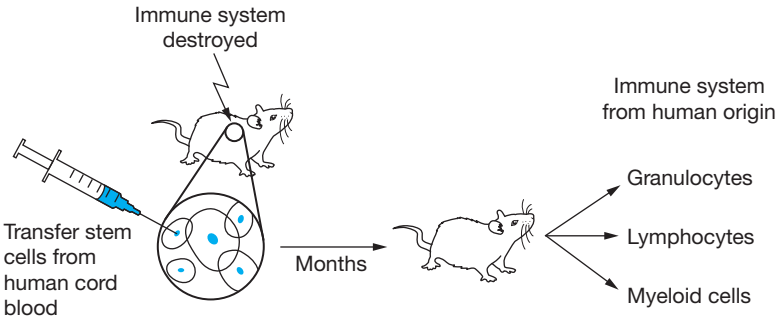


FIGURE 7.2 Mice Tell Us about Ourselves Here, after the immune system of a developing mouse is destroyed, it can be replaced by injecting human stem cells. In this way it is possible to study therapies for humans using mice as models.

TABLE 7.2 Food and Drug Administration Required Testing Phases for Drug Approval

FDA phase testing involves the use of animals for preclinical testing before it is allowed in humans. If the new drug candidate has proven to be nontoxic and has benefit, then it can be awarded an Investigational New Drug (IND) status. If it is successful in the three phases of human testing, it can receive a New Drug Application (NDA) and likely approval for marketing. The FDA continues evaluating the NDA for another 2.5 years, resulting in a total of about 12 years for a successful drug approval.

	Preclinical Testing	Phase I	Phase II	Phase III	FDA	Phase IV
Years	3.5	1	2	3	2.5	12 total
Tested on	**Animals in the lab**	20–80 healthy volunteers	100–300 patient volunteers	1,000–3,000 patient volunteers		
Purpose	Assess safety and biological activity	Determine safety and dosage	Evaluate effectiveness and look for side effects	Verify effectiveness, monitor adverse reactions from long-term use	Review process/ approval	Additional testing after approval required by FDA
	File IND at FDA			File NDA at FDA		
Success rate	5,000 compounds evaluated		5 enter trials		1 approved	

Source: www.fda.gov/cder/handbook/develop.htm

brain, and bone. A good example is the lung on a chip that replicates the active alveolar capillary barrier in the human lung (see **Figure 7.3**). This three-dimensional (3D) system is created when human alveolar tissue is cultured in close proximity with human endothelial tissue on a thin elastomeric membrane. Computer-controlled pressure changes simulate the stretching of alveoli during breathing.

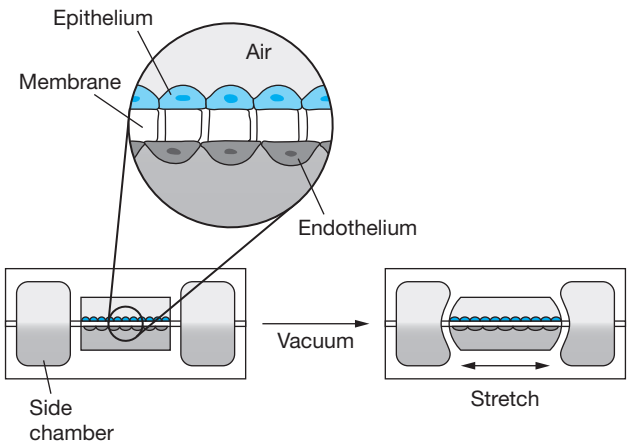


FIGURE 7.3 Lung on a Chip Human alveolar tissue is cultured in close proximity with human endothelial tissue on a thin elastomeric membrane. Computer-controlled pressure changes simulate the stretching of alveoli during breathing, allowing testing of potential drugs on human tissue before phase testing.

Key findings from toxicity screenings with this mouse 3D system were replicated in whole mouse lung, demonstrating the ability to faithfully predict physiological pathology in the lung.

Although animal models emulate physiological complexity, animal surrogates are facing increased scrutiny to show translatability and validity with regard to humans. Organs on a chip have the potential to validate the efficacy, safety, and druggability of potential targets early in the pipeline to increase the likelihood of success in clinical trials. The ability to predict body level responses has been approached by linking multiple microengineered organ models by a fluidic network that allows for their functional integration. One such study combined breast cancer, intestinal, and liver models interconnected to demonstrate the absorption, metabolism, and efficacy of four anticancer drugs. Regulatory agencies have recognized the potential of organ on a chip technology and are watching laboratory findings in preparation for this potential shift in early-phase drug testing.

Regulation of Animal Research

Animal research is heavily regulated. The EU has devised directives regarding animal welfare and the rules are based on the European Convention for the Protection of Animals kept for Farming Purposes. The federal Animal Welfare Act in the U.S. sets specific standards concerning the housing, feeding, cleanliness, and medical care of research animals. Before a study using animals can

begin, researchers are required to prove the need for animals. They also must select the most appropriate species and devise a plan for using as few animals as possible. An oversight committee with the host institution reviews the research while it is in progress to make certain that institutional and federal standards are being upheld. Government agencies regularly monitor conditions in the laboratories. To receive funding for research from the National Institutes of Health (NIH), the FDA, or the Centers for Disease Control and Prevention (CDC), researchers in the U.S. must follow the standards of care set out in *The Guide for the Care and Use of Laboratory Animals,* formulated by the National Academy of Sciences. Given the cost of research, most institutions are eager to follow the guidelines. Researchers are tasked with achieving the "Three Rs" of animal research:

- *Reduce* the number of higher species (cats, dogs, primates) that are used
- *Replace* animals with alternative models whenever possible
- *Refine* tests and experiments to ensure the most humane conditions possible

Veterinary Medicine: Benefits for Humans and Animals

Not all animal research is conducted in laboratories for the sole benefit of humans. Veterinarians, who devote their lives to the care of animals, also participate in research. Research at veterinary clinics has led to new cancer treatments for pets. Because animal and human cancers are strikingly similar, information gleaned from one species might be used to treat the other. For example, the *BRCA1* gene found in 65 percent of human breast tumors is similar to the *BRCA1* gene in dogs. The similarity of breast carcinoma in dogs and humans has been known for a long time, but whether these tumor cells also influence the surrounding tissue in dogs the same way they do in humans was unknown until the University of Zurich started making comparisons in 2017, which proved that some cells in the vicinity of tumors in dogs behave the same way as the corresponding cells in humans. Clinical trials of cancer therapies on pets can pave the way for human trials.

Many of the genes we know about that cause cancer in humans, like the *BRCA* genes in breast cancer, were discovered by studying families that have a high predisposition to cancer. When a particular dog breed gets a high rate of a particular cancer, it means there's something about their genetic makeup that's predisposing them. The approach is behind a new formal partnership between Duke Cancer Institute and the College of Veterinary Medicine at North Carolina State University. The Consortium for Canine Comparative Oncology brings together cancer doctors and researchers and will fund research projects that include both.

7.2 Cloning

The production of an animal from the nucleus of another adult animal was a scientific breakthrough in 1997, when Dolly the sheep was produced in this way, but it also created controversy over whether human cloning would ever be feasible. Here, we examine the methods and issues involved in cloning.

Creating Dolly: A Breakthrough in Cloning

Human DNA, like that of all higher animals, contains genes from two parents. The mixture of genes comes down to chance. To prove this, just look at the children in any large family: Some may have curly hair and others have straight hair; some may be able to "roll" their tongues and others cannot; one child may have a genetic disorder that the others do not have. This uncertainty is eliminated with cloning. When cloning is used to reproduce an animal, there is only one genetic contributor. If the donor sheep has long soft wool, the lamb clone will, too. The offspring have exactly the same genetic programming as the parent.

Embryo twinning (splitting embryos in half) was the initial step toward cloning. The first successful experiment produced two perfectly healthy calves that were essentially twins. To create a clone from an adult, the DNA from a donor cell must be inserted into an egg. In the first step, cells are collected from the donor animal and placed in a low-nutrient culture solution. The cells are alive but starved, so they stop division and switch off their active genes. The donor cells are then ready to be introduced into a recipient egg.

The egg itself is prepared by **enucleation.** A pipette gently suctions out the DNA congregated in the nucleus of the egg. The researcher must then choose a method for delivering the desired genetic payload into the egg. One method is illustrated in **Figure 7.4**. Another method, known as the Honolulu technique, involves injecting the nucleus of the donor cell directly into the middle of the enucleated egg cell.

After the DNA has been delivered to the egg, biology takes over. The new cell responds by behaving as if it were an embryonic cell rather than an adult cell. If all goes well, cell division occurs, just as it would in an ordinary fertilized egg. For the first few days, the embryo grows in an incubator. Then, when the new embryo is ready, it is transferred into a surrogate

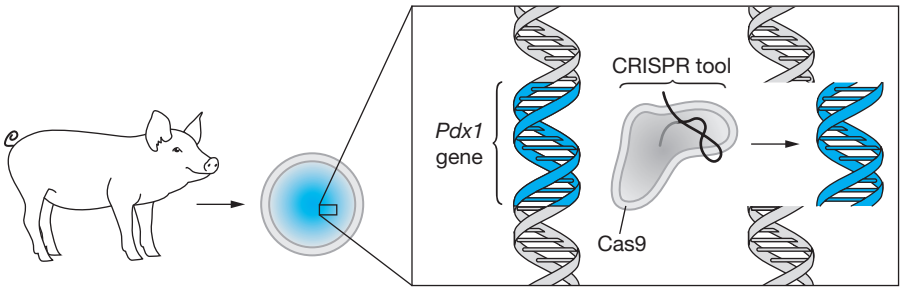


FIGURE 7.4 Human Pancreas Developed in Pigs Scientists add human induced pluripotent stem cells or iPSCs, into the developing embryo after deleting the pig *pdx1* genes, which means that the chimeric embryo can now develop a pancreas (but it will be made of human-like cells).

mother for gestation. The surrogate will give birth to a clone that is genetically identical to the genetic donor. The list of species successfully cloned in this fashion now includes sheep, goats, pigs, cattle, and an endangered cow-like animal called a gaur.

Limits to Cloning

There are limits to the power of cloning technology. First, the donor cell must come from a living organism. Free-ranging dinosaurs à la *Jurassic Park* are likely to remain science fiction. Also, most experts think that the chances of finding a viable mammoth cell, managing to clone it, and succeeding in fostering it in an elephant or other distant relative are laughably remote.

Nuclear transfers can be used to correct errors in human cells, too. Mitochondrial diseases are caused by any of about 200 mutations that affect the genes of mitochondria, tiny powerhouses inside nearly every cell of the body. Depending on the affected genes and cell types, the diseases can cause muscle weakness, liver disease, diabetes, seizures, developmental delays, or vision problems.

Now, researchers at the Salk Institute, California have gotten one step closer to making some cures a reality: They've turned cells from patients into healthy, mutation-free stem cells that can then become any cell type. The team's approach was to move the nucleus of the patient's skin cells, which contains most of his or her genes, into a donor egg cell with healthy mitochondria, then use the new egg cell to generate pluripotent stem cells. When the researchers did this, they found the healthy mitochondria took over, and healthy, genetically similar cells from the patient were successfully generated. For now, this means that researchers can use the healthy cells to generate heart, brain, muscle, or eye cells from the mutation-free stem cells.

Even racehorses, which are carefully bred to have essential genetic characteristics, are shaped by factors other than genetics. The fun of running and the will to win may not be genetically encoded. Looking at the DNA of horses to find good breeding stock may seem obvious, but it goes against centuries of tradition in this most conservative of sports, in which suspicion of applied molecular genetics is rife. The U.K. Jockey Club insists on gene tests to confirm parentage, but bans the use of cloned horses—even though in 2012 the International Federation for Equestrian Sports announced that clones would be allowed to compete in future Olympic Games.

More significantly, the success rate for cloning has improved since Dolly. Dolly the sheep was the result of 277 attempts. Of these, only 29 implanted embryos lived longer than 6 days, and of those 29 embryos, only Dolly was born. Scientists are still trying to understand some of the unexpected problems that arise during the cloning process.

An even more challenging problem is the possibility that clones may become old before their time. This problem came to light when analyses indicated that Dolly's cells seemed to be *older* than she was. This observation bears a little explanation. Every cell in your body contains the chromosomes that determine your genetic makeup. At the end of each chromosome is a string of highly repetitive DNA called a *telomere* (see Chapter 2 for a review). As your cells replicate, the telomere becomes shorter with each division. In this sense, there really is a biological clock inside living cells. A cell's chromosomal age can be read by inspecting the length of its telomeres. As the telomere unravels, signs of aging appear in the organism. Tests showed that Dolly's telomeres were not like those of other lambs born the year she was; instead, they were about the same length as her donor's, or 6 years older than Dolly.

Although eight other species—cow, mouse, pig, goat, rabbit, cat, mule, and horse—have been cloned since Dolly, nuclear transfer is still a very inefficient process (about 2 percent efficient) and very small numbers of clones are available for research. However, telomere length appears to have been exonerated for the inefficiency. Donor cell effect on telomere length is well examined. In a recent study, researchers produced 14 cloned cattle using nuclei of donor cells derived from muscle, oviduct, mammary, and ear skin. All three types (normal, shorter, longer) of telomeres were found when different donor cell types were compared.

The time cells spend in culture may be a factor. Shorter culture time resulted in telomere length similar to that in controls, whereas extremely long culture resulted in extended telomere length. One explanation might be that short time culture allowed the donor cell nucleus to be transferred with relatively long telomeres; that is, they would start out long and remain long. In contrast, extremely long culture time erodes the telomeres so short that the repair mechanisms are triggered and result in the over-elongation of telomeres.

This cloud over cloning has a potential silver lining. Researchers investigating the problem of shortened telomeres in clones learned more about telomerase, an enzyme that regenerates the telomere. This enzyme could eventually serve as a key to understanding and preventing many age-related diseases.

So why are clones necessary? For many reasons, it is advantageous to have multiple animals with the exact genetic makeup. Clones can be used in medical research, in which their identical genetics makes it easier to sort out the results of treatments without the confounding factor of different genetic predispositions. Clones may also provide a unique window on the cellular and molecular secrets of development, aging, and diseases.

In the case of endangered animals, there is a compelling reason to look to cloning to sustain breeding populations. Steps have already been taken to use cloning to preserve animals. Interspecies embryo transfer has been used successfully to provide surrogate mothers for African wildcats (*Felis silvestris lybica*). Noah, the gaur calf, was a clone born to a domestic cow surrogate. Noah later died of an infection unrelated to the cloning process. Despite that disappointment, efforts are now under way to clone pandas, using more common black bears as hosts.

Human Organ Development in GM Animals

Every day, an average of 16 people in Europe and 22 in the United States die while waiting for a replacement heart, liver, or other organ. One way to address the shortage would be to grow replacement organs in the laboratory. Researchers at San Antonio Catholic University of Murcia in Spain and the Moxie Foundation in San Diego, California, have created an international consortium to test their ideas. They began injecting pig embryos with human inducible pluripotent stem cells (iPSCs) in 2015 (see Figure 7.4). As the embryo grows, it forms outer, middle, and inner layers, with each individual cell's precise location determining future growth. Previous work had demonstrated that certain cells within the *inner* layer of the embryo respond to the protein signals in their microenvironment by turning on the gene *Pdxl.* This step in turn activates many other genes that trigger the maturation of the pancreas. A few cells located in the *middle* layer react to external signals by turning on the gene *Six2,* which starts formation of the kidneys. All cells in the body contain the same DNA sequence; during development, these triggers determine which genes are turned on or off and thus what kind of tissue will result. A single gene, such as *Pdxl* or *Six2,* can turn on an entire pathway leading to the formation of a pancreas or a kidney. By deleting the one critical gene needed for growing a pancreas, team members have created pig embryos that will not grow the insulin-producing organ unless they inject enough human stem cells that contain the missing



YOU DECIDE

Can Gene Editing in Chickens Prevent Avian Flu Transfer to Humans?

Chickens are potential hosts that can enable new strains of flu to be transmitted to humans. Preventing virus transmission between chickens should reduce the risk posed to people exposed to the infected birds and the economic impact of the disease. To produce these chickens, Cambridge and Edinburgh scientists introduced a new gene that manufactures a protein that mimics an important control element of the bird flu virus. The replication machinery of the virus is tricked into recognizing the decoy molecule instead of the viral genome that interferes with virus replication. When the transgenic chickens were infected with avian flu, they became sick but did not transmit the infection to other chickens kept in the same pen with them. This is quite different from conventional flu vaccines, which need to be updated in the face of virus evolution, as they tend to protect only against closely matching strains of virus and do not always prevent spread to other birds. Infectious diseases of livestock represent a significant threat to global food security, and the potential of pathogens, such as bird flu virus, to jump to humans and become pandemic has been identified as a top-level national security risk. Considering the benefit of this single gene transfer, is it likely that we could be switching to genetically modified (GM) chickens for their health benefits? How would you decide?

gene. If the added cells develop appropriately, they give rise to a mature organ made entirely of human cells. The rest of the animal will be made up of pig cells. The team still has much to learn about how best to prepare human stem cells and animal embryos so that the chimeras (combined origin embryos) will remain viable throughout pregnancy. A lot could go wrong. But even if we are unable to create fully formed organs, it is expected that within 10 years this process will be accomplished.

Remnants of ancient viral infections, genes from porcine endogenous retroviruses (PERV), are scattered throughout the pig genome, and could infect a person who receives a pig's heart, lung, or kidney as a replacement or temporary organ. Now, a U.S. company, eGenesis, says that it has removed these viruses. Using CRISPR gene editing, researchers from the company and several academic labs created dozens of healthy pigs with no trace of PERV genes. To make PERV-free pigs, they started with genetically normal cells straight from a living pig. In the new work, done with a team of Danish and Chinese collaborators, the eGenesis team applied the CRISPR system to cells derived from the connective tissue of fetal pigs. They inserted the DNA-containing nuclei of these edited cells into egg cells taken from the ovaries of pigs. They allowed each egg to develop into an embryo and implanted it in the uterus of a surrogate mother.

PERVs aren't the only obstacle standing in the way of transplant-ready pig organs. Researchers will need to knock out pig genes that provoke the human immune system, and insert others that will prevent toxic interactions with human blood.

7.3 Transgenic Animals

We've covered transgenics as it applies to plant biotechnology in Chapter 6. The process of introducing new genetic material to an organism is not limited to plants. It can be used on animals as well. We have seen some of the differences between proteins produced from bacterial (prokaryotic) sources and those coming from other sources, as we learned in Chapter 4. Because the structural differences among proteins are minimal, cell and animal cultures offer good solutions to the need for proper structure. Some of the most exciting developments in biotechnology are the result of transgenic research in animals.

The first experiments in animal transgenics were conducted on the cellular level. A new gene was added to a cell in culture and the effects on that one cell were observed. When cloning technology became available, transgenics took a new course. Instead of manipulating a single cell, genes were added to a large number of cells. These cells were then screened to discover which ones exhibited the desired genes. After these cells were selected, each could be used to grow a complete animal from a single cell, using cloning technology (see **Figure 7.5**).



FIGURE 7.5 EnviroPigs
The EnviroPig was modified to secrete phytase in its saliva to reduce the runoff of phosphates downstream of pig farms. The University of Guelph killed the pigs, from the 10th generation of the project, in June 2012 after it couldn't find a new partner to fund the project, even though it was an environmental success.

Transgenic Techniques

A variety of methods can be used to introduce new genetic material into animals, and new methods continue to be developed and refined. Here is a brief overview of some techniques:

- **Retrovirus-mediated transgenics** is accomplished by infecting mouse embryos with retroviruses before the embryos are implanted. The retrovirus acts as a vector for the new DNA. This method is restricted in its applications because the size of the **transgene** (or *trans*ferred *gene*tic material) is limited and the virus's own genetic sequence can interfere with the process, making transgenics a rather hit-and-miss project (retroviruses are discussed in Chapter 3).
- The **pronuclear microinjection** method introduces the transgene DNA at the earliest possible stage of development of the zygote (fertilized egg). When the sperm and egg cells join, the DNA is injected directly into the nucleus of either the sperm or the egg (although the sperm pronucleus is larger). Because the new DNA is injected directly, no vector is required; the foreign DNA must be integrated into the genome prior to the doubling of the genetic material that precedes the first cleavage in order for the animal to be born with a copy of this new information in every cell (see Figure 7.6).
- In the **embryonic stem (ES) cell method,** ES cells are collected from the inner cell mass of blastocysts. They are then mixed with DNA, which has usually been created using recombinant DNA methods. Some of the ES cells will absorb the DNA and be transformed by the new genetic material. Those transformed ES cells will then be injected into the inner cell mass of a host blastocyst.
- A similar method, **sperm-mediated transfer,** uses "linker proteins" to attach DNA to sperm cells. When the sperm cell fertilizes an egg, it carries the valuable genes along with it.
- Finally, gene guns can also be used on animal cells (see Chapter 6).

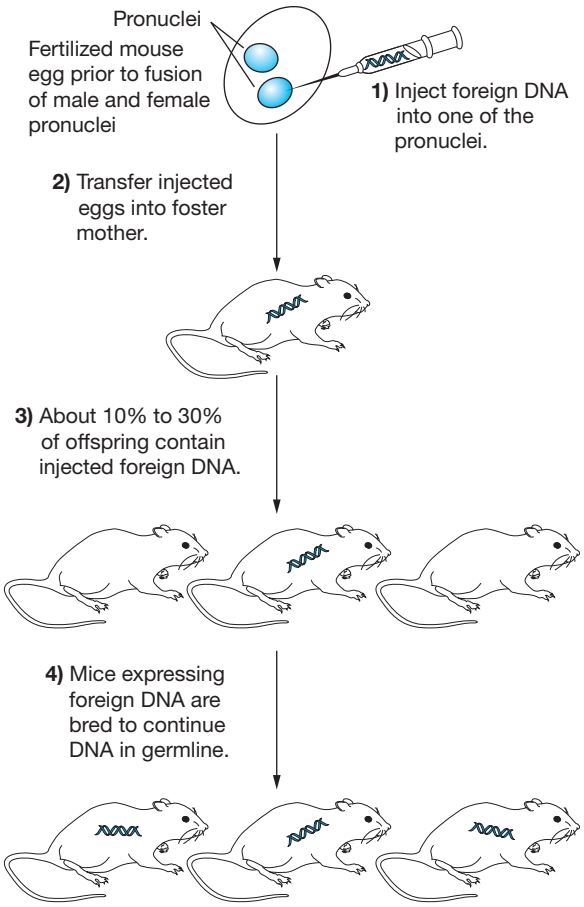


FIGURE 7.6 Pronuclear Microinjection Cloning The process of injecting DNA into one of the pronuclei (the female pronucleus and male pronucleus combine at fertilization) so that it is incorporated can be used to produce clones that bear the new DNA.

GM Animals for the Agriculture Industry

The dairy industry is also a target for genetic improvement. Researchers have used transgenics to increase milk production, make milk richer in proteins, and lower the fat content. Herman, a transgenic bull (produced by transgenics and nuclear transfer), carries the human gene for lactoferrin. This gene is responsible for the higher iron content (essential to the healthy development of infants) found in human milk. Herman's offspring also carry the gene for lactoferrin. Consequently, in much the same way that human polyclonal antibodies can be produced in the plasma of cattle (see Figure 7.7), Herman's daughters may be the first of many cows able to produce milk that is better suited for consumption by human children.

Another important improvement is the reduction of diseases in animals raised for food. During the epidemic of foot-and-mouth disease in England in 2000, herds of dairy and beef cattle as well as sheep and goats were destroyed. The loss of entire herds was catastrophic to farmers and devastating to that nation's agriculture industry. In the United States, concerns over the spread of foot-and-mouth disease resulted in the confiscation and destruction of sheep that had been imported from England, even though they did not necessarily test positive for the disease. Spurred by

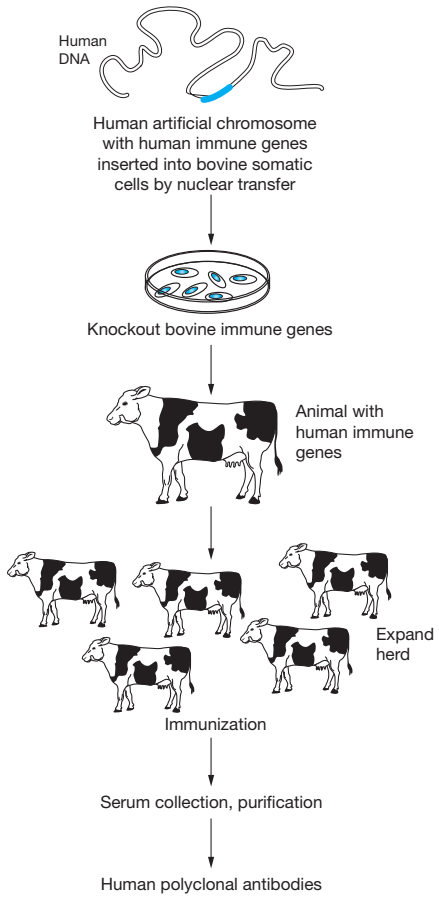


FIGURE 7.7 Human-Like Plasma from Cattle Human plasma for patients is in short supply and could be obtained from cattle cloned to carry human genes for blood factors and antibody genes. Human artificial chromosomes carrying these genes have been successfully transferred by procedures described earlier.

the threat of future disease outbreaks, researchers are studying disease-prevention genes and hope to develop animals that are resistant to foot-and-mouth disease. Similar processes may someday help prevent cholera in hogs and Newcastle disease in chickens.

Researchers at the USDA in Maryland turned to genetic engineering to create dairy cows that could resist mastitis, an inflammation of milk glands caused by bacterial infections. Bacteria such as *Staphylococcus aureus* infect mammary glands, resulting in a highly contagious condition that spreads rapidly and results in losses of billions of dollars for the dairy industry. Scientists created transgenic cows containing a gene from *Staphylococcus simulans,* a relative of *S. aureus,* which produces a protein called lysostaphin, which kills *S. aureus.* Preliminary data indicate that these animals do indeed show resistance to mastitis. But researchers must be cautious. They know that these animals will not necessarily be fully protected from infections by *S. aureus* or other microbes and that these cows represent only a first step toward improving the resistance of cows to the bacteria that cause mastitis. Whether the dairy industry will embrace these and other GM cows remains to be seen.

Researchers at the University of Guelph in Ontario, Canada, recently developed the **EnviroPig** (see Figure 7.5), a transgenic pig expressing the enzyme phytase in its saliva. It is deemed environmentally friendly because it produces substantially less phosphorus in its urine and feces than nontransgenic pigs. Phosphorus is the major pollutant generated on pig farms. Phytase degrades phosphates in the pigs' food, reducing the amounts of phosphorus waste products excreted by about 30 percent.



TOOLS OF THE TRADE Human Therapies for Pets

On average, U.S. pet owners spend an estimated $15.25 billion annually on veterinary care. The Kindred Bio Company estimates it will spend between $3 million and $5 million to develop each pet drug. The company reformulates molecules that are already known to work in humans, lessening the number of times they need to be taken per day and making them more appetizing to animals. Other biologics candidates include feline erythropoietin, to treat anemia in cats; checkpoint inhibitors; and interleukin antibodies. A good example of the market for pet remedies is Zoetis. The company produces medicines and vaccines for pets and livestock. Formerly part of pharmaceutical manufacturer Pfizer Inc., Zoetis operates in more than 100 countries and reported a revenue of $4.8 billion last year. Its latest drug treats fear in dogs. Owners of at least one-third of the 70 million dogs in the United States report problems with fear of loud noises. Dogs are sometimes so frightened they jump through plate-glass windows, destroy doors while trying to escape a room, or run into traffic and get hit by cars. The company's newest product, Sileo, works by blocking norepinephrine, a brain chemical similar to adrenaline that pumps up anxiety. It comes in prefilled plastic syringes. In testing on 182 pet beagles conducted on a recent New Year's Eve, 75 percent of their owners rated its effect good or excellent, which suggests that biotech for pets not only is big business but also may lead to human medications.

The safety of the food that reaches our tables can also be improved by transgenic technology. Human food poisoning may be reduced in the future by transferring antimicrobial genes to farm animals. This application could reduce numerous food poisoning deaths each year and also mean that fewer antibiotics will be used in agriculture. That is good news, because the overuse of antibiotics is leading to the development of resistant strains of *Salmonella, Listeria,* and *Escherichia coli.* The production and use of transgenic animals remain significant to research.

In transgenic pig herd expansion, only those animals capable of transmitting the transgene into the next generation are considered for breeding. Transgenic animals possessing characteristics with greater marketability are used for herd expansion. A three-step prebreeding screening program for transgenic boars is being proposed to select pigs through fluorescence in situ hybridization (FISH) assay combined with common prebreeding screening processes. The first step combines a general transgenic phenotype analysis with FISH to identify the transgenic boars. Individuals producing high-quality semen and transgenic sperm are next identified through the combination of the conventional semen test and FISH. The *in vitro* fertilization embryos are then put through to the final step of screening: identifying the individuals capable of producing transgenic embryos through FISH. Aside from pigs, this program can also be applied to other large animals, and could provide an economical and efficient strategy for transgenic herd expansion.

Transgenic Animals as Bioreactors

Recall that proteins are an important biotechnological product and that whole animals can serve as "bioreactors" to produce those proteins (Chapter 4). An animal bioreactor works as follows: First, the gene for a desired protein is introduced via transgenics to the target cell. Second, using cloning techniques, the cell is raised to become an adult animal. That adult may produce milk or eggs that are rich in the desired protein, thanks to the introduced gene.

An example of an application of this technology is "biosteel," a potential new product that may soon be used to strengthen bulletproof vests and suture silk in operating rooms. Six companies are competing with different versions of spider silk protein as of 2017. Fundamentally, biosteel is made from spiderweb protein, one of the strongest fibers on Earth. In the past, spider silk has never been a viable industrial product because spiders produce too little silk for it to be useful. One company, Bolt Threads, tested every one of the thousands of spider silk genes posted in GenBank before selecting ones for production. With transgenics, the gene that governs the production of spider silk has been successfully transferred into goats, and those goats have reproduced, passing the new gene to their offspring. These goats, like the ones shown in **Figure 7.8**, eat grass, hay, and grain and naturally produce the milk that now contains the proteins that make up spider silk. The silk protein can then be purified from the goats' milk and woven into thread for fabrication into bulletproof vests and other products that will be tested.

Transgenic animals are also being used to produce pharmaceutical proteins. A human gene called *ATryn,*



FIGURE 7.8 Goats with Transgenes That Produce Valuable Proteins in Their Milk ATryn, a recombinant form of human antithrombin developed in goats by GTC Biotherapeutics, was approved by the FDA in 2009 for the prevention of perioperative and peripartum thromboembolic events in patients with hereditary antithrombin deficiency. ATryn is the first-ever transgenically produced therapeutic protein and the first recombinant antithrombin approved in the United States.

which is responsible for an anticlotting agent in humans, is needed by patients with a hereditary deficiency to prevent thromboembolism before or after surgery or childbirth. The company that developed the transgenic goat (GTC) linked *ATryn* to mammary-specific promotors so that it would be expressed in milk. The transgenic method is faster, more reliable, and cheaper than other more complex ways of synthesizing pharmaceutical proteins. The product was approved for use by the FDA in 2009, and although the market for patients with hereditary antithrombin deficiency treatable with *ATryn* is not large, it may prove to have other uses in the operating room. This transgenic system is considerably less expensive using bioreactor animals and is under development for other proteins (like monoclonal antibodies) that are needed in large volumes.

You may be wondering why researchers in transgenics so often use goats. Goats reproduce more quickly and are cheaper to raise than cattle. Because they also produce abundant milk, they are an excellent choice for transgenic product development. Many diseases need short-term treatment of larger-quantity recombinant proteins that can be made amply available from transgenic animal sources. Until gene therapy becomes broadly utilized, transgenic-derived therapeutic proteins are the most attractive alternative for replacement therapy in genetic disorders such as hemophilia. Individuals with hemophilia lack the functional clotting protein factor VIII or factor IX. These proteins are being produced in transgenic goats and being tested for FDA approval. Using transgenic animal bioreactors for the production of complex therapeutic proteins provides lower production costs, higher production capacities, and safer pathogen-free products.

Milk-producing animals are not the only animal bioreactors, however. Eggs are another efficient method of producing biotech products in animals. Because poultry have an efficient reproductive system, it is easy to generate thousands of eggs from a small flock in a relatively short time. An average hen produces 250 eggs per year. With each egg white containing about 3.5 grams of protein, replacement of even a small fraction with recombinant protein creates a highly productive "hen bioreactor." Some medical products such as lysozyme and attenuated virus vaccines are already derived from eggs.

Kidney on a Chip Determines Drug Dosing

Precise dosing in intensive care units (ICUs) is critical, as up to two-thirds of patients in the ICU experience serious kidney injury. Determining a safe dosage, however, can be surprisingly difficult. Today, doctors and drug developers rely mainly on animal testing to measure the toxicity of drugs and determine safe doses. But animals process medications more quickly than humans, making it difficult to interpret test results and sometimes leading researchers to underestimate toxicity. The new technique offers a more accurate way to test medications, closely replicating the environment inside a human kidney. It uses a microfluidic chip device to deliver a precise flow of medication across cultured kidney cells. The use of an artificial device provides the opportunity to run test after test in a controlled environment. It also enables researchers to alter the flow through the device to simulate varying levels of kidney function. In a test of a common antibiotic used in the ICU, they found that a once-daily dose of the medication is significantly less harmful than a continuous infusion (see **Figure 7.9**).

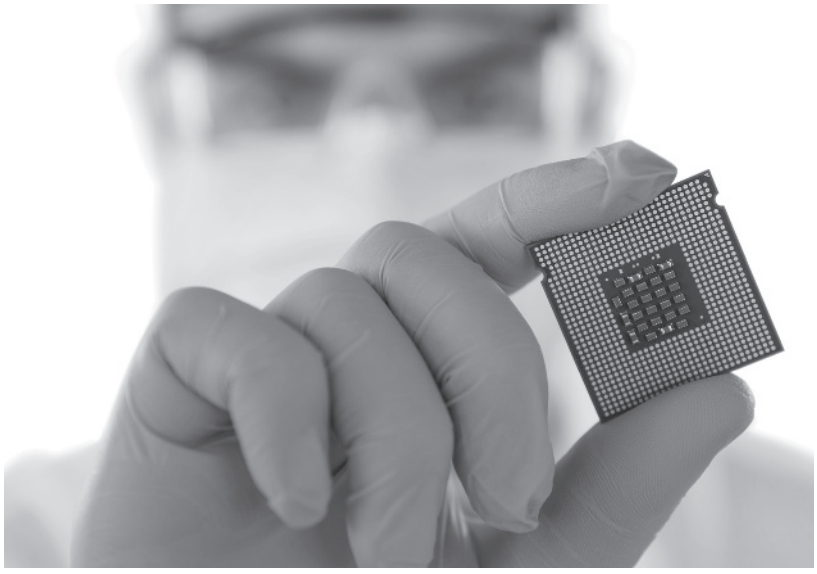


FIGURE 7.9 Kidney on a Chip Determines Drug Dosing A microfluidic chip device to deliver a precise flow of medication across cultured human kidney cells.

Gene Editing Technologies in Animals

Genome engineering has changed significantly with the development of more efficient, user-friendly methods for introducing targeted deletions in single genes. In the past, homologous recombination was the standard, utilizing the technology of chromosome crossover in a time-consuming, error-prone process. Interfering RNA (see RNAi, Chapter 3) rapidly became the research tool for gene silencing following the 2006 Nobel Prize for its discoverers. In fact, we can expect to see many RNAi drugs approved by the FDA in the next few years. These drugs are effective when injected locally in the eye or other specific organs, but are quickly degraded by enzymes when released to the bloodstream and cannot cross cell membranes. Other gene editing methods have been developed that focus on DNA editing. Zinc fingers nucleases (ZFNs) that bind to a DNA cleavage site and recognize specific targeted sites, and transcription activator-like effector nucleases (TALENs), which combine an artificial restriction enzyme with a DNA binding site, have seen considerable research interest but are hard to engineer.

A newer technology that is easier to engineer is beginning to replace other editing technologies. It was adapted from a bacterial defense mechanism that relies on clustered regularly interspaced short palindromic repeats (CRISPR). These repeated sequences were stored by bacteria as a molecular record of hostile genetic sequences previously encountered as viral or plasmid DNA. They allow a precise RNA-based recognition system that directs DNA nuclease enzymes to cleave the complementary DNA sequence. By engineering the RNA guide sequence (against a known gene of interest), it is possible to delete a target gene with precision, by attaching a cleavage enzyme called "Cas"—thus CRISPR-Cas. CRISPR-Cas has swept through animal research labs and rapidly become the commonplace editing technology since its 2012 discovery (as discussed in Chapter 3).

7.4 Producing Human Antibodies in Animals

The power of antibodies places them among the most valuable protein products. Here, we look at how antibodies are produced through the use of animals as bioreactors.

Monoclonal Antibodies

Researchers have long dreamed of harnessing the specificity of antibodies for a variety of uses that target particular sites in the body. In the 1980s, the use of an antibody as a targeting device led to the concept of the "magic bullet," a treatment that could effectively seek and destroy tumor cells wherever they might reside. One major limitation in the therapeutic use of antibodies is the problem of producing a specific antibody in large quantities. Initially, researchers screened **myelomas,** which are antibody-secreting tumors, for the production of useful antibodies. But they lacked a means to program the myeloma to manufacture an antibody to their specifications. This situation changed dramatically with the development of monoclonal antibody technology. **Figure 7.10** illustrates the procedure for

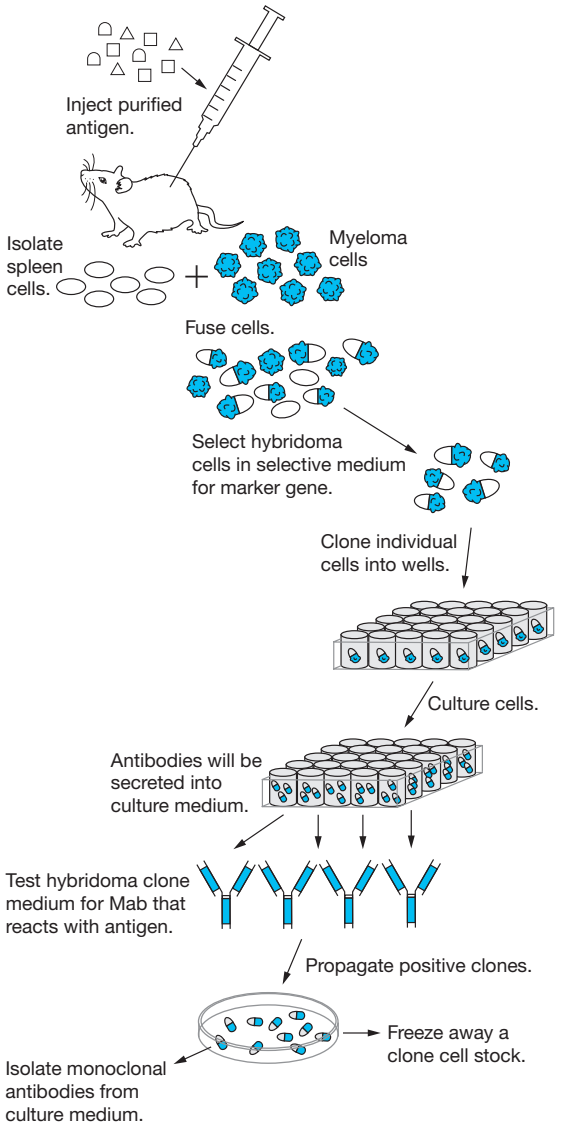


FIGURE 7.10 Monoclonal Antibody Production An antigen-stimulated mouse will provide spleen cells that can be fused with myeloma (cancer) cells to continuously produce monoclonal antibodies in a culture.



YOU DECIDE

Humans to Pets to Humans: Will the Public Accept This Type of Animal Testing?

In 2013 the FDA approved a drug by Pfizer for canine atopic dermatitis. The drug was derived from one approved for human rheumatoid arthritis that blocks the cytokine signaling for inflammatory response. By tweaking the cytokine target to that of dogs, they achieved a new pet medication. The new medication stopped itch within hours and cleared the shelves very quickly. Since biologics represent 6 of the 10 top-selling drugs, the company NexVet has exclusive licenses to adapt human therapeutic antibodies for use in cats and dogs. Another company, MediVet Biologics, has extended stem cell therapy for osteoarthritis to pets. It extracts adipose tissue from pets and turns it into stem cells that are reinjected into the animals. The realization that pets could be a more realistic evaluation for human drugs has caused the National Cancer Institute to develop an initiative to use pet clinical trials for new cancer therapies to treat pets and learn more about human cancers. Do you think the public will accept this kind of animal testing? You decide.

producing monoclonal (made from a single clone of cells) antibodies (Mabs).

First, a mouse or rat is inoculated with the antigen (Ag) to which an antibody is desired. After the animal produces an immune response to the antigen, its spleen is harvested. The spleen houses antibody-producing cells, or lymphocytes. These spleen cells are fused en masse to a specialized myeloma cell line that no longer produces an antibody of its own. The resulting fused cells, or hybridomas, retain the properties of both parents. They grow continuously and rapidly in culture like the myeloma (cancerous) cell and produce antibodies specified by the fused lymphocytes from the immunized animal. Hundreds of hybridomas can be produced from a single fusion event. The hybridomas are then systematically screened to identify the clone, which produces large amounts of the desired antibody. After this clone is identified, the antibody will be produced in large quantities.

Monoclonal antibody products are now used to treat cancer, heart disease, and transplant rejection, but the process has not always been smooth. An early limitation to the success of therapeutic antibodies was immunogenicity. The classic method for producing Mabs using mouse hybridoma cells provoked an immune response in human patients. Mouse hybridomas produce enough "mouse" antigens to still evoke an undesirable immune response in humans. Patients quickly eliminated mouse Mabs, so that the therapeutic effects were short-lived. Researchers called this the **human antimouse antibody (HAMA) response.** The solution is to make Mabs more human. Removing mouse antigens or creating chimeric cells is costly and time-consuming, and the resulting Mabs still provoke a HAMA response. Researchers are working on solving this problem and are producing Mabs in different organisms. In 2010, there were 22 therapeutic Mabs that had been approved by the FDA. All but three of these have been engineered to reduce immunogenicity, and most have some human genetic sequence. Transgenic mice producing humanized Mabs are under study in at least 33 current trials for new drugs.

In 2007, the FDA approved the first monoclonal antibody produced in animals that does not activate the human immune system to rejection. Panitumumab, produced from transgenic mice with knock-in human immune genes, was manufactured by Amgen in Thousand Oaks, California. The process involved inactivating the mouse antibody machinery (heavy- and light-chain proteins of antibodies) and introducing the human equivalent of these antibody-producing genes by homologous recombination into the inactivated (deleted) regions. The work was done in mouse ES cells, followed by the introduction of these cells into mouse embryos to produce founder animals. The transgenic mice (xenomice) produce a fully human antibody against epidermal growth factor receptor (as a treatment for people with advanced colorectal cancer), which can be purified and does not produce the HAMA response. Ever since the discovery that monoclonal antibodies could be generated, scientists have targeted the creation of "fully" human products to reduce the side effects of humanized or chimeric antibodies. As of November 2016, 13 of the 19 "fully" human monoclonal antibody therapeutics on the market were derived from transgenic mice technology. Monoclonal antibodies have been approved to treat cancer, cardiovascular disease, inflammatory diseases, macular degeneration, transplant rejection, multiple sclerosis, and viral infection.

MAKING A DIFFERENCE

The simplicity, high efficiency, and broad applicability of the RNA-guided Cas9 system (CRISPR-Cas) is transforming biological and biomedical research. The ease with which researchers can now make changes in the sequence or expression of any gene makes possible silencing or activation of a gene in virtually any organism or cell type. The ability to express multiple guide RNAs in a single cell magnifies the ability of this technology even further. Although the off-target effects of Cas9 need to be controlled, the high degree of success from gene insertions or deletions make possible analysis without the use of drug selection media required by other methods. Recent advances in developing and optimizing CRISPR-Cas9-based systems for genome editing should propel the technology forward to therapeutic applications, opening the door to diagnosing the mechanisms and new treatments of a wide variety of human diseases. The capacity to adapt this technology to specific problems in animal biotechnology has accelerated progress in this area, and animal models will be the starting point.

QUESTIONS & ACTIVITIES

Answers can be found in Appendix 1.

1. What are the benefits to human drug development of using gene editing to cure diseases in animals?
2. How does immunotherapy extend traditional therapeutic drugs in the treatment of melanoma?
3. Why are goats commonly used for transgenic production of therapeutic proteins?
4. With the entire genome of the pig now completed, what gene targets are likely to be chosen first for research?
5. How does the transparency of zebrafish embryos allow a new dimension to an anti-inflammatory drug study?
6. How can genetically engineered chickens be prevented from transmitting bird flu?
7. If you were to choose a drug to be manufactured in an animal (chicken, rabbit, goat), which would you choose and for what type of drug?
8. How do you construct an organ on a chip so that it is useful for human drug studies?
9. Explain the concept of "reduce, replace, and refine" in the use of animals for biological research.
10. Why are myeloma cells used in hybridomas?
11. Why is gene therapy (that is rarely used in humans *in vivo*) an expanding field in pet biotechnology research?
12. What advantages does "organs on a chip" drug testing have over testing in animals?
13. Why are individual "organs on a chip" being interconnected for drug testing?
14. How does the genetic makeup of pets that have a high incidence of a particular disease (like cancer) provide valuable research potential for the similar disease in humans?
15. Why would it be beneficial to transfer donor nuclei into embryonic stem cells from a different parental source?
16. How can changing to chickens engineered to block the replication of bird flu virus in animals reduce the likelihood of its transfer to humans?
17. Human embryo experimentation has severe limitations. How does R/D (research and development) for human organ replacement alleviate this limitation?
18. What is the cost of developing pet drugs compared to the cost of human drugs, and how does this affect human drug development progress?
19. How can FISH analysis be used to identify animals with desired genetic traits for herd expansion?
20. How is "kidney on a chip" study of a drug helpful in determining drug dosing?

Visit www.pearsonglobaleditions.com for the Companion Website

The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

This case study has been included to give you an opportunity to become familiar with a research example applicable to this field of study. After you have analyzed the data and answered the questions, you can compare them to those provided to your instructor with the text materials.

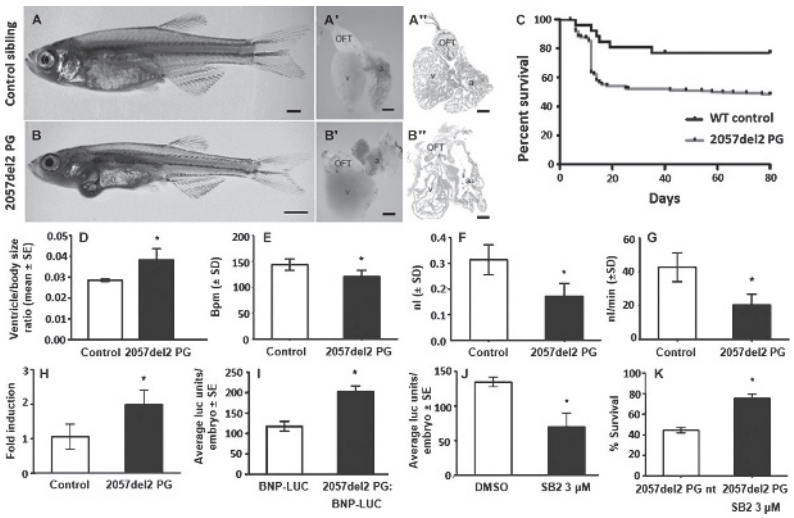
CASE STUDY

Using Zebrafish as a Heart Disease Model

Arrhythmogenic cardiomyopathy (ACM) is a highly arrhythmic form of human heart disease and a significant cause of sudden death in the young. Degeneration of cardiac myocytes (muscle cells) and replacement by fibro-fatty scar tissue develop with disease progression, but arrhythmias are often the earliest feature of ACM, and typically precede structural remodeling of the myocardium (heart muscle). ACM is caused by mutations in genes that encode proteins in the desmosome (cell adhesion sites) of cardiomyocytes. Abnormal localization of the desmosomal protein plakoglobin has been observed in patients with ACM. It has been proposed that ACM develops as a consequence of altered electrical signaling mediated by the mutant desmosomal proteins.

To efficiently explore ACM pathophysiology, researchers developed a stable inducible transgenic zebrafish model. They used the GAL4/UAS transactivation system to drive cardiac myocyte–specific expression of the human 2057del2 (2–base pair deletion) mutation in the gene encoding the desmosomal protein plakoglobin. This mutation is found in certain human cardiomyopathic defects. Transgenic fish expressing this mutation develop abnormal cardiac physiology within 48 hours of fertilization, with subsequent progression within 4 to 6 weeks.

Answer the questions based on the following figure.



Original Research Data from authors. (a and b) Representative images of a 5-week-old control sibling (a) and 2057del2 plakoglobin (PG) zebrafish (b) (left panels); dissected hearts (center panels) [control sibling (a') and 2057del2 plakoglobin (b'); OFT, outflow tract; a, atrium; v, ventricle]; and stained sections (right panels) [control sibling (a") and 2057del2 plakoglobin mutant fish (b"), showing cardiomegaly, wall thinning, and chamber dilatation in early adulthood]. (c) Survival curves for control and 2057del2 plakoglobin mutant fish. Data were pooled from three independent experiments and presented as a total percentage of fish survival as a function of time ($n = 125$; $P < 0.0001$). WT, wild type.

Source: Asimaki A, Kapoor S, Plovie E, et al. Identification of a new modulator of the intercalated disc in a zebrafish model of arrhythmogenic cardiomyopathy. *Sci Transl Med.* 2014 Jun 11;6(240):240ra74. doi:10.1126/scitranslmed.3008008.

Questions

Answers can be found at www.pearsonglobaleditions.com

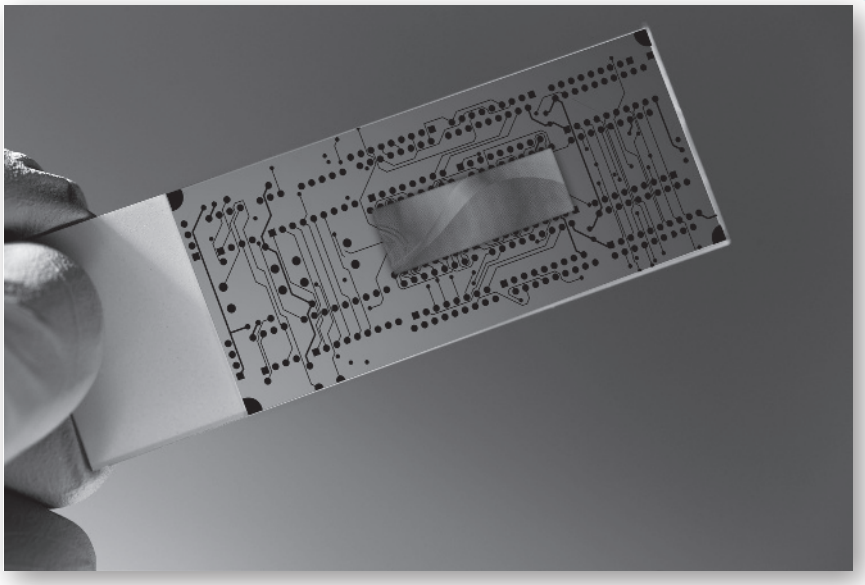
1. Why do zebrafish make good animal models for human arrhythmogenic cardiomyopathy?
2. Why were sibling fish chosen to measure the survival rates for the heart mutation?
3. How does this zebrafish model improve the ability to study potential remedies for this type of mutation in humans with arrhythmogenic cardiomyopathy?
4. What are the likely next steps in this type of research.

CHAPTER EIGHT

DNA Fingerprinting and Forensic Analysis

After completing this chapter, you should be able to:

- Describe the process for producing a DNA profile.
- Describe the significance of contamination to collecting and preparing a DNA sample to be used as evidence.
- Explain why PCR-based DNA analysis has largely replaced RFLP-based analysis.
- Describe how DNA profiles are compared for similarities or differences.
- Explain how "touch DNA" profiles can be easily contaminated.
- Explain the requirements for a DNA fingerprint to be used to exclude a suspect.
- Explain how DNA analysis can be used to detect food/beverage fraud.
- Describe how DNA fingerprinting can be used to identify familial relationships.
- Explain how DNA analysis can improve medical diagnostics.
- Explain how "shotgun proteomics" could be used as comparative evidence to DNA forensics.



Point-of-Care Diagnostics (primarily through microfluidics) is a rapidly growing field enabled by DNA diagnostics resulting from the expanded capacity of microchips. Liquid biopsies for cancer detection and monitoring are becoming a big area of interest. Guardant Health, Exosome Diagnostics, and Illumina have invested millions in this blood-based analysis process. The San Diego–based Illumina announced in 2016 that its blood tests should reach the market by 2019, and cost around $1,000 or less. The new testing concept is referred to as "liquid biopsy." The technique uses high-speed DNA sequencing machines to scour a person's blood for fragments of DNA released by cancer cells.

Forensic science is the intersection of law and science. It can be used to condemn the guilty or exonerate the innocent. Many court cases hinge on scientific evidence provided by forensics. Throughout the years, scientists have developed technologies to uncover facts in criminal investigations, and the law has been quick to embrace these new technologies as they became available.

For example, in the late 1800s, efforts to fight crime were given a boost by a new technology: photography. Photographs were certainly an improvement over verbal descriptions and hand-drawn "wanted" posters, but they still had severe limitations. Criminals found many ways to alter their appearance that could make identification based on a photograph almost impossible.

A little more than 100 years ago, scientists discovered that the tiny arches and whorls in the skin of human fingertips could be used to establish identity. After a single bloody print on the bottom of a cash box helped solve a murder in England, the process of inking a suspect's fingers and collecting a set of prints became routine. The International Criminal Police Organization (Interpol) as well as other law enforcement agencies amassed huge collections of these prints. But fingerprints are not always available: they can be wiped away, and gloves can be worn to avoid leaving them behind.

In 1985, a revolutionary new technology emerged as an important forensics tool. Instead of counting on smudged fingerprints left at the scene of a crime, investigators could look at a new kind of "fingerprint," the unique signature found in each person's genetic makeup. In this chapter, we look at a description of DNA fingerprints and discuss what makes each person's DNA unique. Then we learn the processes used to collect DNA samples and to produce DNA fingerprints for analysis. We compare DNA profiles and learn what it takes for a DNA match to occur. Next we consider examples that illustrate the value—and vulnerabilities—of DNA as evidence. We'll then look at how DNA profiles can be used to establish familial relationships, focusing on the use of mitochondrial DNA to provide proof of kinship. Next we will illustrate how DNA analysis has been extended to diagnose disease at earlier stages owing to the presence of snippets of DNA in blood samples. Finally, because DNA profiles are not limited to humans, we look at the uses of DNA analysis to establish the origin of valuable plant crops, help to enforce laws to protect wildlife, and perform biological research.

FORECASTING THE FUTURE

As DNA sequencing and genome mapping become faster and cheaper, scientists and consumers have wondered about possible broader use, like finding all the mutations hidden in DNA to predict which diseases a person will face decades later. Johns Hopkins University developed a model using registries of thousands of identical twins, who, despite their shared genes, can develop different diseases. The investigators examined 24 ailments, including different types of cancer, heart disease, diabetes, and Alzheimer's disease, to find many DNA differences.

Microfluidics will undoubtedly be used for this type of DNA diagnostic (i.e., fingerprinting) and has an impact on the development of the Point-of-Care (POC) marketplace. The global point-of-care diagnostics market is projected to reach approximately $41 billion by 2022, up from $23 billion in 2016. The ability of lab on a chip devices (see opening image) to be configured in a point-of-need setting has considerable potential for applications in infectious disease agent testing and cardiovascular disease testing, as well as other environmental and biodefense applications, according to a 2016 analysis. DNA fingerprinting will be extending further into health care.

8.1 What Is a DNA Fingerprint?

We know that every human being carries a unique set of genes. The chemical structure of DNA is always the same, but the order of the base pairs in chromosomes differs in individuals. The novel assemblage of the 3.3 billion nucleotides formed into 23 pairs of chromosomes gives each of us a unique genetic identity.

We also know that every cell contains a copy of the DNA that defines the organism as a whole, even though individual cells have different functions (cardiac muscle cells keep our hearts beating, neurons transmit the signals that are our thoughts, etc.). These two aspects of DNA—its uniform nature in a single individual and the genetic variability between individuals—make DNA fingerprinting possible. Because every cell in a body shares the same DNA, cells collected by swabbing the inside of a person's cheek will be a perfect match for those found in white blood cells, skin cells, or any other tissue.

How Is DNA Typing Performed?

Only a tenth of 1 percent of DNA (about 3 million bases) differs from person to person. These variable regions can generate a DNA profile of an individual, using samples from blood, bone, hair, or other body tissues containing a nucleus with DNA. In criminal cases, this generally involves obtaining samples from crime scene evidence and a suspect, extracting the DNA, and analyzing it for the presence of a set of specific DNA regions.

There are two main types of forensic DNA testing: one based on restriction fragment length polymorphism (RFLP) and the other on the polymerase chain reaction (PCR) (see **Figure 8.1** for an illustration of an RFLP). The original method of DNA testing, RFLP testing, requires

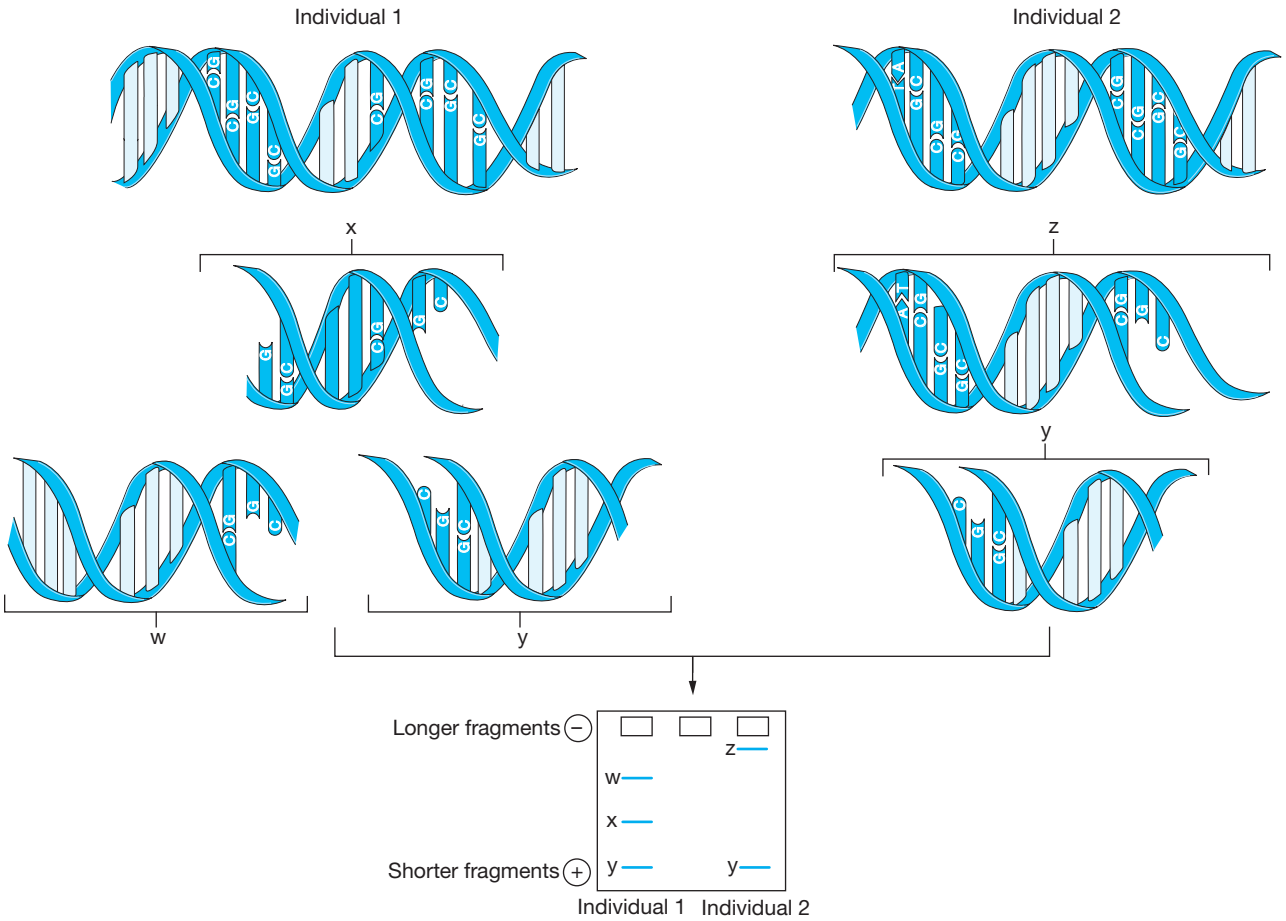


FIGURE 8.1 Restriction Fragment Length Polymorphisms (RFLPs) Can Be Used to Produce DNA Fingerprints Restriction enzymes recognize a short sequence of nucleotides and cut the DNA. Random mutations interfere with enzyme recognition, creating different lengths of DNA (not cut if mutated). The pattern of these cut fragments (when separated on a gel by electrophoresis) can be used to determine similarities and differences. Human DNA fingerprinting has moved to STR analysis largely because of the need for considerably less DNA for the PCR process. Individual 1 in the figure has three cut sites in his DNA, whereas individual 2 has only 2. The banding patterns resulting from the electrophoretic separation shows the differences.

larger samples than does PCR, and the DNA must be intact and undegraded (the FBI accepts RFLPs of four types for case comparison). You may wish to view the animation "Restriction Fragment Length Polymorphisms" on the Companion Website for a visual analysis of the process. Crime scene evidence that is old or present only in small amounts is often unsuitable for RFLP testing. Warm, moist conditions may accelerate DNA degradation, rendering it unsuitable for RFLP testing in a relatively short period of time. Because of this, RFLP is not used as often for DNA testing as it once was.

PCR-based typing requires less DNA than RFLP testing and is still effective if the sample is partially degraded. However, PCR-based tests are also extremely sensitive to contamination by foreign DNA at the crime scene and within the laboratory.

Fortunately, it is not necessary to catalog every base pair in an individual's DNA to arrive at a unique fingerprint. Instead, DNA profiling depends on only a small portion of the genome. Every strand of DNA is composed of both active genetic information, which codes proteins, and inactive DNA, which does not. Some of these inactive portions contain repeated sequences of between 1 and 100 base pairs. These sequences, called **variable number tandem repeats (VNTRs),** are of particular interest in determining genetic identity. Every person has some VNTRs that were inherited from his or her mother and father. No person has VNTRs that are identical to those of either parent (since we receive one allele from each parent, and they may not be the same). The individual's VNTRs are a combination of repeats of those of the

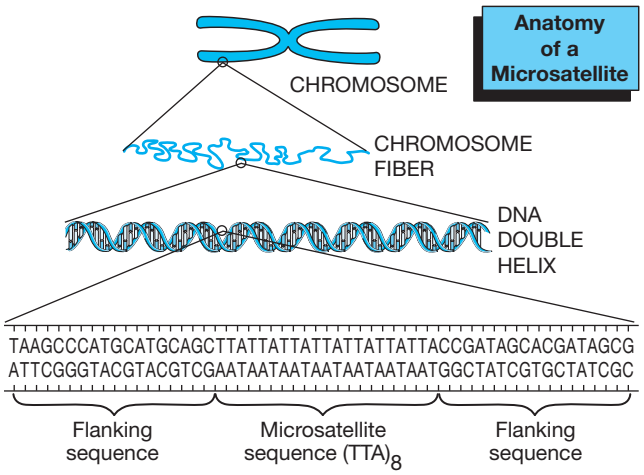


FIGURE 8.2 Anatomy of a DNA Microsatellite
A microsatellite is a variable number of repeated nucleotides (like TTA) that occur in specific locations in the genome. Using primers for the flanking regions on the ends of the microsatellite allows for amplification using PCR (from both directions). Individuals inherit a specific number of these repeats from their parents, but the number of repeats varies for unrelated individuals, forming a distinct pattern or DNA fingerprint.

parents' DNA regions in tandem. VNTRs are broken into two subgroups, the minisatellites, which are the basis for much of the RFLP analysis, and the microsatellites, which are the basis of the current CODIS database. The uniqueness of an individual's VNTRs provides the scientific marker of identity known as a DNA fingerprint.

DNA fingerprints produced by PCR are usually restricted to detecting the presence of **microsatellites** (Figure 8.2), which are one- to six-nucleotide repeats dispersed throughout chromosomes. Because these repeated regions can occur in many locations within the DNA, the probes used to identify them complement the DNA regions that surround the specific microsatellite being analyzed. The small size of these repeated segments has resulted in the term **short tandem repeat, or STR.** The FBI has chosen 13 unique STRs to test for DNA profiles contained within their **Combined DNA Index System,** or **CODIS,** as seen in **Figure 8.3.** In early 2015, the FBI announced that an additional seven loci would be added to the CODIS Core effective January 1, 2017.

8.2 Preparing a DNA Fingerprint

Preparing a DNA fingerprint requires specimen collection, DNA isolation and quantification, and PCR amplification. We discuss these steps in detail on the following pages.

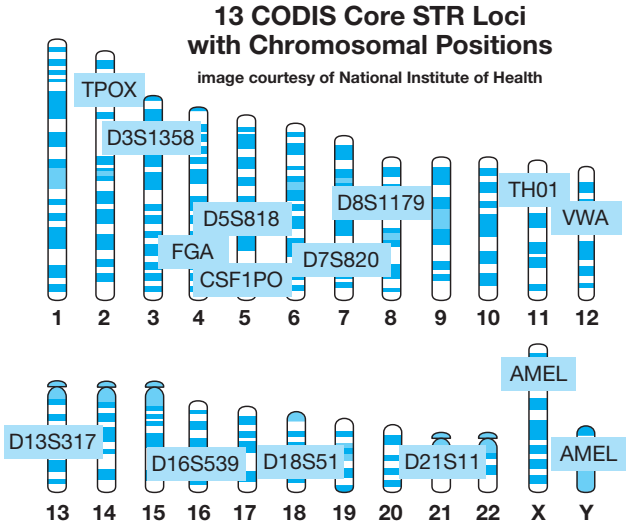


FIGURE 8.3 Human Short Tandem Repeat Patterns
In 1998, the FBI chose 13 unique STR regions (shown on human chromosomes) for analysis and comparison in its library of DNA fingerprints. An additional seven loci were added to the CODIS Core effective January 1, 2017. The additional seven loci—D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, and D22S1045—along with the original 13 loci, will constitute the new CODIS Core Loci. DNA primers for the flanking regions of these sites are commonly found in PCR kits that amplify these sites. After DNA sequencing, the results indicate the number of repeats for each homologous chromosome at that site and constitute a DNA fingerprint for that individual.

Specimen Collection

Crime scene investigators routinely search for sources of DNA: dirty laundry, a licked envelope, a cigarette butt, or anything else that might be a source for human cells left behind. Tiny blood stains or a trace of saliva is often all it takes to crack a case.

Every living thing has DNA, so every crime scene is full of sources of possible contamination. For this reason, scrupulous attention to detail is required in collecting and preserving evidence. To protect the evidence, workers at a crime scene must take precautions (see the FBI website for a full list of precautions).

The enemies of evidence are everywhere. Sunlight and high temperatures can degrade DNA. Bacteria, busy doing their natural work as decomposers, can contaminate a sample before or during collection. A small amount of moisture can damage and ruin a sample.

DNA fingerprinting is a comparative process. DNA from the crime scene must be compared with known DNA samples from the suspect. DNA has been retrieved and successfully analyzed from samples that were a decade old by amplifying the DNA used for comparison by PCR.

Extracting DNA for Analysis

After a sample is collected from the known source, a lab technician is responsible for determining detergents that chemically wash away the unwanted cellular material, followed by protein-and RNA-digesting enzymes and precipitation in ethanol. Next, the DNA must be quantified for accurate comparisons with other samples. Once the DNA is extracted and quantified, the technician must follow several steps to transform the unique signature of that DNA into visible evidence.

PCR and STR Analysis

Several thousand cells are required to do an RFLP analysis, more than can often be collected at a crime scene. Because of this, PCR is now normally used to "grow" or amplify the sample into an amount that can be analyzed.

PCR is much like a photocopier for DNA. The first step involves locating the portions of DNA that can be useful for comparison. Primers, or short pieces of DNA, are the tools used to find those portions on each DNA strand in the 5′ to 3′ direction (see arrows in **Figure 8.4**). The primers (about 20 nucleotides long) complement by DNA base-pairing interactions and bind best when the bases match exactly with complementary sections of the DNA. Once the complementary segment of DNA is located, copying begins, using a device called a *thermal cycler*. Figure 8.4 illustrates PCR as used in DNA amplification for fingerprinting. With the aid of the

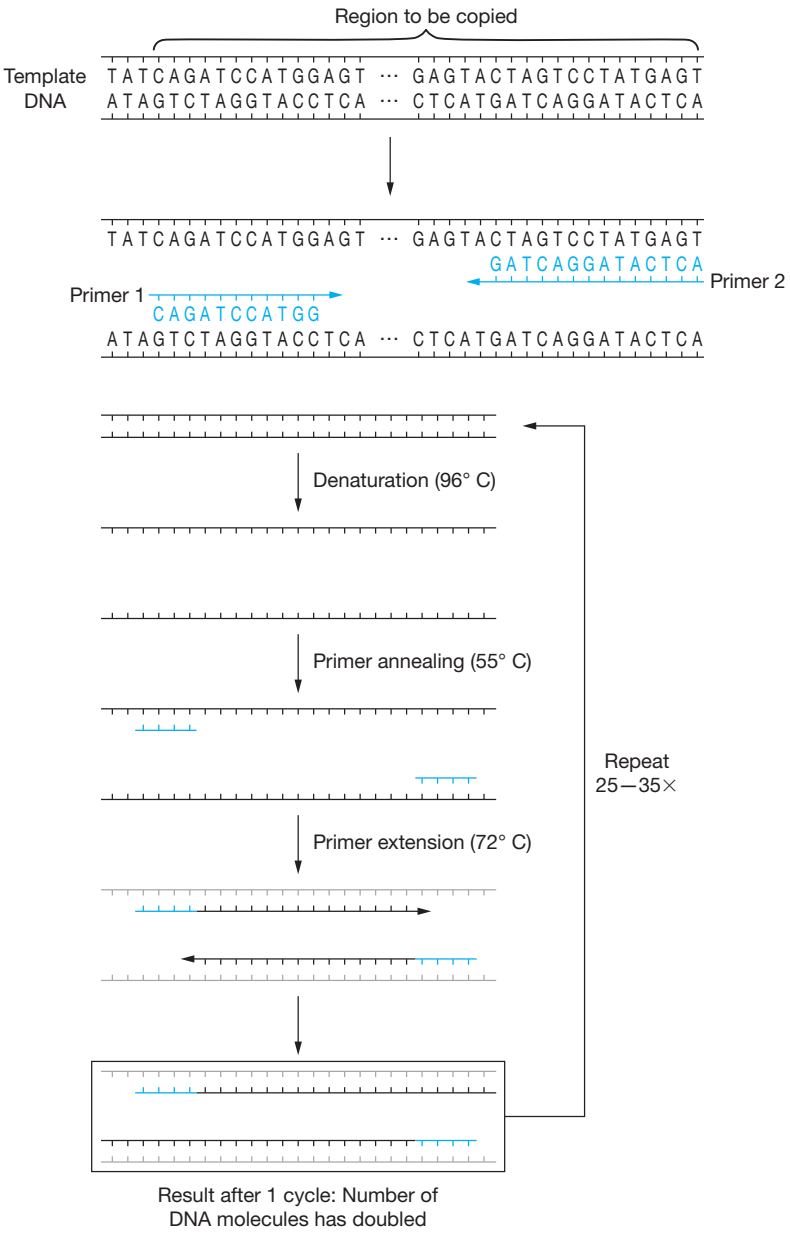


FIGURE 8.4 Amplifying DNA at Specific Sites by PCR Polymerase chain reaction makes many copies of a specific DNA region *in vitro*. PCR relies on a thermostable DNA polymerase, *Taq* polymerase, and requires DNA primers complementarily matched to a specific DNA region. The DNA polymerase enzyme *Taq* makes new strands of DNA, using existing strands as templates. *Taq* polymerase can make DNA only if it's given a primer, a short sequence of nucleotides that provides a starting point for DNA synthesis. When the primers are bound to the template, they can be extended by the polymerase, and the region that lies between them will get copied. The steps are as follows: denaturation (96°C)—heat separates, or denatures, the DNA strands; annealing (55 to 65°C)—cools the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA; extension (72°C)—raises the reaction temperatures so *Taq* polymerase extends the primers, synthesizing new strands of DNA. In PCR, the reaction is repeatedly cycled through a series of temperature changes, which allow many copies of the target region to be produced simultaneously.

enzyme *Taq* polymerase and DNA nucleotides, cycles of heat and cold cause the DNA to separate and then replicate repeatedly. Within hours, the DNA segments can be increased by billions. That rapid growth presents a possible problem: PCR is very sensitive to contamination, and a small error in a field or laboratory procedure can result in the duplication of a contaminating DNA sample. You may wish to watch the animation "Polymerase Chain Reaction (PCR)" on the Companion Website to see how the process is usually performed.

STR Analysis

After STRs are amplified by PCR, the alleles are separated and detected using capillary electrophoresis, which separates the lengths of amplified DNA, allowing the number of repeats in each of the two alleles on homologous chromosomes to be determined. An STR contains repeated numbers of a short (typically three- to four-nucleotide) DNA sequence. The number of repeats within an STR is referred to as an allele. For instance, the STR known as D7S820, found on chromosome 7, contains between 5 and 16 repeats of GATA. An individual with D7S820 alleles 10 and 15, for example, would have inherited a copy of D7S820 with 10 GATA repeats from one parent and a copy of D7S820 with 15 GATA repeats from the other parent. There are 12 different alleles for this STR, creating 78 different possible genotypes, or pairs of alleles, for this one site. The large number of variations for each STR provides the variability that is needed to distinguish different individuals using DNA fingerprints. **Figure 8.5** shows alleles of all 13 STR sites used by CODIS, with their base-pair positions (and the code names for all 20 sites). You may wish to view "Probability" on the Companion Website for a review of the inheritance calculations.

8.3 Putting DNA to Use

DNA fingerprinting is similar to old-fashioned fingerprint analysis in an important way. In both cases, evidence collected from a crime scene is compared with evidence collected from a known source. During evaluation of the samples, analysts are looking for alignment of the bands. If the samples are from the same person (or from identical twins), the bands or dots should line up exactly. All tests are based on exclusion. In other words, testing continues only until a difference is found. If no difference is found after a statistically acceptable amount of testing, the probability of a match

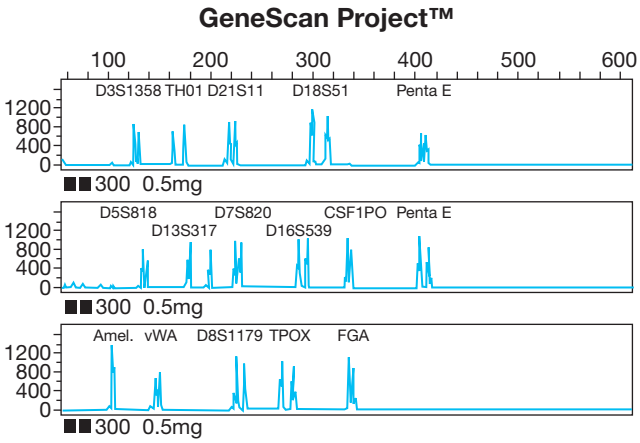


FIGURE 8.5 GeneScan Display of the 13 STRs Accepted by CODIS Utilizing capillary electrophoresis to detect the amplified STR microsatellite DNA sequences, it is possible to determine the number of STRs. By comparing the patterns obtained as evidence, it can be determined if the DNA patterns are exactly the same or different. Note that none of the DNA fingerprints shown are exact matches.

is high. See if you agree with the exclusion of one individual as shown in **Figure 8.6**. You may also wish to use the "Activity: DNA Fingerprinting" on the Companion Website for a visual refresher.

As we will see, such clear-cut visual evidence can be invaluable to crime investigators. Next we consider a few examples of DNA profiling in action.

The Narborough Village Murder Case Led to a New Method of DNA Separation

The first reported use of genetic fingerprinting in a criminal case involved the sexual assault and murder of a schoolgirl in the United Kingdom in 1983. Sir Alex Jefferies and his colleagues, Dr. Peter Gill and Dr. Dave Werrett, developed techniques for extracting DNA and preparing profiles using old samples of human tissue. Gill also developed a method for separating sperm from vaginal cells, which allows fingerprints to be run on vaginal cells first and then sperm cells in order to provide comparison samples (**Figure 8.7**). In this method, a detergent breaks open vaginal cells but leaves sperm cells intact. Without these developments, it would be difficult to use DNA evidence to solve rape cases because sperm cells could not be located to compare them against known samples.

In this case, Jefferies and his colleagues compared DNA evidence collected from the murder scene with a semen sample from an earlier similar rape and murder, and the analysis indicated that both crimes were committed by the same man. At this point, the police had a prime suspect. However, when the DNA

Case Mockup: Results

Sample ID	D3S1358	VWA	FGA	AMEL	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
Jane Doe, Reference Sample	15, 16	16, 19	18, 22	X	11, 12	30, 31.2	14	9, 12	9, 12	8, 11
Dorothy Smith, Reference Sample	15, 18	16, 17	20, 21	X	10, 13	31, 31.2	12, 16	10, 12	11, 12	8
Knife Blade Stain A	15, 16	16, 19	18, 22	X	11, 12	30, 31.2	14	9, 12	9, 12	8, 11
Knife Blade Stain B	15, 16	16, 19	18, 22	X	11, 12	30, 31.2	14	9, 12	9, 12	8, 11

Conclusions: Dorothy Smith CAN be excluded from contributing to the DNA found on the knife blade stains A and B. Jane Doe CANNOT be excluded from contributing to the DNA found on the knife blade stains A and B.

FIGURE 8.6 Who Is Excluded by DNA Comparison? The DNA profiles shown were taken from a crime scene. Knife stains A and B were found on the blade of a knife used in the crime, whereas stain C was found on the handle of the knife. All three stains were amplified for DNA analysis. Two individuals were tested for a match to the DNA profiles. Read the conclusion from the figure and see if you agree with the analysis.

evidence was compared with a DNA sample from the suspect's blood, the DNA did not match. The prime suspect was therefore excluded from consideration.

The investigation continued. The police conducted the world's first mass screening of DNA, collecting 5,500 samples from the district's male population. Using simple blood-typing tests, all but 10 percent of the initial group were quickly excluded (using RFLP analysis methods). After many grueling hours of analysis, the investigation had come to a standstill: none of the remaining profiles matched that of the rapist/killer.

Then came the lucky break. A man was overheard saying that he had given a sample in the name of a friend. When the man who had evaded the mass testing was apprehended, his DNA was analyzed and found to be an exact match for the DNA in the semen specimens from the crimes. The suspect confessed and was sentenced to life in prison.

This case highlights one of the important limitations to the use of DNA evidence: unless there is a known sample to be used as a comparison, identity cannot be established. In the example shown in **Figure 8.8**, the blood samples from the victim and the suspect are known. By comparing these two known DNA fingerprints, investigators were able to place the suspect at the scene of the crime based solely on the DNA fingerprints of the blood found on the suspect's clothes.

How Significant Is Contamination?

In a famous case of crime scene contamination, German police searched for around 15 years for a serial killer they thought was a female linked by traces of DNA to six murders across Germany and Austria. In 2009, the police discovered that a worker at a factory that produced the cotton swabs used in the investigations had been accidentally contaminating them with her own DNA. The importance of controls is emphasized by this example.



YOU DECIDE **Could Genetic Screening Improve Breast Cancer Prevention?**

The Institute of Cancer Research has a test for a wide range of genetic risk factors that could improve doctors' ability to determine which women are at increased risk of developing breast cancer, based on a major study of more than 65,000 women. The research, a collaboration of hundreds of research institutions led by The Institute of Cancer Research, London, and the University of Cambridge, showed that a test for differences in 77 separate letters of DNA code could indicate a woman's risk of developing breast cancer. They found a significant link between the score—called a "polygenic risk score"—and a woman's breast cancer risk. For example, a woman in the top 20 percent for polygenic risk score was 1.8 times more likely to develop breast cancer than the average woman. Analyzing this panel of 77 genetic markers, all of which had previously been linked with slight increases in breast cancer risk on their own, was much more accurate in defining risk than previous tests that used fewer markers. Should DNA forensics for breast cancer become routine? How would medical insurance justify the expense? You decide.

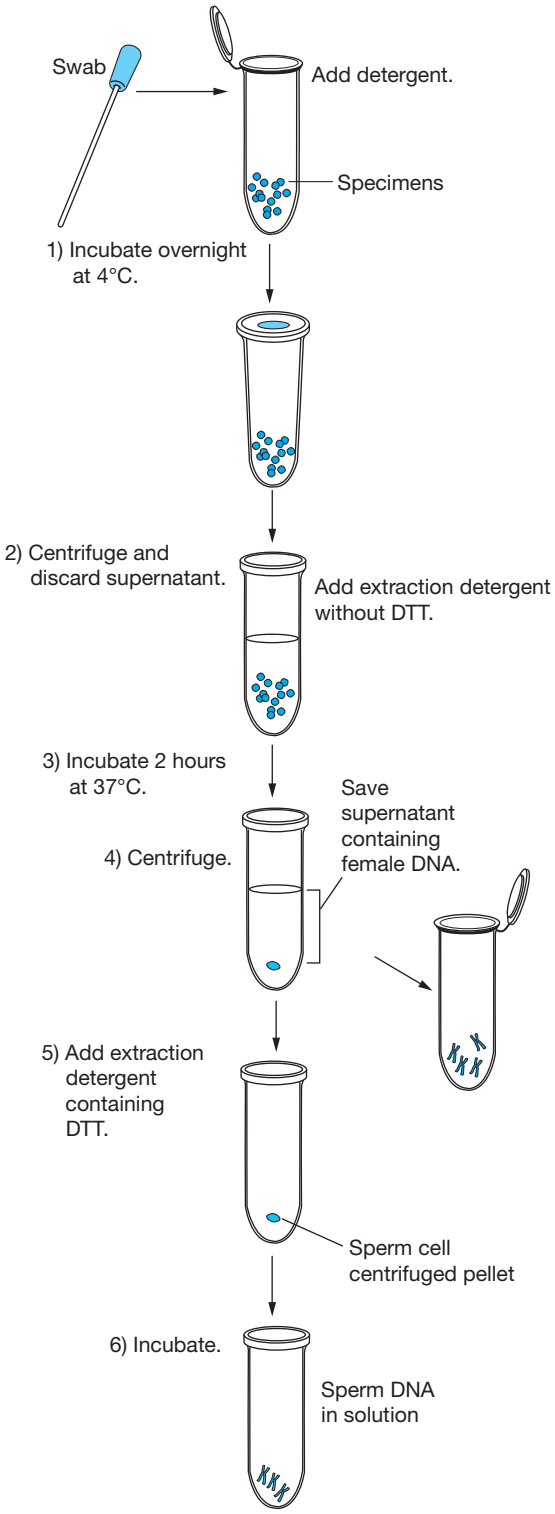


FIGURE 8.7 Isolation of Sperm DNA or Vaginal Cell DNA from Mixed Sources DNA from a sperm cell can be fingerprinted to be compared with the profile obtained from a sperm sample obtained on a vaginal swab. The buffer, containing dithiothreitol (DTT), will break the sperm cell membrane and can be used to detect sperm DNA after vaginal DNA has been analyzed separately in mixed evidence (owing to the ability of the sperm cell membrane to resist disruption until the addition of DTT).

Sample ID	D5S818
Person A	9, 12
Person B	8 (or 8,8)

FIGURE 8.8 Example of an Exclusion Based on One STR from a Suspect At locus D5S818 (chromosome 5), person A has 9 repeats from his mother and 12 repeats from his father. Person B has 8 repeats from both his parents.

World Events Lead to the Development of New Technologies

When major disasters occur and large numbers of people need to be identified, DNA comparisons are not enough. New methods of cataloging evidence have resulted in faster and more efficient comparisons to distinguish individuals.

DNA forensics used after the World Trade Center disaster

Shortly after the twin towers of the World Trade Center in New York City were destroyed by the terror attacks of September 11, 2001, forensic scientists came together and rapidly accelerated efforts to use DNA-based techniques to identify the remains of victims. They were confronted with a tremendous amount of debris at the site, dangerous working conditions, heat and microbial decomposition of remains, and hundreds of thousands of tissue samples from the nearly 3,000 individuals lost. These issues made it evident that new strategies would need to be employed to quickly prepare and organize DNA profiles and compare them with DNA profiles from relatives. How would scientists establish DNA identity for those who perished in over 1.5 million tons of rubble?

State agencies, such as the New York Department of Health and the medical examiner's lab, and several federal agencies, including the U.S. Department of Defense, National Institutes of Health, and the U.S. Department of Justice, immediately responded to help with this task. Within 24 hours after the disaster, the New York City Police Department had established collection points throughout the city where family members could file missing persons reports and provide cheek cell swabs for DNA isolation. Personal items (combs, toothbrushes) from the missing were also collected for DNA profiling.

Myriad Genetics, Inc., Gene Codes Forensics, DNAVIEW, Celera Genomics, Bode Technology Group, and Orchid Genescreen were among the companies assisting



YOU DECIDE

Will Rapid DNA Testing at a Crime Scene Help Law Enforcement?

Technological advances will soon make rapid DNA testing (R-DNA) feasible and practical. But these new "lab on a chip" portable forensic DNA sampling systems also pose new challenges for the forensic DNA typing community. Validation guidelines for today's lab-based DNA typing techniques exist and are employed by forensic specialists. For the past decade, processing each sample using current lab-based forensic DNA typing has taken about 8 to 10 hours. For example, the PCR amplification portion of the workflow alone typically takes approximately 3 hours with standard thermal cycling protocols, whereas the potential applications for rapid DNA devices are many, including at the booking station. Police station personnel could run preliminary tests on incoming suspects quickly and with little training. Saliva samples could be collected in a minimally invasive manner and typed right at the station. Law enforcement would then have a profile that could be instantly searched against a database of known offender profiles. To assist in this effort, a scientific working group for rapid DNA was established at the National Institute of Standards and Technology in 2011. This group was formed to monitor and address quality specifically in regard to the new generation of rapid DNA technologies and to help assess and validate these untested processes. The R-DNA working group will influence policy, define procedures, and discuss the potential implications of usage. Recommendations from this working group will guide the forensic DNA typing community in the proper use of the R-DNA devices. In a lab setting, a trained individual completes the DNA typing, whereas an R-DNA device automatically performs the DNA typing. What should be done to make sure that testing is accurate from R-DNA devices? How would privacy be maintained? You decide.

in this effort. Several other companies were involved in developing new software programs to help match samples submitted by family members to DNA profiles obtained from World Trade Center victims. Available tissue samples primarily consisted of small bone fragments and teeth, which provided fragmented DNA owing to degradation by the intense heat at the site. Because of this, forensic scientists primarily used STR, mitochondrial DNA (mtDNA), and SNP analysis of DNA fragments to develop profiles.

DNA analysis was conducted on over 15,000 tissue samples, although less than 1,700 of the estimated 2,819 people who died at the site were ultimately identified. This tragedy forced the development of new forensic strategies for analyzing and organizing remains recovered from the site and, most importantly, provided closure for a number of families that lost loved ones in the attack.

DNA forensics after the South Asian tsunami

The South Asian tsunami of December 2004 was a tragedy that claimed over 225,000 lives and devastated areas of Indonesia, Sri Lanka, and Thailand. The Michigan-based company Gene Codes modified its software system, called **Mass Fatality Identification System (M-FISys),** to help with the Thailand Tsunami Victim Identification effort. Because M-FISys was essentially built in response to the 9/11 tragedy, Gene Codes did not have to write entirely new software and was able to customize M-FISys as necessary. In addition to analyzing mitochondrial DNA (see Section 8.5), M-FISys incorporated male-specific variations in the Y chromosome, called Y-STRs, to aid in the identification of individuals. Within 3 months, approximately 800 victims had been identified.

Gene Codes also established the DNA Shoah Project (*shoah* is the Hebrew name for the Holocaust), an effort to use M-FISys to establish a genetic database of Nazi-era Holocaust survivors with an overall goal to try and reunite an estimated 10,000 postwar orphans around the world.

8.4 DNA and the Rules of Evidence

Before DNA fingerprints could ever be used in a court of law, DNA fingerprinting had to meet the overarching legal standards regarding the admissibility of evidence. The U.S. Courts use five different standards to determine whether scientific evidence should be allowed in a case. The test used depends on the jurisdiction. When a new technique or method is used to collect, process, or analyze evidence, it must meet one or several of these standards before the evidence is accepted.

- The relevancy test (Federal Rules of Evidence 401, 402, and 403) essentially allows anything that is deemed to be relevant by the courts.
- The *Frye* standard (1923) requires that the underlying theory and techniques used in gathering

evidence have "been sufficiently used and tested within the scientific community and have gained general acceptance." This is known as a general acceptance test.

- The *Coppolino* standard (1968) allows new or controversial science to be used if an adequate foundation can be laid, even if the profession as a whole is not familiar with the new method.
- The *Marx* standard (1975) is basically a common-sense test that requires that the court be able to understand and evaluate the scientific evidence presented. This is sometimes referred to as the no-jargon rule.
- The *Daubert* standard (1993) requires special pretrial hearings for scientific evidence. Under the Daubert standard, any scientific process used to gather or analyze evidence must have been described in a peer-reviewed journal.

The goal is to make certain that the scientific methods and expertise used to provide evidence are trustworthy.

DNA Fingerprinting and the Chain of Evidence

DNA analysis was a relatively new forensic tool when the Los Angeles Police Department used it in the infamous O. J. Simpson trial in 1994. Simpson's ex-wife, Nicole Brown Simpson, and her friend Ronald Goldman were murdered, and Simpson was the primary suspect. Forty-five samples were collected for DNA analysis from the crime scene. During pretrial proceedings, it was announced that the DNA collected at the crime scene matched that of O. J. Simpson.

Defense lawyers immediately attacked the procedures used in collecting, labeling, and testing the evidence. During the trial, the defense showed a videotape of the sample-collection methods and drew on expert testimony to establish doubt about the reliability of the evidence submitted. The defense stressed that contamination could have occurred when a technician touched the ground, when plastic bags were used to store wet swabs, and when sample collection tweezers were rinsed with water between touching samples. While on the stand, one prosecution witness admitted to mislabeling a sample. The possibility that the evidence might be tainted was obvious to both the court and the jury. As a result, the DNA evidence, which had been expected to make the case for the prosecution, was not effective. O. J. Simpson was found not guilty. When the chain of evidence is broken—when the rules of evidence are not followed—DNA samples lose their value in court.

Human Error and Sources of Contamination

One of the greatest threats to DNA evidence is human error. Earlier, we reviewed some of the precautions that crime scene investigators take to preserve and collect DNA evidence. A sneeze, improper storage, failure to label every single sample—these seemingly small mistakes can result in the destruction of evidence. Even if DNA evidence is not degraded by careless handling or bad conditions, it can be disregarded if the "chain of evidence" is suspect, as we saw in the example of the O. J. Simpson trial. The chain of evidence requires that the collection of evidence be systematically recorded, and access to the evidence must be controlled. Crime scene sample collection presents challenges, but even the collection of samples in a controlled environment, such as a morgue, is also problematic. Studies of morgue tables and instruments have found that the DNA of at least three individuals is often present. That DNA could certainly confuse the results of any analysis undertaken on samples collected in that environment.

DNA evidence is also vulnerable to damage during analysis (for example, DNA from the technician's body or from other sources can be inadvertently added to the sample). Defined standards of laboratory practice and procedure can help guard against these errors, however. When DNA evidence was a new idea, laboratories were not regulated and serious errors were made. Consider the case of David Butler, a taxi driver, who was accused of murdering a woman named Anne Marie Foy in 2005. A partial match of his DNA was found on the clothes and under the fingernails of the victim. Butler's lawyer, Michael Wolkind, argued that the procedure of analyzing the DNA under Foy's nails could not be trusted. Moreover, several scenarios explained Butler's DNA being found on Foy without him being her murderer. For instance, on the night of the murder, Foy had received change from Butler, who was suffering from a flaky skin condition that made him deposit an abnormal number of cells. Butler was acquitted eight months later. This case also proves that DNA match alone can no longer be considered adequate proof of a crime.

One step to ensure the reliability of DNA is to make certain that laboratories that process samples adhere to consistently high standards. The Singapore Accreditation Council (SAC), the European Accreditation Advisory Board (EAAB), and the National Accreditation Board for Testing and Calibration Laboratories (NABL) in India all provide accreditation to forensic laboratories. Proficiency testing of technicians has become a basic requirement for employment. These exams

include "blind" tests in which the technician is unaware that the sample being processed is actually a test sample submitted by the certifying organization. MI5, a security service in the UK, has developed a standard operating procedure for the handling and DNA typing of evidence in criminal cases. With clear guidelines in place and thorough training of those responsible for collecting and preserving evidence, the reliability of DNA evidence presented in court proceedings should increase.

DNA Forensics from "Touch DNA"

In addition to the characteristic ridges and whorls used in fingerprint analysis, fingermarks often contain **"touch DNA"** shed by the individual who made it. DNA can also be useful in cases in which only a partial or smudge fingermark is present. Unfortunately, the amount of DNA that can be isolated from a fingermark is usually very small, resulting in poor success rates (5 to 6 percent). In California in 2013, a man called Lukis Anderson was arrested, held for 4 months, and charged with murder after his DNA was found under the fingernails of a homicide victim. Anderson had never met the victim and was in the hospital when the man was killed. The same paramedics who took Anderson to the hospital responded to the murder. Most likely, the paramedics were covered in Anderson's DNA, which they then inadvertently transferred. The charges were dropped. The results highlight again that samples at crime scenes must be gathered with great care. DNA can remain on latex gloves, so they must be changed—or bleached—before and after handling evidence. Forensic science now has a new tool that is better than traditional swabbing for trace DNA. Using a wet vacuum collection system called the **M-Vac,** forensic analysts have pulled enough microscopic trace DNA to run a complete DNA profile that has been used in court cases. The technology works like a "mini-hurricane" of sterile solution combined with vacuum pressure. It sprays the solution onto the subject surface, and then sucks the wet traces back into a container. The solution and DNA material is then run through a filter or a microcentrifuge, and subsequently sequenced.

8.5 Familial Relationships and DNA Profiles

Crime solving is not the only forensic application of DNA fingerprinting. Since even distant family members have a small amount of DNA in common, relationships can be conclusively determined by comparing samples between two individuals. An obvious use of this is in paternity testing (**Figure 8.9**). Every year, roughly 400,000 paternity suits are filed in the United States, Canada, and the United Kingdom. Given samples from the child and adults involved, verifying the child's parentage and resolving child support or custody disputes are relatively easy. However, reproductive technologies—including *in vitro* fertilization, artificial insemination, and surrogacy—have introduced a few quirks into custody and paternity. DNA testing has helped untangle cases in the courts that involved sperm mixups in fertility

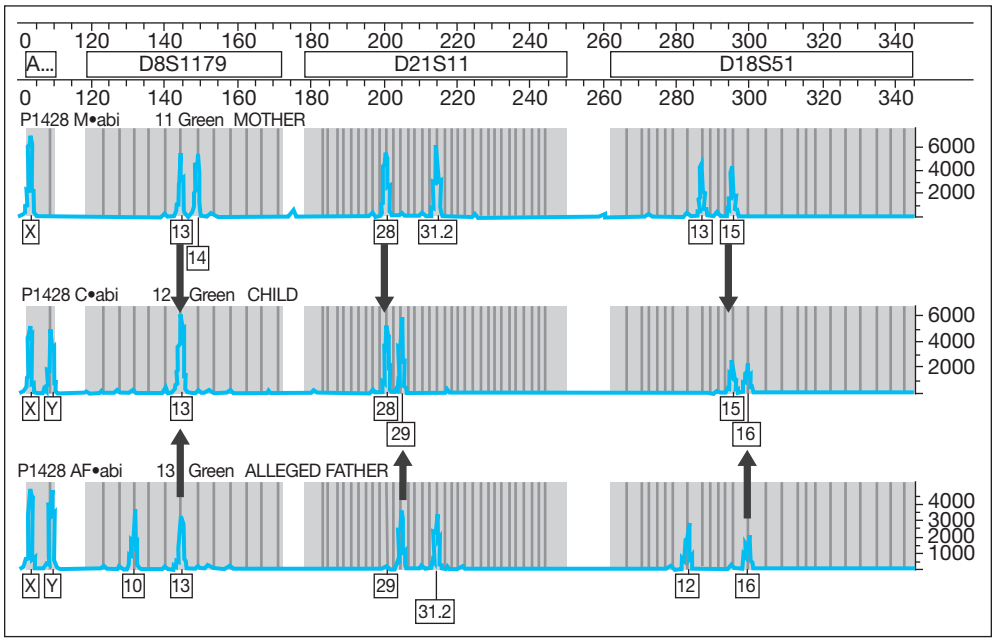


FIGURE 8.9 Paternity Dispute A man has claimed not to be the father of a child, but DNA allelic comparison shows that the child received one allele from each parent. Follow the arrows from the parents to the child to see how each parent contributed one of the two alleles (bands) that show in the DNA fingerprint of the child.

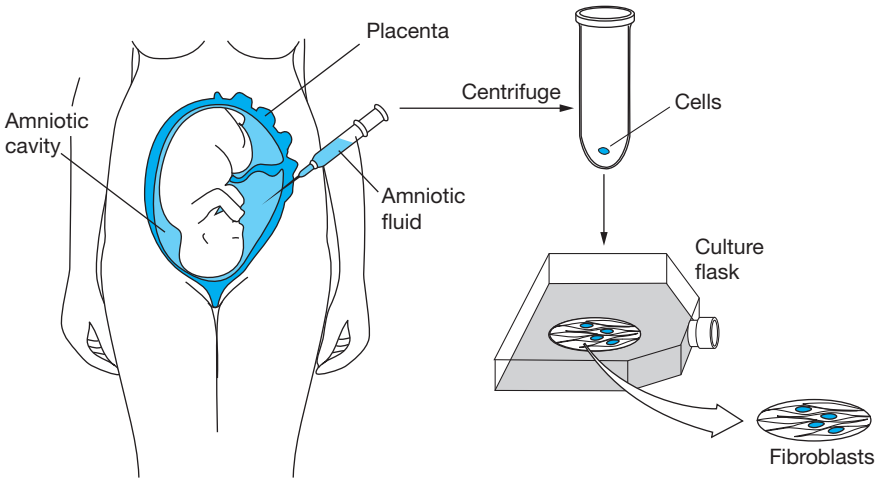


FIGURE 8.10 DNA for Paternity Testing For disputed paternity suits, it is possible to draw a few fetal cells from the fluid that surrounds the fetus (amniotic fluid) without harming the growing fetus. When cultured, these cells can be a source for DNA extraction and fingerprinting. When compared with the DNA of the suspected father, exclusion or inclusion of the individual can be determined.

clinics, for example. Thanks to amniocentesis, as shown in **Figure 8.10**, it is possible to verify a child's parentage even before birth.

Mitochondrial DNA Analysis

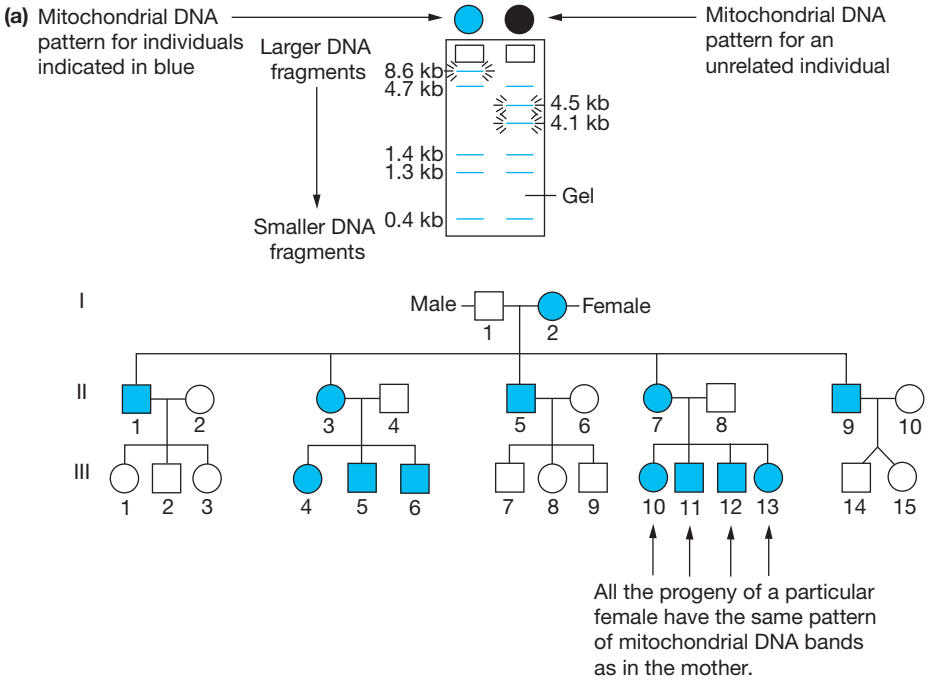
Mitochondrial DNA analysis can be used to examine DNA from samples that cannot be analyzed by RFLP or STR. Nuclear DNA must be extracted from samples for use in RFLP, PCR, and STR, but mtDNA analysis uses DNA extracted from a cellular organelle called a *mitochondrion*. The primary advantage of mtDNA is that it is present in high copy number within cells and numerically more likely to be recovered from highly degraded specimens. A major disadvantage to traditional forensic mtDNA analysis is that it is time-consuming to generate and review the 610 nucleotides of sequence information commonly targeted in hypervariable regions I and II (HVI and HVII) of the control region of the DNA (as seen in Figure 8.11). All daughters have the same mitochondrial DNA as their mothers because the mitochondria of embryos come from the mother's egg cell. The father's sperm contributes only nuclear DNA and no mitochondria. Comparing the mtDNA profile of unidentified remains with the profile of a potential maternal relative can determine whether they share the same mtDNA profile and are related. mtDNA remains virtually the same from generation to generation, changing only about 2 to 4 percent every million years due to random mutation. Consequently, relationships can be traced through the unbroken maternal line, as shown in **Figure 8.11**. The animation "Mitochondrial DNA and Human Disease" on the Companion Website may help to visualize this process.

mtDNA evidence was essential in reuniting families torn apart in Argentina during the Falklands War in the early 1980s. During this repressive regime, the military government routinely arrested and questioned anyone suspected of subversive activity. Among those arrested were a number of young pregnant women. Roughly 15,000 of those arrested and questioned simply disappeared. After the collapse of the government in 1983, the mothers and grandmothers of these "disappeared" women became aware that the horrible crime might well be ongoing: babies born to their daughters while in prison had been taken and were being raised by others—often by the families of those who had murdered their parents.

The mothers sought help from the American Association for the Advancement of Science (AAAS), asking if genetic testing could be used to prove the identity of the stolen children. Through the perseverance of these women and the scientists who volunteered their help, the AAAS was able to prove that at least 51 children had been illegally adopted. DNA evidence was used in court to restore the children to their living relatives.

Y-Chromosome Analysis

The Y chromosome is passed directly from father to son, making the analysis of genetic markers on the Y chromosome useful for tracing relationships among males or for analyzing biological evidence involving multiple male contributors. The Y chromosome can be analyzed with nuclear DNA (usually by PCR) methods. Profiles derived from STRs are used worldwide in paternity testing and individual identification. In complex cases of kinship analysis, autosomal single-nucleotide polymorphism (SNP), X-chromosomal short tandem repeat (X-STRs), and mitochondrial DNA (mtDNA) could be used to complement autosomal STR typing. The probability of kinship is magnified by using all of these tests combined, rather than relying on one individually.



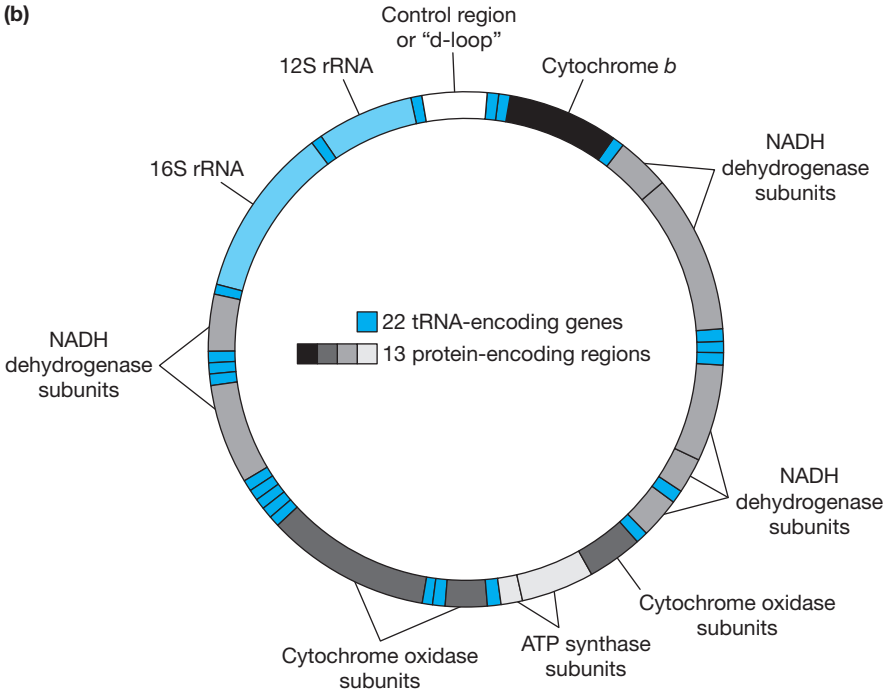


FIGURE 8.11 Maternal DNA Pattern of Inheritance Key genes for mitochondrial function (cell respiration) are located in a small DNA ring in human mitochondria. (a) Because mitochondria are contributed by the egg (only) before fertilization, DNA can be traced through the maternal line with fingerprinting of the mitochondrial DNA. Some genes in mitochondria show variations due to mutations (see top of gel at 8.6, 4.7 vs. 4.5, 4.1 kilobases); others do not. (b) Because mtDNA mutates faster at the third nucleotide, it can provide evidence of close and distant relationships.

8.6 Nonhuman DNA Analysis

Not every legal case is a matter of human identity. Many legal questions—as well as scientific quandaries—have been answered by examining the DNA fingerprints of plants and animals. Expensive plants, animals, and their biological products can be protected using their DNA profiles.

Identifying Plants through DNA

Ginseng is a valuable herbal product; the largest worldwide distributor of it is South Korea with an estimated distribution calculated to be approximately $649 million. Currently, two major herbal products are referred to as ginseng. One is native to North America and the other to Asia. Oddly, Asian ginseng is the type most often sold in American health food stores. Most



TOOLS OF THE TRADE The Verified Identity of King Richard III

A good example of the use of the new tools in forensic DNA science is the recent confirmation of the 529-year-old skeleton of King Richard III. An international research team led by Dr Turi King from the University of Leicester, Department of Genetics, provides overwhelming evidence that the skeleton discovered in Leicester, England, is that of King Richard III. The researchers collected DNA from living relatives of Richard III and analyzed several genetic markers, including the complete mitochondrial genomes, inherited through the maternal line, and Y-chromosomal markers, inherited through the paternal line, from both the skeletal remains and the living relatives. Although the Y-chromosomal markers differ (not direct parental lines), the mitochondrial genome shows a genetic match between the skeleton and the maternal line relatives. The researchers also used genetic markers to determine hair and eye color of Richard III and found probably blond hair and almost certainly blue eyes Richard III looked most similar to one of the earliest surviving portraits of him, found in the Society of Antiquaries in London. Adding this information to a wealth of existing material about Richard III highlights the ways in which forensic DNA methods of human remains can expand understanding of human history.

American ginseng—the rarer and more valuable of the two varieties—is exported to Asia. The two types of ginseng look almost identical, but they have very different reputations. Asian ginseng purportedly boosts energy, whereas American ginseng is prized for its supposed ability to calm nerves. Manufacturers are now using DNA sequencing to help make the distinction between the two varieties. Confirming the origin of the product helps ensure quality control and protects the market for American ginseng products.

This same type of analysis is performed for other plants as well. A DNA profile used in quality control of squash seeds is shown in **Figure 8.12.** This quality control process allows the seed supplier to guarantee a specific degree of genetic hybridity, thus increasing the seeds' value to growers, who depend on these traits to sell their high-value crops.

Protein Analysis May Join DNA Forensics

Lawrence Livermore National Lab Forensic Science Center has used mass spectrometry–based shotgun proteomics to characterize hair shaft proteins in 66 European-American subjects. People do not inherit proteins, but they do inherit the DNA that produces their protein. Protein is not only chemically more robust than DNA, but it also contains genetic variation in the form of single amino acid polymorphisms (SAPs). A total of 596 SNP alleles were correctly imputed in 32 loci from 22 genes of subjects' DNA and directly validated using Sanger sequencing. Currently, the sample preparation, instrument run time, and analysis period for the protein identification method requires about 2 1/2 days, and the team expects the cost to be competitive with that of other similar

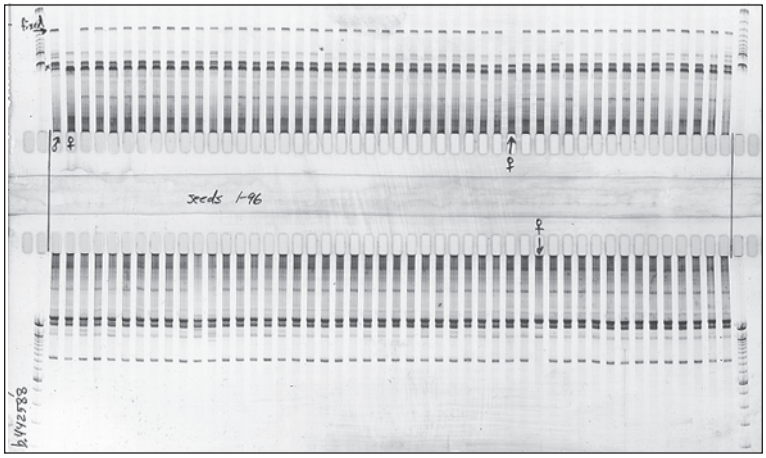


FIGURE 8.12 DNA Profile of Squash Hybrids Used as a Quality Control Analysis The anode is in the center of this gel with cathodes at the top and bottom. When the DNA from 96 seeds is loaded at the center of the gel, it will migrate to the cathodes during electrophoresis. The male parent plant donated the upper DNA band, and the female donated the lower of the two bands of the hybrid offspring plant being tested. The hybrid plants all have both bands (one exception gives 98.9 percent quality assurance of purity in 96 seeds tested). This level of hybridity (quality control) will increase the value of the seed to a grower depending on a high level of hybridity for the growing requirements.

technologies. If the technique is commercialized, it could be used in certain difficult cases.

Animal DNA Analysis

DNA evidence has also been used to generate genetic profiles of animals. For example, in Pennsylvania, DNA fingerprinting was used to prove that a hunter had killed a bear illegally. The bear-hunting season in that state is designed to protect pregnant sows. A law making it illegal to kill bears in their dens ensures that a large portion of the breeding female population will be protected from loss during the season. In this case, a witness reported seeing a hunter discharge his rifle into a bear's den. The hunter had registered his kill at the appropriate check station (without disclosing the circumstances under which it was shot), where one of the harvested bear's premolars was removed, as specified by state hunting regulations, to verify the sex and age of the kill. As part of their investigation of this eyewitness report, authorities collected blood samples from the den and compared the DNA from the den with that from the check station. The tests revealed that the DNA samples were from the same animal, even though the hunter, in an effort to evade prosecution, had claimed to have killed the bear 5 miles from the den. Because of the DNA evidence, he was found guilty.

DNA profiling is regularly conducted by wildlife management authorities in such cases. **Figure 8.13**, for example, shows DNA profiles for four deer that were killed by a hunter with a permit to kill only one deer. Armed with the tools of DNA fingerprinting, wildlife managers have improved their ability to prove that game has been taken beyond the limits set by regulations. The animation "Combing Through 'Fur' ensic evidence" on the Companion Website may provide interesting viewing of this process.

Food Fraud–A Recent DNA Forensics Application

Food fraud can obviously hurt our wallets, but for some, it can cause physical injury. A report issued in February 2013 by the ocean conservation group Oceana found in one study that fully one-third of the 1,215 fish samples tested from 21 different states were mislabeled and 54 percent of retail establishments the group tested were selling mislabeled fish. Fraud is not limited to seafood, either. In 2017, 597 cases of food fraud were reported on the Administrative Assistance and Cooperation System (AAC), which is managed by the European Union Food Fraud Network. Many of these cases involved vegetable oils, milk, honey, spices, and fruit juices. Researchers have a variety of methods to choose from when conducting food fraud investigations, but usually prefer PCR-based approaches. Most proteins in food samples can be degraded by high temperatures during cooking, processing, and packaging, compromising the effectiveness of typical protein analysis methods such as mass spectrometry and chromatography. But PCR looks at DNA, which is a more robust molecule capable of enduring high heat and remaining largely intact. For this reason, investigators will often sequence the PCR product and align the resulting sequence data with

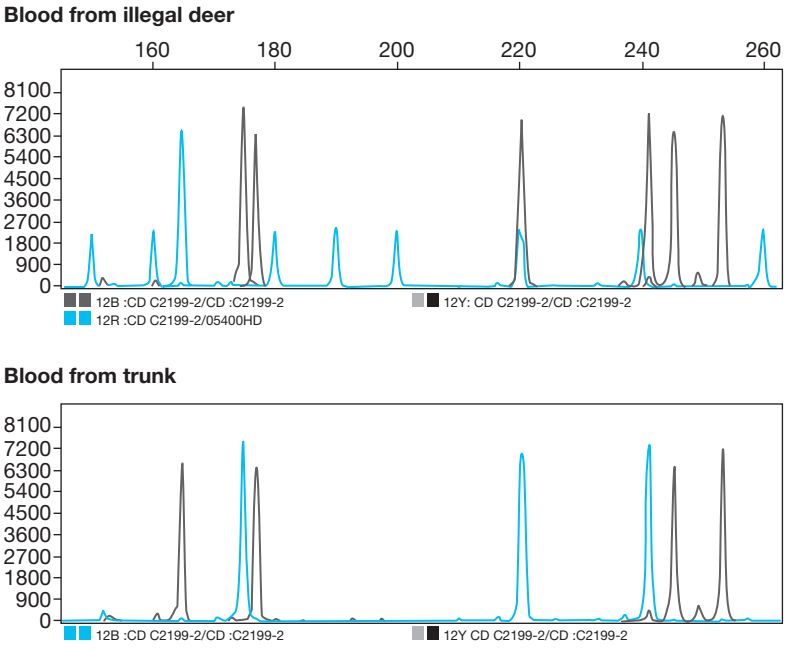


FIGURE 8.13 Validation of Microsatellite Evidence in a Deer Poaching Case Two deer (illegally shot) were found by the side of the road. A suspect had a blood stain (deer) in the trunk of his car. The DNA matched one of the deer, and the suspect was convicted, fined $2,000, and spent a year in jail. The microsatellite STRs of California mule deer had to be discovered to perform the analysis shown.

known species sequences in publicly available databases to unequivocally confirm the validity of a claim. Because PCR is an exponential process that can amplify a sequence up to a million-fold, it was ideal for working with such a tiny sample. Food allergies are increasingly prevalent, with responses ranging from asthma to anaphylactic shock and death.

Genetically Modified Organism (GMO) Testing

Whether or not a GMO is present in a sample can be tested by quantitative PCR (qPCR) and multiplex PCR. Multiplex PCR uses multiple, unique primer sets within a single PCR reaction to produce amplicons of varying sizes specific to different DNA sequences. Currently, it is unlikely that the presence of unexpected or even unknown GMOs will be detected, since either the DNA sequence of the transgene or its product, the protein, must be known for detection. In addition, even testing for known GMOs is time-consuming and costly, as current reliable detection methods can test for only one GMO at a time. The need for developing improved and alternative testing methods exists.

MAKING A DIFFERENCE

Next-generation sequencing (NGS) is an expanding form of DNA forensics. The FDA has recognized the Illumina MiSeqDx instrument platform and the Illumina Universal Kit reagents, two devices that make up the first FDA-regulated test system that allows laboratories and hospitals to develop and validate sequencing of any part of a patient's genome. The Universal Kit reagents isolate and create copies of genes of interest obtained from patient blood samples, and the MiSeqDx platform analyzes the genes. The software compares the patient's genomic sequence to a reference sequence and reports back any differences between the patient and the reference. The sequencing platform differs from the older Sanger method of sequencing by focusing on only specific genes, rather than the whole genome, reducing the time and cost of DNA sequencing. NGS technology does not require species- or transcript-specific probes. It can detect novel transcripts, gene fusions, single-nucleotide variants, indels (small insertions and deletions), and other previously unknown changes. The capacity to read and interpret large segments of DNA very quickly in a single test is being utilized for screening mutations related to cystic fibrosis and blood disorders. It represents a new form of forensic DNA analysis.

QUESTIONS & ACTIVITIES

Answers can be found in Appendix 1.

1. Suppose that you decided to get a gene scan for a genetic disease that has occurred in your family in the past. Will a complete DNA sequence be needed? Why?
2. How can touch DNA lead to complications in forensic crime scene studies?
3. How would you design a CDx PCR kit for patients expecting to undergo treatment for multiple myeloma?
4. Why is traditional swabbing for trace DNAs not as robust as wet vacuum system?
5. How is the polygenic risk score an improvement over the HER2+ screening for breast cancer?
6. Why do food fraud tests through PCR methods usually require very small amounts of DNA from the food sample?
7. How many STR bands found in a child's DNA should occur in the DNA fingerprint of the father? The mother?
8. What is the one downside of using PCR-based tests during DNA profiling?
9. How many errors can you count when you watch an episode of *CSI: Crime Scene Investigation*?
10. Give at least one example of how mtDNA evidence has been used in forensics.
11. How can identical twins have different genetics, since they derive from the same embryonic source?
12. Why do you think the European Network of Forensic Science Institutes (ENFSI) recommended to increase the number of genetic loci in the European Standard Set of DNA database?
13. How does a short tandem repeat function to produce a DNA fingerprint?
14. How could insurance companies benefit from genetic testing of women for breast cancer markers?
15. Why did forensic scientists primarily use STR, mtDNA, and SNP analysis of DNA fragments to develop profiles after the World Trade Center disaster, rather than standard DNA fingerprints of CODIS loci?
16. How would contamination potential limit the benefits from rapid DNA testing at police stations in the future?
17. How does the M-Vac system for touch fingerprinting reduce the contamination potential from current swap sampling?

18. How has mtDNA testing for paternal relationships been improved by adding other DNA tests?
19. Why is it unlikely that shotgun mass spec proteomics will be added to the current DNA profiling used for food fraud testing?
20. How has DNA profiling been extended to hospital testing for some diseases?

Visit www.pearsonglobaleditions.com for the Companion Website

The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

This case study has been included to give you an opportunity to become familiar with a research example applicable to this field of study. After you have analyzed the data and answered the questions, you can compare them to those provided to your instructor with the text materials.

CASE STUDY

Mouse Cell Line Contamination ID Using DNA Forensics

The scientific community has responded to the misidentification of human cell lines with validated methods to authenticate these cells, but few assays are available for nonhuman cell line identification. Researchers from the Biosystems and Biomaterials Science Division, National Institute of Standards and Technology, have developed a multiplex polymerase chain reaction assay that targets nine tetranucleotide short tandem repeat (STR) markers in the mouse genome. They developed unique DNA profiles from 72 mouse samples that were used to determine the allele distribution for each STR marker.

To detect human cell line contaminants, primers for two human STR markers were incorporated into the multiplex assay to facilitate detection of human and African green monkey DNA. This multiplex assay was the first of its kind (2014 publication) to provide a unique STR profile for each individual mouse sample and is now being used to authenticate mouse cell lines (and can be used to ID contamination in human samples). The power of discrimination is based on the probability that a random match (contamination) is 1 in 5.7 million using the nine STR markers in the multiplex assay.

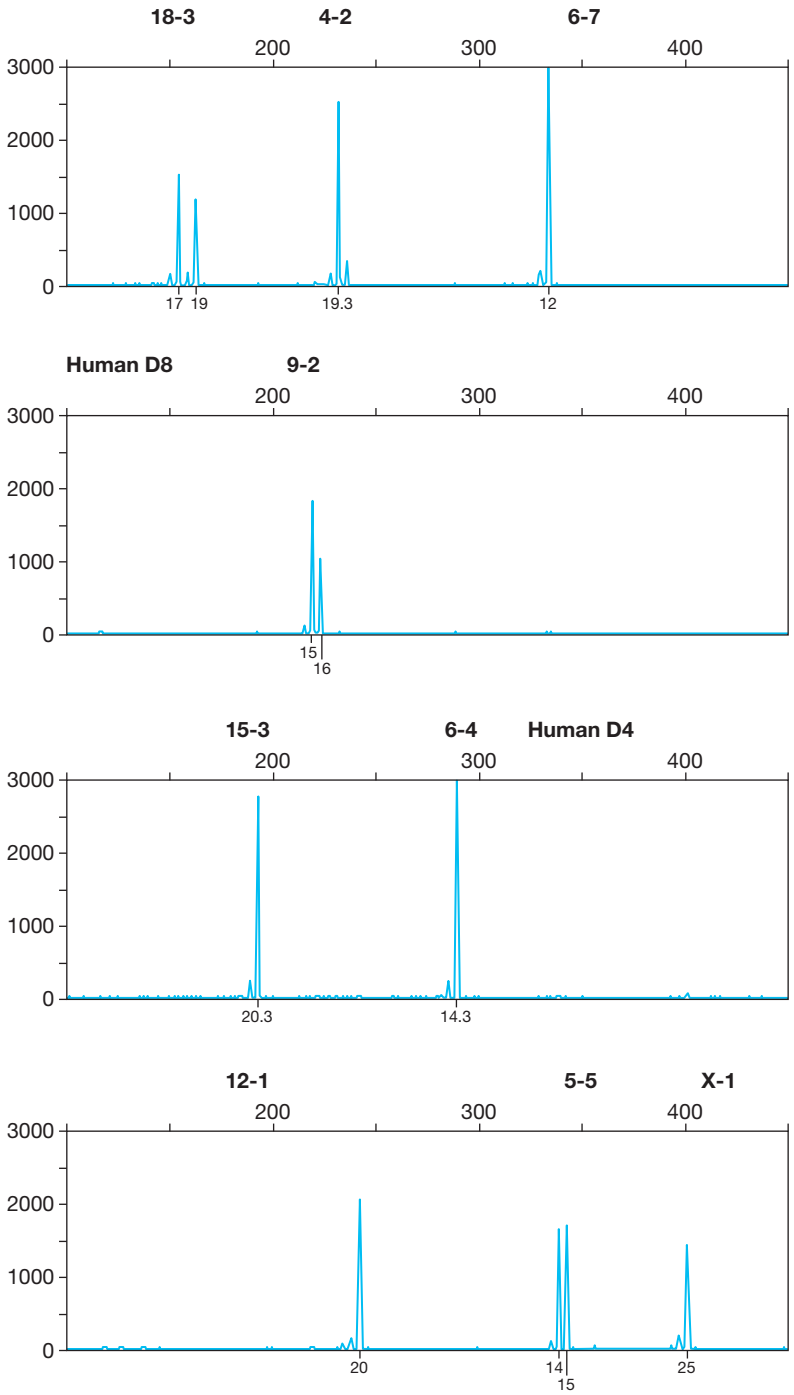
Analyze the following mouse STR profiles to answer the questions that follow.

Questions

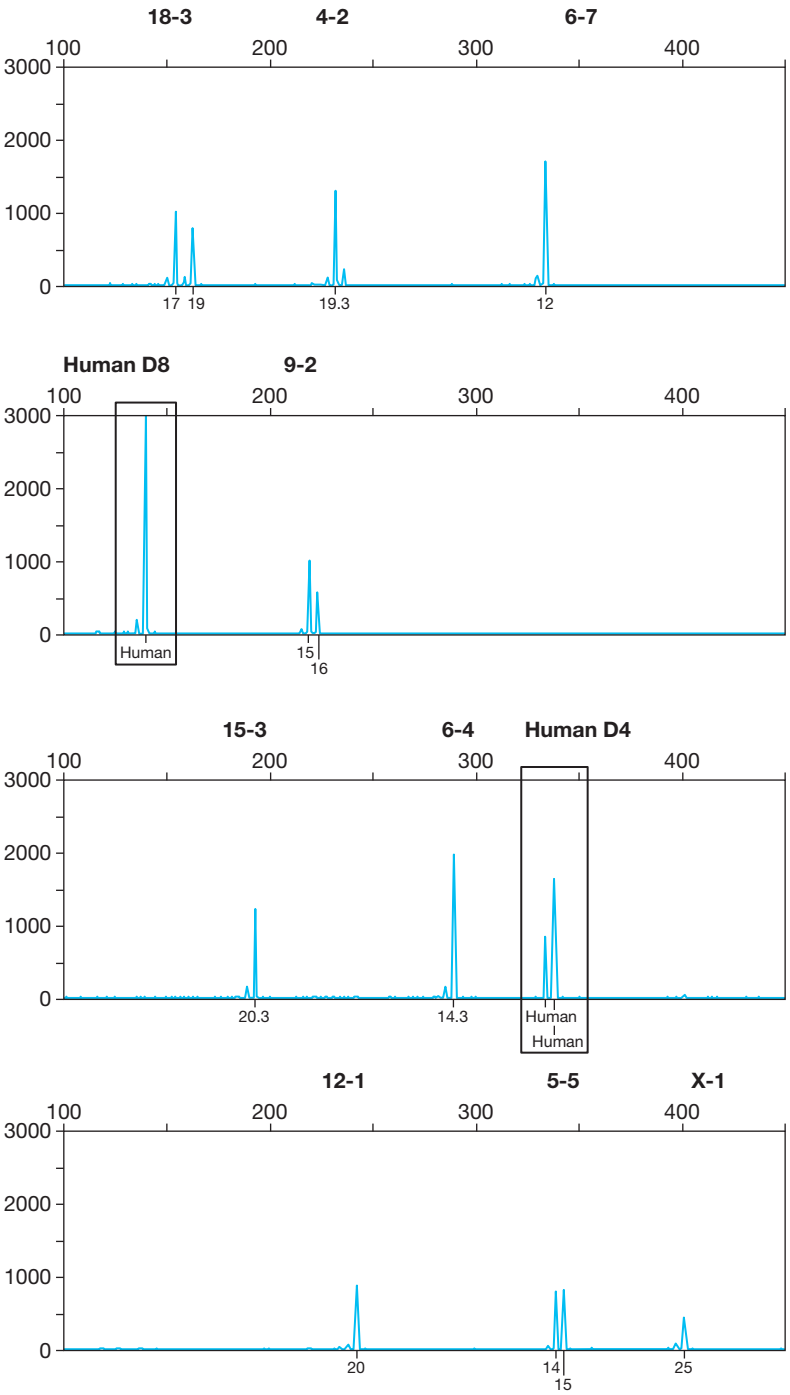
Answers can be found at www.pearsonglobaleditions.com

1. Do the STRs match for the mouse NIH3T3 for the two profiles shown? How do you explain the difference in height of the marker 12 in the two profiles?
2. Were the human STRs D4, D8 overlapping any mouse STRs? How did the choice assist detection?
3. How were the researchers able to say that the probability of a random match was 5.7 million?

Source: Almeida JL, Hill CR, Cole KD. Mouse cell line authentication. *Cytotechnology*. 2014;66(1):133–147. Published online February 22, 2013. doi:10.1007/s10616-013-9545-7.



Genetic profile of the NIH3T3 cell line using the mouse multiplex assay (1 ng DNA). Human D8, D4 locations are shown in the mouse profile for the presence of suspected STRs if human contamination should occur, as in the next profile.



Human contaminant detected in NIH3T3 STR profile (1:1 ratio of DNA from NIH3T3 and HeLa cell lines).

CHAPTER NINE

Bioremediation



Landfills around the world are overflowing with tons of garbage that are generated each day. Bioremediation has great potential for cleaning up chemicals in the environment, reducing waste in landfills, and even producing energy from society's garbage.

After completing this chapter, you should be able to:

- Explain what bioremediation is and describe why it is important.
- Describe advantages of bioremediation strategies over other types of cleanup approaches.
- Name common chemical pollutants that need to be cleaned up in different zones of the environment.
- Distinguish between aerobic and anaerobic biodegradation and provide examples of microbes that can contribute to bioremediation.
- Explain why studying genomes of organisms involved in bioremediation is an active area of research.
- Understand how applications of phytoremediation can be used to clean up the environment.
- Discuss how in situ and ex situ approaches can be used to bioremediate soil and groundwater.
- Provide examples of how genetically modified organisms can be used in bioremediation.
- Understand why scientists are interested in bioremediation applications for generating energy from wastes.
- Describe ongoing opportunities for new areas of bioremediation research and applications, including an understanding of challenges presented by macro- and microplastics in the environment.

Our environment is being threatened with alarming frequency. The air we breathe, the water we drink, and the soil we rely on to grow plants for food are contaminated as a direct result of human activities. On average, approximately 0.74 kg of municipal solid waste is generated per person per day worldwide, resulting in over 2 billion tons of waste produced every year. Globally, more than two-thirds of this waste is currently dumped (33 percent) or disposed of in the form of a landfill (37 percent). Only 19 percent is recovered through recycling and composting, and the remaining 11 percent is incinerated for ultimate disposal. However, as astounding as the numbers are, municipal solid waste is a relatively small part of the problem. Despite regulations to minimize pollution, pollution from industrial manufacturing wastes, chemical spills, household products, pesticides, and other sources has led to contamination of the environment in most of the industrialized world. An increasing number of toxic chemicals are presenting serious threats to the health of environments throughout the world and to the organisms that live there. In developing countries, the prevalence of environmental pollutants is an even greater problem.

Just as biotechnology is considered a key to identifying and solving human health problems, it is also a powerful tool for studying and correcting the poor health of polluted environments. In this chapter we consider how biotechnology can help solve some of our pollution problems and create cleaner environments for humans and wildlife through bioremediation.

FORECASTING THE FUTURE

There are many exciting potential future directions for bioremediation. Among the most likely areas of substantial progress will be global efforts in bioprospecting to find previously undiscovered metabolizing microbes and studying the genomics of these microbes to design pollution-degrading bacteria and plants. For example, in this chapter we discuss how scientists are analyzing oil-degrading microbes discovered in the Gulf of Mexico at the site of the *Deepwater Horizon* oil spill. Creating genetically engineered microbes and plants to degrade particularly toxic and harmful pollutants such as heavy metals and radioactive compounds will continue to be a focus of bioremediation, as will using bioremediation microbes as an energy source. Also, keep an eye out for applications of synthetic biology to artificially create new types of metabolizing microbes with synthetic genomes including genes customized for degrading specific pollutants.

9.1 What Is Bioremediation?

Bioremediation is the use of living organisms such as bacteria, fungi, and plants to break down, or degrade, chemical compounds in the environment. It takes advantage of natural chemical reactions and processes through which organisms break down compounds to obtain nutrients and derive energy. Bacteria, for example, metabolize sugars to make adenosine triphosphate (ATP) as an energy source for cells. But in addition to degrading natural compounds to obtain energy, many microbes have developed unique metabolic reactions that can be used to degrade human-made chemicals. Bioremediation cleans up environmental sites contaminated with pollutants by using living organisms to degrade hazardous materials into less toxic substances.

Bioremediation is not a new application. Humans have relied on biological processes to reduce waste materials for thousands of years. In the simplest sense, the outhouse—which relied on natural microbes in soil to degrade human wastes—was an example of bioremediation. Sewage treatment plants have used microbes to degrade human wastes for decades. Similarly, if you use composting at home or at school to decompose food scraps, leaves, lawn clippings, and other materials, into soil, you are also applying bioremediation. But as you will learn in this chapter, modern applications in bioremediation involve a variety of new and innovative strategies to clean up a wide range of toxic chemicals in many different environmental settings.

Taking advantage of what many microbes already do is only one aspect of bioremediation. One of its key purposes is to improve natural mechanisms and increase rates of biodegradation to accelerate cleanup processes. In this chapter, we explore some of the ways in which scientists can stimulate microbes and other organisms to degrade a variety of wastes in many different situations. Another important aspect of bioremediation is the development of new approaches for the biodegradation of waste materials in the environment, which can involve using plants and genetically modified microorganisms.

Why Is Bioremediation Important?

Our quality of life is directly related to the cleanliness and health of the environment. Pollution can affect human health in many ways. We know that environmental chemicals can influence our genetics and that some chemicals can act as mutagens, leading to human disease conditions. Clearly, there is reason to be concerned about both short- and long-term chemical exposure and the effects of environmental chemicals on humans and other organisms.

According to some estimates, about 600 million tons of hazardous materials are produced each year around the world. Accidental chemical spills can and do occur, but these events typically are contained and cleaned up rapidly to minimize their impact on the environment. More problematic, however, are illegal dumping practices and sites contaminated through

neglect, such as abandoned warehouses, where stored chemicals may leak into the environment. In 1980, the U.S. Congress established the **Superfund Program** as an initiative of the **U.S. Environmental Protection Agency (EPA)** to counteract careless and even negligent practices of chemical dumping and storage as well as to express concern over how these pollutants might affect human health and the environment. The primary purpose of the Superfund Program is to locate and clean up hazardous waste sites to protect U.S. citizens from contaminated areas. To learn more about the Superfund Program, visit the Superfund website, which is listed on the Companion Website.

A recent survey conducted in China revealed that about 16 percent of the soil samples collected from over 1500 surveyed areas were polluted by a range of organic and inorganic substances. In 2016, the State Council of China passed a Soil Pollution Prevention and Control Action Plan to deal with this. The plan is poised to remediate 95 percent polluted farmland soil, making it safe for human use by 2030. Although the Plan is based on the polluter-pays-principle, which is the underlying mechanism of the Superfund program, the implementation of any long-term remediation strategies must deal with significant economic challenges. Estimates for the remediation costs of the currently identified polluted farmland in China are over $1.1 trillion. Waste management and pollution control have become pressing issues in not just China but almost all Asian countries.

Through the National Institute of Environmental Health Sciences, a division of the National Institutes of Health, the United States started a program called the **Environmental Genome Project.** A primary purpose of this project is to study and understand the impacts of environmental chemicals on human disease. This includes studying genes that are sensitive to environmental agents, learning more about detoxification genes, and identifying single-nucleotide polymorphisms (SNPs) that may be indicators of environmental exposures and impacts on human health. Genome data from projects such as this enable scientists to carry out epidemiological studies that will help them to better understand not only how the environment contributes to disease risk but also how specific diseases are influenced by environmental exposure to chemicals.

However, there are many ways to clean up pollutants, so why use bioremediation? We could physically remove contaminated material such as soil or chemically treat polluted areas, but these processes can be very expensive and, in the case of chemical treatments, can create more pollutants that themselves require cleanup. A major advantage of bioremediation is that most approaches convert harmful pollutants into relatively harmless materials such as carbon dioxide, chloride, water, and simple organic molecules. Because living organisms are used for the cleanup, bioremediation processes are generally cleaner than other types of cleanup strategies.

Another advantage of bioremediation is that many cleanup approaches can be conducted at the site of pollution. This is called **in situ bioremediation.** Because the contaminated materials do not need to be transported to another site, a more complete cleanup is often possible without disturbing the environment. In the next section, we consider some of the basic principles of bioremediation in terms of common chemical pollutants and the environments they pollute. We also discuss some of the microbes and reactions that are important for bioremediation.

9.2 Bioremediation Basics

Naturally occurring marshes and wetlands have excelled at bioremediation for hundreds of years. In these environments, plant life and microbes can absorb and degrade a wide variety of chemicals and convert pollutants into harmless products. Before we discuss how living organisms can degrade pollutants, we will first consider areas of the environment that require cleanup and take a look at some of the common chemicals that pollute the environment.

What Needs to Be Cleaned Up?

Unfortunate as it may be, the answer to this question is that almost everything needs to be cleaned up. Soil, water, and sediment (a combination of soil and decaying plant and animal life located at the bottom of a body of water) are the most common treatment environments that require cleanup by bioremediation, although new bioremediation approaches are being developed to detect and clean up air pollution. Each area presents its own complexities for cleanup because the type of bioremediation approach used typically depends on site conditions. For instance, it should not surprise you that approaches for cleaning up soil can be very different from those used to clean up water. Similarly, surface water often needs to be treated differently than groundwater (water underground in soil or in spaces around rock).

Pollutants enter the environment in different ways and affect diverse components of the environment. In some cases, pollutants enter the environment through a tanker spill, a truck accident, or a ruptured chemical tank at an industrial site. Of course, depending on the location of the accident, the amount of chemicals released, and the duration of the spill (hours versus weeks or years), different parts or zones of the environment may be affected. **Figure 9.1** provides an example of a leaking chemical tank at an industrial plant. It may initially contaminate only surface and subsurface soils;

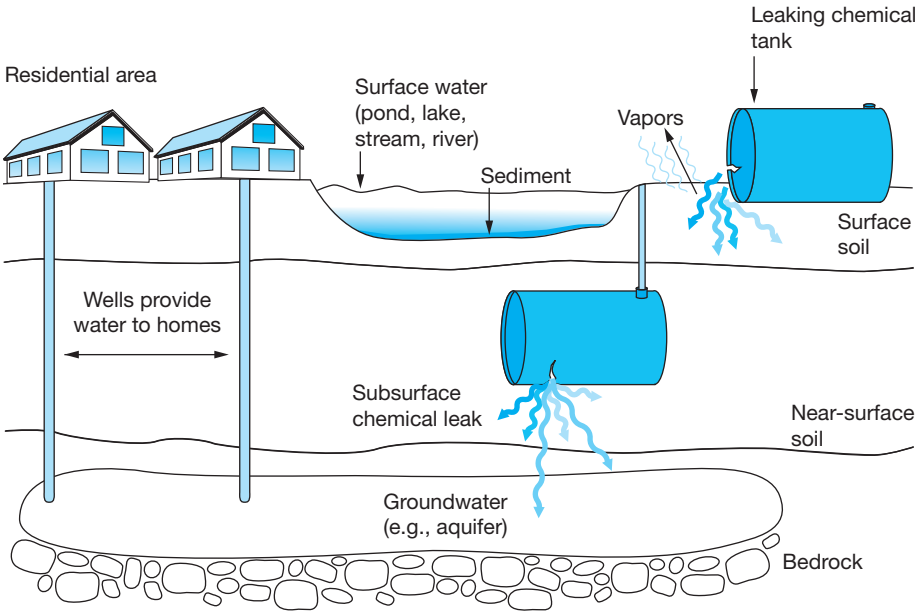


FIGURE 9.1 Treatment Environments and Contamination Zones Chemical spills can create a number of treatment environments and zones of contamination that may be targets for bioremediation. A spill from a leaking chemical tank, which may be located above the ground or below the surface, can release materials that contaminate surface soil, subsurface soil, surface water, and groundwater. In this example, pollution of the aquifer threatens the health of individuals living in houses adjacent to the spill, who rely on the aquifer as a source of drinking water.

however, if large amounts of chemicals are released and the leaky tank goes undetected for a long time, chemicals may move deeper into the soil. Following heavy rains, these same chemicals may create runoff that can contaminate adjacent surface water supplies such as ponds, lakes, streams, and rivers. Chemicals may also leak through the ground, creating **leachate.** Leachate can cause contamination of groundwater, including aquifers—deep pockets of underground water that are a common source of drinking water.

Of course, chemicals also enter the environment through the release of pollutants into the air, which can become trapped in clouds and contaminate surface water and then the groundwater when it rains. This is what causes **acid rain,** for example. Pollutants from industrial manufacturing, landfills, illegal dumps, pesticides used for agriculture, and mining processes also contribute to environmental pollution. As mentioned earlier, because the bioremediation approach used to clean up pollution depends on the treatment environment, cleaning up soil is very different from cleaning up water. But how bioremediation is used also depends on the types of chemicals that need to be cleaned up.

Chemicals in the Environment

Techniques for using bioremediation to degrade human wastes at a sewage treatment plant are quite different (and somewhat simpler) than those used to degrade the variety of chemicals that exist in the environment. Everyday household materials such as cleaning agents, detergents, perfumes, caffeine, insect repellents, pesticides, fertilizers, perfumes, and medicines appear in our wastewater. Increasingly, researchers are also finding that U.S. waterways contain prescription and over-the-counter drugs, including contraceptives, painkillers, antibiotics, cholesterol-lowering drugs, antidepressants, anticonvulsants, and anticancer drugs. Other chemicals that make their way into the environment are the products of industrial manufacturing processes or, as discussed earlier, the result of accidents.

Numerous chemicals from many different sources are common pollutants in the environment. Table 9.1 lists some of the most common categories of chemicals in our environment that require cleanup. Many of these chemicals are known to be potential mutagens and **carcinogens**—compounds that cause cancer. Although we do not discuss the health effects of chemical pollutants in any detail, most of these chemicals are known to cause illnesses, including, for example, asthma, skin rashes and other forms of inflammation, birth defects, weakening of the immune system, and different types of cancer, and they can poison animal and plant life.

One category of environmental pollutants gaining more attention comprises the **endocrine disruptors**—compounds that interfere with natural hormones produced by the body. From Table 9.1 dichloro-diphenyl-trichloroethane (DDT), polychlorinated biphenyls (PCBs), and synthetic estrogens are all endocrine disruptors. Endocrine disruptors are known to affect the health of humans and aquatic species in particular (see **Figure 9.2**). Birth defects in reptiles and amphibians such as extra limbs in frogs as shown in Figure 9.2, as well as sex reversal of fish, are among examples scientists hypothesize are correlated with exposure to endocrine disruptors. Quite

TABLE 9.1 Common Chemical Pollutants in the Environment

Chemical Pollutant	Source
Benzene	Petroleum products used to make plastics, nylon, resins, rubber, detergents, and many other materials
Chromium	Electroplating, leather tanning, corrosion protection
Creosote	Wood preservative to prevent rotting
Cyanide	Mining processes and manufacturing of plastics and metals
Dioxin	Pulp and paper bleaching, waste incineration, and chemical manufacturing processes
Methyl *t*-butyl ether (MTBE)	Fuel additive, automobile exhaust, boat engines, leaking gasoline tanks
Naphthalene	Product of crude oil and petroleum
Nitriles	Rubber compounds, plastics, and oils
Perchloroethylene/tetrachloroethylene (PCE), trichloroethylene (TCE), and trichloroethane (TCA)	Dry-cleaning chemicals and degreasing agents; TCE is present in some 34% of U.S. water supplies and 60% of Superfund sites
Perfluorochemicals (PFCs)	Teflon surfaces, water and fire-resistant textiles, fire-fighting foams
Pesticides (atrazine, carbamates, chlordane, dichloro-diphenyl-trichloroethane (DDT) and herbicides	Chemicals used to kill insects (pesticides) and weeds (herbicides)
Phenol and related compounds (chlorophenols)	Wood preservatives, paints, glues, textiles
Polychlorinated biphenyls (PCBs)	Electrical transistors, cooling and insulating systems
Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated hydrocarbons	Incineration of wastes, automobile exhaust, oil refineries, and leaking oil from cars
Polyvinylchloride	Plastic manufacturing
Radioactive compounds	Research and medical institutions and nuclear power plants
Surfactants (detergents)	Manufacturing of paints, textiles, concrete, paper
Synthetic estrogens (ethinyl estradiol)	Female hormone (estrogen)–related compounds created by a variety of industrial manufacturing processes
Toluene	Petroleum component present in adhesive, inks, paints, cleaners, and glues
Trace metals (arsenic, cadmium, chromium, copper, lead, mercury, silver)	Car batteries and metal manufacturing processes
Trinitrotoluene (TNT)	Explosive used in building and construction industries

simply, the presence of pollutants in an environment leads to an overall decline in the environment along with the health of the organisms living within it. In Section 9.7 we discuss plastic products as endocrine disruptors.

For some of the chemicals listed in Table 9.1, regulations have been in place to limit or ban their applications, but even after their use completely stops, often these chemicals persist in the environment for decades. In addition to the type of spill and the cleanup environment, the type of chemical pollutant also affects the types of cleanup organisms and approaches that can be used for bioremediation. Throughout this chapter we consider strategies for cleaning up many of the pollutants listed in Table 9.1.



FIGURE 9.2 Endocrine Disruptors Present a Significant Hazard for Aquatic Organisms and Humans

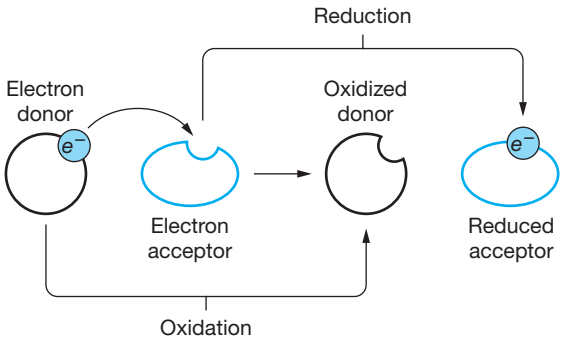


FIGURE 9.3 Oxidation and Reduction (Redox) Reactions Redox reactions are important for the bioremediation of many chemicals. In this oxidation reaction, an electron is transferred from molecule A to molecule B. Molecule B is acting as an electron acceptor or oxidizing agent. In redox reactions, molecule A is oxidized, and molecule B, which gains an electron, is reduced.

Fundamentals of Cleanup Reactions

Microbes can convert many chemicals into harmless compounds through either **aerobic metabolism** (reactions that require oxygen [O_2]) or **anaerobic metabolism** (reactions in which oxygen is not required). Both types of processes involve *oxidation* and *reduction reactions*. You should have a basic familiarity with oxidation and reduction reactions if you are to understand biodegradation.

Oxidation and reduction reactions

Oxidation involves the removal of one or more electrons from an atom or molecule, whereas during **reduction,** an atom or molecule gains one or more electrons. Both reactions can alter the structure and properties of a molecule. In the case of a chemical pollutant, oxidation or reduction can make the chemical harmless by changing its properties. Because oxidation and reduction reactions frequently occur together, these electron transfer reactions are often called **redox reactions** (see Figure 9.3).

During redox reactions, molecules called **oxidizing agents**—also known as electron acceptors, because they have a strong attraction for electrons—remove electrons during the transfer process. When oxidizing agents accept electrons, they become reduced. Oxygen (O_2), iron (Fe^{3+}), sulfate (SO_4^{2-}), and nitrate (NO_3^-) are often involved in redox reactions of bioremediation. Redox reactions are important for many cellular functions. For example, human cells and many other cell types use oxidation and reduction reactions to degrade sugars and derive energy.

Aerobic and anaerobic biodegradation

In some environments, such as surface water and soil where oxygen is readily available, aerobic bacteria degrade pollutants by oxidizing chemical compounds. In aerobic biodegradation reactions, O_2 can oxidize a variety of chemicals including **organic molecules** (those that contain carbon atoms), such as petroleum products (Figure 9.4). In the process, O_2 is reduced to produce water. Microbes can further degrade the oxidized organic compound to make simpler and relatively harmless molecules such as carbon dioxide (CO_2) and methane gas. Bacteria derive energy from this process, which is used to make more cells; scientists refer to this increase

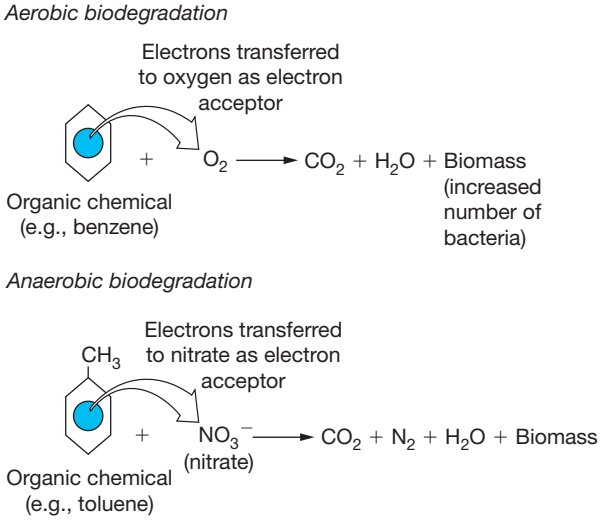


FIGURE 9.4 Aerobic and Anaerobic Biodegradation Aerobic bacteria (aerobes) use oxygen (O_2) as an electron acceptor molecule to oxidize organic chemical pollutants such as benzene. During this process, oxygen is reduced to produce water (H_2O), and carbon dioxide (CO_2) is derived from the oxidation of benzene. Energy from degrading the pollutant is used to stimulate an increase in bacterial cells (biomass). Similar reactions occur during anaerobic biodegradation, except that anaerobic bacteria (anaerobes) rely on iron (Fe^{3+}), sulfate (SO_4^{2-}), nitrate (NO_3^-), and other molecules as electron acceptors to oxidize pollutants.

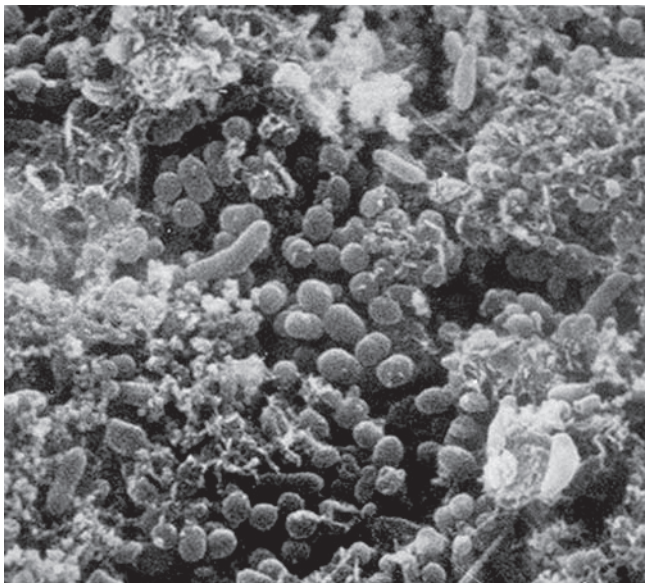


FIGURE 9.5 Anaerobic Bacteria Degrading Dry-Cleaning Chemical The dry-cleaning chemical called perchloroethylene (PCE) is a common contaminant of groundwater; however, anaerobic bacteria can use PCE as food. By growing bacteria on small particles of iron sulfide, which serve as electron acceptors providing the proper chemical environment for anaerobes, bacteria grow rapidly and thrive on PCE.

in cell number as an increase in **biomass.** Some aerobes also oxidize **inorganic compounds** (molecules that do not contain carbon), such as metals and ammonia.

In heavily contaminated sites and deep subsurface environments such as aquifers, the concentration of oxygen may be very low. In subsurface soils, oxygen may diffuse poorly into the ground, and any oxygen that is there may be rapidly consumed by aerobes. Even though it is sometimes possible to inject oxygen into treatment sites to stimulate aerobic biodegradation in low-oxygen environments, biodegradation may take place naturally via anaerobic metabolism (**Figure 9.5**). Anaerobic metabolism also requires oxidation and reduction; however, anaerobic bacteria (anaerobes) rely on molecules other than oxygen as electron acceptors. Iron (Fe^{3+}), sulfate (SO_4^{2-}), and nitrate (NO_3^-) are common electron acceptors for redox reactions in anaerobes (Figure 9.4). Some microbes can carry out both aerobic and anaerobic metabolism. When the amount of oxygen in the environment decreases, they can switch to anaerobic metabolism to continue biodegradation. As you will learn in the next section, aerobes and anaerobes are both important for bioremediation.

The Players: Metabolizing Microbes

Scientists can use microbes—especially bacteria—as tools to clean up the environment. The ability of bacteria to degrade different chemicals effectively depends on many conditions. The type of chemical, temperature, zone of contamination (water versus soil, surface versus groundwater contamination, and so on), nutrients, and many other factors all influence the effectiveness and rates of biodegradation.

At many sites, bioremediation involves the combined actions of both aerobic and anaerobic bacteria to decontaminate the site fully. Anaerobes usually dominate biodegradation reactions that are closest to the source of contamination, where oxygen tends to be very scarce, but sulfates, nitrates, iron, and methane are present for use as electron acceptors by anaerobes. Farther from the source of contamination, where oxygen tends to be more abundant, aerobic bacteria are typically involved in biodegradation (**Figure 9.6**).

The search for useful microorganisms for bioremediation is often best carried out at polluted sites themselves. Often some organisms living in a polluted site will develop resistance to the polluting chemicals and may be useful for bioremediation. **Indigenous microbes**—those found naturally at a polluted site—are often isolated, grown, and studied in a lab and then released back into treatment environments in large numbers. Such microbes are typically the most common and effective "metabolizing" microbes for bioremediation. For instance, strains of bacteria called *Pseudomonas*, which are very abundant in most soils, are known to degrade hundreds of different chemicals. Certain strains of *Escherichia coli* are also fairly effective at degrading many pollutants.

A large number of lesser-known bacteria have been used and are currently being studied for potential roles in bioremediation. For instance, in Section 9.7 we discuss possible applications of *Deinococcus radiodurans*, a microbe that shows an extraordinary ability to tolerate the hazardous effects of radiation. Similarly, researchers at the University of Dublin discovered *Pseudomonas putida,* a strain of bacteria that can convert styrene, a toxic component of many plastics, into a biodegradable plastic.

Scientists believe that many of the microbes that are most effective at bioremediation have not yet been identified. The quest for new metabolizing microbes is an active area of bioremediation research. In 2015, Rutgers University scientists reported the isolation of a bacterial strain in the Betaproteobacteria class from soil of an old uranium ore mill in Colorado. Through unknown processes, this strain reduces uranium to a form that no longer dissolves in water. Keeping uranium out of surface and groundwater would be a major advantage over trying to remove uranium from water supplies, especially sources that are used for drinking water. Scientists are also experimenting with strains of algae and fungi that may be capable of biodegradation. Waste-degrading fungi such as *Phanerochaete chrysosporium* and *Phanerochaete sordida* can metabolize toxic chemicals such as creosote, pentachlorophenol, and other pollutants that bacteria degrade poorly or not at

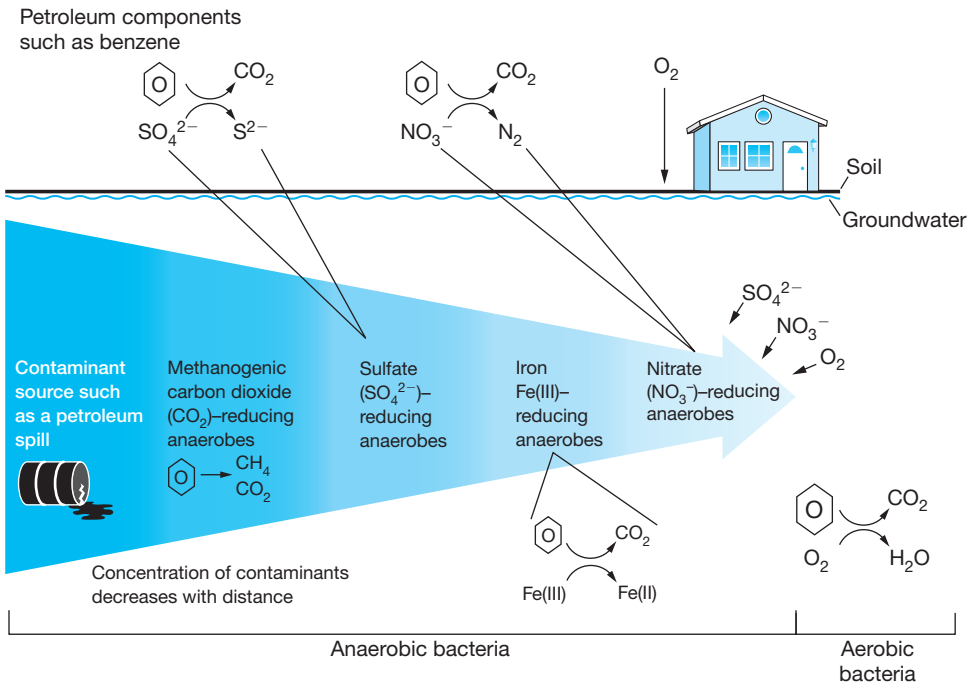


FIGURE 9.6 Anaerobic and Aerobic Bacteria Contribute to Biodegradation of Contaminated Groundwater

all. Asbestos and heavy metal–transforming fungi include *Fusarium oxysporum* and *Mortierella hyalina*. Fungi are also very valuable in composting and degrading sewage and sludge at solid-waste and wastewater treatment plants, PCBs, and other compounds previously thought to be highly resistant to biodegradation. Also, in Chapter 10, we will briefly consider how aquatic organisms can be used for bioremediation.

Bioremediation Genomics Programs

It should not surprise you to learn that many scientists are studying the genomes of organisms that are currently used or may be used in the future for bioremediation. Through genomics, it will be possible to identify novel genes and metabolic pathways that bioremediation organisms use to detoxify chemicals. Scientists are sequencing genomes for entire communities of microorganisms and identifying new genes on chromosomes and in plasmids that encoded enzymes involved in bioremediation. This information will help scientists develop more effective cleanup strategies, including improved strains of bioremediation organisms through genetic engineering (see Section 9.5). It may also be possible to combine detoxifying genes from different microbes into different recombinant bacteria capable of degrading multiple contaminants at the same time—for example, PCBs and mercury. See Table 9.2 for a few examples of completed genomics projects involving bioremediation organisms. Be sure to visit the Genomes to Life link on the Companion Website, which you can use to access genomics studies of bioremediation microbes.

Stimulating bioremediation

As discussed previously, bioremediation scientists typically take advantage of indigenous microbes to degrade pollutants. Depending on the pollutant, many indigenous bacteria are very effective at biodegradation. Scientists also use numerous strategies to make microorganisms more effective in degrading contaminants, depending on the microorganisms involved, the environmental site being cleaned up, and the quantities and types of chemical pollutants that need to be decontaminated.

Nutrient enrichment, also called **fertilization,** is a bioremediation approach in which fertilizers—similar to the phosphorus and nitrogen that are applied to grass on lawns—are added to a contaminated environment to stimulate the growth of indigenous microorganisms that can degrade pollutants (**Figure 9.7**). Because living organisms need an abundance of key elements such as carbon, hydrogen, nitrogen, oxygen, and phosphorus for building macromolecules, adding fertilizers provides bioremediation microbes with essential elements to reproduce and thrive. In some instances, manure, wood chips, and straw may be added to provide microbes with sources of carbon as a fertilizer. Fertilizers are usually delivered to the contaminated site by pumping them into groundwater or mixing them in the soil. The concept behind fertilization is simple. By adding more nutrients, microorganisms replicate, increase in number

TABLE 9.2 Examples of Genome Projects Completed for Bioremediation Organisms

Microorganism	Number of Genes	Bioremediation Applications
Accumulibacter phosphatis	4,790	Major microbe used in wastewater treatment plants for removing high phosphate loads from wastewaters and sludge
Alcanivorax borkumensis	2,755	Hydrocarbon-degrading marine bacterium very effective at breaking down many components of crude and refined oil
Dechloromonas aromatica	4,250	Facultative anaerobe bacterium isolated from Potomac River sludge; anaerobically degrades benzene
Dehalobacter restrictus	2,826	Dechlorinates perchloroethylene (PCE) and trichloroethene
Dehalococcoides ethenogenes	1,591	Used to degrade hydrogen and chlorine—the only known organism to fully dechlorinate perchloroethylene (PCE) and trichloroethene (TCE); PCE and TCE cleanup in wastewaters; polychlorinated dioxin degradation
Deinococcus radiodurans	3,212	Bacterium that can tolerate exposure to radiation; of significant interest for bioremediation of radioactive wastes (see Section 9.7)
Geobacter metallireducens	3,676	Used for subsurface metal reduction, carbon cycling, and to generate electricity (see Figure 9.16 on page 255)
Paracoccus denitrificans	3,744	Identified from coal mine tailings in India; potential for bioremediation of various industrial pollutants through denitrification
Populus trichocarpa (poplar tree)	45,555	First tree genome sequenced (2006); potentially useful for reducing atmospheric carbon dioxide

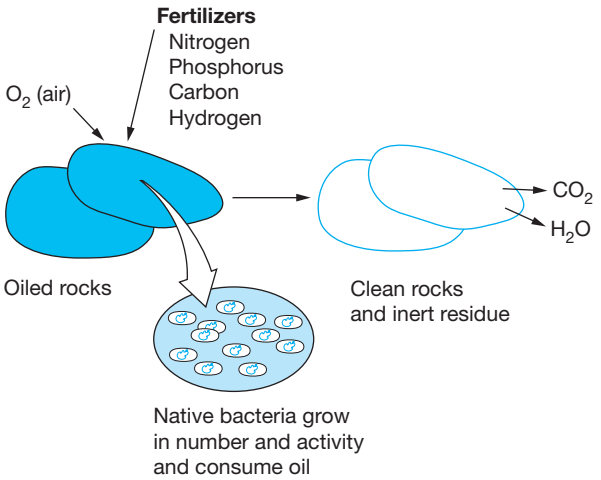


FIGURE 9.7 Fertilizers Can Stimulate Biodegradation by Indigenous Bacteria Bioremediation of chemicals such as those present in oil can be accelerated by adding fertilizers. Fertilizers stimulate the growth and replication of indigenous bacteria, which degrade oil into inert (harmless) compounds such as carbon dioxide (CO_2) and water (H_2O).

(biomass), and grow rapidly, thus increasing the rate of biodegradation.

Bioaugmentation, or **seeding,** is another approach that involves adding bacteria to the contaminated environment to assist indigenous microbes with biodegradative processes. Sometimes seeding involves applying genetically engineered microorganisms with unique biodegradation properties. Bioaugmentation is not always an effective solution, in part because laboratory strains of microbes rarely grow and biodegrade as well as indigenous bacteria, and scientists must be sure that seeded bacteria will not alter the ecology of the environment if they persist after the contamination is gone.

Phytoremediation

A growing number of applications are utilizing plants to clean up chemicals in the soil, water, and air—an approach called **phytoremediation.** An estimated 350 species of plants naturally take up toxic materials. Poplar and juniper trees have been successfully used in phytoremediation, as have certain grasses and alfalfa. In phytoremediation, chemical pollutants are taken in through the roots of the plant as they absorb contaminated water from the ground (**Figure 9.8**). As an example, sunflower plants effectively removed radioactive cesium and strontium from ponds at the Chernobyl nuclear power plant in Ukraine. Water hyacinths have been used to remove arsenic from water supplies in Bangladesh. This is a significant technology, considering that arsenic concentrations exceed health standards in 60 percent of the groundwater in Bangladesh.

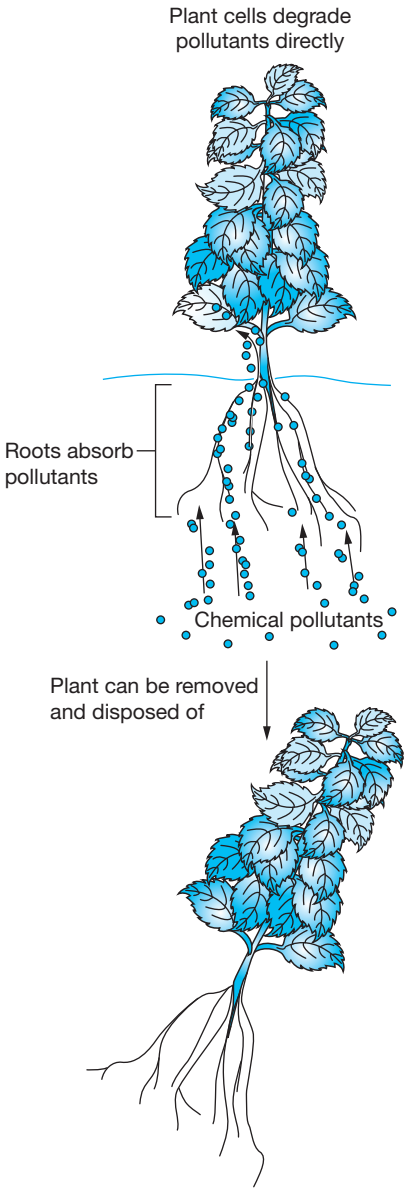


FIGURE 9.8 Phytoremediation Plants can be a valuable addition to many bioremediation strategies. Some plants degrade environmental pollutants directly; others simply absorb pollutants and must later be removed and disposed of.

After toxic chemicals enter the plant, the plant cells may use enzymes to degrade the chemicals. In other cases, the chemical concentrates in the plant cells so that the entire plant serves as a type of "plant sponge" for mopping up pollutants. For example, applications of aquatic ferns for quickly soaking up oil at accidental spills have shown potential. Leaves of these plants are very water repellent but they can absorb large volumes of oil, and they also grow quickly.

Sometimes contaminated plants are treated as waste and may be burned or disposed of in other ways. Because high concentrations of pollutants often kill most plants, phytoremediation tends to work best where the amount of contamination is low—in shallow soils or groundwater.

Scientists are also exploring ways in which plants can be used to clean up pollutants in the air, something many plants do naturally: for example, removing excess carbon dioxide (CO_2), the principal greenhouse gas released from burning fossil fuels, which contributes to global warming. The first genome for a tree, the black cottonwood (a type of poplar), was sequenced in 2006. Poplars are commonly used for phytoremediation, and genetically engineered poplars have shown promise for capturing high levels of CO_2. Scientists have harvested TCE-degrading bacteria from black poplars and transferred these microbes to young trees prior to planing them at TCE-contaminated Superfund sites. Microbe-enriched trees grew trunks nearly 30 percent wider than untreated trees indicating healthy growth by these trees in contaminated soil. Three years later, soil around the microbe-enriched trees had 50 percent more chlorine ions (harmless by-products of TCE degradation) than untreated trees.

Phytoremediation can be an effective, low-cost, and low-maintenance approach for bioremediation. As an added benefit, phytoremediation can also be a less obvious and more eye-appealing strategy. For instance, planting trees and bushes can visually improve the appearance of a polluted landscape and clean the environment at the same time. Currently, however, most phytoremediation approaches still involve removing soil from the polluted site for treatment at another site. Two main drawbacks of phytoremediation are that only surface layers (to around 50 cm deep) can be treated, and cleanup typically takes several years. In the next section, we examine specific cleanup environments and different strategies used for bioremediation.

9.3 Cleanup Sites and Strategies

A variety of bioremediation treatment strategies exist. Which strategy is used depends on many factors. Of primary consideration are the types of chemicals involved, the treatment environment, and the size of the area to be cleaned up. Consequently, some of the following questions must be considered before starting the cleanup process:

- Do the chemicals pose a fire or explosive hazard?
- Do the chemicals pose a threat to human health, including the health of cleanup workers?
- Was the chemical released into the environment through a single incident, or was there long-term leakage from a storage container?

- Where did the contamination occur?
- Is the contaminated area at the surface of the soil? Below the ground? Does it affect water?
- How large is the contaminated area?

Answering these questions often requires the combined talents of molecular biologists, environmental engineers, chemists, and other scientists who work together to develop and implement cleanup plans.

Soil Cleanup

Treatment strategies for both soil and water usually involve either removing chemical materials from the contaminated site to another location for treatment, an approach known as **ex situ bioremediation,** or cleaning up at the contaminated site without excavation or removal, called **in situ** (a Latin term that means "in place") **bioremediation.** In situ bioremediation is often the preferred method of bioremediation, in part because it is usually less expensive than ex situ approaches. Also, because the soil or water does not have to be excavated or pumped out of the site, larger areas of contaminated soil can be treated at one time. In situ approaches rely on stimulating microorganisms in the contaminated soil or water. Those in situ approaches that require aerobic degradation methods often involve **bioventing,** or pumping either air or hydrogen peroxide (H_2O_2) into the contaminated soil. Hydrogen peroxide is frequently used because it is easily degraded into water and oxygen to provide microbes with a source of oxygen. Fertilizers may also be added to the soil through bioventing to stimulate the growth and degrading activities of indigenous bacteria.

In situ bioremediation is not always the best solution. This approach is most effective in sandy soils, which are less compact and allow microorganisms and fertilizing materials to spread rapidly. Solid clay and dense rocky soils are not typically good sites for in situ bioremediation, and contamination with chemicals that persist for long periods can take years to clean up in this way.

For some soil cleanup sites, ex situ bioremediation can be faster and more effective than in situ approaches. As shown in **Figure 9.9**, ex situ bioremediation of soil can involve several different techniques, depending on the type and amount of soil to be treated and the chemicals to be cleaned up. One common ex situ technique is called **slurry-phase bioremediation.** This approach involves moving contaminated soil to another site and then mixing the soil with water and fertilizers (oxygen is often also added) in large bioreactors, where the conditions of biodegradation by microorganisms in the soil can be carefully monitored and controlled. Slurry-phase bioremediation is a rapid process that works fairly well when small amounts of soil need to be cleaned and the composition of chemical pollutants is well known.

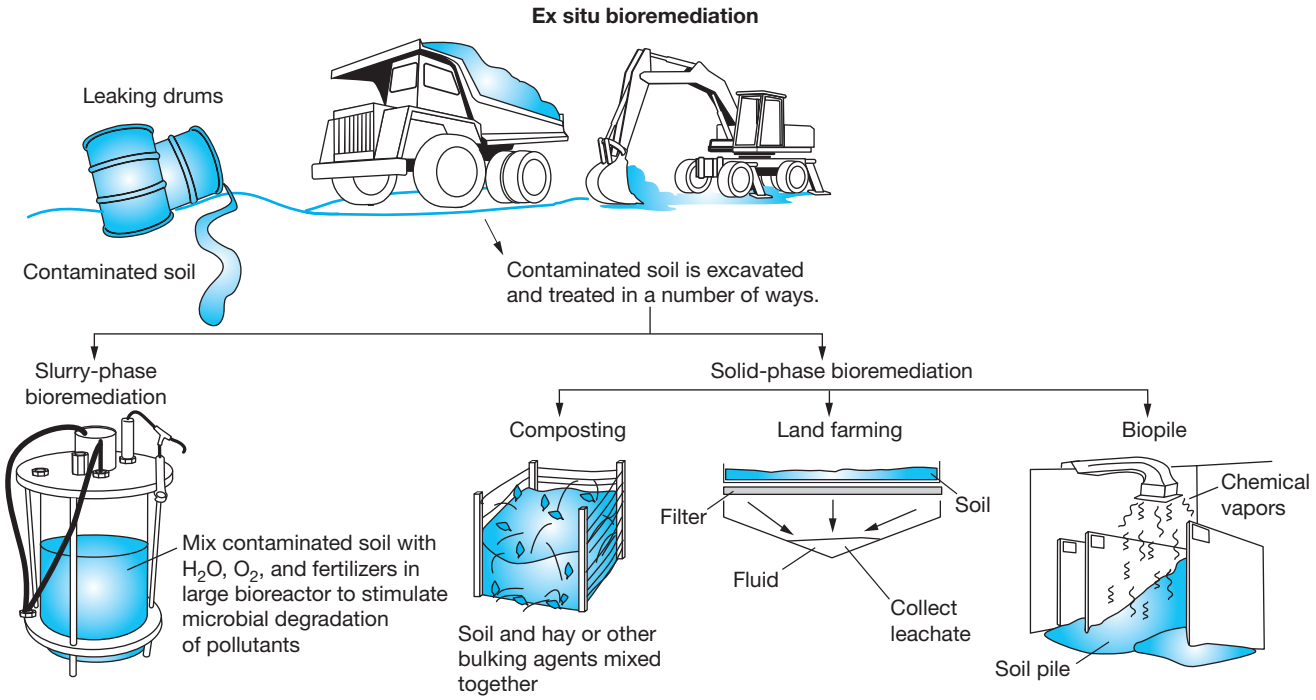


FIGURE 9.9 Ex Situ Bioremediation Strategies for Soil Cleanup Many soil cleanup approaches involve ex situ bioremediation, in which contaminated soil is removed and then subjected to several different cleanup approaches.

For many other soil cleanup strategies, **solid-phase bioremediation** techniques are required. Solid-phase processes are more time-consuming than slurry-phase approaches and typically require large amounts of space; however, they are often the best strategies for degrading certain chemicals. Three solid-phase techniques are widely used: composting, land farming, and biopiles.

Composting can be used to degrade household wastes such as food scraps and grass clippings; similar approaches are used to degrade chemical pollutants in contaminated soil. In a compost pile, hay, straw, or other materials are added to the soil to provide bacteria with nutrients that help bacteria degrade chemicals. **Land farming** strategies involve spreading contaminated soils on a pad so that water and leachates can leak out of the soil. A primary goal of this approach is to collect leachate so that polluted water cannot further contaminate the environment. Because the polluted soil is spread out in a thinner layer than it would be if it were below the ground, land farming also allows chemicals to vaporize from the soil and aerates the soil so that microbes can better degrade pollutants.

Soil **biopiles** are used particularly when the chemicals in the soil are known to evaporate easily and microbes in the soil pile are rapidly degrading the pollutants (Figure 9.10). In this approach, contaminated soil is piled up several meters high. Biopiles differ from compost piles in that relatively few bulking agents are added to the soil and fans and piping systems are used to pump air into or over the pile. As chemicals in the pile evaporate, the vacuum airflow pulls the chemical vapors away from the pile and either releases them into the atmosphere or traps them in filters for disposal, depending on the type of chemical. Almost all ex situ strategies for cleaning up soil involve tilling and mixing soil to disperse nutrients, oxygenating the dirt, and increasing interaction of microbes with contaminated materials to increase biodegradation.



FIGURE 9.10 Soil Biopiles Polluted soil that has been removed from the cleanup site can be stored in piles and bioremediation processes monitored to ensure decontamination of the soil before determining whether the soil can be returned to the environment.

Bioremediation of Water

Contaminated water presents a number of challenges. In Section 9.6, we consider how surface water can be treated following large spills, such as an oil spill. Wastewater and groundwater can be treated in many different ways, depending on the pollutants that need to be removed.

Wastewater treatment

Probably the best-known application of bioremediation is wastewater (or sewage) treatment to remove human sewage (fecal material and paper wastes), soaps, detergents, and other household chemicals. Both septic systems and municipal wastewater treatment plants rely on bioremediation. In a typical septic system, human sewage and wastewater from a single household move through the plumbing system out to a septic tank buried below ground next to the house. In the tank, solid materials such as feces and paper wastes settle to the bottom, to be degraded by microbes, while liquids flow out of the top of the tank and are dispersed underground across an area of soil and gravel called the septic bed. Within the bed, indigenous microbes degrade waste components in the water.

One commercial application of bioremediation recommended to prevent septic tanks from becoming clogged is to add products such as Rid-X (Figure 9.11), which are flushed into the system periodically. These products contain freeze-dried bacteria that are rich in enzymes such as lipases, proteases, amylases, and cellulases, which in turn degrade fats, proteins, sugars, and cellulose in papers and vegetable matter, respectively. Adding microbes this way is an example of the bioaugmentation we discussed in the previous section, and this treatment helps the septic system degrade cooking fats and oils, human wastes, tissue paper products, and other materials that can clog the system.

Wastewater treatment plants are fairly complex and well-organized operations, and now more than 400,000 plants are used throughout the world (Figure 9.12). Water from households that enters sewer lines is pumped into a treatment facility, where feces and paper products are ground and filtered into smaller particles, which settle out into tanks to create a mud-like material called **sludge.** Water flowing out of these tanks is called **effluent.** Effluent is sent to



FIGURE 9.11 Septic Tank Additives Stimulate Bioremediation of Household Wastes

aerating tanks, where aerobic bacteria and other microbes oxidize organic materials in the effluent. In these tanks, water is sprayed over rocks or plastic covered with biofilms of waste-degrading microbes that actively degrade organic materials in the water.

Alternatively, effluent is passed into activated sludge systems—tanks that contain large numbers of waste-degrading microbes grown in carefully controlled environments. Usually these microbes float freely within the water, but in some cases they may be grown on filters through which contaminated water flows. Eventually effluent is disinfected with a chlorine treatment before the water is released back into rivers or oceans so it can become part of the water cycle again.

Sludge is pumped into anaerobic digester tanks in which anaerobic bacteria further degrade it. Methane-producing and carbon dioxide gas–producing bacteria are common in these tanks. Methane gas is often collected and used as fuel to run equipment at the sewage treatment plant. Tiny worms, which are usually

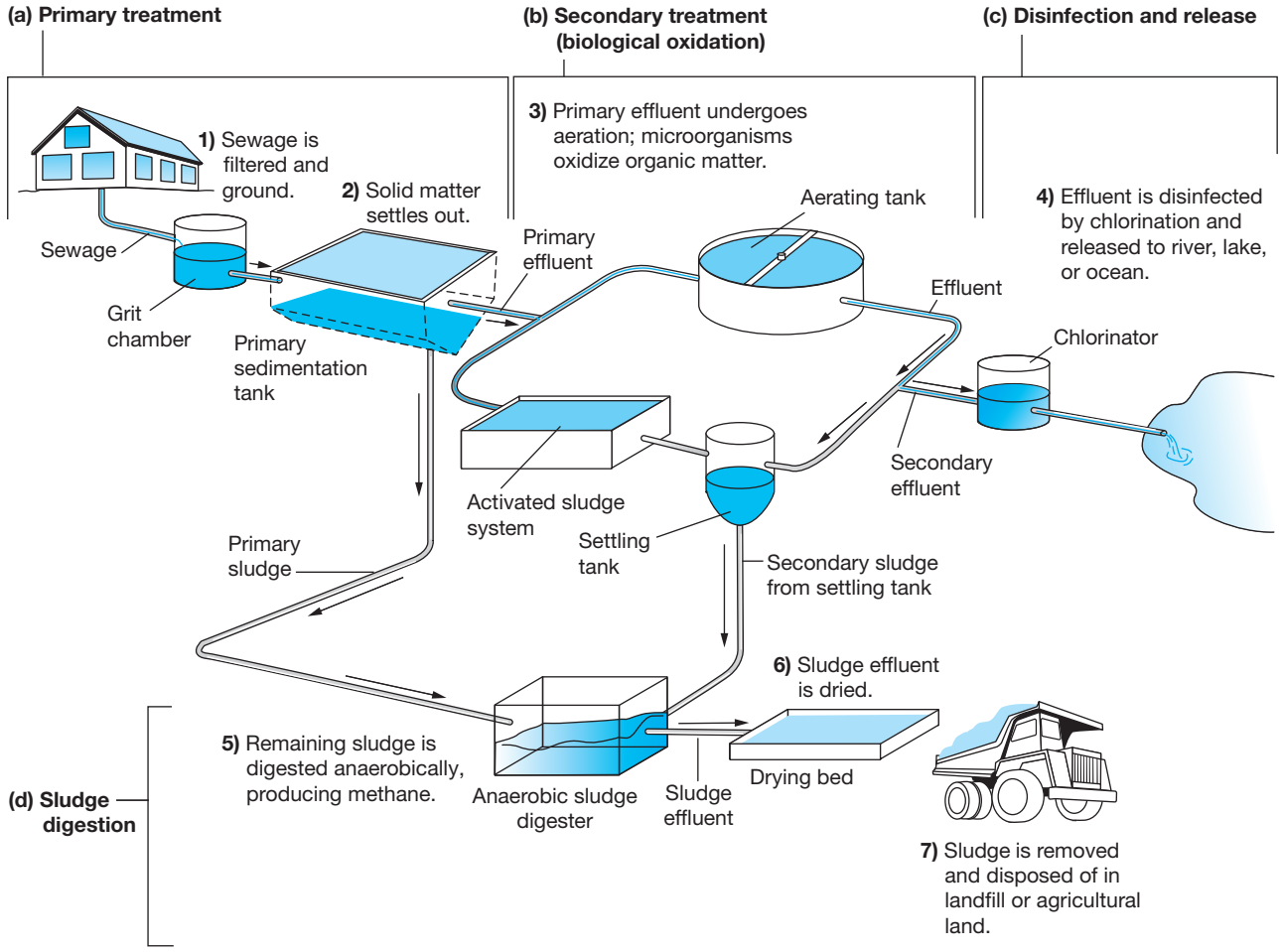


FIGURE 9.12 Wastewater Treatment Wastewater or sewage treatment facilities are well-planned operations that use aerobic and anaerobic bacteria to degrade organic materials such as human feces and household detergents in both the sludge and water (effluent).

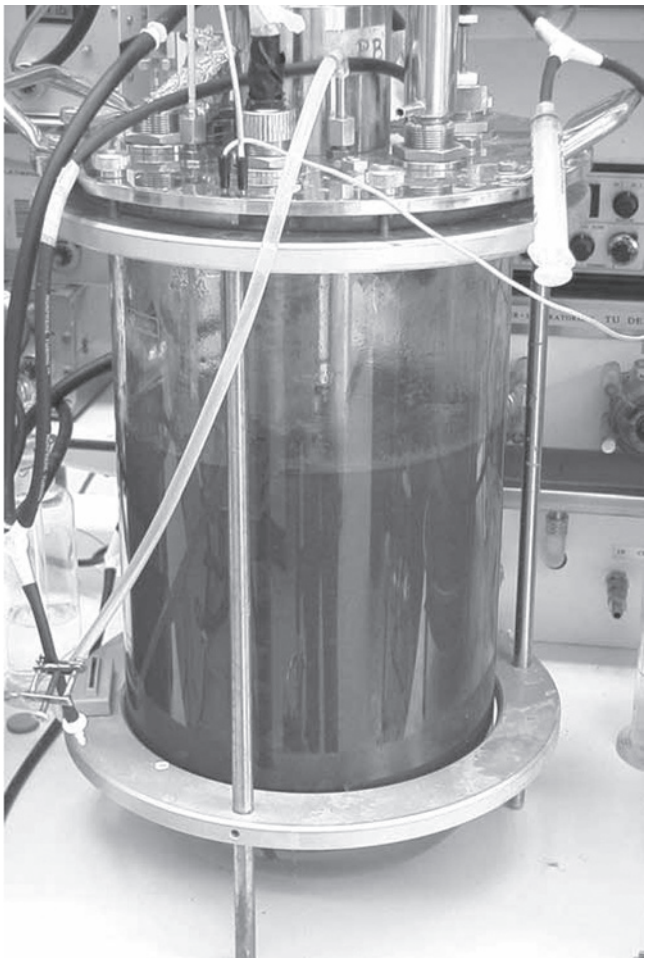


FIGURE 9.13 Bioreactor Containing *Candidatus Brocadia anammoxidans*, Anaerobic Bacterium That Can Degrade Ammonium Novel metabolic properties enable these anaerobes to degrade ammonium from wastewater in a single step.

present in the sludge, also help break down the sludge into small particles. Sludge is never fully broken down, but once the toxic materials have been removed, it is dried and can be used as landfill or fertilizer.

Scientists have discovered a bacterium called *Candidatus Brocadia anammoxidans* that possesses a unique ability to degrade ammonium, a major waste product present in urine (**Figure 9.13**). Removing ammonium from wastewater before the water is released back into the environment is important because high amounts of ammonium can affect the environment by causing algal blooms and diminishing oxygen concentrations in waterways. Typically, wastewater plants rely on aerobic bacteria such as *Nitrosomonas europaea* to oxidize ammonium in a multistep set of reactions. However, *Candidatus Brocadia anammoxidans* is capable of degrading ammonium into nitrogen gas in a single step under anaerobic conditions, a process called anammox (short for anaerobic ammonia oxidation). Wastewater treatment plants in many countries have started to use *Candidatus Brocadia anammoxidans* to remove ammonium from wastewater more efficiently.

Because of the large volumes of water processed daily around the world through wastewater treatment plants, scientists are interested in trying to recover valuable chemicals from wastewater. This includes forms of carbon, nitrogen, and phosphorus—all of which could be recycled as fertilizers—in addition to valuable metals, as we will discuss in Section 9.7.

Groundwater cleanup

With the exception of accidents near coastal beaches, most large-volume chemical spills, such as oil spills, occur in marine environments, often far from heavily populated areas. However, freshwater pollution typically occurs closer to populated areas and poses a serious threat to human health by contaminating sources of drinking water, either groundwater or surface water such as reservoirs. Groundwater contamination is a common problem in many countries. In India, nearly 80 percent of the rural and 50 percent of the urban drinking water supply rely on groundwater sources. However, a large part of Indian groundwater supplies contains elevated concentrations of inorganic pollutants (such as fluoride and arsenic) and organic pollutants (such as pesticides). In China, 70 percent of drinking water comes from groundwater sources, and studies have indicated that over 60 percent of China's groundwater supplies contain pollutants that may affect human health. Polluted groundwater can be very challenging to clean because many pollutants tightly adsorb on to low-permeable soil matrices.

Ex situ and in situ approaches are often used in combination. For instance, when groundwater is contaminated by oil or gasoline, these pollutants rise to the surface of the aquifer. Some of this oil or gas can be directly pumped out, but the portion mixed with groundwater must be pumped to the surface and passed through a large-volume bioreactor (**Figure 9.14**). Inside the bioreactor, bacteria in biofilms growing over a screen or mesh degrade the pollutants. Fertilizers such as wood chips and oxygen are often added to the bioreactor. Clean water from the bioreactor containing fertilizer, bacteria, and oxygen is pumped back into the aquifer for in situ bioremediation (Figure 9.14).

In some cases, large-volume bioreactors can be made fairly simply by digging trenches or pits, which become colonized by indigenous bacteria from the soil. This approach can be used on farms to reduce nitrogen pollution from farm animal waste and overfertilization and to prevent pollution of local ponds, streams, and lakes, which can cause algal blooms and fish kills. The trenches are filled with wood chips as

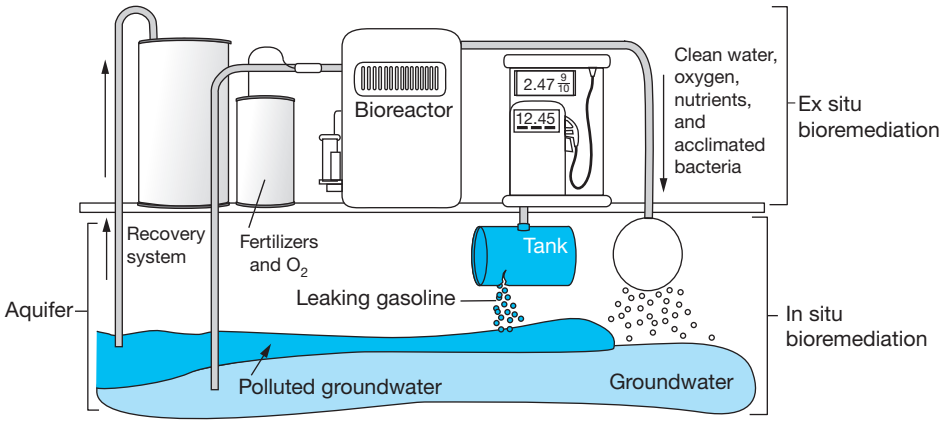


FIGURE 9.14 Ex situ and in situ Bioremediation of Groundwater Gasoline leaking into an underground water supply can be cleaned up using an above ground (ex situ) system in combination with in situ bioremediation.



TOOLS OF THE TRADE Microcosms Provide Major Benefits

Bioremediation scientists are working on innovative ways to biodegrade different chemical compounds under a myriad of environmental conditions. Industry is continually creating new kinds of chemicals, and many of these will inevitably make their way into the environment. To stay one step ahead of new environmental pollutants, bioremediation researchers must continue to develop new cleanup strategies.

How can scientists study bioremediation of a new chemical that has never made it into the environment? They obviously cannot pollute large areas or wait for a large-scale disaster like the *Deepwater Horizon* oil spill (discussed in Section 9.6) before testing their theories about how to clean up this new chemical. One of the most practical approaches to learning about new bioremediation strategies is to make a **microcosm,** an artificially constructed test environment designed to mimic real-life environmental circumstances. Some microcosms consist of small bioreactors—about the size of a 5-gallon bucket—containing soil, water, pollutants, and microbes to be tested for their bioremediation abilities. A microcosm may be as small as a few grams of soil in a test tube or vial, but they are more often scaled up to resemble larger environments. For example, large ponds, which may be indoors or outdoors, or soil plots that are prepared to prevent the escape of pollutants outside the test facility, can be used as microcosms. By carefully designing microcosms, bioremediation researchers can attempt to simulate, on a small scale, a site that needs to be cleaned up.

When indigenous or genetically engineered organisms with bioremediation potential have been identified, studies in bioreactors or on small isolated areas of land or water can be crucial for determining whether these organisms will effectively clean up pollution in larger settings. By carefully manipulating the environmental conditions in the microcosms, scientists can test the ability of organisms to degrade different pollutants under varying conditions, including different levels of moisture, temperature, nutrients, oxygen, and pH and in different soil types. Often *microbial consortia*, communities of two or more different microorganisms cultured together and that often cannot be grown individually, may be used to test the degradation of large organic compounds such as PCBs. Thus microcosm experiments allow scientists to test which combinations of symbiotic organisms are best suited for degrading particular pollutants.

Sometimes microcosms may involve testing experimental microbes on polluted groundwater or contaminated soil that is placed into the microcosm so that the rates of degradation can be monitored and the cleanup time evaluated. Scientists can also carry out experiments to study the bioremediation of mixtures of pollutants at the same time.

In an attempt to produce the best cleanup results, scientists will analyze the data they have gathered and design new experiments. A cleanup approach that demonstrates success in a microcosm is not guaranteed to succeed in the field. Nevertheless, by testing bioremediation approaches in microcosms, much valuable time and money can be saved before deciding whether a cleanup approach is likely to have any chance of success in cleaning up a polluted environment in the field.

fertilizer and then surface water is routed through these trenches. Bacteria use carbon in the chips to increase biomass and degrade nitrates in the water, releasing nitrogen gas.

9.4 Turning Wastes into Energy

Landfills around the world are stressed to their limits, literally overflowing with trash from homes and businesses. The bulk of our household trash consists of food scraps, boxes, paper waste, cardboard packing containers from food, and similar items. A variety of chemical wastes such as detergents, cleaning fluids, paints, nail polish, and varnishes also make their way into the trash, despite the fact that most states are trying to reduce the amount of chemical waste that can be disposed of as regular garbage.

Scientists are working on strategies to reduce waste, including bioreactors containing anaerobic bacteria that can convert food waste and other trash into soil nutrients and methane gas. Methane gas can be used to produce electricity, and soil nutrients can be sold commercially as fertilizer for use by farms, nurseries, and other agricultural industries. Scientists are also working on seeding strategies for landfills, such as adding bioremediation microbes, to degrade chemicals, which often seep through the ground and contaminate local groundwater and surface water. If successful, these applications of biotechnology may help to reduce the amount of waste and greatly increase the usable space in many landfills.

In 2016, a team of biochemists from Utah State University reported promising results using the bacterium *Rhodopseudomonas palustris* to convert carbon dioxide into methane in one step through a single enzyme. Because carbon dioxide is a very stable molecule, it is not easy to degrade. Converting harmful carbon dioxide emissions from the combustion of fossil fuels into valuable fuels is an exciting finding because of its potential to help reduce greenhouse gases. It will be very interesting to see if this research progresses into commercial applications.

Bioremediation scientists are also studying polluted sediments in sewage sludge and at the bottoms of oceans and lakes as an untapped source of energy. Sediment in lakes and oceans is rich in organic materials from the breakdown of decaying materials such as leaves and dead organisms. Within this "muck" are anaerobes that use organic molecules in the sediment to generate energy. The term *electrigens* or electrogenic microbes describes electricity-generating microbes that have the ability to oxidize organic compounds to carbon dioxide and transfer electrons to electrodes. Under certain conditions, electrigens can cluster and interconnect to form nanowires that conduct

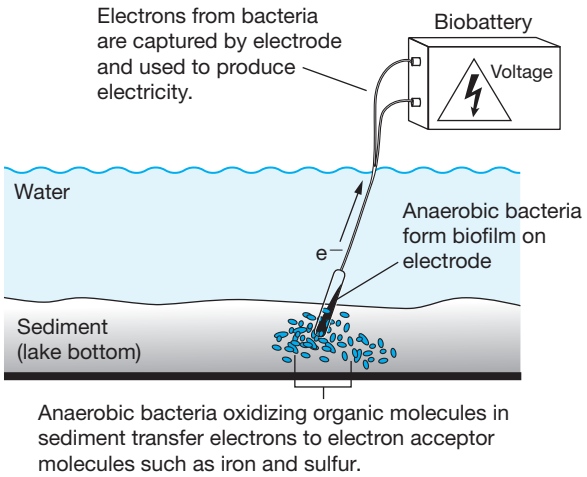


FIGURE 9.15 Polluted Sediments May Be an Untapped Source of Energy Scientists have found that anaerobic bacteria in sediment may be a source of energy. Because these bacteria use redox reactions to degrade molecules in sediment, electrons can be captured by electrodes, which can transfer electrons to generators to create electricity.

electrons! Such strains may even be used to make electricity from manure and common household wastes.

Desulfuromonas acetoxidans is an anaerobic marine bacterium that uses iron as an electron acceptor to oxidize organic molecules in sediment. Researchers are exploring ways in which electrons can be harvested from *D. acetoxidans* and other bacteria such as *Geobacter metallireducens* and *Rhodoferax ferrireducens* as a technique for capturing energy in **microbial fuel cells,** also called *bacterial biobatteries,* that can provide a source of electricity in laboratory environments (**Figure 9.15**). The U.S. Navy continues to fund ongoing research into electrogenic microbes. One application of interest is whether electrogenic microbes in marine sediments could be harnessed to generate power for undersea instrumentation.

In the wild and in laboratory environments, electrogenic microbes have been shown to organize into biowires, whereby long chains of microbes in contact with each other can pass electrons to one another. Some of these chains can act as electronic oscillators—they produce periodic pulses of electrical currents (some species bioluminesce synchronized with each pulse), which they use to communicate with each other when sensing chemicals such as arsenic or iron-containing molecules. Although biobattery technology is improving in the laboratory and this technique clearly has some promise, much more research needs to be done before highly efficient sources of energy can be produced from bioremediating electrogenic bacteria (**Figure 9.16**).

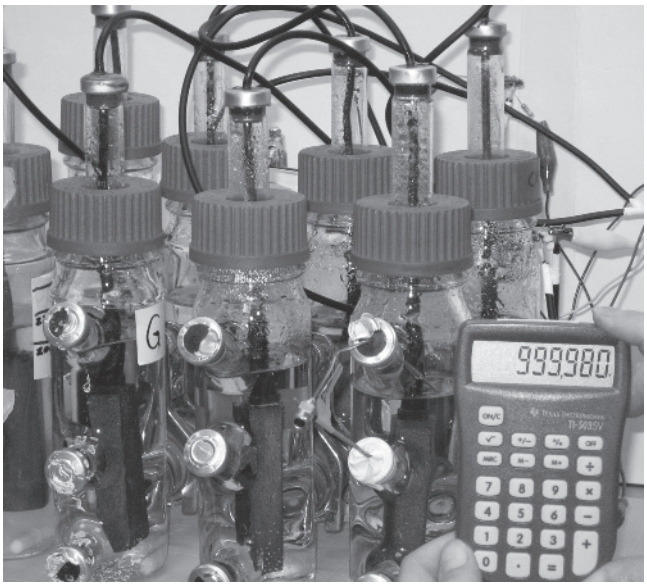


FIGURE 9.16 Microbe-Powered Fuel Cells Researchers at the University of Massachusetts have demonstrated that *Geobacter* fuel cells can effectively convert sugars into electricity.

9.5 Applying Genetically Engineered Strains to Clean Up the Environment

Bioremediation has traditionally relied on stimulating naturally occurring microorganisms. However, many indigenous microbes cannot degrade certain types of chemicals, especially when the polluted environment contains high concentrations of very toxic substances, such as heavy metals, radioactive compounds, and chlorine-rich organic molecules, because these compounds typically kill microbes. To clean up particularly toxic pollutants, we may need to use bacteria and plants that have been genetically altered. The development of recombinant DNA technologies has enabled scientists to create genetically modified organisms (GMO) with the potential to improve bioremediation processes.

Petroleum-Eating Bacteria

The first effective GM microbes for use in bioremediation were created in the 1970s by Ananda Chakrabarty and his colleagues at General Electric. This work was carried out before DNA cloning and recombinant DNA technologies were widely available. So how did Chakrabarty do this? He worked with strains of *Pseudomonas* from soils contaminated with different types of chemicals, including pesticides and crude oil. Chakrabarty identified and isolated strains that showed the ability to degrade such organic compounds as naphthalene, octane, and xylene. Most of these strains could grow in the presence of these compounds because they contained plasmids that encoded genes for breaking down each component.

Chakrabarty mated these different strains and eventually produced a strain that contained several different plasmids. Together, the combined proteins produced by these plasmids could effectively degrade many of the chemical components of crude oil. For his work, Chakrabarty was awarded the first U.S. patent for a GM living organism. However, this patent decision was very controversial and was held up in the courts for about 10 years. The primary issues being debated were whether life forms could be patented and whether Chakrabarty's recombinant bacterium should be considered a product of nature or an invention. Eventually the U.S. Supreme Court ruled that the development of recombinant *Pseudomonas* was an invention worthy of a patent.

Chakrabarty's approach was not as effective as it sounds. Crude oil contains thousands of compounds, and his GM bacteria could degrade only a few of these. The majority of the chemicals in crude oil remain largely unaffected by recombinant organisms. Consequently, developing GM bacteria with different degradative properties is an intense area of research. In the future, a useful approach for cleaning up crude oil may be to release multiple bacterial strains, each with the ability to degrade different compounds in the oil. To date, field applications of GM microbes for bioremediation have been fairly limited, in part because of regulatory obstacles and public concerns over the release of GM bacteria. But GM microorganisms are also often ineffective in the environment because indigenous microbes often outcompete them.

Engineering Microbes to Clean Up Heavy Metals

Heavy metals including copper, lead, cadmium, chromium, and mercury can critically harm humans and wildlife. Mercury is an extremely toxic metal. It is used in manufacturing plants, batteries, electrical switches, medical instruments, and many other products. Mercury, and a related compound called methylmercury (MeHg), can accumulate in organisms through a process called **bioaccumulation.** In bioaccumulation, organisms higher up on the food chain contain higher concentrations of chemicals than organisms lower on the food chain. For instance, in a water supply, mercury may be ingested by small fish, which may then be eaten by birds, larger fish, otters, raccoons, and other animals, including humans. Large fish and birds need to eat a lot of small fish; therefore, they accumulate more mercury in their systems than small fish and birds that eat less.

Similarly, if a person were to eat large fish as a primary source of food, that person would accumulate high amounts of mercury over time. Regular consumption of fish and shellfish contaminated with mercury and MeHg poses serious health threats to humans, including birth defects and brain damage. For these reasons, in many areas of the United States, health officials suggest that pregnant women and young children eat only small amounts of certain types of fish, such as swordfish and fresh tuna, and restrict these meals to no more than one serving a week.

Because it is toxic at very low doses, most existing strategies for removing mercury from contaminated water supplies do not remove enough of it to meet acceptable standards. Scientists have developed genetically engineered strains of *E. coli* that may be useful for cleaning up mercury and other heavy metals. Naturally occurring metal-binding proteins in plants and other organisms have also been identified and may have a role in bioremediation of mercury and other heavy metals. Two of the best-characterized types of proteins—metallothioneins and phytochelatins—have a high capacity for binding to metals. For these proteins to function, however, the metals must enter cells. Scientists have engineered *E. coli* to express transport proteins that allow for the rapid uptake of mercury into the bacterial cell's cytoplasm, where the mercury can bind to metal-binding proteins.

Some of these genetically altered bacteria can absorb mercury directly; others that bind mercury can be grown on **biofilms** to act as sponges for soaking up mercury from a water supply. The biofilms must be changed periodically to remove mercury-containing bacteria. Similarly, genetically engineered single-celled algae containing metallothionein genes and bacteria called cyanobacteria have shown promise for their ability to absorb cadmium, another very toxic heavy metal known to cause many serious health problems in humans.

Genetically Modified Plants and Phytoremediation

In recent years, scientists have been working on genetically modifying plants to improve their phytoremediation capabilities. Currently, using phytoremediation to clean up MeHg is a very active area of research. Plants engineered to contain mercury-detoxifying genes from bacteria have shown some potential for accumulating MeHg, and eventually they may be used for phytoremediation.

Another promising area of research has been the development of GM plants to remove chemicals from military explosives and weapons firing ranges that have contaminated soil and groundwater. Hexahydro-1,3,4-trinitro-1,3,5-triazine (commonly called royal demolition explosive, or RDX) and 2,4,6-trinitrotoluene (TNT) are two of the most common chemical contaminants that result from the production, use, and disposal of explosives. Both RDX and TNT are highly toxic to most organisms and pose significant health threats to wildlife and humans. Notice that TNT was one of the top chemicals listed in Table 9.1 and that the EPA lists both TNT and RDX as priority chemicals to be removed from the environment. These contaminants are a major pollution problem worldwide. Incredibly, hundreds of tons of these compounds are found in sites around the world, including many millions of acres of U.S. military bases with estimated cleanup costs of up to $165 billion.

A few bacteria and plants have been shown to weakly degrade TNT with low efficiency; degradation of RDX is even less effective because of its chemical structure. In the past few years, however, scientists have used genetic engineering to create transgenic plants that may turn out to be very effective for the phytoremediation of TNT and RDX. A transgenic strain of tobacco containing a nitroreductase gene from *Enterobacter cloacae* effectively converts TNT to less toxic chemicals (**Figure 9.17**). Scientists have incorporated a gene called *xplA* from the bacterium *Rhodococcus rhodochrous* into *Arabidopsis thaliana*. The *xplA* gene produces an RDX-degrading enzyme called cytochrome P450, which can degrade RDX once it is absorbed into the plant.

Recently, researchers from the University of Washington and the University of York created two transgenic perennial grass species, switchgrass (*Panicum virgatum*) and creeping bentgrass (*Agrostis stolonifera*), to contain two genes from bacteria that degrade RDX. In test experiments, these transgenic grasses removed all RDX from contaminated soils in less than 2 weeks, and no remnants of RDX were present in the leaves or stems of grasses following phytoremediation. In the future it may also be possible to make plants that can degrade both TNT and RDX. Moreover, since the poplar genome has been sequenced, bioremediation scientists working on genetically modified plants are very enthusiastic about the possibility of making transgenic poplars and other fast-growing, deep-rooted trees that can remediate TNT and RDX well below the surface soil.

Biosensors

For years, the bioluminescent marine bacterium called *Vibrio fischeri* (discussed also in Chapter 5) have been used as **biosensors** to test for the presence of toxic chemicals in environmental samples. For example,

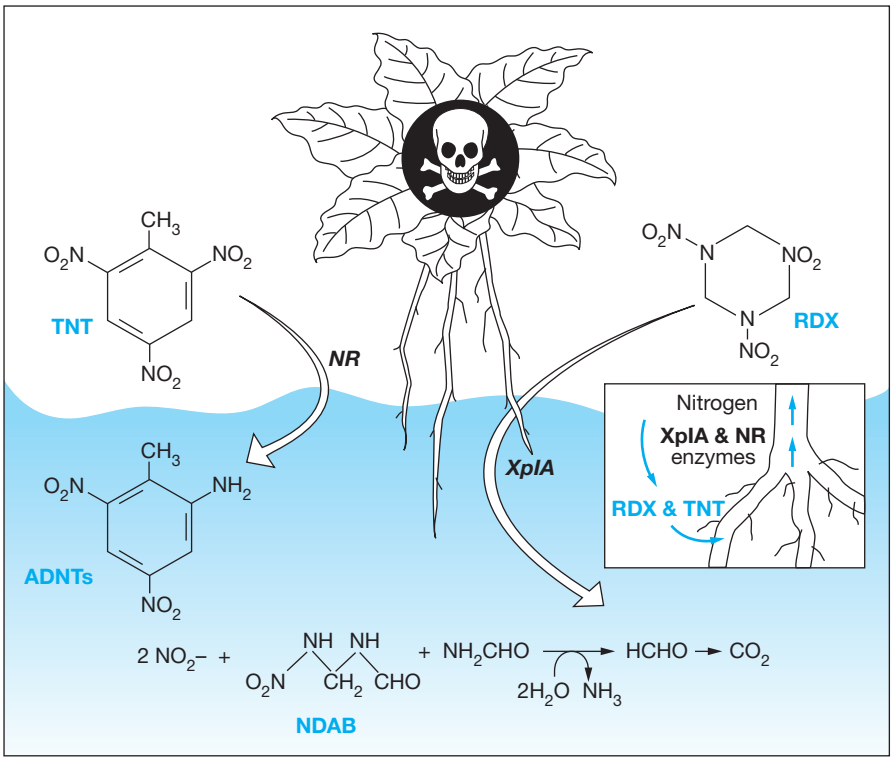


FIGURE 9.17 Phytoremediation of Toxic Explosives Using Transgenic Plants Transgenic plants engineered with the *XplA* or *NR* gene to degrade either RDX or TNT, respectively, absorb the explosive chemicals through their roots and then degrade them into less toxic compounds (aminodinitrotoluenes [ADNTs] or 4-nitro-2,4-diazabutanal [NDAB]) or nontoxic compounds (NH_3, CO_2). Plants engineered with the *XplA* gene use the degradation products from RDX to stimulate their growth.

when mixed with sediment samples, light released by bacteria will decline as chemicals from the sediment kill bacterial cells. Thus scientists can measure the drop in bioluminescence and determine the concentration of toxic chemicals in the sediment.

But more emphasis is now being placed on developing GM-organisms as biosensors. Researchers have developed genetically engineered strains of the bacterium *Pseudomonas fluorescens,* which can effectively degrade complex structures of carbon and hydrogen called polycyclic aromatic hydrocarbons (PAHs) and other toxic chemicals. Using recombinant DNA technology, scientists have spliced bacterial genes encoding specific enzymes (which can metabolize these contaminants) to reporter genes such as the *lux* genes from bioluminescent marine bacteria. Recall that *lux* genes are often used as reporter genes because they encode the light-releasing enzyme luciferase (see Chapter 5). As PAHs are degraded, bacteria release light that can be used to monitor biodegradation rates. Similar techniques are being used to develop biosensors from recombinant bacteria containing *lux* genes. Biosensors have proven to be valuable in the assessment of environmental pollutants such as heavy metals. In the future, GM microbes are expected to play a greater role as biosensors. In the next section, we consider two of the best-studied and most highly publicized examples of bioremediation in action.

9.6 Environmental Disasters: Case Studies in Bioremediation

Most bioremediation approaches involve the cleanup of contaminated areas that are relatively small, perhaps several hundred acres in size. Nevertheless, a great deal has been learned about the effectiveness of different bioremediation strategies by studying large-scale environmental disasters that have been treated by bioremediation.

The *Exxon Valdez* Oil Spill

The world relies heavily on petroleum products. These include crude oil, natural gas, various liquefied refinery gases, and refined petroleum products such as gasoline and diesel fuel.

Petroleum products are used not just as gasoline and diesel fuel to power automobiles but also as the basis for hundreds of everyday products including plastics, paints, cosmetics, and detergents. According to the U.S. Energy Information Administration, as of 2016, the United States alone uses in excess of 7.9 billion barrels (1 barrel = 42 U.S. gallons, or ~159 liters) of petroleum products annually, approximately 19.6 million barrels per day, of which over approximately 10.1 million barrels per day are imported from about 70 different countries.



(a)



(b)

FIGURE 9.18 Oil Spills Pose Serious Threats to the Environment (a) Oil spills typically have the greatest impact on wildlife. (b) Containment booms are used initially to control the spread of oil and minimize pollution of the surrounding area.

The U.S. National Academy of Sciences reports that roughly 1.3 billion liters of oil are released into the world's seas annually! When crude oil is transported, some amount of accidental leakage and spills almost always occurs—this accounts for approximately 12 percent of the total. Oil consumption and release by ships and discharges from land account for about 37 percent, but the largest volumes (46 percent) of oil enter our seas through naturally occurring seepage from the ocean floor.

Oil spills have a tremendous impact on the environment, specifically on large numbers of wildlife (**Figure 9.18a**). Typically, large spills do not have a great effect on human life directly. This is because most large spills occur in open oceans or bays far removed from swimming beaches and water supplies. Humans are often more affected by small local spills, such as a gas station's leaking underground tank that may threaten local drinking water supplies.

In 1989, the *Exxon Valdez* oil tanker ran aground on a reef in Prince William Sound off the coast of Alaska, releasing approximately 42 million liters (11 million gallons, or about 260,000 barrels) of crude oil and contaminating over 1,000 miles of the Alaskan shoreline. Many experimental approaches for bioremediation were implemented to clean up this spill. Prince William Sound became a field laboratory for trying bioremediation cleanup strategies.

As is done at most sizable oil spills, physical cleaning measures were first used to contain and remove large volumes of oil. These measures included the use of containment booms or skimmers—surface nets, fences, or inflatable devices like buoys that float on the surface—which are fixed in place or towed behind a boat to collect and contain oil (**Figure 9.18b**). Then vacuums were used to pump oil from the surface into disposal tanks. But these efforts only recovered approximately 14 percent of the oil. Beaches and rocks were washed with fresh water under high pressure to disperse oil. By diluting and dissolving the oil in the sea, the oil was gradually dispersed. In some areas rocks were steam cleaned with high-pressure hot water, but this likely killed indigenous microbial populations and limited bioremediation initially. Another biotechnology cleanup application used citrus-based products to bind crude oil and allow it to be collected on absorbent pads. But after using all these physical approaches to remove the bulk of the oil, millions of gallons of oil still remained attached to sand, rocks, and gravel both at the surface and below the surface of contaminated shorelines. This is when bioremediation went to work.

As the first step in the bioremediation process, nitrogen and phosphorus fertilizers were applied to the shoreline to stimulate oil-degrading bacteria, mostly strains of *Pseudomonas*, that were living in the sand and rocks (**Figure 9.19**). These indigenous bacteria immediately showed signs that they were degrading the oil. When microbes degrade petroleum products, PAHs are formed and eventually oxidized into carbon chains that can be broken down into carbon dioxide



FIGURE 9.19 Applying Fertilizers to Stimulate Oil-Degrading Microbes Cleanup workers spray nitrogen-rich fertilizers onto an oil-soaked beach in Alaska following the *Exxon Valdez* oil spill. The fertilizers greatly accelerate the growth of indigenous bacteria that will degrade the oil.

and water. Over time, chemical tests on oil from the shoreline soil showed significant changes in chemical composition, indicating that natural degradation by indigenous bacteria was working. However, oil seeped into sediments and other low-oxygen layers below shoreline rocks, where biodegradation is relatively slow. It may take hundreds of years for the oil spilled by the *Exxon Valdez* to be fully cleared, and some areas of the Alaskan environment may never return to the state in which they were prior to the spill.

Oil Fields of Kuwait

The deserts of Kuwait have served as a laboratory for studying bioremediation. During the Iraqi occupation of Kuwait and the Gulf War (August 1990 to February 1991), large areas of Kuwait's desert remained soaked in oil. Countless oil fields were destroyed and burned, releasing an estimated 950 million liters of oil into the deserts—more than 20 times the amount of oil spilled in the *Exxon Valdez* accident. Kuwaiti scientists found that spilled oil has severely affected plant and animal life in many contaminated areas. Some plant species have been completely eliminated, and the long-term biological impacts will not be known for many more years.

In contrast to the *Exxon Valdez* spill, bioremediation of desert soils poses a number of different problems. There are no waves to help disperse and dissolve oil. Dry soil conditions of the desert also tend to harbor fewer strains of oil-metabolizing microbes, and adhesion of oil to sand and rocks slows natural degradation processes. Preliminary studies suggest that novel strains of oil-degrading bacteria are slowly working to break down oil below the surface of the sand.

The Kuwaiti government developed a $1 billion program to address one of the world's largest bioremediation projects. There are no previously studied sites of this size to use as a model for cleaning up arid desert environments, so bioremediation scientists from around the world are studying Kuwait's deserts in hopes of developing strategies for cleaning up these oil-drenched sands. Information that scientists learn from studying this region will certainly be of value for treating oil spills in other sandy environments. For example, several dozen strains of crude oil–degrading bacteria (most belong to a group called *Corynebacterium variabile*) isolated from contaminated sand have been shown to increase biomass and efficiently degrade a majority of crude oil within 1 week under laboratory conditions. In addition to stimulating these microbes to improve bioremediation in situ, they may also be of value for bioremediation at other cleanup sites with similar environmental conditions.

The *Deepwater Horizon* Oil Spill

On April 20, 2010, British Petroleum's (BP) oil-drilling rig the *Deepwater Horizon* exploded, releasing an undersea geyser of oil that lasted for 87 days, gushing millions of gallons of crude oil into the Gulf of Mexico about 65 kilometers off the Louisiana coast (**Figure 9.20a**). Although estimates of oil flow varied widely, more than 600 million liters (at least 5 million barrels) were released into the Gulf before the broken well was capped in mid-July, resulting in the largest marine oil spill in history. Yet, given the size of the spill, far less oil than many predicted made it to the coast. Oil that was not removed by cleanup crews was dispersed by wave action and through the use of chemical dispersants that broke up the slick. Controlled burns and surface oil evaporation of volatile materials such as PAHs also contributed to oil cleanup, but bioremediation played a big role accounting for the degradation of an estimated 50 percent of the oil released.

Studies by oceanographers from the Woods Hole Oceanographic Institute in Massachusetts reported that during the first 5 days of the spill, microbes had not significantly degraded the oil. Over time, an underwater plume of oil that was at least 22 miles long developed. Within weeks, microbes appeared to be flocking to the plume, replicating rapidly, so that bacteria were twice as dense inside the plume as outside the plume

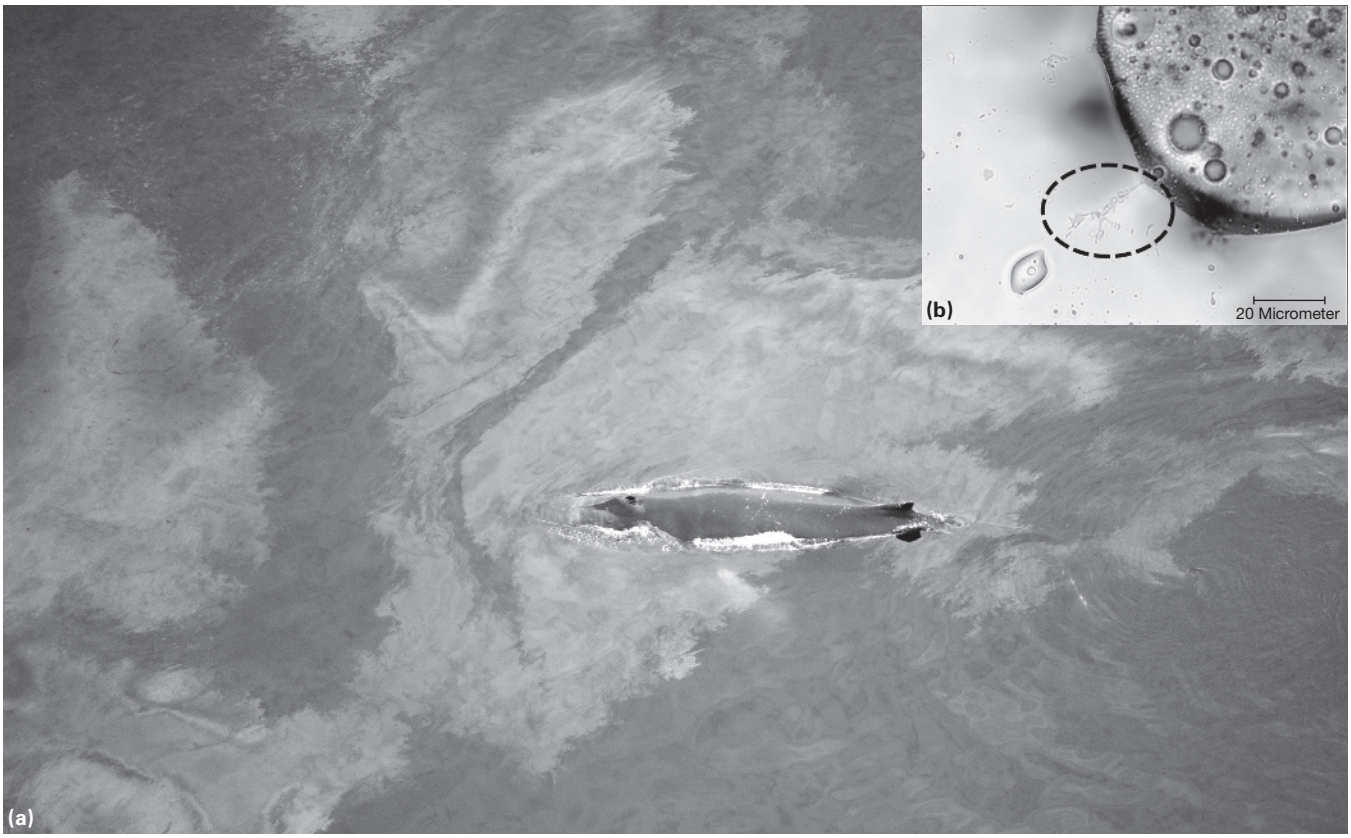


FIGURE 9.20 Microbes Played a Major Role in the Bioremediation of Oil from the *Deepwater Horizon* Oil Spill in the Gulf of Mexico (a) Shown here is a whale swimming through a surface plume of oil in the Gulf. Indigenous microbes that were very abundant in surface and subsurface plumes of oil emanating from the *Deepwater Horizon* spill were essential for bioremediation. (b) An oil droplet magnified 100 times and metabolizing microbes (circled).

(Figure 9.20b). Research on these indigenous microbes in the plume revealed over 1,500 genes encoding proteins designed to degrade hydrocarbons. Clearly, hydrocarbon-degrading microbes were highly enriched in the plume, and these microbes were actively breaking down oil. It was also determined that microbes degraded an estimated 200,000 tons of methane that spewed into the Gulf of Mexico during the spill. Ethane and propane were also major hydrocarbons released and these, too, were degraded by microbes.

The warm waters of the Gulf and components in the plume—such as natural gas, which contains propane and ethane—helped stimulate biodegradation by indigenous microbes. Fertilizers such as iron, nitrogen, and phosphorus were applied at the site to stimulate rates of biodegradation. Gradients in dissolved oxygen levels in the plume also contributed to differences in rates of biodegradation throughout and along the plume. Estimates have suggested that these microbes were reducing oil amounts in the plume by half nearly every 3 days.

Where did they come from? Petroleum-degrading bacteria have existed for eons, thriving on oil that seeps naturally through the sea floor. Each year about 79 million liters of oil leak onto the floor of the Gulf of Mexico through naturally occurring seepage. Within the *Deepwater Horizon* plume, researchers have detected over 900 subfamilies of bacteria, including newly discovered species. It is also likely that chemical dispersants used to break up the stream of oil gushing from the broken well may have created microscopic particles that increased the surface area between oil droplets and water, allowing for greater contact with oil-degrading bacteria. Other studies, however, reported that the dispersants likely suppressed the hydrocarbon-degrading bacteria and favored dispersant-degrading bacteria, which likely explains why bioremediation rates of oil were lower than expected given the density of microbes in the plume.

Supported as part of a $500 million pledge from BP to fund research in the Gulf, many ecological genomics projects are under way. Scientists are using whole-genome sequencing, microarray analysis, and

transcriptome analysis to study how genomes for different organisms are adapting to the stresses of the oil spill. One major finding is that bioremediation required complex communities of different microbes with different degradative capabilities. These categories of microbes thrived on various chemical components in this oil buffet. Some microbes degraded alkanes, and others metabolized aromatic hydrocarbons. The impact of oil that moved into Louisiana's wetlands and long-term impacts on marine ecosystems in the Gulf region will be closely evaluated for decades.

The final section provides a glimpse of how potential applications of bioremediation may be able to solve cleanup problems that have been difficult to treat.

9.7 Challenges for Bioremediation

As you have learned, biotechnology is a significant tool in our fight to rehabilitate areas of the environment that have been polluted through accidents, industrial manufacturing, and mismanagement of ecosystems. Bioremediation is a rapidly expanding science. Scientists around the world are carrying out research aimed at developing a greater understanding of the microorganisms involved in biodegradative processes, including the identification of novel genes and proteins involved in these breakdown processes. Researchers are studying microbial genetics to create genetically engineered microbes that



YOU DECIDE **The PCB Dilemma of the Hudson River**

Winding through upstate New York, the Hudson River Valley comprises some of the most beautiful country in the Northeast. But serious problems lurk below the glimmering surfaces of the Hudson's blue water. From 1947 to 1977, the General Electric Company released over 1.2 million pounds of toxic chemicals called polychlorinated biphenyls (PCBs) into the river from facilities in Hudson Falls and Fort Edward, New York. PCBs were commonly used in transformer boxes, capacitors, and cooling and insulating fluids of electrical equipment manufactured before 1977. PCBs are no longer used in manufacturing in the United States, and they have been banned in most of the world.

PCBs are highly toxic to humans and wildlife because these fat-soluble chemicals gradually accumulate in fatty tissues. Hudson River fish are contaminated with PCBs at concentrations in excess of safe levels. Consumption of fish from most areas of the Hudson is banned or restricted, but some people ignore these publicized restrictions because the water looks so clear and the fish do not *look* polluted. Exposure to PCBs is known to cause cancer, reproductive problems, and other medical conditions affecting the immune system and thyroid gland. Children are particularly susceptible to the health effects of PCBs.

High concentrations of PCBs still lurk in the Hudson River and other environments. In the Hudson River, most PCBs are located in the sediment at the bottom of the river. To get rid of these persistent chemicals, the EPA proposed a project to dredge a 200-mile-long section of riverbed to remove contaminated sediment, which would contain more than 100,000 pounds of PCBs. This dredging plan was highly controversial, and opposition over the project delayed its implementation for many years.

Critics of the plan suggested that dredging would only release more PCBs into the water by stirring up the sediment. Instead of dredging, many believed that leaving the sediment in place and letting natural current flows disperse the chemicals, combined with bioremediation through bacterial degradation of PCBs, was the best way to reduce the load of PCBs in the long run. PCB-degrading anaerobes are present in Hudson River sediments. Some anaerobic bacteria are involved in the first step of breaking down PCBs by cleaving off chlorine and hydrogen groups. Aerobic bacteria in the water can further modify PCBs, and others can then convert them into water, carbon dioxide, and chloride.

Eventually, in 2009, the project moved forward. After 6 years of dredging, nearly 2.8 million cubic yards of polluted sediment were removed, and the project was considered completed by the EPA in 2015. Even if dredging was a good plan, where did the dredged sediments go? How will these chemicals be cleaned up? Some people believe that placing PCB-laden sediments in a sealed landfill, a completely anaerobic environment, will slow down the degradative processes. Should these chemicals have been left in the mud to be broken down slowly over time through natural bioremediation, or should humans have intervened in an effort to speed up nature's cleanup effort? The EPA estimates that it will take 70 years before PCB levels in fish will decline to the point that people can safely eat fish from the river once a week. What evidence would you want to evaluate to determine if fish are safe for human consumption? What are the pros and cons of taking action or doing nothing? You decide.

may be able to degrade new types of chemicals, and they are developing novel biosensors to detect and monitor pollution. Here we briefly spotlight a few other challenges that bioremediation scientists are trying to find solutions for.

Recovering Valuable Metals

The recovery of valuable metals such as copper, nickel, boron, and gold is another area of bioremediation that has yet to reach its full potential. Through oxidation reactions, many microbes can convert metal products into insoluble substances, called metal oxides or ores, which will accumulate in bacterial cells or attach to the bacterial cell surface. Some marine bacteria that live in deep-sea hydrothermal vents have also shown potential for precipitating precious metals.

Using bacteria as a way to recover hazardous metals as part of industrial manufacturing processes is one potential application, but scientists are interested in finding ways in which these bacteria can be used to recover valuable precious metals. For instance, many manufacturing processes use silver and gold plating techniques; these processes produce waste solutions with suspended particles of silver and gold. Microbes may be used to recover some of these metals from these waste solutions. Similarly, microbes may also be used to harvest gold particles from underground water supplies and cave water found in gold mines. Many bacterial strains that live in these environments are actively being studied for potential metal-recovery applications.

Municipal wastewater treatment plants are also of interest to scientists trying to recover valuable metals from sewage, particularly platinum, silver, and gold, and rare elements such as palladium and vanadium that are used in electronics. Estimates suggest that sewage waste from 1 million Americans may contain as much as $13 million worth of metals! Plants may also provide a way to harvest metals from the environment. The wild mustard *Thlaspi goesingense*, native to the Austrian Alps, is known to accumulate metals in storage vacuoles.

Bioremediation of Radioactive Wastes

Another area of active research involves developing bioremediation approaches to remove radioactive materials from the environment. Uranium, plutonium, and other radioactive compounds are found in water from mines where naturally occurring uranium is processed. Radioactive wastes from nuclear power plants also present a disposal problem worldwide. In France, the National Radioactive Waste Management Agency (ANDRA) is mandated to publish a geographical inventory of sites polluted by radioactive substances every 3 years and the latest report listed 70 polluted sites. Radioactive waste sites often have a complex mix of radioactive elements such as plutonium, cesium, and uranium along with mixtures of heavy metals and organic pollutants such as toluene.

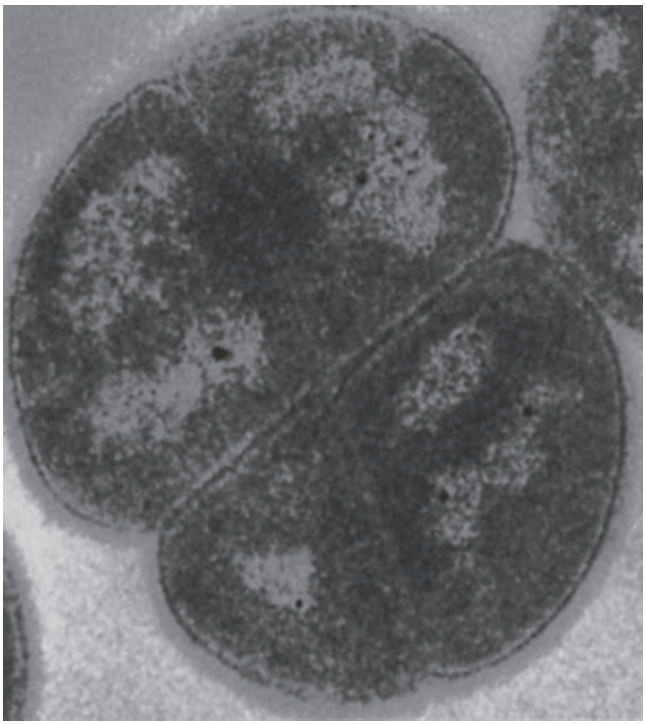


FIGURE 9.21 ***Deinococcus radiodurans*** **Bacteria Are Highly Resistant to Damage by Radiation**

Although most radioactive materials kill a majority of microbes, some strains of bacteria have demonstrated a potential for degrading radioactive chemicals. Some species of *Geobacter* can reduce soluble uranium in groundwater into insoluble uranium, effectively immobilizing the radioactivity. To date, however, no bacterium has been discovered that can completely metabolize radioactive elements into harmless products.

One bacterial strain in particular, called **Deinococcus radiodurans** (Figure 9.21), is especially fascinating. Its name literally means "strange berry that withstands radiation." Named the world's toughest bacterium by *The Guinness Book of World Records*, *D. radiodurans* was first identified and isolated about 50 years ago from a can of ground beef that had spoiled, even though it had been sterilized with radiation. Subsequently, scientists discovered that *D. radiodurans* can endure doses of radiation over 3,000 times higher than other organisms can, including humans. High doses of radiation create double-stranded breaks in

DNA structure and cause mutations, yet *D. radiodurans* shows incredible resistance to the effects of radiation. Although its resistance mechanisms are not entirely known, this microbe clearly possesses elaborate systems for folding its genome to minimize damage from radiation, and it also uses novel DNA repair mechanisms to replace damaged copies of its genome. The genome for *D. radiodurans* has been completely sequenced, and ongoing analyses of gene functions are expected to provide valuable insight into its unique DNA repair genes.

The DOE is very interested in using *D. radiodurans* and another bacterium, *Desulfovibrio desulfuricans*, for bioremediation of radioactive sites. Researchers at the DOE and the University of Minnesota created a recombinant strain of this microbe by joining a *D. radiodurans* gene promoter sequence to the gene encoding the enzyme toluene dioxygenase, which is involved in toluene metabolism. This strain demonstrated the ability to degrade toluene in a high-radiation environment. In an effort to immobilize radioactive elements, scientists hope to use this same strategy to equip *D. radiodurans* with genes from other microbes that are known to encode metal-binding proteins.

Finally, it is important that biodegradation scientists continually look ahead so that they can be prepared to predict how new chemicals may affect the environment and thus be able to develop technologies to clean up new pollutants. Bioremediation will not be able to rid all pollutants from the environment, but well-planned bioremediation approaches are an important component of cleanup efforts. We conclude this chapter by introducing a significant cleanup problem that has gained much more attention in the United States and internationally in recent years.

Degrading Macro- and Microplastics in the Environment

What do toothpaste, clothes, laundry detergents, face scrubs, plastic bags, tires, and plastic bottles all have in common? All contribute to significant pollution of aquatic environments around the world as sources of **macroplastics** (plastic debris larger than 5 cm) and **microplastics** (small plastic particles ~100 nm to 5 mm in size; see **Figure 9.22**). Annually, the average person uses 100 kg of plastic in Western Europe, 60 kg in New Zealand, and 20 kg in Asia. Approximately 300 million tons of new plastic are currently produced annually of which China is the biggest producer. Only 20 percent of this is collected for recycling, which results in a global economic loss of over $100 billion annually. But perhaps the most worrisome statistic is that each year nearly 9 million tons of plastic makes its way into the world's oceans. If this trend continues, scientists estimate that by 2050 plastics will outweigh fish in the ocean—a very sad prospect.



FIGURE 9.22 Macro- and Microplastic Pollution of Marine Environments Worldwide Is an Emerging Concern and an Opportunity for Bioremediation Research

The Great Pacific garbage patch, also called the Pacific trash vortex, is an ocean raft of plastic debris discovered in the late 1980s. Estimates of its size vary widely, from 700,000 square kilometers (270,000 square miles, roughly the size of Texas) to several times larger based on how it is measured and prevailing currents. It is a combination of large trash and microplastics suspended beneath the surface of the ocean. A similar patch exists in the Atlantic Ocean. These patches are found in areas far removed from industrialized countries, emphasizing that local pollution creates global problems. Also, tons of plastic are washing up on uninhabited islands such as Henderson Island, a remote location in the South Pacific more than 5,000 km away from the nearest sizable city.

The problems are enormous. Estimates suggest that more than 8 trillion microbeads enter U.S. waterways each day! Some can be removed by wastewater treatment plants. There are several hundred thousand tons of microplastics on the surface of Earth's ocean. Chemicals such as PCBs, DDT, and PAHs are all known to attach to marine plastics, adding to their toxicity. Macro- and microplastics wreak havoc on marine life. Well documented are studies of dead marine animals with stomachs full of plastic debris. Birds and sea turtles can mistake plastic bags for jellyfish. Investigators estimate that approximately 90 percent of seabirds have plastic in their digestive systems. Filter feeders such as clams, mussels, and oysters can ingest significant amounts of microplastics, which interfere with digestion and act as endocrine disruptors, among many other health effects. Similarly, chemicals associated with

microplastics can bioaccumulate in the food chain as small, contaminated organisms are consumed in large amounts by predator species. It has even been estimated that humans who regularly eat seafood may consume as many as 11,000 microplastic particles each year.

Unlike organic contaminants such as crude oil, which can biodegrade, plastics are highly resistant to bioremediation. *Photodegradation*, the breakdown of plastics from exposure to sunlight (you have observed photodegradation if you have seen parts on a car such as the bumpers or headlight covers fade as a car ages), disintegrates plastics into even smaller particles; it doesn't rid the environment of plastics. One problem is that plastic polymer materials are large and do not easily enter bacterial cells.

Of course, ridding the environment of macro- and microplastics by reducing their production and developing biodegradable materials to replace traditional plastics is a long-term necessity. Some manufacturers are already taking steps to remove microplastics from skin care products such as exfoliants, replacing them with sand and shell particles. As of January 1, 2020, California will enact a ban on the sale of personal care products containing microplastics.

Such measures will take time to have an impact on new sources of pollution. But can bioremediation help to address the macro- and microplastic contamination problem that exists currently? Polyethylene terephthalate (PET) is a polymer present in plastic. Until 2016, no organisms were known to degrade PET. Then Shosuke Yoshida and colleagues in Japan reported isolation of a novel bacterium, *Ideonella sakaiensis*, that completely degrades PET and uses it as a carbon source! *I. sakaiensis* is a Gram-negative aerobic bacterium identified by screening several hundred microbial samples from a PET bottle recycling site. *I. sakaiensis* synthesizes two key enzymes involved in PET metabolism. PET bioremediation is a slow process. In the lab small amounts of PET take about 6 weeks to biodegrade.

In Spain and England, researchers are working on larvae from the greater wax moth (*Galleria mellonella*) which have shown potential to consume and degrade PET-containing shopping. Research such as the examples mentioned here provide optimism that future potential applications of biotechnology will address the bioremediation of environmental plastics.

MAKING A DIFFERENCE

In Hanahan, South Carolina, a suburb of Charleston, a leak of approximately 80,000 gallons of kerosene-based jet fuel occurred in 1975. Despite a number of cleanup measures, the spill could not be kept from soaking through the sandy soil and contaminating the water table. Ten years later, contamination reached a residential area. Excavation of the contaminated soil was impractical because of the large area involved, and removal of the contaminated groundwater would not eliminate the problem. Scientists at the U.S. Geological Survey (USGS) determined that indigenous microbes in the soil were degrading toxic compounds in the jet fuel; they also found that adding fertilizers to these microbes dramatically stimulated rates of biodegradation. This site was one of the first field applications of bioremediation. USGS scientists added nutrients to the groundwater and simultaneously removed some of the contaminated groundwater. By the early 1990s, contamination in the groundwater supply was reduced by 75 percent and bioremediation had demonstrated its potential worth as an effective cleanup strategy.

QUESTIONS & ACTIVITIES

Answers can be found in Appendix 1.

1. Describe three specific examples of a bioremediation application. Include in your description the organisms likely to be involved in the process, and discuss potential advantages and disadvantages of each application.
2. What are indigenous microbes and why are they important for bioremediation?
3. Compare and contrast oxidation and reduction reactions (redox reactions), and explain why redox reactions are important for bioremediation.
4. What is the purpose of adding fertilizers and oxygen to a contaminated site that is undergoing bioremediation?
5. Demonstrate that you understand the concept of phytoremediation by describing three advantages and three disadvantages of this bioremediation approach.
6. In this chapter you learned about in situ and ex situ approaches to bioremediation. What factors are important for determining which cleanup strategy might be most effective?
7. What are endocrine disruptors, what are their sources, where are they in the environment, how do they enter the environment, and why are scientists concerned about health impacts of endocrine disruptors?
8. Suppose you learned that a gas station had leaking underground gasoline storage tanks. Once the

gas station was closed, the site was cleaned up by bioremediation. As a city planner, would you recommend developing this site into a residential housing area? Consider potential problems associated with this scenario.

9. Lead is very toxic and not easily degraded in the environment. Lead pipes, paint containing lead, car batteries, and even lead fishing sinkers are all potential sources for the release of lead into the environment. Propose a strategy for identifying bacteria that may play a role in degrading lead.

10. Suppose your town was interested in building a chemical waste storage facility in your neighborhood. Consider "lessons learned" from past failures of waste storage, dumping, environmental contamination, and successful applications of bioremediation, and then develop a list of priorities town officials should consider before building the site and questions that should be addressed if the site were to be used.

11. Access some of the websites listed on the Companion Website. Use these sites to search for information about the most prevalent pollutants found in the environment. Make a list of five pollutants and describe where they are found, how they enter the environment, their short- and long-term health effects (in humans or other organisms), their environmental effects, and what types of microbes may degrade these pollutants.

12. Visit the United Nations Statistics Division's Environment Statistics webpage (**http://unstats.un.org/unsd/environment/interlinks.htm**) and explore its international and regional data sources related to air pollution, greenhouse gases (GHGs), and waste to see if you can find relevant information regarding your country.

13. Visit the EPA Superfund site and search for environmental cleanup violations reported in certain countries in the United States. Who was responsible for these violations? What fines (if any) were levied? What efforts are planned to remediate the site or sites?

14. In October 2011, the container ship *Rena* ran aground on the Astrolabe Reef off the coast of Tauranga, New Zealand. Approximately 1,700 tons of bunker oil and 200 tons of marine diesel oil were spilled. Conduct an Internet search to see if you can find any information about bioremediation at this site.

15. Scientists monitoring bioremediation at the *Deepwater Horizon* oil spill in the Gulf of Mexico were concerned that microbial degradation of oil could lead to zones of hypoxia, which might result in massive fish kills. Why might this be a concern? As it turns out, this did not appear to be a major problem. Propose reasons why these predictions may have been incorrect.

16. Anammox process has been a very active area in the field of wastewater treatment. Visit Google Scholar (**http://scholar.google.com/**) and conduct a search using the keyword *anammox* to find recent publications in this area.

17. In late 2010, a highly publicized paper was published online in *Science* describing a potentially unprecedented microbe that was reported to metabolize arsenic and incorporate arsenic into its DNA. Many scientists have been skeptical and highly critical of this work. Do an Internet search for articles on the latest viewpoints of this work.

18. In Section 9.7 we presented the concept of macro- and microplastic contamination of marine environments and problems they present. Do an Internet search on the Great Pacific garbage patch and the North Atlantic garbage patch to learn more about these accumulations of marine debris and the latest new scientific information available.

19. Do you think bioremediation will eventually be of value for degrading environmental microplastics? Why or why not? Explain your answers. Propose a strategy you would use to try to develop a bioremediation approach for degrading microplastics.

20. For more information about the novel PET-degrading microbe *I. sakaiensis*, read the paper by Yoshida et al. listed on the Companion Website.

Visit www.pearsonglobaleditions.com for the Companion Website

The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

This case study has been included to give you an opportunity to become familiar with a research example applicable to this field of study. After you have analyzed the data and answered the questions, you can compare them to those provided to your instructor with the text materials.

CASE STUDY

Turning Outhouses into Lighthouses?

In this chapter we provided an introduction to many basic applications of bioremediation. Scientists around the world continue to work on novel applications not only to degrade wastes but also to convert them into useful products. In 2016, a team of Spanish scientists funded by the Bill and Melinda Gates Foundation reported a prototype urinal system that incorporates many elements of topics we discussed in this chapter.

Curious? To learn more, review the news report link from *Science Daily* (**https://www.sciencedaily.com/releases/2016/07/160706092216.htm**), which provides free access to the abstract for a publication by Leropoulos et al. (*Environmental Science Water Research and Technology*. 2016;2(2):336. doi:10.1039/C5EW00270B). The full publication can also be accessed free of charge. From this news report and the *Environmental Science Water Research and Technology* paper, answer the following questions.

Questions

Answers can be found at www.pearsonglobaleditions.com

1. What is the useful application being described by this work?
2. What is the role of microbes in this technology and why are they important?
3. Why were the prototypes for these initial field trials designed for use by men only?
4. What areas of the world would be likely locations for early implementation of this technology? Explain your answers.

CHAPTER TEN

Aquatic Biotechnology



AquAdvantage® transgenic salmon, the first GM animal approved by the FDA for human consumption, overexpress growth hormone genes and weigh nearly 10 times more than similarly aged non-transgenic salmon. Shown here is a non-transgenic Atlantic salmon in front of an AquAdvantage salmon of the same age.

After completing this chapter, you should be able to:

- Discuss important goals, benefits, and practices of aquaculture, and appreciate its global impact.
- Discuss controversies surrounding aquaculture and describe its limitations.
- Understand how novel genes from aquatic species may be beneficial to the biotechnology industry.
- Provide examples of transgenic and polyploid aquatic species and their uses, and understand how these GM organisms are created.
- Explain why scientists are actively bioprospecting aquatic organisms around the world.
- Provide examples of medical and nonmedical products and applications of aquatic biotechnology.
- Describe biofilming and explain how scientists are looking to marine organisms for a natural way to minimize biofilming.
- Understand how marine organisms may be used for biosensing and bioremediation of environmental pollutants.

Given that water covers nearly 75 percent of the earth's surface, it should not surprise you that aquatic environments are a rich source of biotechnology applications and potential solutions to a range of problems. Aquatic organisms exist in a range of extreme conditions, such as frigid polar seas, extraordinarily high pressures at great depths, high salinity, exceedingly high temperatures, and low light conditions. As a result, aquatic organisms have evolved a fascinating number of metabolic pathways, reproductive mechanisms, and sensory adaptations. They harbor a wealth of unique genetic information and potential applications. In this chapter, we consider many fascinating aspects of **aquatic biotechnology** by exploring how both marine and freshwater organisms can be used for biotechnology applications.

FORECASTING THE FUTURE

There are many exciting potential future directions for aquatic biotechnology. Among the most likely areas of substantial progress will be global efforts in bioprospecting to find previously undiscovered aquatic species that may have commercial value. Aquatic environments such as the world's oceans are incredibly rich sources of biological diversity; to survive, many marine organisms have to cope with extremes in pressure, temperature, salinity, and other environmental conditions. Hence the potential for finding novel proteins and other bioactive compounds of commercial value is very high. For example, over 40 companies are bioprospecting in the Arctic alone, hoping to exploit novel properties of Arctic organisms in the ice and in the waters and soils below the ice. Aquaculture, particularly practices involving genetically modified (GM) species, will continue to be a rapidly developing area of aquatic biotech as countries strive to provide protein-rich food sources for their populations, including areas of the world where finfish and shellfish species are nonexistent or overharvested.

10.1 Introduction to Aquatic Biotechnology

Oceans have been a source of food and natural resources for millennia. However, human population growth, overharvesting of fish and other marine species, and declining environmental conditions have caused the collapse of various fisheries globally, which puts pressure on many species and strains ocean resources. Although scientists have learned a great deal about ocean biology, the vast majority of marine organisms—particularly microorganisms—have yet to be identified. It has been estimated that greater than 80 percent of the earth's organisms live in aquatic ecosystems.

The obvious need to utilize the potential wealth of the majority of the earth's surface combined with increasing populations, human medical needs, and environmental concerns about our planet makes aquatic science an emerging frontier for biotechnology research. In the United States, less than $50 million is spent annually for research and development in aquatic biotechnology. In contrast, Japan spends between $900 million and $1 billion annually. The success of Asian countries that have invested in basic science research on aquatic biotechnology and the financial value of the resulting products have encouraged other countries to invest significant amounts of time and resources in aquaculture.

In countries such as the United States, several research priorities have been identified to explore the seemingly endless possibilities of utilizing aquatic organisms. These include the following:

- Increasing the world's food supply
- Restoring and protecting marine ecosystems
- Identifying novel compounds for the benefit of human health and medical treatments
- Improving the safety and quality of seafood
- Discovering and developing new products with applications in the chemical industry
- Seeking new approaches to monitor and treat disease
- Increasing knowledge of biological and geochemical processes in the world's oceans

From fish farming to isolating new medical products from marine organisms, these aspects of aquatic biotechnology are among the range of issues we consider in this chapter. The next section discusses aquaculture, a large and rapidly expanding application of aquatic biotechnology.

10.2 Aquaculture: Increasing the World's Food Supply through Biotechnology

Two fishermen strain to hoist a net overflowing with catfish that are the ideal size and health for human consumption. Every fish in the net is a keeper. When prepared for market, these catfish will possess a highly desired consistency, smell, color, and taste. This scenario does not take place on a fishing dock or a commercial fishing boat but instead describes fish farmers



FIGURE 10.1 A Net Full of Farm-Raised Catfish Ready for Market Aquaculture is an effective way of raising large quantities of fish or shellfish that are ready for market consumption in a relatively short time. Shown here are market-size catfish, called 103 by the U.S. Department of Agriculture, which grow significantly faster than other catfish.

clearing nets from a fish-rearing tank (Figure 10.1). The cultivation of aquatic animals, such as finfish and shellfish, and aquatic plants for recreational or commercial purposes is known as **aquaculture.** Specifically, marine aquaculture is called **mariculture.** Although aquaculture can be considered a form of agricultural biotechnology, it is typically considered a form of aquatic biotechnology. In this section, we primarily discuss farming of both marine and freshwater species of finfish and shellfish.

The Economics of Aquaculture

The global population of 7.5 billion people in 2017 is projected to increase to 9.7 billion by 2050. Additionally, people with higher standards of living have a tendency to eat more meat and seafood. Fish are a critical source of protein and micronutrients such as vitamins, iron, zinc, and omega-3 fatty acids. In many developing nations, entire populations are highly dependent on fish and extremely vulnerable to malnutrition if the food demand cannot be met.

It has been estimated that, in the near future, worldwide demand for seafood of all kinds will exceed what wild stocks can provide by approximately 50 to 80 million tons. Global warming, overharvesting, loss of habitat, and depressed commercial fishing industries are all contributing to the decline in production of wild seafood supplies. According to the Food and Agriculture Organization (FAO) of the United Nations' *2016 Report on the State of World Fisheries and Aquaculture*, more than half of all fish stocks have been deemed fully exploited and another 30 percent or so deemed overexploited, depleted, or recovering. If demand continues to rise and wild catches continue to decline, we will see a deficit of consumable fish and shellfish. Thus, worldwide demand for aquaculture products is expected to grow by at least 70 percent during the next 30 years. Aquaculture, together with better resource-management practices, can, in part, help overcome this problem.

Worldwide aquaculture production in the year 2000 was approximately 33 million metric tons, having more than doubled since 1984. In 2016, total world fisheries produced more than 171 million metric tons annually, which includes 80.03 million tons from aquaculture and 90.91 million tons from natural sources. Of this, 88 percent was used for human consumption directly, resulting in a per-capita consumption of 20.3 kg. For all species, finfish and shellfish, production from conventional commercial fishing methods was about 93 million metric tons per year, its plateau since the mid 1990s, and for aquaculture approximately 74 million metric tons per year. Aquaculture production (in millions of metric tons) consisted of finfish (54.1), molluscs (17.1), crustaceans (7.9), and other aquatic animals including frogs (9.3). Some sources estimate that aquaculture has surpassed cattle ranching in terms of the volume of food produced.

Many countries are actively engaged in aquaculture. In 2014, farmed fish for human consumption exceeded wild-caught fish in at least 35 countries. China, where aquaculture has been practiced for thousands of years, is the single largest producer, accounting for more than 60 percent of the world's aquaculture production. Chinese people eat 50 percent more fish than Americans, and total consumption is higher than the next 10 largest countries combined. As a consequence of this demand, China generates over 60 million metric tons from wild and fish farms annually. In the top 15 producers, China is followed by Indonesia, India, Vietnam, the Philippines, Bangladesh, Republic of Korea, Norway, Chile, Egypt, Japan, Myanmar, Thailand, Brazil, and Malaysia.

Because of location, climate, and other factors, different countries often lead in particular areas of aquaculture. For example, Greece is a leading producer of farmed sea bass in the world, producing in excess of 100,000 tons annually, which accounts for over 90 percent of aquaculture production in Europe. Norway is a leading producer of salmon, as is Canada. The rapid growth and revenue success of the aquaculture industry in small countries such as Chile has many countries asking, "If Chile can do it, why can't we?" As a result, expanding markets are under way in Algeria, Ecuador, Argentina, Scotland, Iceland, the Faroe Islands, Ireland, New Zealand, Colombia, Peru, and many other nations.

Aquaculture in the United States is an industry producing more than 600 million pounds of food

valued at more than $1.2 billion annually. Yet the United States provides only about 5 percent of the world's seafood supply. U.S. aquaculture production has more than doubled over the past 10 years and produces more than 100 different species of aquatic plants and animals. This increase is expected to continue, and similar increases in aquaculture are occurring globally. For example, over half of the salmon sold worldwide is produced by aquaculture, with a nearly 50-fold increase in production of fish-farmed salmon over the past two decades.

Aquaculture in the United States became a major industry in the 1950s, when catfish farming was established in the Southeast; today, aquaculture facilities are found in every state. Some of the most successful examples of the business potential of U.S. aquaculture include the Alabama and Mississippi Delta catfish industry, salmon farming in Maine and Washington (~75 percent of the salmon Americans eat is farmed), trout farming in Idaho and West Virginia, and crawfish farming in Louisiana. Similarly, Florida, Massachusetts, and other states have established successful shellfish farms that have brought benefits to struggling commercial fishers. Cape Cod, which houses approximately 75 percent of the Massachusetts aquaculture industry, has successfully raised a number of different shellfish species, including quahogs, soft-shelled clams, blue mussels, and oysters.

Many of the countries most actively engaged in developing aquaculture industries are doing so because local waters have been overfished to the point where natural stocks of finfish and shellfish have been severely depleted. Through aquaculture, markets can be created in areas where the natural resources have been lost. Aquaculture also provides the benefit of creating seafood industries in areas of the world that, because of geographic location, are not normally known for their fisheries. For instance, a successful fish-farming industry has been developed in the deserts of Arizona, where recycled water from aquaculture is also being used to irrigate crop fields. Fish are even being raised in irrigation ditches. Similar efforts are successful in areas of Australia and many other locations around the world.

In theory, increased productivity in the raising of fish should lead to decreased retail prices for consumers. In some ways fish farming is more economical than animal farming or commercial fishing. For example, it takes approximately 7 pounds of grain to raise 1 pound of beef, but less than 2 pounds of fishmeal are needed to raise approximately 1 pound of most fish. As another example, farm-raised catfish grow nearly 20 percent faster in fish farms than catfish in the wild, and they are ready for market sale in approximately 2 years. For fish species that are fed genetically engineered feed at around 10 cents a pound, the return is



TOOLS OF THE TRADE — Environmental DNA (eDNA) Sampling

In other "Tools of the Trade" we spotlight well-established techniques that have been important in biotechnology. But in this example, we introduce eDNA sampling as an emerging but largely unproven approach. eDNA sampling is an environmental monitoring approach for sequencing and analyzing DNA from aquatic species that is present in water. As aquatic organisms shed cells, these cells contain DNA that can be sequenced. Similarly, eDNA sources also include feces, damaged tissues, and dying organisms.

From a water sample, scientists can take advantage of modern DNA sequencing technology to sequence all of the DNA in the sample, then use bioinformatics to create a survey of biological communities based on the presence or absence of DNA from different organisms in the water. Thus, eDNA has the potential to be a valuable tool for understanding and monitoring biodiversity in aquatic environments. This may eventually help improve environmental management practices. For instance, if eDNA indicates shifts in biodiversity (for example, the absence of previously abundant populations for a particular fish species based on DNA data), especially when these shifts are confirmed with population studies, it can help policy makers implement environmental laws accordingly. Detecting invasive (non-native species) is another potential application.

Many questions must be answered if eDNA is to be accepted as a reliable and well-validated tool. For example, how does the presence of eDNA in a water sample equate to the number of organisms (population) for any species in the wild? How far can eDNA travel from its source and what challenges does this present for understanding biodiversity of resident versus transient species? How sensitive and reliable is eDNA for accurately determining the species present in a water body? How long does eDNA persist in different environments? The current thinking is that sequence-quality eDNA only lasts for a few days.

often 70 to 80 cents a pound on the raised fish—a good return on investment. But as you will learn in this chapter, the economics of aquaculture are not always so favorable.

Although worldwide increases in aquaculture will continue, it is unlikely that fish farming can fully replace wild catches in the near future. One reason is that many species are not amenable to fish farming. Because open-water species such as tuna and swordfish roam over large areas of water and require lots of food, they cannot be raised in fish farms. We discuss other barriers to aquaculture later in this chapter. The purpose of presenting the statistics in this section is not to provide you with a remedy for insomnia but rather to help you develop a perspective on the current value and importance of aquaculture and its future potential.

Fish-Farming Practices

In many ways, aquaculture is an extension of the conventional land-based agricultural techniques that have been practiced for decades. But culturing organisms for human consumption is only one purpose of aquaculture. Organisms are raised for many other reasons, such as providing bait fish for commercial and recreational fishing. Small fish such as anchovies, herring, and sardines are harvested to make fishmeal and oils used in animal feed for poultry, cattle, swine, and other fish. Growing pearls, culturing species to isolate pharmaceutical agents, breeding ornamental fish including goldfish and rare tropical fish, and propagating fish to stock recreational areas are among the many applications of aquaculture.

For example, in New Jersey, fisheries biologists breed hatchery-raised trout to create a "put-and-take" fishery for trout. Each spring the state stocks over 600,000 rainbow trout in streams, rivers, ponds, and lakes throughout the state. Many streams and rivers will not sustain trout throughout the year because they get too warm in the summer or because they are of poor water quality for trout. But through stocking ("put"), anglers are encouraged to keep what they catch ("take") for table fare. Many other states have similar stocking programs for a number of warm- and cold-water species, including panfish, bass, catfish, muskellunge, and hybrid striped bass, the last of which were created by breeding freshwater white bass and saltwater striped bass. Such programs also provide angling opportunities for people who live in urban areas with relatively little access to rural fisheries.

As shown in Table 10.1, a variety of fish and marine organisms have been cultured successfully. In many countries, this list will soon be expanded. For example, marine species such as lobsters and crabs have been farmed on a limited basis but are not yet widely available commercially.

TABLE 10.1 Important Aquaculture Organisms

Freshwater Organisms*	Marine Organisms*
Catfish	Oysters
Crawfish	Clams (hard- and soft-shell)
Trout	Marine shrimp
Atlantic salmon	Flatfishes (turbot, flounder)
Pacific salmon	Sea bass
Bait fish (fathead minnows, golden shiners, mullet, sardines)	Mussels
	Abalone
Tilapia	Quahogs
Hybrid striped bass	
Sturgeon	
Carp (silver, grass)	
Yellowtail	
Bream	
Goldfish	

*Species are ranked approximately according to total production in metric tons from highest to lowest quantity. Production of freshwater organisms exceeds that of saltwater organisms; therefore, the listing of species on the same row does not indicate equal production.

Aquaculture practices vary widely, depending on factors such as the species being farmed, life cycles of the species, environmental requirements for growth, and the length of time needed to achieve maturity for market sale. **Figure 10.2** provides an overview of fish farming. For fish such as salmon and trout, eggs (roe) and milt (sperm) are manually harvested from breeder stocks of adult fish. In an attempt to control the quality and health of the fish produced, parents used for breeder stocks are often fish that display the desired growth characteristics and physical appearance. Fish growth rate, health, quality, and the color of the meat are among the many features considered in the selection of breeder fish.

Eggs are fertilized in small tanks or containers and hatched embryos, called *fry,* are eventually transferred to larger aquarium tanks with flowing water. Most of this culturing initially occurs indoors in a "nursery." Fish typically leave the nursery when they reach *fingerling* size (several inches in length, about the length of a

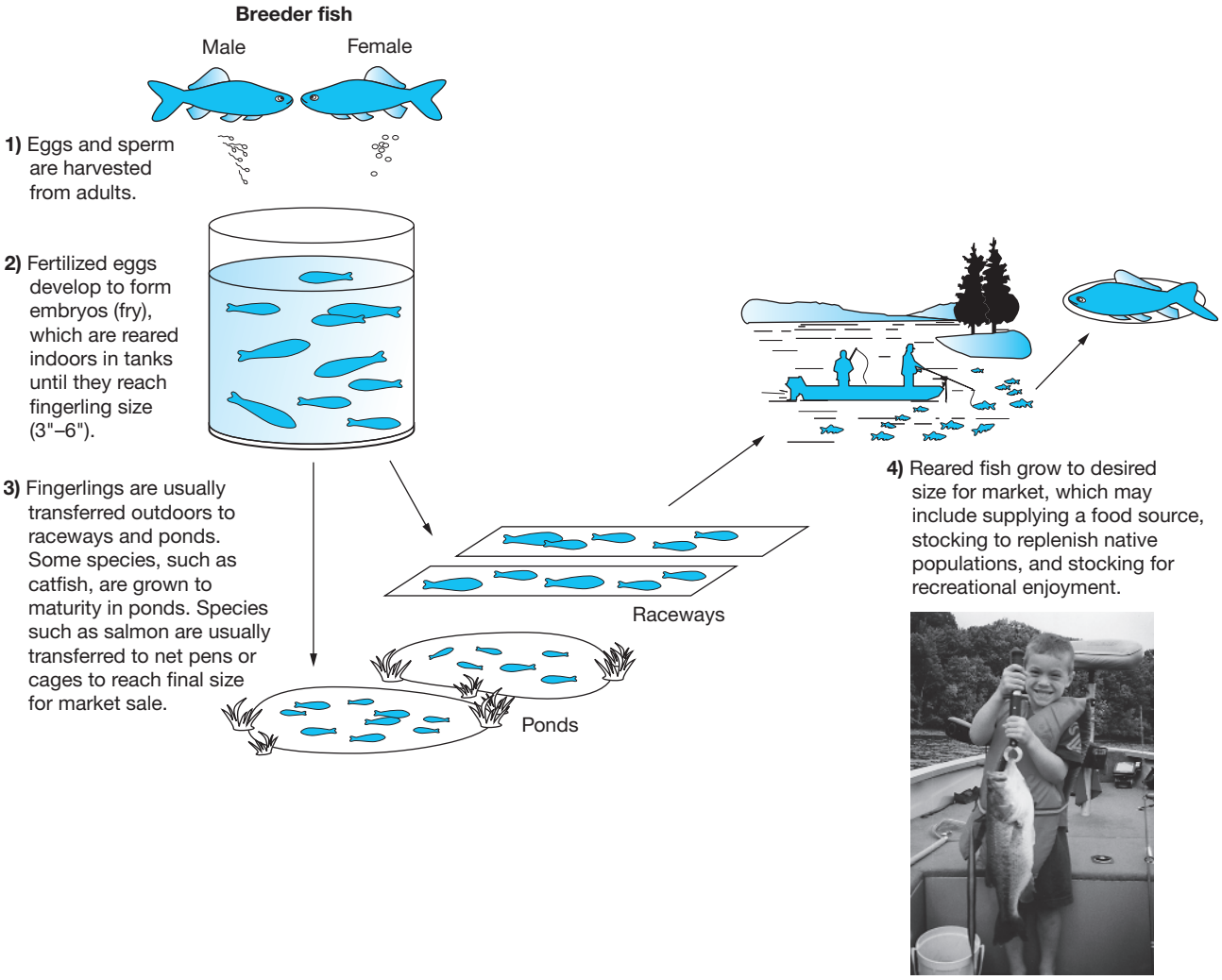


FIGURE 10.2 An Overview of Fish Farming

human finger). Fingerlings, sometimes called *smolts,* are usually moved into concrete tanks called *raceways,* which may be indoors or outdoors (**Figure 10.3**). Raceways are often designed as a series of interconnected tanks that allow for the continuous flow of water through the tanks. This is particularly important for species like salmon and trout, which are accustomed to battling the current to survive in streams and rivers. By mimicking environmental conditions, aquaculturists attempt to allow these species to develop physical characteristics similar to those of wild fish, such as the development of musculature and survival instincts.

Some fish species are raised in raceways until they reach market size, or they may be transferred to small shallow ponds for further growth before being harvested. For certain species, including salmon, after they reach a modest size, they can be raised to maturity in net pens, cages, and other enclosures that are placed in lakes, ponds, or estuaries. Shellfish farmers often use rack or cage systems that are "seeded" with large numbers of tiny nursery-raised immature shellfish such as oysters or clams that adhere to the racks. Racks are then placed in estuarine environments to allow the shellfish to grow to maturity in a natural marine environment. Typically, such racks allow for much greater production of shellfish than natural habitats. Aquaculturists are constantly modifying culturing techniques in an effort to increase the yield of a species in a given area and to minimize the expense-to-profit ratio.

Salmon farming is an excellent example of the fish-rearing process. Eggs and sperm milked from adults bred to grow quickly are used for fertilization, and the resulting embryos are raised for 12 to 18 months in tanks until they reach the fingerling stage. Fingerlings are usually transferred to mesh-enclosed pens placed in oceans or bays close to the shoreline. Throughout this process, the salmon diet often consists

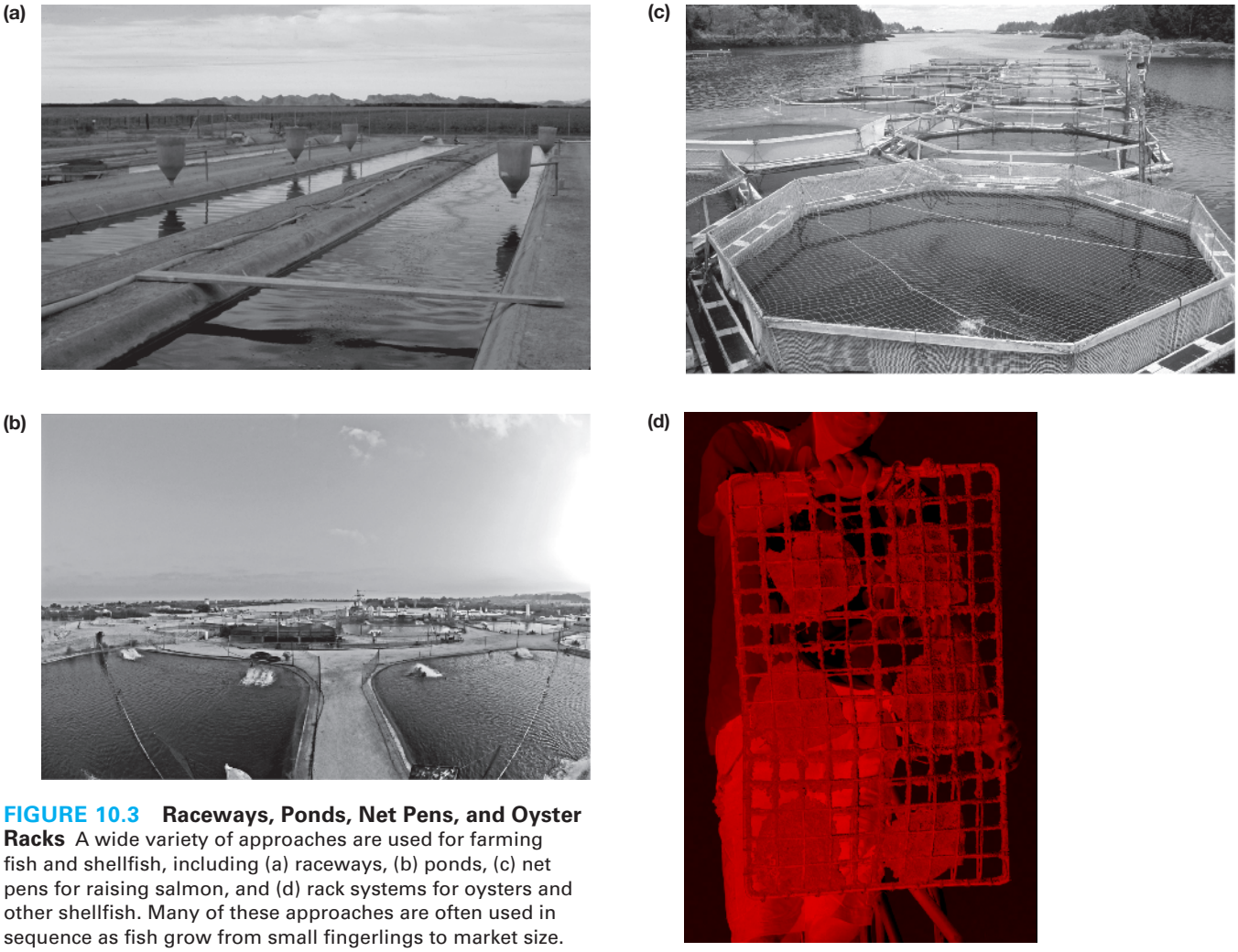


FIGURE 10.3 Raceways, Ponds, Net Pens, and Oyster Racks A wide variety of approaches are used for farming fish and shellfish, including (a) raceways, (b) ponds, (c) net pens for raising salmon, and (d) rack systems for oysters and other shellfish. Many of these approaches are often used in sequence as fish grow from small fingerlings to market size.

of pellets made from ground-up herring, anchovies, fish oil, and animal and poultry by-products (bone, excess meat). The food may also contain antibiotics, as well as additive dyes to make the salmon flesh pink, and fish may be placed in pesticide baths to remove sea lice and other parasites. Throughout the culturing process, aquaculturists can change the taste of farm-raised species by experimenting with food sources such as vegetable-based foods versus foods derived from fish products. Ultimately, unblemished fish of the appropriate size are packaged for market sale.

Innovations in fish farming

Many innovative approaches for raising fish are under development. For example, in West Virginia, fisheries biologists are taking advantage of an abundance of abandoned coal mines. The cool mountain groundwater and water from freshwater springs seep into and fill many mines, providing high-quality environments for rearing cold-water species such as rainbow trout and arctic char. This approach also provides an important use of abandoned mines that would typically serve no purpose otherwise.

Some companies have experimented with **polyculture,** also called *integrated aquaculture*—that is, raising more than one species in the same controlled environment. Raising species that have different nutrient requirements and feeding habits is one way to optimize water resources. Polyculture can involve different fish species cocultured, that is, fish and shellfish raised together, as well as animal and plant polyculture. For example, raising carp in the presence of plants such as lettuce is an effective approach. Roots from the lettuce absorb nutrients and fish waste products (such as nitrogen) from the water and use it as fertilizer to support lettuce growth. By minimizing the cost of artificial filtration and removal of wastes, polyculture limits the amount of wastewater effluent for discharge into the surrounding environment. Another aquaculture approach involves the use of **hydroponic systems.** These systems are small-volume water-flowing systems in which

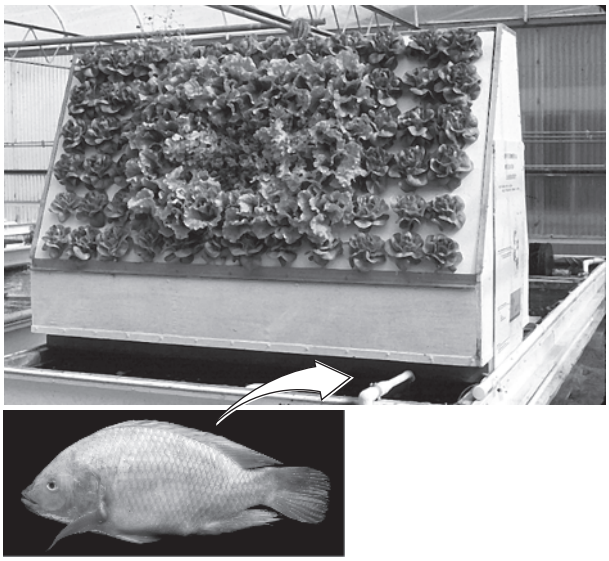


FIGURE 10.4 Polyculture of Lettuce and Tilapia in a Hydroponic System The hydroponic system of lettuce growing in racks above a tank containing tilapia (inset). Water from the tilapia tank, which contains nitrogenous wastes from the tilapia, is cycled through the racks of lettuce. Lettuce will use wastes in the water as fertilizer, thus making efficient use of the water resource.

vegetables (like tomatoes and broccoli) or herbs (like basil and chives) are cultured in racks through which wastewater from fish tanks can flow. Polyculture and hydroponic systems are frequently used together when fish such as catfish, carp, and tilapia are being raised (Figure 10.4).

Improving Strains for Aquaculture

In Section 10.3, we discuss some of the strategies used to improve strains for aquaculture through genetic engineering. Here we consider how finfish or shellfish can be improved for aquaculture through selective breeding to raise species with desirable characteristics. For instance, in a population of catfish, not all fish grow at the same rate or have the same body characteristics, such as muscle mass compared with the percentage of body fat. Scientists can use a number of techniques to identify fish that show fast growth rates and heavy muscle mass. They can use ultrasound machines to measure muscle mass as a means to estimate fillet yield. Fish that show the highest yield are then bred to produce new generations with increased muscle mass.

Scientists at the U.S. Department of Agriculture (USDA) Catfish Genetics Research Unit in Mississippi have selectively bred a variety of catfish called USDA 103 (a name worthy of a government-issue catfish!) that grows 10 to 20 percent faster in ponds, trimming the time from birth to market to between 18 and 24 months (refer to Figure 10.1). Some USDA 103 catfish can reach sexual maturity a full year faster—by 2 years of age—than other species. On the negative side, USDA 103 catfish consume more feed than other varieties of catfish.

In Arizona, researchers have used selective breeding to identify shrimp that are acclimated to growing in low-salinity water. Starting with larval shrimp grown in fresh water with added salt, scientists culture these shrimp through tanks of water with progressively lower salt concentrations. This allows for the farming of high-quality shrimp without the need for saltwater, and low-salt water can then be recycled to irrigate fields of fruits and vegetables. Thus, shrimp can be raised in the desert of Arizona hundreds of miles from natural sources of saltwater.

Enhancing the Quality and Safety of Seafood

Many fish-farming efforts involve activities designed to improve certain qualities, such as growth rate, fat content, taste, texture, and color of the finfish or shellfish being raised. Aquaculture can be combined with cutting-edge techniques in molecular biology to create finfish and shellfish species that have the characteristics consumers want. In addition, scientists are working to make seafood safe, so that it is free of pathogens and chemical contaminants.

Igene Biotech of Columbia, Maryland, has used recombinant DNA technology to mass produce **astaxanthin,** the pigment that gives shrimp their pink color. By including recombinant astaxanthin in fish feed, scientists can create salmon and trout with a rainbow of hues in flesh color. Astaxanthin is also thought to have potential value as an antioxidant to be used in nutritional supplements for humans. Most people like salmon with a pink color. Consumer polls suggest that many people believe the redder the salmon, the higher its quality. Dark red salmon is highly prized in the best sushi bars in Japan. Astaxanthin has no effect on the taste of salmon, but farmers want to grow what consumers prefer. The Swiss drug company Roche Holding AG, a leading producer of astaxanthin, distributes a "salmofan," which resembles a paint-color chart. Aquaculturists can pick shades ranging from light pink to dark crimson and then purchase food with the concentration of astaxanthin that will produce fish with the flesh color they desire.

A variety of approaches to enhance seafood quality and safety are currently being used, and many more are in development. From detecting contaminated seafood to enhancing the taste and shelf life of seafood, aquatic biotechnologists are working to apply innovative technologies. Agilent Technologies Inc., an American public research, development, and manufacturing company,

produces a DNA microarray (chip) that can be used to identify fish species in food products. Port inspectors, government agencies, seafood processors, distributors, and others can use this technology to determine the fish species present in frozen and fresh samples of fish. Similar techniques are used to fight illegal fish-catching poachers by identifying species that were illegally harvested or intentionally mislabeled (see Chapter 8 for forensic identification of fish species).

Marine scientists are using molecular biology techniques to identify and learn more about genes encoding toxins produced by marine organisms; this can help them to understand how these toxins cause disease and to minimize the negative health impacts of toxin exposure. Many molecular probes and polymerase chain reaction (PCR)–based assays are being developed for the detection of bacteria, viruses, and a host of parasites that infect finfish and shellfish. Gene probes have been developed for detecting viral diseases in shrimp, and companies are developing gene probes for detecting and assessing the effects of environmental stresses on fish and shellfish.

A handheld antibody test kit has been developed to detect *Vibrio cholerae* in oysters. It uses antibodies to detect proteins from *V. cholerae*, the bacterium that causes **cholera**—a very serious illness characterized by severe diarrhea; in severe cases, this can lead to dehydration and death. Cholera is particularly a problem in developing nations with water supplies that are contaminated because of inadequate sewage treatment facilities. This kit has been widely and successfully used in South America to detect contaminated seafood and minimize cholera infections. Similar approaches have been used in Hawaii to develop a dipstick monoclonal antibody test for ciguatoxin, which affects finfish from tropical areas that are sold in the aquarium industry.

Aquatic biotechnologists are also interested in developing detection kits or vaccines for a number of pathogens that pose serious threats to fish raised by aquaculture. Several companies are piloting vaccines for infectious salmon anemia, a deadly viral condition that causes internal bleeding and destroys internal organs in salmon and leads to the death of hundreds of thousands of salmon worldwide each year. A PCR-based test is available to detect the anemia virus, but there is still no vaccine that is widely available.

Similar work is being carried out to combat sea lice, such as *Caligus elongatus*. This tiny parasite attaches to salmon, feeding on salmon blood and creating exposed lesions that render salmon susceptible to deadly infections. Scientists in the United States have developed a subunit vaccine against the virus causing infectious hematopoietic necrosis (IHN), which is responsible for the loss of large numbers of commercial trout and salmon each year. The vaccine was developed from an IHN protein expressed in bacteria. This vaccine, which protects fish against IHN, is injected into juvenile fish to stimulate the production of antibodies against the IHN virus.

Barriers and Limitations to Aquaculture

Although aquaculture is firmly established as a global application of aquatic biotechnology that is having an impact on billions of people, there are concerns and obstacles. Not all species are ideally suited for aquaculture. For some species, it is difficult to maintain water with the proper flow rate to deliver adequate concentrations of nutrients and remove rapidly accumulating waste products. This is especially true for many highly prized marine species such as tuna that require large areas of ocean for roaming and do not survive when confined to small living spaces. In addition, marine organisms often have long, complicated life cycles involving a series of larval stages, each of which may have different food requirements before marketable size can be achieved. To raise adults of these species, the loss of young fingerlings can be very high and thus prohibitively costly. Aquaculture is easiest with species that have few larval stages.

Aquaculturists are constantly faced with the problem of minimizing the effects of disease on their fish populations. Given the dense, crowded conditions in which many fish are raised, farmed fish are often more susceptible than native stocks to stress and disease caused by bacterial and viral pathogens as well as lice. Because farmed fish have less genetic diversity and disease resistance, infections can spread rapidly. Most fish in a farmed population will have the same resistance and susceptibility to disease; therefore, a large supply of fish can be wiped out quickly if disease is not controlled (see Question 7 at the end of this chapter for an example).

There is concern that the aquaculture industry is consuming excessive amounts of wild bait fish, such as anchovies and herring. Although some bait fish are raised by aquaculture, in many areas of the world, bait fish used to make fishmeal are still netted from wild stocks. For every pound of salmon, several pounds of other typically wild-caught fish—such as anchovies, herring, and mackerel—are used to raise the salmon. Growing 1 pound of salmon can require 3 to 5 pounds of wild-caught fish. Scientists are looking for ways to change the carnivorous feeding habits of some farmed species (including alligators!) by switching them to vegetable-based diets, but aquaculturists are concerned about the growth rates and food quality of these fish. Supporters of vegan diets for farmed fish claim that some people may like salmon that tastes less fishy.



YOU DECIDE

The Risks of Fish Pollution and Genetic Pollution: Controversies of Aquaculture and Genetically Engineered Species

The term *fish pollution* describes the escape of aquaculture species that can harm natural ecosystems by altering or reducing biodiversity; introducing new parasites; competing with native species for food, habitat, and spawning grounds; breeding with wild fish; and destroying habitats. Farm-raised fish do escape. Many examples of fish pollution and its effects have been documented. For example, in some waterways in Florida, tilapia—a rapidly growing aquaculture panfish with a voracious appetite—have outcompeted native species such as bream for spawning areas and food. As a result, some native fish in tilapia-polluted areas have virtually disappeared. Similarly, nonnative species of Pacific white shrimp farmed along the Gulf Coast of Texas and the Atlantic Coast of South Carolina have escaped and are free swimming in these areas.

We know that farm-raised Atlantic salmon are breeding in waters of the Pacific Northwest. Twenty-six stocks of Pacific salmon and steelhead are currently listed as endangered or threatened. Many scientists believe that the loss of these native species is due to the spread of lice and other parasites from farmed fish. In Norway, an analysis of genetic markers in more than 21,000 wild Atlantic salmon from nearly 150 locations demonstrated that wild fish in about half of these locations contain genetic material (in some cases more than 42 percent) from farmed salmon.

A December 2000 northeaster in Maine—where salmon farming is the second-largest fishery behind lobstering—caused the uprooting of steel cages containing farm-raised salmon and released over 100,000 fish into Machias Bay. This accident is thought to have been the largest known escape of aquaculture fish in the eastern United States. Many are concerned that this spill will weaken the genetic potential of future generations of wild salmon in Maine rivers. The U.S. government has already listed wild salmon in eight Maine rivers as endangered as a result of concerns about the impact of aquaculture in the area. In some spawning rivers in New Brunswick and Maine, farmed escapee salmon greatly outnumber wild salmon.

The escape of farmed fish has caused environmentalists and other groups to rally for a moratorium on new fish farms in many areas of the world and for regulation of the aquaculture industry. Many aquaculture species are similar to domestic farm animals in that they have become reliant on humans and are poorly adapted to life in the wild. Evidence indicates that farmed salmon produce smaller and perhaps less fertile eggs than wild stocks. Research suggests that aquaculture selects for genes and traits that allow fish to grow in confinement and this puts animals at a disadvantage in the wild. A study published in 2017 reported that fast-growing salmon farmed in Norway, Chile, Scotland, Canada, and Australia were three times more likely than native, slower growing fish to have a deformity in the inner ear that can cause fish to lose up to 50 percent of their hearing. What effect a hearing deficit may have on fish survival is unclear but there is concern that this deficiency may be passed to wild stocks by farmed fish that escape into the wild.

Even stronger concerns have been raised about the escape of genetically modified species such as transgenics and triploids. Although most of these species cannot reproduce, no technique ensures that all modified fish will be sterile. Once in the wild, oceans cannot be drained to retrieve them. Transgenic stocks that escape and grow in areas outside their intended growing range often do not grow as well as they do in their intended environment. As a result, if they breed with native species, mixed stocks often lose biological fitness (the ability to reproduce) and grow more slowly. By introducing transgenic species, it is possible to erode adaptations that have occurred over thousands of years and reduce the fitness of native stocks. Alternatively, fish with enhanced growth capabilities may be able to outcompete native fish for food and spawning sites. In 2001, Maryland became the first state to ban the raising of genetically modified fish in ponds and lakes connected to other waterways.

Critics also cite historic examples of the spread of nonnative species as reason for concern. For example, European zebra mussels—thought to have entered the United States in ballast water of oceangoing ships entering the Great Lakes during the 1980s—have created significant problems. Colonies of these prolific shellfish have smothered habitat for other species; through filter feeding, they have caused the decline of native plankton in the Great Lakes, blocked pipes, and coated the hulls of ships. Costs associated with controlling zebra mussels in the Great Lakes region alone are estimated to exceed $400 million annually.

Even though the U.S. Food and Drug Administration (FDA) is involved in regulating the safety of transgenic fish as a food source, no clear policies exist for regulating the release of aquaculture species (including transgenics) in the United States. The USDA is looking closely at ways to assess the risk of bioengineered species and the safety of introducing genetically modified organisms into the environment in both marine and nonmarine environments. Many believe that aquaculture and genetic engineering are necessary to produce enough food for an increasing human population, but are the risks of these technologies greater than the risks of doing nothing? You decide.

Runoff of animal feces and wastes from traditional land farming is a problem that has significant impacts on the environment. Similarly, there is concern about pollution from fish-farming industries. The waste-laden effluent water from aquaculture operations—which contains untreated feces, urine, and uneaten food—is typically discharged into natural waterways. These wastes are rich in nitrogen and phosphorus and can lead to algal blooms and other problems. Dying algae rob water of oxygen, which can lead to fish kills. Fish wastes also harbor strains of bacterial pathogens such as *Salmonella* and *Pseudomonas*, which can affect human health; however, the likelihood of disease transmission from fish wastes to humans has not been well established. At present, it is generally thought that waste production from aquaculture has a small overall effect on water quality, but this may change as aquaculture efforts increase worldwide.

The extermination of "pest" species at aquaculture facilities is another concern. A number of fish-eating birds (such as cormorants, pelicans, herons, egrets, and gulls) as well as mammals (such as seals and sea lions) can be subjected to authorized and unauthorized capture and extermination. However, many facilities employ nonlethal methods (including visual harassment with the use of lights, reflectors, scarecrows, human presence, and auditory devices such as predator distress calls, sirens, and electronic noisemakers) to deal with these "pest" species. Underwater acoustic and explosive devices may also be used, along with perimeter fencing and protective netting to discourage predators from feeding at fish farms.

Concern has also been raised about the discharge of chemicals commonly used in many aquaculture operations. These include antibiotics, pesticides, herbicides (plant killers), algicides (algae killers), and chemicals used to control parasites. In addition, residual chemicals from other antifouling compounds (such as those used to reduce the growth of barnacles, algae, and other organisms) and anticorrosives may be absorbed by fish and shellfish and passed to other marine organisms and humans. A comprehensive survey of farmed salmon has found that they contain significantly higher levels of polychlorinated biphenyls (PCBs) and other, similar compounds correlated with increased risk of cancer than their wild relatives. This is a major concern for the industry, and fish farmers are working on ways to reduce pollutants in farmed salmon.

A number of laws regulate aquaculture and its potential effect on environment. The United Nations Environment Program continues to address the management of marine natural resources through the Law of the Sea treaty. Few countries like the USA, Canada, New Zealand, Norway, France, and Ireland have developed a variety of legislations that directly deal with aquaculture and fisheries. Several other countries such as Portugal have provisions on aquaculture in the form of a section in the basic Fisheries Act.

The visual effects of aquaculture on the landscape are also problematic. For instance, in some areas of Scotland, there are concerns that the abundance of shellfish cages along the Scottish coastline detracts from the natural, aesthetic appeal of coastal landscapes. Finally, a very serious issue raised by both aquaculturists and naturalists is the potential threat to native species by farm-raised fish that accidentally escape into the wild (refer to the "You Decide" box, on the previous page).

So far, we have taken a comprehensive look at the aquaculture industry—one of the oldest applications of aquatic biotechnology. As you have learned, aquaculture is not without its controversies. In the next section, we explore an area in its infancy: the molecular genetics of aquatic organisms.

10.3 Genetic Technologies and Aquatic Organisms

One purpose of improving our understanding of the genomes of aquatic organisms involves manipulating the genetics of these species for biotechnology applications. Scientists are working to improve the survival and growth of aquaculture species, and molecular biology is being used to learn more about the immune systems of aquatic species and their susceptibility and resistance to disease-causing organisms, including disease transmission and the life cycles of the pathogens themselves.

Analysis of Novel Genes from Aquatic Species

Genomic analysis of aquatic organisms covers many interesting areas. Basic knowledge of gene expression and regulation in aquatic organisms and an understanding of genes involved in processes such as reproduction, growth, development, and survival in extreme environmental conditions will be critical for applications such as maintaining populations of endangered marine species, limiting populations of harmful species, and advancing the genetic manipulation of aquatic organisms for aquaculture. In addition, as mentioned in Section 3.2, pathogens that affect finfish and shellfish result in large financial losses each year.

Many research groups are involved in identifying mutations associated with diseases in fish. Eventually, such information will be used in the development of disease-free breeder stocks of fish with selected characteristics. By identifying deleterious genes that may have negative influences on fish growth, health, and longevity, scientists anticipate that many of these genes can be modified or removed to produce improved species for aquaculture. Discovering genes that can be used as probes for the identification of microscopic marine organisms such as phytoplankton and zooplankton—important food sources for many marine species—will help scientists look at environmental effects on the genetics of these microorganisms. This is a critically important issue for understanding how these microscopic organisms affect organisms higher up in the food chain.

Genes are being identified as DNA markers that can be used to distinguish wild stocks of fish from hatchery-reared stocks and markers to identify disease-resistant strains that are more receptive to fish farming. Such markers will also play important roles as scientists attempt to determine the effects of farm-raised escapees on native stocks. Many of these marker genes are species-specific, and they have enabled fish and wildlife officers to apply PCR and sequencing techniques in investigating cases of illegal harvest and retail of fish that are subject to quotas. For example, in the northeastern United States, blackfish are prized table fare. But the overharvesting of blackfish has resulted in strict size limits and restricted seasonal dates for legally harvesting blackfish. If wildlife officials encounter someone they suspect to be a poacher harvesting blackfish during the closed season but the poacher has only fillets in the boat and not intact carcasses, how can officials determine what species the fillets came from? Using blackfish-specific primers, PCR assays can be run on DNA isolated from fillets to determine if the fillets are from blackfish or not.

Cloning the gene for **growth hormone (GH)** is an excellent example of how the gene discovery process can lead to advances in marine biotechnology. We have discussed how the cloning of human GH led to treatments for dwarfism (Chapter 3). Recall that GH is a hormone produced by the pituitary gland that stimulates the growth of bone and muscle cells during adolescence. Cloning of the salmon GH gene has led to the development of **transgenic** species of salmon that demonstrate greatly accelerated growth rates compared with native strains. We consider the GH example in more detail in the next section. As another example, University of Alabama–Birmingham researchers have cloned the gene for molt-inhibiting hormone (MIH) from blue crabs. Molting (shedding) is triggered by a decrease in MIH, leading to the production of soft-shelled crabs that can be eaten whole. Current research is under way to block the release of MIH as a way to produce soft crabs on demand for the seafood industry.

Antifreeze proteins

One of the most successful examples of the identification of novel genes with promising applications involves genes for **antifreeze proteins (AFPs).** A/F Protein, Inc. of Massachusetts is a leader in the production of antifreeze proteins. Many of the first AFPs were isolated from bottom-dwelling fish species such as northern cod, which live off the coast of eastern Canada, and Antarctic fish called teleosts, which live in extremely cold ice-laden waters—some of the most severe environments on earth. Subsequently, AFPs have been isolated from a number of other cold-water species, including winter flounder (*Pleuronectes americanus*), sculpin (*Myoxocephalus scorpius*), ocean pout (*Macrozoarces americanus*), smelt (*Osmerus mordax*), and herring (*Clupea harengus*). Interestingly, similar proteins have been discovered in insects, bacteria, and other organisms that can survive cold temperatures.

Several types of AFPs have been discovered. Structurally, most AFPs have extensive alpha helices and are held together by large numbers of disulfide bridges. AFPs function to lower the freezing temperature of fish blood and extracellular fluids, thus protecting fish from freezing in frigid marine waters. Seawater freezes at approximately –1.8°C. AFPs typically lower the freezing point of fish body fluids by approximately 2°C to 3°C. Currently, the majority of AFPs are isolated from fish blood. To meet the heavy demands that are expected for applications of AFPs in the future, scientists have successfully cloned and expressed recombinant AFPs from different species in bacterial and mammalian cells.

AFPs protect living organisms from freezing in a variety of ways. They can bind to the surfaces of ice crystals to modify or block ice crystal formation, lower the freezing temperature of biological fluids, and protect cell membranes from cold damage. Because of these unique abilities, a number of innovative applications for these cryoprotective proteins are being developed. AFPs are being used to create transgenic fish and plants with enhanced resistance to cold temperatures and freezing. For instance, salmon cannot produce antifreeze molecules; thus, they die when exposed to near-freezing water. Waters off the coast of eastern Canada, too cold for wild species of salmon, are being considered as potential aquaculture habitat for freeze-resistant species of transgenic salmon containing AFP genes.

AFP gene promoter sequences are also being used in recombinant DNA experiments to stimulate the

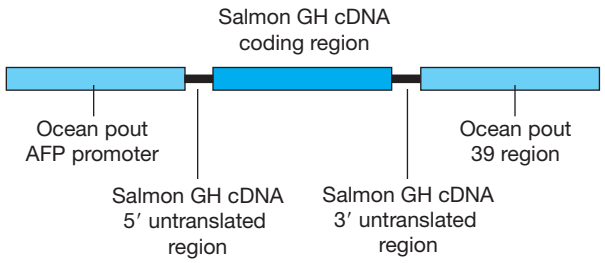


FIGURE 10.5 AFP Promoters Stimulate Gene Expression in Transgenic Fish A recombinant DNA plasmid construct containing an antifreeze protein gene promoter from ocean pout attached to the complementary DNA (cDNA) for salmon growth hormone can be used to produce rapidly growing fish for aquaculture. Transcription from the AFP promoter is stimulated by cold conditions; therefore, transgenic fish containing this construct synthesize large amounts of growth hormone when they are raised in cool water. Increased production of growth hormones causes transgenic fish to grow faster than native non-transgenic strains.

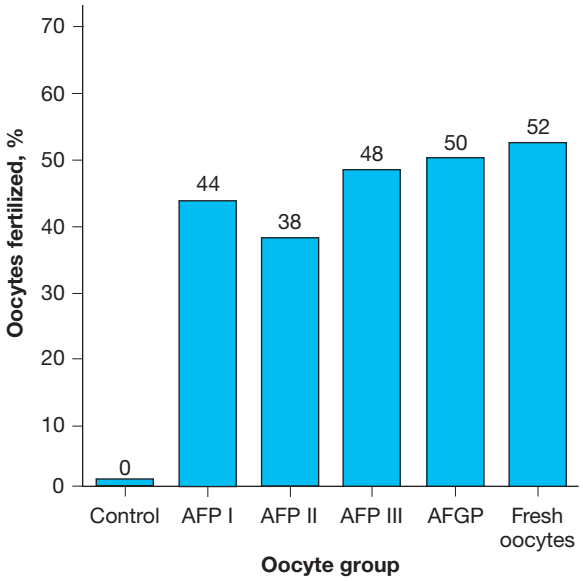


FIGURE 10.6 AFPs as Cryoprotectants of Human Tissues Bovine oocytes were incubated for 24 hours at 4°C in the absence (control) or presence of different types of AFPs or antifreeze glycoprotein (AFGP), a carbohydrate-rich type of AFP, and then fertilized with bovine sperm. Notice how the AFP- and AFGP-treated oocytes show fertilization rates similar to those of fresh oocytes, thus indicating that AFPs can provide cold protection of oocytes that will maintain their ability to be fertilized.

expression of transgenes, including GH in salmon. Transcription from AFP promoter sequences is stimulated by cold temperatures. By ligating genes of interest to the 3′ end of AFP promoter sequences, AFP promoters can be used to stimulate the transcription of these "downstream" genes under cold-water conditions (Figure 10.5). Thus, AFP promoter gene constructs can serve as very effective transcription vectors to transcribe foreign genes, leading to the increased production of protein. Such constructs may be very effective for the genetic engineering of fish, as we discuss in the next section.

For many popular crops such as wheat, coffee, fruit (e.g., citrus, apples, pears, cherries, and peaches), soybean, corn, and potatoes, crop damage as a result of cold temperatures is a problem worldwide. AFPs have been introduced into plants such as tomatoes to produce cold-hardy transgenic strains, but these are not widely available yet because commercial crop plants do not produce cryoprotective proteins as effective as AFPs from fish.

Cryoprotection of human cells, tissues, and organs is a promising medical application of AFPs. As shown in Figure 10.6, cold storage of oocytes used for *in vitro* fertilization is one current application. Studies have demonstrated that AFPs may prove useful for the storage of a number of human tissues, including blood, and for the development of new protocols for the cryogenic storage of human organs such as the heart and liver prior to their use in transplantation surgery.

Finally, scientists are investigating ways that AFPs may be used to improve the shelf life and quality of frozen foods, including ice cream. Changes in the quality of frozen foods often occur during thawing and refreezing, as when you bring home food from the store and put it in your home freezer. It is possible that AFPs can be used to alter the ice crystallization properties of frozen foods to improve their overall quality. Scientists have even proposed using AFPs to control ice formation on aircraft and roadways. As you can see, AFPs may be useful for a number of interesting applications. Undoubtedly, the ocean harbors species containing many other novel genes that may be exploited to benefit biotechnology in the future.

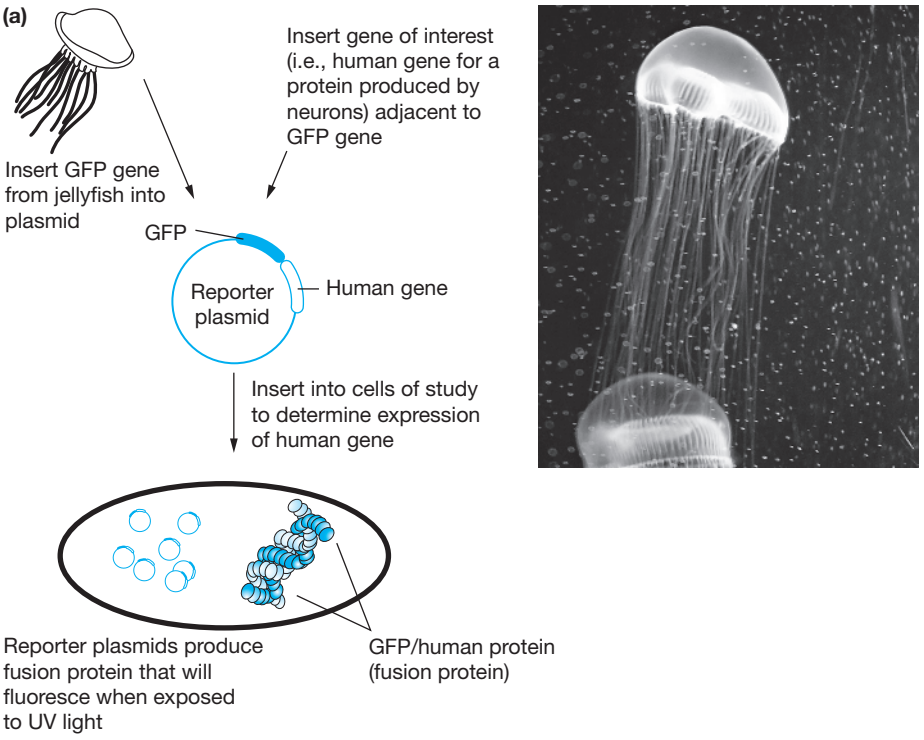
"Green genes"

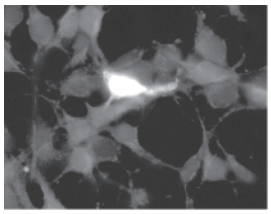
An outstanding example of an aquatic biotechnology research application, and perhaps the most well known modern application of aquatic biotechnology, involves a novel gene from the bioluminescent jellyfish *Aequorea victoria*. *A. victoria* can fluoresce and glow in the dark because of a gene that codes for a protein called **green fluorescent protein (GFP).** GFP produces a bright green glow when exposed to ultraviolet light. It has been estimated that nearly three-fourths of all marine organisms have bioluminescent abilities. Bioluminescence is often used as a way for fish and other organisms to find each other in the dark environments of the ocean during mating activities. Genes for red, orange, and yellow fluorescent proteins have been cloned from sea anemones, expanding the color palette of proteins available for researchers. Previously, we mentioned

that the fluorescence of some marine species is created by bioluminescent bacteria such as *Vibrio harveyi* and *Vibrio fischeri* (Chapter 5). Osamu Shimomura, Martin Chalfie, and Roger Tsien shared the 2008 Nobel Prize in Chemistry for the discovery and development of GFP.

Scientists have taken advantage of the fluorescent properties of GFP and other similar proteins to create unique **reporter gene** constructs. Reporter genes allow researchers to detect the expression of genes of interest in a test tube, cell, or even a whole organ. As shown in **Figure 10.7a**, reporter gene plasmids are created by ligating the GFP gene to a gene of interest and then introducing the reporter plasmid into a cell type of choice. Once inside cells, these plasmids are transcribed and translated to produce a fusion protein that fluoresces when exposed to ultraviolet light. Only cells producing the GFP fusion protein fluoresce. In this manner, these plasmids serve to detect or "report" where the gene of interest is being expressed.

This approach has been widely used to study basic processes of gene expression and regulation. Developmental biologists have used GFP-expressing embryonic stem cells to track their differentiation into different cell types during the development of embryos. Scientists have also used GFP reporter gene constructs in many innovative ways to advance medical diagnostics and disease treatment (**Figure 10.7b**).





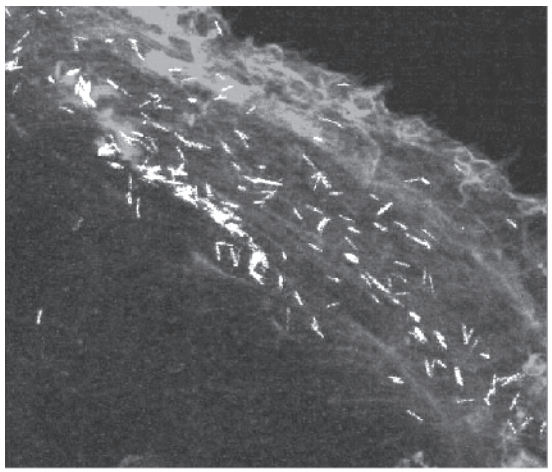


FIGURE 10.7 The GFP Gene Is a Valuable Reporter Gene (a) GFP reporter gene constructs can be created in plasmids by ligating the GFP gene to a gene of interest. In this example, a human gene encoding a protein produced by neurons is attached to the GFP gene and the plasmid is introduced into cells in culture. These cells will then express mRNA molecules, which will be translated to produce a fusion protein in which GFP is attached to the cloned human protein. Cells producing this fusion protein will fluoresce when exposed to ultraviolet light, "reporting" the location of the expressed protein. (b) A GFP reporter gene used to detect a protein in human neurons involved in the neurodegenerative condition Huntington's disease. (c) GFP reporter plasmids used to detect disease-causing bacteria in the human digestive tract, such as the *Campylobacter jejuni* species shown here infecting human intestinal cells. *C. jejuni* is a common contaminant of chicken, causing approximately 2.4 million cases of food poisoning in the United States each year.

For example, GFP genes have been used to pinpoint tumor formation in mice, to follow the progress of bacterial infections in the intestines, to monitor the death of bacteria following antibiotic treatment, and to study the presence of food-contaminating microorganisms in the human digestive tract (Figure 10.7c).

Sequencing genomes of marine pathogens

Marine biologists are interested in sequencing genomes of a variety of marine pathogens that affect wild and farmed species as a way to learn about genes these organisms use to reproduce and cause disease. Chilean scientists have sequenced the genome of the bacterium *Piscirickettsia salmonis*. *P. salmonis* infects salmon and causes a disease called rickettsial syndrome, which affects the livers of infected salmon, leading to their death. Combating this syndrome is a worldwide problem for salmon farmers. Currently, no effective treatment exists. In Chile's salmon industry alone, this syndrome causes financial losses of more than $100 million per year. Armed with genome information about pathogens, scientists are trying to bolster the immune systems of farmed species to improve their resistance to pathogens. For example, it may be possible to inject genes or proteins from *P. salmonis* into salmon as vaccines to stimulate their immune systems to defend against infection by these bacteria.

Scientists are working on ways to use molecular approaches in the battle against diseases and parasites that have essentially eliminated commercial fishing for oysters in several areas of the United States. Across the country, oysters have been under siege. As a result of overharvesting, pollution, habitat destruction, and parasitic and viral diseases, oyster stocks are nonexistent in many areas formerly known as rich sources of oysters for human consumption. Protozoans have caused substantial damage to oyster populations in the eastern United States. The parasites Dermo (*Perkinsus marinus*), SSO (*Haplosporidium costale*), and MSX (*Haplosporidium nelsoni*) have devastated eastern oyster (*Crassostrea virginica*) populations in many areas of the country, such as the Chesapeake Bay in Maryland and the Delaware Bay in New Jersey. Similar problems have occurred along the Gulf Coast and California.

Until recently, part of the difficulty in combating these diseases was a lack of sufficient numbers of parasites to study. Cell culture techniques have been used to overcome this problem, and researchers now know a great deal about the genetics of Dermo, SSO, and MSX. Molecular techniques have been used to learn about the life cycle of these parasites, and molecular probes are available for their detection in the field and in aquaculture facilities. For instance, PCR-based approaches have proven very effective for the rapid and sensitive detection of Dermo, SSO, and MSX, allowing aquaculturists to screen and identify diseased oysters before widespread infections occur (Figure 10.8).

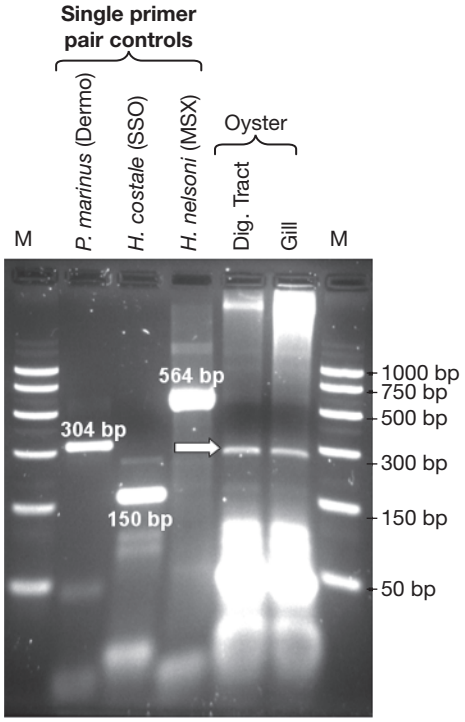


FIGURE 10.8 PCR Can Be Used to Detect DNA from Oyster Pathogens Dermo, SSO, and MSX are significant causes of mortality in oysters. PCR can be used to detect DNA from these pathogens in oyster tissues, helping scientists determine when these parasites are present, in an effort to control infection that can decimate large populations of these shellfish. In this photograph of an agarose gel, notice that DNA samples from the digestive tract and gill of an oyster show the presence of DNA for Dermo (indicated by the arrow). M, DNA size markers.

A greater understanding of genes involved in the oyster's immune system has also led to new strategies for transferring genes for disease resistance from species such as the Pacific oyster (*Crassostrea gigas*) into the more susceptible species of eastern oysters, such as *C. virginica*. In the future it may be possible to create transgenic species of oysters with genes that will provide resistance to damage by these and other parasites.

Genomics and aquatic organisms

Not surprisingly, genomics is being used to sequence and study genomes of a number of aquatic species ranging from metagenomics projects for large and diverse communities of marine microbiomes to analyzing the octopus genome. An international team of scientists completed the sequencing of the sea urchin's genome. Sea urchins are echinoderms, a group of marine animals that include starfish and sea cucumbers. Urchins are believed to have originated over 540 million years ago. Their genome has been of great interest to molecular biologists because urchins share a

common ancestor with humans. From this ancestor a superphylum of animals called the deuterostomes arose; included in this phylum are echinoderms, humans, and other vertebrates. Because deuterostomes are more closely related to each other than to animals not in this superphylum, genome scientists expected that sea urchins would be genetically similar to humans. Comparative genomic studies have shown that we share approximately 7,000 genes with urchins, including genes involved in hearing, balance, immunity, and development. We therefore expect that urchins will continue to be very valuable experimental model organisms for molecular biologists studying both aquatic genomes and the human genome.

Genetic Manipulations of Finfish and Shellfish

Biologists throughout the world are using genetic techniques to create breeds of finfish and shellfish with such desired characteristics as improved growth rates and disease resistance. Table 10.2 shows examples of different species that have been genetically engineered for a variety of purposes.

Genetically engineered species: transgenics and polyploids

In Chapters 6 and 7, we have discussed how **transgenic organisms (transgenics)** can be created. Transgenic fish contain DNA from other species, and interest in transgenic fish has increased along with the aquaculture industry. Aquaculturists are interested in genetically engineering fish designed to grow faster and healthier. As shown in Table 10.3, many species have been genetically modified for potential applications in the aquaculture industry. Foreign genes have been inserted into finfish and shellfish to accelerate growth, increase cold tolerance and disease resistance, and alter flesh qualities to improve table quality.

Cuba is perhaps the most progressive country in the world when it comes to the use of genetically modified foods, particularly seafood. Genetically modified tilapia are sold for human consumption in Cuba. Similarly, Norwegian companies have developed genetically modified farm-raised tilapia that grow nearly twice as fast as wild tilapia. Although genetically modified strains of corn, soybeans, tomatoes, and other crops have been sold in the United States for more than 20 years, until 2013 no transgenic animal had been approved by the FDA for human consumption.

AquaBounty Technologies in Massachusetts has produced transgenic Atlantic salmon containing a GH gene and a gene regulatory sequence from Pacific Chinook salmon (*Oncorhynchus tshawytscha*)—a species that demonstrates more rapid growth and larger adult-size capabilities than most Atlantic salmon, as well as a regulatory sequence from an eel-like organism called ocean pout (*Zoarces americanus*). Atlantic salmon normally stop growing in the winter; not so with these transgenics, called the AquAdvantage® salmon, which produce GH all year long and grow twice as fast as non-transgenic farmed salmon, even though they eat approximately 10 percent less food (see Chapter Opening Figure). AquAdvantage salmon reach market size in 18 months instead of the traditional 36 months. Founded in 1991, AquaBounty has spent more than $67 million to develop these fish. The company initiated discussions with the FDA in 1993, but at that time the agency did not have a process for reviewing GM food animals. In 1995, the company applied for FDA approval. By 2013, AquaBounty was

TABLE 10.2 Genetically Engineered Fish and Shellfish

Fish Species	Shellfish
Atlantic salmon	Abalone
Bluntnose bream	Clams
Channel catfish	Oysters
Chinook salmon	
Coho salmon	
Common carp	
Gilthead bream	
Goldfish	
Killifish	
Largemouth bass	
Loach	
Medaka	
Mud carp	
Mummichog	
Northern pike	
Penaeid shrimp	
Rainbow trout	
Sea bream	
Striped bass	
Tilapia	
Walleye	
Zebrafish	

Source: Adapted from Goldberg R, Triplett T. *Something Fishy.* Environmental Defense, 2000; www.environmentaldefense.org.

TABLE 10.3 Examples of Genetically Modified Organisms Being Tested for Use in Aquaculture

Organisms	Foreign Gene	Desired Effect and Comments	Country
Abalone	Coho salmon GH + various promoters	Increased growth	United States
Atlantic salmon	AFP	Cold tolerance	United States, Canada
	AFP + Chinook salmon GH	Increased growth and feed efficiency	United States, Canada
Channel catfish	GH	33% growth improvement in culture conditions	United States
Chinook salmon	AFP + Chinook salmon GH	Increased growth and feed efficiency	New Zealand
Coho salmon	AFP + Chinook salmon GH	After 1 year, 10- to 30-fold growth increase	Canada
Common carp	Salmon and human GH	150% growth improvement in culture conditions; improved disease resistance; tolerance of low oxygen levels	China, United States
Cutthroat trout	Chinook salmon GH + AFP	Increased growth	Canada
Goldfish	GH AFP	Increased growth	China
Oysters	Coho salmon GH + various promoters	Increased growth	United States
Rabbit	Salmon calcitonin-producing gene	Calcitonin production to control calcium loss from bones	United Kingdom
Rainbow trout	AFP + Chinook salmon GH	Increased growth and feed efficiency	United States, Canada
Salmon	Rainbow trout lysosome gene and flounder pleurocidin gene	Disease resistance; still in development	United States, Canada
*Strawberries and potatoes	AFP	Increased cold tolerance	United Kingdom, Canada
Striped bass	Insect genes	Disease resistance; still in early stages of research	United States
Tilapia	AFP + Chinook salmon GH	Increased growth and feed efficiency; stable inheritance	Canada, United Kingdom
	Tilapia GH	Increased growth and stable inheritance	Cuba
	Modified tilapia insulin-producing gene	Production of human insulin for persons with diabetes	Canada

*Non-aquaculture species but involve use of AFP gene.
Notes: The development of transgenic organisms requires the insertion of the gene of interest and a promoter, which is the switch that controls expression of the gene. AFP, antifreeze protein gene (Arctic flatfish); GH, growth hormone gene.

still waiting for FDA approval, and without raising additional money the company had relatively little operating funds left to continue its work.

It took more than 20 years to receive FDA approval for AquAdvantage salmon despite data indicating no difference in nutritional value, appearance, or taste of these fish compared with wild salmon. In 2010, the FDA concluded that AquAdvantage salmon were safe to eat, but the FDA did not finally grant approval until 2015. A major reason for the delay in making an approval decision was a risk assessment about potential environmental impacts of these salmon if they escaped and bred with wild fish. AquaBounty says that all of these transgenic salmon are female and approximately 99.8 percent of them are sterile *triploids* (see the discussion of triploids beginning on the next page). These fish are also raised in inland pens isolated from natural waterbodies. In 2016, AquAdvantage salmon were approved as the first GM animal for sale in Canada.

In 2004, Yorktown Technologies of Austin, Texas, made popular news headlines when they announced they had created the GloFish®, a transgenic strain of zebrafish containing the red fluorescent protein gene from sea anemones. When ultraviolet light illuminates GloFish, they fluoresce bright pink; they were advertised as the first genetically modified pet sold in the United States. Yorktown has expanded its product line to include GM versions of other popular aquarium fish, such as tetras and barbs, and the use of other transgenes to produce fish that fluoresce yellow, green, red, blue, or purple. Although this fish was originally developed to detect environmental pollutants, a topic we introduced in Chapter 9, a number of anti-biotechnology groups continue to voice their protests about this novelty use of genetic engineering.

A number of **polyploid** aquatic species have been created. Polyploids are organisms with an increased number of *complete sets* of chromosomes. Most animals and plants are **diploid** (abbreviated $2n$, where n = number of chromosomes), meaning that they have two sets of chromosomes in their somatic cells and a single, or **haploid,** number (n) of chromosomes in their gametes. In humans, the haploid number of chromosomes is 23; therefore, the diploid number of chromosomes in human somatic cells is 46 ($2n = 46$).



YOU DECIDE

Transgenic Salmon for Human Consumption: Safe or Not?

In 2013, the FDA approved AquAdvantage® salmon as the first GM animal for human consumption. Given that AquaBounty Technologies spent over $67 million to develop these salmon, it will need to generate significant sales to eventually become a profitable company. Visit the AquaBounty Technologies website (**http://aquabounty.com/**) to review the company's descriptions of the science behind these fish and their safety as a food source.

States such as Alaska and Oregon, which export wild-caught salmon, tried to block approval of these GM fish. The international grocery chain Whole Foods will not sell AquAdvantage salmon. Mothers in the United States purchase about 85 percent of the food consumed by U.S. families. Does AquaBounty need to market its salmon effectively to moms in the United States for this fish to become a successful food product? The FDA determined that AquAdvantage salmon are as safe and nutritious as non-GM Atlantic salmon. Because data from analysis of these salmon determined that they are not different from non-GM Atlantic salmon, the FDA determined that no additional labeling of food from AquAdvantage salmon is required. Should food derived from these salmon be labeled as GM? Why or why not?

As a condition of FDA approval, AquaBounty was initially limited to manufacturing its salmon at two locations: an egg-production site on Prince Edward Island, Canada, and an aquaculture facility in Panama. If AquaBounty needs to expand facilities or develop new facilities to increase production to meet sales expectations, it needs to get permission from the FDA. As *Introduction to Biotechnology* was going to press the FDA granted permission to raise AquAdvantage salmon at a land-based facility near Albany, Indiana. Will such expansion increase the risk that AquAdvantage salmon can escape into the wild? What factors do you think the FDA should consider in determining whether or not the company can expand its production facilities?

A/F Protein, Inc., has requested approval from the FDA to market transgenic fish containing AFP genes, but no other companies are seeking FDA approval for the sale of transgenic fish for human consumption. Will the FDA's approval of AquAdvantage salmon encourage other companies to seek approval or will the lengthy approval process discourage other companies from moving forward? Countries such as India and China are investing heavily in producing GM fish for human consumption. Might these countries become leaders that the United States eventually depends upon to import GM species from?

Here we pose many questions to consider about AquAdvantage salmon, a historic application of biotechnology. Would you eat AquAdvantage salmon? You decide.

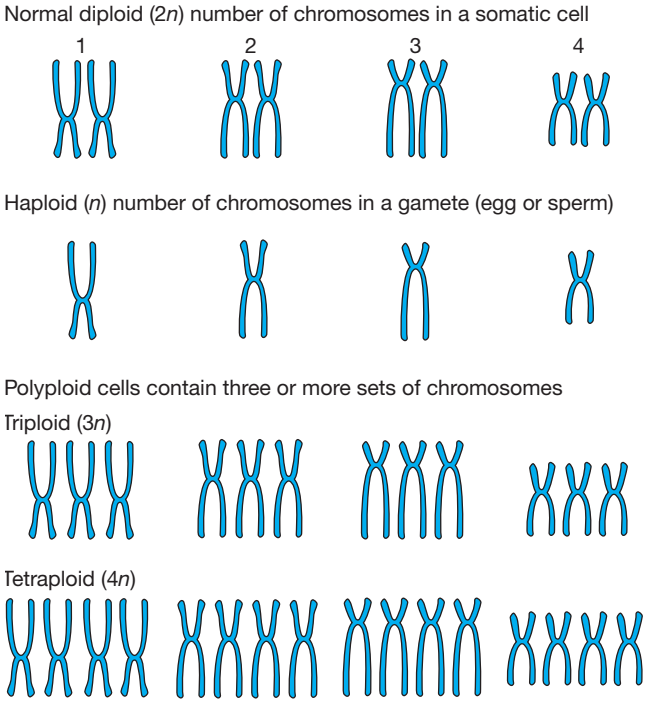


FIGURE 10.9 Polyploid Species Have Variations in Complete Sets of Chromosomes Somatic cells from many animal and plant cells have a diploid number (2*n*) of chromosomes, whereas gametes have a single set, or haploid number (*n*), of chromosomes. Chromosomes from an organism with a 2*n* of eight chromosomes are shown. Polyploids contain three or more sets of chromosomes. Although tetraploid (4*n*) and pentaploid (5*n*) organisms can be created, the vast majority of marine polyploid species are triploids (3*n*).

Figure 10.9 is a simple representation of the differences among haploid, diploid, and polyploid species.

The majority of polyploid aquatic species created to date are **triploids.** Triploid organisms contain three sets of chromosomes (3*n*). A number of different techniques can be used to create triploids. Triploids are usually derived by subjecting fish eggs to a temperature change or chemical treatment to interfere with egg cell division. Eggs treated in this way mature with an extra set of chromosomes.

For instance, treating eggs with **colchicine**, a chemical derived from the crocus flower (*Colchicum autumnale*), is one common approach to creating polyploids (Figure 10.10a). Colchicine blocks cell division by interfering with the formation of microtubules necessary for cell division. As a result, the chromosomes replicate in treated egg cells, but these cells are incapable of dividing. Therefore, these eggs have a diploid number of chromosomes, twice as many chromosomes as normally found in gametes. Fertilization of such an egg cell by a normal haploid sperm cell results in a triploid organism. Another approach to producing triploids involves fertilizing a normal haploid egg with two spermatozoa (Figure 10.10b). The resulting embryo is a triploid containing one set of chromosomes from the egg and one set from each of two different sperm.

Polyploidy usually influences the growth traits of an organism. As you learned in Chapter 6, many fruits we consume, such as bananas and strawberries, are polyploids. Triploids typically grow more rapidly, in most species 30 to 50 percent faster, and larger than their normal diploid counterparts. But most triploids are sterile because they produce gametes with an abnormal number of chromosomes. In some cases triploids may not produce any gametes.

One of the first widely used triploid strains of fish was the triploid grass carp (*Ctenopharyngodon idella;* Figure 10.11a). Grass carp have a voracious appetite for many types of aquatic vegetation. In the early 1960s, the U.S. Fish and Wildlife Service imported diploid grass carp, which are native to Malaysia. In the early 1980s, triploid grass carp were developed by temperature-shocking eggs to create diploid eggs and fertilizing these eggs with normal haploid sperm. Triploids grow rapidly and can exceed 25 pounds. Because of their ability to consume large amounts of vegetation, grass carp became very popular for controlling the growth of aquatic weeds in freshwater ponds and lakes throughout the United States. Triploid grass carp became an instant favorite among pond and lake managers, who could stock these fish in lakes as a "natural" way to control weed growth, minimizing the need to use large doses of chemical herbicides (Figure 10.11b).

Not everyone has been thrilled with triploid grass carp, however. In some waterways, because of grass carp overpopulation (grass carp have few natural predators), they have been so effective at eating their way through aquatic vegetation that water quality has changed substantially. Dramatic decreases in weeds used as habitat by many sport fish, such as trout and bass, have led to a decline in fishing in previously productive fishing waters. Also, many triploids have escaped into waters adjacent to their original stocking body, leading to the loss of vegetation in areas such as marshes and natural lakes not originally targeted for weed control. Finally, reproduction from these presumably sterile fish has also been documented.

Another polyploidy success story involves the triploid oyster, credited with reviving a diminishing oyster industry on the West Coast. Oyster production is seasonal because it is strongly influenced by weather, breeding patterns, and habitat. Not only has the development of a triploid strain of the Pacific oyster provided for year-round harvesting, but the triploid oysters grow substantially larger than their diploid

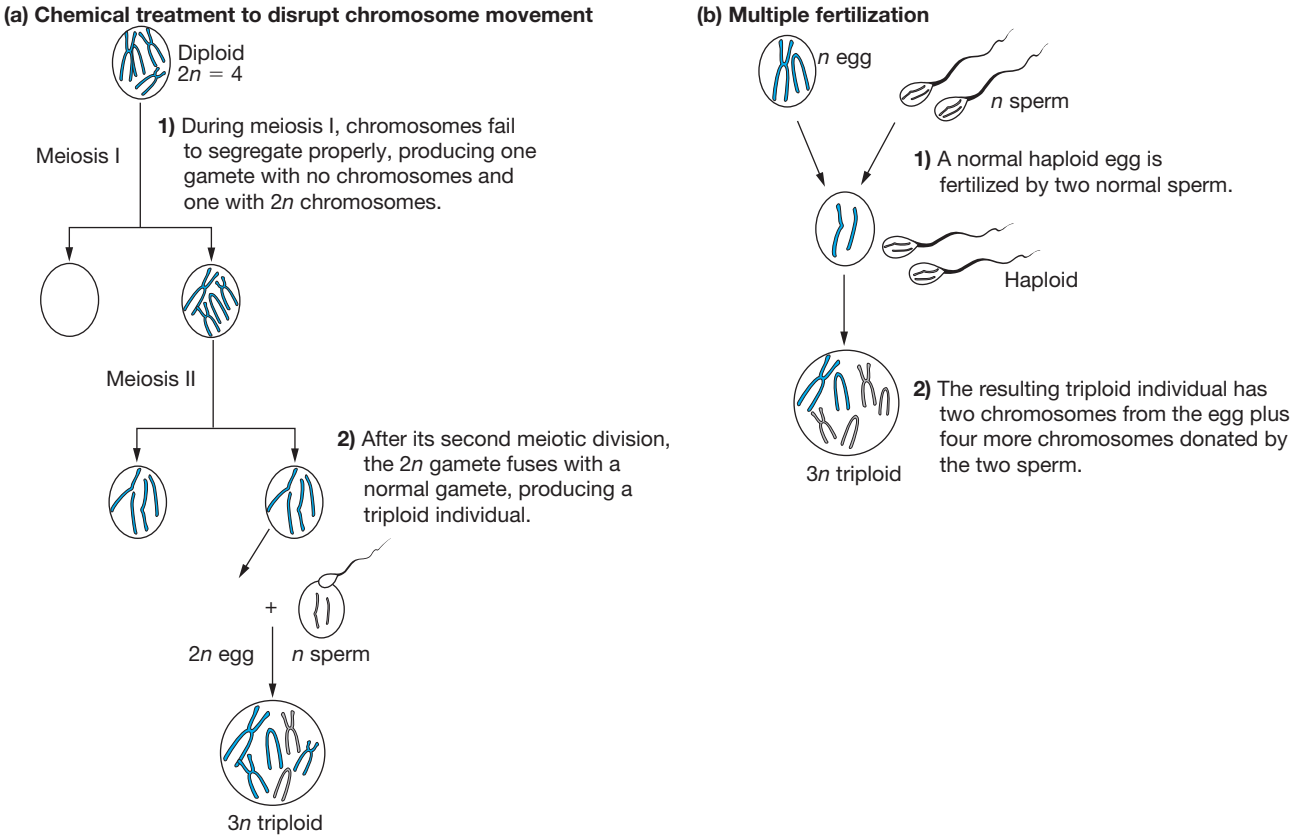


FIGURE 10.10 Producing a Triploid (a) Egg cells can be chemically treated to block chromosome movement during cell division to produce diploid eggs. (b) When these eggs are fertilized by a normal haploid sperm cell, triploid offspring are produced. A triploid can also be created by the fertilization of a normal haploid egg by two normal haploid sperm.

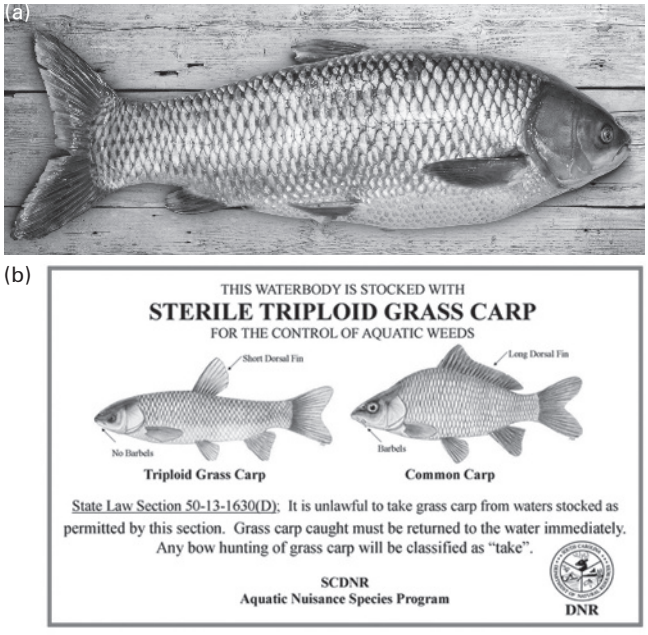


FIGURE 10.11 Triploid Grass Carp (a) Triploid grass carp grow quickly and can consume large amounts of aquatic vegetation, hence their use for weed control in freshwater lakes. (b) Notice the sign here indicates that these triploids are presumed sterile.

cousins (Figure 10.12). Diploids normally store sugars in their tissue and then become lean in the summer while expending energy to spawn. Lean oysters are undesirable for market sale. Because triploids do not spawn, they grow fat all summer, making them market-ready. Recently, scientists have developed new approaches to produce tetraploid molluscs, including abalone, clams, oysters, scallops, and mussels. In the future, these polyploids may become widely available for human consumption.

In the next section we introduce you to some of the valuable ways in which aquatic organisms are being used for important medical applications.

10.4 Medical Applications of Aquatic Biotechnology

Relatively few medical products have been derived from aquatic organisms, but this is rapidly changing. Aquatic scientists believe that oceans and freshwater habitats possess near limitless opportunities for the identification of new medicines. New and effective

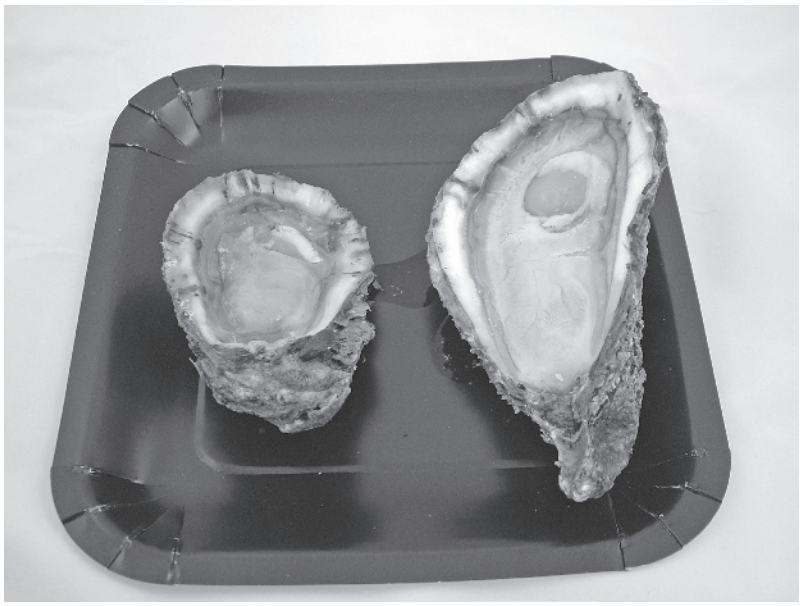


FIGURE 10.12 Triploid Oysters Have Revived Pacific Oyster Fisheries A triploid oyster (right) grows larger than its diploid counterpart (left) and shows greater resistance to disease.

drugs have already been derived from aquatic organisms and used for human benefit, and marine species are also being used as biomedical model organisms to understand, diagnose, and treat human diseases.

Bioprospecting to Isolate Medicines from the Sea

A wide number of marine species contain or are suspected to contain compounds of biomedical interest, including antibiotics, antiviral molecules, anticancer compounds, and insecticides. These species include sea sponges, members of the phylum Cnidaria (hydras, jellyfish, sea anemones, and a variety of corals), members of the phylum Mollusca (snails, oysters, clams, octopuses, and squid), and sharks, among many others. The wealth of organisms under study is broad and impressive. **Bioprospecting,** searching for potentially valuable products (such as drugs) from plant and animal species, on aquatic species is ongoing around the world. There have been several successful applications to date, particularly from marine organisms, and we have many reasons to be optimistic that the waters of the world will continue to yield novel medical treatments in the future. Let's consider a few current and potential medical applications of aquatic products.

Osteoporosis, a condition characterized by a progressive loss of bone mass, creates porous, brittle bones that can lead to fractures of the hips, legs, and joints, thus severely hindering an individual's lifestyle. Over 90 percent of the roughly 25 million Americans affected by osteoporosis are women. A common treatment for osteoporosis is estrogen therapy. This medication is ineffective for many women, and the long-term health effects of estrogen are a concern. Other individuals are treated with human recombinant **calcitonin,** a thyroid hormone that stimulates calcium uptake and bone calcification and inhibits bone-digesting cells called osteoclasts. Researchers have discovered that some species of salmon produce a form of calcitonin with a bioactivity that is 20 times higher than that of human calcitonin. Cloned forms of salmon calcitonin are now available for delivery as an injection form and a nasal spray.

The exquisite patterns of coral reefs around the world are created by the skeletons of corals. These structures partially consist of **hydroxyapatite (HA),** an important component of the matrix that constitutes bone and cartilage in animals, including humans. Biotechnology companies have developed technology that allows HA implants to be cut into small cubes and used to fill gaps in fractured bones. The cubes are ultimately invaded by local connective tissue cells that speed repair. As a result, patients avoid the necessity of bone grafts derived from other parts of their bodies. These implants may also serve to fill bone material lost around the root of a tooth.

A number of adhesives have been identified in glue-like resins produced by mussels and other shellfish. Mussels (*Mytilus edulis*) are a favorite dish in many seafood restaurants. These hinged-shelled molluscs live in physically demanding environments. They typically adhere to rocks or pilings at the edges of oceans and bays. Day after day, mussels are pounded by waves. They dry out during low tides and then, as the tide rises, are submerged again and pounded by waves. How do they maintain their contacts to rocks and other structures without being crushed or pulled off the

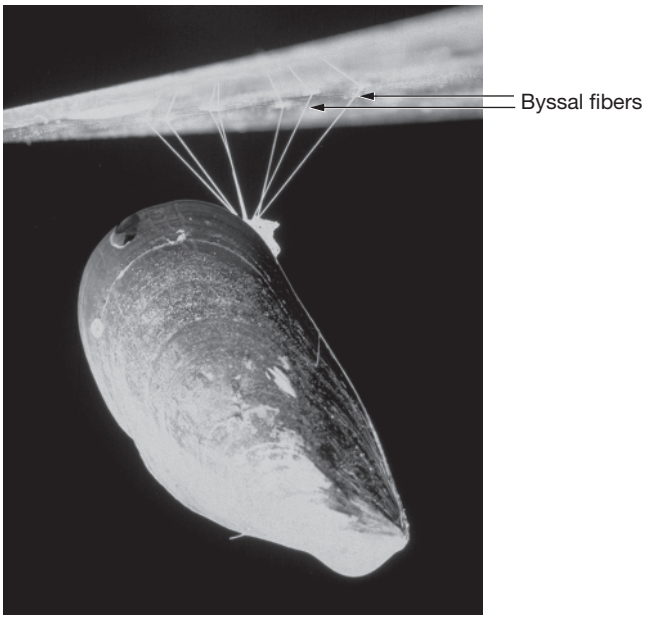


FIGURE 10.13 Mussels Produce Unique Adhesives
Mussels and other shellfish cling tightly to rocks and other surfaces by producing a unique type of adhesive called byssal fiber. Byssal fibers are remarkably strong. They can withstand tremendous physical forces such as those created by waves in the water where mussels live.

rocks? The answer lies in a unique form of protein-rich superadhesive called **byssal fiber** (Figure 10.13).

Byssal fibers are several times tougher and more extensible than human tendons, which themselves are tougher than steel. The adhesive and shock-absorbing elastic properties of byssal fibers absorb energy and stretch as waves tug away at the mussels. Although it would be cost-prohibitive to isolate byssal fibers from mussels directly (nearly 10,000 mussels would be required for 1 gram of adhesive), scientists are using recombinant DNA techniques to express byssal fiber genes in bacteria and yeast to manufacture bioadhesives from byssal fiber proteins on a large scale. In addition, because the amino acid composition and structural organization of byssal fibers are now fairly well known (although how the fibers assemble with mussels is still a mystery), adhesive glues have been produced from the chemical synthesis of byssal fiber protein components. Adhesives based on byssal fiber proteins are being considered and implemented for a variety of diverse applications, from automobile tires to shoes, glue for plywood, bone- and tooth-repair strategies, soft body armor for soldiers, surgical sutures and sealing incisions, and artificial tendons and ligaments to be used as grafts.

A type of marine worm, called the sandcastle worm (*Phragmatopoma californica*), which glues together little shelters from sand grains, is also being studied as a potential source of bioadhesives. Similarly, in 2017 researchers from Harvard announced development of super-strength, tissue-binding bioadhesives derived from the Dusky Arion (*Arion fuscus*), a slug found in Europe and the United States. This slug releases an adhesive mucus that allows it to bond to surfaces. Trials with pig and rat tissues have demonstrated that slug adhesives maintain strength in both wet and dry applications to bond together skin, cartilage, heart, and other tissues for several weeks.

One broad category of drugs from the sea includes several successful medicines and encompasses anti-inflammatory, analgesic (pain-killing), and anticancer compounds, as well as treatments for asthma and arthritis. Table 10.4 lists recent examples of medical compounds isolated from aquatic organisms. Since 1969, the FDA has approved six medicines based on marine organisms. The first product, cytarabine (Cytosar-U), derived from a sea sponge that lives near the Caribbean, has been widely used for the treatment of certain forms of leukemia and lymphoma.

Over a dozen different anticancer compounds have been isolated from marine invertebrates, particularly sea sponges, tunicates, and molluscs. Many of these compounds are in various stages of clinical trials that will ideally lead to new and effective drugs on the market. Several groups of researchers are studying venomous marine creatures in the hope of identifying substances that may be used to treat nervous system disorders.

For example, marine cone snails, a potentially lethal species, produce conotoxins, venomous molecules that can target specific neurotransmitter receptors in the nervous system. In 2004, the FDA approved the drug **Prialt,** a peptide conotoxin purified from the marine cone snail *Conus magus* by the Elan Corporation of Ireland. 1,000 times more potent than morphine, conotoxins such as Prialt represent a promising new source of neurotoxins with the ability to act as strong painkillers by blocking neural pathways that relay pain messages to the brain. Still the only approved venom-derived drug, Prialt has been successfully used to treat chronic, severe forms of pain, such as back pain. Other toxins produced by cone snails that are close relatives of *C. magus* are currently in clinical trials. In cone snails alone, there may be thousands of potential drugs to explore. It has been estimated that less than 0.1 percent of the venom proteome of cone snails (estimated at ~100,000 peptides) have been studied!

In 2010, Halaven, derived from a marine sponge, was approved by the FDA for the treatment of metastatic breast cancer. Halaven works as a chemotherapy by interrupting microtubule lengthening and shortening, thus interfering with cell division. In 2015, Yondelis, an antitumor drug isolated from the sea squirt

TABLE 10.4 Examples of Medical Compounds from Aquatic Organisms

Organism	Medical Product	Application
African clawed frog (*Xenopus laevis*)	Magainins	Antimicrobial peptides first discovered in frog skin. Used to treat bacterial infections.
Coho salmon (*Oncorhynchus kisutch*)	Calcitonin	Hormone that stimulates calcium uptake by bone cells. Used to treat osteoporosis.
Hammerhead shark (*Sphyrna lewini*)	Neovastat (AE-941)	Antiangiogenic compound (blocks blood vessel formation). Used to treat cancer.
Leech (*Hirudo medicinalis*)	Hirudin	Saliva peptide from leeches used as an anticoagulant to thin blood.
Marine cone snail (*Conus magus*)	Prialt (ziconotide)	Synthetic peptide toxin used as a pain reliever to treat chronic severe pain and arthritis.
Sea anemone (*Stichodactyla helianthus*)	Stichodactyla toxin	Peptide that blocks potassium channels; potential applications for treating autoimmune diseases including multiple sclerosis.
Sea sponge (*Halichondria okadai*)	Halaven (eribulin mesylate)	Chemotherapy compound for metastatic breast cancer treatment.
Sea squirt (*Ecteinascidia turbinata*)	Yondelis (trabectedin)	Antitumor agent developed for the treatment of advanced soft tissue sarcoma.

Note: Brand (trade) names for drugs are shown in table with chemical names in parentheses, as appropriate.

Ecteinascidia turbinata, was approved by the FDA for the treatment of soft tissue sarcomas (in adipose tissue and smooth muscle) that are either metastatic or cannot be removed by surgery. Yondelis is also in clinical trials for the treatment of prostate, breast, and ovarian cancer. This drug binds in the minor groove of DNA and inhibits cell division by blocking transcription and DNA repair proteins.

Other researchers are developing culturing systems to provide adequate supplies of marine organisms such as single-celled plankton called dinoflagellates, which contain components useful in the treatment of tumors and cancers. A marine invertebrate called *Bugula neritina* has been shown to contain minute amounts of a compound that is active against certain types of leukemia. Because large amounts of this compound will be needed for human studies, molecular cloning techniques will be important if this compound is to be produced in mass quantities.

Similarly, the Japanese pufferfish, or blowfish (*Fugu rubripes*), has been getting a lot of attention for several decades (**Figure 10.14**). *Fugu* is famous for its ability to swallow water and "puff up" when threatened and to produce a potent nerve cell toxin called tetrodotoxin (TTX). TTX is one of the most toxic poisons ever discovered (nearly 10,000 times more lethal than cyanide). Hollywood has often depicted *Fugu* in movies, showcasing its ability to produce its deadly toxin. In Japan, *Fugu* is a prized and very expensive delicacy for many sushi lovers who enjoy the food quality of this tasty fish despite the risk (eating *Fugu* kills nearly 100 people, mostly in Japan, each year).



FIGURE 10.14 Marine Organisms Such as Pufferfish Are Helping Scientists Discover New Ways to Treat Cancer and Chronic Pain in Humans

Scientists have used TTX to develop a greater understanding of how proteins called sodium channels help neurons to produce electrical impulses. TTX is a deadly poison because it blocks sodium channels and prevents nerve impulse transmission. An understanding of how TTX affects sodium channels has led to the development of new drugs that are being tested not only as anesthetics to treat patients with different types of chronic pain but also as anticancer agents in humans. The *Fugu* genome contains nearly the same number of genes as that of humans but is much smaller (390 Mb). Because it has far less non-coding DNA (introns) than the human genome, some scientists consider it an ideal model organism for studying the importance of introns.

A steroid called squalamine, first identified in dogfish sharks (*Squalus acanthias*), appears to be a potent antifungal agent that may be used to treat life-threatening fungal infections that can kill patients with conditions such as AIDS and cancer. Sharks rarely develop cancer (although it is a myth that sharks do not get cancer), and shark cartilage has been proposed as a rich source of anticancer agents. Although no compounds from shark cartilage have demonstrated effectiveness in controlled clinical trials, shark cartilage extracts possess *antiangiogenic* compounds, and cartilage extracts are in various stages of clinical trials. **Angiogenesis,** or the formation of blood vessels, is a process that is often required for growth and development of many types of tumors. By blocking blood vessel formation, antiangiogenic compounds derived from marine species show promise for inhibiting the growth of certain tumors.

In addition, because many aquatic organisms live in harsh environments, scientists are optimistic that we can learn from the adaptations these organisms have developed. For example, researchers are currently studying marine organisms that show tolerance to ultraviolet light; these may prove to be a source of natural sunscreens. As another example, alligator wounds are remarkably resistant to infection, even though alligators swim in microbe-infested waters. Scientists have found that alligators produce antimicrobial peptides capable of killing such microbes as MRSA (methicillin-resistant *Staphylococcus aureus,* responsible for about 70 percent of lethal infections in hospitals), which have become immune to modern antibiotics.

Even discarded crab shells from the commercial crabbing industry play a role in medical applications of aquatic biotechnology. The outer skeleton, or exoskeleton, of members of the phylum Arthropoda—which includes crabs, lobsters, shrimp, insects, and spiders—is a rich source of *chitin* and *chitosan***.** These complex carbohydrates are structurally similar to cellulose, which forms the tough outer layer of the cell wall in plants. Cellulose is widely known as a source of dietary fiber. Similarly, **chitin** and **chitosan** are also sources of fiber. Eating vegetables and fruits to get fiber is much easier on your digestive tract than eating crab shells. Nonetheless, ground-up extracts of crab shell can be purchased as a powder in many nutrition stores. Many skin creams and contact lenses also contain chitin, and chitin has been used to create nonallergenic dissolvable stitches that appear to stimulate healing when surgeons place them in humans.

From the examples we presented in this section, hopefully you now have a greater understanding of medical applications from aquatic organisms that have been, or will be, achieved through biotechnology.

10.5 Nonmedical Products

To further appreciate the biotechnology potential of our world's waters, we now consider a number of nonmedical products derived from aquatic organisms, from research reagents to food supplements, which have had an impact on the biotechnology industry. We conclude this section by discussing biomass and bioprocessing as potential new applications in the future.

A Potpourri of Products

We have discussed the importance of *Taq* polymerase, isolated from the archaebacterium *Thermus aquaticus*, found in hot springs, which allowed for the development of the PCR as a powerful tool in molecular biology (Chapter 3). The ocean also has proved to be an excellent source of enzymes and other products that have played an important role in basic and applied research. For example, bacteria living near hydrothermal vents (hot-water geysers on the ocean floor) have yielded a second generation of heat-stable enzymes for use in PCR and DNA-modifying enzymes, including ligases and restriction enzymes.

Other enzymes produced by marine bacteria possess a variety of interesting properties that may result in important applications in the future. Some enzymes are salt-resistant, which renders them ideal for industrial scale-up procedures involving high-salt solutions. We have discussed the role of the bioluminescent bacterium *Vibrio harveyi* in detecting environmental pollution (Chapter 5). Marine species of *Vibrio* produce a number of proteases, including several unique ones that are resistant to the detergents used in many manufacturing processes. As a result, these detergent-resistant proteases may have potential applications for degrading proteins in cleaning processes, including their use in laundry detergent for removing protein stains in clothes. *Vibrio* is also a good source of **collagenase,** a protease used in tissue culturing. When

scientists are looking to grow cells in culture, such as liver cells, they can use collagenase to digest the connective tissues holding cells together so the individual cells can be dispersed into cell culture dishes.

Another product is **carrageenan,** a common ingredient in preserved foods, toothpaste, and cosmetics. This sulfate-rich polysaccharide, extracted from red seaweeds, has been in use for over 60 years. There is a large family of carrageenan polysaccharides. They have the ability to form gels of varying densities at different temperatures. As a result, carrageenans have been used as thickening agents and for improving the way foods "feel" in our mouths when we eat them. Some of the most common applications of carrageenans include their use as stabilizing and bulking agents in chewing gum, chocolate milk, beers and wine, ice cream, salad dressings, syrups, sauces, processed lunch meats, adhesives, textiles, polishes, and hundreds of other products.

The Philippines is the world's largest producers of red seaweeds (Rhodophyta), from which many carrageenans are derived. Red algae are also prized for their use in nori, a paper-thin seaweed product used to wrap sushi. Improvements in farming seaweeds have allowed for increased production of other algal polymers, including agar and agarose, which are used, respectively, to make agar gels for plating bacteria and to create agarose gels for electrophoresis (Chapter 3).

Aquatic Biomass and Bioprocessing Applications

One newly emerging area of aquatic biotechnology involves the exploration of marine biomass. A mat of aquatic weeds of algae represents biomass, as does a school of fish. As you know, plants are responsible for the production of oxygen through the process of photosynthesis, in which carbon dioxide and water are converted into carbohydrates and oxygen. Marine plants (including seaweeds, grasses, and planktons) use photosynthesis to capture and convert a tremendous amount of energy (nearly 30 percent of all energy produced worldwide) from the sun into chemical energy. Can chemical energy from such biomass be harvested?

Scientists are examining ways in which algae and aquatic plants may be used to produce alternative fuels. It may be possible to take advantage of the rapid biosynthetic capabilities of marine algae—with their ability to mass-produce hydrocarbons and lipids in extraordinary quantities—to provide alternative sources of materials that are normally cost-prohibitive to produce or to isolate from natural materials. Similarly, it may be possible to convert marine biomass such as seaweed into fuels such as ethanol.

Scientists are also investigating potential ways to use plankton as underwater "fuel cells." Plankton at the water's surface release energy as they undergo photosynthesis, whereas plankton closer to the sediment at the bottom of the ocean (where there is less oxygen) use other reactions to generate energy. As a result, these plankton create a natural voltage gradient from the surface to the ocean floor that can be harvested to produce an indefinite source of electricity. In the future, the ocean may turn out to be a valuable resource for providing energy. Lastly, scientists are exploring ways in which biomass of marine algae may be used to increase the absorption of carbon dioxide and decrease greenhouse effects on the earth.

A bacterium called *Prochlorococcus* is the smallest but most abundant photosynthetically active cell in the ocean that has been discovered to date. Some estimate that this tiny microbe accounts for approximately 5 percent of global photosynthesis! Different strains of *Prochloroccocus* have an estimated 80,000 genes. What can we learn from *Prochlorococcus* that might be important for reducing carbon pollution in the environment and bolstering oxygen production? Stay tuned.

Marine scientists are exploring ways in which **bioprocessing** may involve marine products to produce a biological product such as a recombinant protein. Algae may potentially be very valuable for expressing recombinant proteins. Researchers have found that they can make an abundance of proteins, such as antibodies, in marine algae because they can be grown on a very large scale. Marine biologists are exploring how marine organisms may be used to synthesize a variety of polymers and other biomaterials, which may find a place in industrial manufacturing processes. For instance, as nontoxic, biodegradable alternatives to currently used materials, oyster shell proteins are being considered as additives in detergents and other solvents.

We conclude this chapter by discussing how aquatic organisms are being considered for use in applications to clean up the environment.

10.6 Environmental Applications of Aquatic Biotechnology

Unfortunately, the world's oceans have long served as dumping grounds for the wastes of humanity and industrialization, with too little thought given to the effect of pollution on fish stocks, marine organisms, and the environment. Clearly, oceans do not have an infinite ability to accept waste products without consequences, and we are seeing the results of this as wetlands and other estuarine environments—which are

critical habitats for the spawning of many marine species and the growth of young marine organisms—are showing signs of severe decline due to pollution and human impact. In this section, we consider how aquatic organisms can be used to control, detect, and help clean up pollution in the environments in which they live.

Antifouling Agents

Biofilming, also called biofouling, refers to the attachment of organisms to surfaces. These surfaces include the hulls of ships, inner linings of pipes, cement walls, and pilings used around piers, bridges, and buildings. Biofilming also occurs on the surfaces of marine organisms, especially shellfish. If you have spent any time around boat docks and piers located in marine environments, you have undoubtedly seen the effects of biofilming on structures that are in the water. In marine environments, barnacles, algae, mussels, clams, and bacteria are among the most common organisms responsible for biofilming (**Figure 10.15**). As the term *biofilming* suggests, these organisms literally grow to create a layer, or usually multiple layers, that create a "film" over the surfaces to which they adhere.

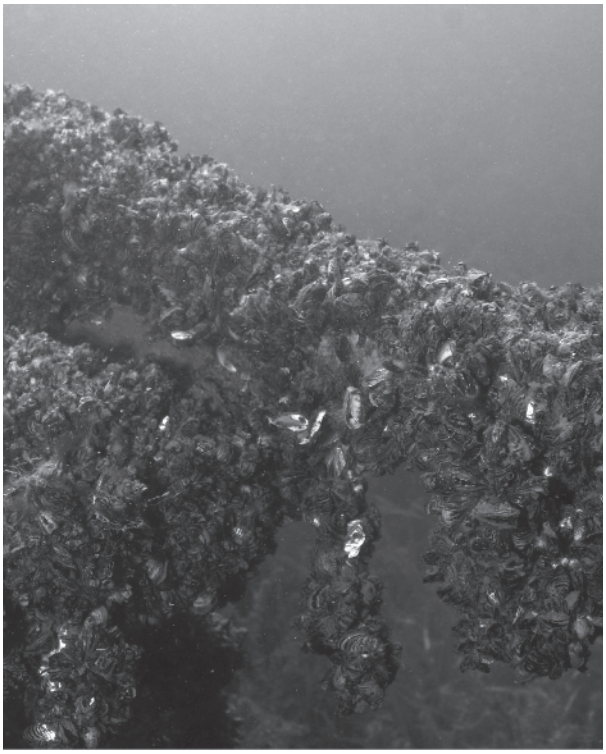


FIGURE 10.15 Unwanted Invaders Create Biofilms That Have Serious Economic Impacts Mussels, barnacles, and other organisms can create hard biofilms, shown here covering a pipe.

However, biofilming is not exclusively a marine problem. Biofilming occurs in the plumbing of your home, on contact lenses, and in your mouth. Bacteria that coat your teeth and adhere to implanted surgical devices and prostheses are examples of biofilming. Although brushing your teeth regularly minimizes biofilming in your mouth, such simple remedies are not available for marine environments.

Biofilming can create significant problems. The attachment of marine organisms to the hull of a ship can greatly increase the resistance of the ship as it moves through the water, slowing its travel time and reducing its fuel efficiency. A progressive accumulation of biofilms can clog pipes, block water intake and filtration systems for ships, and corrode metal surfaces. Furthermore, the colonization of surfaces with invertebrates and molluscs creates "hard" biofouling that is difficult and costly to remove (Figure 10.15).

Traditionally, most antifouling agents have employed toxic chemicals such as copper- and mercury-rich paints to minimize the growth of fouling organisms. However, the metals that leach from these paints can contribute to environmental pollution. As a result, researchers are investigating the natural mechanisms that many organisms use to prevent biofouling on their own surfaces. If biofilming is a problem for both manufactured surfaces and the surfaces of marine organisms, how do clams, mussels, and even turtles minimize biofilming and thus prevent their shells from being completely closed by biofilming organisms? Scientists are using molecular techniques to find the answer. Some organisms produce repelling substances, while others appear to produce molecules that block the adhesion of biofilming organisms (**Figure 10.16**). Marine seagrasses—such as the eelgrass *Zostera marina*—and algae produce compounds that prevent bacteria, fungi, and algae from adhering or that neutralize them. Identifying and producing antifouling compounds is an area of active research. In the future, these compounds may be used to produce protective coatings for covering ship hulls, aquaculture equipment, and other surfaces susceptible to biofilming.

Biosensors

Scientists are working to explore the use of aquatic organisms as biosensors to detect low concentrations of pollutants and toxins in waterways. As you learned in Chapter 5, bioluminescent strains of bacteria can be used as biosensors. Some species use bioluminescence to illuminate their environment; others use it to find mates in the dark depths of the ocean. Most bioluminescent deep-sea fish are involved in symbiotic relationships with bioluminescent bacteria such as *Vibrio fischeri*.

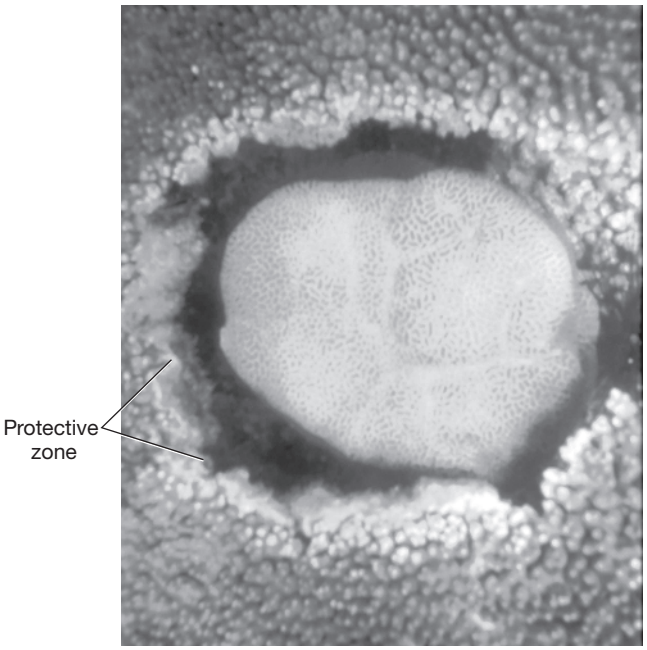


FIGURE 10.16 Many Marine Organisms Have Natural Antifouling Mechanisms Many stationary marine organisms release defensive chemicals to create a protective zone (indicated by the dark-colored band around this marine coral) around them. These chemicals may deter fouling microorganisms and protect the organism from predators.

V. fischeri and other bioluminescent strains (such as *V. harveyi*) express *lux* genes, which code for the light-emitting enzyme luciferase. In response to changing environmental conditions, the intensity of light emitted by *Vibrio* organisms can change. Because of this ability, *Vibrio* bacteria have been used as biosensors to detect pollutants such as organic chemicals and nitrogen-containing compounds in marine environments.

Not only are some marine organisms useful for detecting environmental pollutants, but many marine species are also believed to possess metabolic pathways for degrading a range of substances both natural and manufactured. Much research is dedicated to characterizing the biochemical pathways involved in degradative processes to determine how marine organisms may be used to remediate, or clean up, the environment of a variety of hazardous substances that enter marine environments.

Environmental Remediation

In Chapter 9, we discussed how native microorganisms or genetically engineered strains can be used to degrade chemicals through bioremediation. Marine organisms also possess unique mechanisms for breaking down substances including toxic organic chemicals such as phenols and toluene, oil products found in harbors and adjacent to oil rigs, and toxic metals.

One of the earliest techniques used in marine remediation involved increasing the quantity of shellfish in polluted areas. Scientists introduced racks of shellfish into polluted waters to take advantage of the natural feeding method of bivalves such as clams, oysters, and mussels. Because these organisms strain the water, they act as a form of estuarine filters to remove wastes such as nitrogen compounds and organic chemicals. These chemicals, in turn, are absorbed in the tissues of the shellfish. After periods of time, these shellfish can be harvested, thus removing wastes from the water. As an example, a recent test with floating rafts of mussels attached to anchored platforms in the Bronx River estuary of New York City revealed the animals had removed about 6 kg of nitrogen from the river in a year. This is only a tiny fraction of the amount of nitrogen pollution in the river and scientists recognized it would take hundreds or thousands of rafts to have a significant impact on improving water quality.

Heavy metal contamination of marine waters is the result of many industrial manufacturing processes. As a solution to this problem, scientists have isolated marine bacteria that oxidize metals such as iron, manganese, nickel, and cobalt. Some of these bacteria can also be used to extract important metals from low-grade ores. Additionally, some marine bacteria and single-celled algae express metallothioneins, a family of metal-binding proteins. These species thrive in water contaminated with cadmium and other heavy metals, where they literally mop up cadmium from the surrounding environment and then degrade toxic metals into harmless by-products. Scientists are looking at ways to use these organisms to extract, recover, and recycle important and expensive metals such as gold and silver from manufacturing processes.

By using aquatic organisms or their products, it is possible to stimulate waste degradation in natural environments or in bioreactors seeded with these organisms in much the same way that sewage treatment plants rely on bacteria to degrade fecal wastes. For example, microbiologists at the USDA have experimented with growing nitrogen-metabolizing algae on large mats, called scrubbers, so that they can be used as natural filters. These scrubbers work like charcoal filters in an aquarium in that they bind nitrogenous wastes. Water contaminated with farm animal wastes is passed over the scrubbers, and the algae absorb and metabolize the wastes. These wastes provide nutrients for the algae, which grow into thick mats. The algae are periodically harvested by cutting them back, but they are allowed to regrow like grass. Water cleaned in this way has been used for irrigation, and some of the

harvested algae have even been used as livestock food. Similar experiments have been done in Florida using water hyacinths to clean water rich in phosphorus, nitrogen, and other nutrients.

MAKING A DIFFERENCE

During early spring and summer along many beaches on the East Coast of the United States, a common scene has repeated itself for centuries. Large numbers of horseshoe crabs (*Limulus polyphemus*) invade shallow bays to mate. Horseshoe crabs preceded dinosaurs on the earth. In some ways, these "living fossils" have changed very little from their initial appearance over 300 million years ago. *L. polyphemus* was one of the first marine organisms to be used successfully for medical applications. In the early 1950s, it was discovered that *Limulus* blood contains cells that kill bacteria. From this simple observation, a very powerful medical application of marine biotechnology was developed. The **limulus amoebocyte lysate (LAL) test** is an extract of blood cells (amoebocytes) from the crab that is used to detect bacterial **endotoxins.** Endotoxins, also called lipopolysaccharides, are part of the outer cell wall of many bacteria such as *Escherichia coli* and *Salmonella*. Endotoxins can cause instant death to many cells. In humans, exposure to endotoxins from certain bacteria can result in mild symptoms ranging from joint pain, inflammation, and fever to more severe conditions such as a stroke. Certain endotoxins can be lethal.

Researchers discovered that horseshoe crab blood would clot when exposed to whole *E. coli* or purified endotoxins. They later determined that amoebocytes—which are similar to human white blood cells—in horseshoe crab blood could be lysed, centrifuged, and freeze-dried to create a lysate that can be used in an LAL test. The LAL test, a rapid and very effective assay for endotoxins in human blood and fluid samples, is also used to ensure that endotoxins are not present in biotechnology drugs such as recombinant therapeutic proteins. It is also used to detect bacteria in raw milk and beef. In addition, many medical companies and hospitals use the LAL test to make sure that surgical instruments, needles used for drawing blood and cerebrospinal fluid, and implanted devices such as pacemakers are endotoxin-free.

Amoebocytes are collected from crabs, which are then released. The destruction of the crabs' nesting habitat, predation, and harvesting are reducing the *Limulus* population, causing concern about the long-term sustainability of this species. But without question the LAL test is an outstanding example of the power of aquatic biotechnology to benefit humanity, and it has "made a difference" by ensuring the safety of medical products given to millions of humans, pets, farm animals, and others.



Horseshoe crabs (*Limulus polyphemus*) are a source of blood cells for an important test used to ensure that foods and medical products are free of harmful contaminants.

QUESTIONS & ACTIVITIES

Answers can be found in Appendix 1.

1. Provide examples of finfish and shellfish species that are important for aquaculture.
2. Create a list of benefits and problems associated with aquaculture.
3. In this chapter, we primarily focused on applications of aquatic biotechnology that benefit humans. But many aquatic biotechnologists are also interested in using biotechnology to improve native populations of aquatic organisms (finfish or shellfish) around the world. Suggest at least three ways in which biotechnology may be used to restore fish stocks.
4. There is concern that aquaculture consumes excessive amounts of wild bait fish. Describe potential pros and cons of switching farmed fish

from bait fish to vegetable-based diets. Do an Internet search to reveal recent examples of debates and discussions about this topic.

5. What is "fish pollution" and why is this a concern for the aquaculture industry?

6. The Food and Agriculture Organization (FAO) of the United Nations produces an annual report called *The State of World Fisheries and Aquaculture,* which is the key resource for facts, statistics, and trends on native fisheries and aquaculture. Go to the FAO homepage (**http://www.fao.org/about/en/**) and use the Search tool to search for the most current version of the report, which will be available as a pdf. Review the report to examine trends in fish production, examine the tables of seafood production showing fish caught in the wild (saltwater and fresh water) and raised on farms, and aquaculture production trends by countries globally, along with any other topics of interest to you.

7. A few years ago, a disease decimated large numbers of trout being raised by aquaculture at a New Jersey hatchery. To learn more about this, visit **http://www.njfishandwildlife.com/fishhealth_hatcheries.htm**, then answer the following questions: What were these trout raised for? What disease and organism was involved and where did it originate from? What impact did this disease have on the trout population at the hatchery? How did this incident affect applications involving these trout?

8. Describe the differences between transgenic fish and triploids, and discuss how each type of GM fish can be created.

9. The AquAdvantage® salmon is a biotechnology pioneer. Why? What is this salmon, and what makes it unique and controversial?

10. Suggest ways to assess the risk of GM marine species. Consider this from the standpoint of risks associated with the consumption of GM species as well as risks associated with introducing these species into natural environments.

11. The GloFish® has been marketed as the world's first genetically modified pet. Even though the GloFish was not designed for human consumption, many different groups (including scientists) have voiced strong concerns against this use of transgenic animals. According to Yorktown Technologies, the company that produced the GloFish, the Environmental Protection Agency (EPA), USDA, U.S. Fish and Wildlife Service, and FDA all claimed that there was no need for any of these agencies to regulate or approve sale of the GloFish. Why do you think federal agencies decided that it was not necessary to regulate the GloFish? Describe possible reasons why the GloFish is so controversial. What might some of the primary concerns be about this fish?

12. Visit the Yorktown Technologies website (**www.glofish.com**) to learn more about GloFish and different products the company has created. Did reviewing the website change your thinking on whether or not you support this use of biotechnology? Explain your answer.

13. What properties of an aquatic organism might be attractive to aquatic biotechnologists interested in bioprospecting novel compounds for medical applications?

14. Describe an example of a medicine derived from an aquatic organism. In your answer be sure to mention the source organism and the medical application of the drug.

15. Access the Marinebiotech.org website from the Companion Website. Use the Resources tab to learn more about dozens of compounds derived from marine organisms. This site is also a good resource for many topics in marine biotechnology.

16. Do an Internet search to learn more about byssal fibers produced by mussels. From your search, see if you can identify the rare amino acid present in byssal fiber proteins and explain how the chemical properties of this amino acid help mussels bind to surfaces even though cations (positive charges) in saltwater interfere with adhesion.

17. What are biofilms? Give examples of biofilms in aquatic environments as well as in humans. How may aquatic organisms be used to help scientists combat biofilming?

18. How might aquatic organisms be used for bioremediation?

19. What is the limulus amoebocyte lysate (LAL) test? Briefly describe how it works and its uses.

20. Describe the most interesting aspect of aquatic biotechnology you learned about in this chapter. What topic did you choose? Why did you find it interesting?

Visit www.pearsonglobaleditions.com for the Companion Website

The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

This case study has been included to give you an opportunity to become familiar with a research example applicable to this field of study. After you have analyzed the data and answered the questions, you can compare them to those provided to your instructor with the text materials.

CASE STUDY

Massive Fish Escape Jeopardizes New Salmon Farm

In August 2017, over 300,000 farm-raised Atlantic salmon escaped from a net pen near Cypress Island in Washington state into local rivers and waterways. This escape triggered significant concerns about the impact of farmed fish on native salmon stocks. State officials encouraged locals to catch as many of these fish as possible. Tribal officials from the Lummi Nation of Native Americans declared a state of emergency.

Visit **https://www.npr.org/sections/thesalt/2017/08/24/ 545619525/environmental-nightmare-after-thousands-of-atlantic-salmon-escape-fish-farm** to learn more about this escape and to answer the following questions.

Questions

Answers can be found at www.pearsonglobaleditions.com

1. What weather-related event was blamed for the escape? Do you think there is any way to completely prevent this type of problem again in the future?
2. Locals were encouraged to harvest as many farmed salmon as possible, but how would people distinguish farmed Atlantic salmon from native Pacific salmon?
3. Based on what you have learned about these farmed salmon, is there any clear way to distinguish them genetically from wild salmon?
4. The Canada-based company Cooke Aquaculture was proposing to build 14 new floating net pens in the area near the Strait of Juan de Fuca. How has this escape affected Cooke's plans?

CHAPTER ELEVEN

Medical Biotechnology



Customized medicine is one goal of medical biotechnology.

After completing this chapter, you should be able to:

- Appreciate and explain why model organisms are important for medical biotechnology.
- Describe different techniques for detecting chromosomal abnormalities and for genetic testing, and understand how these approaches help scientists and physicians diagnose diseases.
- Explain potential impacts of personal genomics on genetic testing.
- Understand what precision medicine is and describe specific examples including pharmacogenomics, nanotechnology, and others.
- Discuss how monoclonal antibodies and immunotherapy may be used for treating disease.
- Understand the purpose of gene therapy, different therapy strategies, and recognize limitations of gene therapy.
- Describe how genome editing is providing new and promising strategies for gene therapy.
- Define regenerative medicine and provide examples of how cell and tissue transplantation and tissue engineering may be used for therapeutic purposes.
- Understand what stem cells are, their sources, and provide examples of potential stem cell therapies.
- Compare and contrast therapeutic and reproductive cloning, and consider ethical issues associated with stem cell applications and cloning.

There is no topic in biotechnology that provokes greater optimism and debate than **medical biotechnology.** Applications of medical biotechnology have existed for decades. For instance, 100 years ago, leeches were commonly used to treat illness by so-called bloodletting. Some doctors believed that by using leeches to suck blood out of a patient, diseases were being removed from the body. Since then, of course, much better ways of treating disease have been developed thanks to biotechnology. It is an exciting time to learn about medical biotechnology because advances in this field are occurring at a mind-boggling rate. However, even the leech is getting attention again—not for bloodletting but for enzymes found in its saliva that can dissolve blood clots and possibly be used to treat strokes and heart attacks!

Medical biotechnology incorporates many topics that we have already discussed. From diagnosing genetic disorders, to developing new drugs, to gene therapy including genome editing, to stem cells and cloning, the possibilities of medical biotechnology are extraordinary but also ethically challenging to many people, including scientists.

In this chapter, we consider a wide range of different applications of medical biotechnology and discuss many potential impacts of this very exciting area. We begin by providing an overview of how molecular biology techniques can be used to detect and diagnose disease, and consider different innovative applications of precision medicine. We then present an introduction to gene therapy before we discuss regenerative medicine. The chapter concludes by examining stem cells and their potential applications.

FORECASTING THE FUTURE

Without question medical biotechnology is one of the most rapidly changing disciplines in biotechnology, and the possibilities for affecting human life positively are amazing. Because of the range of different technologies being developed and the new applications in medical biotechnology, it is challenging to predict what the next major future discoveries will be. In this "forecasting," we pose several questions that are among the major challenges being addressed by medical biotechnologists.

- To what extent will personalized genomics and sequencing of individual genomes affect disease diagnosis and treatment in the future?
- What new medicines and treatments will result from our understanding of the genome and epigenome?
- What roles will nanotechnologies play for sensing and treating disease?
- Immunotherapy has produced some remarkable results for treating certain cancers. Will these approaches live up to expectations?
- What new treatments will emerge from regenerative medicine and the ability to engineer cells, tissues, and organs?
- Will gene therapy techniques advance to become more routine options for treating genetic diseases? Will genome editing have the impact it is projected to have on significantly advancing successful gene therapy approaches?
- Will stem cells become reliable, safe, and affordable options for treating disease?

11.1 Animal Models of Human Disease

Many of the applications you will learn about in this chapter are possible because of **model organisms.** As you learned in Chapter 7, in particular, mice, rats, chicks, yeasts, worms, flies, and even the zebrafish, a common fish in home aquariums, have played essential roles in helping scientists study human genetics. Model organisms are critically important to scientists, in part, because we cannot manipulate human genetics for experimental purposes. It is, of course, unethical and illegal to force humans to breed or to remove their genes to learn how they function. However, these approaches are widely used to study genes in model organisms.

We think of ourselves as unmatched by other species not only for our ability to communicate through speech and writing but also for walking upright, creating music, making good pizza, and exploring distant planets. But we are not really unique at the genetic level. From yeasts and worms to mice, we share large numbers of genes with other organisms, and a number of human genetic diseases also occur in model organisms. Many important genes are highly conserved from species to species. If we identify key genes in model organisms, we can form hypotheses and make predictions about how these genes may function in humans. Therefore, scientists can use model organisms to identify disease genes and test gene therapy and drug-based therapeutic approaches to evaluate their effectiveness and safety in preclinical studies before using them for **clinical trials** in humans.

Many genes identified in different model organisms have been shown to be related to human genes based on DNA sequence similarity. Such related genes are called **homologues.** A gene thought to play a role in human illness can be eliminated in model organisms through gene knockout or genome editing. The effects on the organism can then be studied to learn

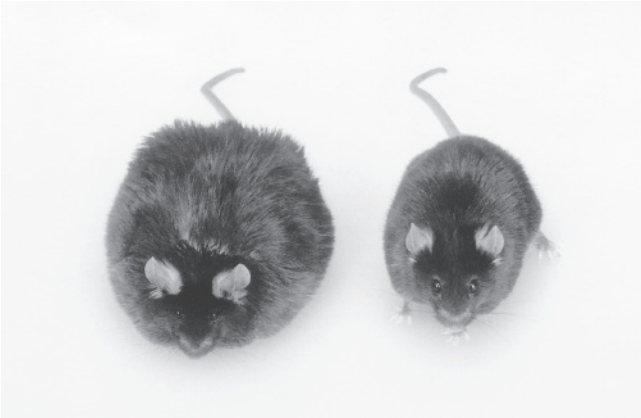


FIGURE 11.1 Obese Mice Model organisms are valuable for learning about human disease genes. The mouse on the left has been genetically engineered by gene knockout to lack the leptin (*Lep*) gene, which produces a protein hormone called leptin, from the Greek word *leptos*, meaning "thin." The *Lep* knockout mouse weighs almost five times as much as its normal sister (right).

about the functions of the gene. For instance, scientists discovered that mice can become obese if they lack a single gene called *Lep* (Figure 11.1). *Lep* codes for a protein hormone called leptin, which is produced by fat cells (adipocytes) and travels through the bloodstream to the brain to regulate hunger, essentially telling the brain when the body is full. The subsequent discovery of a human homologue for leptin has led to new areas of research providing insight into fat metabolism in humans and the genetics that influences weight disorders. Some childhood diseases of obesity are affected by mutation of the human *LEP* gene. Extremely obese children in England have been treated with leptin and have responded very well in preliminary studies.

The Human Genome Project and studies in comparative genomics have clearly demonstrated that we share a large number of genes with other organisms. Can you believe that you share approximately 50 percent of your genes with the pesky fruit flies you bring home on fruit from the grocery store? It may seem hard to believe that it takes only about twice as many genes to make a human as it does a fruit fly. And plants, such as rice, have even more genes than we do. We share nearly 40 percent of our genes with roundworms and 31 percent of our genes with yeast—the same yeast we use to help make bread rise and ferment alcoholic beverages. We share even more genes with mice; approximately 90 percent of our genes are similar in structure and function.

Many genes that determine our body plan, organ development, and eventually our aging and death are virtually identical to genes in fruit flies. Moreover, mutated genes that are known to give rise to disease in humans also cause disease in fruit flies. Approximately 61 percent of genes mutated in 289 human disease conditions are found in the fruit fly. This group includes the genes involved in prostate cancer, pancreatic cancer, cystic fibrosis, leukemia, and many other human genetic disorders. Heart disease is another example of a condition that scientists are studying in model organisms. Nearly 1 million people die each year in the United States from heart disease.

And, as we have discussed throughout this book, the use of genome editing applications such as Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated proteins (**CRISPR-Cas**) is a significant tool for editing genes in model organisms to study different diseases. This research area is progressing very rapidly. As one example, researchers have used gene knockouts to develop "heart attack mice" deficient in certain genes required for cholesterol metabolism. These mice have elevated blood cholesterol levels similar to those that occur in atherosclerosis—hardening of the arteries—so that they can be used to test therapies to combat heart disease.

11.2 Detecting and Diagnosing Human Disease Conditions

Because of the Human Genome Project and related advances in genomics, researchers have been making swift progress in identifying genes involved in genetic diseases and developing powerful techniques for detecting human genetic diseases. This is one of the most rapidly changing areas of medical biotechnology. Such technologies have had major impacts on the diagnosis of disease and are revolutionizing medical treatments. Here we provide an overview of different approaches to detecting and diagnosing human disease conditions.

Biomarkers for Disease Detection

In theory, with the right diagnostic tools, it may be possible to detect almost every disease at an early stage. For many diseases, such as cancer, early detection is critical for providing the best treatment and improving the odds of survival. One detection approach is to look for **biomarkers** as indicators of disease. Biomarkers are typically proteins produced by diseased tissue or proteins whose production is increased when a tissue is functioning abnormally. Many biomarkers are released into body fluids such as blood and urine as a product of cell damage—released by dead and dying cells such as cells undergoing apoptosis. For example, a protein called **prostate-specific antigen (PSA)** is released into the bloodstream when the prostate gland is inflamed, and elevated levels can be a marker for prostate inflammation and even prostate cancer.

One area of intense interest for biomarker development is to identify indicators of traumatic brain injuries such as concussions. As you may know, concern has been raised about long-term effects of concussions in athletes such as football players, which has revealed a degenerative brain condition called chronic traumatic encephalopathy (CTE). Currently CTE can only be detected by analyzing the brain after a person has died, thus a biomarker for CTE would be of value in diagnosing this illness and for potentially treating people showing effects of CTE.

Detecting individual genes or gene expression patterns also provides scientists with biomarkers for disease, and many biotechnology companies are actively involved in searching for better biomarkers that can be used for early detection and disease diagnosis. It is also now known that for some cancers, when tumor cells die they release DNA (called *circulating tumor DNA* or *ctDNA*) into the bloodstream, and fragments of the cancer cell genome can sometimes be detected by DNA sequencing. Research here is far too preliminary to know whether analyzing ctDNA can be an accurate and reliable way to diagnose cancer early enough to help patients.

Protein microarrays are another option for disease diagnosis. They are used in much the same way as DNA microarrays. These microarrays can contain hundreds or thousands of antibodies spotted on a chip. Similarly, scientists have recently developed a way to detect *autoantibodies* (antibodies produced against one's own, diseased cells) in a single drop of blood as an accurate way to predict the onset of Alzheimer's disease and other conditions. It is hoped that early detection may lead to new drugs that can delay or possibly even eliminate progression of the disease.

Devices such as Fitbits already exist for monitoring vital signs such as blood pressure and heart rate. Research is progressing rapidly with smartphones and wearable devices that may be used for detecting biomarkers in the future. Finally, researchers from Harvard University and the Massachusetts Institute of Technology have developed ink for so-called smart tattoos that can sense changing levels of biomarkers such as glucose. Eventually, technology such as this may enable a way for continuous monitoring of disease conditions.

The Human Genome Project Revealed Disease Genes on All Human Chromosomes

Prior to completion of the Human Genome Project, only about 100 genetic diseases could be tested for. Now there are over 2,500 diseases for which genetic tests are available, and the molecular basis for over 5,500 diseases is known, yet therapies exist for only around 500 of these diseases. Through the Human Genome Project, scientists developed complex "maps" showing the locations of normal and diseased genes on all human chromosomes. **Figure 11.2** shows simplified chromosome maps, highlighting one or two prominent genes on each chromosome that are known to be involved in human genetic disease.

The Human Genome Project also led to the development of several follow-up projects, such as **The Cancer Genome Atlas Project (TCGA),** a comprehensive effort to identify genomic changes involved in a variety of different cancers. Scientists are particularly interested in genetic changes that trigger normal cells to become cancerous cells in the brain, mammary glands, ovaries, pancreas, liver, and lungs because cancers of these organs affect large numbers of Americans. As you will soon learn, information from TCGA and other genomics projects is having a great impact on diagnosing and treating cancer.

Genetic Testing: Detecting Chromosomal Abnormalities and Defective Genes

Molecular biology techniques have proven to be extremely valuable for detecting many different genetic diseases. Genetic testing was one of the first successful applications of recombinant DNA technology, and currently nearly 1,000 gene tests are in use. Increasingly, scientists and physicians can directly examine an individual's DNA for mutations associated with disease. These tests usually detect gene alterations associated with single-gene disorders such as sickle cell anemia, cystic fibrosis, Huntington's disease, hemophilia, and muscular dystrophy. But only about 3900 protein-coding genes (~0.3% of the human genome) are involved in disorders created by a single gene. As you will learn later in this section, DNA sequencing is increasingly being used to provide a comprehensive analysis of the genome for genetic testing applications.

Genetic testing is used for prenatal, childhood, and adult *prognosis* and *diagnosis* of genetic diseases and to identify genetic diseases in embryos created by *in vitro* fertilization, among other applications. For genetic testing of adults, DNA from white blood cells is commonly used. Alternatively, many genetic tests can be carried out on DNA from cheek cells, collected by swabbing the inside of the mouth, or on hair cells. Some genetic testing can be carried out on gametes. What does it mean when a genetic test is performed for *prognostic* purposes, and how does this differ from a *diagnostic* test? A prognostic test predicts a person's likelihood of developing a particular genetic disorder. A diagnostic test for a genetic condition identifies a particular mutation or genetic change that causes the disease or condition. Sometimes a diagnostic test identifies a gene or mutation associated with a condition, but the test will not be able to determine whether the gene or mutation is the cause of the disorder or is a genetic variation that results from the condition.

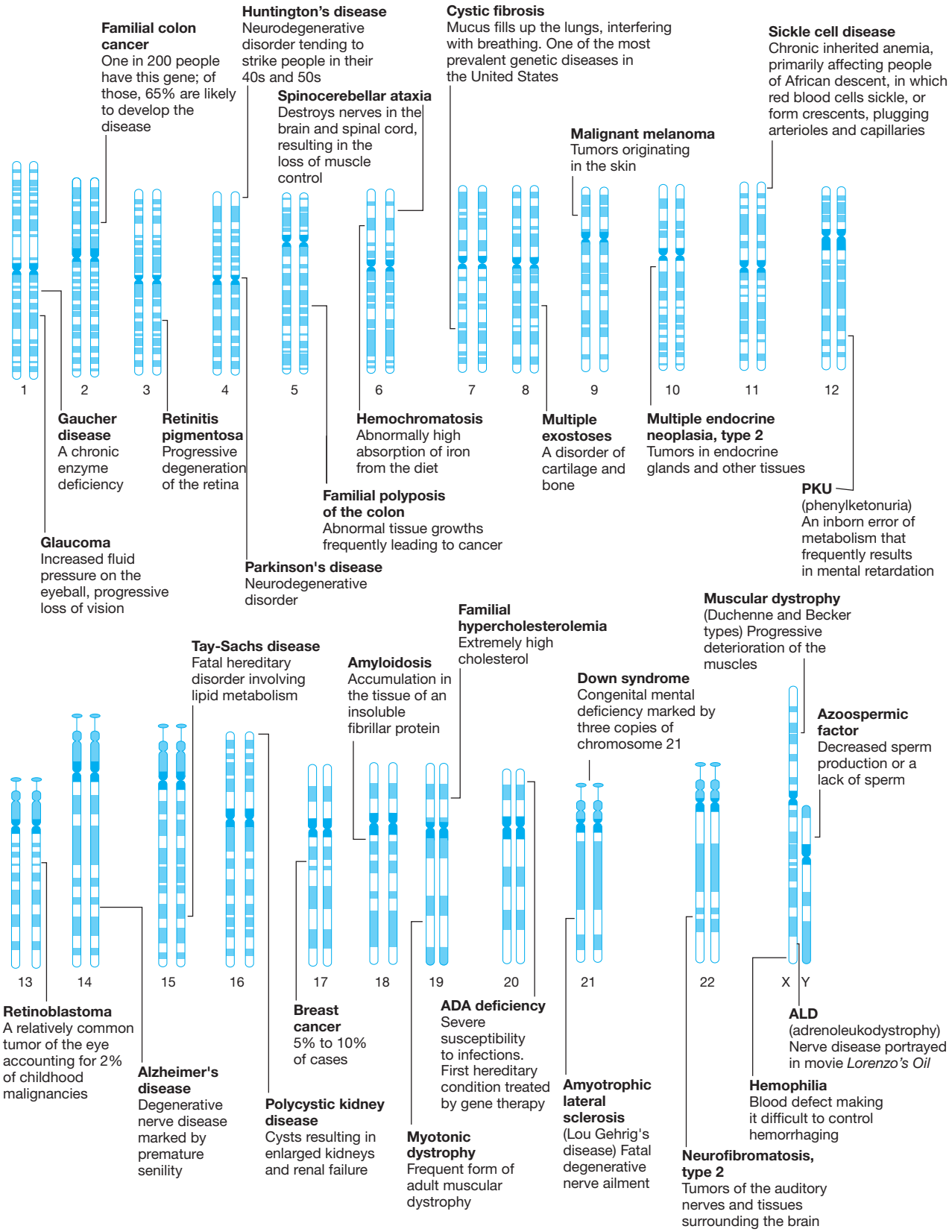


FIGURE 11.2 Disease Gene Maps of Human Chromosomes Maps show one or two representative genes on each human chromosome that are involved in a genetic condition. Many more genes than shown in this figure are located on each chromosome. Note: Chromosomes are not drawn to scale.



TOOLS OF THE TRADE

Using the Internet to Learn about Human Chromosomes and Genes

We have discussed the importance of bioinformatics for analyzing genetic information and creating databases as tools that scientists around the world can use to compile, share, and compare DNA sequence information. The wealth of freely available databases that catalog information about human chromosomes and genes resulting in part from the Human Genome Project is a great example. An excellent way to learn about what the Human Genome Project has revealed is to review some of the chromosome maps available on the Internet. For instance, if you are interested in the Y chromosome, you could review maps and descriptions of the genes found on it to discover why this chromosome is partially responsible for making male humans.

We encourage you to use the websites mentioned here and available through the Companion Website to follow a chromosome or gene of interest for a few months to see what kinds of information you can uncover. These sites are great resources and among the best sites for learning about human disease genes and chromosome maps. You can, for example, use them to learn more about a rare disease gene related to a disease condition affecting someone close to you. These sites present up-to-date information that cannot be found in even the most recent books. If you cannot find a gene of interest at these sites, it probably has not been identified yet!

The Department of Energy's Human Genome Program Information site provides excellent chromosome maps of identified genes and good historical information about the Human Genome Project. The Online Mendelian Inheritance in Man site (OMIM) is a great database of human genes and genetic disorders. In the keyword box, type in the name of a gene or disease that interests you. For example, type in "breast cancer" and then click the search button. When the next page appears, you will see a list of genes implicated in breast cancer, along with corresponding access numbers highlighted in blue as links. Clicking on one of the links will take you to a wealth of information about that gene, including background information, links to scientific papers about the gene, gene maps, and even nucleotide and protein sequence data (when available). You might also want to search this site to see if a gene has been found for a particular behavioral condition (for example, alcoholism or depression).

The National Center for Biotechnology Information (NCBI) sponsors the Genomes and Maps website, which allows you to access detailed maps of chromosomes and disease genes using a feature called Map Viewer.

Until relatively recently, most genetic testing occurred on fetuses for the purpose of identifying the sex of a child or to detect a small number of genetic diseases. Most of these procedures involved testing for genetic conditions that occur as a result of alterations in chromosome number or large structural abnormalities of chromosomes. If there are problems with chromosome separation during the formation of sperm or egg cells, a fetus may contain abnormal numbers of chromosomes. One of the best-understood examples of a disorder created by an alteration in chromosome number is **Down syndrome.** Most individuals with this condition have three copies of chromosome 21 (trisomy 21). Affected individuals show a number of symptoms, including cognitive impairments, short stature, and broadened facial features.

Fetal testing for Down syndrome is fairly common, particularly in pregnant women older than 40 years, because the incidence of Down syndrome is related to the age of eggs produced by the woman. Trisomy 21 and other abnormalities in chromosome number can be tested in a fetus to provide parents with information that may be used to determine if they want the pregnancy to continue. If a defect is detected, genetic tests also provide information that can be used to treat fetuses during pregnancy and after the child is born.

So, how is a developing fetus tested for Down syndrome? Two different techniques are commonly used, **amniocentesis** and **chorionic villus sampling (CVS).** Amniocentesis is performed when the developing fetus is around 16 weeks of age. A needle is inserted through the mother's abdomen into the pocket of amniotic fluid surrounding and cushioning the fetus (**Figure 11.3**). This fluid contains cells shed from the fetus, such as skin cells. Isolated cells are then cultured for a few days to increase cell numbers, after which the cells are treated to arrest them in mitosis to facilitate the viewing of mitotic chromosomes, which are spread onto a glass slide. The chromosomes are stained with different dyes that bind to proteins attached to the DNA, creating patterns of alternating light and dark bands on each chromosome. Based on the size of

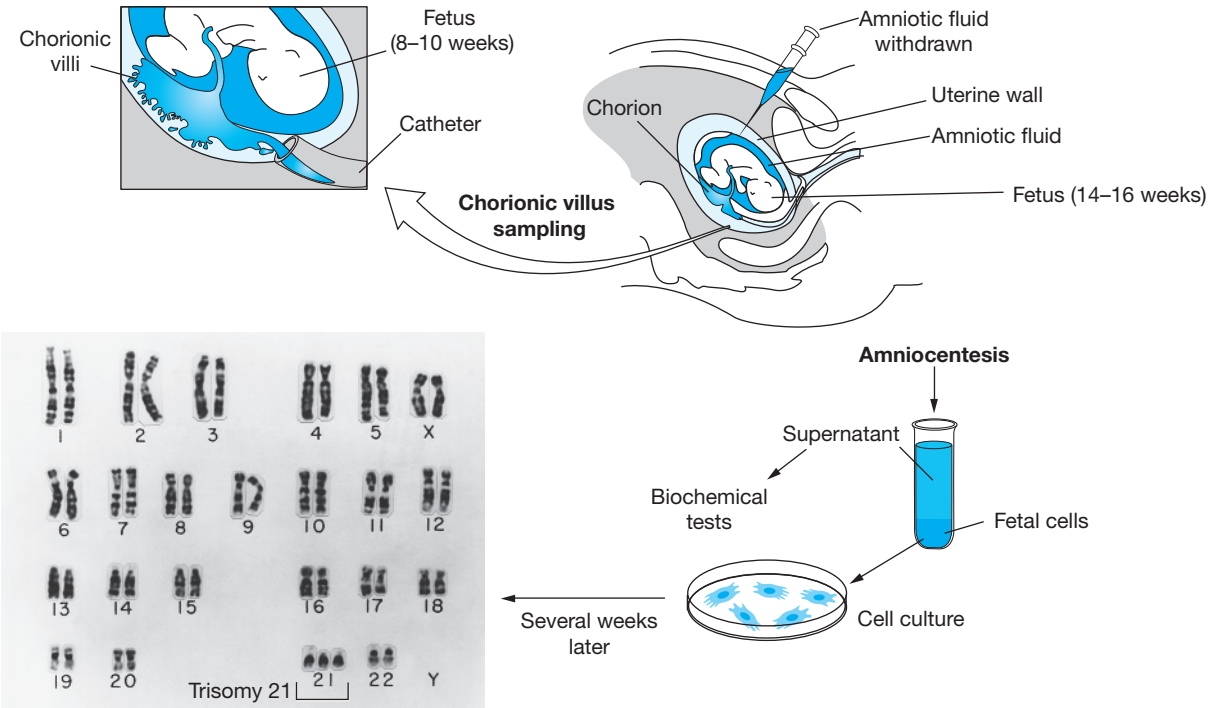


FIGURE 11.3 Amniocentesis and Chorionic Villus Sampling Fetal testing for chromosomal abnormalities is most commonly achieved through either amniocentesis or chorionic villus sampling. This karyotype from a person with Down syndrome shows three copies of chromosome 21 (trisomy 21).

each chromosome and its banding pattern, chromosomes can be aligned into pairs. This procedure is called **karyotyping;** it can also be used to determine a child's sex based on the presence of the sex chromosomes (X and Y) (see Chapter 2).

During CVS, a suction tube is used to remove a small portion of a layer of cells called the chorionic villus, fetal tissue that helps form the placenta (Figure 11.3). An advantage of CVS over amniocentesis is that enough cells are obtained so the sample can immediately be used for karyotyping. Another advantage of CVS is that the procedure can be done earlier in the pregnancy, around 8 to 10 weeks. Because the fetus is so small at that stage, CVS carries a higher risk than amniocentesis of disturbing it and causing a miscarriage (overall, about 1 percent of amniocentesis and CVS procedures cause miscarriages). Later we will discuss sequencing individual genomes, but the genomes for several children in utero have been sequenced from CVS samples and this may become a routine technique in the future.

Noninvasive prenatal genetic diagnosis (NIPD) procedures are also being developed for prenatal testing of fetal DNA. These procedures reduce the risk to the fetus. Circulating in each person's bloodstream is DNA that is released from the person's dead and dying cells. The blood of a pregnant woman also contains snippets of DNA from the developing fetus. It is estimated that approximately 3 to 6 percent of the DNA in a pregnant mother's blood belongs to her baby. It is now possible to analyze these traces of fetal DNA to determine if the baby has certain types of genetic conditions such as Down syndrome. Such tests require about a tablespoon of blood.

Tests for fetal genetic analysis based on maternal blood samples started to arrive on the market in 2011. Sequenom of San Diego, California, was one of the first companies to launch such a test—*MaterniT® 21 PLUS*, a Down syndrome test that can also be used to test for trisomy 13 (Patau's syndrome) and trisomy 18 (Edwards' syndrome). For Down syndrome, MaterniT® 21 PLUS analyzes 36–base pair (bp) fragments of DNA to identify chromosome 21 from the fetus. Sequenom claims that this test is highly accurate, with a false-positive rate of just 0.2 percent. The test can be done as early as week 10 (about the same time CVS sampling can be performed, which is about 4 to 6 weeks earlier than amniocentesis can be performed). Other companies have followed the Sequenom approach, and it has been estimated that the future market for these tests could be greater than $1 billion. As discussed later in the You Decide on page 307, many ethical issues are associated with genetic testing.

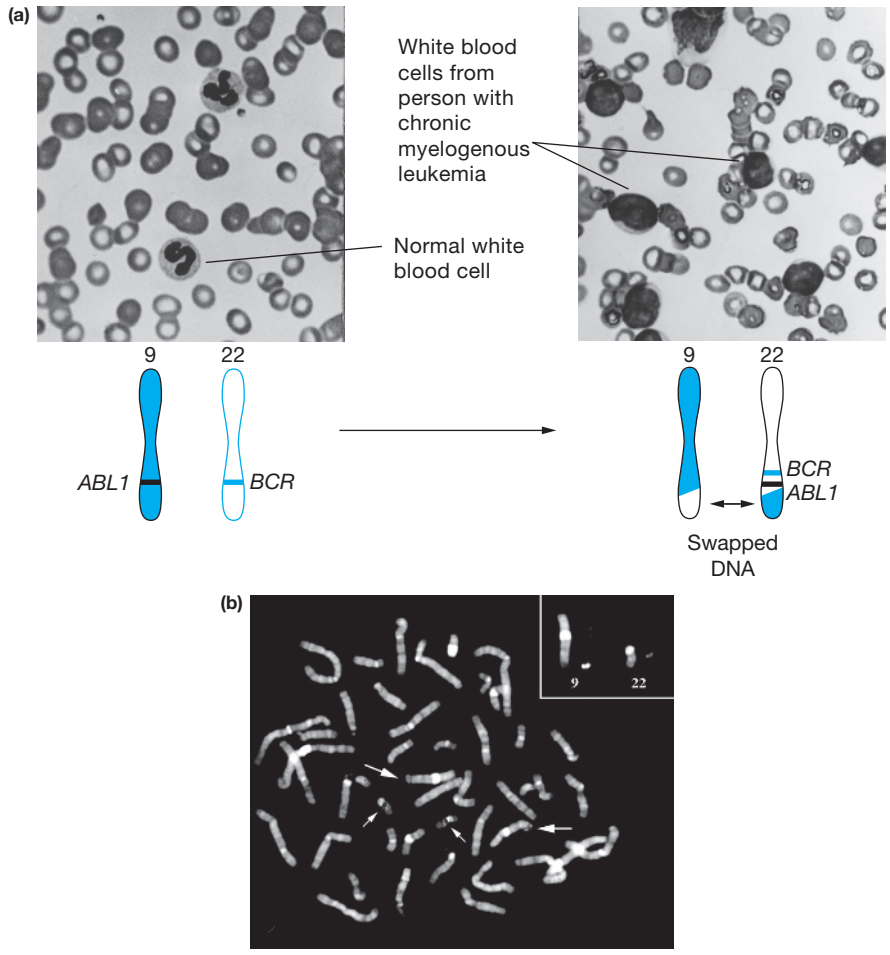


FIGURE 11.4 FISH Can Be Used to Detect Chromosomal Defects (a) Chronic myelogenous leukemia, a cancer of white blood cells created when genes on chromosome 9 and 22 are swapped, can be detected by FISH. (b) FISH analysis showing probe for *BCR* gene binding to chromosome 22 (small arrows) but also to chromosome 9 (large arrows) suggesting the presence of a region of homology between the two chromosomes.

Karyotyping is easily carried out on adults to check for chromosomal abnormalities. Typically, blood is drawn from an adult and the white blood cells are used. A modern technique for karyotyping in both fetuses and adults is **fluorescence in situ hybridization (FISH).** In FISH, a chromosome spread is prepared on a slide, and then fluorescent probes are hybridized to each chromosome. Each probe is specific for certain "marker" sequences on each chromosome. In some cases, FISH can be performed with probes that fluoresce different colors—a procedure called **spectral karyotyping.** FISH is very useful for identifying missing chromosomes and extra chromosomes, but in particular FISH makes it much easier than conventional karyotyping to detect defective chromosomes.

A number of human genetic diseases due to chromosomal abnormalities occur when a portion of a chromosome is deleted or a piece of chromosome is swapped from one chromosome to another because of problems in chromosomal replication. For instance, in a type of leukemia (a cancer of the white blood cells) called chronic myelogenous leukemia, DNA is exchanged between chromosomes 9 and 22, so that genes from 9 are swapped onto 22 and vice versa (Figure 11.4). This exchange, called a *translocation*, can be detected by FISH using different-colored fluorescent probes for each chromosome.

More genetic diseases result from mutations in specific genes than abnormalities in the numbers or structures of chromosomes. As a result of the Human Genome Project, more sophisticated techniques have been developed to detect individual diseased genes in both fetuses and adults. For example, **allele-specific oligonucleotide (ASO) analysis** allows for the detection of a single-nucleotide change in a gene. In this technique, DNA is isolated from human cells, usually white blood cells, and then amplified by the polymerase chain reaction (PCR) using primers that flank a disease gene of interest. Amplified DNA is then blotted onto nylon filters and hybridized separately to two different ASOs as probes. ASOs are small single-stranded oligonucleotide sequences, usually around 20 nucleotides in length. An ASO that will hybridize to a normal gene and another for the mutant gene are used. Figure 11.5 shows an example of how ASO analysis can be used to test for **sickle cell anemia,** which

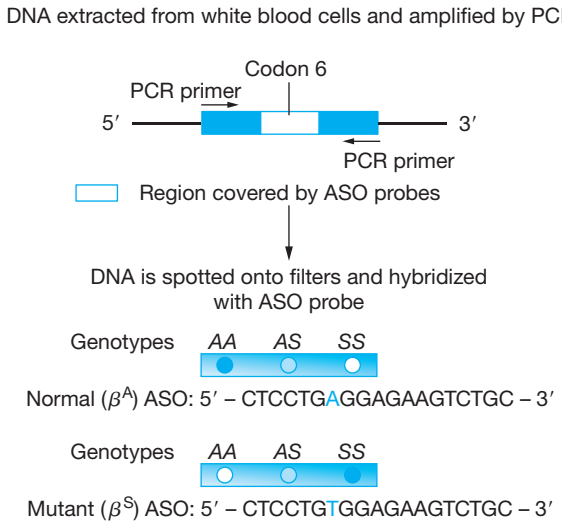


FIGURE 11.5 Using PCR and ASOs to Test for the Sickle Cell Gene ASO tests are valuable for detecting single-nucleotide mutations, such as the one that causes sickle cell anemia (see also Figure 2.18). In this example, DNA from white blood cells is amplified by PCR, blotted onto nylon membrane, and hybridized to fluorescently labeled ASO probes (probe binding shown as blue spots). The ASO for the normal hemoglobin gene ($ß^A$) will bind to DNA on the nylon only if the normal gene is present; the ASO for the mutant hemoglobin gene ($ß^S$) will bind to the defective gene only if it is present on the nylon. In this figure, probe binding is represented by blue DNA from individuals who are homozygous for the normal gene (AA) and have two copies of the $ß^A$ allele; their DNA will bind only to the normal $ß^A$ ASO. Individuals who are homozygous for the sickle cell hemoglobin gene (SS) have two copies of the $ß^S$ allele, and their DNA will bind only to the $ß^S$ ASO and not the normal $ß^A$ ASO. Heterozygous individuals (AS) have one normal copy of the gene and one mutant copy; as a result, their DNA will bind to both probes but produce a lighter hybridization signal than that from homozygous individuals.

occurs when a person has two mutant versions of the ß-globin gene. Mutant copies of ß-globin protein produce an abnormal form of hemoglobin that affects the size and shape of red blood cells, giving them a characteristic "sickled" appearance.

One major advantage of PCR is its high sensitivity for detecting defects in small amounts of DNA. Consequently, PCR and ASO analysis as well as FISH are also used to screen for gene defects in single cells from 8- to 32-cell embryos created by *in vitro* fertilization (IVF) through an approach called **preimplantation genetic diagnosis (PGD) or preimplantation genetic testing (PGT)**. PGD is sometimes referred to as embryo selection or screening, because it allows parents to screen for certain genetic diseases prior to selecting an embryo for implantation. PGD has been used for over 25 years, typically when there is concern about inheritance of a particular genetic defect. In the United States PGD is used in approximately 25% of IVF cases. Also, as you will learn in the next section, it is now possible to carry out whole-genome sequencing (WGS) on individual cells, and this method is being applied for PGD of single cells from embryos created by IVF. By far, PGD has the largest share of the genetic testing market, and the PGD market alone is estimated to be worth $542 million by 2022.

Currently, several hundred defective genes, including most of the genes involved in diseases noted in Figure 11.2, can be tested for in adults or fetuses using many of the techniques we have described.

Single-nucleotide polymorphisms

If a segment of chromosomal DNA from two different people is compared by DNA sequencing, approximately 99.9 percent of the DNA sequence will be exactly the same. One of the many intriguing findings of the Human Genome Project was the discovery that **single-nucleotide polymorphisms** (**SNPs;** pronounced "snips") represent one of the most common forms of genetic variation among humans. SNPs are single-nucleotide changes in DNA sequences that vary from individual to individual (see Figure 1.15).

SNPs have been found on all human chromosomes. It has been estimated that SNPs make up about 90 percent of human genetic variation and occur approximately every 100 to 300 bp in the human genome. Most SNPs are less likely to have an effect on a cell because they occur in non–coding regions (introns) of the genome. But when a SNP occurs in a gene sequence, it may cause a change in protein structure that produces disease or influences traits in a variety of ways, including conferring susceptibility for some types of disease conditions.

SNPs represent variations in DNA sequences that may ultimately influence how we respond to stress and disease. The first SNP discovered to be associated with a disease condition was for sickle cell disease. Because SNPs occur frequently throughout the genome, they serve as valuable genetic markers for identifying disease-related genes. SNPs are being used to predict susceptibilities to ailments such as stroke, diabetes, cancer, heart disease, behavioral and emotional illnesses, and a host of other disorders with a genetic basis.

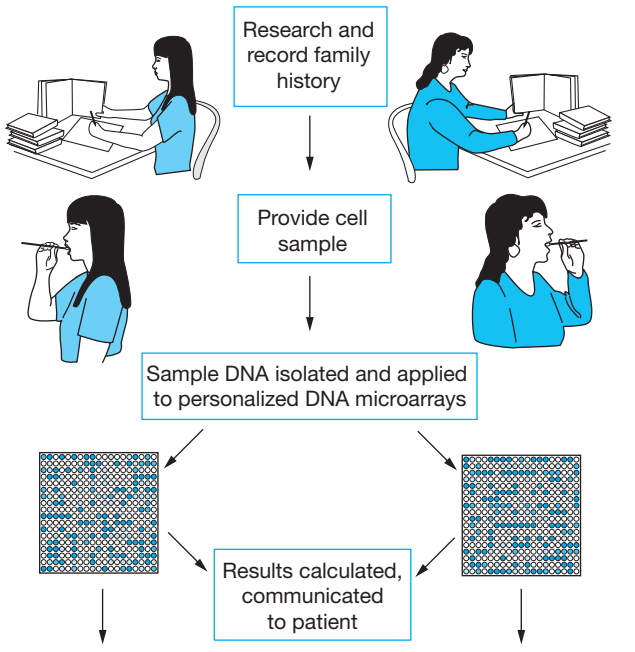
Pharmaceutical companies have invested millions of dollars in collaborative partnership among companies, academic institutions, and private foundations to identify and catalog chromosomal locations (loci) of the more than 1.4 million SNPs that are present in the 3 billion bp of the human genome and to understand the roles of SNPs in disease diagnosis and treatment. As you just learned, ASO analysis is one way to detect SNPs, as is DNA sequencing, which we will discussion in Section 11.3.

Identifying sets of disease genes by microarray analysis

For over 20 years another key technique for genetic testing has been **DNA microarrays,** also called gene chips (Chapter 3). A single microarray can contain probes for thousands of genes. Researchers can use microarrays to screen a patient for a pattern of genes that might be expressed in a particular disease condition. As shown in **Figure 11.6**, microarray data can then be used to predict the patient's risk of developing disease based on the patient's expressed genes for the disease. For example, microarrays have been used to identify genetic differences in patients with various types of cancer, and then treatment strategies are designed based on subtle differences in the expression of cancer-causing genes.

To do this, DNA or RNA is isolated from a patient's tissue sample—a blood sample or even a scraping of cells lining the cheeks. The patient's DNA is tagged with fluorescent dyes and then hybridized to the chip. Spots on the microarray where the patient's DNA bound are revealed by fluorescence (refer to Figure 3.17). Binding of a patient's DNA to a gene probe on the chip indicates that his or her DNA has a particular mutation or SNP. Companies have even developed handheld chip devices to get nearly instant information about a patient's genetics. Since the beginning of microarray technology, significant questions have been raised about the importance of quality control and consistency measures in using microarrays for genetic testing. In response to such concerns, the U.S. Food and Drug Administration (FDA) initiated guidelines for microarrays (and DNA sequencing) known as *microarray quality control* (*MAQC*) designed to provide measures for the research community to avoid experimental problems that can provide inaccurate results and to help the public understand the limitations of microarray data.

Although microarrays have been valuable for examining numbers of genes or SNPs involved in disease, increasingly DNA sequencing (and even RNA sequencing) is rapidly replacing microarray analysis, which in the near future may become obsolete for genetic testing purposes.



Susan's Genetic Profile

Trait	Risk
Addictive behavior	: Greater than general population
Lung cancer	: Greater than general population
Colon cancer	: Less than general population
Alzheimer's disease	: Less than general population

Lisa's Genetic Profile

Trait	Risk
Cystic fibrosis	: 100% diagnosis
Type II diabetes mellitus	: Less than general population
Cardiovascular disease	: Greater than general population

FIGURE 11.6 Using Gene Microarrays to Create a Genetic Profile

11.3 Sequence Analysis of Individual Genomes

Because of the relatively low cost of quickly and accurately sequencing individual genomes—what we call **personal genomics**—the ways that scientists and physicians evaluate a person's genetic information is rapidly changing. **Whole-genome sequencing (WGS)** is being utilized in medical clinics at an accelerating rate. In the future, WGS may become the routine method for almost all forms of genetic testing.

Recently, WGS has provided new insights into the genetics of anorexia, Alzheimer's disease, and autism, among other disorders. Already there have been some very exciting success stories whereby WGS of individual genomes has led to improved treatment of diseases in children and adults. Although personal genomics is rapidly becoming more widely implemented, it is not yet a routine practice, but it may be in the future. However, important issues about the quality control standards of clinically relevant sequencing data for use in disease diagnosis must be addressed to ensure the accuracy of sequence data and their interpretations.

Similarly, very significant data privacy issues are associated with personal genomics. There have been several highly publicized examples in which names and addresses of individuals participating in personal genome projects have been revealed from demographic data disclosed in online databases. This demonstrates that anonymity is often easily breached—revealing highly



YOU DECIDE **Genetic Testing: Issues to Consider**

Since genetic testing first became available, many concerns have been raised about how genetic information could be used and what it may be used for beyond personal and private decisions. Consider some of these issues:

- What are the psychological effects of a false result, which may indicate erroneously that a healthy person has a disease gene or a gene defect that goes undetected in a person with a genetic disorder?
- How do we ensure privacy and confidentiality of genetic information and avoid genetic discrimination?
- Who should have access to your genetic information? How could your genetic background be used to discriminate against you? How could your health or life insurance company's access to your genetic information affect your premiums? Could your premiums be raised based on "genetic" risk in the same way that premiums are raised based on other risks, such as how old you are and the car you drive?
- What are your obligations to inform others, such as a potential spouse or employer, of your knowledge about a possible genetic disorder?
- If genes are discovered for undesirable human behaviors, how would these genes be perceived in courts of law if accused criminals use genetics as their basis for a plea of not guilty by reason of genetics?
- Errors in genetic testing can have tragic consequences. The Organization for Economic Cooperation and Development (OECD), the European Commission (EC), and World Health Organization (WHO) are working to develop consensus around international standards and best practices to ensure the quality of genetic services in their member countries. What quality control requirements should be in place to minimize errors?

In addition to the previous questions, prenatal and fetal testing raises other ethically challenging questions such as:

- Should we test unborn children or adults for genetic conditions for which there is currently no treatment or cure?
- What are acceptable consequences if parents learn their unborn child has a genetic defect?
- Would society implement mechanisms to prevent or dissuade individuals with genetic defects from having children?
- Most insurance companies are not yet paying for WGS of maternal blood, which can cost as much as $1,000 for a single test. Should such tests be covered by routine health insurance plans?

As you can see, genetic testing is certainly not without its controversies and there are few easy answers to these issues. Visit the "Your Genes, Your Choices" website listed on the Companion Website for a thought-provoking series of ethical dilemmas created by genetic testing and genetic technologies. What would you do if you had to face the scenarios presented at this site? You decide.

sensitive information, including predispositions to genetic conditions.

Whole-Exome Sequencing

In Chapter 3 recall that we introduced the concept of **whole-exome sequencing (WES)**. This alternative to WGS has also produced promising results in clinical settings. For example, from the time he was born, Nicholas Volmer had to live with unimaginable discomfort from an undiagnosed condition that was causing intestinal fistulas (holes from his gut to outside his body) that were leaking body fluids and feces and requiring constant surgery. By 3 years of age, Nicholas had been to the operating room more than 100 times. A team at the Medical College of Wisconsin decided to have Nicholas's exome sequenced. Applying bioinformatics to compare his sequence to that of the general population, they identified a mutation in a gene on the X chromosome called *X-linked inhibitor of apoptosis* (*XIAP*). *XIAP* is known to be linked to another condition that can often be corrected by a bone marrow transplant. In 2010 a bone marrow transplant saved Nicholas's life and largely restored his health. Shortly thereafter, the popular press described Nicholas as the first child saved by DNA sequencing.

In the future, you will continue to hear about progress with WES for diagnosing disease. In late 2017 the FDA approved a WES assay by Foundation Medicine of Cambridge, Massachusetts; this examines coding sequences for 354 genes and can be used to identify any of five cancer tumor types (non-small cell-lung, melanoma, breast cancer, colorectal, and ovarian) in patients.

The National Institutes of Health (NIH) has an initiative called the *Undiagnosed Diseases Network*. Its goal is to use WES and WGS to help diagnose rare diseases (often called orphan diseases) of unknown genetic basis—which may include more than 7,000 conditions. These diseases often do not attract the attention of large companies because relatively few people have each disease (defined in the United States as affecting less than 200,000 individuals per disease condition) compared to diseases such as heart disease, but it is estimated that over 300 million people around the world have rare diseases. In this program, exome sequences from an individual with a disorder can be compared to exome sequences from healthy family members and reference sequences to identify mutations that may be involved in the disease. To date, the program has diagnosed over 300 cases. Unfortunately for many of these rare conditions, a cure or treatment has often not been developed.

Genomic Analysis of Single Cells by DNA and RNA Sequencing

We now have the ability to sequence the genome from a *single cell*! **Single-cell sequencing (SCS)** typically involves isolating genomic DNA from a single cell that is then subjected to PCR to produce sufficient DNA to be sequenced. Amplification of the genome without introducing errors remains a major challenge that researchers are working on so that SCS can become a more reliable and accurate technique for genetic testing.

Genomic sequencing from single cells is valuable for analyzing both *somatic cell mutations* (for example, mutations that arise in somatic cells, such as in a skin cancer, which are not heritable) as well as *germ-line mutations* (heritable mutations that are transmitted to offspring via gametes). SCS allows scientists to explore genetic variations from cell to cell. These studies are revealing that different mutant genes can vary greatly between individual cells. In particular, cancer cells from a tumor often show genetic diversity, a fact that is being appreciated more and more by researchers and clinicians. Understanding variations in genetic diversity and gene expression by individual cells within a tumor will lead to better and more specific treatment options.

The contribution of individual cells to the phenotype of a tissue or organ affected by a genetic disorder is increasingly of interest. Thus, **RNA sequencing (RNA-seq)** is becoming a powerful tool for transcriptome-wide analysis of genes expressed by cells within a population, allowing researchers to differentiate genetic variations between cells. Until recently, researchers or clinicians had to analyze the genome and transcriptome from cells independently. But now, it is possible to isolate DNA and RNA from the same cells, sequence the DNA, and, thanks to **single-cell RNA sequencing (scRNA-seq)**, sequence RNA from these same cells. This enables a comparison of the genes present in a cell and the relative levels of expression for each transcript encoded by the genome.

Many disease treatments are designed to target cells, such as those in a tumor, as if all cells are homogeneous in genotype and phenotype. In fact, often such cells are quite heterogeneous genetically. scRNA-seq is now being applied to reveal the heterogeneity of cell types in tumors and other conditions, to then help plan better treatment approaches based on genetics of the cell types and their relative abundance. scRNA-seq provides quantitative data about RNA expression, similar to gene-expression microarray analysis. But scRNA-seq reveals all transcripts expressed in a cell, whereas the transcripts analyzed by microarrays are limited by the probes present on the array. This is one reason why scRNA-seq is likely to eventually replace microarrays for transcriptome analysis.

Genome-Wide Association Studies Identify Genome Variations in Populations

Many of the genetic testing approaches we have discussed so far have focused on analyzing genes in individuals or relatively small numbers of people. Microarray-based genomic analysis and WGS have led geneticists to employ a powerful strategy called **genome-wide association studies (GWAS)** in their quest to analyze populations of people for disease genes.

GWAS of relatively large populations of people for diagnostic or prognostic purposes often enable scientists to identify multiple genes that may influence disease risk. During the past decade, the number of GWAS being reported has dramatically expanded. For example, GWAS for autism, obesity, diabetes, macular degeneration, myocardial infarction, arthritis, hypertension, several cancers, bipolar disease, autoimmune diseases, Crohn's disease, schizophrenia, amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS) are among the many GWAS that have been widely publicized in the scientific literature and popular press. Behavioral traits such as intelligence have also been analyzed by GWAS.

In a GWAS, the genomes from several hundred, or several thousand (if available), often unrelated individuals with a particular disease are analyzed, and results are compared with genomes of individuals without the disease. In some cases, related individuals are the focus of GWAS. For example, GWAS of female identical twins, but one with MS and one without MS, have helped researchers identify gene variants linked to a higher risk of developing MS. The goal is to identify genes, genetic variations (including SNPs and



YOU DECIDE — Genetic Testing: Destiny Tests?

A few companies are now promoting the ability to do *preconception testing* and thus make "destiny predictions" about potential phenotypes of hypothetical offspring based on computational methods for analyzing sequence data of parental DNA samples. The company 23andMe has been awarded a U.S. patent for a computational method called the *Family Traits Inheritance Calculator* to use parental DNA samples to predict a baby's traits, including eye color and the risk of certain diseases. This patent includes applications of technologies to screen sperm and ova for IVF.

Currently, gender selection of embryos generated by IVF is very common. But could preconception testing lead to the selection of "designer babies"? Fear of *eugenics* surrounds these conversations, particularly as genetic analysis starts moving away from disease conditions to nonmedical traits such as hair color, eye color, other physical traits, and potentially behavioral traits. The patent has been awarded for a process that will compare the genotypic data of an egg provider and a sperm provider to suggest gamete donors that might result in a baby or hypothetical offspring with particular phenotypes of interest to a prospective parent.

A company called GenePeeks claims to have a patent-pending technology for reducing the risk of inherited disorders by "digitally weaving" together the DNA of prospective parents. GenePeeks plans to sequence the DNA of sperm donors and women who want to get pregnant to inform women about donors who are most genetically compatible for the traits they seek in offspring. The company's proprietary computing technology is intended to use sequence data to examine "virtual" eggs and sperm from donor–client pairings to estimate the likelihood of about 10,000 specific diseases in hypothetical offspring from prospective parents.

Will technologies such as this become widespread and attract consumer demand in the future? What do you think? Would you want this analysis done before deciding whether to have a child with a particular person? What assurances or quality control measures should be in place to validate the accuracy of such destiny tests? You decide.

other variations) that may confer risk of developing a disease. Changes in the epigenome, such as methylation patterns, may also be used in GWAS. By determining which genes, SNPs, epigenome, or other changes are present in individuals with the disease, scientists can calculate the disease risk associated with each variation. Analysis of GWAS results requires statistical analysis to predict the relative potential impact (association or risk) of a particular genetic variation on development of a disease phenotype.

Figure 11.7 shows one representation, called a Manhattan plot, of how results from GWAS are commonly reported when there are a large number of data points. These data are from a study designed to identify genes implicated in extreme longevity (>90 years of age). Different loci in the genome are plotted on the *x*-axis; in this case loci on each chromosome are plotted. The results of a genotypic association test are plotted on the *y*-axis. There are several ways that associations can be calculated. Shown here is a negative log of *p* values for genes determined to be significantly associated with longevity from a population of over 7,000 individuals aged 90 or older. The control group was more than 16,000 individuals aged 65 or younger. The dotted line in this plot establishes a threshold value for

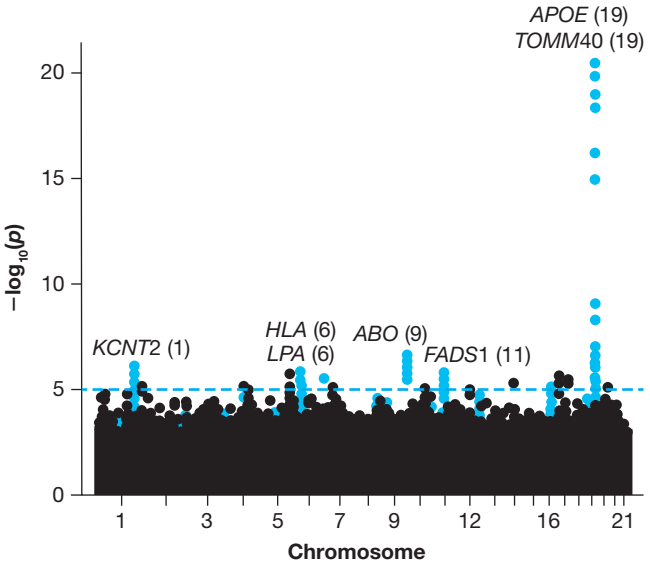


FIGURE 11.7 Genome-Wide Association Study (GWAS) for Human Longevity Results from a GWAS from populations of aging individuals. The dotted blue line indicates statistically significant correlations ($p < 0.05$), meaning there is a 95 percent or greater chance that a particular gene is correlated with aging. Notice here that seven genes were found to be significantly associated with aging in this study, as indicated by gene symbol with chromosome number in parenthesis.

significance. Genes with significance levels exceeding the threshold p value of 10^{-5}, corresponding to 5.0 on the y-axis, are likely disease-related sequences. Based on statistical analysis of these data, eight genes associated with extreme longevity include the *APOE* gene and a closely linked gene *TOMM40*. Variants of these genes are associated with an increased risk of Alzheimer's disease and coronary artery disease.

GWAS are showing us that, unlike single-gene disorders such as sickle cell anemia, many human genetic diseases involve a multitude of genetic factors contributing to the total risk for developing a condition. We need such information to make meaningful progress in disease diagnosis and treatment, which is ultimately a major purpose of what GWAS are all about. In some cases, risk data revealed by GWAS may help patients and physicians develop diet and exercise plans designed to minimize the potential for developing a particular disease. But often GWAS can reveal dozens of DNA variations, and much remains to be understood about the effects these variations have individually or collectively on risk for a particular disease over an individual's lifetime.

In the next section we consider how biotechnology is changing medicine through highly personalized treatment approaches.

11.4 Precision Medicine and Biotechnology

In the previous section we discussed how genetic testing, sequencing, and other approaches are helping scientists detect genetic differences that contribute to disease, with the hope that this will lead to new treatment options and cures that improve the quality of life for patients. The approaches we discussed in Section 11.3 are all important for understanding **precision medicine.**

Here we provide an overview of precision medicine and consider a few representative examples, concluding with immunotherapy—one of the most progressive and promising areas of medical biotechnology to emerge in the past few years. Recognize that many other topics are part of the precision medicine discussion, including gene therapy, which we will cover separately in Section 11.5 because of the amount of information to discuss.

What Is the Precision Medicine Initiative?

During a State of the Union address in January 2015, President Barack Obama announced the **Precision Medicine Initiative (PMI).** The mission statement of the PMI is "to enable a new era of medicine through research, technology, and policies that empower patients, researchers, and providers to work together toward development of individualized treatments."

Biotechnology has major roles in the PMI, from the identification of biological, behavioral, and environmental influences on disease through the research, development, and applications of new treatments. Precision medicine focuses on individualized treatments that are highly personalized based on individual differences in genetics, lifestyle, environment, and other factors. This is opposed to many current treatment strategies that are one size fits all, whereby most people with the same disease condition are treated the same way.

One initiative, through partnership with the NIH, is to recruit at least 1 million American volunteers from whom a range of biological sample data, including DNA sequencing, microbiome, epigenome data, and other data, will be collected and analyzed to provide new insights into health and disease at individual, community, and population levels. Understanding biological data in the context of behavioral issues, such as diet and exercise patterns (captured via wearable devices such as a Fitbit), as well as social and environmental factors, will be essential. Each person in the PMI will generate billions of data points, so handling, analyzing, storing, and protecting the privacy of these data are "big data" challenges for scientists.

Not everyone agrees that the PMI is an effective use of resources, and questions abound about the costs of the PMI (investment of more than $215 million is planned), as well as about issues such as data privacy and security, among others. See the Companion Website for the link to the PMI website.

Pharmacogenomics for precision medicine

Throughout the book we provide examples of how the biotechnology industry is identifying novel drugs and developing new ways to treat disease—from therapeutic proteins produced by recombinant DNA technology to *small molecule inhibitors*, drugs that can bind to proteins and block their function. Similarly, researchers are working on drugs that can serve as "activators," binding to and stimulating important proteins that may be used to fight disease.

The Human Genome Project and discovery of SNPs is partially responsible for the rapidly emerging field called **pharmacogenomics**—customizing medicine by designing treatment strategies based on the specific genetic profile of a particular patient. Pharmacogenomics is based on the idea that individuals can react differently to the same drugs, which can have varying degrees of effectiveness and side effects in part

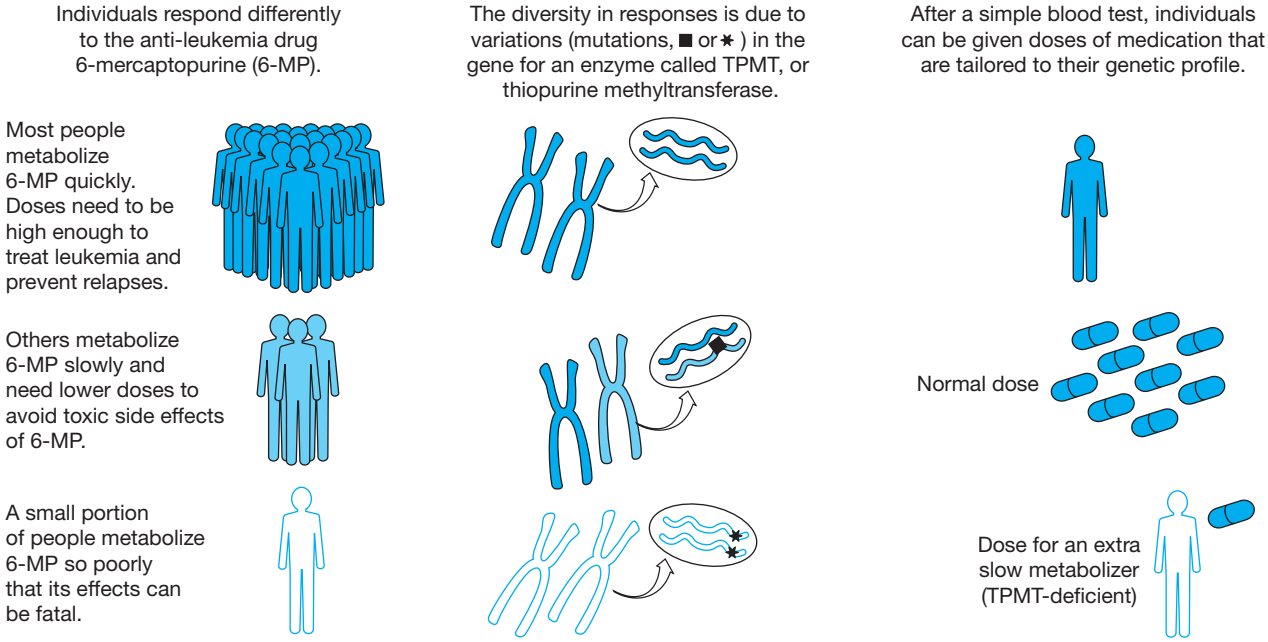


FIGURE 11.8 Pharmacogenomics Different individuals with the same disease often respond differently to a drug treatment because of subtle differences in gene expression. The dose that works for one person may be toxic for another—a basic problem of conventional medicine. This example shows patients' responses to a chemotherapy compound called 6-mercaptopurine (6-MP), which has long been used to treat children with acute lymphocytic anemia (ALL), a form of blood cancer. Since it was found that genetic variations of the gene for the 6-MP–degrading enzyme thiopurine methyltransferase (TPMT) are important for determining how patients respond to 6-MP, physicians now routinely run genetic tests (or blood tests to measure the enzyme levels) on ALL patients before determining the proper dosage of 6-MP.

because of genetic variations (**Figure 11.8**). Each year in the United States alone, over 100,000 deaths occur from the adverse effects of properly prescribed medications. It remains unclear whether pharmacogenomics will be a cost-effective broad approach to medicine or whether it will primarily be used for very specific treatments; nonetheless, this area of medical biotechnology holds great potential and has already demonstrated success for treating some conditions.

Many drugs currently used in **chemotherapy** can be effective against cancer cells because these drugs target rapidly dividing cells; however, these drugs also affect normal body cells that regularly reproduce, such as hair and skin cells and cells in the bone marrow, the last of which are responsible for making blood cells. As a result, hair loss, dry skin, changes in blood cell counts, and nausea are all related to the ways in which chemotherapy affects normal cells. Researchers have been looking for "magic bullet" drugs that destroy only cancer cells without harming normal cells. If such drugs were designed, patients might get well faster because the drugs would have little or no effects on normal cells in noncancerous tissues.

Consider the following example. Breast cancer is a disease that shows familial inheritance for some women. Women with defective copies of the genes called *BRCA1* or *BRCA2* have an increased risk of developing breast cancer, but other forms of breast cancer do not exhibit a clear mode of inheritance. In the past, all of these women would be treated with the same form of chemotherapy, with very mixed results. Since the development of pharmacogenomics, if a woman has a breast tumor thought to be cancerous, a small piece of the tissue is used to isolate RNA or DNA for genetic testing, microarray analysis, or sequencing, which could then serve to determine which genes are involved in a particular woman's form of breast cancer. Armed with this genetic information, a physician can then design a drug treatment strategy—based on the genes involved—that would be *specific* and *most effective* against this woman's cancer. A second woman with a different genetic profile for her breast cancer might undergo a different treatment.

Scientists at Genentech used this strategy to develop **Herceptin,** a type of **monoclonal antibody** (discussed in the next section) approved by the FDA in

1998. Herceptin binds to and inhibits HER-2, a protein produced by the human epidermal growth factor receptor 2 gene, which is overexpressed in about 25 to 30 percent of breast cancer cases. Women with HER-2–positive tumors (HER-2 overexpression) typically develop aggressive breast cancer with a greater likelihood of metastasis (spreading) and poorer prognosis for survival. Herceptin has proven to be effective in some women, but in others, tumors become resistant to the antibody. A similar problem has occurred with other pharmacogenomics drugs developed for treating other cancers. Approximately 15 to 20 percent of breast cancers are categorized as "triple negative" because tumors do not express three key proteins: HER2, estrogen receptor, and the progesterone receptor. These tumors aggressively metastasize and there are no current treatments; thus, significant research is under way to identify specific genes or proteins that can be targeted for treatment in these women.

One of the first successful examples of pharmacogenomics involved a drug called **Gleevec,** introduced by Novartis in 2001 and used to treat **chronic myelogenous leukemia (CML)**, the condition discussed in Figure 11.4. Gleevec targets the BCR-ABL fusion protein, which is created by an exchange of DNA between chromosomes 9 and 22 that occurs in CML; in doing so, Gleevec has proven to be a relatively effective way of treating the disease. Before Gleevec and other related treatments, the median survival length for a CML patient was 4 to 6 years. Now patients have a near-normal life expectancy, and the survival rate after 10 years is approximately 90 percent of individuals.

Gene expression data from DNA microarrays are being used to diagnose disease based on the genes patients express; then patients may be eligible for pharmacogenomics treatment (if available) based on those genes. **Figure 11.9** shows an example of microarray data for individuals diagnosed with different forms of leukemia. Notice how the patients can be grouped into different genetic categories of leukemias based on the clusters of genes most actively expressed. Because each group of patients expressed large numbers of different genes and proteins, there is no reason to think that they would all respond well to the same chemotherapy. With this knowledge,

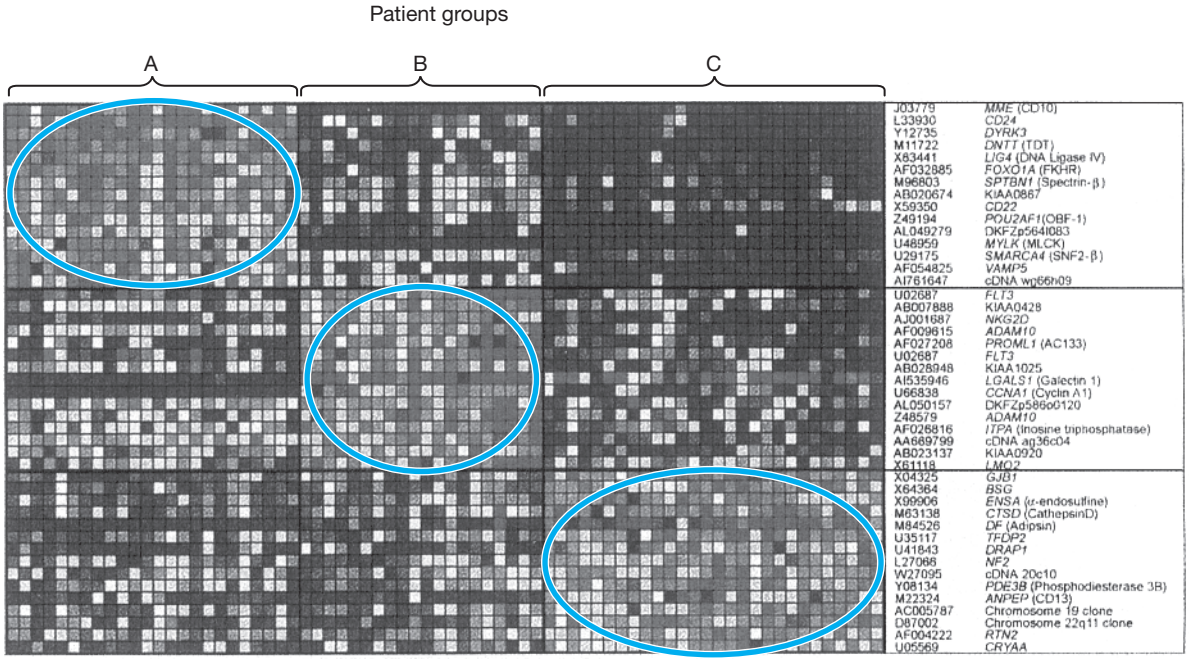


FIGURE 11.9 Microarrays for Gene Expression Analysis and Pharmacogenomics
Shown here are microarray results indicating gene expression profiles in leukemia patients. Each column represents data for one patient, and each row is a different gene (gene symbols and names are on the far right side of the figure). Because this black-and-white image was reproduced from a color image, gray spots shown here represent highly expressed active genes. Blue ovals highlight clusters of actively expressed genes (gray and white spots) that can be used to categorize patients into different groupings based on expressed and relatively inactive (dark spots) genes.

different chemotherapy approaches can be customized for each category of patients. As a result, patient survival rates have greatly increased. Several promising clinical trials are under way for pharmacogenomics treatments of melanoma and many other cancers. There are now approximately 1,200 FDA-approved pharmacogenomics drugs for a range of different diseases.

Recall from our discussions in Section 11.3 that, increasingly, whole-genome sequencing and personal genomics will probably become routine, or at least more commonly used than microarray analysis, so it will be interesting to follow what happens with personalized genomics and the impact this will have on pharmacogenomics. Similarly, the epigenome is being analyzed for its role in diseases and as a target for new personalized treatment approaches.

Nanotechnology and nanomedicine: Biotechnology at the nanoscale

Nanotechnology is an area of science involved in designing, building, and manipulating structures at the nanometer scale. A nanometer (nm) is one-billionth of a meter. For reference, a human hair is approximately 200,000 nm in diameter (and thus can fit about 3,000 nanoparticles across its width!), and DNA is about 2 nm in diameter. Nanotechnology is a big business, with applications in materials manufacturing, energy, electronics, and engineering, but **nanomedicine**—applications of nanotechnology for improving human health—is of particular interest for medical biotechnology.

Nanoparticles can be made from lipids, silica, gold, graphite, nanocrystal of semiconductor materials (called quantum dots), and even DNA. Nanoparticles can be coated with a variety of molecules to help them bind to proteins and carbohydrates on the surface of cell targets. Sometimes peptide or oligonucleotide coating of nanoparticles that bind to specific target molecules are referred to as *aptamers*. Because of their potential to better target specific cells in the body, unlike conventional drugs (such as chemotherapy, for example), we consider nanoparticles as part of our discussion about precision medicine.

Scientists envision tiny devices in the body carrying out a myriad of medical functions, including nanodevice sensors to monitor blood pressure, blood oxygen levels, and hormone concentrations, and nanoparticles that can unclog blocked arteries and detect and eliminate cancer cells. Prototypes of these examples and many others are under development. Sometimes even a well-designed drug is not as effective as desired because of delivery problems—getting the drug to where it must function. For instance, if a drug to treat knee arthritis is taken as an oral pill, only a small amount of the drug will be absorbed by the body and transported via blood to tissues of the knee joint. Other factors that influence drug effectiveness are a drug's solubility (its ability to dissolve in body fluids), drug breakdown by body organs, and drug elimination by the liver and kidneys. Many companies are working on nanotechnologies to develop innovative ways to improve drug delivery approaches, drug effectiveness, and ways to precisely measure how much drug has been released from a nanoparticle to a cell at a particular time.

Microspheres, nanoparticles between 1 and 100 nm that can be filled or coated with drugs, may be one way to improve drug delivery and effectiveness. These particles are often made of lipid materials that closely resemble the phospholipids in cell membranes. Delivery of microspheres as a mist sprayed into the airways through the nose and mouth has been used successfully for treating lung cancer and other respiratory illnesses such as asthma, emphysema, tuberculosis, and flu (**Figure 11.10**). Refer to Section 11.5 for a discussion of how microspheres called liposomes are used in gene therapy. Researchers are also investigating ways to package anticancer drugs into microspheres

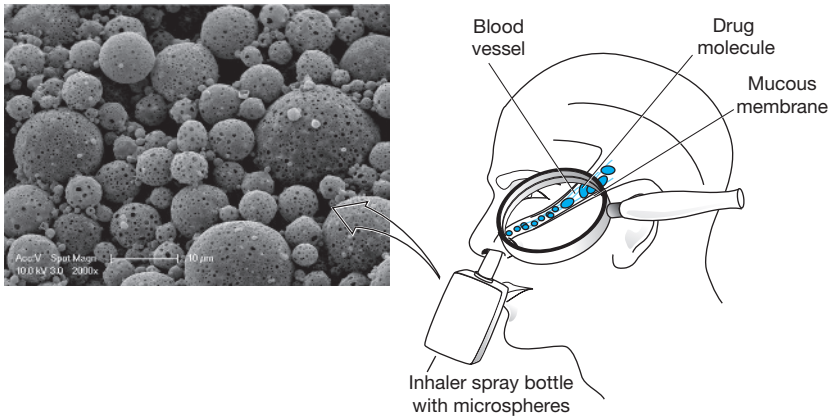


FIGURE 11.10 Microspheres for Drug Delivery Microspheres can deliver drugs to specific locations in the body or be distributed throughout the body, depending on how they are used. In this example, drug-containing microspheres are sprayed into the nose, where they will enter blood vessels and rapidly enter the bloodstream to travel throughout the body.

for implantation in the body adjacent to growing tumors; they are also working on anesthetics for pain management and adding microspheres to wafers and patches that can be used for drug delivery.

In 2006, the FDA approved Exubera, an inhalable version of insulin produced by Nektar Therapeutics of San Francisco and sold by the pharmaceutical giant Pfizer. Exubera is a recombinant form of insulin delivered as an inhalable powder; it offered diabetic patients the first alternative to needle-based delivery of insulin. But after barely a year, Pfizer stopped selling Exubera because of slow sales. Among the reasons given for Exubera's failure were the unwillingness of physicians and patients to try something new, a bulky inhaler used to deliver the particles, and a poor marketing strategy.

Scientists have developed "smart drugs" using gold nanoparticles that are introduced into the body to seek out and target viruses or specific cells, such as cancer cells; they then deliver a cargo intended to treat or destroy those cells rapidly, effectively, and silently, with few side effects (**Figure 11.11**). Currently, several dozen nanoparticle-based drugs are in clinical trials in the United States, and most of these target cancers. BIND Biosciences of Cambridge, Massachusetts, is one of a number of companies involved in various stages of clinical trials with drug-bearing nanoparticles coated with ligands to bind to cell surface proteins or receptors associated with diseased cells. Most of these particles are designed to target tumors in lung, prostate, and other cancers.

Many are optimistic about the powers of nanotechnology for drug delivery and therapeutics, so stay tuned!

Harnessing the Immune System for Treating Disease: Antibodies and Immunotherapy

As you learned in Chapter 5, vaccines can be used to stimulate the body's immune system to produce antibodies and provide a person with protection against infectious microbes. Certainly vaccination has been very effective for protecting us from pathogens that cause polio, tetanus, typhoid, and dozens of other diseases. Development of vaccines against some of the most-deadly pathogens is a very active and important area of ongoing research. Many scientists hope that vaccination may also be useful against conditions such as Alzheimer's disease and many different types of cancers, but vaccination for these purposes is still mostly unproven in humans.

Cancer vaccines are being experimented with as therapeutic treatments that are not preventative (as in the case of Gardasil) but designed to treat a person who already has cancer. In this approach, a person is injected with cancer cell antigens in an effort to stimulate the patient's immune system to attack existing cancer cells. In 2010 the FDA approved the first vaccine in the United States to treat cancer. Sipuleucel-T (Provenge) is

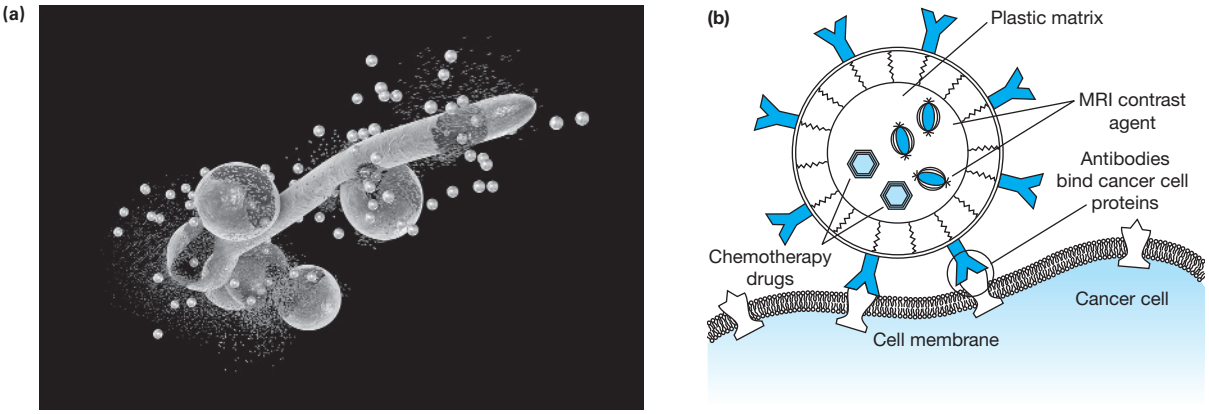


FIGURE 11.11 Tumor-Seeking and Tumor-Killing Nanoparticles (a) Silver nanoparticles coated with antifungal drugs shown binding to *Candida albicans*, a yeast that lives naturally in and on the human body. Overgrowth of *Candida* can cause infections. (b) Multifunctional nanoparticles have been developed that have shown promise for seeking out and destroying tumor cells. This example shows a plastic nanoparticle covered with antibodies against cancer cell proteins, which allow the particle to bind to cancer cells. Inside the particle are contrast agents, which can be used for magnetic resonance imaging or x-ray approaches to detect the tumor. The nanoparticle can also contain chemotherapy drugs that can diffuse out of it to kill tumor cells.



YOU DECIDE Direct-to-Consumer Genetic Tests

The past decade has seen dramatic developments in **direct-to-consumer (DTC) genetic tests,** an industry expected to grow to more than $340 million by 2022 in the United States alone. A simple web search will reveal many companies offering such tests. 23andme is among the most well-known and widely used companies with more than 2 million customers. There are approximately 2,000 diseases for which such tests are now available (in 1993 there were about 100 such tests). Most DTC tests require that a person mail a saliva sample, hair sample, or cheek cell swab to the company. For a range of pricing options, DTC companies largely use SNP-based tests such as ASO tests to screen for different mutations. For example, in 2007, Myriad Genetics, Inc., began a major DTC marketing campaign of its tests for *BRCA1* and *BRCA2*. Mutations in these genes increase the risk of developing breast and ovarian cancer. DTC testing companies report absolute risk, the probability that an individual will develop a disease; but how such risk results are calculated is highly variable and subject to certain assumptions.

Such tests are controversial for many reasons. For example, the test is purchased online by individual consumers and requires no involvement of a physician or other health care professionals, such as a nurse or genetic counselor, to administer the test or to interpret results. There are significant questions about the quality, effectiveness, and accuracy of such products because the DTC industry is currently largely self-regulated. The U.S. Food and Drug Administration (FDA) does not regulate DTC genetic tests.

Whether the FDA will oversee DTC genetic tests in the future is unclear. However, at the time of publication of this edition, the FDA has not revealed any definitive plans to regulate or oversee DTC genetic tests. But because some DTC genetic testing companies, such as 23andMe, offer health-related analyses or health reports, they do fall under FDA regulations. The FDA continues to issue warnings to DTC testing companies to provide what the FDA considers to be appropriate health-related interpretations of genetic tests. There are varying opinions on the regulatory issue. Some believe that the FDA has no business regulating DTC tests and that consumers should be free to purchase products based on their personal needs or interests. Others insist that the FDA must regulate DTCs in the interest of protecting consumers.

There is currently no comprehensive way for patients to make comparisons and evaluations about the range of tests available and their relative quality. Most companies make it clear they are not trying to diagnose or prevent disease, nor are they offering health advice. So what is the purpose of the information that these tests provide? Websites and online programs from DTC companies provide information on what advice a person should pursue if positive results are obtained. But is this enough? If the results are not understood, might negative tests provide a false sense of security? Just because a woman is negative for *BRCA1* and *BRCA2* mutations *does not* mean that she cannot develop breast or ovarian cancer.

The National Institutes of Health has created a **Genetic Testing Registry (GTR)** designed to increase transparency by publicly sharing information about the utility of their tests and research with the general public, patients, health care workers, genetic counselors, insurance companies, and others. The GTR is intended to allow individuals and families access to key resources to let make them better-informed decisions about their health and genetic tests. But participation in the GTR by DTC companies has not been made mandatory yet. Therefore, will companies involved in genetic testing participate? Should DTC genetic tests be more carefully regulated? You decide.

used to treat advanced prostate cancer in patients who do not respond to hormone therapy. This vaccine is not a cure for prostate cancer, but it can help extend patients' lifespan. To make this vaccine, immune cells are harvested from a patient's blood, then treated to induce them into immune cells called *dendritic cells*. The dendritic cells are also exposed to a protein called prostatic acid phosphatase (PAP), which causes the cells to stimulate an immune response against prostate cancer cells when put back into the body. The dendritic cells are returned to the patient's bloodstream by intravenous injection, and several treatments are necessary. In the patient, the dendritic cells also stimulate other immune cells to attack the prostate cancer.

We mention vaccines here because they are one way to use the immune system to treat disease. But in recent years, the use of antibodies for a variety of immunotherapies has brought about great excitement and promise for treating human disease, as we discuss next.

Monoclonal antibodies

The primary purpose of vaccination is to stimulate antibody production by the immune system and thus to help ward off foreign materials, but antibodies themselves can be used as treatments. Using antibodies in some types of therapy makes good sense because antibodies are very specific for the molecules or pathogens to which they are produced and can find and bind to their target with great affinity. Since their development in 1975, **monoclonal antibodies (mAbs),** purified antibodies that are very specific for certain molecules, have been considered "magic bullets" for disease treatment. To make a mAb, a mouse or rat is injected with the purified antigen to which researchers are trying to make antibodies. Figure 11.12 shows production of mAbs specific for proteins from human liver cancer cells. After the mouse makes antibodies to the antigen, a process that usually takes several weeks, the animal's spleen is removed. The spleen is a rich source of antibody-producing B lymphocytes, commonly called B cells. In a culture dish, B cells are mixed with cancerous cells, called myeloma cells, which can grow and divide indefinitely. Under the right conditions, a certain number of B cells and myeloma cells will fuse together to create hybrid cells called **hybridomas**.

Hybridoma cells grow rapidly in liquid culture because they contain antibody-producing genes from B cells. These cells are literally factories for making antibodies. Hybridoma cells secrete antibodies into the liquid culture medium surrounding the cells. Chemical treatment is used to select for hybridomas and discard unfused mouse and myeloma cells, so that researchers have pure populations of fused, antibody-producing cells. Hybridomas can be transferred to other culture dishes and frozen at ultralow temperatures so that a permanent stock of cells is always available. Antibodies can be isolated from hybridoma cultures in large batches by growing hybridoma cells in batch culture using bioreactors.

There are many variations to how mAbs can be produced. For example, transgenic mice engineered with different genes can be used to produce mAbs including **humanized antibodies,** which have been genetically engineered to increase their similarity to human antibodies (as we discussed in Chapter 4). About half of the commercially available, FDA-approved mAbs are humanized antibodies. Phages can also be used to express antigens for producing mAbs, and increasingly many companies are taking this approach.

Monoclonal antibodies can be injected into patients to seek out and target the antigens to which the mAbs

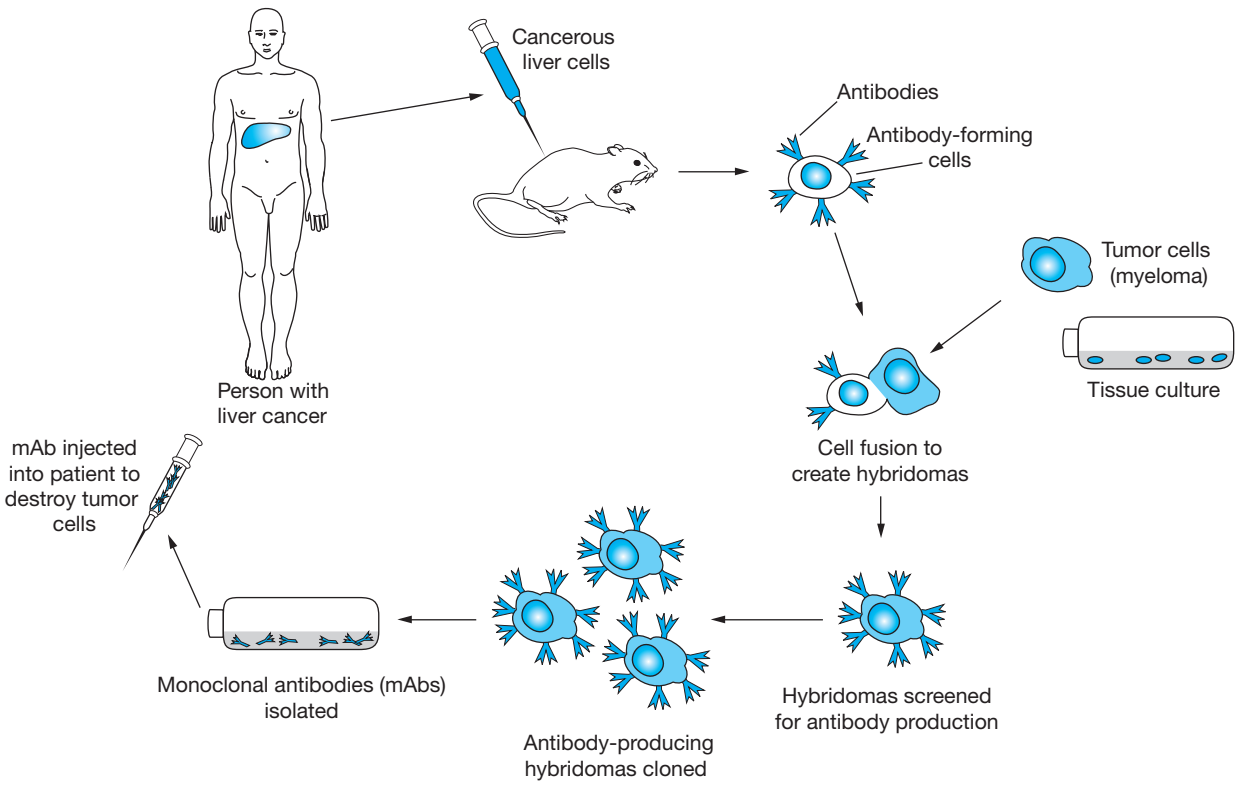


FIGURE 11.12 Making Monoclonal Antibodies

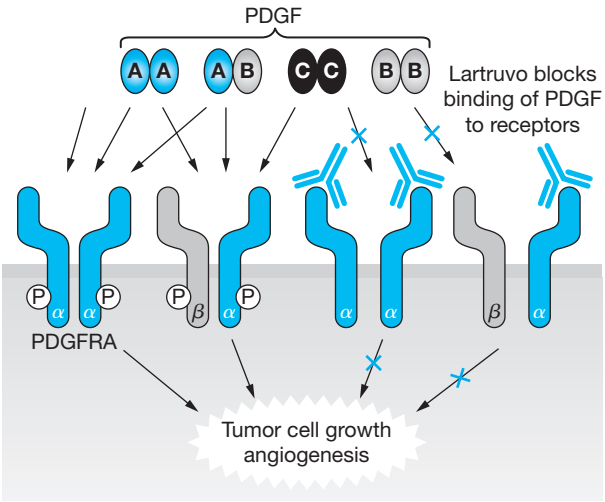


FIGURE 11.13 Lartruvo Is a Monoclonal Antibody Used to Treat Soft Tissue Sarcoma Lartruvo binds to the extracellular domain of the protein called platelet-derived growth factor receptor alpha (PDGFRA), which is important for protein signaling in cells. PDGFRA binds to the different forms of growth factor PDGF, which stimulates its growth. Lartruvo blocks the receptor preventing binding to PDGF and thus blocks growth of the tumor.

were produced. The mAbs in Figure 11.12 would bind to liver cancer cells and work on destroying the tumor. Figure 11.13 shows an example of how olaratumab (notice that the suffix for this drug name ends in "mab"—this tells you a drug is a monoclonal antibody), or Lartruvo, works against soft tissue sarcoma.

In 1992, the FDA approved the first monoclonal antibody, OKT3, which was used to treat organ transplant rejection. Subsequently mAbs were developed to treat breast cancer (Herceptin), lymphoma (Rituxan), and other diseases. As of January 2017, 68 mAbs had been approved by the FDA. For several reasons, the past few years have seen a resurgence in mAb research and new products. For example, the FDA approved 10 new mAbs in 2015, a high since 1992, and then another 10 mAbs in 2016.

Scientists even envision attaching chemicals or radioactive molecules to mAbs in the hope that these might target damaged or cancerous cells and use their payloads to kill these cells. Therapeutic antibody strategies may also be of value for treating people addicted to harmful drugs, such as cocaine and nicotine. In 2016, 275 million people worldwide used drugs at least once a year. Scientists believe that it may be possible to stimulate antibody production to drugs such as cocaine. These antibodies would then bind to the drug as the antigen, trapping and preventing the drug from affecting brain cells. Monoclonal antibodies have also been used for several years in common tests for conditions such as strep throat, and most home pregnancy kits use mAbs to detect hormones produced during pregnancy.

Monoclonals for disease treatment have still not lived up to their initial hype, and there have been some setbacks in the field. For example, mAb treatment of Alzheimer's disease patients produced severe inflammation in several people owing to a human antimouse antibody response. Increasingly, it appears that mAbs will continue to be valuable tools for medicine in the twenty-first century.

Immunotherapy shows great promise as a cancer treatment

Although in development for several decades (the U.S. government alone has provided well over $200 million in research and development support), in the past few years, **immunotherapy,** the use of a patient's immune system to attack and destroy diseased cells, has emerged as one of the most promising ways to treat cancer. As an example of this, in 2016 the FDA approved many new clinical trials involving immunotherapies for treating advanced forms of lymphoma, lung, kidney, bladder cancer, and other diseases. Immunotherapy harnesses a patient's own immune system to kill tumors, and some approaches have led to remarkable therapeutic effects in clinical trials in which patients have gone into remission lasting years and tumors have seemingly disappeared.

The two main strategies for immunotherapy are to create **chimeric antigen receptor (CAR)-T cells** engineered to express receptors that can directly recognize antigens on the surface of the tumor cell without requiring T cell activation by antigen-presenting cells (Figure 11.14a) or to recombinant **T cell receptors (TCRs)** that specifically recognize antigens on or within cancer cells (Figure 11.14b). Figure 11.14b demonstrates how **neoantigens**—novel, cancer-specific antigens from an individual patient—can be identified and exploited so the immune system recognizes these antigens and attacks cancer cells. Figure 11.15 shows one approach for isolating a patient's immune cells from blood, engineering cells to create CAR-T cells (as shown in Figure 11.14a) or TCR cells, culturing altered T cells in the laboratory, and then returning CAR-T or TCR cells to the patient, where they seek out and destroy cancer cells. Gene editing by CRISPR-Cas is also enhancing the use of engineered T cells for the treatments of many diseases. We will return to this concept in Section 11.5, Gene Therapy.

Most of the first patients treated by immunotherapy were offered this approach as a last option because they had a life-threatening illness and other, conventional treatments were unsuccessful. In August 2017, after a panel of physicians and researchers who advise the FDA unanimously recommended approval of Novartis'

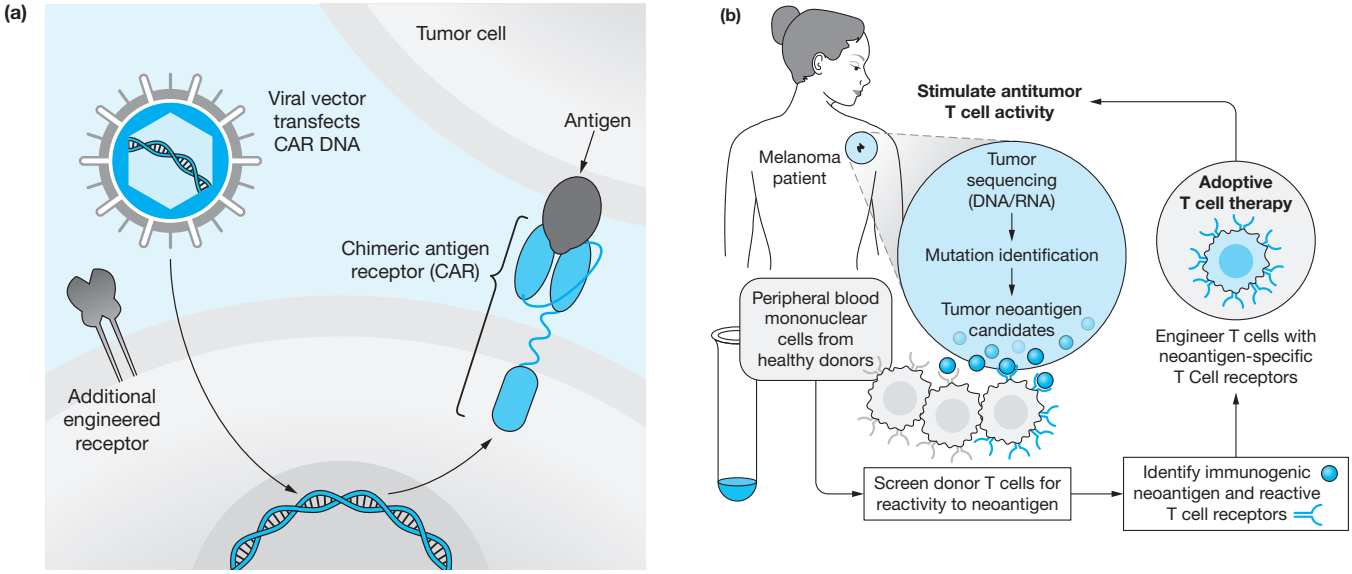


FIGURE 11.14 Modifying T Cells for Immunotherapy (a) Chimeric antigen receptor (CARs) proteins recognize cancer-specific antigens. By introducing genes that encode CAR proteins into T cells isolated from a patient to create CAR-T cells, the cells are programmed to precisely recognize and destroy tumor cells that would not normally be recognized by the immune system. At the same time, CAR-T cells will not target normal cells. Additional receptors may be engineered into CAR-T cells to help them bind to tumor cells. (b) Circulating T cells are removed from a patient with melanoma and engineered with genes for neoantigens to create adoptive T cells. Cells are cultured to increase in number (expansion) before being infused back into the patient.

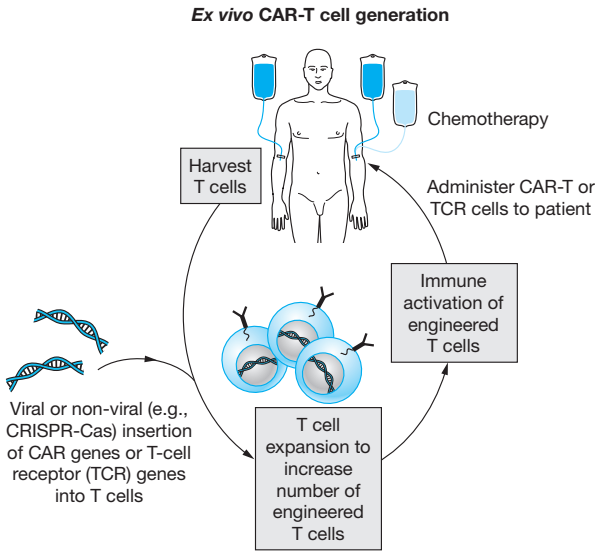


FIGURE 11.15 Process for Preparing CAR-T Cells from a Patient Circulating T cells are removed from a patient, transfected with a gene for a CAR delivered via a viral vector (or by CRISPR-Cas), activated to recognize tumor cells, and grown in culture to increase the number of cells before being infused back into the patient. Often, prior to infusion of CAR-T cells, the patient receives chemotherapy to deplete endogenous T cells.

brand name drug **Kymriah** (CTL019) CAR-T treatment for children and young adults with B cell acute lymphoblastic leukemia (ALL), the FDA approved Kymriah as the first CAR-T therapy. ALL is the most common childhood cancer in the United States, and patients who relapse or fail to respond to chemotherapy have a low survival rate. But over 80 percent of 68 pediatric or adult patients went into remission almost immediately after treatment began, and most remained cancer-free 6 months after treatment. Kymriah is approved for ALL patients up to 25 years of age. This first approval was a milestone. Seven weeks later a second CAR-T therapy, called Yescarta, created by Gilead Sciences for treating non-Hodgkin's lymphoma in adults was approved. As of late 2017 there were more than 240 CAR-T clinical trials registered at clinicaltrials.gov and it was expected several new immunotherapies in the pipeline would be approved in 2018. Kymriah also made headlines for its sticker price of approximately $475,000 for a single treatment and estimates that the total cost of care with this drug could exceed $1.5M.

Several companies are using engineered viruses for immunotherapy. For example, **oncolytic viruses** are engineered to selectively bind to and infect cancer cells. Usually these viruses have been stripped of genes that the virus normally uses to make people sick. The viruses are genetically engineered to contain surface proteins that bind to and recognize receptors

on cancer cells and not healthy non-cancerous cells, which them stimulates the immune system to attack the cancer cells. This form of immunotherapy is challenging for many reasons, but several oncolytic viruses are continued to progress through clinical trials.

Many questions and challenges need to be addressed before immunotherapy is a routine, reliable treatment option. For example, why do some patients respond well to immunotherapy, while others do not? Of those selected for immunotherapy, less than half of the patients respond well or respond for only a short time. So far, immunotherapy is more effective for blood cancers and less effective for solid tumors, which account for the majority of cancers, such as breast, brain, lung, ovarian cancers, and others. It can take several weeks to produce engineering T cells from each patient. Are there ways to speed this up and shorten the time to make these cells? Immunotherapy can stimulate immune reactions resulting in death, and the FDA has halted several trials because of patients' deaths.

Treatment costs are also an issue, with CAR-T treatments as high as $400,000 to $600,000 and currently not covered by insurance (although patients in clinical trials often have costs waived because they are volunteering for the trial). Engineered T cells destroy cancer cells but are short-lived. Are there ways to make these cells last longer? Scientists are working hard to address these and other questions, so keep a close eye on immunotherapy.

In the next section we consider another example of precision medicine by exploring gene therapy, a promising and controversial topic of medical biotechnology.

11.5 Gene Therapy

Gene therapy involves the delivery of therapeutic genes into the human body to correct disease conditions created by a faulty gene or genes. Think about the awesome power and potential of gene therapy—providing a person with normal genes to supplement defective genes and cure disease, using genome editing to remove a disease gene, or even replacing a faulty gene with a normal gene. In actuality, gene therapy was one of the first precision medicine approaches to treating disease that existed decades before the term *precision medicine* was ever used. Gene therapy has not lived up to its initial hype, but as you will learn here, the field is undergoing a dramatic resurgence largely because of recent successes and the promise of genome editing approaches such as CRISPR-Cas.

How Is It Done?

The two primary strategies for gene delivery are ***ex vivo* gene therapy** and ***in vivo* gene therapy** (Figure 11.16). In *ex vivo* therapy (*ex* means "out of"; *vivo* is Latin for "something alive"), cells from a person with a disease condition are removed from that person, treated in the laboratory using techniques similar to bacterial transformation, and then reintroduced into him or her. Technically speaking, introducing DNA into animal or plant cells is called **transfection.** For instance, liver cells from a patient suffering from a liver disorder would be surgically removed and cultured. Appropriate therapeutic genes would then be

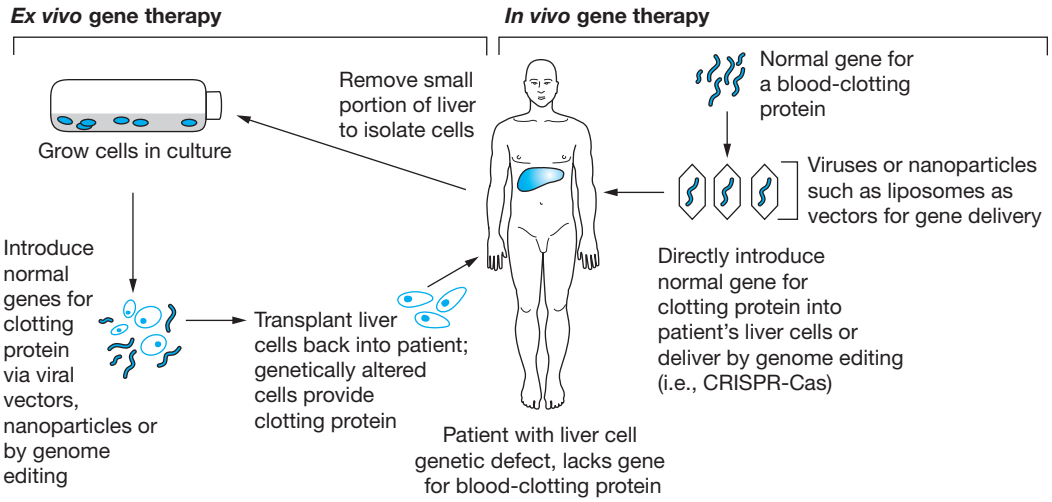


FIGURE 11.16 *Ex Vivo* and *in Vivo* Gene Therapy in a Patient with a Liver Disorder
Ex vivo gene therapy involves isolating cells from the patient, introducing normal genes for the clotting protein into these cells, and then transplanting cells back into the body, where these cells will produce the required clotting protein. *In vivo* gene therapy involves introducing DNA directly into cells while they are in the patient. In either mode of gene therapy, genes may be introduced into cells as DNA packaged into viruses as vectors or as naked DNA.

delivered into these cells using vectors and other approaches discussed in the next section. These genetically altered liver cells would then be transplanted back into the patient without fear of rejection of the tissue transplant because these cells came from the patient initially (Figure 11.16).

In vivo gene therapy does not entail removal of a patient's cells; DNA is introduced directly into cells and tissues in the body (Figure 11.16). One challenge of *in vivo* gene therapy is delivering genes only to the intended tissues and not to tissues throughout the body. Scientists have primarily relied on using viruses as vectors for gene delivery, but in some cases genes have been directly injected into some tissues. So far, *ex vivo* strategies have generally proven to be more effective than *in vivo* approaches.

Delivering the payload: Vectors for gene delivery

A major challenge that must be overcome if gene therapy is to become a reliable tool for treating disease is achieving a safe and effective delivery of therapeutic genes—the payload. Depending on the genetic condition to be treated, some therapeutic strategies may require long-term expression of a corrective gene, whereas others may require rapid expression for shorter periods of time. A majority of gene delivery strategies, both *ex vivo* and *in vivo*, rely on viruses as vectors to introduce therapeutic genes into cells.

A viral vector uses a viral genome to carry a therapeutic gene or genes to "infect" human body cells, thereby introducing the therapeutic gene (Figure 11.17a). Scientists use various viruses—such as **adenovirus,** which causes the common cold; a related virus called **adeno-associated virus (AAV);** influenza viruses, which cause the flu; and herpes viruses, which can cause cold sores and certain of which cause sexually transmitted diseases—as potential **vectors** for gene delivery. For any viral vector to work, scientists must be sure that these vectors have been genetically engineered and inactivated so they neither produce disease nor spread throughout the body and infect other tissues.

Most viruses infect human body cells by binding to and entering cells and then releasing their genetic material into the nucleus or cytoplasm of the human cell. This is usually DNA, but some viruses contain an RNA genome. The infected human cell then serves as a host for reproducing the viral genome and producing viral RNA and proteins. Viral proteins ultimately assemble to create more viral particles that break out of the host cells so they are free to infect other cells and repeat the life cycle (Figure 11.17a).

It may seem strange that viruses would even be considered for carrying genes to cure human diseases. However, since viruses are very effective at introducing their genomes into cells, scientists reasoned that if viruses could be disabled so that they would not cause disease and genetically altered to deliver therapeutic

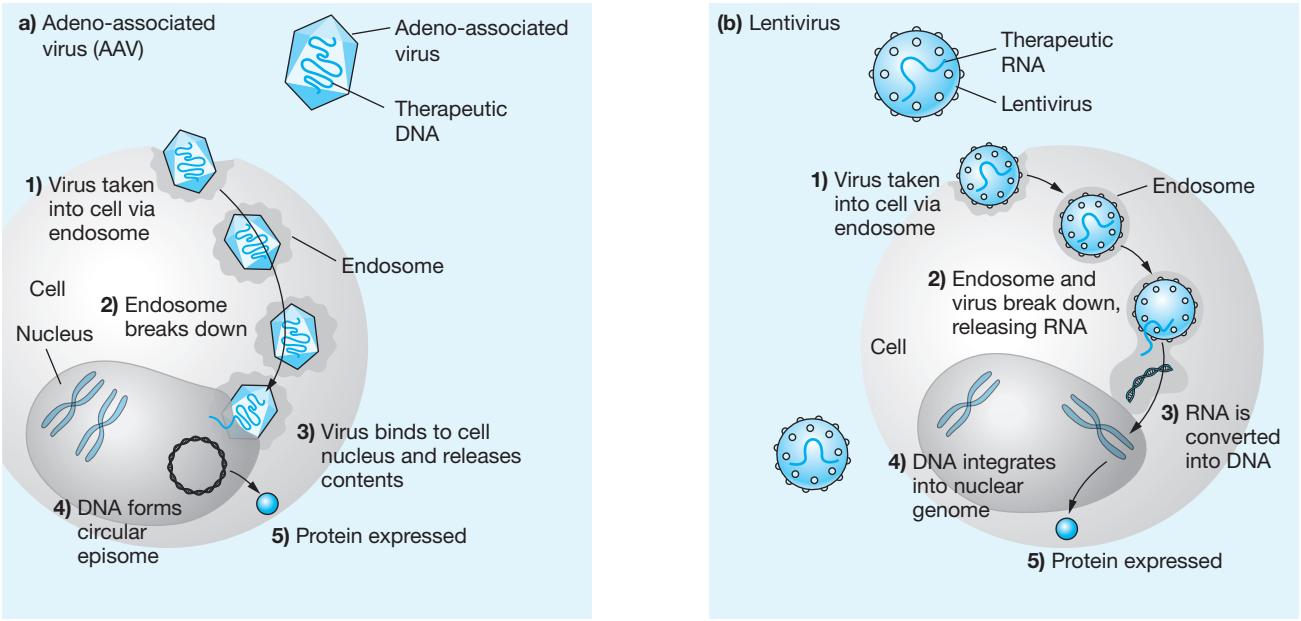


FIGURE 11.17 Delivering Therapeutic Genes with Viral Vector (a) Viruses such as modified adeno-associated virus (AAV) deliver therapeutic genes into cells without integrating them into the genome of target cells. (b) Viruses such as lentivirus, an RNA virus, deliver therapeutic genes into the cytoplasm, where reverse transcriptase converts RNA into DNA. DNA then integrates into the genome, ensuring that it will be passed to daughter cells during cell division.

genes safely, we could use viruses for beneficial purposes. In many ways, viruses are perfectly designed as gene therapy vehicles or vectors for gene delivery. For instance, adenovirus—which approximately 80 to 90 percent of the population has been infected with in childhood because it causes the common cold—can infect many types of body cells fairly efficiently.

Retroviruses such as **lentivirus,** including even HIV, are of interest as vectors because, on entering a host cell, they copy their RNA genome into DNA and then randomly insert their DNA into the genome of the host cell, where it remains permanently, a process called *integration*. A main reason why retroviruses are used for gene delivery is that they can integrate therapeutic genes into the DNA of human host cells, allowing permanent insertion of genes into the chromosomes of a patient's cells as a way of providing lasting gene therapy (Figure 11.17b). For example, researchers at the University of Paris and Harvard Medical School have reported that 2 years after gene therapy treatment for ß-thalassemia, a blood disorder that involves a defect in the ß-globin chain of hemoglobin that reduces the production of hemoglobin, a young man no longer needs transfusions and appears to be healthy. A modified, disabled HIV-derived lentivirus vector was used to carry a copy of the normal gene and delivered via blood stem cells.

Viruses have also been widely studied as gene therapy vectors because some viruses infect only certain body cells. This strategy has been used for *targeted* gene therapy—the ability to deliver genes only to the tissues infected by a certain virus. For instance, a strain of herpesvirus (HSV-1) primarily infects cells of the central nervous system. This strain has been used for targeted gene therapy of cells in the nervous system, which may be an effective way to treat genetic disorders of the brain such as Alzheimer's disease and Parkinson's disease. Researchers are also investigating ways that the genetically altered herpesviruses can be used to destroy brain tumors. Preliminary trials of this approach in humans have shown some promise.

Most human cells do not take up DNA easily. If they did, it would then be possible to transfect cells by simply mixing them with DNA in a tube in much the same way that transformation is achieved with bacterial cells. However, some success has been demonstrated for both *in vivo* and *ex vivo* strategies using "naked" DNA. Naked DNA is simply DNA by itself, without a viral vector, which is injected directly into body tissues. Small plasmids containing therapeutic genes are often used for this approach. Cells of certain tissues will take in some of the naked DNA and express genes delivered in this way.

Delivery techniques for naked DNA have been somewhat effective in the liver and in skeletal muscle. One of the major problems with transfecting human cells *in vivo* is that because a relatively small number of cells take up the injected DNA, there may not be enough cells expressing the therapeutic gene for gene therapy to have any effect on the tissue. Scientists are working on ways to overcome these problems and deliver naked DNA more effectively. For example, electroporation (recall that in Chapter 5 we discussed how electrical stimulation is used to move plasmids into bacterial cells) can be used to stimulate movement of DNA into cells.

One approach to deliver DNA without viral vectors involves **liposomes,** small-diameter hollow microspheres made of lipid molecules, similar to the fat molecules in cell membranes. Liposomes are packaged or coated with genes and then injected into tissues or sprayed onto them. A similar technique involves coating tiny gold nanoparticles with DNA and then shooting these into cells using a DNA gun, a pressurized air gun that delivers gold or liposome particles through cell membranes without killing most cells. A variety of different types of nanoparticles are being used as gene-carrying vectors. Short-term expression of genes through "gene pills" is also being explored. In this approach, a pill delivers DNA to the intestines, where it is absorbed by intestinal cells; these then express therapeutic proteins encoded by the DNA and secrete these proteins into the bloodstream.

Therapeutic RNA approaches for gene therapy

We have discussed the use of **antisense RNA technology** as a way to block translation of mRNA molecules to silence gene expression (Chapter 3). This approach was used to create the Flavr Savr tomato (see Figure 6.7). The basic concept of antisense RNA technology is to design an RNA molecule that will serve as a complementary base pair to the mRNA you want to inhibit, thus blocking it from being translated into a protein (Figure 11.18). This approach for shutting off a gene is frequently called **RNA** or **gene silencing.** Since the development of antisense RNA technologies in the 1970s, scientists have thought that RNA silencing approaches would be highly effective ways to turn off disease genes as a gene therapy approach, but so far RNA-based therapies have not lived up to their initial promise.

But it is very likely that RNA-based therapies will become much more successful and more widely adopted within the next decade. In the mid 2000s, many companies dropped this approach to gene therapy because of problems associated with delivering antisense RNA oligonucleotides across cell membranes and keeping them from being degraded while in the circulatory system. However, recent advances in RNA oligonucleotide chemistry have helped overcome these hurdles, and hundreds of clinical trials involving antisense RNAs are in the planning stages.

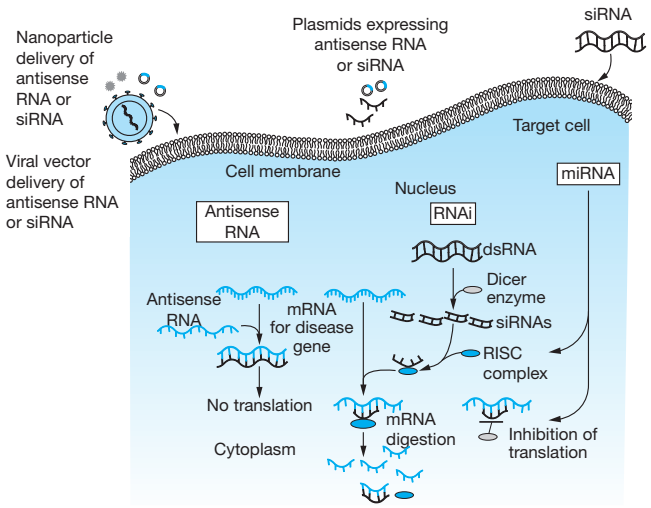


FIGURE 11.18 Antisense RNA and RNAi Approaches for Gene Therapy by Gene Silencing Antisense technology and RNAi are two ways to silence gene expression and turn off disease genes. In antisense technology (left) an antisense RNA molecule binds to the mRNA for the disease gene and prevents it from being translated into a protein. With RNAi technology, double-stranded RNA (dsRNA) molecules are delivered to the cell. The dsRNAs are then cleaved by Dicer into short interfering RNAs (siRNAs), which are escorted by the RISC protein complex to bind to their target mRNA, causing its degradation and thus preventing translation.

Within a 6-month span in late 2016 alone, antisense oligonucleotide trials were approved by the FDA for familial cholesterolemia, Duchenne muscular dystrophy, and **spinal muscular atrophy (SMA).** For example, the antisense RNA called **Spinraza (nusinersen),** produced by Ionis Pharmaceuticals of Carlsbad, California, was approved as the first treatment for SMA. Affecting 1 in 10,000 to 12,000 children born, this disease is characterized by the loss of motor neurons in the spinal cord, resulting in progressive muscle weakness, and is a leading genetic cause of death for infants (more than 90 percent of infants die before age 2). SMA patients have a mutation in the *SMN1* gene that prevents production of the functional SMN protein required for normal motor neuron development (**Figure 11.19**). The therapeutic RNA is delivered into the cerebrospinal fluid. It is an 18-nucleotide (nt) antisense RNA that targets *SMN2*, a homologue of *SMN1*. *SMN2* is normally mis-spliced at exon 7 to produce a truncated, largely nonfunctional SMN protein.

Spinraza binds to pre-mRNA of the *SMN2* gene, altering splicing to include exon 7 in the mature transcript and leading to translation of a functional copy of the SMN protein (Figure 11.19). The drug showed such promise in two trials that the trials were halted early and considered successful because it was deemed unethical to continue to deny the drug to SMN-affected children in placebo groups. This antisense approach to alter mRNA splicing for gene therapy has also generated excitement because it has potential for treating Huntington's disease, amyotrophic lateral sclerosis (ALS), and other neurological conditions. Since Spinraza, recent gene therapy trials delivering *SMN1* via AAV have proven to be effective in clinical trials for over a dozen infants with SMA.

The recent emergence of **RNA interference (RNAi)** as a method to control mRNA stability and protein synthesis has reinvigorated gene therapy approaches by gene silencing (refer to Figure 3.18).

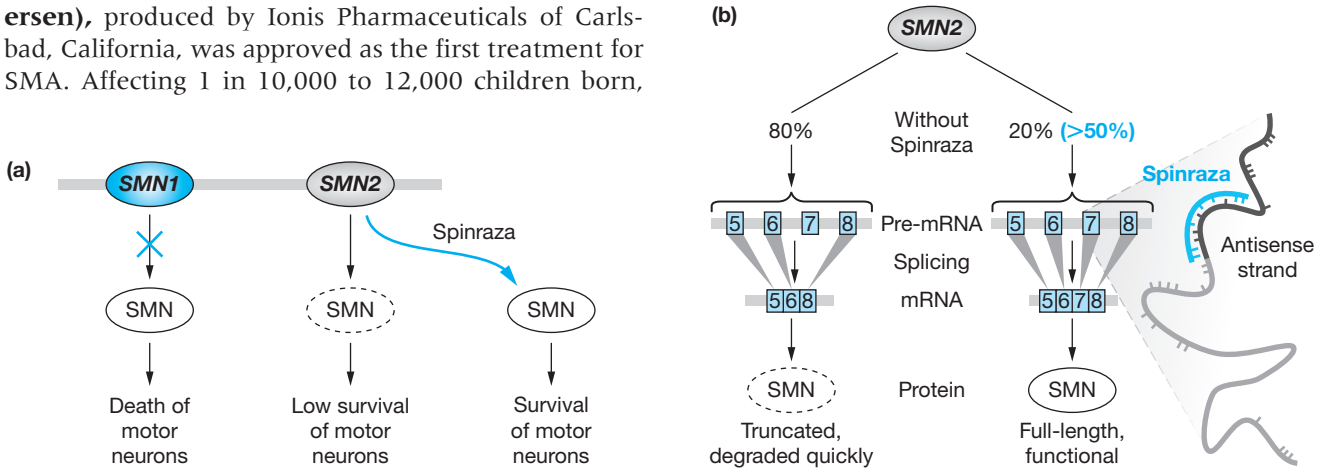


FIGURE 11.19 Spinraza Is an Antisense RNA Therapy that Affects Splicing of the *SMN* Gene (a) In SMA, expression of the *SMN1* gene leads to death of motor neurons. *SMN2* typically makes little functional SMN protein. Spinraza increases SMN protein production to increase survival of motor neurons. (b) Typically protein from *SMN2* is nonfunctional because ~80% of the time a mis-splicing error of SMN2 mRNA occurs excluding exon 7, a region that encodes critical amino acids required for SMN function, whereas normal splicing occurs about 20% of the time. But by altering splicing, Spinraza blocks splicing so that exon 7 is included in the functional mRNA more than 50% of the time thus significantly increasing the levels of functional SMN.

With RNAi, double-stranded RNA molecules are delivered into cells where the enzyme Dicer chops them into 21-nt-long pieces called **small interfering RNAs (siRNAs;** Figure 11.18). The siRNAs then join with an enzyme complex called the **RNA-induced silencing complex (RISC),** which shuttles the siRNAs to their target mRNA, where they bind by complementary base pairing. The RISC complex degrades the siRNA-bound mRNAs so they cannot be translated into protein.

Using gene therapy to stimulate naturally occurring **microRNAs (miRNAs)** that inhibit gene expression is another area of active investigation (Figure 11.18). A main challenge to RNAi-based therapeutics so far has been sustained *in vivo* delivery of the antisense RNA, dsRNA, miRNA, or siRNA to the target tissues. RNAs degrade quickly in the body. It is also hard to get them to penetrate cells and to target the right tissue. Two common delivery approaches are to inject the antisense RNA or siRNA directly or as a plasmid that is taken in by cells to be transcribed to make antisense or RNAi molecules (Figure 11.18). Nanoparticle, liposome, and lentivirus delivery mechanisms, and attachment of siRNAs to cholesterol and fatty acids, are also used to deliver silencing RNAs. Another problem is that most complex diseases are not caused by just one gene. For example, as mentioned earlier in this chapter, the gene *BRCA2* is overexpressed in about 50 percent of breast cancer cases, and antisense RNA technology can silence this gene *in vitro;* but breast cancer is a multigene disease, and it is not yet possible to silence all of the genes involved in it.

Several dozen clinical trials using RNAi are under way in the United States. Diseases that have been targeted by RNAi include several different cancers, macular degeneration (a form of blindness), diabetes, multiple sclerosis, arthritis, and neurodegenerative diseases.

Stem cells for delivering therapeutic genes

We will discuss **stem cells** in great detail in Section 11.6. Increasingly, viral and nonviral vectors are being used to deliver therapeutic genes into stem cells, usually *in vitro*, and then the stem cells are either introduced into the patient or differentiated *in vitro* into mature cell types before being transplanted into the correct organ of a patient being treated. In particular, *hematopoietic stem cells* (*HSCs*), which are found in bone marrow and give rise to blood cells, are widely used for gene therapy in adults and children. There are many advantages to using HSCs: They are easily accessible and often taken from the marrow of the patient to be treated to avoid complications with tissue rejection by the immune system when they are reintroduced; they replicate quickly *in vitro;* they are fairly long-lived; and they differentiate into both red blood cells and white blood cells (leukocytes). And as you will also learn, stem cells are being used in CRISPR-Cas and gene-silencing approaches, thus demonstrating their value for different gene therapy applications.

Genome Editing Approaches for Gene Therapy

Historically, most gene therapy approaches focused on adding normal copies of a gene in an effort to override the effects of a defective gene. **Genome** or **gene editing** is a general term for approaches designed to precisely modify specific genes in the genome. Recognize that editing approaches are designed to correct or replace a defective gene or genes. Editing approaches can now also include replacing defective genes with a correct version of a gene. In the early days of gene editing, researchers attempted to use specific types of DNA-editing nucleases called **zinc-finger nucleases (ZFNs)** or TALENS (transcription activator-like effector nucleases). Using plasmids encoding ZFNs to "edit" out and replace defective genes has been an area of intense research in gene therapy. For example, Sangamo Therapeutics has implemented a ZFN-based gene therapy trial for patients with HIV in an attempt to disrupt the *CCR5* gene. This gene encodes a protein that HIV uses to enter cells and the trial has shown promising results in halting spread of the virus. More than 100 people have now been treated with this therapy. But because these nucleases have generally had limited success, we will focus our gene editing discussion on **CRISPR-Cas**.

No gene therapy method has created more excitement than the gene editing technique known as CRISPR-Cas or simply CRISPR. We have discussed various applications of CRISPR-Cas throughout the book. Identified in bacterial cells, the CRISPR system functions to provide bacteria and archaea immunity against invading bacteriophages and foreign plasmids. First introduced in 2013, a CRISPR craze unfolded that has revolutionized genome-engineering applications including gene editing for gene therapy.

CRISPR is based on delivering a single-stranded "guided" RNA sequence (sgRNA) that is complementary to the target gene sequence in the genome and attached to the endonuclease called Cas9 **(Figure 11.20).** sgRNAs are relatively easy to design and synthesize. At the same time as the sgRNA sequence is delivered, a DNA template strand coding for a replacement sequence is delivered. The sgRNA-Cas9 complex binds to the target DNA sequence, and Cas9 generates a blunt, double-stranded break in the DNA. CRISPR recognition of DNA cleavage sites is determined by RNA-DNA base pairing and a protospacer-adjacent motif (PAM), a three-nucleotide sequence adjacent to the complementary sequence. As cells repair the DNA damage caused by Cas9, repair enzymes incorporate template DNA into

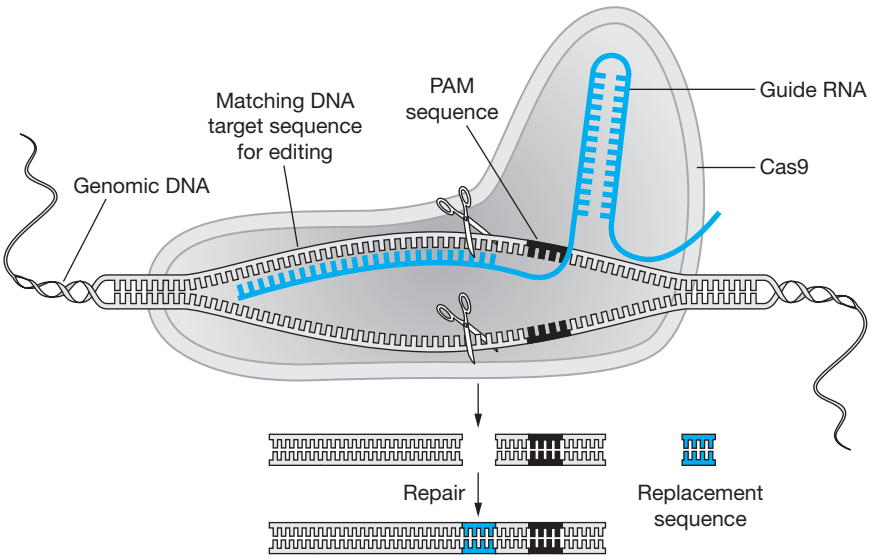


FIGURE 11.20 CRISPR-Cas for Genome Editing

the genome at the CRISPR-Cas site, thus replacing the target DNA sequence. As we also discussed in chapter 3, but not shown in figure 11.20, CRISPR can also be used to remove a target DNA sequencing by removing it and then end-joining fragments of DNA together without replacing the deleted sequence.

Part of the power of CRISPR is that editing can be done directly in a living, adult animal. Within months of the technique being widely available, researchers around the world used CRISPR to target specific genes in human cells, mice, rats, bacteria, fruit flies, yeast, zebrafish, and dozens of other organisms. A team from the Massachusetts Institute of Technology (MIT) recently cured mice of a rare liver disorder, type I tyrosinemia, through gene editing by CRISPR. In tyrosinemia, a condition affecting about 1 in 100,000 people, mutation of the *FAH* gene encoding the enzyme fumarylacetoacetase prevents breakdown of the amino acid tyrosine. After an *in vivo* approach with a one-time treatment, roughly 1 in 250 liver cells accepted the CRISPR-delivered replacement of the mutant gene with a normal copy of the gene. But about 1 month later these cells proliferated and replaced diseased cells, taking over about one-third of the liver, which was sufficient to allow mice to metabolize tyrosine and show no effects of disease. Mice were subsequently taken off a low-protein diet and a drug normally used to disrupt tyrosine production.

In late 2016, a team of Chinese scientists provided the first report of CRISPR-Cas treatment in humans with a trial to treat an aggressive form of cancer called metastatic non–small cell lung cancer. An *ex vivo* approach was used to edit a gene called *programmed cell death 1* (*PD-1*), which expresses a protein on the surface of T cells that can often be neutralized by cancer cells to minimize immune responses and ward off T cell destruction of a tumor. Edited T cells were then reintroduced into patients with the hope that these cells would target tumors in the lung for destruction without being disabled by the cancer cells. Results from this trial were expected by the end of 2018, after this edition of *Introduction to Biotechnology* was published.

In the United States, as of late 2017, only one CRISPR trial had been recommended for approval so far. An advisory group from the NIH approved trials for CRISPR-Cas9 editing of T cells from patients with melanoma, multiple myeloma, and other cancers not responding to traditional therapies. This protocol then had to be reviewed and approved by the FDA. Several more gene editing trials are scheduled to begin at different centers around the world, targeting kidney, bladder, and prostate cancer, among others.

On an almost daily basis, headline-grabbing examples of successful CRISPR-Cas applications in model organisms and humans that highlight the potential of this approach for gene therapy are being reported. These include:

- AAV delivery of CRISPR-Cas9 to remove a defective exon from the *Dmd* gene in a mouse model (*mdx* mice) of **Duchenne muscular dystrophy (DMD)** significantly restored muscle function in treated mice. It has been estimated that this approach has the potential to cure approximately 80 percent of human cases of DMD.
- After successful trials in mice, the human ß-globin (*HBB*) gene in HSCs was repaired *in vitro* to treat sickle cell disease and ß-thalassemia in humans. This work has shown great promise and may lead to clinical trials within the next 5 years.
- CRISPR-Cas9 was used to target and replace the defective clotting Factor IX gene in liver cells to cure mice of hemophilia B.



YOU DECIDE Human Embryo and Germline Editing?

Gene editing of somatic cells to prevent or treat diseases holds great promise. But even approaches to editing somatic cells have been controversial. For example, should this be used for genetic enhancement? Gene editing to treat muscular dystrophy is one thing, but what about editing to enhance muscle mass and strength in an otherwise healthy male? No potential application of CRISPR-Cas has generated as much attention as the possibility of embryo and germline editing. The use of CRISPR-Cas to edit human embryos and the human germline (sperm and egg cells) has become the most controversial potential application of this technology, although similar concerns existed when ZFNs and TALENS were first being used.

Editing an early-stage embryo, before cells are fully differentiated, would result in edits to the human germline (sperm and egg cells), and thus embryo editing would affect subsequent generations and allow parents with a known disease to have offspring without passing the mutation to their children. This raises many safety issues including concerns about unintended consequences such as, what if a gene editing approach makes changes that are undetected? Currently, in the United States there is a ban on the use of federal funding for embryo editing. The FDA has convened a committee to define criteria under which such editing could be permitted if the ban were to be lifted in the future.

In 2015, a team of Chinese scientists reported using CRISPR-Cas9 to edit the *HBB* gene in 86 human embryos donated previously for *in vitro* fertilization. Two days after CRISPR-Cas treatment, 71 embryos had survived, but only 4 of them carried the intended change to the *HBB* gene. Unexpectedly, many other embryos had acquired mutations in genes other than *HBB* as a result of the treatment. From this the Chinese research team concluded that gene editing technology is not sufficiently developed for use in embryos. In 2016, the second published report of gene editing of human embryos, also from a team in China, described the use of CRISPR-Cas9 to introduce a mutation in the *CCR5* gene to confer resistance to HIV infection. In this study, 4 of 26 embryos were successfully edited but others contained undesirable mutations as a result of the treatment. Although this demonstrated some proof of concept for creating HIV-resistant embryos, like the 2015 work, it also clearly showed that gene editing of embryos is neither precise nor safe at this point.

In 2017, researchers at Oregon Health and Science University published work claiming to repair a gene defect in human embryos without causing unwanted changes elsewhere in the genome. This was done with embryos created through IVF and targeted a gene called *MYBPC3*, which leads to an enlarged heart and can cause sudden cardiac arrest in seemingly healthy young adults. After editing, the embryos were not implanted but destroyed. As previously noted, this work was not supported by federal funding.

These and other studies have stimulated significant ethical discussions about genetic engineering of embryos. Currently, about two dozen countries (including the United States and the United Kingdom) have a strict ban on human germline and embryo modification, but more permissive regulations exist in other countries. This continues to prompt widespread concern from scientists, ethicists, politicians, and patients. Countries such as China, India, and Japan have guidelines, not true legislation, banning genetic modifications, but these are generally considered unenforceable or are largely ignored. Recently an advisory group formed by the U.S. National Academy of Sciences and the National Academy of Medicine endorsed embryo editing for genes known to cause "serious diseases and disabilities" and only if there is no "reasonable alternative." Should embryo editing be permitted? If so, what restrictions or considerations would have to be in place? What genetic conditions should be eligible for embryo editing and what conditions should not be? What safety and efficacy measures should be in place to ensure that editing will not produce unintended changes in the genome? You decide.

- CRISPR editing of a mutation in the gene *PCSK9* present in some individuals with dangerously high levels of low density lipoprotein (LDL or "bad cholesterol") could reduce the risk of cardiovascular disease.
- A CRISPR-Cas approach to treat Leber's congenital amaurosis (LCA) type 10 (a form of blindness) is expected to enter clinical trials in 2018.
- CRISPR-Cas editing of mutations involved in genetic forms of hearing loss in mice have shown early potential.

Recall that in Section 11.4, during our discussions about precision medicine, we mentioned a promising way to treat cancer called immunotherapy and approaches in which a patient's T cells are genetically engineered *in vivo* before being returned to the body.

Gene editing of T cells by CRISPR-Cas is dramatically enhancing the field of immunotherapy. So as you can see, in many ways immunotherapy as a form of precision medicine also incorporates and depends on elements of gene therapy.

As is the case with other gene therapy approaches, there is concern about CRISPR-Cas creating mutations at non-target locations in the genome. But so far, CRISPR-Cas is clearly the most promising tool for gene editing and gene therapy that has ever been developed, and in a short time, the pace of progress with this technique in animals and humans is remarkable. Stay tuned!

Curing Genetic Diseases: Targets for Gene Therapy

Most gene therapy researchers are focusing on genetic disorders created by single-gene mutations or deficiencies—such as sickle cell disease—because in theory these conditions may be easier to cure by gene therapy than genetic diseases involving multiple genes that interact in complex ways. Current estimates indicate there may be more than 3,000 human genetic disease conditions caused by single genes. Worldwide, over 2,300 gene therapy trials have been approved since 1989, with approximately two-thirds of these occurring in the United States.

Nearly every major category of genetic diseases has been targeted by gene therapy. Many recently approved clinical trials are for cancer treatment. Gene therapy approaches are currently being investigated for the treatment of hereditary blindness; cystic fibrosis; deafness; arthritis; neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS); aging (by attempting to minimize telomere shortening); cardiovascular disease; muscular dystrophy; hemophilia; infectious diseases such as HIV; and many other conditions, including depression and drug and alcohol addiction.

Pfizer launched its first Phase I clinical trials for boys with DMD receiving infusions of the *DMD* gene delivered by AAV in January 2018. Early data from this trial is expected to be available soon. Here we consider a few well-studied, successful examples of gene therapy in action that reflect progress in this field.

The first human gene therapy

The first human gene therapy was carried out in 1990 by a group of researchers and physicians at the National Institutes of Health in Bethesda, Maryland, led by W. French Anderson, R. Michael Blaese, and Kenneth Culver. The patient, 4-year-old Ashanti DaSilva, lacked a functional immune system because of a defect in a gene called adenosine deaminase (*ADA*). This genetic disorder is called **ADA–severe combined immunodeficiency (ADA-SCID)** and often referred to as "bubble boy" disease. *ADA* produces an enzyme involved in metabolism of the nucleotide deoxyadenosine triphosphate (dATP). Mutation of the *ADA* gene results in the accumulation of dATP, which, at high concentration, is toxic to certain types of T cells, resulting in a near-complete loss of these cells in patients with ADA-SCID. This condition is appropriately called "severe combined" immunodeficiency because mutations in the *ADA* gene deliver a knockout blow to the immune system's ability to make antibodies and fight off disease. Without functioning T cells, B cells cannot recognize antigen and make antibodies. Prior to gene therapy, most SCID patients did not live past their teens because their immune systems simply could not fight off infections.

To treat Ashanti, the normal gene for *ADA* was cloned into a vector that was then introduced into a retrovirus. An *ex vivo* gene therapy approach was used in which a small number of T cells were isolated from Ashanti's blood and cultured in the lab. Her T cells were then infected with the ADA-containing retrovirus, and the infected T cells were further cultured. Because retroviruses integrate their genome into the genome of host cells, the retrovirus was integrating the normal *ADA* gene into the chromosomes of Ashanti's T cells during this culturing. After a short period of culturing, these ADA-containing T cells were reintroduced back into Ashanti (**Figure 11.21**).

Ashanti received 11 treatments over about 2 years. Within a few months after gene therapy, the T cell numbers in Ashanti began to increase. After 2 years, Ashanti's ADA enzyme activity was relatively high, and she was showing near-normal T cell counts, with about 20 to 25 percent of her T cells showing the added ADA gene. Ashanti is currently enjoying a healthy life.

Subsequent gene therapy treatments for ADA-SCID have focused on using bone marrow stem cells and *in vitro* approaches to repopulate the number of ADA-producing T cells. Gene therapy has restored the health of over 100 people affected by ADA-SCID, mostly children, making it by far the most successful example of gene therapy.

Gene therapy for treating different forms of blindness

More than 200 genes are associated with retinal disorders, so treating eye disorders by gene therapy is a significant opportunity. Several dozen gene therapy trials have been completed or are ongoing for various forms of blindness, including age-related degenerative causes of blindness that cause progressive vision loss. Because of the small size of the eye and the relatively

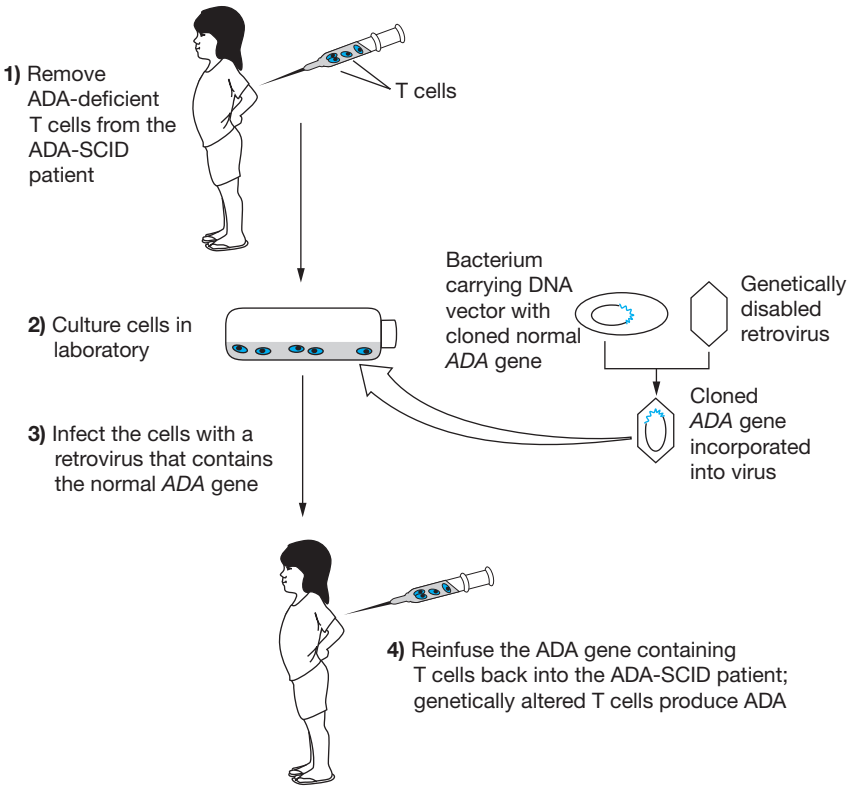


FIGURE 11.21 The First Human Gene Therapy An *ex vivo* gene therapy strategy was used in a 4-year-old ADA-SCID patient.

small number of cells that need to be treated, the prospects for gene therapy to become routine treatment for eye disorders appears to be very good. Retinal cells are also very long-lived; thus, AAV delivery approaches can be successful for long periods even if the gene does not integrate. Gene therapy treatments for several forms of blindness were originally pioneered in dogs, and then based on their success, protocols were adapted and applied to human trials.

University of Pennsylvania and Children's Hospital of Philadelphia researchers reported beneficial results for treatments of **Leber's congenital amaurosis (LCA),** a degenerative disease of the retina that affects 1 in 50,000 to 100,000 infants each year and causes severe blindness. LCA is caused by alterations to photoreceptor cells (rods and cones), light-sensitive cells in the retina, due to 18 or more genes. One gene in particular, *RPE65*, has been the gene therapy target of choice. The protein product of the *RPE65* gene metabolizes retinol, which is a form of vitamin A that allows the rod and cone cells to detect light and transmit electrical signals to the brain. Young adult patients with defects in *RPE65* were given injections of the normal gene delivered via AAV. After a single treatment, most patients are still legally blind but they can see more light, some of them can read the lines of an eye chart, and when used in children many have gained sufficient vision to be ambulatory. In late 2017 the FDA issued the first approved gene therapy for an inherited eye condition to Spark Therapeutics of Philadelphia for its product Luxturn, which delivers *RPE65 in vivo* to treat LCA.

Gene therapy trials for **choroideremia,** a rare inherited form of blindness caused by a mutation in the *CHM* gene that affects pigment cells in the retina, have also generated exciting results. Retinal injections of AAV carrying functional copies of *CHM* have significantly improved the abilities of adult patients to see light, and for several to read lines on an eye chart, for more than 2 years after a single treatment.

Gene therapy for hemophilia B

Recent gene therapy trials, also by Spark Therapeutics, have demonstrated success for **hemophilia B**, a blood clotting disorder caused by defects in the *FIX* gene and which occurs predominantly in men. *FIX* encodes a protein called Factor IX which is essential for blood clotting. This trial used an AAV-mediated gene therapy to deliver *FIX* to liver cells. Of 10 men treated, all 10 were producing normal levels of clotting factor proteins, and nine of the 10 no longer needed treatment a year after the initial gene therapy. This study provides the most successful hemophilia gene therapy outcome reported for hemophilia as of the date of publication of *Introduction to Biotechnology*.

Challenges Facing Gene Therapy

Scientists have always been concerned about the potential risks associated with gene therapy and the safety of these procedures. Discussions about the safety of gene therapy greatly intensified after 18-year-old Jesse Gelsinger died during a gene therapy clinical trial at the University of Pennsylvania in 1999. Jesse's death was directly attributed to complications related to the adenovirus vector used to deliver therapeutic genes to treat him for a liver disorder (ornithine transcarbamylase deficiency) that affected his ability to break down dietary amino acids.

Jesse's death was triggered by a severe inflammatory response to a modified adenovirus vector bearing the *ornithine transcarbamylase* (*OTC*) gene that had been injected into his hepatic artery. The vectors were intended to enter liver cells and result in the production of OTC protein in the hope that this treatment would cure him of his liver disease. Within hours of his first treatment, a massive immune reaction surged through Jesse's body. He developed a high fever, his lungs filled with fluid, multiple organs shut down, and he died 4 days later of acute respiratory failure.

During inquiries into this tragedy, it was learned that clinical trial scientists had not reported other adverse reactions to gene therapy and that some of the scientists involved in this trial were affiliated with private companies that could benefit financially from the trials. It was found that serious side effects seen in animal studies were not explained to patients during informed-consent discussions. The FDA then scrutinized gene therapy trials across the country, halted a number of them, and shut down several. Other research groups voluntarily suspended their gene therapy studies. Jesse Gelsinger was the first person in a gene therapy clinical trial to die as a result of his treatment. His death raised more questions about using viral vectors, placed greater emphasis on the development of nonviral vectors, and called for greater scrutiny of gene therapy and tighter restrictions on gene therapy trials.

In 2002, concerns about gene therapy involving retroviruses were further elevated as a result of trials in France and the United Kingdom for treating X-linked severe combined immunodeficiency syndrome (X-SCID). In these trials, 5 of 20 children treated developed leukemia because of the therapy, and 1 of them died in 2004. They had received injections of bone marrow cells that had been treated (*ex vivo*) with a retrovirus-delivered therapeutic gene. Two of these children had returned home to a normal life, seemingly cured, until they developed a leukemia-like cancer about 2½ years later. Their cancer was caused by retrovirus vectors that randomly integrated the therapeutic gene into a critical location of the genome containing the promoter region for a gene called *LM02*, which encodes a transcription factor required for the normal formation of white blood cells. This integration led to aberrant transcription of the *LM02* gene and overexpression of *LM02*, which triggered the uncontrolled division of mature T cells.

This tragedy resulted in the temporary cessation of a large number of gene therapy trials, and the FDA completely stopped most retroviral studies. Trials eventually resumed, but with greater patient monitoring and significant reassessment of protocols for gene therapy with retroviruses.

Glybera approved as the first gene therapy product in the Western world and subsequently withdrawn

In 2012, Glybera (alipogene tiparvovec) made history when the European Medicines Agency of the European Union approved it as the first gene therapy product to win commercial approval in the Western world. Glybera is an AAV vector system for delivering therapeutic copies of the *LPL* gene to treat patients with a rare disease called lipoprotein lipase deficiency (LPLD; also called familial hyperchylomicronemia). LPLD patients have high levels of triglycerides in their blood. Elevated serum triglycerides are toxic to the pancreas and cause a severe form of pancreatic inflammation called pancreatitis. Glybera was developed by Amsterdam-based company uniQure BV, which, for many reasons, including cost, decided not to seek approval by the U.S. FDA. Glybera was one of the most expensive drugs in history, perhaps the most expensive drug, at a cost of over $1 million per treatment. But by 2017, Glybera had failed to be widely used by any European country and since inception had been used in only one patient—with a unique source of reimbursement funding for the treatment. Thus uniQure announced it would not renew European marketing authorization after October 2017.

Future Challenges to Address

Currently, there are still more barriers to gene therapy than solutions to medical problems. These include the following:

- How can expression of the therapeutic gene be controlled in the patient? What happens if therapeutic genes are overexpressed or if a gene shuts off shortly after it has been introduced?
- How can scientists safely and efficiently target only the cells and tissues that require the

therapeutic gene without affecting other cells in the body where the gene is not needed?

- How can gene therapy be targeted to specific regions of the genome to prevent the random integration problems encountered in the French trials or instability and movement of the inserted DNA? This is one area in which CRISPR-Cas approaches have shown great promise.
- How can gene therapy provide permanent treatment without frequent administration of the therapeutic gene?
- How can rejection of the therapeutic gene be avoided? Whenever gene therapy is used, it is not always known if the recipient's immune system will reject the protein produced by therapeutic genes or reject genetically altered cells containing therapeutic genes.
- What percentage of cells in an organ or tissue must express the therapeutic gene to treat the condition effectively? This will vary depending on the disease condition, but it remains to be determined if a majority of diseased cells must be affected by the therapeutic gene or if a disease can be treated by correcting only a small number of cells.
- Will it be possible to use gene therapy to treat diseases that involve multiple genes?
- Will gene editing approaches eventually become effective, safe, and widely used for gene therapy?

These and other barriers must be overcome before gene therapy becomes a safe and reliable treatment approach, but scientists are making excellent progress in this field. They are also making incredibly rapid advances in another hot area of medical biotechnology called regenerative medicine, the final topic of this chapter.

11.6 The Potential of Regenerative Medicine

Currently, physicians treating most human illness are primarily limited to approaches such as surgical techniques, radiation treatment, and drug therapy. Although these approaches all have a place in medicine to treat certain human conditions, they do not offer the ability to regenerate tissue or restore the functions of damaged organs. For example, when a person has a heart attack or stroke, tissue damage often results. When an organ is damaged, the only way to restore its functions fully is to replace the damaged tissue with new ones—something that often does not occur naturally in heart or brain tissue.

When organ development occurs in the embryo, changes in the expression of many genes must occur in an ordered sequence. Unwanted changes that even drugs cannot fix may occur in cells, and no one drug can stimulate the growth and repair of new tissue when an organ is severely damaged. Even with new knowledge gained from the Human Genome Project, it is highly unlikely that any one drug or even a few drugs could be used to stimulate hundreds of changes in gene expression with the proper timing required for tissue regeneration and the restoration of organ function.

Regenerative medicine, growing cells and tissues that can be used to replace or repair defective tissues and organs, is an exciting field of biotechnology that holds the promise and potential for radically changing medicine and the delivery of health care as we know it. As you will learn, clearly many of the topics we will discuss here could be considered under the category of precision medicine based on the person-specific nature of these different technologies.

Cell and Tissue Transplantation

Transplantation is not a new idea, but applications that involve transplanting specific cells and tissues to replace or repair damaged tissues are relatively new aspects of medical biotechnology research.

Fetal tissue transplants

After demonstrated success in rodents, **fetal tissue transplants** have been used since the late 1980s. Most human fetal tissue comes from embryos or fetuses obtained from accident victims and legally aborted embryos. We mention traditional fetal tissue transplantation work here but keep in mind that in the future it is expected that stem cells, and not embryos or fetuses, will become the primary source of cells for such transplantations.

Neurodegenerative diseases occur gradually, leading to progressive loss of brain functions over time. Alzheimer's disease and Parkinson's disease are perhaps the two best-known examples. These diseases rank first and second, respectively, as the most common neurodegenerative disorders. For Parkinson's disease alone, approximately 50,000 cases are diagnosed yearly, and an estimated 500,000 Americans currently have the disease. Parkinson's disease is due to the loss of cells in an area deep inside the brain called the *substantia nigra*. Neurons in this region produce a chemical called *dopamine*, a neurotransmitter or chemical used by neurons (nerve cells) to signal one another.

Loss of these dopamine-producing cells causes tremors, weakness, poor balance, loss of dexterity, muscle rigidity, a reduced sense of smell, inability to swallow, and speech problems, among other effects. Most treatments involve drugs that increase the production or accumulation of dopamine in the brain; however, after about 4 to 10 years of drug treatment, the disease progresses and the effectiveness of these drugs diminishes, leading to a poor quality of life for the patient, who typically dies of complications related to the disease.

Similarly, over 250,000 individuals have been paralyzed by trauma to the spinal cord, and nearly 2 million people worldwide are living with spinal cord injuries. Each year approximately 85,000 people suffer spinal cord injuries, including roughly 10,000 in the United States. Treating neurodegenerative diseases and spinal cord injuries with fetal tissue transplantation has been among the most widely used applications of fetal tissue. Unlike fetal neurons, which can divide, most adult neurons will not repair themselves when damaged, and most neurons do not undergo cell division. Patients receiving fetal transplants have shown varying degrees of improvement, including relief of symptoms in over 40 percent of patients and, in some cases, the almost complete elimination of most symptoms even several years later, but fetal transplants have not provided full recovery.

Many transplantation strategies have been used in attempts to repair spinal cord injuries. One approach has been to graft nerve fibers from fetal or adult neurons into the damaged area of the spinal cord so as to bridge the parts of the cord that were severed. Such bridge implants have shown promise in dogs and rats. As scientists learn more about the inflammatory chemicals that hinder nerve growth and the factors that stimulate it, it may be possible to use such molecules to minimize scar tissue formation, reduce damage caused by scar tissue to supporting cells called glial cells, block growth inhibitor molecules, and stimulate neuron regeneration at the same time.

Organ transplantation

Organ transplantation can and does save lives. In 2016 alone, 135,860 solid organs were transplanted worldwide. This reflects a 7.25 percent increase in organ transplantation as compared to 2015.

Autografting—that is, the transplantation of a patient's own tissue from one region of the body to another—can alleviate some transplantation problems. For example, coronary artery bypass operations involve removing segments of a vein from the patient's leg and connecting it surgically to arteries in the heart as a bypass around obstructed vessels. But if a patient needs a heart or a liver transplant, another person who can donate an organ for the recipient must be found. Even when a human donor who appears to be a match is found, organ rejection is a major problem. Rejection typically occurs when the recipient's immune system recognizes that the donor organ is foreign. Matching organs for transplantation involves tissue typing to check if a donor organ is compatible for a recipient.

Tissue typing is based on a large group of proteins found on the plasma membrane of every cell in the body, called the **major histocompatibility complex (MHC)**. MHCs are aptly named because *histo* means "tissues," and MHC molecules must be matched between donor and recipient to have a compatible organ for transplantation. There are many different types of MHC proteins. One common group, the human leukocyte antigens (HLAs, named because they were first discovered on white blood cells, or leukocytes), are found on virtually all body cells. Immune system cells such as B and T cells recognize HLAs on all body cells present since birth as "self" (belonging to the same individual), whereas any other cells are "nonself," or foreign cells that may be attacked by the immune system and destroyed. Some common HLAs are found on most human tissues, and others are unique to a given individual. To have a successful transplantation of an organ from one human to another requires a close match of several types of HLAs between the donor organ and the recipient's cells; otherwise the recipient will reject the transplanted organ.

Since the first human liver transplant in 1963, transplant surgeons have been using immunosuppressive drugs to weaken the recipient's immune system and minimize organ rejection. Most transplant recipients must use immunosuppressive drugs for the rest of their lives. One obvious problem with this approach is that patients on immunosuppressive drugs can and do develop infections, which, because of their weakened immune systems, can be life-threatening. The lack of sufficient human organ donors and the problem of organ rejection are major reasons why scientists are looking at other ways to provide donor organs.

Xenotransplantation—the transfer of organs from different species—may one day become a viable alternative to human-to-human organ donation, thus helping relieve the tremendous need for human donor organs. Baboons were once considered the animal of choice for providing organs to human recipients. The first animal-to-human organ transplant in a child, carried out in 1984 by doctors at Loma Linda University Medical Center in California, involved transplanting a baboon heart into Baby Fae, a 12-day-old girl. Baby Fae

lived with the baboon heart for 3 weeks before she died of complications related to organ rejection. Similar transplants have been performed without great success.

Many researchers are focusing on pigs as organ sources. Pigs may be a good choice because they are easy to breed, relatively inexpensive, and many pig organs are also similar in function and size to human organs. Progress on using pig organs for transplantation in humans has been slowed by concerns that viruses may be transmitted from pigs to humans, causing the transplanted organs to be rejected and creating other health problems. To date, efforts to engineer cloned pigs to provide human immune system rejection-free or pig virus-free organs for transplantation have met with limited success. But in 2017, the biotechnology company eGenesis reported they had used CRISPR to eliminate all 62 copies of endogenous retrovirus (PERV) genes in the pig genome and created embryos that were developed into healthy piglets (**Figure 11.22**). Other pig genes that stimulate the immune system also need to be modified (as we discussed in Chapter 6), but now that CRISPR is available for genome editing this research warrants close attention in the future.

Xenotransplantation does not always have to involve the transfer of a whole organ, as you will learn in the next section. Scientists are working hard to develop ways to deliver small clusters of cells as a technique for cell and tissue transplantation.

Cellular therapeutics

Cellular therapeutics involves using cells, instead of whole tissues or organs, to replace defective tissues or to deliver important biological molecules. One alternative for avoiding organ rejection of transplants is to use living cells that have been encapsulated into tiny plastic beads or tubes called **biocapsules** or microcapsules. Biocapsules may also contain genetically engineered cells designed to produce therapeutic molecules such as recombinant proteins.

Biocapsules have tiny holes in their walls, making them permeable to nutrient exchange and allowing molecules produced by the encapsulated cells to escape from the capsule and enter the bloodstream or surrounding tissues (**Figure 11.23**). For instance, microcapsules containing insulin-producing cells (beta cells) from the pancreas, implanted in patients with type 1 diabetes, produce insulin that can travel out of the capsule into the bloodstream of the patient to all body organs requiring insulin. Microcapsules containing beta cells, including cells derived from stem cells, are expected to move into clinical trials in the next few years. Another important feature of biocapsules is that they protect cells from being attacked by the recipient's immune system by hiding them within capsules, where immune cells and antibodies cannot reach and destroy them. Immune system attack of beta cells is the problem in type I diabetes; thus, encapsulated cells may be a successful strategy. Although not a permanent cure, biocapsules can provide lasting release of molecules into the body. This approach would likely require that biocapsules be changed every few months; however, in the case of diabetes, this might be a better alternative than daily injections of insulin.



FIGURE 11.22 Genome Edited Pigs Could Potentially Save the Lives of Patients Waiting for a Transplant These piglets have been engineered by CRISPR-Cas to remove endogenous retroviruses, potentially providing a source of virus-free organs for human transplantation.

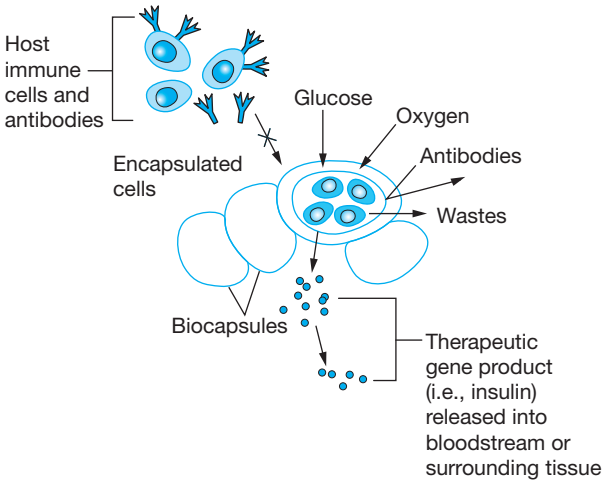


FIGURE 11.23 Biocapsules Encapsulated cells may provide valuable ways to deliver therapeutic molecules. Biocapsules allow molecules produced by the cells to leave the capsule and provide therapeutic benefits for the host. At the same time, cells in the biocapsule are protected from attack by immune cells and antibodies of the host. This example illustrates how insulin-producing cells could be used to provide a patient with diabetes with a source of insulin.

Tissue Engineering

Whether you pay $30,000 or $300 for your first car, one thing is certain: over time parts will wear out and break. A trip to the local mechanic can repair and replace some car parts, but eventually the car wears out to the point of no return, and it is time to buy another car. Wear and tear on human body parts also takes its toll. Over time, organs do not work as well as they should; in some cases, an organ may stop functioning altogether. Even if a person lives a relatively healthy life, the wear and tear of aging or a sudden event such as a stroke or heart attack will lead to a decline in organ function and perhaps organ failure. But our bodies are not like cars: we cannot go to a warehouse of body parts for replacements.

In the future, however, the emerging science of **tissue engineering** may provide tissues and organs that can be used to replace damaged or diseased tissues. This small but growing industry—there are over 75 biotechnology companies involved in tissue engineering in the United States alone and experts predict that the field will grow very rapidly over the next decade—is actively involved in research to engineer human tissues and organs as replacements for worn-out and damaged tissues. As we age, we outlive the functional abilities of our organs. Tissue engineering and regenerative medicine approaches are also predicted to lead to substantial cost savings for care of chronic diseases such as congestive heart failure and diabetes. In the case of congestive heart failure, if regenerated heart tissues restored cardiac function for even a small percentage of individuals, cost savings could be several billion dollars annually worldwide.

In the 1990s, tissue engineering pioneer Charles Vacanti and colleagues made headlines around the world when they revealed a mouse with an engineered ear growing on its back. In this example, a biodegradable scaffold in the shape of an outer ear was attached to a mouse and then seeded with cartilage cells from cows. The cartilage cells infiltrated the scaffolding and produced cartilage as they grew; then, as the scaffolding degraded, the cartilage developed enough strength to support itself. The human ear–looking tissue was never transplanted onto a human. Because it was made of cow cells, it would have been rejected by the human immune system. Also, this tissue was just the outer ear without the inner ear structures that actually detect sound, but it provided strong evidence that tissue engineering could work.

Tissue engineering scientists often begin by designing and constructing a biomaterial framework or scaffold made of biological substances such as calcium, collagen, a polysaccharide called alginate, or biodegradable materials (**Figure 11.24a**). The scaffold is shaped as a mold of the tissue or organ to be made, and its purpose is to create a three-dimensional framework onto which cells are placed. Growing human cells on the scaffolding is called *seeding* because the cells literally act as "seeds" to create more cells that will grow over the scaffold. Scaffolds seeded with cells are bathed in a nutrient-rich medium and, over time, cell layers build up over the scaffolding material to assume the shape of the scaffold. Engineered sheets of human skin have proven useful for treating severe burn victims who have lost substantial portions of

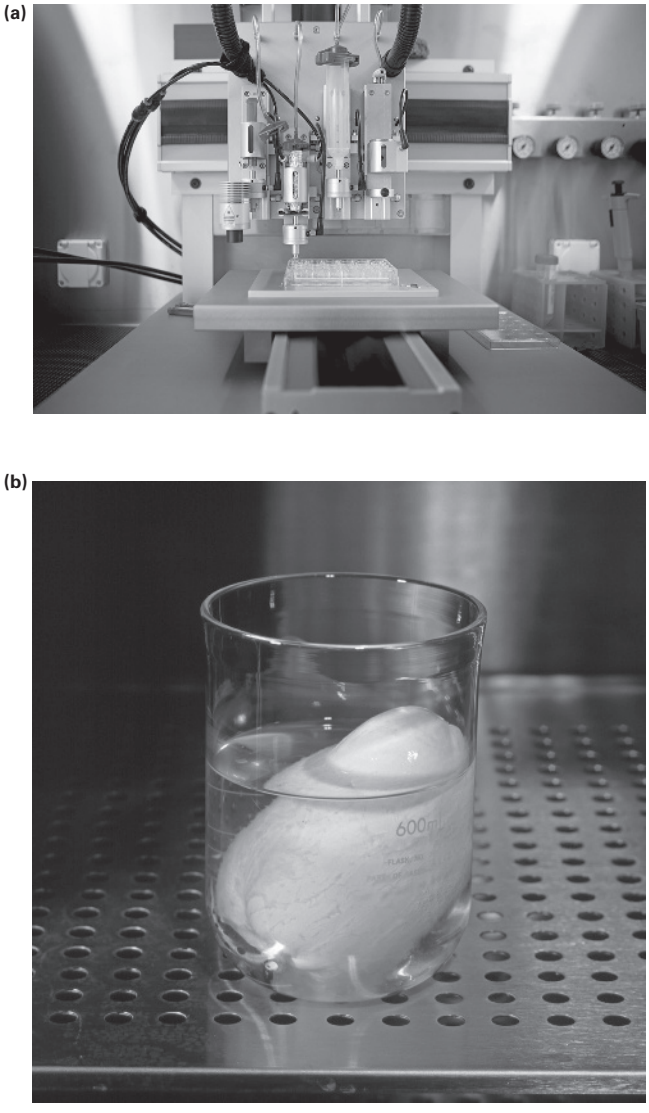


FIGURE 11.24 Tissue Engineering (a) 3D Bioprinting muscle cells. (b) A urinary bladder created from stem cells.

their skin, a very painful and life-threatening condition. Engineered bone structures for healing bone fractures have also worked fairly well. Scaffolding to engineer teeth and blood vessels has shown promise, and many products of tissue engineering are FDA-approved and being used to treat patients or in clinical trials.

Three-dimensional bioprinting of tissues

Three-dimensional (3D) printers have become very popular for creating all kinds of prototypes, particularly from plastics. But for over a decade, scientists and engineers have used **3D bioprinting** to produce human tissues! As farfetched as it sounds, printing tissues from cells works much like printing letters from ink (**Figure 11.24b**). Cells in solution, like particles of pigments in ink, can be sprayed through inkjet-like nozzles to print different tissue types—often by spraying cells onto a prepared scaffold. Layer by layer, different cells can be positioned precisely to create 3D tissue and organ structures. Scientists at the Wake Forest Institute for Regenerative Medicine have even used magnetic resonance imaging (MRI) scans of the urinary bladder to then print scaffolds with the proper 3D shape to grow individual bladders for implantation into cancer patients.

Many technical challenges are associated with 3D bioprinting that researchers are working to overcome. The slowness of the bioprinting process is one such challenge; growing readily available sources of cells for printing, constructing complex functional organs, and ensuring that a printed tissue has sufficient vascularization (blood vessel supply) and innervation (nerve supply) exemplify other challenges. But in a relatively short time the field has progressed rapidly, and there is no doubt that multidisciplinary research approaches will help 3D bioprinting become an important tool for regenerative medicine.

Engineering organoids and organs

Developed in the past decade, **organoids,** miniature organs produced from stem cells and grown in culture, have become valuable tools for tissue engineers. Much like the way organs develop *in vivo*, mixtures of cells grown under the right conditions *in vitro* can be coaxed to self-assemble as cells differentiate and organize to form the organoid. In addition to helping scientists understand organ development, organoids are valuable for drug testing, disease modeling, and potentially for organ replacement in the future. Organoids from patients' stem cells have been made for the cerebral cortex of the brain, heart, intestine, kidney, liver, lung, pituitary gland, prostate, stomach, and others.

Sometimes organoids are grown on glass or plastic slides. Referred to as **"organs-on-chips,"** these miniature models of human organs are becoming very valuable as biotechnology and pharmaceutical companies use these *in vitro* systems for drug testing and development. Organs-on-chips are thought to be better models for drug testing than traditional cell culturing of layers of cells on a plate, and in some cases they can replace animal testing. For example, a recently created organ on a chip model of uterine tissue that functionally mimics the female menstrual cycle is helping scientists study causes of miscarriages.

When it comes to engineering entire organs for transplantation, skin is a relatively simple organ to produce, as are hollow organs such as tracheas and bladders, both of which have been engineered and transplanted into patients. Creating large and complex organs such as the liver, heart, and kidney has proven to be much more difficult, although fetal tissue has been used to grow a rudimentary kidney in rats that was able to produce a urine-like fluid. At least two phase II clinical trials are under way in which human bladders were created using tissue biopsies from patients' bladders to seed scaffolding and produce new bladders (**Figure 11.24c**). To date, scientists have also engineered blood vessels, including bioengineered blood vessels that have been used in patients with kidney failure who require dialysis, corneas, retinas, thyroid tissue, urethras, and vaginas (implanted in teenage girls born with absent or underdeveloped vaginas), among others.

The field of tissue engineering is in its infancy. But progress is advancing at an incredibly rapid rate and, as discussed in the next section, applications involving stem cells are adding to this progress. There is every reason to expect that engineered organs will become a reality.

Stem Cells

Since first being reported to the public in 1998, the tremendous promise and controversy surrounding stem cells has made stem cell research and related topics regular themes of front-page news items and TV headlines. Stem cells evoke emotional and controversial responses from scientists, clergy, politicians, and the general public. Potential applications of these cells engender excitement, fear, anger, and a range of other emotions.

What are stem cells?

There are many different types of **stem cells,** but in general all stem cells have two basic characteristics that set them apart from other cell types: **self-renewal** and **differentiation** into specialized cells:

- Self-renewal: Stem cells grow and divide (proliferate) indefinitely by mitosis to create populations of identical stem cells.
- Differentiation: This is a complex process involving many genes that must be activated and silenced, and differentiating cells rely on chemical signals such as growth factors and hormones from other cells to help them change. Stem cells are special because they can eventually differentiate to form all of the more than 200 cell types that make up the human body. Stem cells are called **pluripotent** because they have the potential to develop into a variety of different types.

To understand what stem cells are, we consider development of the human embryo and what occurs when *in vitro* **fertilization (IVF)** is carried out. IVF first gained public attention in 1978, when Louise Brown, the first test tube baby, was born. To create a child by IVF, sperm and egg from donor parents are mixed together in a culture dish to produce an embryo. After several days of division, the embryo is surgically implanted in the uterus of a woman, usually the egg donor, who has been treated with hormones to prepare her uterus for implantation. When a couple agrees to undergo IVF, several embryos are usually created, but often only one is implanted during each procedure. The remaining embryos are frozen for future use as needed. Potentially, the leftover embryos can be a source of **human embryonic stem cells (hESCs** or **ES cells**), but they are also the source of a great deal of controversy.

An embryo goes through a predictable series of developmental stages (**Figure 11.25**). After an egg cell is fertilized, it is called a **zygote.** The zygote divides rapidly and, after 3 to 5 days, first forms a compact ball of about 12 cells, called a **morula,** meaning "little mulberry." Around 5 to 7 days after fertilization, the embryo consists of a small, hollow cluster of approximately 100 cells, known as a **blastocyst.** The blastocyst is approximately one-seventh of a millimeter in

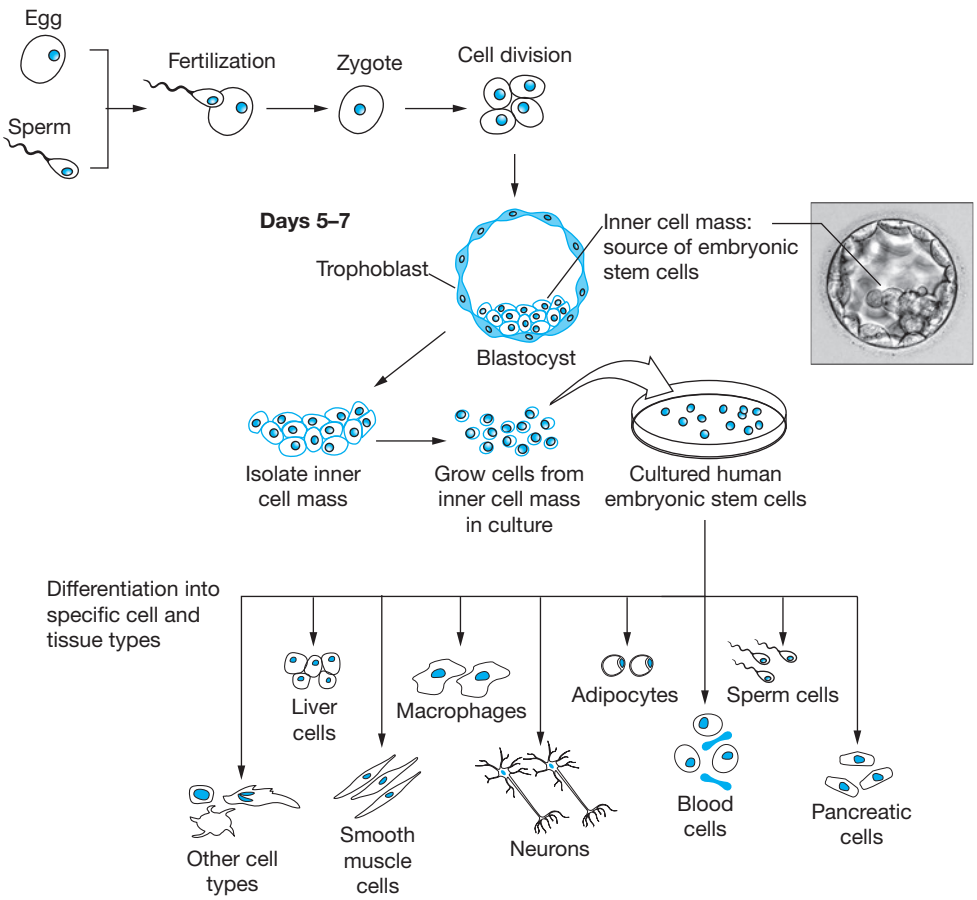


FIGURE 11.25 Isolating and Culturing Human Embryonic Stem Cells (hESCs) Cells isolated from the inner cell mass of human embryos can be grown in culture as a source of hESCs. Under the proper growth conditions, hESCs can be stimulated to differentiate into virtually any cell type in the body.

diameter. It contains an outer row of single cells called the trophoblast; this layer develops to form part of the placenta, which nourishes the developing embryo. The area of cells of primary interest to stem cell biologists is a small cluster of around 30 cells tucked inside the blastocyst, which form a structure known as the **inner cell mass,** the source of hESCs (Figure 11.25).

Cells of the inner cell mass develop and undergo differentiation to form the embryo itself. Successful isolation and culture of the first hESCs from a human blastocyst was reported in 1998 by James Thomson of the University of Wisconsin at Madison, who had cultured hESCs from rhesus monkeys 2 years earlier. Also in 1998, John Gearhart and colleagues at Johns Hopkins University isolated embryonic germ cells, primitive cells that form the gametes—sperm and egg cells—from human fetal tissue and demonstrated that these cells could develop into different cell types. These discoveries followed the work of other scientists who had isolated stem cells in species such as mice, pigs, cows, rabbits, and sheep. In fact, stem cell researchers credit much of what is known about isolating hESCs from pioneering work initiated in mice in the 1980s—another outstanding example of how model organisms contribute to the advancement of science.

Human ESCs avoid senescence and show no signs of aging, in part because they express high levels of telomerase. Several groups have maintained stem cells for years and over 600 rounds of division without apparent problems. Cultured cells such as these, which can be maintained and grown successively, are called **cell lines.** Stem cells also grow rapidly and can be frozen for long periods and retain their properties. Under the right conditions, when they are stimulated with different molecules including hormones and substances called **growth factors,** stem cell lines can be coaxed to differentiate into different types of cells. This *directed differentiation* of stem cells into specific differentiated cells of interest is key to creating tissues for regenerative medicine applications. A major focus of stem cell research is experimentation to determine what controls the pluripotency of stem cells and to identify the factors that stimulate their differentiation into discrete cell types. These signals include substances called growth factors, hormones, and peptides, which stimulate differentiation in tissue-specific ways.

For example, signaling systems that involve transforming growth factor-ß (TGF-ß), bone morphogenetic proteins (BMPs), and other growth differentiation factors act on a gene for a transcription factor called *Nanog*. NANOG is one key protein that maintains hESCs in an undifferentiated pluripotent state. Once stem cells are produced and isolated, scientists use a number of tests to determine their pluripotency. In the laboratory under the proper culturing conditions, ESCs from humans, mice, rats, primates, and other species have been shown to differentiate into a myriad of cells including skin cells; brain cells (both neurons and glial cells, which support, nourish, and protect neurons); cartilage (chondrocytes); oocytes (that can be fertilized to give rise to normal offspring); spermatozoa; osteoblasts (bone-forming cells); liver cells (hepatocytes); insulin-secreting pancreatic beta cells; muscle cells including smooth muscle, which forms the walls of blood vessels; skeletal muscle cells, which form the muscles that attach to and move the skeleton; cardiac muscle cells (myocytes), which form the muscular walls of the heart; and many other cell types.

How do researchers obtain hESCs? hESCs for research purposes are derived from the blastocysts of embryos that are no longer needed for IVF or from human embryos created by IVF from sperm and egg cells donated in order to provide embryos for research purposes. Typically, leftover blastocysts would be either destroyed or frozen indefinitely, but with the consent of the couple who provided the sperm and egg, they can be used to derive hESCs, as described in Figure 11.25. In U.S. fertility clinics alone, estimates indicate more than 1 million human embryos remain frozen with nearly 100,000 of these being abandoned (not intended for future use), and several thousand eggs are discarded annually.

Other sources of stem cells

Research on hESCs is very controversial because of their source—an early embryo. Scientists have discovered **adult-derived stem cells (ASCs),** cells that reside in mature adult tissue and could be cultured and differentiated to produce other cell types. ASCs appear in very small numbers and have been isolated from the heart, brain, intestine, hair, skin, pancreas, bone marrow, fat, mammary glands, teeth, muscle, blood, and other tissues. It is thought that ASCs are present in all adult tissues.

Opponents of hESC research often claim that ASCs are a more acceptable alternative than using hESCs because isolating ASCs does not require the destruction of an embryo. ASCs can be harvested from people by fine-needle biopsy, through a thin-diameter needle inserted into muscle or bone tissue. It may even be possible to isolate ASCs from cadavers. ASCs are present in fat (adipose) tissue, which provides an outstanding source of stem cells, especially if you consider that over 500,000 L of fat tissue collected by liposuction and other cosmetic surgery techniques are discarded in the United States each year.

Experiments have shown that ASCs from one tissue can differentiate into another different specialized

cell type. For instance, an ASC isolated from muscle tissue could be used to develop into a blood cell. But other studies have demonstrated that ASCs may not be as pluripotent as hESCs. Ongoing research is required to determine whether ASCs can be as valuable as hESCs might be.

There are other types of stem cells. Stem cells can be isolated from human amniotic fluid, the protective fluid that surrounds a developing fetus. In the lab, these **amniotic fluid–derived stem cells** have been coaxed to become neurons, muscle cells, adipocytes, bone, blood vessels, liver cells, and others. **Cancer stem cells (CSCs),** also called tumor-initiating cells, have been identified and implicated in the development of cancers, tumor progression, tumor metastasis, and the recurrence of cancers. Like normal stem cells, CSCs can self-renew and differentiate to form the tissues from which they were derived. Certain CSCs grow slowly in clusters, or *niches,* within a tissue. It is not clear what properties CSCs may have besides the ability to form a tumor. Researchers are also not sure whether CSCs are derived from normal cells or if they are involved in cancer tumor resistance to chemotherapies, but these cells are a focus of intense research and potential therapeutic treatments for the treatment of cancers.

Creating stem cells by nuclear reprogramming of somatic cells

Research on stem cell biology is an extremely active field. A primary focus continues to be alternative approaches for producing pluripotent stem cells without destroying an embryo. One of the most promising new approaches for doing this involves a technique called **nuclear reprogramming of somatic cells.** The basic concept of this approach is to use genes involved in cell development to push a somatic cell back to an earlier stage of development and affect gene expression and thus to reprogram the somatic cell genetically to return to a pluripotent state characteristic of the stem cells from which it was derived. It was previously thought that once cells differentiated to become specific, specialized cell types—for example, a skin cell—their differentiation fate was irreversible. But we now know that this is not the case. One of the first successful reprogramming techniques involved fusing hESCs with skin cells called *fibroblasts*. The hybrid cells generated in this way displayed several properties of hESCs both *in vitro* and *in vivo*.

There are several different approaches to nuclear reprogramming, and this method has greatly advanced stem cell research. One initial approach, first reported in 2006 by Shinya Yamanaka and colleagues from Japan, involved using retroviruses to deliver four transgenes—*OCT3/4*, *SOX2*, *c-MYC*, and *KLF4*—into fibroblasts (**Figure 11.26**). Expression of these four genes, which encode transcription factors involved in cell development, "reprograms" the fibroblasts back to an earlier stage of differentiation. Such reprogrammed cells are called **induced pluripotent stem cells (iPSCs or iPS cells)**. Yamanaka shared the 2012 Nobel Prize in Physiology of Medicine with Sir John Gordon of the United Kingdom (who, decades earlier, pioneered groundbreaking work on reprogramming cells).

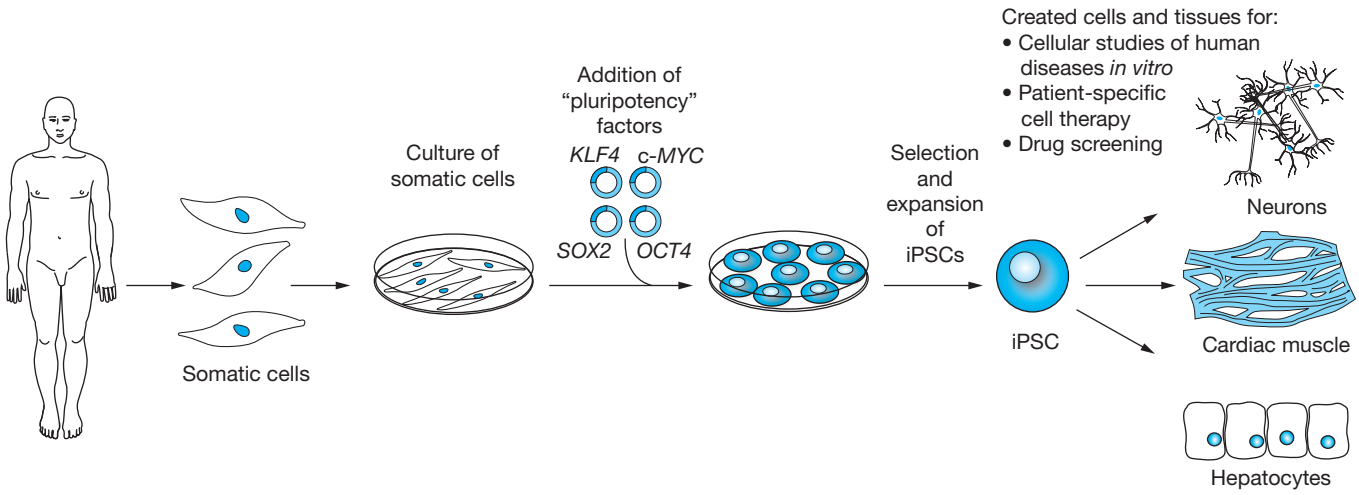


FIGURE 11.26 Nuclear Reprogramming of Somatic Cells to Produce Induced Pluripotent Stem Cells The introduction of four transcription factor genes (*OCT3/4*, *SOX2*, *c-MYC*, *KLF4*) into somatic cells such as fibroblasts results in the formation of induced pluripotent stem cells (iPSCs). Invariably a number of these cells are defective in reprogramming and must be selected out during culture. They must also be selected for the expression of endogenous marker genes (such as *Nanog* and *Oct*) known to be expressed in pluripotent stem cells.

iPSCs demonstrate many properties of hESCs, such as self-renewal and pluripotency, and are often indistinguishable from hESCs. iPSCs have been produced from human, mouse, rat, pig, monkey, and other organisms. Subsequently, a cocktail of RNA molecules for the four genes described previously has also been used to create iPSCs from somatic cells. iPSCs have been produced without using the *c-MYC* gene. This is a potentially important advance because *c-MYC* is a known oncogene. Also, human neural stem cells have been reprogrammed to iPSCs by introducing only *OCT4*.

These iPSCs express genes such as *Nanog* and *Oct,* which are characteristic markers known to be expressed in undifferentiated hESCs. Researchers have demonstrated that iPSCs can differentiate into a range of cell types including neural cells, cardiac muscle cells, liver cells, and many others. Nuclear reprogramming may be a way to generate patient-specific iPSCs without the need for an embryo and hESCs. iPSCs have been successfully derived by reprogramming human skin cells from patients. Reprogrammed skin cells taken from the face of a 36-year-old woman, connective tissue cells from the joints of a 69-year-old man, and skin cells from the foreskin of a newborn boy are examples. Oocytes and spermatozoa have been derived from mouse iPSCs (and ESCs). Fertility scientists are very interested in producing human eggs and sperm both to study infertility and to help infertile couples have children.

Stem cells derived by reprogramming could be used for patient-specific cell therapies—another example of precision medicine! In addition, with iPSCs, it is theoretically possible to create disease-specific stem cells from individual patients. For example, one could take a tissue biopsy, such as skin, from a person with a particular disease and reprogram those cells into stem cells that could then be used to create cell types for combating the disease. Patient-specific iPSCs could be used for cell-based therapies without the risk of immune rejection. For patient-specific stem cells of any type, CRISPR-Cas now also makes it possible to consider correcting mutations in a patient's cells and then reintroducing the corrected cells.

But even the most optimistic iPSC researchers believe that such cell therapies will not be ready for at least another decade or more. In 2017, a report on the first use of iPSCs in humans to treat age-related macular degeneration (a progressive form of blindness) determined the treatments were safe. This trial did not improve vision in patients but it did stop progression of the disease. Trials will soon be under way for using iPSCs to treat Parkinson's disease patients, among many other trials expected to occur over the next several years.

Scientists are also making exciting advances with reprogrammed cells from patients; these cells can be used to study "diseases in a dish." Cultured reprogrammed cells from diseased patients are being studied to help scientists better understand human disease progression and disease processes. They are also being utilized for drug screening tests to determine the effectiveness of potential drug treatments for diseased cells. Labs around the world are making iPSCs from patients with a disease and differentiating these cells (including to make organoids) to become the tissues affected by a particular disease so that these diseases can be modeled *in vitro* and cells can be used for drug treatments.

On the surface, iPSCs circumvent some of the legal and ethical controversies associated with hESCs. But as promising as iPSCs are, there are challenges associated with them that will have to be addressed. For example, scientists still do not fully understand how pluripotent iPSCs may be and how to best control the potency of these cells. In addition, iPSCs:

- Are relatively inefficient to produce (only about 1 in 1,000 somatic cells exposed to most reprogramming approaches becomes an iPSC)
- Require constant feeding to maintain viable cell lines
- Show low viability compared with other cell types once they have been stored frozen
- Can be prone to forming tumors
- Occasionally show spontaneous differentiation into mature cell types when in culture
- Can sometimes be difficult to use for directing differentiation into particular cell types

Research with iPSCs is progressing at an astonishing pace as scientists work to better understand the properties and capabilities of these cells, and their potential for generating patient-specific stem cells without the need for an embryo. Watch for exciting new developments in the next few years involving nuclear reprogramming and iPSCs.

Potential applications of stem cells

Physicians and surgeons recognize that only stem cells have the potential to replace diseased cells with healthy cells, tissues, and, hopefully, organs. In the future, stem cell research may affect the lives of millions of people throughout the world. There are many potential applications for stem cells—from growing healthy tissues, to studying diseases, to genetic manipulation for delivering genes in gene therapy approaches, to creating whole tissues in the laboratory by tissue engineering. Many scientists believe that stem cell technologies will play key roles in developing treatments for diseases such as stroke,

TABLE 11.1 Stem Cell–Based Therapies May Potentially Benefit Millions of People

Disease Condition	Number of Patients in the United States
Cardiovascular disease	58 million
Autoimmune diseases	30 million
Diabetes	16 million
Osteoporosis	10 million
Cancers (urinary bladder, prostate, ovarian, breast, brain, lung, and colorectal cancers; brain tumors)	8.2 million
Degenerative retinal disease	5.5 million
Phenylketonuria (PKU)	5.5 million
Severe combined immunodeficiency (SCID)	0.3 million
Sickle cell disease	0.25 million
Neurodegenerative diseases (Alzheimer's and Parkinson's diseases)	0.15 million

Source: Adapted from *Stem Cells and the Future of Regenerative Medicine*, www.nap.edu/catalog/10195.html.

heart disease, Parkinson's disease, Alzheimer's disease, Lou Gehrig's disease, diabetes, and other conditions (refer to Table 11.1).

Potential and *promise* are frequently used words when stem cell applications are being discussed, but use of these cells for treating disease is still largely unproven. Here we consider some of the most promising examples of stem cell applications to date. Patients with leukemia frequently require chemotherapy or radiation treatment to destroy defective white blood cells. As a result, the patient's immune system is greatly weakened. Leukemia treatments may also involve blood transfusions to replace white blood cells and red blood cells damaged by chemotherapy. The use of stem cells to make white blood cells has already become an effective way to treat leukemia. Stem cells from umbilical cord blood have also been used to provide red blood cells for patients with sickle cell disease and those with other blood deficiencies. The isolation of stem cells from cord blood is becoming so popular that, in many U.S. states, parents can opt to pay to have cord blood stem cells frozen indefinitely should their child need them at some time in the future.

You should approach any reports of stem cell treatment successes with caution. There have been well-publicized and hyped stories of patients showing seemingly remarkable recoveries from stroke, spinal cord injuries, arthritis, and other conditions after receiving stem cell treatment. Most of these are unproven treatments that have not been subjected to rigorous evaluation to determine their efficacy or safety. However, there have been a number of well-evaluated treatments in animal models as well as in human clinical trials using stem cells for tissue repair. For example, stem cells from fat have been used to form bone tissue in the human skull.

The repair of heart tissue has shown strong potential. Stem cells might be used to replace dead and dying cells following trauma, such as a heart attack. Ischemic heart disease and stroke are recorded as the leading causes of death in the last 15 years. In 2016, these diseases alone caused 15.2 million deaths worldwide. The death of cardiac muscle cells weakens the heart and can prevent it from beating with the proper strength to maintain normal blood flow. Adult cardiac muscle cells do not repair themselves well.

Several groups of researchers have injected ASCs from different sources, such as bone marrow or heart tissue, into damaged areas of the heart. One of the first successful approaches using mice is described in **Figure 11.27**. Often various molecules that serve as growth-activating signals for cardiac cells are also injected into the damaged heart along with the stem cells. A percentage of transplanted stem cells develop into cardiac muscle cells, form electrical connections with healthy muscle cells, and improve heart function by over 35 percent. Researchers have reported improved pumping efficiency in mice at least a year after treatment. With this work, however, there are still some questions about whether many of the ASCs develop into cardiac muscle cells or whether the ASCs release molecules that stimulate the healing of cells surrounding the damaged tissue.

Similar approaches have been used to transplant hESCs, iPSCs, or hESC-derived cardiomyocytes into the ventricular walls of damaged hearts in pigs, in rats, in mice, and in humans involved in clinical trials. In some cases, synthetic scaffolds are placed at sites in the injured heart to assist engraftment of transplanted cells. In these types of experiments, transplanted stem cells differentiated to form cardiac muscle cells, which can then restore a significant percentage of electrical activity and contractility to the damaged areas.

Several human clinical trials are under way at different stages, and scientists are optimistic that these approaches may significantly counteract the damaging effects of cardiovascular disease in the future. Consider this exciting possibility: in the future, a surgeon may order a few grams of cardiac muscle cells from a regenerative medicine lab to transplant into a heart attack patient in much the same way that surgeons routinely

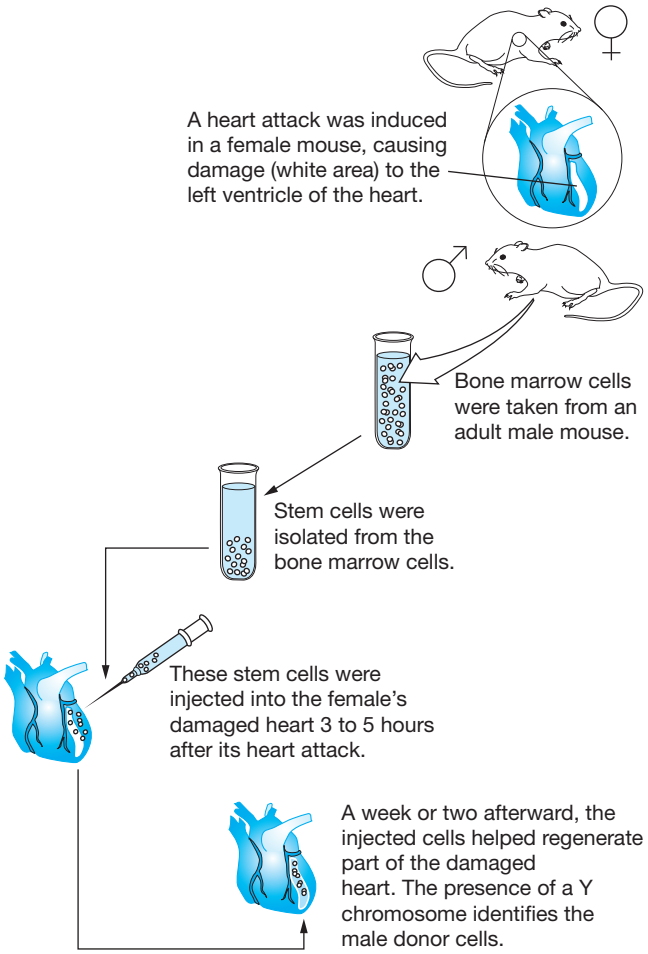


FIGURE 11.27 Repairing a Damaged Heart with Adult Bone Marrow Stem Cells Mouse adult bone marrow stem cells can be used to repair areas of the mouse heart damaged by a heart attack.

order blood from a blood bank for a transfusion during a surgical procedure!

A potentially promising and innovative treatment using iPSCs to correct sickle cell anemia in mice has been demonstrated (**Figure 11.28** on the next page). In this work, iPSCs were produced from skin cells of transgenic mice that express a mutated version of the human sickle cell hemoglobin gene and display sickle cell disease. These iPSCs were genetically engineered to correct the hemoglobin gene mutation. The corrected iPSCs were then induced to form blood stem cells and transferred into donor sickle cell mice, which produced functional red blood cells that, in turn, corrected the disease condition. Similar results have been reported using CRISPR to edit the sickle cell hemoglobin gene. These are incredibly exciting results, combining aspects of stem cell technologies and gene therapy.

Earlier in this chapter, we discussed problems faced by patients who suffer from spinal cord injuries. Stroke is another nervous system injury for which scientists and physicians are seeking treatments. In the past few years, researchers have disproved a long-standing belief that the human brain and spinal cord cannot grow new neurons. Adult stem cells have been isolated from the human brain and used to make neurons in culture, and scientists have already demonstrated that ESCs can be differentiated to form neurons that can be injected into mice and rats to improve neural function in those with spinal cord injuries.

In some of the earliest studies, researchers at Johns Hopkins University demonstrated that human stem cell transplants can enable mice with paralyzed hind limbs to walk. These studies were carried out on mice that were paralyzed after they were infected with a virus similar to the poliomyelitis virus that causes polio. But we know that nervous system injuries in animal models repair more readily than they do in humans. So there is still much work to be done in the field of spinal cord repair and regeneration, but researchers are optimistic that adult neural stem cell transplants may be ready for human clinical trials in the next few years, offering hope to the many individuals who are affected by spinal cord injuries.

In late 2017 one of the most extraordinarily successful applications of stem cell therapy was reported. This approach combined gene therapy with stem cells to regenerate the epidermis of a 7-year-old boy with a genetic condition called junctional epidermolysis bullosa, who had lost nearly two-thirds of his skin due to bacterial infections (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). A team of stem cell scientists in Italy led by Dr. Michele De Luca from the Center for Regenerative Medicine at the University of Modena and Reggio Emilia used a small biopsy of skin from the boy and introduced copies of the *LAMB3* gene via a retroviral vector, then grew cells in culture into sheets of skin. Through a series of three operations spanning 4 months, a surgical team attached the skin sheets to the boy's body. After 21 months, skin from these sheets had grown normally and replaced prior wounds, thus reconstructing ~80% of the boy's skin with healthy tissue.

Many fundamental questions about stem cells must still be answered before stem cell technologies can become viable treatment strategies. Studies that have used pluripotent stem cells of any source and introduced those into animals or patients have largely been plagued by problems. A main problem is controlling the differentiation of pluripotent stem cells into desired tissues of interest. For example, if the stem cells are injected into a tissue, say, skeletal muscle, it is difficult to control how they will respond to differentiation cues *in vivo*. As a result, such cells often develop into many

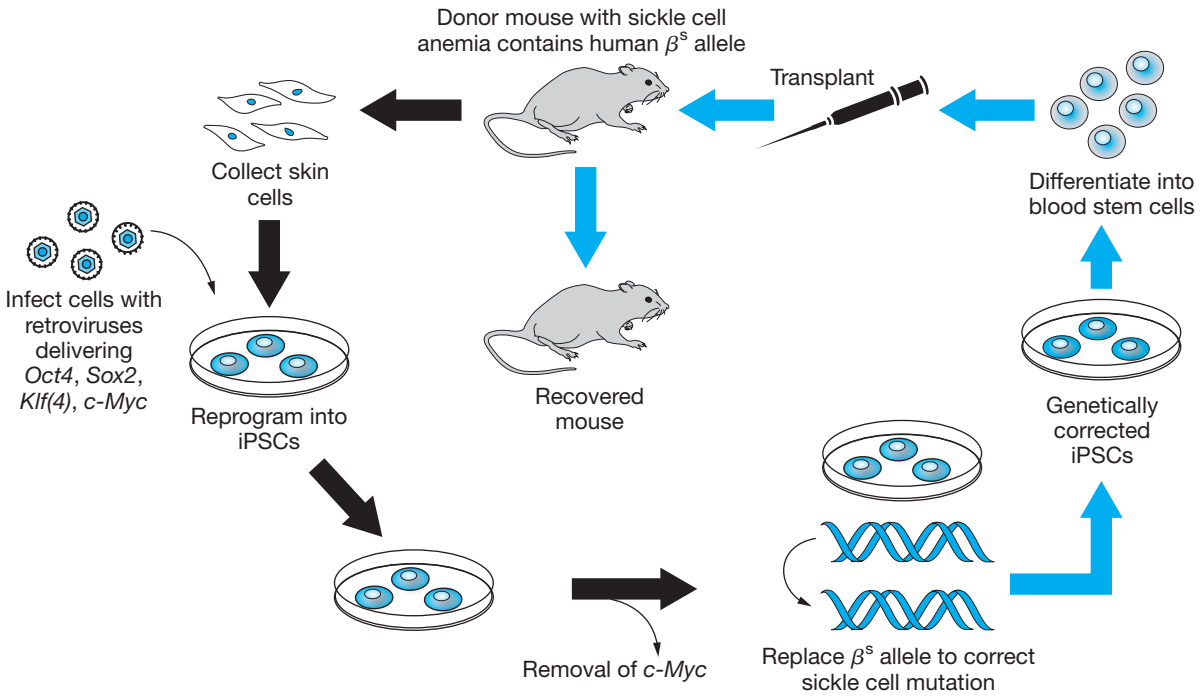


FIGURE 11.28 A Mouse Model for Correcting Sickle Cell Anemia Using iPSCs

different and undesirable cell and tissue types other than the types that were intended. Injected hESCs can form tumors, including types of tumors called *teratomas,* which contain mixtures of differentiated tissues such as teeth, bone, and hair, all in one tumor.

When stem cells instead of differentiated adult tissues are injected, scientists also cannot control the spread of the cells to other places in the body. Another problem is avoiding chromosomal abnormalities that are known to occur when stem cells differentiate. For example, alterations in chromosome number (trisomy 12, trisomy 17, and others) frequently occur when stem cells differentiate. Patients in unregulated stem cell clinics in China, Thailand, Korea, Romania, and other countries have died as a direct result of complications after having received injections of stem cells. It is important to recognize that the most effective and safe stem cell treatments in the future are likely to involve differentiating stem cells *in vitro* into desired cell and tissue types and then introducing those cells into a patient as appropriate, instead of injecting them directly.

Other important questions to be answered include:

- Why do stem cells self-renew and maintain an undifferentiated state?
- What factors trigger the division of stem cells?
- What are the growth signals (chemical, genetic, environmental) that influence the differentiation of stem cells?
- What factors affect the integration of new tissues and cells into existing organs?
- Can nuclear reprogramming of somatic cells or other approaches that do not require an embryo become reliable techniques for producing pluripotent stem cells with the properties of hESCs?
- Which diseases can be most effectively treated by stem cell technologies?
- What strategies will be most effective for delivering stem cell treatments?

Answers to these and many other questions will help scientists and physicians in their quest to develop stem cell–based applications for treating human disease.

Cloning

We have discussed different types of cloning in this book. Remember that *cloning* refers to making a copy of something—a gene, a cell, or an entire organism. Recombinant DNA technology is used for gene cloning. When bacteria or cultured cells divide in a petri dish or bioreactor, clones of cells are being produced. But clearly no aspect of cloning is as controversial as animal cloning. With the announcement of the cloning of Dolly the sheep in 1997, the world was immediately faced with the prospect that advances in biotechnology could lead to human cloning. Dolly's creation generated a great increase in public awareness and

additional discussion about cloning. In this section, we consider the scientific implications of human cloning applications.

Therapeutic Cloning and Reproductive Cloning

There are two main approaches to cloning: **reproductive cloning** and **therapeutic cloning** (Table 11.2). The intent of reproductive cloning is to create a baby. Dolly was the first of many mammals to be produced by reproductive cloning. Unlike reproductive cloning, therapeutic cloning provides stem cells that are a genetic match to a patient who requires a transplant. In therapeutic cloning, the nucleus from a patient's cell (for instance, skin cells) are injected into an enucleated egg—an egg that has had its nucleus removed—that is then stimulated to divide in culture to create an embryo (**Figure 11.29** on the next page). The embryo produced will not be used to produce a child; instead, it will be grown for several days until it reaches the blastocyst stage so it can be used to harvest stem cells. Stem cells isolated from this embryo can be grown in culture and then introduced into the donor patient (Figure 11.29).

Prior to the development of iPSCs, therapeutic cloning was seen as a potential way to provide patient-specific stem cells that could be used to treat disease without fear of immune rejection by the recipient

TABLE 11.2 Comparison of Stem Cells, Therapeutic Cloning, and Reproductive Cloning

	Embryonic Stem Cells (ESCs)	Adult Stem Cells (ASCs)	Induced Pluripotent Stem Cells (iPSCs)	Therapeutic Cloning (Somatic Cell Nuclear Transfer)	Reproductive Cloning
Final or "end" product	Undifferentiated stem cells (isolated from fetal or embryonic tissue such as an embryo at the blastocyst stage) growing in culture	Undifferentiated stem cells (isolated from adult tissue such as bone marrow cells) growing in a culture dish	Undifferentiated stem cells created from somatic cells; may be patient-specific	Undifferentiated stem cells growing in a culture dish (obtained from the person who will also serve as the recipient of these cells)	"Cloned" human
Purpose/ application	Source of stem cells for research and for treating human disease conditions such as replacing diseased or injured tissue	Source of stem cells for research and for treating human disease conditions such as replacing diseased or injured tissue	Potential source of patient-specific stem cells for studying disease and for treating human disease conditions	Source of stem cells that are genetically matched to recipient for treating human disease conditions such as replacing diseased or injured tissue	Create, duplicate, or replace a human by producing an embryo for implantation, leading to the birth of a child
Embryo required	Yes	No	No	Yes	Yes
Surrogate mother required	No	No	No	No	Yes
Human created	Depends on how one defines an embryo	No	No	Depends on how one defines an embryo	Yes
Time frame	A few weeks of growth in culture	A few weeks of growth in culture	Weeks to months	A few weeks of growth in culture	9 months, the duration of a normal biological pregnancy (after growth of the embryo in culture)

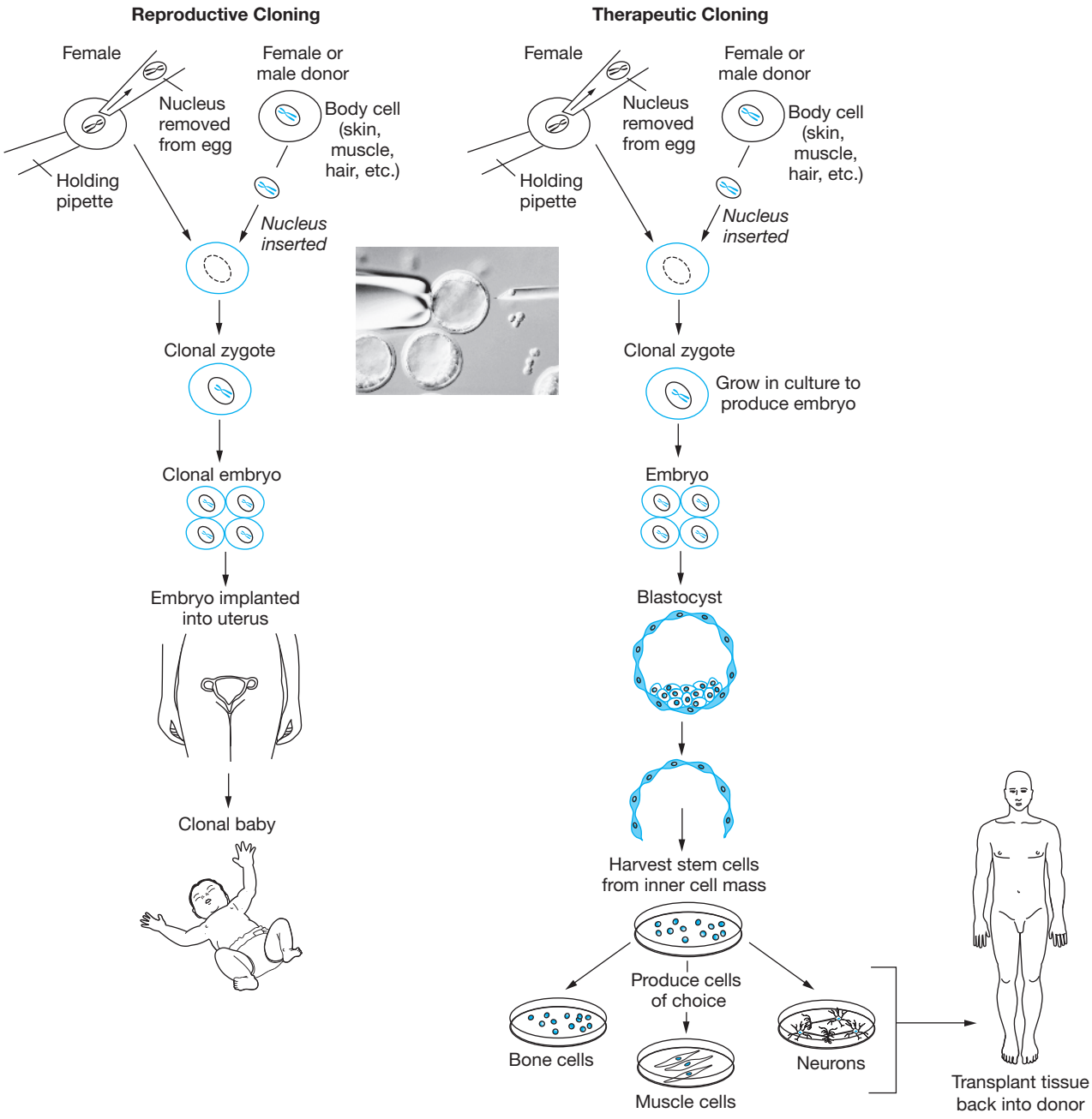


FIGURE 11.29 Reproductive Cloning and Therapeutic Cloning If reproductive cloning was carried out on humans, the goal would be to produce a cloned baby. In therapeutic cloning, stem cells that are genetically identical to the cells taken from a patient are produced to provide patient-specific stem cell therapy. The photo shows a holding pipette (left side of egg) holding an egg while the nucleus is being removed with a glass micropipette (right).

because he or she was the original source of the cells. As mentioned earlier, in theory, stem cells from a patient can also be used to create cell lines from humans with genetic diseases to provide scientists with unprecedented potential to study and learn more about human disease conditions.

Many scientists do not like the term *therapeutic cloning* because it implies creating a human clone. Creating stem cells to treat human diseases is not the same as cloning a human being. Most stem cell researchers prefer the term **somatic cell nuclear transfer (SCNT)** because *nuclear transfer* truly describes the

biological processes taking place. Recall that we discussed the details of SCNT earlier (Chapter 7). For therapeutic cloning, the blastocyst would be used as a source of stem cells and then destroyed. In 2013, at least two different research teams published papers demonstrating that they could clone human embryos and produce hESCs by SCNT. Embryos and hESCs have also been produced from people with diseases. The first example involved a patient with type 1 diabetes and insulin-producing pancreatic cells that were differentiated from hESCs, bringing scientists a step closer to treating patients with their own insulin-producing cells. It has not been proven that therapeutic cloning in humans can work as a treatment, but clinical trials are in progress.

For reproductive cloning, the blastocyst would be implanted in a woman's uterus to allow it to grow and develop to form a baby, which would take the normal 9 months. Reproductive cloning by nuclear transfer is a very inefficient process at best. In the case of Dolly the sheep, it took 277 nuclear fusion attempts to produce only one successfully implanted embryo that developed completely and formed Dolly (Chapter 7).

Many scientists think that reproductive cloning of humans is unethical, immoral, and scientifically unsafe; others think that it may be inevitable at least somewhere in the world. There have been several well-publicized announcements from a few private groups about their ongoing plans to produce humans by reproductive cloning. These claims have generated much skepticism and have been widely disproved and strongly denounced by most of the scientific community. At the time this book was printed, no definitive proof existed that a human has ever been cloned.

Regulations Governing Embryonic Stem Cell and Therapeutic Cloning in the United States

Although there are international guidelines on the ethical use of stem cells, there is currently no international policy governing stem cell use. Shortly after hESCs were first isolated in the United States, the National Bioethics Advisory Committee and the National Institutes of Health (NIH) began working on guidelines for hESCs research. In 2001, President George W. Bush announced a ban on the use of federal funds to create an embryo for the purpose of isolating ESCs. This ban did provide for the use of federal funds for research on 78 cell lines that had already been established and were available through the NIH prior to this date.

However, only 21 of these lines became available for research, and many of the lines turned out to be far less valuable than initially believed. Some failed to grow without differentiating. Others showed genetic instability with abnormalities in chromosome number. Some lines were contaminated by mouse feeder cells (which were commonly used to provide key nutrients to growing stem cells in the early days of ESC work). Many scientists and stem cell advocacy groups fought hard to lift the ban on federal funding for the creation of new hESC lines.

In 2006, a bill to lift the ban on federal funding passed the Senate but was subsequently vetoed by President Bush. Congress tried again with a bill in 2007 that was also quickly vetoed by President Bush. In 2009, President Barack Obama issued an executive order to have the ban lifted and charged the NIH to develop guidelines for governing federal funding of ESC work, but not for the use of federal funds to create embryos to derive hESCs.

Many private foundations are providing hundreds of millions of dollars for stem cell researchers, and several states have enacted legislation to create stem cell research institutes and provide state-funded support for ESC research. Some of the more active states include California, New Jersey, Connecticut, Illinois, Maryland, and Wisconsin. California in particular was a pioneer. In 2004, citizens approved a budget of nearly $300 million in bonds and over $3 billion overall to support stem cell research and create the California Institute for Regenerative Medicine (CIRM). As CIRM funding runs out in 2017, the state is faced with deciding whether another public proposition will be passed if put to a vote or whether CIRM will need to be supported primarily by private funding. Around the world, regulations vary on the production of new hESC lines and policies on therapeutic cloning. For instance, production of new lines and therapeutic cloning are legal in the United Kingdom, Israel, South Korea, China, and Singapore. Therapeutic cloning is banned in Brazil, Australia, and the European Union (although in member nations that allow it, hESCs can be derived from unused embryos from *in vitro* fertilization procedures).

Legitimate stem cell research centers have been established around the world in countries considered major powers as well as in many relatively small countries (Belgium, Sweden, Turkey, Israel, Switzerland, etc.). But as mentioned earlier, many stem cell clinics have also sprouted up around the world, and patients desperate for cures have taken to traveling to other countries seeking stem cell–based cures, which are often overhyped and promoted as successful despite baseless information and adverse side effects. Often called "stem cell tourists," patients have been known to travel around the world for unproven stem cell treatments, and the number of individuals involved in



YOU DECIDE Stem Cell and Cloning Debates

Frequently, when science and medicine produce innovative discoveries, society is not prepared for the consequences of new technology. In 1850, the development of anesthesia was considered very controversial. Many worried about unanticipated adverse reactions from anesthesia. Over 150 years later, few dispute anesthesia as important for complex surgeries and even routine procedures such as having a wisdom tooth extracted. Recall from Chapter 3 that when recombinant DNA technology was developed, there was fear and speculation about what would result from such experiments.

Not unlike the anesthesia and recombinant DNA controversies, clergy, politicians, researchers, and the general public currently debate the merits of stem cells and cloning. At the root of these debates is the source of human stem cells, in particular, hESCs and their potential uses and abuses. hESCs are controversial because of their source—the early human embryo. ESCs are perhaps the most controversial scientific issue ever debated by the public and by politicians. Knowing that hESCs may have enormous potential for treating and curing many devastating diseases and providing people with an opportunity for healthier, longer lives, what do you think about their use? The list of questions surrounding stem cells and cloning is seemingly endless.

- Is it acceptable to produce a human embryo for the sole purpose of destroying it for other uses?
- If you agree with creating human embryos for research, should there be a time limit for growing and studying these embryos before ethical concerns set in? Most Western countries, including the United States, have requirements that human embryos grown in the lab should be studied only up to 14 days after creation.
- Some fear that stem cell research will cause a great need for human eggs. Is it acceptable to pay women to collect their eggs surgically?
- What is the moral status of early embryos created by therapeutic cloning?
- What rights does a donor have to stem cell lines or technologies created from cells they have donated? Should tissue donors share in the monetary rewards of a stem cell line created from their cells?

Some people believe that a person is formed at the moment an egg is fertilized, so they consider therapeutic cloning the equivalent of killing a child deliberately for the benefit of another person. Others believe that the early embryo is a cluster of living cells with the *potential* for forming a person but that the early embryo itself is not a human being.

- Should we justify destroying embryos that are developed IVF?
- Why not use these embryos in an attempt to reduce pain and suffering in other humans?

Scientists define life in many ways. Biologists agree that the cluster of cells called the blastocyst is alive at the cellular level. Although all life forms deserve respect, the blastocyst is not a person because it does not have limbs, a nervous system, organs, or other physical features of a human individual. So a major source of debate continues about whether we should assign moral status to human embryos and, if so, at what stage.

- Does the moral value of an embryo increase as it develops or is it equal to that of a baby or adult? If an embryo is deemed a living person, then it has all the rights of other living persons. Consequently, to destroy an embryo intentionally is immoral.

Citizens must decide how their tax money will be spent and what they believe is ethical, responsible, and safe research. The scientific establishment must share its knowledge to ensure that citizens make well-informed decisions on such topics as ESCs and cloning.

Human reproductive cloning is illegal in at least 13 U.S. states and illegal in more than 40 countries, but many countries have less restrictive policies. A number of countries that banned reproductive cloning are advocating cloning for research purposes. United Kingdom, Sweden, China, and Israel have authorized the creation of human clonal embryo for medical research. Now, countries such as the United States, France, Germany, and Canada are being encouraged by the biomedical community to allow the same.

Even if therapeutic cloning is eventually approved in these countries, will its acceptance make it more likely that people will accept reproductive cloning? Probably not. Therapeutic cloning, which is intended to be used to treat illness, is a very different issue than creating a new human. If creating iPSCs turns out to be a viable way to produce stem cells, scientific and ethical debates about therapeutic

cloning and the use of hESCs may become irrelevant because stem cells could be generated without an egg or embryo.

In 2018, public opinion polls have indicated that 66 percent of the people worldwide are morally accepting of medical research using stem cells obtained from human embryos, whereas only 29 percent are still opposed to the idea. If scientists in countries that approve therapeutic cloning use the technology to research treatments for Parkinson's disease, Alzheimer's disease, and others, how will the public from other countries feel about not having access to such technologies? Some have even argued that reproductive cloning is a fundamental right of people. How would you feel if you were part of an infertile couple who could not have a biologically related child any way other than through reproductive cloning? Are there proper and ethically acceptable applications of using early embryos and their stem cells? Can the same be said for cloning? You decide.

this "tourism" continues to grow at an alarming rate. The European Union has laws similar to FDA regulations in the United States that govern stem cell usage.

The FDA has basic guidelines regarding the purity of stem cells and their applications in clinical trials and medical products. But even in the United States, FDA regulations are poorly enforced and open to interpretation, and estimates suggest that there are nearly 600 stem cell clinics operating in the United States. It is clear that better regulations must be established to govern safe applications of stem cells that comply with good manufacturing practices (cGMPs) for the production of clinical-grade cells, and to avoid the fraudulent and tragic cases of their inappropriate and unethical use. Such abuses and the resulting tragedies will only impede progress and erode confidence in the legitimate treatments being developed.

MAKING A DIFFERENCE

In 1968, about 40 years before hESCs were isolated, physicians performed the first successful bone marrow transplant. Bone marrow contains ASCs, specifically hematopoietic stem cells, which can produce all of the different cell types present in blood. Since this first transplantation, bone marrow transplantation has become routine; it is commonly used to treat a variety of blood and bone marrow diseases, blood cancers such as leukemia, blood clotting disorders, and immune disorders of the bloodstream. Many of these diseases were once thought to be incurable and, in the early days of bone marrow transplants, there were many skeptics and many challenges to be overcome. In some ways research with hESCs and iPSCs is facing some of these same challenges, and the future success of these technologies remains to be seen. In the United States alone, some 10,000 patients are treated by bone marrow transplants each year, and the success of such treatments is "making a difference" for many patients and their families.

QUESTIONS & ACTIVITIES

Answers can be found in Appendix 1.

1. Briefly describe how the Human Genome Project has led to advances in medical biotechnology.
2. Do an Internet search for the *MECP2* gene and see if you can find research papers or articles that describe research on this gene in model organisms. What human genetic conditions are associated with this gene based on research with model organisms?
3. What techniques can be used to identify genetic disease conditions in fetal or adult humans? Provide at least two examples.
4. Which of the following examples of genetic testing are prognostic tests? Which are diagnostic?
   a. Whole-genome sequence analysis (personal genomics) shows mutations associated with Parkinson's disease.
   b. ASO testing determines that an individual is a carrier for the mutant ß-globin allele ($ß^S$) found in sickle cell anemia.
   c. DNA sequencing of a breast tumor reveals mutations in the *BRCA1* gene.
   d. Genetic testing in a healthy teenager identifies an SNP correlated with Down syndrome.
5. Would you have your genome sequenced? Why or why not? If you answered yes, would you make your genome sequence publicly available? How might such information be misused? Who should have access to your sequence data?
6. In 2013, the actress Angelina Jolie elected to have prophylactic double-mastectomy surgery to prevent breast cancer based on a positive test for

mutation of the *BRCA1* gene. What are some potential positive and negative consequences of this high-profile example of acting on the results of a genetic test?

7. What is the Precision Medicine Initiative (PMI)? Describe how the PMI is expected to improve health care in the future. Visit the PMI website at **https://allofus.nih.gov/** to learn more.
8. What is pharmacogenomics? Explain how it has already affected drug treatments by describing a specific example.
9. Visit ClinicalTrials.gov and search for mAb treatments currently in clinical trials. Visit the American Society of Gene and Cell Therapy site (**http://www.asgct.org/**) to search for gene therapy clinical trials currently in progress.
10. It is hard to watch almost any TV show without seeing ads for the monoclonal antibody Humira, the most widely used and highest-selling mAb to date. What is Humira designed to treat? Do an Internet search to learn more about this mAb.
11. CAR-T treatments and other forms of immunotherapy have produced remarkable results for some patients undergoing cancer treatment. Create a diagram that details how CAR-T cells are made from a patient and how these cells are engineered to target a specific tumor.
12. What is gene therapy? Explain the differences between *ex vivo* and *in vivo* gene therapy, and give an example of a human genetic disease treated by each approach. Provide two examples of how therapeutic genes can be delivered into cells.
13. Discuss the challenges scientists face in making gene therapy an effective technique for treating human genetic disease conditions.
14. Describe two gene-silencing techniques and how they may be used for gene therapy.
15. Explain why genome editing approaches have the potential to radically change how gene therapy may be carried out in the future.
16. Why is germline editing so controversial? Explain your answers.
17. What is regenerative medicine? Describe potential ways in which tissue engineering may be used to treat disease.
18. Compare and contrast different types of stem cells, including embryonic stem cells, adult stem cells, and induced pluripotent stem cells. Include an explanation of where each type of stem cell comes from and how each type can be isolated. Give two examples of how stem cells may be used to help treat human diseases.
19. In some countries, the use of government funds for embryonic stem cell research has been highly controversial. What types of funding are utilized for carrying out experiments with hESCs? Are any of the major funding bodies funding this research? If not, then what types of resources are available to the laboratories and scientists working with hESCs? Do a search on the Internet to know more.
20. Compare and contrast therapeutic cloning and reproductive cloning by preparing a table listing the pros and cons of each technology.

Visit www.pearsonglobaleditions.com for the Companion Website

The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

This case study has been included to give you an opportunity to become familiar with a research example applicable to this field of study. After you have analyzed the data and answered the questions, you can compare them to those provided to your instructor with the text materials.

CASE STUDY

Personal Genomics Helps Elizabeth Davis Walk Again

Around age 6 years, Elizabeth Davis suddenly started walking on her toes, and then began experiencing symptoms such as leg and arm weakness, muffled voice, and knee knocking when she walked. Her toes curled under her feet, and by adulthood she was often confined to a wheelchair and unable to engage in basic everyday activities such as shopping on her own. After 30 years of misdiagnosis by traditional physicians, with no treatment, Davis enrolled in a trial at the University of North Carolina at Chapel Hill to have her exome sequenced. Sequencing revealed a mutation in the *GCH1* gene, and this diagnosis suggested Elizabeth might respond well to the drug levodopa, which elevates dopamine levels in the brain and is a common treatment for patients with Parkinson's disease. Subsequently, this treatment allowed Elizabeth to walk again.

Elizabeth's story reveals an example of how genome sequencing is offering an extraordinary ability to diagnose genetic diseases and help treat patients. To learn more about Elizabeth's story, including a video testimonial from Elizabeth, go to: **http://endeavors.unc.edu/the-cure-code/**

Questions

Answers can be found at www.pearsonglobaleditions.com

1. What is the *GCH1* gene and what do we know about the protein it encodes and its functions?
2. What genetic conditions are associated with the *GCH1* gene?
3. Consider ethical dilemmas if the exome sequencing of Elizabeth's genome revealed genes associated with other genetic conditions such as cancer or mutations for which there are no treatments.

CHAPTER TWELVE

International Biotechnology Regulations

After completing this chapter, you should be able to:

- Understand the context of international regulation of biotechnology and how this intersects with national level regulation.
- Describe the combined role of the Food and Agriculture Organization (FAO), the World Organisation for Animal Health (OIE), and the World Health Organization (WHO) in regulating biotechnology products to protect plant, animal, and human health.
- Describe how genetically modified organisms (GMOs), which might pose risks to biological diversity, are regulated.
- Explain the process of access, exchange, and benefit-sharing of plant, animal, and microbial genetic resources.
- Describe how international trade rules apply to biotechnology products and how patents are protected under international regulation.
- Describe how human rights and bioethical principles should shape biotechnology research.
- Understand the role that scientific expertise—on an individual or collaborative basis—can play in the development and implementation of international regulations.



Specialized agencies of the United Nations—such as the United Nations Educational, Scientific and Cultural Organization (UNESCO) and the Food and Agriculture Organization (FAO)—are some of the international bodies that regulate biotechnology.

The use of a mislabeled drug or consumption of a contaminated food item can cause death. Medicines and food are regulated by a country's government. These regulations have profound effects on the biotechnology industry. It can take years to test a new drug to confirm its safety and effectiveness; in the meantime, lives may be lost that the new drug might have saved. There is a cost if food must be discarded, just as there is a cost if agricultural productivity is diminished because potentially toxic herbicides are not used. Clearly, a conflict exists between ensuring safety and reducing costs. New and innovative biotechnology products in medicine and agriculture promise benefits but also pose risks. When former U.S. President Bill Clinton and former U.K. Prime Minister Tony Blair pledged to limit gene sequence patents by (among other things) sharing information gathered by researchers working on the Human Genome Project, panic spread in the biotech industry. Many applaud a restriction on the patenting of gene sequences because they argue that such patenting is morally unacceptable. At the heart of this debate is what it means for something to be "patentable." Later in this chapter, we discuss patents and how patents on biotechnology products are regulated.

FORECASTING THE FUTURE

Synthetic gene drives utilize genetic engineering technology and increase the chances of a particular trait being passed on to one's offspring from the usual 50 percent probability that results from sexual reproduction to almost 100 percent. This can be achieved by adding, deleting, or modifying genes (see **Figure 12.1**). The use of CRISPR-Cas9 as a method for developing synthetic gene drives was suggested by scientists in 2014. Gene drives are likely to be most efficient in organisms with short reproductive cycles. There is a growing interest in the use of gene drives for controlling vector mosquito populations that spread malaria and other vector-borne diseases and for combatting invasive species. The main regulations that currently apply to gene drives are the ones that apply to genetically modified organisms (GMOs). The prospective development of gene drives signals the need for suitable regulations as gene drives do not fit well within the existing regulations on the contained use of GMOs and the deliberate release of GMOs into the environment. Regulations on contained use apply to the stage in which all activities concerning GMOs are carried out in research laboratories or production facilities with adequate physical, chemical, and biological control measures to prevent their release into the environment. Such existing regulations tend to focus on the risk assessment of the organism for establishing the control measures required for an appropriate level of containment. They may not consider the nature of the transformation that could pose an additional level of risk. In contrast, regulations on deliberate release apply to the stage in which GMOs are intentionally released into the environment for field trials or similar experiments and before being marketed. These regulations tend to assume that the potential of GMOs to spread and persist in the environment is an undesirable trait, even though this is likely to be a valued feature of gene-drive organisms. So, if these organisms were to be regulated under existing regulations on deliberate release, they probably would not be cleared for use. Substantial work is needed on regulatory development and societal consultation before such technology is deployed.

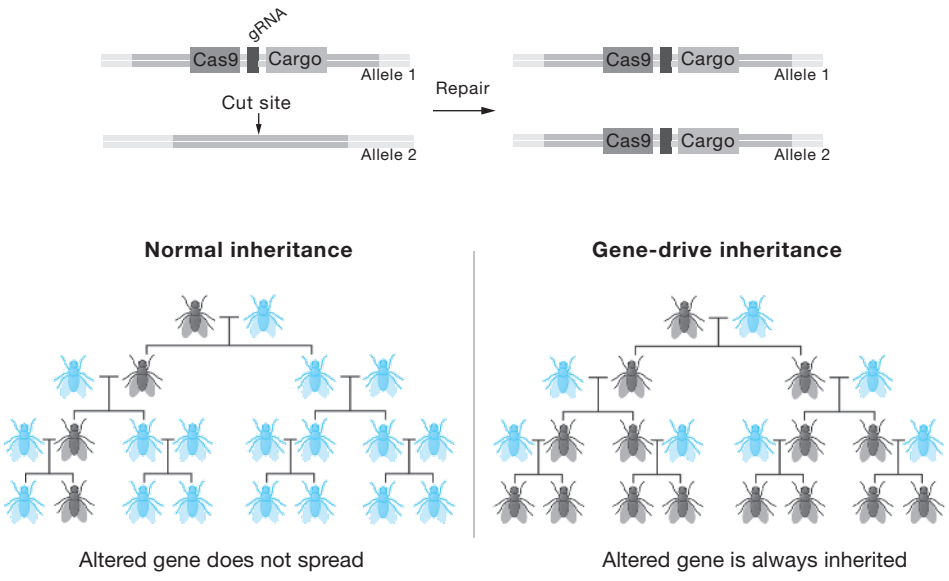


FIGURE 12.1 **An illustration of the difference between the probability of inheriting a trait through normal inheritance and gene-drive inheritance.**

12.1 Overview of International Regulations

The applications of biotechnology intersect with several areas in which the international community has long-standing common interests, such as global public health and food security. Biotechnology has the potential to contribute to the management of a range of global challenges. This includes combatting emerging infectious diseases and adapting agricultural crops to changing climate conditions. International regulations can play a facilitating role in managing such challenges. As for the risks associated with the research, development, and use of biotechnology, there is a range of international guidance relevant to the assessment, management, and communication of such risks. These regulations are supposed to serve the interests of all states (and humanity) and should facilitate capacity building in science and technology as well as in the associated regulatory bodies of developing countries to promote locally appropriate technology development and a broader distribution of technological benefits.

International regulations aim to coordinate state activities in areas where separate action by individual states may be insufficient to achieve common interests or to address common concerns. Some of these common areas are the protection of human, animal, and plant health; environmental protection; sustainable development; trade; agriculture and food systems; intellectual property rights; and arms control. In this chapter, we'll use the term "state" to refer to a country or its government.

International rules are created by states, and states retain authority over their development and implementation. This work is usually supported by an international (inter-governmental) organization that provides secretariat services for meetings, coordinates information exchange systems and expert networks, and facilitates regulatory review processes. The member states of such an international organization retain decision-making authority, which is exercised through governing bodies. The nature of the international system is such that for international rules to have a practical effect, implementation measures are generally required at the national level. International regulations should assist the harmonization of such implementation measures (by providing minimum standards, for example), but national-level regulations will often need to be consulted to understand how international rules are applied within a particular context.

The international regulations that apply to biotechnology may have been developed as part of a broader scope. For example, the **Biological Weapons Convention** or BWC (see Section 12.7) prohibits the use of biology for harmful purposes. The states party to the convention commit to providing international cooperation in the further application and development of biological sciences for only peaceful purposes. The states have affirmed that the convention applies to all relevant scientific and technological developments, so it clearly includes biotechnological advances within its scope. However, other regulations have been specifically designed to address biotechnology products, such as the Codex Alimentarius Principles and Guidelines relating to food safety assessment and modern biotechnology. New provisions have also been introduced into other regulations such as the *Terrestrial Animal Health Code* (see Section 12.2).

International regulations come in two main forms: "hard law" and "soft law" instruments. Hard law refers to international treaties and conventions that are legally binding on state parties, whereas soft law refers to international standards, guidelines, and codes for which compliance is voluntary. Hard law is not necessarily more effective than soft law. This is because: (a) most international treaties usually lack enforcement mechanisms to ensure compliance and (b) soft-law instruments are usually easier and quicker to update. This makes them relevant in areas of rapid scientific advancement. An example of this could be seen in the chapters recently added to the OIE's *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* on topics like "high-throughput sequencing, bioinformatics, and computational genomics" and "biotechnology in the diagnosis of infectious diseases," and "the application of biotechnology to the development of veterinary vaccines."

International regulations are a core tool of international policy-making, but they exist alongside a range of other governance mechanisms like surveillance and monitoring systems, clearing house mechanisms, and capacity building funds. These mechanisms support coordinated state action. Many international organizations and the rules they oversee incorporate processes for science advice or review. These can be standing or ad hoc bodies and there is considerable variability in their composition, roles, and requirements for expertise. Such processes are discussed in Section 12.8. They provide a key route for the input of expert advice in international policymaking.

There are some areas in which international regulations are not keeping pace with scientific and technological advances. In biotechnology, one of the current debates is the extent to which existing regulations cover or may need to adapt to the digitization of biology. Most existing regulations focus on the exchange or movement of biological materials between and across states.

As the use of data such as digitized sequence information becomes more prevalent, such a focus may no longer be appropriate or sufficient. Any effort to extend the scope of data regulation needs to understand the context of scientific practice so that it does not unintentionally impede global research efforts by obstructing, delaying, or substantially increasing the costs of accessing and exchanging such information.

The main international organizations that oversee regulations with relevance to biotechnology are the World Health Organization (WHO), the **World Organisation for Animal Health** (OIE), the **World Trade Organization** (WTO), the **Secretariat of the Convention on Biological Diversity** (SCBD), the **Food and Agriculture Organization** (FAO), and the **United Nations Educational, Scientific and Cultural Organization** (UNESCO) (see Table 12.1). In the United States, the **U.S. Department of Agriculture** (USDA), the **Environmental Protection Agency** (EPA), and the **U.S. Food and Drug Administration** (FDA) monitor the use of biotechnology products.

TABLE 12.1 Major International Regulations and Associated Organizations

International Regulations	Associated International Organizations
Protection of Human, Animal, and Plant Health	
International Health Regulations	WHO
Terrestrial Animal Health Code, and *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*	OIE
International Plant Protection Convention	FAO
Laboratory Biosafety Manual	WHO
Biorisk Management: Laboratory Biosecurity Guidance	WHO
Guidance on Regulations for the Safe Transport of Infectious Substances	WHO
Codex principles and guidelines on foods derived from modern biotechnology	CAC
Biodiversity Conservation	
The Convention on Biological Diversity	SCBD
Cartagena Protocol on Biosafety	SCBD
Management of Genetic Resources	
Nagoya Protocol on Access and Benefit-Sharing	SCBD
International Treaty on Plant Genetic Resources for Food and Agriculture	FAO
Pandemic Influenza Preparedness Framework	WHO
Trade and Intellectual Property Rights	
Agreement on Technical Barriers to Trade	WTO
Agreement on the Application of Sanitary and Phytosanitary Measures	WTO
Agreement on Trade-Related Aspects of Intellectual Property Rights	WTO
Convention for the Protection of New Varieties of Plants	Union for the Protection of New Varieties of Plants
Human Rights	
Universal Declaration on the Human Genome and Human Rights	UNESCO
International Declaration on Human Genetic Data	UNESCO
Universal Declaration on Bioethics and Human Rights	UNESCO
Biological Weapons Convention	

"International regulations" is used here to describe the rules agreed between states at the international level. These are open to any state to subscribe to regardless of their geographical or economic status. The discussion in this chapter does not extend to regional-level rules (such as those of the European Union) nor to bilateral agreements between states or national-level rules. Visit the Companion Website to know about drug approval and marketing authorization processes in the United States and the European Union.

12.2 Protection of Human, Animal, and Plant Health

The three main international organizations that address human, animal, and plant health are WHO, the OIE, and FAO. They oversee a range of regulations, draw on extensive networks of expertise, and have created many joint initiatives. Of their work, there are three areas of particular relevance to biotechnology:

- disease control, surveillance, and response,
- safe handling, transfer, and use of biological materials, and
- food safety.

Biotechnology for Disease Control, Surveillance, and Response

Biotechnology strengthens capabilities for disease control, surveillance, and response by developing improved diagnostic tools, novel vaccines, and treatments.



FIGURE 12.2 The OIE Protects Animal Health The OIE strives to promote good governance and quality of veterinary services.

The international organizations responsible for the protection of health bring together collaborating laboratory networks that contribute to surveillance and response. These are WHO collaborating centers and expert advisory committees, the OIE collaborating centers and reference laboratories, WHO's Global Outbreak Alert and Response Network and **GISRS**, the OIE's World Animal Health Information System, FAO's Emergency Prevention and Response Systems, and the joint FAO and OIE network of expertise on animal influenza (OFFLU). These systems often collaborate with external companies because they do not have the internal capacity to manufacture vaccines and treatments. WHO has been working to enhance the relationship among states, its expert networks, and industry, after concerns were identified about inequity in access to vaccines and treatments during international public health emergencies (see the Pandemic Influenza Preparedness Framework in Section 12.4).

Safe Handling, Transfer, and Use of Biological Materials

Both WHO and the OIE provide guidance on the safe handling, transfer, and use of biological materials like clinical samples and vaccine strains, particularly those that are or could be pathogenic. They aim to protect the people working in laboratory facilities, the wider public and animal health, and the environment. The guidance extends to security and safety considerations and can be found in WHO's *Laboratory Biosafety Manual*, *Biorisk Management: Laboratory Biosecurity Guidance*, *Guidance on Regulations for the Transport of Infectious Substances*, and in the OIE's *Terrestrial Animal Health Code* and *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. The guidance is broadly applicable to any laboratory work involving biological materials. Before undertaking work in a laboratory, a risk analysis should be conducted. This risk analysis should include assessment of the organism to be worked on, available equipment and facilities, local practices and procedures, and conditions in the surrounding environment (such as the presence or absence of disease vectors). The outcome of this analysis will indicate the requirements—for containment measures, operating practices, etc.—applicable to the work.

Chapter 16 of WHO's *Laboratory Biosafety Manual* provides guidance tailored specifically to work with recombinant DNA in laboratory facilities. It also includes guidance on the safety assessment of biological expression systems, expression vectors, viral vectors, transgenic and knock-out animals, transgenic plants, and genetically modified organisms.



YOU DECIDE

How Should We Regulate Biotechnology beyond Traditional Settings?

As biotechnology tools and techniques become more widely accessible, opportunities are opening for biology to move from traditional laboratory settings (such as those in public and private research institutions, universities, and clinical facilities) into community spaces such as DIY biology facilities. Existing regulation is designed around traditional institutional structures and workplaces and may not be well suited to these new spaces. So, how might appropriate management of biotechnology be promoted regardless of the operating space?

The International Genetically Engineered Machine (iGEM) competition provides an example of how this can be addressed. This is a student competition where students form teams and apply synthetic biology parts and techniques to real-world challenges. The teams are supposed to adhere to the safety rules of their labs and the biosafety standards of their countries; the iGEM safety program also provides safety guidelines. This is done to ensure that biotechnology experiments outside of traditional spaces also follow a set of safety practices that are similar to national and international-level regulations. This means that from an early stage of their careers, competitors develop not only their scientific and technical skills and knowledge but also a practical understanding of responsible practice.

This is a different approach to the regulation of biotechnology as compared to the regulations set by governments, which have been discussed in the rest of this chapter. What advantages might such community-developed standards, norms, and codes of practice have? Should more biotechnology regulations look like this in the future? You decide.

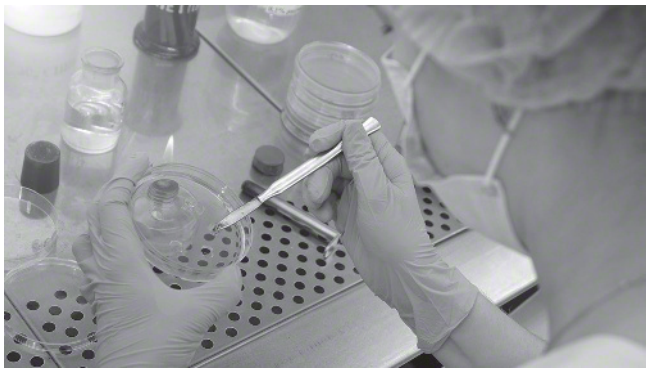


FIGURE 12.3 Laboratories Must Comply with Biosafety and Biosecurity Regulations WHO's *Laboratory Biosafety Manual* is a helpful reference for people working in laboratory facilities.

Food Safety

The food that we eat should be safe. The **Codex Alimentarius Commission** (CAC), a joint body of FAO and WHO, was founded in 1961 to promote harmonization of food safety standards internationally for the protection of consumers and promotion of fair practices in the food trade. The OIE also plays a collaborative role in relation to food safety in animal production.

In 2003, CAC adopted a set of principles that focus on the risk analysis of foods derived from modern biotechnology. It also adopted two standards for the conduct of safety assessment of foods produced using recombinant-DNA microorganisms and foods derived from recombinant-DNA plants. It followed these up in 2008 with a standard for the safety assessment of foods derived from recombinant-DNA animals and in 2010 with a document called *Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Food.* According to these guidelines, a risk analysis should be conducted before a product is marketed. This process involves comparing the GM food to a conventional counterpart, i.e., traditional parental variety from which the GM food was derived and important commercial variants of the parental food. This is done to identify any new or altered hazard to human health or nutrition. Additionally, the analysis includes an assessment based on description and characterization of the donor, host and recipient organisms, and elements and changes introduced in the food. The CAC standards also contain general advice on avoiding the transfer of genes from allergenic foods and discourages the use of techniques that result in antibiotic-resistance genes being present in the food product.

Notably, while the standards of the CAC and the OIE are voluntary in nature, member states are encouraged in the WTO's Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) to use these international standards as a basis for measures to protect human, animal, and plant health while avoiding unjustified trade restrictions (see Section 12.5). The SPS Agreement is legally binding.

12.3 Biodiversity Conservation and the Cartagena Protocol on Biosafety

The Convention on Biological Diversity (CBD) was adopted in 1992 at the United Nations Conference on Environment and Development. This international legally binding treaty aims to promote the conservation of biodiversity and sustainable use of its components as well as equitable benefit-sharing from the use of genetic resources.

The CBD focuses on two aspects of biotechnology in relation to its objectives. The first aspect is recognizing the potential benefits of biotechnology for the conservation and sustainable use of biodiversity. The convention promotes technology transfer and collaborative research efforts that utilize biotechnology for such purposes. The second aspect is the potential risks to biodiversity and the environment that might be posed by the release of GMOs. The convention requires the states to have the ability to manage such risks. It also suggests that states ought to collectively consider developing procedures for the safe handling, transfer, and use of such organisms. To ensure this, the Cartagena Protocol on Biosafety was adopted in 2000.

The Cartagena Protocol is a legal agreement that forms an addition to the requirements of the CBD. It applies to transboundary movements (or movements across international borders) of GMOs—also referred to as **living modified organisms** or LMOs in the Protocol text—that might present risks to biological diversity.

The protocol applies to the first movement of any specific LMO into a country for release into its environment. Such transboundary movements are subject to an Advanced Informed Agreement procedure, involving a process for informed consent by the importing state. This process requires that the exporter notify the importing state about the proposed transboundary movement in advance and not proceed unless consent is obtained. The decision about allowing or denying the transboundary movement should be based on an assessment of the risks to the conservation and sustainable use of biodiversity. This decision may also take into account the risks to human health. The exporter may be required to bear the costs of conducting this risk assessment.

The protocol does not apply to LMOs covered by other international regulations or organizations or LMOs that are pharmaceuticals for humans. The AIA procedure does not apply to LMOs in transit or intended for contained use in the importing country. There is a separate procedure for LMOs for direct use in food, feed, or processing as stated in Article 11 of the Cartagena Protocol. Simplified procedures may be adopted by exempting some LMOs from AIA or importing an LMO at the same time when its transboundary movement is notified to the importing country. The **Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress** was adopted in 2010, adding further provisions in those areas.

Most states have become parties to the CBD; the United States is a notable exception, and because it is not a party to the CBD, it cannot be a state party to its protocols.

12.4 Management of Genetic Resources

Genetic resources—defined as genetic material of actual or potential value—are a key input for a range of biotechnology research and development. Genetic material may be of plant, animal, or microbial origin. Biotechnology products and processes may constitute genetic material and would, therefore, be considered genetic resources.

International rules on genetic resources, which mainly address access, exchange, and benefit-sharing, were developed with an initial focus on plant genetic resources and the historic patterns of exchange associated with them. All countries depend on regular exchange of plant genetic resources (in the form of seeds or similar materials) in order to sustain their agricultural productivity. Historically, and particularly during colonial times, a few wealthy countries accessed vast amounts of plant genetic resources from around the globe, adapted, and exploited them without returning these resources or sharing the benefits (in monetary form or through improved varieties) with the countries of origin. Likewise, major agricultural companies were able to access, adapt, and commercialize such resources in the absence of any benefit-sharing requirements. International systems for the exchange of seeds were established in the 1950s, and rules promoting fair benefit-sharing in exchange for facilitated access to plant genetic resources have been under development since the 1980s.

With advances in biotechnology, plant and other forms of genetic resources have become more valuable for a range of research and development purposes and are used in a number of products and industrial processes such as in the production of dyes and feed and food additives, and in areas like mining and waste processing. International approaches to access and benefit-sharing have expanded to cover most types of genetic

resources (including marine, aquatic, livestock, forestry, insect, and microbial resources) but do not extend to human genetic resources.

In the 1980s, attempts were made to frame genetic resources as a "common heritage of mankind," an international legal approach to the management of certain resources emphasizing that they belong to all of humanity. However, a common heritage approach to genetic resources was not established largely due to a controversy over whether it should be applied to **worked genetic resources** as well as **raw genetic resources**. Instead, since 1992, the dominant international approach to the regulation of genetic resources has been based on "state sovereign rights."

State Sovereign Rights

State sovereign rights generally apply to natural resources—such as timber and minerals—within the territorial jurisdiction of a state and have been established in international law since the 1960s. These rights were not extended to genetic resources for a long time. In 1992, the CBD recognized the sovereign rights of states over their natural resources and that the authority to determine access to genetic resources, therefore, lies with national governments. The convention also says that access to genetic resources should be subject to the **prior informed consent** of the provider state, under mutually agreed terms, including benefit-sharing provisions. This is generally done in the form of a material transfer agreement between the provider state and the individual or institution accessing the resource. The Nagoya Protocol adds further detail to this by covering the legislative measures to be applied by provider states and providing examples of monetary (royalty payments, research funding, etc.) and non-monetary forms (sharing research results, participation in product development, etc.) of benefit-sharing.

Exercise of state sovereign rights to manage access to genetic resources does not generally mean that Intellectual Property Rights (IPRs) cannot be claimed on such resources. Although, it is necessary that the work satisfies the general criteria for the awarding of such rights (see Section 12.5). Co-ownership of IPRs or sharing of license fees may be one form of benefit-sharing.

The Nagoya Protocol

In 2010, the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity was adopted in Nagoya, Japan. The "utilization" includes research and development of the genetic and biochemical composition of genetic resources through the application of biotechnology (Article 2). The protocol requires following the CBD's provision of prior informed consent and mutually agreed terms of access.

The system of prior informed consent may sometimes involve lengthy negotiating periods, which has led to concerns that the protocol's applicability to pathogenic genetic resources and similar resources could be damaging to global research efforts. There could be obstructions to the timely sharing of such resources during a public health emergency. The current discussions about whether the scope of the protocol should be extended to include digital sequence information have heightened these concerns, which also extend to other areas of biotechnology research that address global challenges.

International Treaty on Plant Genetic Resources for Food and Agriculture

In 1983, FAO adopted the International Undertaking for Plant Genetic Resources in Food and Agriculture, which was based on the principle that plant genetic resources are a common heritage of mankind. However, this did not gain broad acceptance from states. With the adoption of the CBD in 1992 and its recognition of state sovereign rights over such resources, FAO was asked to revise the undertaking in line with the convention's provisions, and in 2001 the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGR) was adopted. The ITPGR established a Multilateral System for Access and Benefit-Sharing, which covers 64 food and forage crops—based on the criteria of interdependence and importance to food security—and places them in a global pool of genetic resources, designed to be easily accessible to users from its member states.

The treaty facilitates access to the system's resources for utilization and conservation for research, breeding, and training for food and agriculture only. Any benefits from the use of the resources are supposed to be shared fairly and equitably through information exchange, technology transfer, or profit-sharing. The treaty also states that any IPRs claimed on products derived from the resources should not restrict access to them in their original form in the system.

While the treaty only covers plant genetic resources, FAO's Commission on Genetic Resources for Food and Agriculture oversees regular global assessments of the status of the plant, animal, aquatic, forest, micro-organism and invertebrate genetic resources relevant to food and agriculture. It also provides additional standards and guidance on the collection, storage, and characterization of resources and provides a forum for international policy discussions.

The Pandemic Influenza Preparedness Framework

Rapid sharing of viral and other pathogenic genetic resources with international surveillance systems is an essential part of timely and effective responses to disease outbreaks. The sharing of viral samples with WHO's Global Influenza Surveillance Network (now the **Global Influenza Surveillance and Response System** or GISRS, a network of over 150 public laboratories located around the world) was challenged in 2006–07 during an avian influenza outbreak, which was linked to more than 350 human cases and 180 deaths. Indonesia halted its sharing of viral samples at various points during this period. The country stated concerns that the vaccines being manufactured from these samples would subsequently be unaffordable to its population.

WHO and its member states responded with efforts to rebuild trust in the system. This included the development of an **Influenza Virus Traceability Mechanism** in 2010, which enables tracking of samples into, around, and out of the GISRS, and adoption of the Pandemic Influenza Preparedness Framework (PIP Framework) in 2011. The framework acknowledges state sovereign rights over biological resources and creates systems for centralizing access and benefit-sharing for genetic resources, in the context of influenza viruses of human pandemic potential.

The centralized access system uses two forms of standardized agreements to manage the transfer of viral genetic resources. The first is for transfers within the GISRS, and the second is for any transfer from the GISRS to external groups such as biotechnology and pharmaceutical companies, vaccine and diagnostics manufacturers, and other research institutions. The benefit-sharing system incorporates a centralized stockpile of vaccines. These vaccines are to be distributed on the basis of public health risk and need in the early stages of an outbreak and on a per capita basis to those countries that are otherwise unable to access vaccines during a pandemic. Further, benefit-sharing occurs through "Partnership Contributions" where companies that work with the GISRS provide support equivalent to half of its running costs.



FIGURE 12.4 The PIP Framework Promotes Equitable Access to Vaccines in the Case of Pandemic Influenza Outbreaks

12.5 Trade and Intellectual Property Rights

Many biotechnology products are made available through global markets and are subject to international trade rules. These rules—mostly overseen by the WTO—are designed to facilitate international trade. One way of achieving this is by limiting barriers, such as import tariffs, in order to facilitate the free movement of goods. If there are limits to be placed on imported goods to ensure comparable quality or for consumer protection, international rules support fairness in their application. By harmonizing standards in these areas, such rules also promote predictability in trade, which means that an exporter can expect a basic level of consistency in the rules applied by different countries.

Trade in Biotechnology Products

The two agreements of the WTO that have relevance to the import and export of certain biotechnology products are the Agreement on Technical Barriers to Trade (TBT Agreement) and the Agreement on Application of Sanitary and Phytosanitary Measures (SPS Agreement). These are both legally binding on all member states of the WTO and are backed up by an enforcement mechanism that can include trade sanctions where there is noncompliance. Trade sanctions generally refer to measures—often imposed for political or economic reasons—that restrict exports to and imports from a country or set of countries. They can, for example, include bans on the export of arms to certain governments. In the context of trade disputes in the WTO, sanctions are directed towards countries found to be breaching the WTO rules and should be equivalent to the costs their noncompliance imposes on others.

The TBT Agreement covers the technical regulations and quality standards applied to any product in international trade. Such standards are limited to those that are necessary and scientifically justified. Applying different labeling standards or technical standards to imported products instead of the ones applied to domestic products can lead to the violation of this agreement. An example of this is when Mexico complained to the WTO's

Dispute Settlement Body that the U.S. rules regarding "dolphin-safe" labeling on canned tuna products are in disjunction with international laws, and they disqualify Mexican tuna products from carrying the labels. The label has important commercial value as the consumers and retailers of the U.S. market would prefer buying labeled tuna products. Denying Mexico the label puts Mexican tuna products at a disadvantage in the U.S. market. The United States was requested to bring its measures into compliance with international rules. When that was not done, Mexico was authorized to suspend trade concessions on U.S. goods up to the amount of $163 million per annum, equivalent to the trade losses incurred by Mexico because of the labeling requirements.

The TBT Agreement covers all technical regulations and standards except when these are sanitary (human and animal health) or phytosanitary measures (plant health) as defined by the SPS Agreement. The SPS Agreement aims to avoid unjustified trade barriers being created under the guise of protecting health. It is not expected that all states will have identical standards for addressing health concerns in trade, and the agreement allows for the recognition of the equivalence of different measures that achieve the same level of protection. Where they exist, relevant international standards should be used as the basis for national measures and any measures that conform to international standards will be considered compliant with the agreement. The agreement refers to three international bodies whose standards may be particularly relevant in this context: the OIE, FAO (particularly the standards under the International Plant Protection Convention), and the Codex Alimentarius Commission.

Intellectual Property Rights

Intellectual property is a unique creation of human intelligence and Intellectual Property Rights (IPRs) are assigned to the creators of such products. **Patents** are a form of IPRs that gives an inventor or researcher exclusive rights to prevent third parties from making, using, or selling the patented product, using the patented process, or the product obtained directly from that process without the patent owner's consent. The **World Intellectual Property Organization (WIPO)** has adopted two treaties that aim to facilitate applications for patents in more than one country: the Patent Cooperation Treaty and the Patent Law Treaty.

The WTO oversees the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS Agreement), which came into effect on January 1, 1995. As the basis for international harmonization, the agreement provides the minimum standards of protection with respect to the main areas of IPRs such as copyright, trademarks, patents, etc. According to the agreement, an invention must meet three basic requirements to be granted a patent: it must be novel, it must involve an inventive step, and it should be capable of industrial application. Patent applicants are required to disclose the invention in a clear and concise manner so that any person skilled in the art can easily carry it out. The protection lasts for at least 20 years from the filing of an application. During this period, third parties must seek consent to use the process or make, use, or sell the product obtained directly from the process. Where an invention involves a microorganism, tissue culture, or plasmid, a sample may need to be made available to fulfill the disclosure requirement. The Budapest Treaty on the Deposit of Microorganisms for the Purpose of Patent Procedure, overseen by the WIPO, established a system in which deposit of such samples can be made at an International Depositary Authority and recognized for patent procedures.

There have been concerns that the patent rules under the TRIPS Agreement restrict access to essential medicines for populations in developing countries. Some attempts have been made to remedy this through the adoption of the **Doha Ministerial Declaration on the TRIPS Agreement and Public Health** in 2001 and through the creation of a system referred to as the **Paragraph 6 Mechanism**. If one country lacks domestic manufacturing capacity, this mechanism allows the manufacturing of pharmaceuticals in a different country under compulsory licensing.

The application of the TRIPS Agreement to genetic resources has also proven contentious in two main ways. Some countries object on moral grounds to the application of IPRs to lifeforms and associated genetic resources, believing that such resources should not be subject to private appropriation. Then there are other countries who accept that patents may be granted for genetic resources but argue that the origin of the genetic resource and any traditional knowledge relevant to the invention should be disclosed in patent applications. These issues are being discussed within the review of Article 27.3(b) of the TRIPS Agreement, which covers patentability or non-patentability of plant and animal inventions. The WIPO's Intergovernmental Committee on Intellectual Property, Genetic Resources, Traditional Knowledge and Folklore is also working to develop international legal instruments to address some of these issues.

The relationship between IPRs and development is also being addressed. The WIPO has a development agenda that guides some of its work, and it also developed the Doha Development Agenda in 2001. The WTO also participates in the Standards and Trade Development Facility that provides funds to support capacity building for implementation of standards for food safety and animal and plant health in developing countries.



TOOLS OF THE TRADE **What Is the Patent Cooperation Treaty System?**

While the TRIPS Agreement sets out the minimum international standards for IPRs, it does not remove the need to apply separately for a patent in each individual country where the inventor wants patent protection. To simplify the process and reduce the costs for these inventors, the WIPO adopted the Patent Cooperation Treaty (PCT) that established a system to address this. It involves a "unified procedure" to handle the filing of the patent application. When submitted by the applicant in one member state, the filing occurs simultaneously in other member states that the applicant designates. Using the PCT system, a single "international" application can be filed. The states that are parties to this treaty constitute the International Patent Cooperation Union, which cooperates in the filing, searching, and examining of the patent applications. WIPO has also created an International Search Authority that conducts checks for prior art or any information that provides evidence of the invention being publicly known prior to the date of a patent application. Decisions on whether to grant a patent still take place on a national basis but the use of this system can save applicants substantial time and effort. Over 235,000 filings were made through the system in 2018.

Plant Variety Rights

Intellectual property protection for new varieties of plants can be sought through the patent system or through "sui generis" i.e., more specialized systems. At the international level, the alternative system to patents is plant variety rights (PVR), rights given to the breeder of a new variety of plant. These are governed by the International Convention for the Protection of New Varieties of Plants. The criteria for being awarded a plant variety right require the variety to be new, distinct, uniform, and stable. Once granted the right, the right holder's authorization is required for acts of production, reproduction, conditioning for propagation, offering for sale, selling, marketing, exporting, importing, or stocking of the variety. Authorization is not required for private, non-commercial, or experimental uses of the plant variety or use of the material to breed other new varieties. These rights extend for at least 20 years.

12.6 Human Rights

New biotechnological developments have opened the human genome and human genetic data to a range of uses; many of these are proving to be beneficial, such as improved understanding of diseases and identification of new ways to treat them. However, this new knowledge and data may be used for purposes that breach human rights. Employers or healthcare providers may use this data to discriminate against ethnic groups or individuals on genetic grounds. An example of such discrimination would be the refusal to grant health insurance to someone with a genetic predisposition to cancer or heart disease. Risks like these motivated the United Nations Educational, Scientific and Cultural Organization (UNESCO) to develop three declarations that outline how human rights and bioethical principles should shape biotechnology research and applications involving human genome and related data:

- The Universal Declaration on the Human Genome and Human Rights
- The International Declaration on Human Genetic Data
- The Universal Declaration on Bioethics and Human Rights

Such declarations of principles can indicate a level of consensus among states about how certain issues should be framed and addressed, but, unlike standards and guidelines, tend not to have substantive technical provisions. These declarations are not legally binding. Among other topics, they address avoidance of genetic discrimination or stigmatization; the purposes of research that utilizes the human genome and human genetic data; confidentiality of genetic data; collection, processing, storage, and use of data and samples; and respect for privacy, equity, and justice.

Indigenous people have often played a key role in the development and cultivation of genetic resources and hold traditional knowledge relevant to their uses. The CBD and its Protocols and the WIPO Intergovernmental Committee recognize these people as key stakeholders in the access to and management of genetic resources and associated knowledge. This is done by enabling their representation in the prior informed consent and benefit-sharing



YOU DECIDE **Should There Be a Global Ban on Human Genome Editing?**

In November 2018, Professor He Jiankui announced the birth of the world's first gene-edited babies. He had reportedly edited the embryos of the twins to protect them from HIV infection (see Chapter 13). The announcement was widely condemned by scientific and policy communities, and there have been calls for a global ban, or at least a moratorium, on human (germline) genome editing. A moratorium could give the opportunity for the thorough societal discussion about necessary technical, medical, ethical, and other aspects of human genome editing before establishing an international framework.

But achieving global consensus on regulations addressing human genetic technologies has been extremely difficult in the past. For example, in the early 2000s, France and Germany proposed that the United Nations create an international treaty banning human cloning. Although there was a consensus that reproductive human cloning should be banned, establishing a treaty proved impossible. This was due to differences of opinion about whether therapeutic human cloning should be included in the ban. Therapeutic human cloning can be used to produce embryonic stem cells, and states had divergent opinions on the ethics of medical research using such stem cells, generally based on the moral status accorded to human embryos.

A similar problem may occur for attempts to achieve a ban on human genome editing. Divergent perspectives on the morality of human-genome editing are likely to make reaching a consensus extremely difficult. For some, a moratorium would make sense, until its use in humans is proven safe. After that stage, with fuller knowledge of potential risks and benefits, they believe it would be justifiable to use such techniques to cure serious hereditary diseases and so on. For others, who view any instance of human germline editing as ethically unjustifiable, a full and permanent ban is preferred.

Is this the right stage in the development of the technology to move to a global ban? What might the advantages and disadvantages be of regulating human genome editing at the national level instead? You decide.

processes. There are also two international declarations that cover the rights of indigenous people as stakeholders: the United Nations Declaration on the Rights of Indigenous Peoples and the Indigenous and Tribal Peoples Convention overseen by the International Labor Organization.

12.7 Preventing the Hostile Use of Biotechnology

There is a long-standing international norm and prohibition against the use of biology for non-peaceful purposes. This extends to all scientific and technological developments in the life sciences and other relevant fields. The main international treaty addressing this is the BWC, adopted in 1975. In addition to the prohibition on misuse, the BWC promotes international cooperation in scientific research and development and technology transfer for peaceful purposes. It also provides a mechanism for addressing suspected noncompliance and for assistance in the event of a biological warfare attack.

UN Resolution 1540 was adopted in 2004 to prevent the proliferation of biological weapons, as well as chemical, radiological, and nuclear weapons, from state to non-state actors. This has since been followed by a series of resolutions that sustain its provisions. The Australia Group, an informal organization established in 1985, provides a mechanism for its 43 member states for controlling exports related to chemical and biological agents and dual-use items (that can be used for both peaceful and non-peaceful purposes), and associated equipment.

To strengthen and maintain the international norm against the misuse of biology, states have placed increasing emphasis on fostering education and creating awareness regarding safety, security, and ethical responsibilities in the scientific community. Such initiatives are regularly promoted by states that are parties to the BWC and by WHO through guidance documents. An example of this would be the *Responsible Life Sciences Research for Global Health Security*, published in 2010 by WHO. Many science academies are also playing a role in developing these initiatives and promoting awareness among their members.

12.8 Role of Scientists in the Development of International Regulations

International regulations need to stay relevant to ever-changing scientific practices and broader technological contexts. But sometimes the regulations can struggle to adapt in a timely manner, particularly in areas of rapid scientific and technological advancement. Scientific and other expert communities have a role to play in the development and implementation of international policies and regulations that can subsequently have significant implications for their work.

This often happens through intermediary organizations, such as science academies or science funders, but can also be done through individual involvement in the provision of expert advice. The InterAcademy Partnership is a global network that brings together more than 130 national and regional member academies. It produced a report for the 2016 Review Conference of the Biological Weapons Convention and continues to bring together people with expertise in **Dual Use Research of Concern** and the security implications of scientific advances. The Wellcome Trust is another independent foundation that funds scientists from all over the world. The Trust along with other science funders in the United Kingdom provided inputs in a government consultation on how to implement the Nagoya Protocol. Individual experts participate in online discussions about the interactions between synthetic biology and the objectives of the CBD.

Some international organizations and treaties have specific processes or mechanisms for scientific advice and review. These include the Specialist Commissions of the OIE, the Subsidiary Body on Scientific, Technical and Technological Advice of the CBD, and the Roster of Experts drawn on by WHO Secretary-General during public health emergencies. The OIE and WHO also have substantial collaborating networks of laboratories and other research centers that support their work.

MAKING A DIFFERENCE

Alongside the pursuit of a scientific career in biotechnology in the public or private sector, it is possible to play a role in shaping policies and regulations that apply to the field. You may do this as an individual expert by serving on an expert advisory group for an international organization or by participating in policy consultations. International regulations are developed through negotiations involving national governmental representatives, so there will also be opportunities to provide advice to national governments on their negotiating positions. You may also play a role in shaping policy through memberships of scientific associations or industry organizations. Many national organizations have collective bodies providing international representation. For example, the InterAcademy Partnership represents scientific, engineering, and medical academies on a range of international issues including biotechnology and biosecurity (www.interacademies.org). Early career scientists can find similar representation through the Global Young Academy (globalyoungacademy.net). There is also the Biotechnology Innovation Organization (www.bio.org/about), which brings together biotechnology companies and others working on biotechnology research and development across more than 30 nations.

QUESTIONS & ACTIVITIES

Answers can be found at Appendix 1.

1. What are some of the ways in which international regulations serve the function of coordinating state action?
2. How has the management of genetic resources improved resource sharing among states?
3. The prohibition of chemical and biological weapons under the BWC relates to the intent—whether it is peaceful or non-peaceful. It does not ban the research, development, production, stockpiling, and use of biological agents and toxins outright. Why is such an approach important?
4. Some components of international regulations seek to limit or constrain certain activities; other components play a facilitative role in the application of biotechnology. Can you identify examples of both?
5. What does the ITPGR cover?
6. How did the PIP Framework restore the trust of developing countries in the sharing of viral samples?
7. Can you think of how the definition of "utilization" of a genetic resource in the Nagoya Protocol might be interpreted in different ways?
8. State sovereign rights are generally applied to resources within a state's territorial jurisdiction. Why might this make them difficult to apply to some forms of genetic resources?

9. Why did the WTO's Dispute Settlement Body authorize Mexico to suspend trade concessions on U.S. goods?
10. How did the WTO remedy the problem of the TRIPS Agreement restricting access to affordable medicines for populations in developing countries?
11. How does the PCT system ensure that an inventor can seek patent protection in a large number of countries?
12. How are the rights of indigenous peoples possessing knowledge about valuable genetic resources protected by the Nagoya Protocol?
13. The member states of an international organization exercise decision-making authority through governing bodies. Go to the web address **www.who.int/about/governance/world-health-assembly** and discuss some of the functions of the World Health Assembly, a governing body.
14. Go to the web address **www.who.int/features/qa/one-health/en/** and discuss the value of "One Health" approach in the context of biotechnology regulations.
15. Why is it important for scientists (as individuals or collectively) to be engaged in the development and implementation of international regulation?
16. WHO's *Laboratory Biosecurity Guidance* applies to "valuable biological material" and not just to pathogenic organisms that have generally been associated with the need for biosafety and biosecurity measures. What are some of the other forms of valuable biological material you might come across in your work?
17. Go to the web address **www.unog.ch/80256EE600585943/(httpPages)/E292144E188C41A0C1257B98004A04A0?OpenDocument** and pick a couple of examples from the listed recent developments in science and technology and summarize their implications for Biological Weapon Convention's objectives.
18. Go to the web address **www.oie.int/en/scientific-expertise/biological-threat-reduction/** and explain some of the common approaches that can be used to counter natural disease threats and also to fight deliberate threats like bioterrorism.
19. Go to the web address **www.who.int/csr/resources/publications/HSE_GAR_BDP_2010_2/en/** and explain the value of a guidance document like the Responsible Life Sciences Research for Global Health Security.
20. Go to the web address **bch.cbd.int/synbio/open-ended/discussion/** and look at the discussions on synthetic biology in the CBD's open-ended online forum. Do you think this is a useful mechanism for engaging scientific expertise?

Visit www.pearsonglobaleditions.com for the Companion Website

The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

CASE STUDY

How Should the Safe Transport of Infectious Substances Be Ensured?

In February 2019, Gang Li of Guelph, Ontario was fined $20,000 by a Canadian court for breaching animal health regulations. He had transported undeclared samples of four animal viruses in his checked baggage and had no permits or documentation for them. See **www.inspection.gc.ca/about-the-cfia/newsroom/prosecution-bulletins/2019-02-20/eng/1550673296677/1550673298754**.

Consult the *Guidance on Regulations for the Safe Transport of Infectious Substances* published by WHO to answer the following questions:

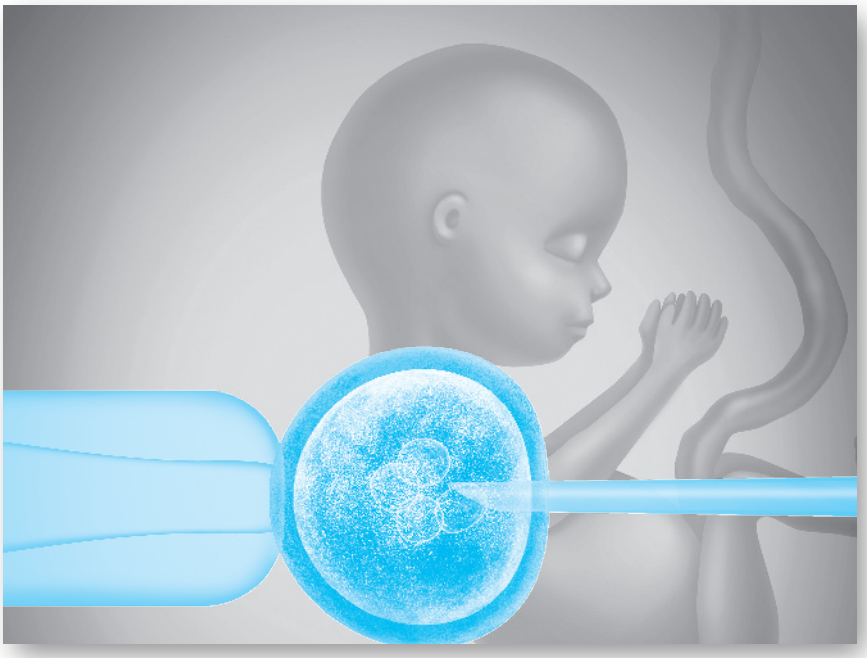
Questions

Answers can be found at www.pearsonglobaleditions.com

1. Did the samples of animal viruses meet the definition of infectious substances as stated in these regulations?
2. Would the samples be classified as Category A (capable of causing permanent disability, life-threatening, or fatal disease in otherwise healthy humans and animals) or Category B (other infectious substances)?
3. Who is responsible for ensuring the safe transport of infectious substances?

CHAPTER THIRTEEN

Ethics and Biotechnology



In November 2018, a Chinese scientist, He Jiankui, announced the birth of the world's first genome-edited twins. He went on to present his work at the Second International Human Genome Editing Summit in Hong Kong. Jiankui claimed to have used CRISPR-Cas9 technology to edit CCR5, the gene that allows HIV to infect human cells, and asserted that the girls are immune to HIV infection for life. There was an immediate international outcry against his claim as he flouted international ethical considerations by allowing the edited embryo to proceed through development.

After completing this chapter, you should be able to:

- Define bioethics and explain how it relates to biotechnology.
- Understand and apply different approaches to ethical thought.
- Identify potential ethical dilemmas associated with biotechnology, including the use of evidence that relies on a scientific understanding to evaluate.
- Pose questions and approaches that address the ethical problems identified in this chapter.
- Identify outcomes and pitfalls associated with different ethical approaches.
- Discuss interactions among science, economics, communication, and public policy for ethically-challenging issues in biotechnology.
- Understand and explain controversies and ethical issues surrounding genetic testing, stem cells, genetic modified organisms, cloning, regenerative medicine, genome editing, and other bioethical topics.
- Analyze the ethical issue of genetic privacy from more than one perspective to demonstrate an understanding of bioethical concepts.

In some ways, **ethics** might seem like plain old common sense. Of course it is unethical to intentionally cause cancer in a group of people without their knowledge just to test your new anticancer drug. But in many cases the choices are not so clear-cut. Often the decisions must deal with potential trade-offs, compromises, or possibly even sacrifices. Bioethics deals with some of the most fundamental questions confronting our society. And in many respects the decisions made now can affect the future of science, humanity, and the world in which we live. The intent of this chapter is not to tell you *what* to think about bioethics. Instead, the intent is to get you to understand *how* to think about bioethics—to encourage you to ask questions, to think about how to ask the right questions, acquire all the facts, and make decisions based on information rather than emotional reactions.

FORECASTING THE FUTURE

Ethics and related issues in all areas of science are increasingly gaining attention among the public, scientists, politicians, and others. As new biotechnology applications emerge, discussions and increased awareness of ethical concerns associated with these applications will almost always precede the actual development of the application. Using stem cells as an example, well before any stem cell applications were proven and ready to be implemented, ethicists were discussing *potential* ethical issues of stem cell applications. With the way information is rapidly communicated now, there will be few situations in which a biotechnology application or discovery is not preceded by ethical debate. So rather than trying to do the nearly impossible—that is, to forecast the most immediately pressing ethical issues that biotechnology will encounter over the next few years—we want to emphasize that, increasingly, ethical thinking and decision making will be important skills for the future. Almost every application you have learned about in this book, including those we forecast as hot areas in the future, will have significant ethical components to them.

13.1 What Is Ethics?

Ethics identifies a code of values for our actions, especially toward other humans. In simple terms, ethics could be considered a guide to separate right from wrong and good from evil. The area of ethics that deals with the implications of biological research and biotechnological applications, especially regarding medicine, is called **bioethics**. It considers social and moral aspects and potential outcomes of the use of biological and medical technologies.

It is important for you to appreciate that ethics is a *dilemma-based discipline*. Ethical dilemmas arise when an important problem or situation requires careful consideration and thought to make what one believes to be sound ethical decisions.

One fundamental question that should be asked in dealing with bioethical issues is not "Can this be done?" but "Should this be done?" And if something should be done, the question becomes "How can it be done in the right way?" Such questions are important for everyone to consider, especially in areas of biotechnology, where discoveries and their applications can have a great impact on human health and the environment. These questions get to the heart of not only society but also of science and its role in society.

For example, consider the photo shown in Figure 13.1. Scientists have implanted mouse cells into chicken embryos to demonstrate that the mouse cells could receive signals from the chicken embryo to induce development of teeth—something that does not normally occur in chickens. Chick embryos started to form teeth, but they were not allowed to develop into adults. But some people are outraged at experiments such as this because it creates images of "Frankenstein-like" mutant chickens with teeth that aren't normal. The rooster image shown in Figure 13.1 was created by photographers for shock value and headlines; it is not real. So just because technology is



FIGURE 13.1 Growing Teeth in Chickens. Should This Be Done? The image shown here is an artificial depiction of a rooster with teeth; no such animal actually exists. This is a deliberately inaccurate image presented for its shock value and to stimulate your thinking about bioethics.

available to create a chicken embryo with teeth, should it be done in the first place? Should the embryo be allowed to grow into an adult chicken to see if it will develop teeth? Is this ethical? What do you think? For more information on the research we refer to here, see the paper by Mitsiadis et al. in the reference list on the Companion Website.

Approaches to Ethical Decision Making

Before we explore bioethical issues, we first examine some of the more important methods of ethical thought. The study of ethics is as old as humanity itself. Questions of our duties and responsibilities to other members of the human community have been with us for ages. Hippocrates (c. 460–361 B.C.) might be considered the first **bioethicist.** He emphasized the patient rather than the disease in his practice of medicine, viewing the worth of the individual and the sanctity of human life as of primary importance. For years, physicians have pledged to follow the central tenets of the **Hippocratic Oath**—"do not kill," "to help, or at least do no harm"—in their duty to patients and to their profession.

Ethical thoughts and methods to approach problems can often be divided between two main viewpoints (although there are certainly other approaches as well). The **utilitarian approach,** or consequential ethics, started with the Scottish philosopher Jeremy Bentham (1748–1832) and the English philosopher John Stuart Mill (1806–1873). This approach states that something is good if it is useful, and an action is moral if it produces the "greatest good for the greatest number." The second main approach—the **deontological (Kantian) approach,** or duty ethics—comes primarily from the German philosopher Immanuel Kant (1724–1804) and focuses on certain imperatives, or absolute principles, which we should follow out of a sense of duty and should dictate our actions.

Modern bioethics can be traced to more recent times and is primarily the work of two ethicists in the 1970s: Joseph Fletcher and Paul Ramsey. These men refined the two primary approaches to ethical thought mentioned—Fletcher for **utilitarianism,** also termed situational ethics, and Ramsey for deontology, or **objectivism.**

Utilitarianism emphasizes consequences, not intentions. Another way to phrase this emphasis is that "the ends justify the means." The idea is to calculate what the consequences of an action would be and to weigh different consequences against one another. If we can analyze various courses of action to determine which will have the greatest positive effect on the greatest number of people, then we can provide an answer to the question of what we ought to do. In some ways, this is an elegant method for making decisions. All avenues can be assessed for potential benefit, and the decision becomes more quantitative. The disadvantage of this calculation is that we must assign a value to all of the things considered. How do we assign these values? Some would say that some of the most important things in life (love and family) are not easily quantified, whereas other things (material goods and life span) could be emphasized in the calculations because they are quantifiable. Another source of concern could also be the individual doing the calculating and assigning values. For example, the utilitarian calculation would be less than ideal if done by someone who believed that males are worth more than females or that the primary consideration should be the profit margin.

Deontology, or objectivism, starts from the point of view that there are at least some absolutes (definitive rules that cannot be broken) and that we have a moral obligation or commitment to adhere to these absolutes. One absolute usually mentioned is for the value of human life, expressed by Kant in this way: "Act in such a way that you always treat humanity, whether in your own person or in the person of any other, never simply as a means, but always at the same time as an end." Another way to say this is in terms of respect for others—that we ought to treat others as ends in themselves rather than as means to an end.

This approach has often been associated with religious traditions, but it is a mistaken notion to assume that an objective ethical approach is solely a religious approach or even that all religious approaches to ethical issues begin with the same absolutes. Deeply held convictions are just that—personal points of reference to which an individual adheres. An advantage of objectivism is that it gives firm guidelines in many situations in which ethical decisions are required, providing a clear-cut ethical formula for decision making. A disadvantage of this approach is that it may be, or at least may be perceived to be, too rigid in its decision-making process, not taking what may be important factors into account or considering possible changes in values. And there may be situations or issues in which no clear conviction or absolute exists, requiring further examination of the issue to define the moral imperatives and discern the course of action.

To illustrate the difference between utilitarianism and objectivism, consider a situation in which a person is desperately hungry and has no money to buy food. Wandering by a store, the person sees a loaf of bread left out on a table. A utilitarian calculation might take into account the person's need, the available food, and the minuscule loss in value to the store and consider that it would be okay to take the bread. An objectivist approach might have the absolute "it is wrong to steal" and consider it unethical to take the bread.

Note that other ethical approaches and methods beyond the two main approaches mentioned here are possible, and even these two approaches to ethical decision making can sometimes be blended. In approaching ethical decisions, a key objective is to gather information, consider the facts, and make a thoughtful, informed decision. And in debates on ethically contentious issues, it is neither wise nor polite to deride or belittle another person's decision. Be sensitive to the effects of your own conduct. Aim to understand and defend your own ethical decisions rationally, and strive to consider the decisions of others.

The idea of the likelihood of an event, the **statistical probability,** is another important concept to keep in mind in making ethical decisions. It is crucial to determine accurately what chance exists for a "bad" event to happen. Another consideration might be just how negative the effect of the possible event would be. Because many consequences take time to develop, the probability of an outcome occurring must be part of the consideration.

Bioethicists and scientists often use statistical measures to determine **risk assessments,** the likelihood that something harmful or unintended will happen. Risk assessment is part of your decision-making process every day. For instance, you know that each time you drive your car there is a risk that you might be in an accident. Assuming that you are not afraid of driving, your risk assessment probably tells you that the likelihood of an accident does not outweigh the need to get where you want to go. Cell phone use; air travel; drinking alcohol; the risk-benefit of taking antibiotics, cold medicines, vaccines, or other drugs (versus the side effects); and making poor dietary choices are other good examples of risk assessments in your regular life.

Ethical Exercise Warm-Up

What follows is a typical scenario regarding tough ethical decision making. Although the situation may seem a bit harsh because it deals with a life-or-death scenario, some of the biotechnologies being developed also deal with issues of life and death and have far-reaching consequences.

A family pulls up to the Grand Canyon in their car. The parents get out to check out a refreshment stand and lock the car doors, leaving three young children asleep in the back seat. But they do not set the brake well enough. After they've moved some distance away, the car slowly begins to roll toward the edge of the cliff. You are the only one who notices. A large man is standing close to where the car is headed. You could push him in front of the car, which would stop its rolling, although he would either be crushed or pushed over the edge himself. What would you do?

First, do you have all the facts in this situation? So far we have painted the scenario so you have only been given two choices: to let the car go over the edge or to sacrifice the one man for the three children. The question seems to be simply, "Can you trade his life for theirs?" A utilitarian approach might consider this is an ethical trade, one life for three, and also consider that the three are young lives; an objectivist approach might consider that it is wrong to endanger or kill any human life, no matter the eventual consequences. Are there other possible choices or alternatives?



TOOLS OF THE TRADE

Careful Thought and an Open Mind Are Powerful Tools for Bioethicists

In contrast to the various laboratory and industrial applications of biotechnology, bioethics requires no specialized equipment. The main tools of the trade are logic and an open, inquisitive mind. Bioethicists must be able to assess a situation carefully, considering the possibilities from many different angles. They must include medical, religious, philosophical, legal, scientific, and social concerns and outcomes in their assessments. Bioethicists must be able and willing to consider many different viewpoints, which are often conflicting. Bioethicists must also be inquisitive, able to ask probing questions, and must determine the many factors that underlie the concerns. They may also find it necessary to do extensive literature searches, delving into the background of a topic from numerous perspectives. Language skills can be an additional asset, because not all perspectives or literature may be accessible in a single language. Sorting through possible outcomes and pointing out potential pathways for the resolution of concerns requires reason and logic as well as patience. In the modern world, knowledge of regulatory agencies and rules is also extremely important. This means that bioethicists must not only be able to understand the regulations and laws currently in place but also be able to interact with policy makers to facilitate the creation of sound regulations and laws. Finally, good interpersonal and communication skills (both oral and written) are essential for making the concerns, questions, and outcomes clear to all involved.



YOU DECIDE **Right or Wrong?**

The chief executive officer (CEO) of a small startup biotech company is involved in negotiating a multimillion-dollar merger of his company with a larger biotech company because of a promising stem cell cloning technology his company has developed. However, the CEO is also aware of a significant problem with this technology that has recently turned up. Does the CEO reveal the problem and risk jeopardizing the merger, or does the CEO allow the merger to move ahead without discussing the problem? You decide, basing your answer on objectivism and utilitarianism.

13.2 Examples of Ethics and Biotechnology

Bacteria routinely swap plasmids, and recombination and mutation occur, allowing the expression of new genes or new combinations of genes that were not present before. However, the game changes when humans get involved and create new genetic combinations. As we discussed in Chapter 3, because of concerns with recombinant DNA technology as a new method, scientists met at a conference in Asilomar, California, in 1975 and called for a **moratorium** (a temporary but complete stoppage of any research) until the safety of the technique and possible consequences could be assessed. Recall that a primary concern was that genetically engineered bacteria would escape from the laboratory into the environment, possibly creating new diseases, spreading old diseases in a more virulent manner, or creating ecosystem changes that might lead to decimation of some species. Guidelines were developed for different levels of biosafety containment, depending on the inherent dangers of the experimental system used. For example, experiments with nonpathogenic bacteria and nonpathogenic gene sequences require only minimal safety equipment, whereas experiments with known pathogens, human cells, or potentially pathogenic genes require more stringent containment procedures.

Cells and Products

As we have discussed throughout this book, both bacteria and eukaryotic cells can be genetically engineered to express foreign genes and proteins. This has proven to be an indispensable approach for producing medically valuable products. Think about the ethical challenges involved with the genetic modification of individual cells and the products that have emerged as a result of these changes.

When a product concerns human application, such as a therapeutic recombinant protein, there is not only the obvious issue of safety but also the issue of **efficacy,** or effectiveness in its intended use. This is obviously important for an intended patient because the patient wants an effective treatment. But efficacy is also important for the manufacturer; if the product is not effective, there can be tremendous economic waste. For any drug, an important consideration will be the dose at which the drug is effective, with minimal side effects and toxicity. Consequently, it will also be important to establish whether the drug poses any **carcinogenic** or **teratogenic** hazards. These considerations prevent future problems—such as finding out that the drug cures the disease at one dose but kills the patient or causes cancer or birth defects at another dose—because they raise the ethical concern of harming rather than helping the patient and the potential problems involved in using the patients themselves as guinea pigs to test drug effectiveness.

We must be mindful of the **humane treatment** of animals in preclinical studies. We need to determine how many experimental animals will be the minimum needed to test the drug, what types of treatments will be necessary for the tests, and whether rodents or primates must be used. If possible, the target organs can be tested with the drugs (organ on a chip) before any animal studies. The choice of species can affect the action of the drug. One of the best examples of species difference in drug action occurred with the drug **thalidomide,** which was originally designed as a mild sedative. It was tested in standard laboratory rodents and found to be safe. However, many of the pregnant women who took this sedative gave birth to babies with severe birth defects. When the drug was tested on marmosets (a type of monkey), birth defects similar to those seen in humans resulted. It turns out that drug metabolism can vary among species. We consider the question of human experimentation in detail later, but the possible differences in effects on humans versus various animal species must always be kept in mind.

GM Crops: Are You What You Eat?

A key advance in genetic engineering, and also one of the biggest controversies, has come from the production of **genetically modified (GM) organisms,** particularly GM crops. The aim is to produce plants that can resist pests, disease, or harsh climates, facilitating the production of crops. Yet many people are opposed to GM crops and have an aversion to ingesting genetically modified food.

GM crops and other genetically modified plants present several areas of concern. One concern involves the plant itself. Scientists must determine whether the alterations in the plant's genetics provide a benefit to the plant or at least do not produce a less vigorous plant. One question that might be worth answering is whether the integrity of the species (maintaining the original genetic composition of a species without major change, so that it is still essentially the same species) is somehow preserved along with the alteration. You should seek to define for yourself whether such **species integrity** is important or whether creating a "better" plant species is more desirable than trying to maintain an "old" species. In doing so, determine for yourself whether the genetic modification of organisms, in this case plants, violates any ethical codes.

Another question, on a broader scale from the first, is the possible effect of altered plants on the ecosystem and on overall **biodiversity** (the range of different species present in an ecosystem). We must determine the effect of the introduction of a genetically modified plant on the local environment. Because we are focusing on crop plants, the desired effect will likely be not only to increase the growth and production of the GM crop plant but also to reduce the harm that might be caused by potential pests and diseases.

One example is Roundup-Ready soybeans. This soybean is genetically modified to resist Roundup herbicide, allowing a farmer to spray the crop and kill noxious weeds that would interfere with growth of the crop without harming the soybeans. Another example is Bt corn (**Figure 13.2**). Recall from Chapter 6 that this GM crop is engineered to produce a toxin from the bacterium *Bacillus thuringiensis,* which kills corn borer larvae and other insect pests that feed on the plant.

Some research has suggested a possible toxic effect of Bt crops on monarch butterflies, even though they were not a target insect and do not feed on corn. It would thus be important to know if the toxin affects specific species or groups of insects and whether nontarget insects can also be affected. One question that was considered was whether the toxin could be spread or was confined solely to the corn plant. Because corn is wind-pollinated, the pollen might be carried to other plants and be toxic to some insects at a distance. Researchers had to determine the likelihood of this happening. For the monarch butterflies, the study indicated that corn pollen could be spread by wind to milkweed plants (which are a food source for monarchs) located next to the GM cornfield. Monarchs feeding on the milkweed could then ingest the corn pollen (and the toxin). Scientists had to ask whether this was a likely occurrence and how much pollen and toxin it would take to kill a monarch butterfly. Think about the types of experiments you might design to test these questions. In recent years, several long-term studies have shown no adverse effects of monarch exposure to Bt crops.

There might be possible effects of cross-pollination between Bt corn and the transfer of engineered genes



YOU DECIDE — Should GM Wheat (for Gluten Sufferers) Be Approved Quickly?

The most common source of gluten is wheat. Gluten proteins trap air as bread ferments, giving it the elastic, chewy taste that we associate with bread. Unfortunately, gluten is toxic to a small number of people who suffer from celiac disease, and a much larger number who are allergic to gluten. Barley and rye are other grains that contain gluten, but they are not as common in foods as wheat. Attempts have been made to breed out the gluten-containing genes, but they exist on multiple chromosomes and most wheat is hexaploid, which makes it difficult. The Institute for Sustainable Agriculture in Spain has taken a different approach, using RNA interference (RNAi) to silence all the gliadin-producing genes in wheat before they combine with glutenin to produce gluten. The bread that is produced from this silenced wheat appears to have the texture and taste of bread made from current wheat flour. Trials are currently determining if it can be tolerated by gluten sufferers.

Since commercialization of this low-gliadin wheat will require separate mills and distributors, it is likely that North American investors will be needed for the commercial enterprise. The approval process in the United States and European Union will also take time and money. Should GM (gluten-free) wheat receive a faster approval than other GM foods? Should other beneficial GM foods be included in the approval? You decide.

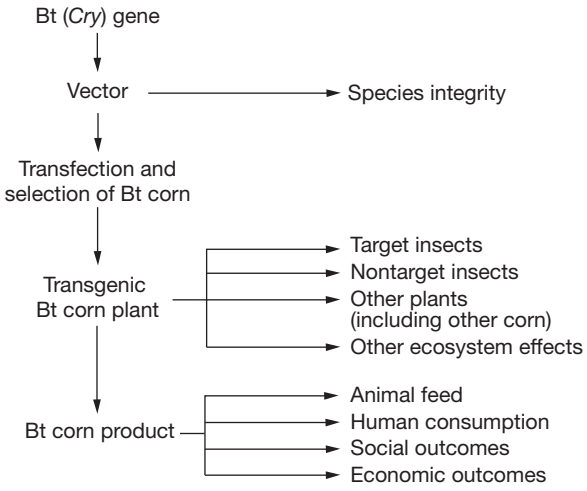






FIGURE 13.2 Possible Interactions and Considerations for a GM Crop, Bt Corn Bt-modified crops were designed to protect plants against pests such as the cotton bollworm (top photo) and corn borer; but soon after their use, concerns were raised about possible effects on nontarget insects such as monarch butterflies (bottom photo).

to other noncrop species (for example, other plants that might acquire the toxin gene and kill desirable insects, such as monarch butterflies). You would have to consider the likelihood of such an occurrence. The whole question of introducing GM plants into the natural environment needs careful scrutiny to consider possible long-term changes to the ecosystem and the effects on biodiversity. Another consideration is the spread of GM plants to other areas beyond where they are cultivated.

Another question to consider regards the product of the GM crop: we must consider how it will be used, whether it is safe to feed to animals, and whether it is safe for humans. We must also gather all the facts and make informed evaluations. Because the toxin is directed against insects, it seems unlikely that it would affect animals or humans. But making this assumption is not enough; evidence and tests are needed that could verify its safety. Think of some experiments you could do to test the safety of a GM product.



YOU DECIDE

Should the Public Be Consulted about the Release of Genetically Modified Insects?

In 2010, some 6,000 GM mosquitoes were deliberately released into an uninhabited forest in Malaysia—to the surprise of locals and international followers. The Malaysian trial was an effort to control dengue fever, a virus-based disease transmitted by the mosquito *Aedes aegypti*. The dengue virus has spread to over 100 countries, and the World Health Organization (WHO) considers the at-risk population for dengue infection to comprise nearly 2.5 billion people. In some parts of the world, dengue fever is a major cause of death in children. Dengue fever causes severe and painful flu-like symptoms in adults, which can sometimes lead to lethal complications. British biotech company Oxitec had previously released 3.3 million GM insects in trials in the Cayman Islands in 2009 and 2010 in the world's first GM mosquito field trial designed to control dengue-carrying carrier insect populations. The company claims that their Cayman Island trials reduced that insect population by about 80 percent.

In 2016, a branch of Google's parent company, Verily, announced that it was using robots to create GM infertile male mosquitos and planned to release these in California to help combat the spread of insect-borne diseases such as Zika virus infection and dengue fever. More than 20 million of these GM insects have been released in Fresno, California, in Verily's first field study.

In these examples, the public and even researchers in the field were generally unaware of the releases until after results of the trials were announced. There are no policies on informing the public about such experiments. Should there be? Is it sufficient to simply inform the public that GM insects have been released? You decide.



YOU DECIDE **What Would Be the Effect of Banning GM Organisms?**

A study conducted by researchers from Purdue University reveals that a global ban on GM crops would raise food prices and add the equivalent of nearly a billion tons of carbon dioxide to the atmosphere. If, instead, countries planting GM crops matched the rate of GM crop plantings in the United States, global greenhouse gas emissions would fall by an equivalent 0.2 billion ton of carbon dioxide, and would allow 2 million acres to return to forests and pastures. Some of the same groups that want to reduce greenhouse gas emissions also want to ban GM organisms (GMOs). What objectivism and utilitarianism arguments would you use to explain the benefit of GMOs to the global environment?

We should not only focus on the particular gene in question but also ask whether other genes or products present in the GM crop might have to be considered. For example, in many cases antibiotic-resistance genes are used as selection markers for genetically engineered cells. Are the genes still present in the GM crop? And if so, can these genes be transferred from ingested food to gut bacteria? You should again ask what the likelihood is that DNA or proteins would survive digestion. Figure 13.2 shows some of the possible interactions and considerations. We must also determine whether the product from a GM crop should be quarantined after the crop has been cultivated. This again comes back to our question of safety, especially regarding human exposure to or consumption of the product.

Some countries strictly limit the importation of GM crops, and there is a movement to label foods to designate their origin in terms of potential genetically modified plants (**Figure 13.3**). Generally, there is concern that GM foods are unnatural. Some people believe that, based on the data, these restrictions are not valid and may unduly frighten consumers. What would be your reaction to finding a "GM" label on your pizza or cornflakes? Because of CRISPR (Clustered Regularly Interspaced Palindromic Repeats), new crops known as gene-edited, rather than genetically modified, are coming to the market. Should these crops be labeled as GM?

Social and economic questions also arise from the potential use of GM crops. The ability to modify plants for better, less costly production could drastically change the agricultural industry. Potentially, more abundant food could be available at a reduced cost both to the farmer and to the consumer. These advantages may be offset by potential disadvantages, however, such as the safety concerns described previously. Other possible uses for GM crops include the production of medically useful compounds. Safety and efficacy again become key considerations in the ethical assessment for use of these compounds. Current U.S. policy requires not only the usual range of tests for safety and efficacy of medical compounds but also that growth of the GM plants be restricted. The EU members and Australia also have stringent policies regarding the production of GM Crops. The commercial production of a GM pharmaceutical crop has not been approved in any of these countries.



FIGURE 13.3 Do Consumers Have a "Right to Know" If Foods They Eat Contain Genetically Modified Organisms?



YOU DECIDE **How Much Return on the Investment?**

Monsanto, a company based in St. Louis, Missouri, created Roundup-Ready soybeans, which are genetically engineered to withstand Monsanto's Roundup weed killer, a commonly used herbicide that kills almost all plants. Roundup-Ready seed costs several dollars per bushel more than conventional soybean seeds. Monsanto has used private investigators to check out reports that farmers in different locations had been recycling seed harvested from Roundup-Ready soybeans planted the previous year. Using seed harvested from a previous crop is a common practice for many farmers and allows them to save money on seed for planting a new crop. Because Monsanto invested so much money in their technology, they wanted farmers to buy new Roundup-Ready seeds each year, but farmers claim they were never told their seed could not be replanted. Monsanto claims farmers are using its high-tech expensive product for free.

Should the farmers be allowed to continue their traditional practice of recycling seed? Or should biotechnology companies be able to enforce restricted uses of their products? You decide.

Animal Husbandry or Animal Tinkering?

Genetic modification of animals raises many of the same questions posed by the genetic modification of plants. Early applications of biotechnology to animal husbandry have included antibiotic supplements in feeds and injections of growth hormone or steroids to increase growth of the animals. Consider the application of these supplements and injections from an ethical standpoint. First, because these are agricultural animals, the effects of genetic modification on the products from the animals (milk, meat, and so on) and the safety of these products for human consumption were the main concern. One consideration was the length of time the supplements (especially hormones) would persist within the animal—that is, whether they would still be present when the animal products were consumed by humans. If so, scientists must also determine whether there would be any effects on the consumer and whether the hormone, if present, would survive any cooking or the digestive process.

Little concern has been expressed about the effect of GM agricultural animals on the environment, but there are still questions about species integrity and the health of the animal as well as the safety of animal products for human consumers. Similar considerations are in order regarding animals that have been genetically engineered to produce medically useful products. These could include transgenic animals modified to produce a clotting factor in their milk, transgenic animals such as pigs engineered with human genes so their organs could be transplanted into humans without being rejected, or cloned cows to be used for meat. Chinese scientists have created transgenic cows that express human milk. At what point would you consider such alteration of an animal to be unethical?

Be sure to consider whether there is a point at which the animal might acquire enough human genes, cells, or attributes that you would consider it human and whether this would change your ethical viewpoint on using the animal for research. Refer back to



YOU DECIDE **Animal Organ Acceptance**

A study published recently in the journal *Nature* reported the entire sequence of the domestic pig genome and identified genes and protein sequences that have similarities with those found in humans (some of which are implicated in human diseases). The product of a broad international collaboration, this study reveals the first draft of the pig's genome sequence, which could provide a reference tool for exploring the pig as an experimental model for specific human diseases, as pig physiology is very similar to that of humans (and for swine livestock improvement). The pig genome sequence would be a valuable resource in using pigs in both agricultural production and biomedical research for potential organ donation, called xenotransplantation. Since some religions prohibit killing of certain animal species, how should animal organs be made available to people on the donor list? How would an ethical argument justify accepting an animal organ by a patient with religious or other ethical objections? You decide.

Figure 13.1. English and French scientists implanted mouse cells into chicken embryos to demonstrate that mouse cells could recognize developmental signals from chicken cells to stimulate tooth formation. Biologists are interested in this in part because although the genes involved in these developmental signals are not active in birds (teeth were lost in birds about 70 to 80 million years ago), it indicates that these genes can be activated and can stimulate tooth development when the proper cells are present. So what? Who wants to make chickens with teeth? These are good questions, and you may certainly ask whether these types of experiments are ethical uses of animals. But also consider that information from these experiments can be fundamentally valuable to clarify tooth development and potentially in the future to develop novel treatments for tooth formation, replacement, and regeneration in humans—the main reasons for this research.

Recombinetics in St. Paul, Minnesota, has produced hornless dairy cattle by inserting a gene from naturally hornless beef cattle into a breed of the same species that is used in milk production (**Figure 13.4**). The animals could help to reduce the practice of surgical "dehorning," a controversial practice that has raised animal welfare concerns. Recombinetics told the Food and Drug Administration (FDA) that it intended to market food from its cows without FDA approval, and with a label reading "generally recognized as safe." The company's decision was bolstered by the U.S. Department of Agriculture's announcement in April 2016 that it would forgo regulation of a mushroom that has been genetically modified to resist browning. What ethical objections from animal rights activists can you foresee from this improved treatment of animals? Is this application of genetic engineering justified by objectivism or utilitarianism or both?

Synthetic Genomes and Synthetic Biology

Research on synthetic genomes has resulted in transplantation of a synthetic genome into a bacterial strain to change the recipient microbe into the organism of the donor synthetic genome (Chapter 5). These and other future applications of synthetic biology are raising many ethical questions about what should and should not be done with synthetic genomes and their potential uses to create novel life forms.

Drug Trials with Human Patients

Many of the thorniest questions regarding biotechnology, and some of the most contentious debates, revolve around humans. Even simple scientific procedures can evoke strong emotions and stir profound controversy when humans are the subjects. Why do you think the use or potential use of humans experimentally causes such strong reactions? Later we discuss how the mere definition of what is human has become an area for debate.

For now, we look at a simple example based on a potential anticancer drug generated through biotechnology. Suppose the drug has moved along to the point where it was ready for clinical trials. We must decide to whom we will administer the drug as a test. Because it is an anticancer drug, we will naturally give it to patients who have the type of cancer targeted by

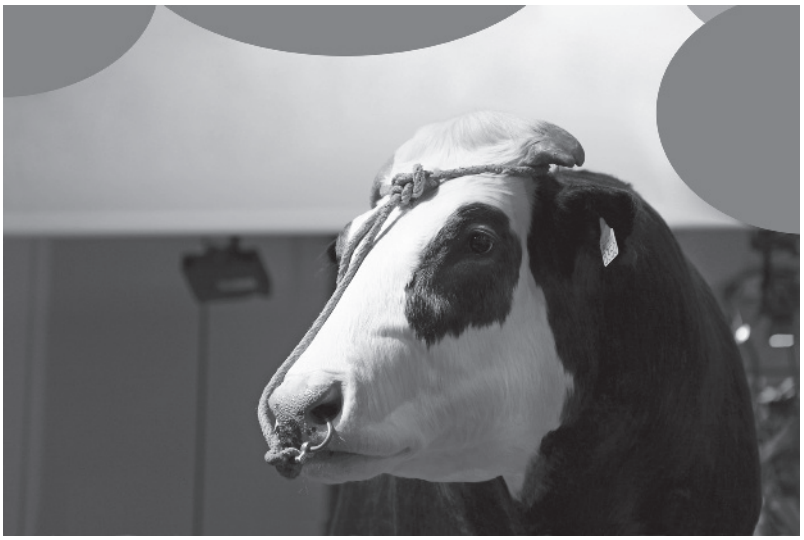


FIGURE 13.4 Hornless Bull Calf Created by Gene Insertion.

this drug. We must decide, however, whether to give it to all patients with that type of cancer or only to those in the most advanced stages of disease—those who may have exhausted all other means of treatment and for whom the new experimental drug is a last resort. The rationale is that, for this subset of patients, there is no other possible treatment; therefore, the experimental treatment presents at least some hope. However, the drug may work better with patients who are earlier in the progression of the cancer and thus might be more effective. Nonetheless, the patients who are in earlier stages of the disease have other alternatives that have already shown safety and efficacy (at least to some extent).

After you have picked the patient group for the clinical trial, another dilemma arises. Patients have a right to be informed fully of the potential effects of the experimental treatment, both good and bad. Only when so informed can they be willing participants in the trial. This is termed **informed consent.** Patients give their consent to proceed with the experiment, fully informed of the potential benefits and risks that lie ahead. The concept of informed consent is vital to any procedure involving a human. If a patient is unable to give consent on her or his own (because the individual is too young or in a coma, for example), a family member or guardian can give proxy consent. Determine for yourself why informed consent is so vital.

Placebos present another problem as far as experimental procedures on humans are concerned. In a placebo-controlled study, standard scientific practice involves using an experimental group (in this case, patients who would receive the drug) and a placebo-controlled group (in this case, patients who would receive a placebo, a safe but noneffective treatment such as a sugar pill or saline injection). In a completely randomized **double-blind trial,** neither the patients nor the doctors administering the treatment would know who received the real drug and who received the placebo. Informed consent should play a role in this type of experiment. Using placebos is part of objective science, but we must look at whether it is ethical. Objective science may not always be the best approach or the ethical approach.

What Does It Mean to Be Human?

Many of the ethical debates about stem cells and cloning revolve around the moral status of the human embryo. As we discussed in Chapter 11, stem cells may hold the potential for tremendous breakthroughs in **regenerative medicine,** making possible the repair or replacement of damaged and diseased tissue in many diseases such as heart disease, stroke, neurodegenerative diseases, diabetes, and others. As you know, many possible sources of stem cells are available, including embryos, fetuses, umbilical cord blood, adult tissues, and even "tamed" tumor cells (**Figure 13.5**). These sources of stem cells are in addition to nuclear reprogramming options, such as making induced pluripotent stem cells from human somatic cells. Much of the stem cell debate has centered on the scientific question of the abilities of different stem cells to transform into other tissue types



YOU DECIDE

Should Clinical Trial Data Be Public or Do They Belong to the Drug Company?

In 2002, the National Institutes of Health (NIH) launched Clinicaltrials.gov to help patients and physicians find information on nearby clinical trials. The current registry contains information on over 260,000 clinical trials in all 50 U.S. states and in 201 countries. This registry has been voluntarily utilized by most biotech and drug companies because it excludes early-stage trials, in which sensitive business information could be lost. For several years, a congressional bill (S.467—the Fair Access to Clinical Trial Act; FACT Act) to call for mandatory disclosure of the results of clinical trials in a public database was debated but never passed as a law. Some claim that the information could help patients determine whether to participate, but some in the drug industry say it is unnecessary and will stifle innovation.

The bill proposed to require open access to information from phase 1 trials, which enroll healthy patients in small numbers. According to some industry spokespersons, however, such information would be of little value to patients. The advocates for the bill claimed that companies could benefit from sharing early-stage trials to prevent new drugs from repeating the same mistakes that had already produced negative results. In defense of the industry, biotech companies rely on venture capital funding, and if early data are shared with other companies, there is concern that funding for innovation would dry up. Is the benefit worth the risk that it may reduce funding for drug research? Is there a better solution? You decide.

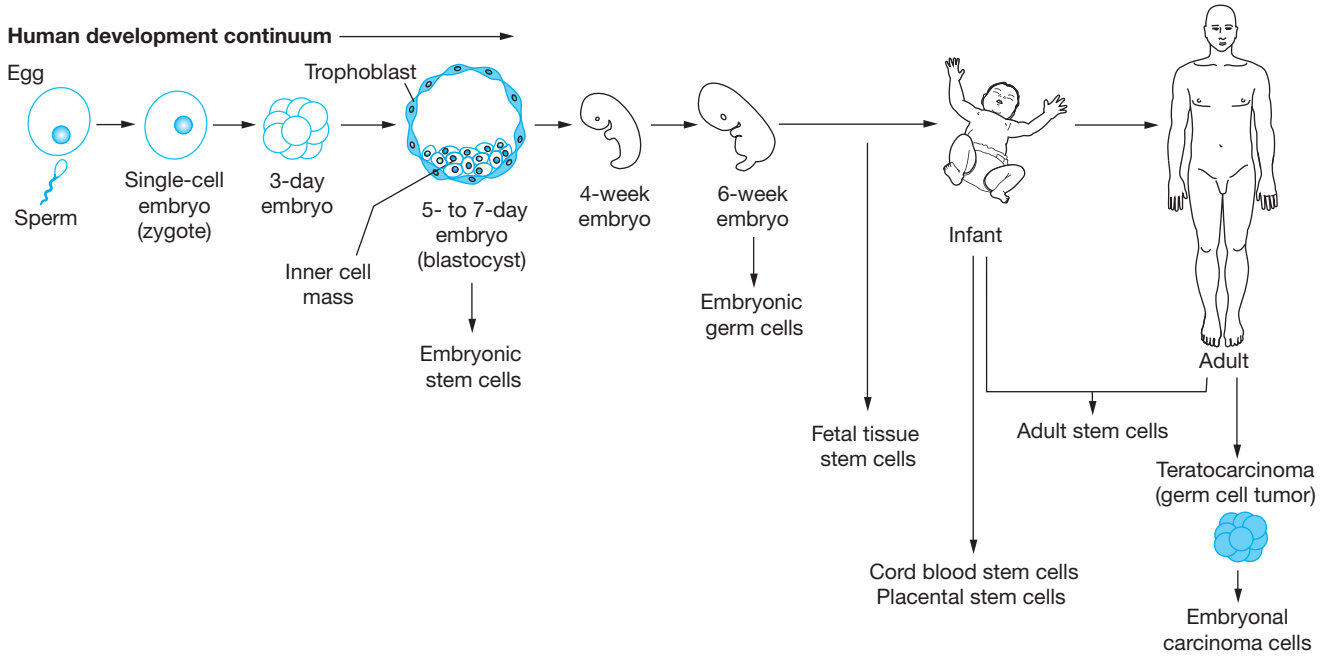


FIGURE 13.5 Sources of Stem Cells Human embryos, fetal tissue, infants, and adults are all potential sources of stem cells.

for the treatment of diseases; however, key elements in the debate have been ethical questions about embryos as a source of stem cells.

As a society, we have not been able to agree on whether it is ethical to destroy early-stage human embryos for research that may potentially benefit thousands of patients. Unlike organ donation, in which an individual may donate one of two paired organs while alive or after his or her death, the process of harvesting embryonic stem cells destroys the donor (the embryo; Figure 13.5). The use of human embryos as a source of stem cells is a very controversial topic, in part because it raises questions such as the following: When is the embryo considered to be a person? Is it ethical to destroy a human embryo to harvest stem cells that may benefit many other humans?

We must resolve the focal question, which examines the moral or ethical status of a human embryo. Biologically, the embryo is a human being, species *Homo sapiens*, just starting out on the developmental journey. We must weigh the relevance of different developmental milestones for being considered human against the simple biological fact that the embryo is a member of our own species. Biologically,



YOU DECIDE

Regenerative Medicine: For the Rich Only?

Molecular mechanisms responsible for differences in longevity between animal species are relatively unknown. The longest-lived rodent, the naked mole rat, has more accurate protein translation than the mouse. Researchers from the Institute of Evolution, Israel, have shown that the naked mole rat has a unique fragmented ribosomal RNA structure and speculate that it may change the folding or dynamics of the large ribosomal subunit, modifying the interaction of the large subunit with transfer RNA (tRNA) and affecting protein synthesis.

Their results show that naked mole rat cells produce fewer aberrant proteins, supporting the hypothesis that the more stable proteome of the naked mole rat contributes to its longevity. It is expected that further study of the fidelity of protein translation will improve understanding and lead to testable therapies. Since this research is likely to be expensive, therapies may be affordable only for the rich. Should public funding be restricted, so that the rich will pay for its benefits? Consider utilitarianism and objectivism responses. You decide.

an embryo is a member of the human species, but the question of the moral status of a human embryo goes beyond the biological and also revolves around what some have termed the status of **personhood.** This term has been used to define an entity that qualifies for protection based not on an intrinsic value but rather on certain attributes, such as self-awareness. List for yourself the advantages and disadvantages of this concept of personhood. One concern with it might be the question of who decides which attributes count in evaluating whether a particular human being can be valued as a person.

Regarding embryo research, some have taken this attitude: "Not a person, not a problem." They reason that a human embryo is microscopic, not yet possessing a beating heart, brainwaves, arms, or legs. Of course those things develop later, but at a very early stage the embryo lacks what we usually associate with our concept of humanity. There are obviously various views of the status of the human embryo, and no consensus. Some say it is simply a clump of cells, just like a chunk of skin. Others believe it is a form of human life deserving of profound respect—a potential person. Still others maintain that an embryo has the same moral value as any other member of the human species. Consider the question of whether *any* human cell deserves respect as a potential person. When combined, an egg cell and a sperm cell form an embryo, and any somatic cell can contribute a nucleus to form a cloned embryo. Consider, then, whether there is something special about having a complete human genome in an egg cell.

Considered in the context of using human embryos to isolate embryonic stem cells, the debate regarding the necessary destruction of the embryo is contentious. Certainly, embryonic stem cells can theoretically be used to form any tissue, with the potential for transplants to repair or replace damaged or diseased tissue. This might suggest that it would be ethical to destroy human embryos for research if, from that destruction, it meant that such research could lead to treatments for disease. However, based on current published evidence, one question that first must be asked is whether embryonic stem cells are as good for potential treatments as claimed.

Another consideration that has both ethical and scientific components is whether other viable alternatives, such as adult stem cells or induced pluripotent stem cells, are just as good. The ethical questions then take on a new form, asking, on the one hand, whether research on embryos is necessary if science is to explore all possible avenues for medical breakthroughs or, on the other, whether the calculation now indicates that the alternative makes ethically contentious research unnecessary. Of course one ethical position would be that even if there are no alternatives, the ethical cost is too high to justify the destruction of embryos.

Interestingly, even some people who view a human embryo as a potential person and not a realized individual oppose destruction of human embryos on ethical grounds. The concern is not directly with the embryo and its status but rather with how society views any human life. From their point of view, we are embarking on a slippery slope in which the destruction of human beings for medical use or experimentation might move from the use of embryos to the use of born individuals. Once again, we return to the question of personhood, especially as a societal construct that might rank different humans based on their quality of life and on their usefulness to society.

Spare Embryos for Research versus Creating Embryos for Research

As we consider the question regarding the moral status of the embryo, there are varying views based on the original purpose for which the embryo was created or on the method by which the embryo was created. Contrary to a common misconception that aborted fetuses are used for stem cell research, the primary source of embryos for this research is "excess" embryos from ***in vitro* fertilization** (**IVF**, Chapter 11). These embryos, left in frozen storage after a couple has used other embryos for implantation and ideally pregnancy and birth, may be donated (with the couple's consent) for research. Depending on the IVF clinic or the country, frozen embryos may be discarded after a certain period of time. List for yourself the necessary ethical considerations in this instance. Some say that it is ethically valid to use these embryos for research if they would otherwise be discarded—that some ethical good could be salvaged from their existence if they were to contribute to a potential therapy or new scientific knowledge. Others say their destruction for research crosses an ethical line inconsistent with the purpose for which they were originally created.

Another potential source of human embryos is the specific creation of embryos for research purposes. Some have argued that the use of spare embryos for research is ethically valid (reasoning that this could be salvaging a potential good out of certain destruction) but that the specific creation of embryos for research is not. To determine whether this argument is ethically consistent, consider whether there is any difference in the embryos biologically or only in a view of the intent or use of the embryos.

Should Humans and Other Animals Be Cloned for Any Reason?

Creation of embryos by somatic cell nuclear transfer cloning raises many of the same questions encountered with stem cell research, with the added complexity of the technique and the potential "identity" of the clone (Figure 13.6). A cloned embryo created for transfer into a uterus and implantation faces many risks and safety factors for its own development and growth, both before and after birth. The success rate for live births and the subsequent survival of the clones is extremely low. As we discussed in Chapters 7 and 11, we know that there can be a number of problems, some subtle and some very severe, with the health of clones.

One consideration is whether creating a cloned human embryo with the intent of initiating a pregnancy and live birth should be considered another type of assisted reproductive technology similar to IVF or whether (because of the safety risks and low success rates) it should be considered unethical human research. Societal questions regarding the identity of a born human clone must also be taken into consideration. For example, if a couple decided to create a cloned child using a donor cell from the wife, the clone would not be a genetic daughter but rather the wife's sister, a late-born twin, and would not be related to the husband at all.

Ethical considerations related to the clone include how the lack of relatedness to one parent might change kinship and family relationships. Given that the clone is a "copy" of one of the "parents," the clone, once born, might be expected to "live a better life" than the person who was cloned. Another potential concern arises if a previously existing person, now deceased, had been cloned. The genetic makeup of the clone would already be known, already dictated, because the process of cloning reproduces a previously existent individual. A clone may be expected to live up to that genetic legacy, with heightened expectations by the parents and others based on what was achieved by the donor of the clone's genetic material.

Our genes determine many of our physical characteristics and predispose us to various diseases or behaviors, but after we are born there are many experiences and environments that make us who we are and who we will become. Those experiences cannot be duplicated, so the clone will grow up differently than the one who was cloned and may behave quite differently. A clone of Einstein might become an artist instead of a scientist. A great deal more affects us and our makeup than just our genetics, including our environment and experiences.

So far we have considered the creation of a cloned human embryo with the intent of producing a live-born child. However, this is not the only proposal for the creation of cloned human embryos. Creating human embryos for **therapeutic cloning** could lead to matched embryonic cells for patients (Figure 13.6) and to valuable human research models for the study of genetic diseases and cancer. Although this might sound like a potentially valuable and ethically valid reason for creating cloned human embryos, others argue that such embryos should not be produced.

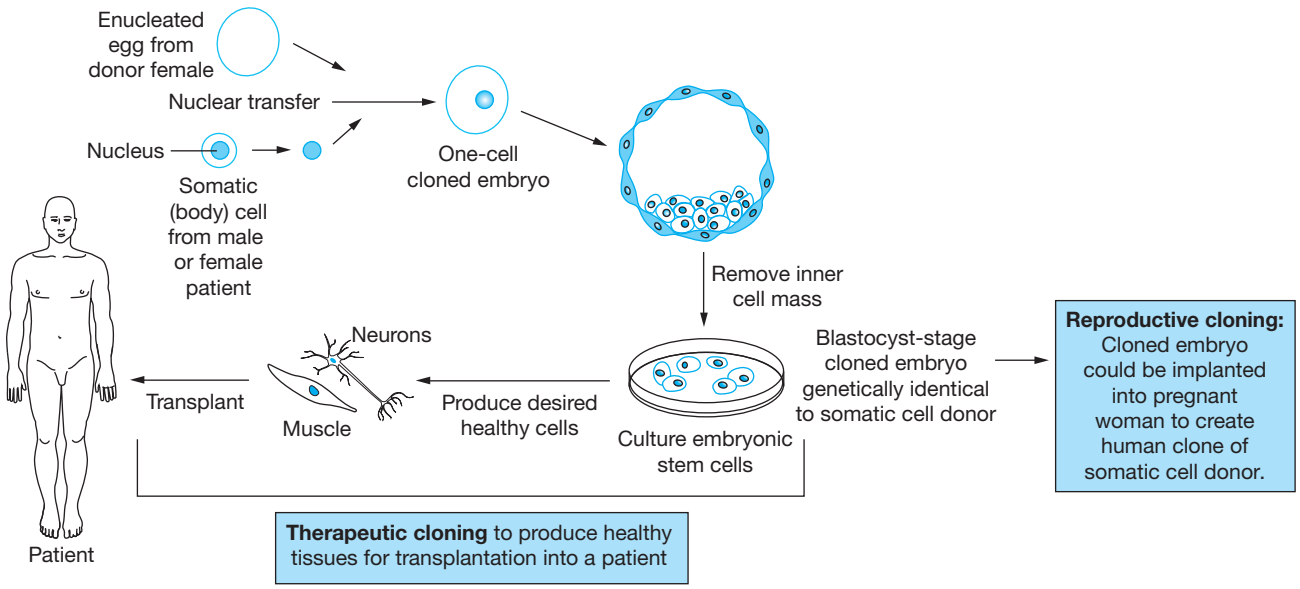


FIGURE 13.6 Theoretical Scheme for Human Cloning or Therapeutic Cloning to Produce Tissues for Transplantation

One argument against the creation of cloned human embryos is not based on the embryo's inherent value as a human or person but on the argument that the creation of human embryos for such purposes could lead to human commercialization, making any human life a commodity to be bought, sold, and used, thus cheapening life in the process. Still others have argued that we should not be creating human embryos in a manner that manufactures human life, the so-called *designer embryos*.

One argument in favor of creating cloned human embryos relies on the assumption that if an embryo is not created by normal means (in this case meaning by fertilization), it is not human. Each of these arguments is based on different definitions of what it means to be human and the value placed on human life. Interestingly, nearly 30 years ago, similar ethical debates about what it means to be human arose when Louise Brown, the first so-called test tube baby, was conceived by IVF. Over the years, IVF has been accepted as a completely ethical way in which to conceive an embryo.

Mitochondrial Replacement Therapy

Approximately 1 in 5,000 humans have a **mitochondrial DNA (mtDNA)**–based disease or are at risk for developing such a genetic disorder. Many mtDNA-based genetic disorders can be detected by genetic testing, but currently no cures for these disorders are available. In 2010, scientists demonstrated that the mtDNA genome can be replaced in the eggs of a non-human primate, the rhesus monkey (*Macaca mulatta*). This was accomplished by transplanting the nuclear genome from an egg of one mother to an enucleated recipient egg of another female with a normal content of mitochondria (**Figure 13.7**). The reconstructed eggs were fertilized and implanted in the uterus of the original mother. The result was the production of "three-parent offspring" containing nuclear DNA from the transplanted maternal nuclear genome of one female, mitochondria from the recipient egg of another female, and a paternal genome from the sperm donor. As these monkeys have progressed into adulthood, so far they appear to be normal.

Referred to as **mitochondrial replacement therapy (MRT)** or three-parent IVF, in 2012 MRT was applied to human eggs. The successful zygotes developed normally to the blastocyst stage, and these embryos were used to develop cultured embryonic cell lines, which were tested in numerous ways. These cell lines behaved and tested similarly to those that had not undergone MRT. Thus, this technology may potentially offer a future treatment option for preventing mtDNA disease transmission in families with known histories of mtDNA-based disorders.

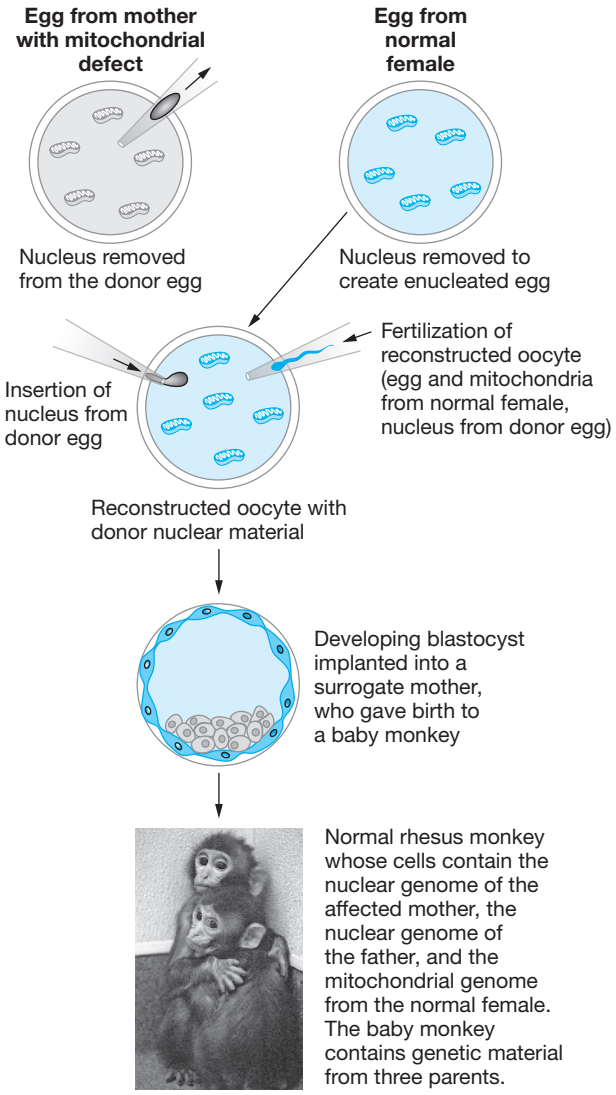


FIGURE 13.7 Mitochondrial DNA Swapping with Oocytes from Rhesus Monkeys The nucleus from one oocyte (left) with defective mitochondria was removed and transferred to an enucleated egg containing normal mitochondria. The father's sperm was injected, and following fertilization, the developing blastocyst was implanted in a surrogate mother. A healthy monkey resulted.

Several technical and ethical hurdles need to be overcome if MRT is to be used for humans. For instance, current techniques do not fully eliminate the transfer of some defective mitochondria into the donor egg. Whether enough defective mitochondria are transferred to affect the health of the embryo is not clear. Also, is it ethically acceptable to produce offspring with genetic contributions from three parents? In 2015, the United Kingdom became the first nation to legalize MRT techniques, designed to prevent children from inheriting

diseases caused by mutations in mtDNA. Thus, women with mtDNA-based diseases could possibly bear healthy children derived from their own nuclear DNA and mitochondria derived from a third party.

Patient Rights and Biological Materials

Consider how you would feel if the following scenario happened to you. You are being treated for a disease such as leukemia and you donate blood, bone marrow, and spleen cell samples for analysis as part of your treatment. You later learn that the physicians treating you developed cell lines from your tissues, which they patented and then used to bring substantial financial rewards. Such situations have in fact occurred and resulted in a number of lawsuits brought by patients. In these cases, patients have claimed that prior to providing their **informed consent** to extract the cells, they were not told that their physicians would conduct research on their cells and that they would benefit financially. Patients and lawyers have claimed that these examples represent a breach of the doctor–patient relationship and an example of a "principle of unjust enrichment."

In most of these cases, patients have sought a share of financial compensation for their cells. In several high-profile cases, courts have ruled that physicians do have a duty to disclose their personal interest in research and potential economic matters unrelated to patient treatment. But courts have also ruled that donors of cells and other biological materials do not have ownership rights of their biological materials, and physicians and scientists who develop patents and receive financial benefits from these materials are not liable for patient compensation.

Many donors are unaware that they do not own their own cells and they relinquish a large degree of control over their tissues when they donate them. Related to this, there have even been situations in which donated sperm samples were used to fertilize eggs and produce children without the consent of the individual who donated the sperm, thus making men fathers without their consent. Overall, U.S. regulations are increasingly favoring written informed consent without coercion for any donor subject. As stem cells and regenerative medicine technologies become more common, undoubtedly the issue of patients' rights regarding biological materials will become even more complex and require greater scrutiny and ethical consideration.

Genetic Information and Genetic Privacy

The Human Genome Project has led to the identification of genes responsible for or contributing to many disease states. As you know, the project has also led to new strategies for genetic testing, treating genetic disease, and for personal genomics. But because the story told in our DNA could become so easily read, there is a growing concern over the privacy of that genetic information. One's DNA sequence can be a truly unique identifier. Researchers especially must take care to guard the confidentiality of those who have donated DNA for sequencing and testing. Because a free flow of scientific information ensures rapid dissemination of ideas and facilitates scientific advances, we next look at some of the ways in which scientists can safeguard genetic information to assure individual research subjects or groups of subjects that their privacy will be maintained. This is important not only to reassure research subjects but also for scientists to maintain the continued trust of people and their willingness to participate in research projects.

A sampling of ethical questions about genetic information include the following (refer back to Chapter 11 for a more extensive discussion and further questions about genetic testing):

- Should we test people for genetic disorders for which there are no effective treatments?
- What ethical obligation do physicians and scientists have to divulge genetic testing results if analysis of a person's DNA reveals mutations unrelated to the original reasons for the test?
- A negative result from a genetic test does not always rule out the future development of certain diseases, nor does a positive result always mean that an individual will develop a disease. How do we effectively communicate the results of genetic tests and actual risks to the person being tested?
- **Incidental findings** are results that were not the primary purpose of the test. For example, if you had an x-ray done because of a chest injury while playing a sport and it revealed a lung tumor (the incidental finding), you would be told you had a tumor. Should individuals be told about incidental findings during genetic testing (currently, there are no requirements to do so)?
- As preimplantation genetic diagnosis and fetal testing become more widespread, ethical questions will continue to arise. When are parents justified in discarding embryos? Is it acceptable for all diseases? For diseases that develop in adulthood? What if a gene only increases the risk of a disease?
- Should individuals who were tested as newborns have access to their genetic information when they become adults? In the future, this could include results from fetal DNA sequencing.
- Should it be possible for someone to be tested for non-disease-related genes affecting such traits as intelligence, skin color, height, or weight?



YOU DECIDE

Genome Hackers and "Anonymous" Genomes Identify Individual DNA Donors

Using only publicly available Internet search tools, scientists at the Whitehead Institute for Biomedical Research in Massachusetts determined the identity of 50 individuals whose genetic information was part of the 1000 Genomes Project. Whitehead researcher Yaniv Erlich demonstrated he could discover the identities of people who participated in sequencing research by analyzing short tandem repeats from Y chromosome sequences publicly available for the 1000 Genomes Project and then cross-referencing data from the study with information on two public genealogy databases. Others have shown that the names and addresses of hundreds of people involved in supposedly anonymous genome projects can be revealed by comparing demographic data in different databases and cross-checking public records containing ZIP codes, dates of birth, and gender information.

Even those who agree to allow their DNA data to be public knowledge may have information about themselves they would not want others to know about. When James Watson, the co-discoverer of DNA structure, had his DNA sequenced he did not want information about whether he had a variant of the apolipoprotein E gene made publicly available. This gene is linked to an increased risk for Alzheimer's disease. For people to participate in genetic testing and genomics studies, they need to trust this work and not fear that their genetic information may be vulnerable and misused.

Erlich published the results of his work but did not detail the step-by-step processes he used. Was this ethical? Why or why not? What should the genomics research community do to ensure the privacy of genetic information in genome studies? What assurances would you expect if you were to have your genome sequenced? What ethical obligations do researchers have to ensure the privacy of genetic information? You decide.

Identifiability, the potential for disseminated genetic data to be associated with (or reveal the confidential identity of) specific individuals, is a major concern, especially as more individuals are subjected to whole-genome sequence analysis or personalized genomics, and, increasingly, precision medicine. As you know, a completed sequence generates a great deal of data, which must be stored electronically and which can be part of an electronic medical record kept by a physician, shared by e-mail, or stored in the cloud. The Health Insurance Portability and Accountability Act (HIPAA) offers protection for personal health information and establishes privacy standards, including for electronic records. Can electronic medical record keeping prevent identifiability even when patients agree to share or release certain aspects of their medical records? The concept of "good genomic practices" has been proposed (similar to good laboratory and good manufacturing practices in other areas of biotechnology) to ensure the proper and ethical handling of genome data.

The HeLa tissue culture cell line is one of the most widely used and distributed cell lines in the world. HeLa cells are named after Henrietta Lacks, a poor mother of five from Baltimore, whose cells were removed from a cervical tumor and cultured without her permission prior to her death in 1951. Recently, the HeLa cell genome was completed and posted online without the consent of the Lacks family, raising concerns that genetic information about the family had been released without their permission, thus violating their privacy.

We have discussed controversies associated with **direct-to-consumer genetic tests** (Chapter 11). As the identification of genetic traits becomes more routine in clinical settings, physicians will have to ensure their patients' privacy. There are significant concerns as to how genetic information could be used negatively by employers, insurance companies, government agencies, or through perceptions acquired by the general public. Genetic privacy and the prevention of genetic discrimination will be increasingly important in the coming years. As we discussed in Chapter 11, the **Genetic Information Nondiscrimination Act (GINA)** prohibits discrimination based on genetics or the improper use of genetic information in health insurance and employment.

Visa, Slack Technologies, and Instacart are three companies in the United States that recently offered subsidized genetic testing for breast and ovarian cancer to their employees. Although recognized as a valuable perk, some claim that it could lead to privacy invasion and employee discrimination for other genetic testing. Obesity testing through genetic tests can indicate those with mutations that create a tendency to hold weight and adversely affect appetite. Type 2 diabetes can cost companies $12,000 per year per employee. One company that provides genetic testing, Newtopia, claims that it does not provide individual test results, only total employee participation and total weight loss

information. Are these safeguards adequate to protect individual privacy and prevent discrimination? What other safeguards are needed?

More or Less Human?

The first successful example of gene therapy involved children suffering from adenosine deaminase severe combined immunodeficiency syndrome (ADA-SCID; Chapter 11). Individuals with ADA-SCID are born without an effective immune system, primarily because of one defective gene in their immune cells. Successful treatments have used adult stem cells from the children's bone marrow, using genetic engineering to add the correct gene and replacing the engineered stem cells back into the patients (refer to Figure 11.16).

Think about some of the ethical considerations associated with gene therapy technology. Gene therapy treatments such as those used to correct ADA-SCID seem wildly successful, so one might think there should be no concerns. However, because these are experimental medical procedures, there are certainly issues of informed consent, safety, and efficacy. One safety issue that has arisen is the potential for cancer formation. As discussed in Chapter 11, an adenovirus vector used for gene therapy led to leukemia in several children. This consequence created considerable concern about future genetic therapies, especially using this particular viral vector. So one ethical consideration might be the risks associated with altering an individual's genetics, especially the specificity of the targeting for insertion of the replacement gene.

Current and proposed somatic gene therapies involve the treatment of existing genetic diseases. However, we should consider the possibility of genetic treatments for conditions in which there is only a genetic *tendency* toward a particular disease and there is no certainty that the disease will occur. One possibility is breast cancer, in which mutation in genes such as *BRCA1* and *BRCA2* increases the risk for development of breast and ovarian cancer. We must consider the difference between a genetic disease and a genetic attribute. For a genetic disease such as ADA-SCID, it is known that an individual with such a genetic problem will definitely develop the disease. However, a genetic *attribute* does not mean that the disease condition already exists or that it will inevitably develop. If we consider attributes that may not necessarily lead to a disease or may not even be associated with an increased risk for disease, we extend our considerations of genetic engineering beyond the therapeutic and begin to contemplate whether we will consider other potential genetic modifications as ethically acceptable, going beyond treatment for an existing condition to alterations and even enhancements of the human genetic composition.

Consider whether **genetic enhancement**, perhaps to increase muscle mass or the oxygen-carrying capacity of red blood cells, might also be considered medically necessary for the health of the individual, even if it were really just a personal preference. The prohibition of such genetic alterations could be regarded as an infringement of individual rights. Because this individual's genome would be altered to something other than the normal, we must reconsider the question of whether he or she would still be considered human as well as just what we might include in that definition. Many questions surround the possibility of **gene doping** in athletes—that is, using gene therapy for genetic modification to enhance human traits important for sports. Genetic techniques applied to animals have demonstrated enhanced muscle function, increased blood cell production and oxygen content, and enhanced metabolism and endurance performance.

Genome Editing and Germline Gene Modifications

The question of what makes an organism human becomes more pressing when we look at the potential for **genome editing.** As we discussed in Chapter 11 and elsewhere in the book, genome editing by approaches such as CRISPR-Cas engender a wide range of ethical considerations. CRISPR makes it possible to edit genomes precisely and with much greater ease than other approaches. CRISPR has already been used to edit the genomes of mice, rats, and monkeys, and few scientists doubt that it would work the same way in people.

Currently, no topic elicits more attention than the dangers of genome editing for human germline gene modification—genetically altering human sperm, eggs, or embryos that will last through the life of the individual and be passed on to future generations. Germline editing can affect every cell in the individual's body, making the genetic alteration inheritable. Potentially, this could mean the elimination of some genetic diseases, because those genes could be removed from the human gene pool. Of course, any problems resulting from the genetic alteration could also be inheritable, as could any genetic enhancements. Consider whether this type of genome editing, which would affect not only the treated individual but also future generations, might be ethically acceptable. Some of the potential outcomes of removing or adding genes in the human gene pool could include the elimination of genetic diseases or disease susceptibility, enhanced



YOU DECIDE **The Same or Different?**

Consider the following scenario. An embryo is created by IVF for a couple who want a child. However, it is known that the couple carry a genetic disease, and there is a 100 percent certainty that the embryo will have the disease. Scientists working with the couple isolate embryonic stem cells from the embryo. In culture, they are able to repair the genetic problem in the cells by genome editing. Some of the embryonic stem cells are then packaged in the form of tetraploid embryonic cells, creating an embryo that is implanted into the woman and carried to term. The infant that is born does not have the genetic disease, and neither will any of that child's offspring. In addition to the several ethical questions that can be discussed regarding this scenario, consider this one: Is the born child the same human/individual/person as the original embryo? Consider utilitarianism and objectivism responses. You decide.

human performance, increased or decreased human life span, and increased cancer risks.

One Nobel laureate has been an advocate of this type of research because it could lead to the development of a "better human being"; but we might pause to consider just what constitutes "better" and whose definition should be utilized in these decisions. Another potential outcome sometimes mentioned for germline genetic alteration could be the creation of a new, superior species of human—the basic concept behind **eugenics.** One potential negative associated with this outcome could be splitting humans into different genetic social classes. In 2015, a group of leading biologists called for a worldwide moratorium on the use of this new genome-editing technique that would alter human DNA in a way that can be inherited.

In the United States, one survey (see the paper by Scheufele et al., 2017, in Chapter 13 references on the Companion Website) reported that approximately 65 percent of respondents saw both somatic and germline editing as acceptable. But when asked about germline enhancement, lower levels of acceptance for germline editing (26 percent, with 51 percent finding it unacceptable) were reported, compared to somatic enhancement (39 percent, with 35 percent finding it unacceptable).

Refer to the You Decide: Human Embryo and Germline Editing in Chapter 11 for an ethics discussion about this topic.

13.3 Economics, the Role of Science, and Communication

We cannot escape the fact that money plays a major role in research decisions. Private investments as well as government funds fuel research and development, and individuals and companies alike seek to use biotechnology for discoveries that will be profitable. Biotechnology is a business, after all.

Not only is scientific research expensive, but projects, companies, and careers may live or die depending on the funding for and profitability of the research. There is clearly not enough money to fund every scientific proposal, whether the proposal is from a university faculty member doing research or a company working to develop a new product. Research funding is often evaluated based on whether it will increase our knowledge about fundamental aspects of science or produce a discovery or product that improves our lives.

Many college and university researchers seeking funding submit research proposals to funding agencies. These proposals are then reviewed by panels of scientists who make recommendations to the funding agencies about which projects should be funded. But the amount of money and even some of the decisions on funding direction also come from those providing the money, especially the government. Most of the decisions are based on the potential success of the science, its novelty, and its potential application to health and general knowledge. However, some of the decisions may also be affected by how well the scientists are known by the reviewers or by political pressure.

We should consider not only how research proposals are evaluated and their chances of success but also the possibility of their contributing to the breadth of scientific knowledge (because in science, it is difficult to know where the next major breakthroughs might come from and what background knowledge might lead to such breakthroughs). Additional considerations are the costs of and access to any treatments that might be produced. Funding for research might be so costly that only the rich would be able to benefit from it, or it might prove to be a colossal waste of



YOU DECIDE **What to Treat Using Gene Therapy?**

In Chapter 11, we discussed some of the unresolved scientific questions surrounding gene therapy. There are also many ethical concerns about gene therapy and the risks associated with it. Do the patient and his or her family members understand the risks associated with gene therapy trials? For instance, Jesse Gelsinger (recall from Chapter 11 that Jesse died as a result of his gene therapy) had a relatively mild form of a disease that was being successfully treated with medication. Should he have been a gene therapy trial participant? Gene therapy is currently very expensive. Should everyone have access to gene therapy regardless of the cost? What types of disease and conditions should be subject to gene therapy (for example, only deadly diseases)? What about treatable diseases for which gene therapy would be used so patients would not have to take daily but effective medications? What about cosmetic conditions such as baldness? You decide.

money and time; it might simply suggest that other avenues of research would have a better chance of producing useful results. Because the possible sources of funding for research are limited, funding decisions may have to be weighed in terms of whether funding one type of research might decrease the funding of another, potentially more successful line.

A biotechnology company with a potentially profitable idea typically has to seek funding from venture capitalists and other investors willing to fund the research and development (R&D) necessary for product development. If such investors are risk-averse but seeking a profit, who will fund risky experimental research that is expensive but has great potential for saving lives or leading to some new and useful technology?

We could even extend the discussion of economics to ethical issues such as whether it is acceptable to pay women to donate eggs for stem cell research. The lack of quality eggs left over from IVF, coupled with the increased demand for eggs as stem cell research becomes more common, is driving a need for human eggs. In 2009, New York became the first state in the USA to allow public money to be used in this way—up to $10,000! Some of the ethical issues here revolve around the risk associated with hormonal stimulation, potential complications, and the invasive approaches required to harvest eggs. Many economic and noneconomic questions revolve around this issue:

- Should women be paid as egg donors?
- If so, should there be limits on such payments?
- Should there be a limit on how many times a woman can donate eggs over a particular period of time or should she be allowed to serve as a "serial" donor, giving dozens of eggs over time?
- Will paying for eggs create a market wherein women use egg donation as a form of employment?

Intellectual property rights are also important as we consider the ethical implications of research and its funding. The **patenting** of intellectual property—whether it be applications involving particular DNA sequences, new cell types, GMOs, or even embryos—can be lucrative for the discoverers but may also pose ethical and scientific dilemmas. For example, consider the possibilities for a human gene that has been isolated and characterized—and then patented by the discoverer. The person or company holding the patent could require that anyone attempting to do research with the patented gene pay a licensing fee for its use. Should a diagnostic test or therapy result from the research, more fees and royalties might be required. This could make it difficult or expensive to carry out research on some genes or limit the clinical use of the patented gene.

Most recently, significant controversy has arisen over who should be awarded the first patent for use of the CRISPR-Cas system in genome editing. In 2014, the U.S. Patent and Trademark Office (PTO) awarded the first patent to Feng Zhang of the Broad Institute and MIT for application of CRISPR-Cas to eukaryotic cells. But a patent application by Jennifer Doudna of the University of California–Berkeley and Emmanuelle Charpentier, who co-discovered the CRISPR-Cas system as a genome editing tool, was submitted 7 months earlier than Zhang's. Zhang, however, had applied for a fast-track patent, which awarded the patent 6 months after he applied. The U.S. PTO decided that Zhang's application was distinctly different from Berkeley's more broad patent on genetic engineering by CRISPR. The CRISPR patents have led to many companies licensing the technology for a range of applications in medicine, agriculture, and industry, and by 2017 the collective value of these companies had already exceeded $100 million.



FIGURE 13.8 Ethical Decision Making Is an Essential Part of Science

Some physicians have already complained that they cannot afford to pass on the charges of certain genetic tests to their patients owing to licensing restrictions. However, limiting or preventing patents for genes or genetic tools could remove the incentive for pursuing such research, especially for companies that hope to profit from their research. Compile a list of potential ethical problems associated with patenting of genes or cells and consider possible alternatives or compromises that might be reached to allow research to continue.

Ethical questions regarding biotechnology also touch on science's role in society (**Figure 13.8**). Science and technology have provided discoveries and inventions that make our lives happier and healthier. But it is important to consider whether scientists should have unlimited freedom for research. It is often difficult to know what the source of the next major breakthrough will be, and sometimes, new discoveries arise from unexpected sources and paths that many scientists may not even consider worth following. We must determine how science can best serve society. The whole concept of regulating something as unpredictable and free-flowing as scientific discovery is difficult. It is also hard to know who should decide these questions—scientists because they know how science works, policy makers because they must set the rules, or society because it is most affected by the decisions. Consider what types of regulations might best serve the needs both of science in furthering discovery and of society in furthering health care and ethical interests. Bioethics becomes critically important to the biotechnology industry because ethical discussions and debate are often the driving force for making laws in general (laws that govern the population's behavior and certainly laws that will regulate the biotechnology industry) once it becomes a general consensus of a particular population that a law is necessary.

Accurate, honest communication is vital to the success of science in general and biotechnology in particular. Scientists must be willing and able to communicate openly and candidly with other scientists but also, and more importantly, with the general community. A public that cannot understand and appreciate the importance of the contributions that science makes to their daily lives will not support its endeavors. Straightforward communication is necessary, without overstating the potential of the research or the imminence of the results and without using confusing terminology. If the public and the policy makers believe they have been misled, the outcomes might be disastrous for both science and society. If the public believes scientists are insensitive to ethical questions in their research, scientists will have a hard time earning the public's trust. Consideration of all the facts is important. Integrity in research is essential, but so is integrity in the communication of science.

Many of these ethical questions involve difficult decisions affecting not only yourself but other lives as well, both now and into the future. It would always be easier if the decisions, especially the tough ones, could be made for you. But the latter approach assumes that the decision maker has all the facts or has objectives that match your own. It also involves giving up your individual freedom in making those decisions, as well as your individual responsibility. With freedom comes responsibility—if you make choices, you are responsible for those choices, and your choices affect not only you but others as well. A person who is clever knows the best means to achieve an end; a person who is wise knows which ends are worth striving for.

MAKING A DIFFERENCE

The human immune system holds the key to preventing and controlling a broad spectrum of infectious diseases, cancers, autoimmune diseases, and allergies. The Human Vaccines Project is a public-private collaboration to identify human immune responses associated with optimal vaccine protection. Insights gained from the project will guide the development of potentially improved vaccines against diseases such as influenza, dengue, HIV, and other infectious illnesses, as well as cancer. By bringing together leading vaccine researchers, institutions, and biopharmaceutical companies, and harnessing recent technological advances in molecular and cellular biology and bioinformatics, the project may

enable accelerated development of vaccines and immunotherapies for some of the most devastating diseases of our time. The most recent company to join the project is Pfizer. What ethical questions do you think will arise if this project leads to new vaccines that can be administered to the public to prevent disease? Do you think bioethicists should be part of the application regulations?

QUESTIONS & ACTIVITIES

Answers can be found in Appendix 1.

1. What are the two leading approaches to ethical thought, and how do they differ?
2. "Last September a little girl from California named Molly received a lifesaving transplant of umbilical cord blood from her newborn brother, Adam. Molly, who was 8 years old, suffered from a potentially fatal genetic blood disorder known as Fanconi anemia. But what made the procedure particularly unusual was that Adam might not have been born had his sister not been sick. He was conceived through *IVF*, and physicians specifically selected his embryo from a group of others for implantation into his mother's womb after tests showed that he would not have the disease and he would be the best tissue match for Molly." Was this ethical?

   Adapted from Kline RM. Whose blood is it, anyway? *Scientific American*. 2001;284(4):42–49.
3. When drug companies find out that a drug target is not responding to their new drug, do they have a legal obligation to inform other drug companies?
4. Make a table with a list in one column of all the possibilities you can think of for interactions a GM plant might have with the ecosystem. Then, in the second column, list the possible outcomes, good or bad, for each of the listed interactions.
5. If a GM crop was engineered to produce growth hormone, what are some of the possible ethical outcomes and questions that must be anticipated?
6. As you learned in Chapter 6, the National Bioengineered Food Disclosure Standard passed in 2016 requires biotech foods containing GMOs to be labeled. Explain the pros and cons of labeling GM foods and possible ethical issues associated with this discussion.
7. If a food allergen can be removed from food products by using a recombinant plant food source and a consumer is harmed by the unwillingness of a producer to utilize the GM source, could the company be found liable for the harm created?
8. If you support releasing GM organisms such as plants or insects into the environment, develop a plan for controlling the spread of these organisms.
9. If a company develops a vaccine for a deadly disease without a cure but early studies show that the vaccine is effective in only about 40 percent of patients, should the company wait to bring the vaccine to market while it works to improve the vaccine's efficacy (which may not be possible) and people suffering from the disease die, or should it be available immediately?
10. Is there a difference between a human being and a person? Why or why not?
11. Suppose that gene therapy could be used to lower levels of cholesterol and saturated fat in the blood, thus allowing people to consume fat-rich fried foods with little risk of heart disease. However, this treatment might not work for everyone, and there would be no way of knowing in advance who would benefit. As a result, some people would inevitably die prematurely from heart disease. Should such gene therapy be approved for use in humans? Answer this question by briefly describing how a bioethicist who believes in a utilitarian approach might view this scenario, and contrast this perspective with a deontological viewpoint.
12. If a single cell is removed from an embryo *in vitro* and kept to be used for future organ regeneration but the embryo is not harmed and can continue to develop normally, is this an ethical use of human embryonic tissue?
13. Michelle, a 21-year-old doctoral candidate in sciences at one of the best universities in the United States, is an attractive woman. To earn a little extra cash, she responds to an ad in a local newspaper from a fertility clinic seeking women eager to donate eggs for infertile couples seeking to have children. Michelle will be paid for these eggs, about $5,000 each. Over a period of several years she donates eggs for infertile couples and eggs for somatic-cell nuclear transfer procedures used to create embryos for deriving embryonic stem cells. During this time, Michelle earns around $45,000 for her donated eggs. She will never know if any of her eggs actually led to a successful pregnancy. Is this ethically OK with you? Is it acceptable to pay someone for her eggs? What information do you think potential egg donors should be told about before or after making a donation?
14. There is never enough money available, even from the government, to support every scientific research proposal that is submitted to funding agencies. How should decisions be made about

which of these proposals are a waste of money and which will yield discoveries that may change human life forever? When competing scientists are applying for research grants, what principles should guide decisions on how the available tax money is to be spent? Should ethical concerns be a part of such funding decisions?

15. What is mitochondrial replacement therapy (MRT) and how can it be used to treat mtDNA diseases? Is producing children by MRT and IVF ethical? Use objectivism or utilitarianism approaches to answer this question. Based on what you know about MRT, is "three-parent babies" an appropriate way to describe offspring born from this approach? Explain your answers. Do an Internet search with the search terms Nuffield Council on Bioethics and MRT. Where is the Nuffield Council located, and what statements have the Council made about the ethics of MRT?
16. GINA (Genetic Information Nondisclosure Act) covers medical insurance but not life insurance. Should life insurance be included? Why or why not? Explain.
17. What ethical issues are associated with genome editing and germline gene modification? Describe the risks and ethics of editing human embryos.
18. What ethical issues can be associated with patenting a biotech product or application? Often, companies attempt to gain approval for patents that are overly broad in scope. Why might this be advantageous for a company? Are there instances in which patenting can interfere with the progress of research and development? Explain your answers.
19. At a school in Newtown, Connecticut, in 2013, a man shot 20 children, 6 school staff, his mother, and himself. In the wake of this horrific tragedy, geneticists at the University of Connecticut in Farmington analyzed the shooter's genes. What ethical issues might be associated with genetic variations that might be revealed about this individual? Describe your answers.
20. Based on what you have learned about biotechnology, what do you think are the top 5 or 10 issues or applications that raise the most ethical concerns? Which one of these issues is of most interest to you? Why is this so?

Visit www.pearsonglobaleditions.com for the Companion Website

The Companion Website includes quiz questions, flashcards, study tools, Internet and literature references, and biotech career information, including Career Profiles contributed by professionals working in the biotechnology industry.

This case study has been included to give you an opportunity to become familiar with a research example applicable to this field of study. After you have analyzed the data and answered the questions, you can compare them to those provided to your instructor with the text materials.

CASE STUDY

The GTEx Project, Cadavers, and Family Rights

The Genotype-Tissue Expression (GTEx; see **https://www.gtexportal.org/home/**) project is a $100 million effort funded by the NIH in the USA to examine gene expression variations in different parts of the body and across multiple individuals. While families or individuals gave consent for cadavers that have been donated for the project, most families did not realize that they would not have access to genetic findings from GTEx, even those that may affect donors' families by revealing predispositions to genetic diseases.

Questions

Answers can be found at www.pearsonglobaleditions.com

1. Should family members be informed of incidental findings?
2. Should disclosure of any experimental results be a requirement of providing consent to allow a loved one's body to be used as a cadaver for research?
3. If you were in this situation, would you be unhappy to know that researchers might have access to genetic information about you and your family that is relevant to your health but not disclose this information to you?
4. Dead people are not considered human subjects. What ethical questions does this case study raise for you about research involving cadavers?

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APPENDIX 1

Answers to Questions & Activities

Answers for Case Studies can be found at www.pearsonhighered.com/biotechnology

Chapter 1

1–2. Open-ended activities; answers variable.

3. Bioremediation.

4. Precision medicine involves prescribing a treatment strategy based on specific information about the individual's particular disease. For example, the treatment strategy could be based on the genetic profile of a patient—fitting the drug to the patient's genetics. An RNA or DNA sample from a patient with a medical condition can be analyzed to determine whether the genes that person expresses match the genetic profile of a particular disease process. If they do, a specific treatment approach can be designed according to the best-known treatment strategy based on the patient's genetic profile, with drugs that target specific protein(s) encoded by those genes.

5. Open-ended activity; answers variable.

6. No product can leave a biotechnology company until it passes rigorous tests by the internal unit called quality control (QC). Quality assurance (QA) is responsible for monitoring everything that enters and leaves a company to ensure product safety and quality and tracing sources of problems identified by complaints as specified by regulatory agencies, such as the U.S. Food and Drug Administration (FDA). QA and QC measures are important for every step of research and development (R&D) as well as for product manufacturing, including packaging and marketing.

7–10. Open-ended activities; answers variable.

11. Open-ended questions; answers variable about potential advantages and disadvantages and ethical issues. But one primary reason why scientists are interested in this approach is that it could reduce the need to grow and farm animals for meat production.

12–13. Open-ended questions; answers variable.

14. Biotechnology companies often begin with a discovery by a scientist working in academia. For example, a gene or protein is found to be important in a disease process and may have potential applications as a recombinant DNA product. Starting a biotechnology company, a "start-up" company, to develop this protein as a product then requires significant initial funding to do the R&D, such as preclinical research in animals and then ultimately FDA clinical trials (if the protein is to be used in humans). Sometimes a company starts with initial "seed money," which may come from a few individual investors. Then if the product shows potential, the person or persons starting the company will seek venture capital (from corporations or groups wishing to provide money for early-stage, high-risk, potentially high-reward-earning companies) or "angel" investors (individuals who provide capital for a start-up in exchange for ownership and profit making in the company).

15. The pharmaceutical industry is primarily involved in producing, selling, and marketing drugs for treating humans. This is one similarity to many (medical) biotechnology companies; however, the way in which pharma as opposed to biotech makes such drugs differs. Pharma companies primarily use synthetic chemical processes to make drug compounds, whereas biotech companies use living cells to produce a drug. Pharma companies also often produce medical devices. In recent years many large pharma companies have acquired smaller biotech companies; as a result, the many traditional pharma companies are also doing biotechnology R&D. For example, the pharma company Roche purchased the biotech company Genentech.

16. Open-ended. No specific answer. Students sign up for biotech e-news.

17–20. Open-ended activities; answers variable.

Chapter 2

1. Refer to Table 2.1 and Figure 2.2 for a comparison of eukaryotic and prokaryotic cells and important differences between these types of cells.

2. Genes are sequences of DNA nucleotides—usually from 1,000 to around 4,000 nucleotides long—that provide the instructions (code) for the synthesis of RNA. Most genes produce mRNA molecules encoding proteins, but some genes produce RNAs that do not code for proteins. Genes are contained in chromosomes, which are tightly packed coils of DNA and protein. Chromosomes enable cells to separate DNA evenly during cell division. Chromosomes contain multiple genes, and the number of genes in a chromosome can vary depending on its size.

3. 1′ carbon.

4. The complementary strand will be an antiparallel strand with the sequence 3′ – TAACGTCCTTGGGAAT – 5′.

5. Chargaff's rules state that the percentage of thymines is approximately equal to the percentage of adenines in a genome; the percentages of guanines and cytosines are also roughly equal. This is true because DNA consists of complementary base pairs. Thymines in one strand form hydrogen bonds with adenines in the opposite strand; guanines form hydrogen bonds with cytosines. This knowledge could be applied as follows: If DNA contains approximately

15 percent thymines, then it would also contain approximately 15 percent adenines. Combined, these bases account for approximately 30 percent of the DNA in the bacterium; therefore, the rest of the DNA (70 percent) consists of equal amounts of guanines and cytosines. Therefore, guanine would comprise approximately 35 percent of the genome and cytosine approximately 35 percent.

6. DNA is a double-stranded molecule located in the nuclei of cells. Each strand of DNA is made of building blocks called *nucleotides*, which consist of a pentose sugar, phosphate group, and base. DNA is the inherited genetic material of cells; its genes contain instructions for the synthesis of proteins. RNA is copied from DNA through a process called *transcription*. RNA is a single-stranded molecule, which is an important structural difference compared with DNA. RNA contains not only nucleotides—including the base uracil, which replaces the thymine present in DNA—but also a different pentose sugar (ribose) than that in DNA (deoxyribose). Several major types of RNA are transcribed, including mRNA, tRNA, and rRNA. tRNA and rRNA are included among a major category of non-coding RNAs (ncRNA) that are involved in translation, mRNA splicing, and the regulation of gene expression, among other roles. After transcription, RNA molecules move into the cytoplasm, where they are required for protein synthesis (translation).
7. Refer to Figure 2.8 for a summary of the major components involved in DNA replication.
8. Open-ended question; answers variable.
9. Eleven codons, six amino acids are coded.

   Start Stop

   5′-GGCACCAUGCGUCGAAAAUCAAAGUGAA-ACAAAAA-3′.

   The amino acid sequence is methionine—arginine—arginine—lysine—serine—lysine. Remember that actual mRNAs and their encoded proteins are much longer than shown in this example and that stop codons do not code for an amino acid.
10. (a) Only the bottom strand produces a functional mRNA with a start codon. This sequence is 5′ – GGGAUGCCCUGGACGCGGCGUUAGAU – 3′. The amino sequence of a polypeptide produced from this sequence is:

    Methione—proline—tryptophan—threonine—arginine—arginine

    (b) mRNA copied from the bottom strand of DNA with a T inserted between bases 10 and 11 is 5′– GGGAUGCCCU**A**GGACGCGGCGUUAGAU-3′. This will result in an A (bold) inserted in the mRNA sequence transcribed, which now creates a stop codon (UAG) in the mRNA, which now creates a stop codon (UAG) in the mRNA, which will produce a truncated (shortened) peptide of only 2 amino acids, methionine—proline.
11. Messenger RNA (mRNA) is an exact copy of a gene. It acts like a "messenger" of sorts by carrying the genetic code in the form of codons, encoded by DNA, from the nucleus to the cytoplasm, where this information can be interpreted to produce a protein. Ribosomal RNA (rRNA) molecules are important components of ribosomes. Ribosomes recognize and bind to mRNA and "read" along the mRNA during translation. Transfer RNA (tRNA) molecules transport amino acids to the ribosome during protein synthesis. Each tRNA contains an amino acid and an anticodon sequence that base pairs with codon sequences during translation.
12. Biologists use the phrase "gene expression" to talk about the production of mRNA from a particular gene. From mRNA, cells translate proteins that are responsible for many aspects of cell structure and function.
13. ncRNAs are often involved in regulating gene expression but also play other roles, such as in mRNA splicing. Commonly known ncRNAs include transfer RNA and ribosomal RNA, both of which are involved in translation. Refer to Table 2.4 for a list of ncRNAs and their functions.
14. *Gene (expression) regulation* is a broad phrase used to describe ways in which cells can control gene expression. Gene regulation is an essential aspect of a cell's functions. Cells use many complex mechanisms to regulate gene expression. In this chapter, we highlighted transcriptional control as a regulatory process. Gene regulation allows cells to tightly control the amount of RNA and protein they produce in response to particular needs of the cell. Refer to Figure 2.13 for an overview of gene regulatory mechanisms in eukaryotes.
15. Operons are clusters of genes that are typically regulated simultaneously under the control of a single promoter. Operons are most prevalent in bacterial genomes. Bacteria can use operons to carefully control the expression of gene products (proteins) involved in similar processes, such as nutrient metabolism (the lactose operon is a classic example), depending on the demands of the cell.
16. Transcription regulation involves controlling gene expression by regulating the amount of mRNA produced (transcribed). It is one of the most frequent ways that eukaryotic cells control gene expression. Often, transcriptional regulation involves specific transcription factors that can stimulate gene expression by helping RNA polymerase bind to and transcribe genes. Other proteins can act as negative regulators of transcription.
17. Yes. This is regulating gene expression. Gene expression can often be finely controlled by cells, depending on the amount of RNA and protein product required. Hence, genes can be turned on (increased expression) or turned off (decreased expression—rarely are genes not expressed at all; usually expression may be decreased relative to expression at other times).
18. Mutations that affect protein structure and function include nonsense mutations (which affect a codon by creating a stop codon), missense mutations (which change a codon to code for a different amino acid with different properties than the original amino acid coded for), and frameshifts created by insertions or deletions. Silent mutations have no effect on protein structure and function because these mutations of a codon do not affect the amino acid coded for by the mutated codon.
19. The epigenome comprises structural aspects of DNA that do not involve direct mutation of a nucleotide or changes in DNA sequences. The epigenome includes methylation of histone proteins on chromosomes, acetylation, noncoding RNAs that bind to the genome, and other modifications. Epigenetic modifications of chromosomes affect gene expression. In recent years we have learned that epigenetic

modifications play a role in disease; thus, these modifications are being targeted for new therapeutic approaches to treating disease.

20. CRISPR-Cas provides a novel and fairly simple way to target a specific sequence in the gene for removal or replacement (genome editing). The implications of genome editing are far reaching, from editing animal, plant, and bacterial cells for research to creating genetically modified food to gene therapy in humans.

Chapter 3

1. Gene cloning is the copying (cloning) of a gene or a piece of a gene. The terms *recombinant DNA technology* and *genetic engineering* are often used interchangeably. Technically, recombinant DNA technology involves combining DNA from different sources, whereas genetic engineering involves manipulating or altering the genetic composition of an organism. For instance, ligating a piece of human DNA into a bacterial chromosome is an example of recombinant DNA technology, and placing this piece of recombinant DNA into a bacterial cell to create a bacterium is considered genetic engineering.
2. Yes, 5′-GAATTC-3′ (with its complementary sequence 3′-CTTAAG-3′), the restriction site for enzyme *Eco*RI.
3. Primers are short single-stranded DNA oligonucleotides usually around 20 to 30 nucleotides long. They initiate the DNA synthesis and are complementary to nucleotides flanking opposite ends of the target DNA to be amplified.
4. Refer to Section 3.1.
5. The gene sequence cloned from rats could be used to create primers that might be used to amplify DNA from human cells in an effort to amplify the complementary gene in humans. If one were successful in obtaining PCR products from this experiment, the PCR products could be sequenced and compared with the rat gene to search for similar nucleotide sequences (suggesting that these genes are related). Also, PCR products could be used as probes in library screening experiments to find the full-length human gene or as probes for Northern blot analysis to determine if mRNA for this gene is expressed in human tissues.
6. Approximately 32,768 (2^{n-1}, where n = number of cycles).
7. In RT-PCR, mRNA has to be converted into DNA by the enzyme reverse transcriptase because PCR cannot directly amplify RNA. In qPCR, DNA templates are used in conjunction with one of two real-time quantification approaches, TaqMan Probes or SYBR Green that emit light that is proportional to the number of PCR products. By combining RT-PCR and qPCR, one can monitor the amount of RNA produced by a tissue exposed to a certain medicine or toxin and quantitatively study what genes might be turned on or off to respond to the exposure.
8. Searching for *diabetes* reveals a list of sequences for genes related to insulin-dependent and noninsulin-dependent diabetes mellitus. Clicking on any of the highlighted numbered links will take you to informative pages providing great detail about each gene. Searching with the accession number 114480 reveals information on genes for familial breast cancer.
9. This piece of DNA can be cut once by *Hind*III at the end of the sequence and once by *Eco*RI at the beginning of the sequence. There are no cutting sites for *Bam*HI in this sequence. A search for all enzymes in the database reveals approximately 20 restriction enzymes that can cut this sequence. This demonstration should provide you with an appreciation of how powerful computer programs can be for helping molecular biologists analyze DNA sequences.
10. Corrected answers are available on the website as students work on the questions.
11. It is the first 30 bp of a human insulin gene.
12. Open-ended; answers variable but the outline should generally include a process that involves fragmenting chromosomes into smaller pieces for sequencing and then sequence alignment using bioinformatics software. Third-generation sequencing approaches may involve sequencing without fragmenting.
13. Genome analysis has rapidly advanced in large part because of NGS and TGS approaches (along with bioinformatics to analyze and compare sequence data) that have resulted in computer automated, high-throughput techniques to sequence longer stretches of DNA more quickly, more accurately and at lower costs than Sanger sequencing methods. As a result, the number of genomes being analyzed has increased dramatically due to advances in sequencing technologies.
14. Refer to Section 3.4 for a summary of key findings of the Human Genome Project.
15. MyHeritage provides a genetic testing service, including DNA testing for ancestry analysis.
16. The "omics" revolution of modern biology refers to the rapid expansion of new disciplines of research that have resulted from genomics studies, as reflected by new terms using the suffix -omics or -ome. Generally, such studies involve a large-scale comprehensive analysis. For example, proteomics involves the study of all the proteins in a cell or tissue; metabolomics involves the study of all the proteins and metabolic products involved in a metabolic process, such as carbohydrate (sugar) metabolism. Because of genomics projects such as the Human Genome Project, many other areas of biology have progressed to where scientists can study entire categories of molecules simultaneously or in their entirety. Open-ended answers for omics disciplines and applications.
17. Sequencing an individual genome (personal genomics) can reveal (diagnose) genes involved in disease. Depending on the gene and the disease, it may then be possible to target such genes at the DNA, RNA, or protein level in precise ways to treat or cure diseases.
18. Open-ended question; an important thing to consider would be the company's policies regarding data sharing.
19. Systems biology involves studying relationships between genomic data, proteomic data, metabolic pathways, and

other pathways to better understand how cells function. Understanding complex interactions in a cell involving genomic, transcriptomics, and proteomics, for example, is helping companies decipher key components of a cell involved in disease, which will aid in development of novel treatments (for example, drugs targeting specific pathways).

20. Synthetic biology involves engineering cells with specific purposes. For example, a synthetic genome can be created with specific genes that can be used to create cells with novel properties. One possible application is to create microorganisms that can be used to synthesize biofuels; other applications include creating microbes engineered for bioremediation, the synthesis of new biopharmaceutical products, or the production of chemicals and fuels from sunlight and carbon dioxide.

Chapter 4

1. The public protein database website has archived submitted structures that can be compared.
2. You could subject the bacterial cultures to increasing temperatures and substrate variances, selecting the survivors until a population has evolved with the desirable qualities, then purify your enzyme.
3. Replacing active sites with structures that bind ligands provides information about the binding structure of the active site.
4. The amino acid sequence would dictate the cDNA sequence, which is not the "natural" sequence, since it lacks introns.
5. Prion proteins change normal protein structures so that they aggregate (and accumulate) and cannot be removed by body mechanisms. If the prion process can be understood, therapies can be designed to test effectiveness against Alzheimer's disease.
6. Usually living animal bioreactors are preferable if large quantities of a protein that has significant glycosylations are needed. Industrial bioreactors are faster to produce proteins that are needed in smaller quantities and where other post-translational modifications can be made.
7. The affinity column should have the bound substrate or ligand of the enzyme in its matrix.
8. MS, HPLC, and X-ray crystallography
9. The pI would tell you the most stable pH for the protein, and whether it had an affinity for a cationic or anionic column matrix, or its relative size for a SEC column.
10. If the protein (structure) could be modeled after the shape of the chemical structure that is needed and produced biologically (for testing), then it is likely to be less expensive and faster to produce than the slower chemical synthesis process.
11. XNAzymes are produced from artificial DNAs and are unique structures not produced by natural evolution. Degrading XNAzymes is more difficult for degradation enzymes to find sites for catalytic digestion.
12. The letters stand for the 20 amino acids (See Appendix A2); the colors represent the relative abundance of the different peptides that match EGFR.
13. Cryo EM builds a 3D structure from multiple 2D images and can be used to compare natural protein structures to those constructed from amino acid sequences.
14. Smart bandages have DNA, RNA that directs synthesis of penicillinase, other enzymes that degrade bacteria, and those that can produce vaccines to infectious agents present in the specific area detected by the embedded sensors.
15. Yeast cells are transfected with two plasmids: the bait (protein of interest fused with the DNA-binding domain of a yeast transcription factor) and the prey (a library of cDNA fragments linked to the activation domain of a transcription factor). Transcription of reporter genes signaling interaction does not occur unless bait and prey interact with each other and form a measurable transcription factor. The interaction between proteins can be detected by the presence of the products resulting from the reporter gene expression.
16. Histidine and cysteine have available sulfides for this post-translational modification.
17. You could raise a group of mice with the PRNP mutation and use a sensitive analysis method (e.g., mass spectrometry) to detect the presence of the unusual protein in brain cells.
18. Signal peptides have been identified for the proteins secreted by *B. subtilis.* You would need to find out if they are produced for your protein of interest so you would know that it would be secreted.
19. You would need to replace the salt with a buffer that matches the pI of your protein (probably by dialysis or diafiltration).
20. Multiple sites on the primary antibody for attachment of the secondary antibody would increase the enzyme reaction for the color signal produced for detection.

Chapter 5

1. There are several important structural differences between prokaryotic and eukaryotic cells. Bacteria and Archaea are prokaryotes; eukaryotic cells include plant cells, animal cells, fungi, and protozoa, which are structurally more complex than bacteria. Prokaryotic cells are smaller than eukaryotic cells, do not contain a nucleus, have relatively few organelles, and contain a cell wall. Prokaryotes also have smaller genomes than eukaryotic cells; the prokaryotic genome usually consists of a single circular chromosome. Prokaryotic cells have served many important roles in biotechnology. Bacteria are used as hosts in gene cloning experiments, recombinant proteins produced by bacteria are important for research, some proteins are used in medical applications, microbes are used for manufacturing many foods and beverages, and bacteria are used as sources of antibiotics and as hosts for the production of subunit vaccines, among many other applications.
2. Yeasts are unicellular fungal eukaryotes, and bacteria are prokaryotic cells. Most yeasts have larger genomes than bacteria. Through fermentation, yeasts are used to make breads and dough as well as for brewing beers and wines. Yeasts serve many important roles in research. The yeast two-hybrid system is a valuable technique for studying protein interactions. Because the yeast genome contains many genes similar to human genes, geneticists study it for clues about the functions of many human genes.
3. Open-ended question; answers variable.

4. DNA binds to cells when cells and DNA are chilled on ice. A brief heat-shock step at about 37°C to 42°C causes DNA to enter cells. Electroporation has the advantages of being a very quick procedure, enabling many samples to be processed quickly. Moreover, much smaller amounts of DNA can be used than in calcium chloride transformation, and electroporation can also be used to introduce DNA into plant and animal cells.
5. Fusion proteins are generated by inserting a cloned gene for a protein of interest into an expression vector that allows production of a protein fused to a tag protein such as green fluorescent protein or maltose-binding protein. This fused protein can then be passed over an affinity column, which will bind the tag portion of the fusion protein and allow the fusion protein to be isolated from a bacterial extract of proteins.
6. Anaerobic microbes are those microorganisms that do not require oxygen to convert sugars into energy (in the form of ATP). Under anaerobic conditions, some microbes use lactic acid fermentation; others use alcohol (ethanol) fermentation to derive energy. Lactic acid and ethanol are waste products of anaerobic fermentation that are important components of many foods. Lactic acid is found in cheeses and yogurts; ethanol is found in alcoholic beverages such as wine and beer.
7. One way to control alcohol content is to carefully regulate the amount of oxygen added to a fermentation bioreactor while making wine. When facultative anaerobes (microorganisms that can use cellular respiration or fermentation) are used for fermentation, they will, in the presence of oxygen, degrade sugars by aerobic cellular respiration and produce less alcohol. In the absence of oxygen, such microbes will degrade sugars by fermentation, and thus the alcohol content of the wine will be higher.
8. Lantibiotics are a class of gene-encoded peptides that contain intramolecular ring structures, introduced through unusual thioether amino acids, such as lanthionine and methyllanthionine. Lantibiotics bind to lipid II, a precursor molecule in the bacterial cell-wall biosynthesis. Odilorhabdins (ODLs) are produced by enzymes of the non-ribosomal peptide synthetase gene cluster of the bacterium *Xenorhabdus nematophila*. Unlike lantibiotics, ODLs bind to ribosomal subunits that have not been exploited by any antibiotics previously.
9. All vaccines are designed to stimulate the immune system of the recipient to make antibodies or activated T cells to a pathogen—for example, a bacterium or virus. During antibody production, immune memory cells, such as antibody-producing B cells and memory B cells, are also produced. By creating antibodies and memory cells in the recipient, the goal of vaccination is to provide the recipient with protection against a particular pathogen should he or she be exposed to it. Three major types of vaccines are subunit vaccines, which consist of molecules from the pathogen (usually proteins expressed by recombinant DNA technology); attenuated vaccines, which are made from live pathogens that have been weakened to prevent replication; and inactivated vaccines, which are prepared by killing the pathogen and injecting dead or inactivated microbes into the vaccine recipient.
10. Open-ended questions; answers variable.
11. Open-ended questions; answers variable.
12. A preventative, or prophylactic, vaccine such as Gardasil is designed to provide immune protection before you are exposed to a particular pathogen but will typically not protect or cure you of a preexisting condition.
13. HIV/AIDS, tuberculosis, and malaria.
14. The study of microbial genomes can help scientists in many ways. The sequencing of genomes can reveal new genes, including genes that may be used in biotechnology applications (e.g., genes that encode novel enzymes with important properties for various commercial uses, including bioremediation). Scientists can study genes of disease-causing pathogens (including potential agents of bioterrorism) and then use this knowledge to help fight disease. The study of genomes can help scientists learn more about bacterial metabolism, the relatedness of bacterial strains, and lateral gene transfer (the sharing of genes between species). Metagenomics involves analyzing genomes from environmental samples. Important potential applications include identifying previously unknown microbes, genes, and proteins with commercially valuable properties; learning about the impact of environmental changes on microbial community; and studying the genetic relatedness of communities of microbes in various environmental samples.
15. These were breakthrough experiments in part because they demonstrated that creating a synthetic genome that could be transferred into a cell and be functional is possible.
16. Refer to pages 176–177.

17–20. Open-ended activities; answers variable.

Chapter 6

1. The T-DNA contains genes for encoding enzymes that cause the plant to create specialized amino acid derivatives that are used by *A. tumefaciens* for crown gall formation and are not metabolized by the host organism, resulting in a tumor.
2. One drawback is that facilities with large sterile bioreactors are more expensive to build than engineered plants, and require considerable time to begin producing products. One benefit is that genetically engineered plants can produce pharmaceutical proteins in quantity that can be harvested. Patients with Gaucher's disease can also benefit from the rapid production of their missing enzyme from genetically engineered plants if other sources fail.
3. Evolutionary pressure will select those with genetic variations that convey any resistance, and these will be the survivors that transmit this resistance to their offspring, increasing their numbers in future generations.
4. It is very likely because several countries (e.g., USA, Brazil) have ruled that certain CRISPR-cas9-edited plants should receive fast-track, deregulated approval. This is because such plants have been transformed by inactivating genes and not by introducing foreign genes from some other organisms. Such GM plants may elicit less resistance in the consumer population leading to broader general acceptance.
5. In hybrid stacking of genes, parental lines—each containing one of the genes—are crossed and then an offspring that will inherit both the genes is selected. In the case of molecular stacking, the genes are introduced via genetic engineering, either simultaneously or sequentially.
6. Less-developed countries see the need for greater food production for their populations, and realize that GM innovations can provide them.

7. The Arctic Apple was produced by gene deletion, with no "foreign" genes added, and received de-regulated status.
8. Probably yes, since there are few alternatives to them in drought areas, and other drought-tolerant plants have been present for some time.
9. *Agrobacterium tumefaciens* infects the plant cell through a large, circular double-stranded Ti plasmid. T-DNA, i.e., the portion of the Ti plasmid that integrates into the plant's chromosomal DNA, is modified by removing the genes that cause tumor formation and replacing them with the genes to be inserted into the plant.
10. Markers can be chosen that are expressed before the engineered variation is observed, verifying that genes inserted with the marker will also be expressed.
11. They create pores in the midgut lining, inhibit midgut proteases that could destroy the CRY proteins, and stimulate autodigestion of the insect gut.
12. Chloroplast gene inserts are limited to the photosynthetic plant parts and are not dispersed, limiting environmental transmission.
13. Gene deletion by CRISPR-Cas editing does not introduce genes from other sources that need additional testing, but deletes genes, making them as safe as non-edited plants.
14. The viral DNA that is introduced is for the virus coat, and is non-infectious. The coat protein that is produced usually induces immunity to the virus in the plant.
15. Extensive testing and notification to the USDA and isolated field trials of at least 120 days.
16. This controversy changes, and the search will result in different arguments.
17. Proteins that are needed in larger quantities and on a daily basis by patients (like antibodies, blood products, cytokines, growth factors, hormones, and recombinant enzymes) as well as proteins that are needed to deal with an immediate threat (e.g., rapid ramp-up production).
18. The engineered tomato has the normal (sense) DNA sequence to the PG protein and an inserted complementary (antisense) sequence. When they are transcribed, they combine and cancel PG production.
19. If the parent plants containing the genes have already been approved for commercial use by regulatory agencies, the offspring of hybrid stacking will inherit that approval.
20. Modifying gene promoters can result in greater or lesser expression of proteins that are beneficial commercially.

Chapter 7

1. Veterinary medicines are a valuable product of genetic engineering, and all human drugs must be tested in animals in phase trials required by the FDA.
2. Immunotherapy directs the immune system to target cancer cells while other drugs are attacking the cancer in other ways.
3. Goats breed more quickly and are cheaper to raise than cattle and can release proteins in milk.
4. Animal disease genes with similarity to human disease genes can be used to research for variations associated with disease progression, severity, and drug response.
5. The targeting of immune cells to cancer or other diseases can often be observed through the transparent embryo.
6. By blocking the virus replication mechanism, vaccines against each new flu virus are not needed.
7. Biologic drugs needed in large quantities can be produced in animals without the expense of industrial bioreactors, but must be expressed in the eggs or milk that can be harvested routinely.
8. By combining the appropriate tissues in the proper arrangement so that they mimic the natural human organ relationship, it is possible to evaluate efficacy and safety of drugs before clinical trials at earlier stages in drug development.
9. The humane use of animals in required FDA testing must demonstrate efforts to reduce the number of animals, replace them with lower species, and refine the tests to produce less pain and discomfort for the animals in the tests.
10. Melanoma, as a cancer, can continuously divide, which provides the needed replication of the hybridoma cells that produce the monoclonal antibody.
11. Regulations for approval of gene therapy for pets is less stringent than for humans, and results can be helpful to advance human gene therapy.
12. Organs on a chip are produced from relevant human tissue and allow testing in vitro prior to testing in humans. Animal testing provides valuable data, but does not mimic human organ testing as closely as testing in the relevant tissue, before mandatory phase trials.
13. Connecting individual organs in a way that reflects how interconnections in the relevant animal (e.g., humans) mimic the situation of what most likely will occur in the whole animal provides valuable data before mandatory phase testing.
14. Genetic mutations associated with pet disease provide a "proof of concept" opportunity for research to determine if they have a counterpart in humans.
15. Mitochondria from a different source can be screened for mutations that exist in the original donor cell and can be ruled out as a cause for disease transmission (i.e., mitochondrial).
16. Animal handlers can contract mutated versions of bird flu, which can lead to flu epidemics in humans. Blocking the replication of this virus in chickens reduces the likelihood of transfer to humans.
17. By transferring human embryonic stem cells into animal embryos that have been blocked from organ development by an embryological trigger, the ESCs can produce more human-like organs for study.
18. Pet medications cost $3–5 million, compared to $3.2 billion for human medicines. Pet medications often have potential counterpart applications in humans, reducing the development time/cost.
19. Fluorescent in situ hybridization can be used to identify genetic traits that have been chosen for breed expansion if the fluorescent tags are used in chromosomal analysis of semen or eggs of the potential breeding partners.

20. Comparing drug dosing at different intervals (or continuously) can indicate response rate benefits from different protocols, and alleviate testing (or failures) in humans who are subsequently treated with the drug.

Chapter 8

1. No, only the primers for genes that have been associated with the disease will be needed to PCR those areas to look for key mutations.
2. Touch DNA analyzes the skin cells left behind in a crime scene. A person's DNA may unwittingly travel from one location to another through secondary transfer, without the individual directly touching the surface where the DNA eventually ends up. The presence of such DNA can lead to inaccurate investigation outcomes.
3. Using treatment data from successful responders to specific treatment protocols, find gene variations that are related and develop primers for these genes to sequence for comparison before treatment.
4. Traditional swabbing only picks up DNA found in large quantities, whereas wet vacuum system can pull enough microscopic trace DNA to run a complete DNA profile.
5. It evaluates more (77) genetic markers instead of 1 (for HER 2+).
6. PCR is an exponential process that can amplify a sequence up to a million-fold. So, it is reasonable to begin with only a small amount of DNA from the food sample.
7. One from each of the two parents' relevant STRs.
8. PCR tests are extremely sensitive to contamination by foreign DNA at the crime scene and within the laboratory.
9. Usually, the errors are contaminations of collected material from the crime scene.
10. Parentage disputes primarily, when nuclear DNA profiles are unclear or disputed.
11. Mutations that occur after conception and during their lifetimes can produce genetic variations.
12. More sites will increase the accuracy of DNA fingerprints and were considered necessary based on evidence.
13. STRs vary in number between individuals, but the number of STRs for a given loci is inherited from your parents. This creates a genetically identifiable connection for comparison to the parents' DNA profiles.
14. The cost of care for breast cancer would be reduced by early detection, although the information is prohibited by the Genetic Information Non-disclosure Act (see Chapter 12).
15. The high heat from the disaster created mostly DNA fragments, reducing the accuracy of CODIS fingerprinting methods in some cases.
16. Contaminating DNA produces false results and would be more likely in a non-lab setting. Safeguards would be needed and validated for expansion to rapid DNA testing at police stations.
17. M-Vac uses an application of sterile solution and vacuums it from the touched surface, followed by concentration of the material for DNA amplification. Cotton swabbing has greater potential for contamination than this method.
18. Adding standard DNA profiling (CODIS sites) and X chromosome STRs to the mtDNA tests increases accuracy of paternal DNA tests.
19. Proteomic testing for food fraud is not commonly used due to the protein degradation caused by cooking, which makes it unreliable.
20. Hospital testing for novel transcripts, gene fusions, single nucleotide variants, and indels provides DNA profiles for specific DNA variations associated with specific diseases. Its usage provides valuable indications and potential early detection of disease.

Chapter 9

1. Open-ended activity; answers depend on the processes described.
2. Indigenous microbes are microorganisms, such as bacteria that live naturally (often called native microbes) in a particular environment. Because indigenous microbes are adapted to a particular environment, they are often ideally suited for bioremediation because they have developed adaptive mechanisms for survival in the presence of different pollutants. Many bioremediation approaches rely on techniques for stimulating (such as fertilizing) indigenous microbes to improve their degradative capabilities and accelerate cleanup processes.
3. Oxidation involves the removal of one or more electrons from an atom or molecule, and during reduction, an atom or molecule gains one or more electrons. These reactions often happen together and are thus called redox reactions. Redox reactions can alter the structure and properties of a molecule. For a chemical pollutant, oxidation or reduction can make the chemical harmless by changing its properties. Redox reactions are frequently involved in bioremediation.
4. The addition of fertilizers such as carbon, nitrogen, phosphorus, and potassium is often an important step in many bioremediation processes. Fertilization, also called nutrient enrichment, stimulates the growth and activity (metabolism) of microorganisms in the environment. These microorganisms, usually bacteria, also divide more rapidly to create more bacteria. By stimulating the metabolism of bacteria and increasing their number at a contaminated site, pollutants are usually degraded more rapidly. Adding oxygen to a contaminated site is effective when the microorganisms involved in the cleanup are relying on aerobic biodegradation.
5. Refer to Phytoremediation in Section 9.2.
6. Refer to Section 9.3.
7. Endocrine disruptors are chemicals in the environment that disrupt the endocrine system by mimicking or blocking naturally occurring hormones in organisms, including humans. They are particularly prominent in water, entering the environment from municipal wastewater, as these drugs (for example, by-products of birth control pills) are found in human wastes. Certain industrial chemicals are also endocrine disruptors. Harmful effects of disruptors on the endocrine systems of aquatic organisms, their development, and reproduction are among concerns for both aquatic organisms and potentially for humans.
8. Not all countries require builders to disclose the history of the land to potential future owners. However, it is not recommended to develop a previously contaminated site

into a residential area. In this case, due to the leaking underground storage tanks, the soil matrices and the groundwater aquifers beneath the site might have been heavily polluted. Contaminated groundwater source is difficult to be completely cleaned up even after successful bioremediation. Trace amounts of gasoline may go undetected and the health effects of this may not be fully known. A major problem with redeveloping bioremediation sites for residential use is that it may take many years (or generations) to determine if residents are experiencing health effects from chemicals remaining at the site. Even if residents experience some health problems, it is often very difficult to determine if these effects are caused by pollutants at the site.

9. One approach might involve studying lead-containing structures to find out whether bacteria are growing on them. For instance, one could study lead pipes left in the environment and, over time, determine if bacteria are growing on these pipes. Bacterial growth on a lead surface could be an indication that the bacteria have developed a way to avoid the toxic effects of lead. This could be a sign that these cells may be capable of degrading lead. Such bacteria could then be isolated and experiments using microcosms could be designed to test whether the bacteria could be used to degrade lead.

10–13. Open-ended activities; answers variable.

14. Open-ended activity; answers variable. The Rena Environmental Recovery Monitoring Program has been undertaken, which assesses the recovery of the Bay of Plenty coastal environment after the grounding of *Rena*. So far, no bioremediation activities have been reported. Bioremediation by oil-degrading microorganisms could have happened in the polluted region.

15. It was reasonable to expect that zones of hypoxia might develop because, as aerobic microbes degraded oil, they would consume oxygen in local areas of the ocean. Declining oxygen levels in the water could cause hypoxia, resulting in fish kills. However, given the great depth of the spill, wave and wind action was likely responsible for causing sufficient mixing of the water, thus preventing large zones of hypoxia and resulting fish kills from occurring.

16. Open-ended activity; answers variable.

17. Open-ended activity; answers variable but a primary criticism was to question whether these microbes actually metabolize arsenic and incorporate arsenic into their DNA, as claimed by the scientists publishing this work.

18–20. Open-ended activity; answers variable.

Chapter 10

1. Refer to Table 10.1.

2. Examples of benefits include providing food sources, improving populations of fish and shellfish, creating farming industries in noncoastal regions that do not have fishing or seafood industries, and providing fish for recreational fishing. Examples of problems include some species that are not suitable for aquaculture or too expensive to raise through farming, diseases and illnesses that might decimate fish stocks because most farmed fish are genetically similar, wastes from fish farms that could create pollution problems, and threats to the genetic potential of native species when escaped farmed fish enter the population.

3. Many aquatic biotechnologists believe that biotechnology will play an important role in improving finfish and shellfish populations in the future. One obvious way in which this may be done is to use bioremediation approaches to detect and clean up environmental pollution. Another approach may involve using biotechnology to learn about the pathogens that cause disease in aquatic organisms and develop ways to prevent or treat such diseases. Transgenic and polyploidy approaches may be used to continue to produce aquatic organisms with improved resistance to disease. In addition, aquaculture may be used to grow species that can be stocked, so as to increase dwindling populations of aquatic organisms.

4. One advantage of switching to vegetable-based diets is that the number of baitfish consumed by farmed species would be decreased. Concern has been raised that farmed fish raised on vegetable-based diets have a bland, unnatural taste. Others claim these fish taste less "fishy" and that is desirable.

5. Fish pollution refers to the escape of farmed fish from aquaculture into natural ecosystems. Refer to the "You Decide" in Chapter 10 for discussion about concerns such as interbreeding with native stocks and competing with native stocks, habitat destruction, and so on.

6. Open-ended activity.

7. In the past, rainbow, brown, and brook trout were raised and stocked in rivers, lakes, and ponds for fishermen to enjoy as a "put and take" fishery. In 2013, a bacterial disease called furunculosis, caused by the bacterium *Aeromonas salmonicida,* occurred in the hatchery. The disease was thought to originate from birds feeding on wild fish with the disease and then introducing the disease into the hatchery either through their feces or by feeding in hatchery ponds and raceways. As a result, more than 200,000 fish had to be euthanized instead of stocked. Raceways were steam cleaned and disinfected. Measures were taken to keep birds off the property. Some brook and brown trout were vaccinated to protect them from the disease. The hatchery also focused on rearing and stocking rainbow trout, which have a natural immunity to *Aeromonas salmonicida.*

8. As discussed in Chapter 8, transgenic animals contain genes from another source. The transgenes may be from related species (for example, introducing the salmon gene for growth hormone into trout) or from very different species (for example, introducing the *luciferase* [*lux*] gene from bioluminescent marine bacteria or fireflies into salmon). Transgenic fish can be created using a number of different techniques, but one prominent technique includes microinjecting the transgene into blastocyst-stage embryos to allow incorporation of the transgene into embryonic tissues. Polyploid fish such as triploids, which contain three complete sets of chromosomes, are usually created by either chemical or electrical treatment of sperm or egg cells to produce diploid gametes that can be used to fertilize haploid gametes.

9. This GM salmon is the first genetically modified animal approved by the U.S. Food and Drug Administration for

human consumption. It is a transgenic Atlantic salmon expressing the growth hormone gene from (larger) Chinook salmon so it grows much faster than non-transgenic salmon. Refer to Section 10.3 for controversies surrounding GM salmon.

10. Open-ended activity; answers variable.
11. Federal agencies such as the FDA and the U.S. Department of Agriculture (USDA) believed that there was no need to regulate the GloFish because it posed no threat to public health, since tropical fish are not used for food. The U.S. Fish and Wildlife Service determined that the fish posed little threat to the environment because unmodified zebrafish have been sold in the United States and released into the wild with no known consequences. Many animal rights groups and others have strongly protested development of the GloFish primarily because they perceive it to be an abuse of genetic engineering to create a transgenic species solely for the purpose of its enjoyment as a pet. Concerns have also been raised about the potential release of this genetically modified (GM) pet into the wild. Some people have also raised the point that the GloFish has provided a precedent for the unregulated development of other GM pets and GM animals that may pose environmental threats. Antibiotech groups also cite this as an example to raise public skepticism about biotechnology because if the GloFish can go unregulated, what other areas of biotechnology may go unregulated?
12. Open-ended activity; answers variable.
13. Open-ended activity; answers variable. Answers should, however, mention that organisms that grow under unique or extreme environmental conditions (such as pressure, heat, and ocean depths) are among the most highly studied for the identification of unique molecules that might be potentially valuable. For instance, mussels, which adhere tightly to structures to withstand the constant pounding of waves, produce unique molecules in their byssal fibers that provide adhesive strength.
14. Open-ended activity; answers variable.
15. Open-ended activity; answers variable.
16. The rare amino acid in byssal fibers is l-dopa, which is not usually found in most proteins. It contains a chemical group called catechol, which consists of a benzene ring with two hydroxyl (–OH) groups. The catechol group can form hydrogen bonds with negatively charged (anionic) surfaces on rocks. In laboratory conditions, positively charged ions (cations) interfere with catechol binding. But in saltwater it appears that byssal proteins contain cations that displace cations in saltwater, and allow the proteins to be attracted to and bond to anionic surfaces of rocks.
17. Biofilms are accumulations of living organisms such as bacteria that coat a surface. For example, biofilms occur on teeth, heart implants, and intravenous lines. In marine environments, algae and mollusks growing on the hulls of ships exemplify biofilming. Many aquatic organisms combat biofilming by producing compounds that kill or inhibit the growth of biofilming microbes. Therefore, scientists are interested in identifying these compounds so that they can be used in commercial applications to combat biofilming.
18. Aquatic organisms, especially filter feeders such as clams and mussels, have the potential to help bioremediate water because they can filter and accumulate toxins (as they do naturally). Aquatic algae and bacteria are also valuable for bioremediation.
19. The limulus amebocyte lysate (LAL) test relies on enzymes from the blood of the horseshoe crab (*Limulus polyphemus*) to detect toxins called endotoxins (lipopolysaccharides) produced by toxic microbes. It is used to check the sterility of instruments such as medical devices.
20. Open-ended activity; answers variable.

Chapter 11

1. The Human Genome Project has revealed the location of all human genes, including those involved in normal conditions as well as in disease processes. Identifying these genes was a key to understanding how genes function and how genes are involved in the development of certain diseases. The identification of disease genes has led to genetic tests that can be used to screen individuals for the likelihood that they will inherit a particular genetic condition or for disease-causing genes. Specific forms of medical treatment (precision medicine) have been developed in the form of drugs designed to affect the specific genes and proteins involved in genetic diseases and conditions that have a genetic basis. Such treatments also include gene therapy strategies.
2. The *MECP2* gene encodes a protein MECP2 that modifies chromatin through DNA methylation and helps to turn off or downregulate expression of other genes. It is abundantly expressed in neurons and plays a role in brain function by helping neurons develop and maintain synapses (connections for transmitting electrical impulses). In humans, mutation in *MECP2*, found on the X chromosome, causes Rhett syndrome, an autism-spectrum disorder and progressive neurodegenerative disorder that causes mental retardation, particularly in females. In animal models, mutation of *MECP2* in macaque monkeys results in symptoms associated with autism, and these GM monkeys pass the mutation to offspring. Scientists are hopeful that models such as this may prove valuable for treating Rhett syndrome and autism-spectrum disorders.
3. Amniocentesis and chorionic villus sampling are ways to sample tissue from developing fetuses for genetic testing. Fetal cells or adult tissue (usually white blood cells, or skin, cheek, or hair cells) can be tested by karyotype analysis, including FISH, to check for abnormalities in chromosome number and structure. Molecular techniques such as allele-specific oligonucleotide (ASO) analysis, or microarrays, can be used to detect defective genes in human cells. Increasingly, DNA sequencing, and possibly RNA sequencing, will play a greater role in genetic testing.
4. (a) Prognostic (but could be diagnostic if a person is showing symptoms of Parkinson's disease); (b) Diagnostic; (c) Diagnostic; (d) Diagnostic.

5–6. Open-ended questions; answers variable.

7. The PMI is designed to transform medical treatment through an integrated approach of research, technology, and policies that provide individualized treatments. Biotechnology has major roles in the PMI, from the

identification of biological, behavioral, and environmental influences on disease through the research, development, and applications of new treatments. Precision medicine focuses on individualized treatments that are highly personalized based on individual differences in genetics, lifestyle, environment, and other factors. This is opposed to many current treatment strategies that are one size fits all, whereby most people with the same disease condition are treated the same way. One PMI initiative under way is to recruit at least 1 million American volunteers from whom a range of biological sample data, including DNA sequencing, microbiome, epigenome data, and other data, will be collected and analyzed to provide new insights into health and disease at individual, community, and population levels.

8. Pharmacogenomics is customized or personalized medicine created by analyzing a person's genetics and designing a drug or treatment strategy that is specific for that particular person based on the genes involved in that person's medical condition. Pharmacogenomics is a form of precision medicine that has existed long before the term *precision medicine* came into use. Gleevec and Herceptin for treating CML and breast cancer, respectively, are examples.
9. Open-ended activity; answers variable.
10. Humira is an FDA-approved monoclonal antibody treatment for rheumatoid arthritis, forms of psoriasis, Crohn's disease, ulcerative colitis, and other conditions.
11. Open-ended activity. Refer to Figures 11.14 and 11.15.
12. The purpose of gene therapy is to deliver therapeutic genes into humans to treat or cure disease conditions. Gene therapy may also involve genome editing by techniques such as CRISPR-Cas or techniques for gene silencing, such as RNAi. Ex vivo gene therapy involves removing cells from a patient, inserting a gene or genes into these cells, and then injecting or implanting them into the patient. Treatment of SCID is an example of ex vivo gene therapy. In vivo gene therapy occurs within the body by delivering genes directly into the body (for example, treating retinal conditions by direct injections into the eye). Some techniques for delivering therapeutic genes include using viruses as vectors for gene therapy, injecting naked DNA, and using liposomes to deliver genes.
13. Gene therapy scientists face many challenges, including ensuring the safe delivery of genes (especially when viral vectors are used), targeting the therapeutic gene to the correct cells and tissues, and finding ways to get sufficient expression of the therapeutic gene to cure the given condition. Similar challenges are presented by genome editing, such as ensuring that only target gene sequences are edited.
14. Antisense RNA technologies, RNAi and miRNAs, are gene silencing techniques that could be used to inhibit a gene involved in a disease process. Refer to Section 11.5.
15. A primary advantage of genome editing approaches such as CRISPR is that they provide for the removal or replacement of a sequence such as gene in a highly specific manner by targeting very specific areas of the genome. These techniques are also relatively easier to use than other methods developed to date and they can be used *in vivo* and *in vitro*.
16. Open-ended question; answers variable.
17. Regenerative medicine involves creating cells, tissues, and organs that can be used to repair or replace dead or damaged tissues in a person. This could include growing organs in vitro for implantation into patients.
18. Embryonic stem cells (ESCs) are isolated from early embryos at the blastocyst stage. Blastocysts are usually derived from embryos left over from in vitro fertilization procedures. To isolate and culture ESCs, the inner cell mass is dissected out of the blastocyst, and these cells are then grown in a tissue culture dish. In contrast, adult-derived stem cells (ASCs) are isolated from mature adult tissues. These cells are found in small numbers in many tissues of the body, such as muscle and bone. A small sample (biopsy) of adult tissue is removed (for instance, a needle can be used to perform a biopsy on a sample of adult bone marrow stem cells), and ASCs can then be grown in culture. By treating ESCs or ASCs with different growth factors, scientists can stimulate stem cells to differentiate into different types of body cells. Amniotic stem cells are harvested from the amniotic fluid surrounding a developing embryo. Induced pluripotent stem cells (iPSCs) are produced by reprogramming somatic cells to become stem cells. Stem cell biologists envision many ways in which stem cells can be used to treat disease, and various applications are currently in clinical trials. As examples, stem cells could be implanted in the body to replace damaged tissue, stem cells have been used as vectors for the delivery of therapeutic genes, and they could be used to grow tissues and organs in culture for subsequent transplantation into humans, among other applications.
19. Open-ended questions; answers variable.
20. Refer to Table 11.2 for a comparison of therapeutic and reproductive cloning.

Chapter 12

1. International regulations serve the function of coordinating state action by creating shared principles and harmonizing standards. Other examples include promoting predictability in state behavior, facilitating information sharing, reducing the costs of multiple and separate interactions, and establishing dispute settlement mechanisms.
2. Resource sharing has led to mutual benefit: the people or states using the resources as well as the people or states providing them both enjoy the benefits generating from the utilization of such resources.
3. It is important because the same biological agents and toxins have a dual use. They might be used to cause harm, but they also protect against natural disease threats.
4. A component that seeks to limit or constrain certain activities in biotechnology would be the restriction on the transboundary movements of LMOs in the Cartagena Protocol and one that plays a facilitative role in the application of biotechnology would be the guidance on utilization of new tools and techniques as seen in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*.
5. Sixty-four food and forage crops that are universally dependent for food security.

6. The benefit-sharing system of the PIP framework incorporates a centralized stockpile of vaccines, which are distributed in the early stages of an outbreak to the countries that are otherwise unable to access vaccines during a pandemic.
7. Some of the issues to consider would be whether 'utilization' only applies where there are joint acts of research and development, or whether it applies to both in isolation as well. Does it extend to basic research with no planned commercial application or research undertaken solely for taxonomic or other conservation purposes?
8. It might be difficult to apply state sovereign rights to some forms of genetic resources that are not constrained by geographical boundaries. For example, establishing which country has rights over which particular viral genetic resource could be very difficult as these resources can spread and evolve rapidly.
9. The conditions of the United States regarding "dolphin-safe" labeling on tuna products were not internationally accepted.
10. By allowing the WTO members to issue compulsory licenses so that patented medicines can be exported to countries lacking domestic manufacturing capacity.
11. Under the PCT System, if the applicant submits the filing in any one member state, the filing occurs simultaneously in any other member states the applicant designates.
12. The Nagoya Protocol requires states to inform indigenous peoples and involve them in decisions about access to traditional knowledge associated with genetic resources.
13. Setting budgets and work programs and determining policy.
14. One health approach can bring together multiple sectors dealing with human, animal, and plant health for a more comprehensive understanding of the risks and the benefits of biotechnology and for achieving better public health results.
15. Good scientific evidence may be needed to effectively implement certain regulations. International regulations can also have significant implications for scientific practice, so scientists can help in raising awareness about the nature of these implications.
16. You may come across genetically modified organisms, organisms with significant historic value, important reference samples, vaccines strains, etc.
17. Open-ended question; answers variable.
18. Having good surveillance and monitoring systems so that outbreaks are identified in their early stages, having well-trained veterinary services, and sharing information internationally so that other states can prepare effectively.
19. The document raises awareness among scientists about how responsible working practices will promote the benefits of life sciences research while minimizing risks to global health security in case of serious safety or security failure. The focus on the scientific community and their actions is thought to be a useful complement to 'top-down' governmental regulation.
20. Open-ended question; answers variable.

Chapter 13

1. Utilitarian approach and deontological (Kantian) approach. Utilitarianism tries to weigh all possible outcomes and produce the greatest good for the greatest number, so that the end result justifies the means. The deontological approach starts with certain absolutes that cannot be crossed in order to maintain an ethically correct outcome, no matter how much good might be achieved.
2. Most bioethicists concluded that this was ethically appropriate because Adam was not physically harmed by providing cord blood for his sister Molly. In addition, no evidence indicated that Adam's parents treated Adam poorly or as if he had been conceived only to save his sister. However, what do you think about parents selecting the offspring they want based on genetics (a process that is not uncommon when doing in vitro fertilization)?
3. From an ethics perspective, one could argue that for the overall benefit of improving human health, drug companies should share such information to help facilitate the development of drugs that cure. However, there is no legal requirement for companies to do so.

4–6. Open-ended activities; answers variable.

7. No, currently there is no such legal basis for this liability claim against a food company.

8–10. Open-ended questions; answers variable.

11. A bioethicist following a utilitarian thought process would propose a "greatest good for the greatest number" answer. If this form of gene therapy can help lower cholesterol and saturated fat levels in humans and allow most people to enjoy fatty foods, then it is ethically all right to assume the risk that some smaller number of people may die prematurely because of this therapy.

12–14. Open-ended question; answers variable.

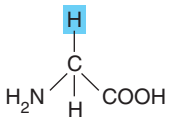
15. See Chapter 13 for a description of MRT. As a student, you should decide if you think producing children by MRT is ethical, and if you think "three-parent babies" is an appropriate name. The Nuffield Council is based in the United Kingdom and has generally declared MRT ethical. Here is one substantive quote from a 2012 Nuffield report on MRT: "Due to the health and social benefits to individuals and families of living free from mitochondrial disorders, and where potential parents express a preference to have genetically-related children, on balance we believe that if these novel techniques are adequately proven to be acceptably safe and effective as treatments, it would be ethical for families to use them, if they wish to do so and have been offered an appropriate level of information and support."
16. Open-ended question; answers variable.
17. Many of the ethical issues associated with genome editing are focused on applications in humans, such as: Is it ethical to modify the genome? If so, under what circumstances (i.e., to cure a disease or for genetic enhancement)? Germline edits are transmitted to offspring, and so this also raises questions about designer babies and producing genetic modifications that can be inherited. Editing human embryos is highly controversial. Refer to Chapter 11 and Chapter 13 for detailed discussions on embryo editing.

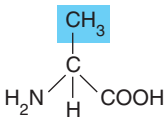
18–20. Open-ended activities; answers variable.

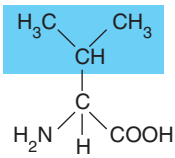
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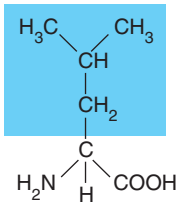
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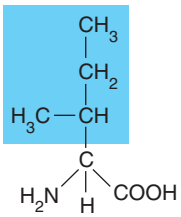
Nonpolar Amino Acids

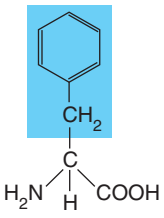

Glycine
Gly
G

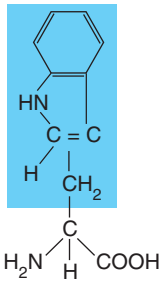

Alanine
Ala
A

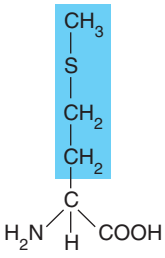

Valine
Val
V

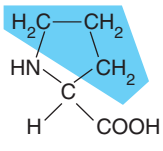

Leucine
Leu
L


Isoleucine
Ile
I

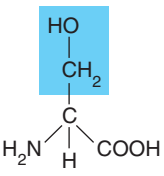

Phenylalanine
Phe
F

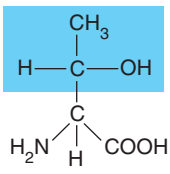

Tryptophan
Trp
W

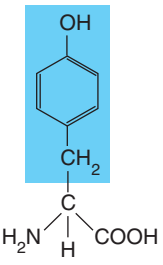

Methionine
Met
M

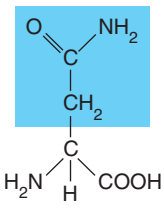

Proline
Pro
P

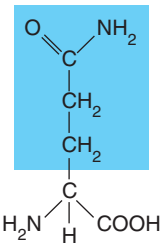
Polar Amino Acids

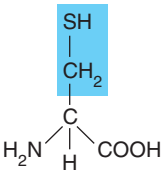

Serine
Ser
S


Threonine
Thr
T

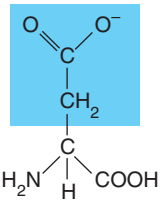

Tyrosine
Tyr
Y

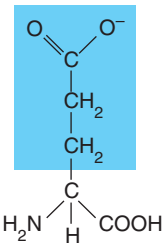

Asparagine
Asn
N


Glutamine
Gln
Q

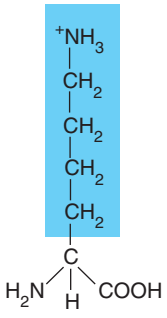

Cysteine
Cys
C

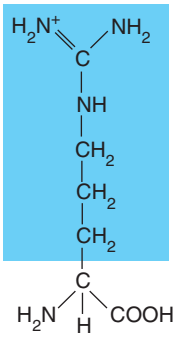
Negatively Charged (Acidic) Polar Amino Acids

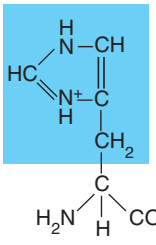

Aspartic acid
Asp
D


Glutamic acid
Glu
E

Positively Charged (Basic) Polar Amino Acids


Lysine
Lys
K


Arginine
Arg
R


Histidine
His
H

The 20 Amino Acids of Proteins Up to 20 different amino acids can be used to synthesize a protein. Each amino acid has a common structure but side chains (shaded) vary from amino acid to amino acid. These side chains determine the chemical properties of amino acids. Notice that some amino acids are nonpolar while others are polar and there are also negatively charged and positively charged amino acids. Both the three letter code and single letter code of each amino acid is also shown.

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Figure 1.19: Biotech Careers.

Figure 1.20: Organizational Structure of a Typical Medium-Size Biotechnology, Ernst & Young, Beyond Borders: The Global Biotechnology Report, 2011., Ernst & Young LLP.

Table 1.1: Data based on the most resource source available at the time of publication:Morrison, C., and Lähteenmäki, R. Public biotech in 2016-the numbers. Nature Biotech., 35: 623–629, 2017.

Table 1.3: Adapted from Genetic Engineering & Biotechnology News, Revenue based on preliminary results reported by companies.

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Figure 7.2: From "A mightier mouse with human adaptive immunity" by Marie Kosco-Vilbois, Volume 22, Issue 6, 2004, *Nature Biotechnology* by Nature Publishing Company.

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Figure 9.3: Pearson Education Inc.

Figure 9.17: Based On "Plants tackle explosive contamination" by Richard B Meagher, Volume 24, Issue 2, 2006, *Nature Biotechnology* by Nature Publishing Company.

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Figure 10.11b: South Carolina Department of Natural Resources. ©Duane Raver.

Table 10.1: Data from Goldberg, R., and Triplett, T. (2000): Something Fishy. Environmental Defense, www.environmentaldefense.org.

Table 10.2: Based On Goldberg, R., and Triplett, T. (2000): Something Fishy. Environmental Defense, www.environmentaldefense.org.

Figure 11.5: Kleinsmith, Lewis J.; Kish, Valerie M., *Principles of Cell and Molecular Biology*, 2nd Ed., (c) 1995, p. 110. Reprinted and Electronically reproduced by permission of Pearson Education, Inc., Upper Saddle River, New Jersey.

Figure 11.7: Based On Fortney K, Dobriban E, Garagnani P, Pirazzini C, Monti D, et al. (2015) Genome-Wide Scan Informed by Age-Related Disease Identifies Loci for Exceptional Human Longevity. PLOS Genetics 11(12): e1005728. https://doi.org/10.1371/journal.pgen.1005728.

Figure 11.8: National Institute of General Medical Sciences.

Figure 11.11: "Nanotechnology takes aim at cancer" by R. F. Service. From *Science* 310: 1132–1134, November 2005.

Figure 11.13: Based On Klug, L.R. and Heinrich, M.C. PDGFRA antibody for soft tissue sarcoma. *Cell* 168, Feb 9, 2017. P. 555, http://www.cell.com/cell/pdf/S0092-8674(17)30113-7.pdf.

Figure 11.14a: Based on Vicki Brower.April 1, 2015.The CAR T-Cell Race.The Scientist.

Figure 11.14b: Based On Yadev, M., and Delamarra, L. *Science*, 352:1275-1337, 2017.

Figure 11.15: Based on Olweus, J. Nature Biotech, 35: 520–521, 2017. Figure 1, right panel. Nature Publishing Group.

Figure 11.17: Klug, William S.; Cummings, Michael R.; Spencer, Charlotte A.; Palladino, Michael A., Concepts of genetics, 11th Ed., ©2015. Reprinted and Electronically reproduced by permission of Pearson Education, Inc., New York, NY.

Figure 11.19: Based on Wadman, M. *Science* 354: 1359–1360, 2016; Wan, L., and Dreyfuss, G. *Cell*, 170:5, 2017.

Figure 11.20: Based on Klug et al. Concepts of Genetics, 11e. ST-5: Gene Therapy Figure ST5-7.

Figure 11.26: Based on From "Brain function and chromatin plasticity" by Catherine Dulac, Volume 465, Is. 7299, 2010.

Table 11.1: Based on Stem Cells and the Future of Regenerative Medicine, www.nap.edu/catalog/10195.html.

Figure 12.5: Based On Intellectual Property Landscape of the Human Genome, *Science* 310: 239. American Association for the Advancement of Science.

Question 13.2: R. M. Kline (2001), Whose Blood Is It, Anyway? *Scientific American*, 284(4): 42–49.

Glossary

100,000 Genomes Project: A United Kingdom government project that is sequencing whole genomes from National Health Service patients. The project is focusing on rare diseases, some common types of cancer, and infectious diseases. Participants give consent for their genome data to be linked to information about their medical condition and health records. The medical and genomic data is shared with researchers, to improve knowledge of the causes, treatment and care of diseases.

3D bioprinting: A technique that allows cells, growth factors and other materials to be printed (sprayed), often onto a solid support matrix, to create tissues or organs.

A (aminoacyl) site: Portion of a ribosome into which aminoacyl tRNA molecules bind during translation.

accession number: Unique identifying letter and number code assigned to every cloned DNA sequence that is catalogued in databases such as GenBank (for example, BC009971 is the accession number for one of the human keratin genes, which produces a protein that is a major component of skin and hair cells). The accession number for any sequence can be used by scientists around the world to retrieve database information on that particular sequence.

acid rain: Rain with a low (acidic) pH created as water vapors in the atmosphere combine with pollutants that create acids which lower the pH of rain water.

acquired immunodeficiency syndrome (AIDS): Acquired immunodeficiency syndrome (AIDS) is the most advanced stage of HIV (human immunodeficiency virus) infection, a HIV destroys the CD4 T lymphocytes (CD4 cells) of the immune system, leaving the body vulnerable to life-threatening infections and cancers.

acquired mutations: These occur in the genome of somatic cells and are not passed along to offspring; can cause abnormalities in cell growth leading to cancerous tumor formation, metabolic disorders, and other conditions.

ADA-severe combined immunodeficiency (ADA-SCID): Genetic condition created by a mutation in the gene encoding the enzyme adenosine deaminase. Affected individuals have no functional immune system and are prone to death by common, normally minor infections. Commonly known as "boy in the bubble" condition because of need for SCID individuals to live in germ-free environments.

Adenine (A): Abbreviated A; purine base present in DNA and RNA nucleotides.

adeno-associated virus (AAV): Virus which infects humans and some other primate species. In humans, the virus causes a very mild immune response. Gene therapy vectors using AAV can infect both dividing and non-dividing cells and persist in an extrachromosomal state without integrating into the genome of the host cell. These features make AAV a very attractive candidate for creating viral vectors for gene therapy and research.

adenosine triphosphate (ATP): A nucleoside-triphosphate that contains the nitrogenous base adenine. ATP is the primary form of energy used by living cells.

adenovirus: Virus that causes the common cold.

adult-derived stem cells (ASCs): Stem cells derived from tissues of an adult, as opposed to embryonic stem cells, which are derived from a blastocyst; can differentiate to produce other cell types.

aerobes: Organisms that use oxygen for their metabolism.

aerobic conditions: Conditions in which oxygen is present.

aerobic metabolism: Metabolism that requires oxygen.

affinity chromatography: A separation technique, based on the unique match between a molecule and its column-bound chemical counterpart (like antigen/antibody), that involves passing proteins or other substances in solution over a medium that will bind to ("have an affinity for") specific components of the solution. It is used to isolate fusion proteins from a mixture of bacterial cell proteins.

agarose gel electrophoresis: See *gel electrophoresis*.

alcohol (ethanol) fermentation: Enzymatic breakdown of carbohydrates (sugars) in the absence of oxygen; products include ATP, CO2, and ethanol (alcohol) as waste products; important type of microbial metabolism used for the production of certain types of alcohol-containing beverages.

allele-specific oligonucleotide (ASO) analysis: Genetic testing technique that involves using PCR with oligonucleotides specific to a disease gene to analyze a person's DNA.

alternative splicing: Splicing sometimes can join certain exons and cut out other exons, essentially treating them as introns. This process creates multiple mRNAs of different sizes from the same gene. Each mRNA can then be used to produce different proteins with different, sometimes unique, functions. Alternative splicing allows several different protein products to be produced from the same gene sequence.

amino acids: Building blocks of protein structure; combinations of 20 different amino acids can join together by covalent bonds in varying order and length to make a polypeptide.

aminoacyl transfer RNA (tRNA): Transfer RNA (tRNA) molecule with an amino acid attached.

amniocentesis: A technique for obtaining fetal cells from a pregnant woman to analyze fetal cells to determine the genetic composition of the cells, such as the number of chromosomes or the sex of the fetus.

amniotic fluid–derived stem cells (AFSs): Stem cells in the amniotic fluid of pregnant women that have been found to have many of the same traits as embryonic stem cells.

amylase: An enzyme that digests starch.

anaerobes: Organisms that do not require oxygen for their metabolism.

anaerobic conditions: Living, occurring, or existing in the absence of free oxygen

anaerobic metabolism: Lacking oxygen; an organism, environment, or cellular process that does not require oxygen.

angel investors: Investors that save your company from financial ruin at the last minute (like an angel).

antibiotic: A substance produced by microorganisms that inhibit the growth of other microorganisms; commonly used to treat bacterial infections in humans, pets, and farm animals.

antibiotic selection: See *selection*.

antibodies: Proteins produced in response to a non-self molecule by the immune system; antigen-binding immunoglobulins, produced by B cells, that function as the effector in an immune response.

antibody-mediated immunity: Portion of the immune system dedicated to producing antibodies that combat foreign materials; also known as humoral immunity.

anticodon: Three-nucleotide sequence at the end of a tRNA molecule. During translation, the anticodon binds to a specific codon in an mRNA molecule by complementary base pairing.

antifreeze proteins (AFPs): Category of proteins isolated from aquatic organisms that live in cold environments; these proteins have the unique property of lowering the freezing temperature of body fluids and tissues.

antigens: Molecules unique to specific surfaces that can stimulate antibody response; substances that trigger antibody production when introduced into the body.

antimicrobial drugs: Chemicals that inhibit or destroy microorganisms.

antisense molecule: See *antisense RNA*.

antisense RNA: An RNA molecule that is complementary to a native RNA.

antisense RNA technology: See *antisense RNA*.

aquaculture: Farming finfish, shellfish, or plants for commercial or recreational uses.

aquatic biotechnology: The use of aquatic organisms such as finfish, shellfish, marine bacteria, and aquatic plants for biotechnology applications.

Archaea: See *domain Archaea*.

artificial or **synthetic genomes:** Artificially (synthetically-created) genome created in a laboratory environment; allows for addition of customized genes to create a novel genome or lifeforms with novel properties.

astaxanthin: A pigment that gives shrimp their pink color. Recombinant astaxanthin is used to change the color of aquaculture species such as salmon.

attenuated vaccine: Vaccine consisting of weakened, live microorganisms.

autografting: The transplantation of a patient's own tissues from one region of the body to another; for example, skin or hair grafting, coronary artery bypass surgery.

autosomes: Chromosomes whose genes are not primarily involved in determining an organism's sex; chromosomes 1–22 in humans.

avian flu (H5N1): "Avian influenza virus" refers to influenza A viruses found chiefly in birds, but infections with these viruses can occur in humans.

B lymphocytes (B cells): White blood cells (leukocytes) that develop in the bone marrow and can mature into antibodyproducing cells called plasma cells.

bacilli: Rod-shaped bacteria.

Bacillus thuringiensis (Bt): A bacterium that produces a crystalline protein that dissolves the cementing substance between certain insect midgut cells.

bacteria: See *domain Bacteria*.

bacterial artificial chromosomes (BACs): Large circular vectors that can replicate very large pieces of DNA; used to clone pieces of human chromosomes for the Human Genome Project.

bacterial biofilms: Accumulations of bacteria organisms that form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings. In bacterial biofilms, cells organize themselves into a coordinated functional community.

bacteriophages: Viruses that infect bacterial cells; often simply called phages.

baculoviruses: Viruses that attack insect cells.

Basic Local Alignment Search Tool (BLAST): Internet-based program from the U.S. National Center for Biotechnology Information (NCBI) that can be used for DNA nucleotidesequence-comparison (alignment) studies and for sequence searches in GenBank and other databases.

basic sciences: Research disciplines that examine fundamental aspects of biological processes, often without obvious direct applications (for curing disease or making a product).

batch (large-scale or scale-up) processes: Growing microorganisms such as bacteria or yeast and other living cells such as mammalian cells in large quantities for the purpose of isolating useful products in a batch.

big data: Current term to describe the generation of large amounts of information (data) such as DNA sequence data, patient healthcare information, clinical trial data, computer files and data saved to the cloud, etc. Modern technologies are resulting in a dramatic expansion of information/data which researchers have access to creating challenges such as storage, protection of data, data mining (analysis of data to solve a problem or to create new knowledge from large datasets such as a database).

bioaccumulation: Progressive concentration or accumulation of a substance, such as a chemical pollutant, as it moves up the "food chain" from organism to organism.

bioaugmentation: Adding bacteria or other microorganisms to a contaminated environment to assist native (indigenous) microbes in the bioremediation processes.

biocapsules: Tiny spheres or tubes filled with therapeutic cells or a chemical substance such as a drug; can be implanted for therapeutic purposes; also known as microcapsules.

biodiversity: The range of different species present in an ecosystem.

bioethicist: A person who studies bioethics.

bioethics: The area of ethics (a code of values for our actions) concerning the implications of biologic and biomedical research and biotechnological applications (particularly with respect to medicine).

biofilming: The attachment of organisms to surfaces; examples include the attachment of shellfish to the hull of a ship and the attachment of microorganisms to teeth.

biofuel: A product of biological organisms that can substitute or enhance existing fuels.

bioinformaticists: Scientists specializing in the bioinformatics.

bioinformatics: Interdisciplinary science that involves developing and applying information technology (computer hardware and software) for analyzing biological data such as DNA and protein sequences; also includes the use of computers for the analysis of molecular structures and creating databases for storing and sharing biological data.

biologic: Any medical preparation made from living organisms or their products.

Biological Weapons Convention (BWC): A legally-binding treaty banning the development, production, and stockpiling of biological agents and toxins for non-peaceful purposes and the weapons, equipment, and means of delivery associated with the hostile use of such agents and toxins.

bioluminescence: The release of light by living organisms.

biomarker proteins: A protein biomarker (see *biomarkers*).

biomarkers: Substances used as indicators of a biologic state. A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

biomass: The dry weight of living material in part of an organism, a whole organism, or a population of organisms.

biopiles: Large piles of contaminated soil that have been removed from the original site. Air and vacuum systems are used to help dry these piles and release chemicals by evaporation. Vacuums are sometimes used to collect chemical vapors and trap them in filters.

bioprocessing: The use of biologic systems to manufacture (process) a product.

bioprospecting: Endeavors to capitalize on indigenous knowledge of natural resources. However, bioprospecting may also describe the search for previously unknown compounds in organisms that have never been used in traditional medicine.

bioremediation: The use of living organisms to process, degrade, and clean up naturally occurring or man-made pollutants in the environment.

biosensors: Living organisms used to detect or measure biological effects of some factor, such as a chemical pollutant, or condition.

biosimilar drugs: Also called "follow-on" biologics. Subsequent version of a recombinant protein product after the original patent has expired. The biosimilar is produced by a different company than the innovator holding the initial patent. As a result, when a biosimilar is made, the exact production processes are not the same as the innovator's hence the product or its manufacturing is "similar" but not identical to the original protein. The equivalent of a generic for a pharmaceutical drug.

biotechnology: A broad area of science involving many different disciplines designed to use living organisms or their products to perform valuable industrial or manufacturing processes or applications that will solve problems.

bioterrorism: The use of biological materials (live organisms or their toxins) as weapons to cause fear in or harm against civilians.

bioventing: Pumping air or oxygen-releasing chemicals such as hydrogen peroxide into contaminated soil or water to stimulate aerobic degradation by microorganisms.

bioweapons: Biological materials used as weapons.

blastocyst: Hollow cluster of approximately 100 cells formed about 1 week after fertilization of an egg.

blue-white selection: A technique for identifying (selecting) bacteria cells containing recombinant DNA; Involves inserting DNA to be cloned into a vector, usually a plasmid, containing the gene that encodes the enzyme β-galactosidase (β-gal). When grown on a specific media, nonrecombinant cells express β-gal and turn blue, recombinant cells containing cloned DNA inserted into the β-gal gene cannot produce functional β-gal and appear white.

blunt ends: Double-stranded ends of a DNA molecule created by the action of certain restriction enzymes.

byssal fiber: Ultrastrong, protein-rich threads created by mussels and other shellfish. Byssal fibers have unique adhesive properties and can withstand great stress from stretching and shearing forces; attach shellfish to substrates such as rocks.

CAAT box: Short nucleotide sequence (CAAT) usually located approximately 80 to 90 base pairs "upstream" (in the 5′ direction) of the start site of many eukaryotic genes; part of promoter sequence bound by transcription factors used to stimulate RNA polymerase.

calcitonin: A thyroid hormone that stimulates calcium uptake by digestive organs and promotes bone-hardening (calcification).

callus: A loose collection of de-differentiated plant tissue.

Cancer Genome Atlas Project (TCGA): NIH sponsored project to identify and map important genes and genetic changes involved in cancer.

cancer stem cells (CSCs): Stem cells which develop to form cancerous tumors.

capital: In reference to the business of biotechnology, capital refers to financial assets (funds available), or the financial value of a company (based on equipment, facilities, stocks, etc.); often used to describe funding available for a company or funding raised from investors to support a company.

CAR-T: Chimeric antigen receptor T cells developed to recognize a chimera of cancer presenting antigens.

carcinogen: A cancer-causing chemical.

carcinogenic: Cancer causing agents as chemicals and X-rays.

carrageenan: Polysaccharide (sugar) derived from seaweeds; wide range of uses in everyday products such as syrups, sauces, and adhesives as a "thickening" or bulking agent.

cDNA: DNA synthesized as an exact copy of mRNA called complementary DNA (cDNA). The mRNA is degraded by treatment with an alkaline solution or enzymatically digested; then DNA polymerase is used to synthesize a second strand to create double-stranded cDNA.

cell lines: An established or immortalized cell line that has acquired the ability to proliferate indefinitely, either through random mutation or deliberate modification. Numerous well-established cell lines are representative of particular cell types.

cell lysis: See *lysis*.

cellular respiration: Metabolic process of individual cells to convert food molecules such as sugars into energy (such as ATP) that can power reactions in cells.

cellular therapeutics: Cell-based therapies. Including, for example, stem cells and implanted cells.

cellulase: Bacterial enzyme that degrades the polysaccharide cellulose (a primary component of the plant cell wall).

Centers for Disease Control and Prevention (CDC): Based in Atlanta, the CDC is the national public health institute of the United States. The CDC works to protect public health and safety through monitoring, controlling and preventing illness from infectious diseases in the U.S. and internationally.

centrifugation: Involves using an instrument called a centrifuge to apply a rotational force to samples to separate components based on their weight. Rotational forces measured in revolutions per minute (rpm) or times gravity (g). Centrifugation has a number of important applications related to separating components of a mixture (for instance, separating proteins from DNA, separating different cell types).

centromere: Constricted region of a chromosome formed by intertwined DNA and proteins that hold two sister chromatids together.

chemiluminescence: Chemical reactions that release photos of light; often enzymes attached to DNA probes and other molecules can be used in chemiluminescent reactions for different detection purposes.

chemotherapy: The treatment of cancer and other diseases with specific chemical agents or drugs that have a toxic effect on diseased cells or disease-causing microorganisms.

chimeric antigen receptor (CAR)-T cells: T cells engineered to express protein receptors on their cell membranes which can recognize surface molecules on specific cells such as cancer cells; CAR-T cells are important for immunotherapy approaches designed to target cancer cells.

chitin: A complex polysaccharide polymer composed of repeating units of a sugar called N-acetylglucosamine. Chitin forms the hard outer shell (exoskeleton) of crabs, lobsters, crawfish, shrimp, and other crustaceans. Also found in insects and other organisms.

chitosan: Polysaccharide polymer derived from chitin. Used in many applications from health care to agriculture to dyes for fabrics and in dietary supplements for weight loss.

cholera: Acute intestinal infection of humans caused by the bacterium Vibrio cholerae that causes severe diarrhea, which can result in dehydration and death, particularly in young children, if not treated. Spreads by contaminated water and food; bacterium often found in water supplies of developing countries with poor sanitary conditions.

chorionic villus sampling: Technique for sampling fetal cells from the placenta to determine the genetic composition of these cells, such as the number of chromosomes or the sex of the fetus.

choroideremia: A rare, inherited form of blindness caused by mutaiton of the *CHM* gene.

chromatin: Strings of DNA wrapped around proteins; present in the nucleus of eukaryotes and the cytoplasm of prokaryotes; coils tightly together to form chromosomes during cell division.

chromatography: A columnar separation method that uses resin beads with special separation capabilities.

chromosomes: Highly folded arrangements of DNA and proteins; they package DNA to allow even separation of genetic material during cell division.

chronic myelogenous leukemia (CML): Form of leukemia (blood cell cancer) created by an exchange of DNA between chromosomes 9 and 22; typically occurs in older adults.

chymosin: See *renin*.

circular RNA (circRNAs): Recently identified category of single-stranded, non-coding RNA molecules; relatively little is known about the function of circRNAs.

clinical trials: Experimental process of testing products before approval of a drug, or treatment plan for widespread use in humans; clinical trials involve several "phases" of carefully planned experiments in different numbers of human participants to test drug effectiveness and safety.

clones: A genetically identical copy of a cell or whole organism; also describes the process of making copies of a gene, cell, or organism.

Cloning: In biotechnology refers to processes used to create genetically identical copies of DNA fragments (molecular cloning), cells (cell cloning), or organisms (organism cloning).

cocci: Bacteria with a spherical shape.

Codex Alimentarius Commission (CAC): A body that develops and maintains the "Codex Alimentarius", a collection of international standards, guidelines, and codes of practice that protect consumer health and promote fairness in the international food trade.

CODIS, Combined DNA Index System: CODIS enables federal, state, and local crime labs to exchange and compare DNA profiles electronically, thereby linking crimes to each other and to convicted offenders.

codons: A codon comprises a combination of three nucleotide sequences in an mRNA molecule. With few exceptions, each codon codes for a single amino acid; 64 possible codons compose the genetic code of mRNA molecules.

cohesive ends: Overhanging single-stranded ends of a DNA molecule created by the action of certain restriction enzymes.

colchicine: Substance derived from crocus flowers that blocks microtubule formation; used to stop cell division (for instance, when creating polyploid organisms).

collagenase: Protease (protein-digesting enzyme) used in a number of biotechnology applications.

Combined DNA Index System, or **CODIS:** The **Combined DNA Index System (CODIS)** is the United States national DNA database created and maintained by the Federal Bureau of Investigation. CODIS includes the Local DNA Index Systems (LDIS) where DNA profiles originate.

Commander: Consists of about a dozen individual proteins. Commander is present in all animal cells, scientists have disrup-ted its components to reveal the way cells are positioned during embryo development, and protein to protein interactions.

comparative genomics: Studies that allow researchers to investigate gene structure and function in organisms in ways designed to understand gene structure and function in other species including humans.

complementary base pairs: Refers to the nucleotides adenine (A) and thymine (T) [or uracil (U) when referring to RNA], and guanine (G) and cytosine (C); in a DNA molecule, A&T and G&C nucleotides join by hydrogen bonds to "complement" each other.

complementary DNA (cDNA): DNA copy of an mRNA molecule; mRNA can be copied into cDNA by the enzyme reverse transcriptase (for instance, when preparing a cDNA library).

complementary DNA (cDNA libraries): DNA copies of all mRNA molecules expressed in an organism's cells; can be "screened" to isolate genes of interest.

composting: Mixing soil with hay, straw, grass clippings, wood chips, or other similar "bulking" materials to stimulate biodegradation by soil microbes; also used to degrade everyday materials such as vegetable scraps, grass clippings, weeds, and leaves.

computer-automated high-throughput DNA sequencing methods: Computer-assisted methodologies for DNA sequencing. Allow for sequencing and analysis of large amounts (high-throughput) of sequence data compared to older manual sequencing methods.

contigs (contiguous sequences): Restriction enzyme–digested pieces of entire chromosomes are sequenced separately, and then computer programs are used to align the fragments based on overlapping sequence pieces called contigs (contiguous sequences).

Contract Research Organizations (CROs): Organization that provide services for the pharmaceutical, biotechnology, and medical device industries in the form of research services (such as clinical trials, marketing studies, animal or cell testing, etc.) outsourced on a contract basis; often it is more cost effective for both large and small biotechnology companies to hire a CRO to provide specialized services in their area of expertise instead of doing the work within the biotech company itself.

Coomassie stain: A sensitive stain that reacts with proteins.

copy number: The number of copies of a particular DNA molecule or gene sequence such as a plasmid in a bacterial cell.

copy number variations (CNVs): DNA segments larger than 1 kb such as deletions, insertions and duplications in the genome that vary between individuals; account for much of the genome diversity identified between humans.

CRISPR-Cas (Cluster Regularly Interspace Palindromic Repeats, or CRISPR-Cas System): CRISPR is an abbreviation of **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats. These are segments of DNA containing short, repetitive base sequences. In a palindromic repeat, the sequence of nucleotides is the same in both directions. Each repetition is followed by short segments of spacer DNA from previous exposures to foreign DNA (e.g., a virus or plasmid). Small clusters of *cas* (CRISPR-associated system) genes are located next to CRISPR sequences. RNA harboring the spacer sequence helps Cas proteins recognize and cut exogenous DNA.

CRISPR-derived RNAs (crRNAs): Proteins expressed from CRISPR-associated (Cas) genes cause expression of CRISPR RNA genes to produce. crRNAs; crRNAs guide a Cas nuclease to specific regions in the genome that aid CRISPR complex recognition and cleavage of the target DNA.

crown gall: A hardened mass of protruding dedifferentiated plant tissue usually resulting from an attack from Agrobacter.

cryo-electron microscopy: Assembles 2D electron microscopic images of proteins to create 3D images.

customer relations specialists: Sometimes work in QA divisions of a company. One function of customer relations is to investigate consumer complaints about a problem with a product and follow up with the consumer to provide an appropriate response or solution to the problem encountered.

cytogenetics: The analysis of chromosome structure and abnormalities.

cytoplasm: The inner contents of a cell, consisting of fluid (cytosol) and organelles; its boundaries are defined by the plasma membrane.

Cytosine (C): Abbreviated C; pyrimidine base present in DNA and RNA nucleotides.

cytosol: Gel-like, nutrient-rich aqueous (water-based) fluid of the cytoplasm.

data scientists or analysts: Position involved in analyzing and interpreting data and communicating relevant findings to help decision making.

Deinococcus radiodurans: Extremophilic bacterium because it can grow and thrive even after exposure to radiation; of interest to bioremediation scientists because it is extremely radiation-resistant.

deontological (Kantian) approach: Developed by German philosopher Immanuel Kant, this approach suggests that absolute principles (of moral or ethical behavior) should be followed to dictate our actions.

deoxyribonucleic acid (DNA): Double-stranded nucleic acid consisting of bases (adenines, guanines, cytosines, thymines), sugars (deoxyribose), and phosphate groups arranged in a helical molecule; inherited genetic material that contains "genes," which direct the production of proteins in a cell.

depolymerization: A process of reducing multiunit structures to single units.

diafiltration: See *dialysis*.

dialysis: A separation of water and a solute involving a semipermeable membrane.

dideoxyribonucleotide (ddNTP): Nucleotides used for certain types of DNA sequencing methods. Structurally different than nucleotides because they lack oxygen atoms at positions 2 and 3 of the deoxyribose sugar. As a result ddNTPs are called "chain-terminating nucleotides" because they cannot form phosphodiester bonds.

differentiation: Cellular maturation process; involves changes in patterns of gene expression that affect the structure and functions of cells. For instance, embryonic stem cells differentiate to produce mature cells such as neurons, liver cells, skin cells, and all other cell types in the body.

diploid: Refers to a cell or an organism consisting of two sets of chromosomes: usually, one set from the mother and another set from the father. In a diploid state, the haploid number is doubled; thus, this condition is also known as $2n$.

diploid number ($2n$): Two sets of chromosomes ($2n$); often used to describe cells with two sets of chromosomes, typically one set from each parent.

direct-to-consumer (DTC) genetic tests: DNA sequence analysis or genetic testing of human DNA for comparison to known sequences that have been associated with abnormal human conditions; DTCs can be purchased directly without prescription.

DNA fingerprinting: An analysis of an organism's unique DNA composition as a characteristic marker or fingerprint for identification purposes, such as forensic analysis, remains identification, and paternity. DNA fingerprinting is also used in biologic research (for example, to compare related species based on their DNA sequences).

DNA helicase: Enzyme that separates two strands of a DNA molecule during DNA replication.

DNA library: See *genomic DNA library* or *complementary (cDNA) library*.

DNA ligase: Enzyme that forms covalent bonds between incomplete (Okazaki) fragments of DNA during DNA replication; routinely used for joining DNA fragments in recombinant DNA experiments.

DNA microarray: A chip consisting of a glass microscope slide containing thousands of pieces of singlestranded DNA molecules attached to specific spots on the slide; each spot of DNA is a unique sequence.

DNA polymerase III: One form of DNA polymerase; the primary DNA polymerase which copies DNA in prokaryotes.

DNA polymerases: Key enzymes that copy strands of DNA during DNA replication to create new strands of nucleotides; these have important applications for synthesizing DNA in molecular biology experiments.

DNA sequencing: Laboratory technique for determining the nucleotide "sequence" or arrangement of A, G, T, and C nucleotides in a segment of DNA.

DNA-based vaccines: A vaccination approach that involves injection of native or genetically engineered DNA so cells directly produce molecules (antigen) resulting in an immune response to provides protection against a particular condition.

Doha Ministerial Declaration on the TRIPS Agreement and Public Health: Adopted by the WTO Ministerial Conference of 2001, the declaration supports the rights of WTO member states to protect public health and to promote access to medicines.

do-it-yourself (DIY) biotechnology: A growing biotechnological social movement in which individuals, communities, and small organizations study biology and life science using the same methods as traditional research institutions. DIY biology is primarily undertaken by individuals with extensive research training from academia or corporations, who then mentor and oversee other DIY biologists with little or no formal training.

domains: Taxonomic categories above the kingdom level: Archaea, Bacteria, and Eukarya.

double-blind trial: Study, such as a clinical trial, in which neither the patients nor the researchers administering the treatment know which patients receive a drug treatment and which patients receive a placebo; researchers learn the treatment of each patient after the study is completed.

Down syndrome: Human genetic disorder first described by John Langdon Down. Most commonly results from individuals having three copies of chromosome 21 (trisomy 21). Characteristics of affected individuals include a flat face; shortness; short, broad hands and fingers; protruding tongue; and impaired physical, motor, and mental development.

downstream processing: Purification processes involved in producing a pure product such as a recombinant protein.

DPT vaccine: Childhood vaccine designed to provide immune protection against bacterial toxins diphtheria, pertussis, and tetanus (contains killed Bordetella pertussis cells). Microbes producing these toxins cause upper respiratory infections, whooping cough, and tetanus (muscle spasm and paralysis), respectively.

Dual Use Research of Concern: A subset of "dual-use research" having a high potential for misuse through bioterrorism or bio-warfare.

Duchenne muscular dystrophy (DMD): A genetic disorder characterized by progressive muscle degeneration and weakness. DMD is caused by an absence of *dystrophin*, a protein that helps keep muscle cells intact.

Ebola virus: One of several viruses in the Ebola family; can produce fatal hemorrhagic fever (Ebola virus disease) in humans.

efficacy: The effectiveness of a particular product such as a drug or medical procedure.

effluent: Water that has been treated to remove chemical pollutants or sewage; treated water as it leaves a treatment facility, such as water leaving a sewage-treatment plant.

electroporation: A process for transforming bacteria with DNA that uses electrical shock to move DNA into cells; can also be used to introduce DNA into animal and plant cells.

ELISA: An enzyme-linked immunosorbent assay where the enzyme converts a dye when an immune reaction recognizes the presence of the target protein being captured by the antibody.

embryo twinning: Splitting embryos in half at the two-cell stage of development to produce two embryos.

embryonic stem cells (ESCs): Cells typically derived from the inner cell mass of a blastocyst; cells can undergo differentiation to form all cell types in the body.

embryonic stem cell method: Embryonic stem cells are collected from the inner cell mass of blastocysts. They are then mixed with DNA, which has usually been created using recombinant DNA methods. Some of the ES cells will absorb the DNA and be transformed by the new genetic material and can be isolated for implantation.

Encyclopedia of DNA Elements (ENCODE) Project: Genome project to identify DNA regulatory sequences.

endocrine disruptors: Compounds that interfere with or disrupt the functions of naturally occuring hormones; present in the environment and present health threats to humans and aquatic organisms.

endotoxins: Molecules that are toxic to cells; endotoxins are part of the cell wall of certain bacteria; endotoxins cause cell death.

enhancers: Specific DNA sequences that bind proteins called transcription factors to stimulate ("enhance") transcription of a gene.

enucleation: Removal of the DNA from the nucleus of a cell.

Environmental Genome Project: NIH program designed to study the impacts of environmental chemicals on human genetics and disease.

EnviroPig: A transgenic pig expressing the enzyme phytase in its saliva. It is deemed environmentally friendly because it produces substantially less phosphorus in its urine and feces than do nontransgenic pigs.

enzymes: Catalytic proteins.

epigenome: Modifications in chromatin structures, which to do not involve mutations in DNA sequence. Some modifications are inherited and can persist in several generations of offspring.

ES cells or ESCs (embryonic stem cells): Stem cells derived from the inner cell mass of a blastocyst, an early-stage pre-implantation embryo. ES cells are distinguished by their ability to differentiate into any cell type (pluripotency) and by their ability to replicate and self-replenish.

ethics: Moral principles that guide individual actions and behaviors.

ES (embryonic stem) cell transfer: Embryonic stem cells (ES cells) are collected from the inner cell mass of blastocysts. Transformed ES cells are usually injected into the inner cell mass of a host blastocyst.

ethidium bromide: Tracking dye that penetrates (intercalates) between the base pairs of DNA. Commonly used to stain DNA in gels because ethidium bromide fluoresces when exposed to ultraviolet light.

eugenics: The creation of a new superior species of human.

eukaryotic cells: Cells that contain a nucleus, including plant and animal cells, protists, and fungi.

exons: Protein-coding sequences in eukaryotic genes and mRNA molecules.

ex situ bioremediation: Removing contaminated soils or water from the site of contamination for clean-up at another location (as opposed to cleaning up pollutants at the contaminated site—a process called in situ bioremediation).

***ex vivo* gene therapy:** Gene-therapy procedure that involves removing a person's cells (such as blood cells) from the body, introducing therapeutic genes into these cells, and then reintroducing the cells back into a person.

expressed sequence tags (ESTs): Small incomplete pieces (tags) of gene sequence such as those derived from a cDNA library; represent mRNAs expressed in a given tissue.

expression vectors: DNA vector such as a plasmid that can be used to produce (express) proteins in a cell.

fast protein liquid chromatography (FPLC): Often used to analyze or purify mixtures of proteins. The buffer flow rate is controlled by a positive-displacement pump and is normally kept constant, while the composition of the buffer can be varied by drawing fluids in different proportions from two or more external reservoirs. The stationary phase is a resin composed of beads, usually of cross-linked agarose, packed into a cylindrical glass or plastic column. The correct balance of buffer and ligand will bind the protein of interest.

fermentation: A metabolic process that produces small amounts of ATP from glucose in the absence of oxygen and also creates byproducts such as ethyl alcohol (ethanol) or lactic acid (lactate). Fermenting microbes (bacteria and yeast) are important for producing a variety of beverages and foods, including beer, wine, breads, yogurts, and cheeses.

fertilization: Addition of nutrients (fertilizer such as nitrogen and phosphorus) to a contaminated environment to stimulate growth and activity of naturally occurring soil microorganisms that will aid bioremediation.

fetal tissue transplants: Transplanted cells or tissues derived from a fetus.

field trials: Tests outside of the laboratory required after a new plant has been engineered in a laboratory; may require several years to investigate everything about the plant, including disease resistance, drought tolerance, and reproductive rates.

5′ end: The end of a strand of DNA or RNA in which the last nucleotide is not attached to another nucleotide by a phosphodiester bond involving the 5′ carbon of the pentose sugar.

finance divisions: Unit responsible for overseeing company finances.

fluorescence in situ hybridization (FISH): Laboratory technique that uses single-stranded DNA or RNA probes labeled with fluorescent nucleotides to identify gene sequences in a chromosome or cell in situ (Latin for "in its original place").

Food and Agriculture Organization (FAO): Specialized agency of the United Nations working to reduce food insecurity and rural poverty and to create sustainable food and agriculture systems.

forensic science: Application of a broad spectrum of sciences to answer questions of interest to a legal system.

frameshift mutation: Mutation resulting from the addition or deletion of nucleotides that causes a shift in the genetic code-reading frame of a gene.

fungi: Eukaryotic organisms that belong to kingdom Fungi.

fusion proteins: A "hybrid" recombinant protein consisting of a protein from a gene of interest connected (fused) to another, well-known protein that serves as a tag for isolating recombinant proteins.

gametes: Haploid cells often called "sex" cells include human sperm and egg cells (ova). Gametes join together during fertilization to form a zygote (see *meiosis*).

GenBank: Renowned public database of DNA sequences provided by researchers throughout the world; resources for sharing and analyzing DNA sequence information.

genes: A specific sequence of DNA nucleotides that serves as a unit of inheritance. Genes govern visible and invisible characteristics (traits) of living organisms in large part by directing the synthesis of proteins in a cell.

gene chip: See *DNA microarray*.

gene cloning: The process of producing multiple copies of a gene.

gene expression: Term generally used to describe the synthesis ("expression") of RNA by a particular cell or tissue. For instance, if mRNA for the fictitious gene korn is detected in a tissue by PCR or Northern blot analysis, that tissue is said to express the korn gene.

Genentech: California company. Name derived from genetic engineering technology. Founded in 1976 and widely recognized as the world's first biotechnology company.

gene regulation: General term used to describe processes by which cells can control gene activity or "expression" (RNA and protein synthesis).

gene stacks: Multiple genes inserted into transgenic plants.

gene therapy: The use of therapeutic genes to treat or cure a disease process; also refers to the delivery of genes to improve a person's health.

generic drugs: Copies of brand-name products that generally have the same effectiveness, safety, and quality but are produced at a cheaper cost to the consumer than the brand name drugs.

genetic code: Information contained in bases of DNA and RNA that provide cells with instructions for synthesizing proteins; consist of three-nucleotide combinations called codons.

genetic doping: The use of genes for genetic improvements to enhance athletic performance.

genetic engineering: The process of altering an organism's DNA. This is usually by design.

genetic enhancement: The use of genes for genetic improvements for non-medical treatment purposes (i.e., to improve appearances).

Genetic Information Nondiscrimination Act (GINA): This act prohibits discrimination based on genetics and the improper use of genetic information in health insurance and employment.

Genetic Testing Registry (GTR): A branch of the NCBI projected to be developed by late 2011, it will provide access to information about genetic tests for inherited and somatic genetic variations, including newer types of tests such as arrays and multiplex panels. GTR information about tests primarily will be based on voluntary data submissions by test developers and manufacturers.

genetically modified (GM) organisms: GM organisms included selectively bred and transgenic organisms. For example, GM crops have been produced to plants that can resist pests, disease, or harsh climates, allowing better production of crops.

genome: All of the genes in an organism's DNA.

Genome 10K plan: Project to create a genomic "zoo" containing the sequences of 10,000 vertebrate species.

genome or gene editing: Specific form of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism. Typically involves DNA cutting enzymes called nucleases such as the CRISPR/Cas system or zinc finger nucleases.

genome-wide association studies (GWAS): An approach for analyzing genomes or specific genes in large populations of individuals with a particular condition, such as a human genetic disease, in an attempt to identify genes, mutations, or loci frequently associated with the condition.

genomic DNA libraries: Collection of DNA fragments containing all DNA sequences in an organism's genome; can be "screened" to isolate genes of interest.

genomics: The study of genomes.

Gleevec: A drug used to treat certain types of leukemia (cancer that begins in the white blood cells) and other cancers of the blood cells. Imatinib (Gleevec) is also used to treat gastrointestinal stromal tumors.

Global Influenza Surveillance and Response System (GISRS): A global network that identifies and responds to influenza outbreaks through real-time virus monitoring and timely virus sharing.

glycolysis: Metabolic process in cells to breakdown glucose into pyruvate; results in the production of two molecules of ATP; during aerobic respiration, pyruvate can be metabolized further to produce additional molecules of ATP.

golden rice: Golden rice is a variety of rice produced through genetic engineering to biosynthesize beta-carotene, a precursor of vitamin A, in the edible parts of rice.

Gram stain: Technique for staining the bacterial cell wall that can be used to divide bacteria into different categories, gram-positive or gram-negative bacteria.

green fluorescent protein (GFP): Protein produced by the bioluminescent jellyfish Aequorea victoria. Protein fluoresces when exposed to ultraviolet light; the GFP gene is used as a reporter gene.

growth factors: Molecules that stimulate cell growth and division.

growth hormone (GH): A peptide hormone produced by the pituitary gland; accelerates the growth of bone, muscle, and other tissues.

Guanine (G): Abbreviated G; purine base present in DNA and RNA nucleotides.

haploid: A single non-paired set of chromosomes.

haploid number (*n*): A single set of chromosomes (n); often used to describe cells with a single set of chromosomes.

hemoglobin: Oxygen-binding and oxygen-transporting protein of red blood cells in vertebrates. Mutation of globin genes that encode hemoglobin results in a number of different human blood disorders with a genetic basis, including sickle-cell disease.

hemophilia B: Blood clotting disease, which occurs predominantly in men, caused by mutation of *FIX* gene which encodes a protein called Factor IX essential for clotting.

hepatitis B: An infectious disease that affects the liver caused by the hepatitis B virus (HBV).

Herceptin: Herceptin is approved for the treatment of earlystage breast cancer that is **H**uman **E**pidermal growth factor **R**eceptor **2**-positive (HER2+) and has spread into the lymph nodes, or is HER2+ and has not spread into the lymph nodes. If it has not spread into the lymph nodes, the cancer needs to be estrogen receptor/progesterone receptor (ER/ PR)-negative or have one high risk feature.

high-performance liquid chromatography (HPLC): High-pressure separation of similar proteins by using incompressible beads with special properties.

Hippocratic Oath: An oath that embodies the obligations and duties of medical doctors; one expectation of the oath is that physicians treat patients with the intent of not worsening human health ("first, do no harm").

histones: DNA-binding proteins that are important for chromosomal structure.

HIV: See *Human immunodeficiency virus*.

homologous pairs: Pairs of chromosomes or genes that occur in organisms with multiple sets of chromosomes.

homologues: Related genes in different species; genes share sequence similarity because of a common evolutionary origin.

horizontal gene transfer: Transfer of DNA, genes, or genomes between different species (for example, from one species of bacteria to another, or from bacteria to an animal species); distinctly different from passing genes from parents to offspring (vertical gene transfer).

human antimouse antibody (HAMA) response: Mouse hybridomas produce enough "mouse" antigens that they still evoke an undesirable immune response in humans.

human embryonic stem cells (hESCs): Stem cells are immature cells that can grow and divide to produce different types of cells such as skin, muscle, liver, kidney, and blood cells. Most stem cells are obtained from embryos (embryonic stem cells or ESCs). Some ESCs can be isolated from the cord blood of newborn infants.

Human Epigenome Project: Project designed to reveal epigenetic changes in different cell and tissue types and to evaluate potential roles of epigenetics in diseases.

Human Genome Project (HGP): An international effort with overall scientific goals of identifying all human genes and determining (mapping) their locations to each human chromosome.

human immunodeficiency virus (HIV): HIV is a lentivirus (a subgroup of retrovirus) that causes HIV infection. HIV infects vital cells in the human immune system such as helper T cells (CD4+T), macrophages, and dendritic cells; loss of $CD4^+$ T cell numbers decline below a critical leve results in a lossof cell-mediated immunity is lost and the body becomes progressively more susceptible to opportunistic infections; HIV is the virus that causes AIDS.

Human Microbiome Project (HMP): Project designed to identify the genomes of all microorganisms (bacterial, yeast, viral) living in or on the human body.

human papillomavirus (HPV): Causes about 70% of cervical cancers (HPV strains 16 and 18) and a large percentage of genital warts (caused by HPV strains 6 and 11). Cervical cancer affects 1 in 130 women, nearly half a million women worldwide, and approximately 70% of sexually active women will become infected with HPV.

human proteome: A federal initiative proposed to study the structures and functions of all human proteins (the proteome).

humane treatment: Compassionate and sympathetic approach, as applied to treating humans or animals.

humanized antibodies: Antibodies from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans.

Humulin: The recombinant form of human insulin, became the first recombinant DNA product to be approved for human applications by the U.S. Food and Drug Administration.

hybridomas: Hybrid cells used to create monoclonal antibodies; created by fusing B cells with cancerous cells called myeloma cells that no longer produce an antibody of their own.

hydrophilic: Water-loving (portion of protein molecule).

hydrophobic: Water-hating (portion of protein molecule).

hydrophobic interaction chromatography (HIC): Chromatographic separation based on binding of hydrophobic parts of proteins to the column.

hydroponic systems: A form of aquaculture in which tanks of flowing water are used to grow plants; in some cases, water from fish aquaculture tanks is used to provide nutrients for plant growth in a polyculture approach.

hydroxyapatite (HA): Structural component of bone and cartilage.

immunotherapy: Precision medicine approach that involves the use of a patient's immune system to attack and destroy diseased cells such as cancer cells.

in situ bioremediation: Cleaning up pollutants at the actual site of contamination; "in place" clean-up as opposed to removing contaminated soils or water from the clean-up site for remediation at another location—a process called ex situ bioremediation.

***in vitro* fertilization (IVF):** Assisted-reproduction technology in which sperm and egg cells are removed from patients and cultured in a dish (in vitro) to achieve fertilization.

***in vivo* gene therapy:** Gene therapy procedure that involves introducing therapeutic genes directly into a person's tissue or organs without removing them from the body.

inactivated (killed) vaccines: Vaccine consisting of killed microorganisms.

incidental findings: Previously undiagnosed or undetected medical or mental health conditions discovered unintentionally (for example, such as when testing for a different condition).

inclusion bodies: Foreign proteins that concentrate in transformed cell.

IND: To legally test a drug on human subjects in the U.S., the maker must first obtain an Investigational New Drug (IND) designation from FDA. This application is based on nonclinical data, typically from a combination of in vivo and in vitro laboratory safety studies, that shows the drug is safe enough to test in humans.

indigenous microbes: Naturally occurring microorganisms living in the environment.

induced pluripotent stem cells (iPSCs or **iPS cells):** Nuclear reprogramming of mouse and human cells, heralded as a revolution in stem-cell biology research. One approach has involved using retroviruses to deliver four transgenes, Oct3/4, Sox2, c-myc, and Klf4, into fibroblasts. Expression of these four genes, which encode transcription factors involved in cell development, "reprograms" the fibroblasts back to an earlier stage of differentiation. iPS cells demonstrate many properties of hESCs, such as self-renewal and pluripotency, and appear to be indistinguishable from hESCs.

industrial biotechnology: The application of biotechnology for industrial purposes, including industrial fermentation. It includes using cells such as microorganisms, or components of cells like enzymes, to generate industrially useful products in sectors such as chemicals, food and feed, detergents, paper and pulp, textiles and biofuels.

influenza: Caused by a large number of viruses that belong to the influenza family of viruses. Influenza kills approximately 500,000 to 1 million people worldwide each year. Because flu viruses mutate so rapidly, no one-size-fits-all vaccine protects against all strains.

Influenza Virus Traceability Mechanism: An electronic system that tracks all influenza viruses of pandemic potential contributed by the WHO Member States to the Global Influenza Surveillance and Response System.

informed consent: Applies to clinical trials in humans. Patients are made aware of potential beneficial and harmful effects of a particular treatment so that they are informed of the risks of a procedure before they agree (consent) to participate.

inherited mutations: A change in DNA structure or sequence of a gene passed to offspring through gametes; can be a cause of birth defects and genetic disease.

initial public offering (IPO): First sale of stock by a formerly private company. It can be used by either small or large companies to raise expansion capital and become publicly traded enterprises.

inner cell mass: Layer of cells in the blastocyst that develop to form body tissues; a source of embryonic stem cells.

inorganic compounds: See *inorganic molecules.*

inorganic molecules: Molecules that do not contain carbon.

insulin: Protein hormone produced by cells of the pancreas; involved in glucose metabolism by cells; deficiencies in insulin production or insulin-receptor production can cause different forms of diabetes.

interactome: Collective term referring to all molecular interactions in a cell (i.e., protein-protein, protein-DNA, etc.).

interfering RNAs (siRNAs; short interfering RNAs or silencing RNAs): Non-coding RNAs used in the molecular technique called RNA interference (RNAi); when delivered into cells siRNAs can block or degrade specific mRNA target sequences to silence those sequences from being translated.

International HapMap Project: International effort to develop haplotype maps of human genetic variation; includes SNP analysis of the genome.

introns: Non-protein-coding sequences in eukaryotic genes and primary transcripts that are removed during RNA splicing.

ion-exchange (IonX) chromatography: The attachment of protein to a column based on its charged side groups.

isoelectric focusing: Migration of a protein until its charge matches the pH of the medium.

isoelectric point (IEP): The pH at which the charge on proteins matches that of the surrounding medium.

karyotype: A technique for analyzing the number and appearance of chromosomes in the nucleus of a eukaryotic cell.

karyotyping: A laboratory procedure for analyzing the number and structure of chromosomes in a cell.

Kingdom Fungi: Kingdom of diverse organisms distinct from plant, animal and bacterial cells'; includes yeasts, mushrooms, molds, mildews and other organisms.

knockout animals: A knockout animal is a genetically modified animal in which researchers have inactivated, or "knocked out", an existing gene or genes.

Kymriah (CTL019): Trade name for the first FDA-approved CAR-T cell therapy to treat B-cell acute lymphoblastic leukemia.

laboratory technicians: Entry-level laboratory jobs with a range of responsibilities such as preparing solutions and mediums, ordering laboratory supplies, cleaning and maintaining equipment; may sometimes involve bench research.

lac operon: A well-characterized bacterial operon that contains a series of genes (lacZ,Y, Z) responsible for metabolism of the sugar lactose.

lac repressor: Inhibitory protein encoded by the lacI gene of the lactose (lac) operon in bacteria; in the absence of lactose, repressor blocks transcription of the lac operon.

lactic acid fermentation: See *fermentation*.

lagging strand: During DNA replication, the strand of newly synthesized DNA that is copied by DNA polymerase in a discontinuous (interrupted) fashion, 5' to 3' away from the replication fork as a series of short DNA pieces called Okazaki fragments.

land farming: Process by which contaminated soil is removed from a site and spread into thin layers on a pad that allows polluted liquids (usually water) to leach from the soil. Chemicals also vaporize from spread-out soil as the soil dries.

leachate: Water or other liquids that move (leach) through the ground from the surface or near the surface to deeper layers.

leading strand: During DNA replication, the strand of newly synthesized DNA that is copied by DNA polymerase in a continuous fashion, 5' to 3' into the replication fork.

leaf-fragment technique: A method of plant cloning from asexual plant tissue.

Leber's congenital amaurosis (LCA): Degenerative disease of the retina that affects 1 in 50,000 to 100,000 infants each year and causes severe blindness; caused by defects in the *RPE65* gene.

legal specialists: In biotechnology companies, typically work on legal issues associated with product development and marketing, such as copyrights, naming rights, and obtaining patents. Staff in this area also address legal circumstances that may arise if problems are found with a product or litigation from a user of a product.

lentivirus: A genus of retroviruses characterized by long incubation periods; infects humans and other mammalian species; HIV, which causes AIDS, is a lentivirus; used as vectors to deliver therapeutic genes for gene therapy.

leuko cyte: White blood cells; important cells of the immune system; include B and T lymphocytes and macrophages.

ligands: Binding components.

limulus amoebocyte lysate (LAL) test: A procedure involving blood cells (amoebocytes) from the horseshoe crab (Limulus polyphemus); an important test used to detect endotoxin and bacterial contamination of foods, medical instruments, and other applications.

lipases: Fat-digesting enzymes.

liposomes: Small hollow spheres or particles made of lipids; can be packaged to contain molecules such as DNA and medicines for use in therapeutic procedures (for example, gene therapy).

Living Modified Organisms (LMOs): A subset of GM organisms, these are living organisms that have a unique combination of genetic material derived through modern biotechnology.

long noncoding RNAs (lncRNAs): Noncoding RNAs greater than 200 nucleotides long; involved in post-transcriptional regulation, RNA splicing, inactivation of the X-chromosome and other functions.

luciferase: A light-releasing enzyme present in bioluminescent organisms.

lyophilization: The process of freeze-drying.

M-Vac: A device that creates a vacuum to remove fingermark DNA.

macrophage: Term literally means big eaters; macrophages are phagocytic white blood cells that engulf and destroy dead cells and foreign materials such as bacteria.

macroplastics: Plastic particles larger than ~5 mm, present in aquatic environments in particular.

major histocompatibility complex (MHC): Tissue-typing proteins present on all cells and tissues; recognized by a person's immune system to determine whether cells are normal body cells or foreign; MHCs must be "matched" for successful organ transplantation.

malaria: Caused by the protozoan parasite Plasmodium falciparum and transmitted by insects. Worldwide, Plasmodium strains are developing resistance to the most commonly used antimalarial drugs.

mariculture: The cultivation or "farming" of aquatic organisms (animals or plants).

marker-assisted selection (MAS): A process whereby a marker used for indirect selection of a genetic trait of interest (e.g., productivity, disease resistance, stress tolerance, and quality).

marketing specialists: Marketing and sales position in biotechnology companies; marketing specialists are often involved in designing ad campaigns and promotional materials to market effectively a company's products.

Mass Fatality Identification System (M-FISys): M-FISys was essentially built in response to the 9/11 tragedy. Gene Codes did not have to write entirely new software, and they were able to customize M-FISys as necessary. In addition to analyzing mitochondrial DNA, M-FISys incorporated male-specific variations in the Y chromosome called Y-STRs to aid in the identification of individuals.

mass spectrometry (mass spec): Separation for identification of chemicals (e.g., proteins) based on charge-to-mass ratio.

maternal chromosomes: The 23 chromosomes inherited from your mother.

medical biotechnology: A diverse discipline of biotechnology dedicated to improving human health; includes a spectrum of topics in human medicine from disease diagnosis to drug discovery, disease treatment, and tissue engineering.

meiosis: A nuclear division process that occurs during formation of gametes. Meiosis involves a series of steps that reduce the amount of DNA in newly divided cells by half compared with those in the original cell.

membrane filtration: Using a thin membrane sheet with small holes (pores) to filter materials (such as large proteins or whole cells) out of a solution.

messenger RNA (mRNA): A template for protein synthesis, is an exact copy of a gene, contains nucleotide sequences copied from DNA that serve as a genetic code for synthesizing a protein, and is then bound and "read" by ribosomes to produce proteins.

metagenomics: The sequencing of genomes for entire communities of microbes in environmental samples of water, air, and soils from oceans throughout the world, glaciers, mines—virtually every corner of the globe.

metallothioneins: Metal-binding proteins.

metastasize: Typically refers to the spread of cancer cells from the site of origin in the body to different (secondary sites).

microbes: Tiny organisms that are too small to be seen individually by the naked eye and must be viewed with the help of a microscope. Although the most abundant microorganisms are bacteria, microbes also include viruses, fungi such as yeast and mold, algae, and single-celled organisms called protozoa.

microbial diagnostics: Methods for identifying microbes for different purposes such as clinical diagnosis of bacteria infections, detecting microbes causing food outbreaks, and detecting bioweapons; many of these techniques are based on molecular analysis of DNA.

microbial fuel cells: The use of microorganisms to generate electrical currents for power sources such as batteries by converting chemicals into electrical energy.

Microbial Genome Program (MGP): U.S. Department of Energy program to map and sequence genomes of a broad range of microorganisms.

microcosms: Test environments designed to mimic polluted environmental conditions; may consist of bioreactors or simply a bucket of polluted soil; allows scientists to test clean-up strategies on a small scale under controlled conditions before trying a particular clean-up approach in the environment.

microfiltration: Removal of particles 40 μm or smaller.

microorganisms: Organisms that are too small to be seen individually by the naked eye; microscopic organism (visualized through a microscope) such as bacteria, viruses, yeasts.

microplastics: Plastic particles smaller than ~5 mm, present in aquatic environments in particular.

microorganisms (microbes): Organisms that cannot individually be seen with the naked eye; include bacteria, yeast, fungi, protozoans, and viruses.

microRNAs (miRNAs): A new class of non-protein coding RNA molecules. MicroRNAs are part of a rapidly growing family of small RNA molecules about 20 to 25 nucleotides in size that play novel roles in regulating gene expression.

microsatellites: Also known as short tandem repeats (STRs), microsatellites are short repeating sequences of DNA usually consisting of one-, two-, or three-base sequences (for example, CACACACACA); microsatellites are important markers for forensic DNA analysis.

microspheres: Tiny particles that can be filled with drugs or other substances and used in therapeutic applications.

microtubules: Component of the cytoskeleton of cells; comprised of long rods consisting of protein subunits called tubulin; provide strength and stability for cell structure; used for movement of organelles with cells and for cellular movement.

missense mutation: A mutation changing a codon to another codon that codes for a different amino acid.

mitochondria: Double-membrane organelles in cells; site of ATP synthesis; powerhouse of the cell.

mitochondrial DNA (mtDNA): Small circular DNA found in mitochondria responsible for proteins unique to mitochondria. Mutations in this DNA can be followed from mother to offspring, because the egg is the predominant source of mitochondria.

mitochondrial replacement therapy (MRT): Form of in vitro fertilization used when the mother carries genes for a mitochondrial disease(s); involves the transfer of mitochondria and mitochondrial DNA (mtDNA) from an egg donor to replace those present in a recipient egg with a nuclear genome.

mitosis: A nuclear-division process that occurs during cell division in eukaryotic somatic cells and prokaryotes; divided into four major stages (prophase, metaphase, anaphase, and telophase). Mitosis results in the even separation of DNA into dividing cells.

MMR: Measles, mumps, and rubella (German measles) vaccine designed to provide immune protection against common childhood diseases.

model organisms: Nonhuman organisms that scientists use to study biologic processes in experimental laboratory conditions; common examples include mice, rats, fruit flies, worms, and bacteria.

monoclonal antibodies (mAbs): Antibody proteins produced from clones of a single ("mono") cell; these proteins are highly specific for a particular antigen.

moratorium: As it relates to science, a temporary but complete stoppage of any research.

morula: Latin term meaning "little mulberry;" solid ball of cells that forms during embryonic development in animals, created by repeated cell division of the zygote.

MRSA (methicillin resistant Staphylococcus aureus): Potentially deadly strain of *S. aureus* resistant to most antibiotics including methicillin one of the most effective for treating staph infections, thus infections of MRSA can be difficult to treat.

mutagens: Physical or chemical agents that cause mutations.

mutation breeding: Mutation breeding, is the process of exposing seeds to chemicals or radiation in order to generate mutants with desirable traits to be bred with other cultivars.

mutations: A change in the DNA structure or sequence of a gene.

myelomas: Antibody-secreting tumors.

Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress: A legally-binding agreement that establishes international liability and redress frameworks for any damage to biological diversity caused by transboundary movements of living modified organisms.

nanomedicine: Applying nanotechnology to improve health; for instance, microsensors that can be implanted into humans to monitor vital values such as blood pressure.

nanotechnology: Engineering and structures and technologies at the nanometer scale.

National Institutes of Health (NIH): Government agency that is the focal point for medical research in the United States; houses world-renowned research centers and agencies that are an essential source of funding for biomedical research in the United States.

neoantigens: Patient-specific, cancer antigens (such as proteins) from an individual person; can be targeted by different therapies for treating cancer.

network map: A diagrammitc representation of interacting, proteins, genes and other molecules in cells; allow for the analysis of key interactions in complex conditions such as metabolism and disease.

next-generation sequencing (NGS): Next-generation sequencing refers to non-Sanger-based high-throughput DNA sequencing technologies. Millions or billions of DNA strands can be sequenced in parallel, yielding substantially more throughput and minimizing the need for the fragment- cloning methods that are often used in Sanger sequencing of genomes.

nitrogenous base: Important component of DNA and RNA nucleotides; often simply called a "base." Nitrogen-containing structures include double-ring purines (which include the bases adenine and guanine) and single-ring pyrimidines (which include the bases cytosine, thymine, and uracil).

non-coding RNA (ncRNA): RNA molecules that do not code for proteins; include transfer RNA (tRNA), ribosomal RNAs (rRNA), microRNAs (miRNAs) and many others.

noninvasive prenatal genetic diagnosis (NIPD): Tests based on fragments of fetal DNA from cells that have died and been digested by enzymes. Free-floating DNA fragments end up in the mother's bloodstream and can be tested using a blood sample from the mother.

nonsense mutations: Mutations that change a codon into a stop codon; these usually produce a shortened, poorly functioning, or nonfunctional protein.

Northern blot: Laboratory technique for separating RNA molecules by gel electrophoresis and transferring (blotting) RNA onto a filter paper blot for use in hybridization studies.

Northern blotting: See *Northern blot analysis*.

nuclear envelope: A double-layered membrane, is typically the largest structure in an animal cell.

nuclear reprogramming of somatic cells: Used for isolating stem cells without creating an embryo. The basic concept of this approach is to use genes involved in cell development to push a somatic cell back to an earlier stage of development and affect gene expression, to reprogram the somatic cell genetically to return to a pluripotent state characteristic of the stem cells from which it was derived.

nucleic acids: Molecules composed of nucleotide building blocks; two major types are DNA and RNA.

nucleotide: Building block of nucleic acids; consists of a five-carbon (pentose) sugar molecule (ribose or deoxyribose), a phosphate group, and the bases adenine (A), guanine (G), cytosine (C), thymine (T), or uracil (U).

nucleus: Membrane-enclosed organelle that contains the DNA of a eukaryotic cell.

nutrient enrichment: See *fertilization*.

nutrigenomics: A new field of nutritional science focused on understanding interactions between diet and genes.

objectivism: Refers to philosophical approaches to ethics and morals which are thought to exist independently of knowledge or perception.

oncolytic viruses: A form of gene therapy involving genetically engineered viruses designed to target and destroy cancer cells.

operator: Region of DNA, such as that located in an operon, that binds to a specific repressor protein to control expression of a gene or group of genes such as an operon.

operons: Gene units common in bacteria; typically consisting of a series of genes, located on adjacent regions of a chromosome, and regulated in a coordinated fashion. Many operons are involved in bacterial cell metabolism of nutrients such as sugars. Refer to the lac operon as a well-characterized bacterial operon.

OPV (Oral Polio Vaccine): An attenuated vaccine for protection against the polio virus, taken orally.

Organ on Chip: Micro engineered biological mimic systems composed of relevant tissues from functioning organs used to test drug effectiveness.

organelles: Small structures in the cytoplasm of eukaryotic cells that perform specific functions.

organic molecules: Molecules that contain carbon and hydrogen.

organoids: An **organoid** is a miniaturized and simplified version of an organ produced in vitro in three dimensions with realistic micro-anatomy. Organoids are an excellent tool to study basic biological processes.

origins of replication: Specific locations in a DNA molecule where DNA replication begins.

Orphan Drug: Drugs produced for a small number of beneficiaries (that permits faster FDA approval).

osteoporosis: Category of bone disorders that generally involve a progressive loss of bone mass.

oxidation: The removal of one or more electrons from an atom or molecule.

oxidizing agents: Atoms or molecules that accept electrons during a redox reaction and cause the oxidation of other atoms or molecules; known as electron acceptors, oxidizing agents become reduced when they accept an electron.

P (peptidyl) site: Portion of a ribosome into which peptidyl tRNA molecules bind during translation.

p arm: "Petit" or small/short arm of a chromosome.

PACE Phage Assisted Continuous Evolution: the use of mutate phage to study effectiveness of new proteins due to their rapid reproduction cycling.

paleogenomics: The analysis of ancient DNA such as DNA from fossils; also called "stone-age genomics."

papain: Protein-digesting enzyme.

Paragraph 6 Mechanism: A mechanism to establish a framework for compulsory licenses to enable the export of patented medicines to WTO members who do not have adequate domestic manufacturing capacity.

patent: Legal recognition that gives an inventor or researcher exclusive rights to a product and prohibits others from making, using, or selling the product for a certain number of years (20 years from the date of filing).

patenting: The process of receiving a patent (see *patent*).

paternal chromosomes: Copies of chromosomes inherited from the father.

pathogens: Disease-causing organisms.

pentose sugar: A five-carbon sugar; important components of DNA and RNA nucleotide structure. The pentose sugar deoxyribose is contained in DNA nucleotides; the pentose sugar ribose is contained in RNA nucleotides.

peptidoglycan: Structure in bacterial cell walls consisting of specialized sugars and short, interconnected polypeptides.

peptidyl transferase: The rRNA enzyme that is part of the large ribosomal subunit; catalyzes the formation of peptide bonds between amino acids during translation.

Personal Genome Project (PGP): An international project to develop personal genomics technologies and practices with several goals such as effective, informative, and responsible management of personal genome data, analysis and open sharing of personal genome sequences, and the establishment of how personal genomes may be used for improving human health and fighting disease.

personalized genomes: Sequencing a genome for an individual.

personalized genomics: A branch of genomics where individual genomes are sequenced and analyzed using bioinformatics tools that may lead to personalized medicines.

personalized or **precision medicine:** The customization of healthcare, with medical decisions, treatments, practices, or products being tailored to the individual patient. Tools employed in precision medicine can include molecular diagnostics, imaging, and analytics.

personhood: A term popular in bioethics that defines an entity that qualifies for protection based on certain attributes not intrinsic (built-in) or automatic values.

phage therapy: The use of bacteriophages (viruses that infect bacteria) for treating human disease conditions.

pharmaceutical companies: Companies creating drugs for the treatment of human health conditions.

pharmacogenetics: See *personalized genetics*.

pharmacogenomics: A form of customized medicine in which disease-treatment strategies are designed based on a person's genetic information (for a particular health condition).

phase testing: A statistically significant number of trials are required on cell cultures, in live animals, and on human subjects in the three-phase testing processes specified by the Food and Drug Administration.

phosphodiester bonds: Covalent bonds between the sugars of one nucleotide and the phosphate group of an adjacent nucleotide; join nucleotides within strands of DNA and RNA.

phytoremediation: Using plants for bioremediation.

placebos: A blank or ineffective treatment. Used in the scientific practice of having a control group in an experiment, such as a drug trial, that receives an ineffective pill or treatment such as a sugar pill or injection of water instead of the actual medication being tested.

plant transformation: Plant transformation produces genetically modified plants, in which the DNA has been modified using genetic engineering methods. In most cases, the aim is to introduce a new trait to the plant which does not occur naturally in the species.

plasma (cell) membrane: A double-layered structure, consisting of lipids, proteins, and carbohydrates, that defines the boundaries of a cell; performs important roles in cell shape and regulating transport of molecules in and out of a cell.

plasma cells: Antibody-producing cells that develop from B lymphocytes after B cells are exposed to foreign materials (antigens).

plasmids (plasmid DNA): Small, circular, self-replicating double-stranded DNA molecules found primarily in bacterial cells. Plasmids often contain genes coding for antibiotic resistance proteins and are routinely used for DNA-cloning experiments.

pluripotent: Term used to describe cells, such as embryonic stem cells, with the potential to develop into other cell types.

point mutations: A single base change in DNA sequence.

polarity: Refers to the 5′ and 3′ ends of DNA and RNA molecules.

polyadenylation: Addition of a short sequence or "tail" of adenine (A) nucleotides to the 3′ end of an mRNA molecule; occurs during RNA splicing in eukaryotes; poly(A) tail is important for mRNA stability in the cytoplasm.

polyculture: Raising more than one aquatic species in the same environment. For instance, cultivating fish together with aquatic vegetation.

polygalacturonase: An enzyme naturally produced by plants that digests tissue, causing rotting.

polymerase chain reaction (PCR): Laboratory technique for amplifying and cloning DNA; involves multiple cycles of denaturation, primer hybridization, and DNA polymerase synthesis of new strands.

polypeptide: A chain of amino acids joined by covalent (peptide) bonds; usually greater than 50 amino acids in length.

polyploid: Organisms with an increased number of complete sets of chromosomes.

posttranslational modifications: Protein modifications that occur naturally after initial synthesis.

precipitate: Combines because of mutual attraction.

precision medicine, the Precision Medicine Initiative (PMI): (See also *personalized medicine*.) An emerging approach for disease prevention and treatment that takes into account people's individual variations in genes, environment, and lifestyle; intended to lead to the creation of specific (precise) individualized treatment approaches to improve the health of populations of humans.

preimplantation genetic diagnosis (PGD or PIGD): The genetic analysis of embryos or oocytes, prior to implantation or prior to fertilization; enables embryo selection as an effort to minimize the likelihood that an embryo will have an inherited genetic disease; also used to select embroyos of a certain sex.

Prialt: FDA-approved peptide conotoxin purified from the marine cone snail *Conus magus* by the Elan Corporation of Ireland. Conotoxins peptides are natural neurotoxins that block neural pathways that relay pain messages to the brain. Prialt is a painkiller used to treat chronic, severe forms of pain, such as back pain.

primary transcript (pre-mRNA): Initial mRNA molecule copied from a gene in the nucleus of eukaryotic cells; undergoes modifications (processing) to produce mature mRNA molecules that enter the cytoplasm.

primase: Enzyme that adds small RNA segments to a single strand of DNA as an early and necessary step for DNA replication.

primers: Oligonucleotides complementary to specific sequences of interest; used in PCR reactions to amplify DNA and DNA-sequencing reactions.

principal/senior scientists: Science leadership position in biotechnology companies; senior scientists are usually Ph.D. or M.D.-trained individuals who plan and direct the research priorities of a company.

Prior informed consent: Permission of the provider state that is required prior to accessing genetic resources.

probe: Single-stranded DNA or RNA molecule (labeled, for instance, with radioactive or fluorescing nucleotides) that can bind other DNA or RNA sequences by complementary base pairing and be detected by a process such as autoradiography; important laboratory technique for such applications as identifying genes and studying gene activity. Protein probes are usually antibodies that bind to structures with high affinity (e.g., antigens) and can be detected by fluorescent tags.

product complaint specialists: A Quality Control (QC) specialist that certifies that a product meets the criteria specified in its package insert or other specifications submitted to regulatory agencies.

prokaryotic cells: Cells that lack a nucleus and membraneenclosed organelles; only examples are bacteria and Archae.

promoter: Specific DNA sequences adjacent to a gene that direct transcription (RNA synthesis); binding site of RNA polymerase to begin transcription.

pronuclear microinjection: This method introduces the transgene DNA at the earliest possible stage of development of the zygote (fertilized egg).

prostate-specific antigen (PSA): A protein released into the bloodstream when the prostate gland is inflamed, and elevated levels can be a marker for prostate inflammation and even prostate cancer.

proteases: Protein-digesting enzymes.

proteins: Macromolecules consisting of amino acids joined by peptide bonds; major structural and functional molecules of cells.

protein chip: See *protein microarray*.

protein microarray: A "chip" similar to a DNA microarray; consists of a glass slide containing thousands of individual proteins attached to specific spots on the slide; each "spot" contains a unique protein.

proteolytic: Protein–lysing characteristic.

proteome: The entire complement of proteins in an organism.

proteomes: Families of proteins.

proteomics: Study of protein families.

protoplast fusion: Fusing of plant cells lacking cell walls to produce a fused cell that can become a clone.

protoplast: A naked plant cell (without cell wall).

PulseNet: Partnership of bacterial DNA fingerprinting laboratories, developed by the U.S. Centers for Disease Control and Prevention (CDC) and the U.S. Department of Agriculture, designed to provide rapid analysis of contaminated food, with the purpose of identifying contaminating microbes and preventing outbreaks of food-borne disease.

q arm: Long arm of a chromosome.

Raw genetic resources: The genetic material in its natural state.

real-time or quantitative PCR (qPCR): New applications in PCR technology make it possible to determine the amount of PCR product made during an experiment. Uses primers made with fluorescent dyes and specialized thermal cyclers that enable researchers to quantify amplification reactions as they occur.

Recombinant DNA Advisory Committee (RAC): NIH panel responsible for establishing and overseeing guidelines for recombinant DNA research and related topics.

recombinant DNA (rDNA) technology: Technique that allows DNA to be combined from different sources; also called gene or DNA splicing. Recombinant DNA is an important technique for many gene-cloning applications.

recombinant proteins: Commercially valuable proteins created by recombinant DNA technology and gene-cloning techniques; examples include insulin and growth hormone.

redox reactions: Combination of oxidation and reduction reactions.

reduction: The addition of one or more electrons to a molecule.

regenerative medicine: A discipline of medical biotechnology that involves repairing or replacing damaged tissues and organs by using tissues and organs grown through biotechnological approaches.

rennin: Protein-degrading enzyme derived from the stomach of milk-producing animals such as cows and goats; used in cheese production; recombinant form called chymosin.

reporter gene: Genes (such as the lux genes) that can be used to track or monitor (report on) expression of other genes.

reproductive cloning: Cloning process that creates a new individual; process typically involves using the genetic material of a single cell to create an individual with the single genetic composition of its creator cell.

research and development (R&D): All of the processes involved in basic research (for example, pre-clinical research) and development of a potential product. The lifeblood of a biotechnology company, R&D is how companies identify new technologies, drugs, etc. for commercialization.

research assistants/associates: Laboratory positions in which individuals are primarily involved in carrying out experiments under the supervision of other scientists such as principal or senior scientists.

restriction enzymes: DNA-cutting proteins found primarily in bacteria. Enzymes cleave (cut) the phosphodiester backbone of double-stranded DNA at specific nucleotide sequences (restriction sites). Commercially available restriction enzymes are essential for molecular biology experiments.

restriction map: An arrangement or "map" of the number, order, and types of restriction-enzyme cutting sites in a DNA molecule.

restriction sites: Specific sequences of DNA nucleotides recognized and cut by restriction enzymes.

retroviruses: Viruses that contain an RNA genome and use reverse transcriptase to copy RNA into DNA during the replication cycle in host cells.

retrovirus-mediated transgenics: Infecting embryos with a retrovirus (usually genetically engineered) before the embryos are implanted. The retrovirus acts as a vector for the new DNA.

reverse transcriptase (RT): Viral polymerase enzyme that copies RNA into single-stranded DNA. This commercially available enzyme is used for many molecular biology experiments, such as creating cDNA.

reverse transcription PCR (RT-PCR): Laboratory technique that involves using the enzyme reverse transcriptase to copy RNA from a cell into cDNA and then amplifying cDNA by the polymerase chain reaction (PCR); a valuable technique for studying gene expression.

RFLP: Restriction Fragment Length Polymorphisms result from mutations that prevent DNA restriction enzymes from cutting DNA, and can be used as a type of 'DNA fingerprint'.

ribonucleic acid (RNA): Single strands of nucleotides produced from DNA. Different types of RNA have important functions in protein synthesis.

ribosomal RNA (rRNA): Small RNA molecules that are essential components of ribosomes.

ribosomes: Organelles composed of ribosomal ribonucleic acid (rRNA) and proteins assembled into packages called subunits. Ribosomes bind to mRNA and tRNA molecules and are the site of protein synthesis in prokaryotes and eukaryotes.

risk assessments: Analysis of potential risks and benefits of a procedure, drug, technology, etc.

RNA-induced silencing complex (RISC): RISC unwinds the double-stranded siRNAs, releasing single-stranded siRNAs that bind to complementary sequences in mRNA molecules. Binding of siRNAs to mRNA results in degradation of the mRNA (by the enzyme dicer) or blocks translation by interfering with ribosome binding.

RNA interference (RNAi): RNA-based mechanisms of gene silencing. These siRNAs are bound by a protein–RNA complex called the RNA-induced silencing complex (RISC).

RNA polymerase: Copies RNA from a DNA template; different forms of RNA polymerase synthesize different types of RNA.

RNA primers: Short, single-strand RNA sequence that binds DNA and is used to start DNA replication by DNA polymerase.

RNA sequencing (RNA-seq): Molecular technique for determining the nucleotide sequence of RNA molecules in cells (as opposed to DNA sequencing).

RNA or gene silencing: General term for techniques that are used to inhibit a gene or RNA molecule from expressing the protein it encodes.

RNA splicing: The removal of nonprotein coding sequences (introns) from primary transcript (pre-mRNA) and joining of protein-coding sequences (exons).

sales representatives: Salespersons in biotechnology companies; sales representatives are "people persons" who work closely with medical doctors, hospitals, and health care providers to promote a company's products.

scale-up processes: The industrial implementation of processes in which chemical or microbiological conversion of material takes place that behave differently on a small scale (in laboratories or pilot plants) and on a large scale (in production).

SDS-PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis is a process where proteins are boiled in the presence of SDS to denature the protein and attach sulfate (negatively charged) groups to each amide link in a protein so that they can be separated by their number of charges by electrophoresis in a gel made of polyacrylimide.

Secretariat of the Convention on Biological Diversity (SCBD): Administrative office, based in Canada, that supports the goals of the Convention on Biological Diversity.

secretome: The protein expression and secretion machinery in bacteria.

seeding: See *bioaugmentation*.

selection: Laboratory technique used to identify bacteria containing recombinant DNA of interest; involves growing bacteria on media with antibiotic or other selection molecules.

selective breeding: Mating organisms with desired features to produce offspring with the same characteristics.

self-renewal: Refers to cells, such as stem cells, that can replicate to produce more cells indefinitely.

semiconservative replication: Process by which DNA is copied; one original (parent) DNA molecule gives rise to two molecules, each of which has one original strand and one new strand.

senescence: Cellular aging process.

severe acute respiratory syndrome (SARS): Severe form of pneumonia caused by the SARS virus; can lead to death due to respiratory failure.

sex chromosomes: Contain majority of genes that determine an organism's sex; the X (female) and Y (male) chromosomes in humans.

short interfering RNA (siRNA): Small (21 or 22 nt) doublestranded pieces of nonprotein coding RNA, so named because they were shown to bind to mRNA and subsequently block or interfere with translation of bound mRNAs.

short tandem repeat (STR): One to six nucleotide repeats that are dispersed throughout chromosomes. Because these repeated regions can occur in many locations within the DNA, the probes used to identify them complement the DNA regions that surround the specific microsatellite being analyzed.

sickle cell anemia: Inherited genetic disorders of red blood cells; caused by a mutation in the hemoglobin gene that codes fo rthe oxygen-carrying protein hemoglobin; produces sickled cells that transport oxygen poorly and cause circulatory problems.

silent mutation: Base-pair substitution that has no effect on the amino acid sequence of a protein.

single-cell RNA sequencing (scRNA-seq): Current technology that can provide RNA sequence information from a single cell as opposed to sequencing RNAfrom a collection of cells; allows for an examination of gene expression (transcriptomes) divesity within populations of cells in the same tissue.

single-cell sequencing (SCS): Current technology that can provide DNA or RNA sequence information from a single cell as opposed to sequencing the genomes from a collection of cells; allows for an examination of genetic diversity within populations of cells in the same tissue.

single-molecule sequencing in real time (SMRT): A third generation sequencing approach that involves sequencing a single molecule of single-stranded DNA.

single-nucleotide polymorphisms (SNPs): Single-nucleotide variations in the gene sequence, or a type of DNA mutation; the basis of genetic variation among humans.

sister chromatids: Exact copies of double-stranded DNA molecules and proteins (chromatin) joined to form a chromosome.

site-directed mutagenesis: With this technique, mutations can be created in specific nucleotides of a cloned gene contained in a vector. The gene can then be expressed in cells, which results in translation of a mutated protein. This allows researchers to study the effects of particular mutations on protein structure and functions as a way to determine what nucleotides are important for specific functions of the protein.

size-exclusion chromatography (SEC): Separation based on molecular size.

sludge: A semisolid material produced from treated sewage waste; consists largely of small particles of feces, waste papers, and microorganisms.

slurry-phase bioremediation: Process in which contaminated soil is removed from a site and mixed with water and fertilizers (and often oxygen) in large drums or bioreactors to create a mixture (slurry) that stimulates bioremediation by microorganisms in the soil.

small interfering RNAs (siRNA): Double-stranded RNA molecules, usually 20-25 bp in length, which can be used for RNA interference (silencing) experiments. Naturally occurring in many species and contribute to gene expression regulation through RNA silencing.

small noncoding RNAs (sncRNAs): RNA molecules that are not translated into a protein. These include transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), microRNAs (miRNA) and others.

solid-phase bioremediation: *Ex situ* soil clean-up strategies that primarily involve composting, landfarming, or biopiles.

somatic cell nuclear transfer (SCNT): DNA transfer process that can be used for reproductive or therapeutic cloning purposes; involves removing DNA from one cell and inserting it into an egg cell that has had its DNA removed (enucleated).

somatic cells: All cell types in multicellular organisms except for gametes (sperm and egg cells).

Southern blot analysis: Laboratory technique invented by Ed Southern that involves transferring (blotting) DNA fragments onto a filter-paper blot for use in probe hybridization studies.

Southern blotting: See *Southern blot analysis*.

species integrity: Generally refers to maintaining the natural functions, abilities, and genetic constitution of a particular species; for example, creating recombinant animals or plants such as transgenic or polyploid organisms may change "species integrity" by making a species less well adapted to life in the wild.

spectral karyotyping: A technique involving probes specific for each chromosome that fluoresces different colors to identify chromosomes according to their color pattern.

sperm-mediated transfer: The DNA is injected or attached directly to the nucleus of the sperm before fertilization.

spinal muscular atrophy (SMA): A disease characterized by the loss of motor neurons in the spinal cord, resulting in progressive muscle weakness. A leading genetic cause of death for infants. Caused by a mutation in the *SMN1* gene that prevents production of the functional SMN protein required for normal motor neuron development.

Spinraza (nusinersen): Trade name for antisense RNA-based therapy to treat spinal muscular atrophy (SMA).

spiral: Refers to corkscrew-shaped category of bacterial cells.

startup companies: Formed by a small team of scientists who believe they may have a promising product to make (such as a recombinant protein to treat disease). The team must typically then seek investors to fund their company so they can buy or rent laboratory facilities, buy equipment and supplies, and continue the research and development necessary to make their product.

statistical probability: Using statistical measures to calculate the possibility (probability) that a particular event will happen.

stem cells: Immature (undifferentiated) cells that are capable of forming all mature cell types in animals and that can be derived from embryos at several days of age or from adult tissues.

stone age genomics: A number of laboratories around the world are involved in analyzing "ancient" DNA. These studies are generating fascinating data from minuscule amounts of ancient DNA from bone and other tissues and fossil samples that are tens of thousands of years old.

substrates: Molecule or molecules on which an enzyme performs a reaction.

subtilisin: Protease derived from Bacillus subtilis, a valuable component of many laundry detergents, where it functions to degrade and remove protein stains from clothing. Several bacterial enzymes are also used to manufacture foods, such as carbohydrate-digesting enzymes called amylases that are used to degrade starches.

subunit vaccines: Vaccine created from components of a pathogen such as viral proteins or lipid molecules.

Superfund Program: Program established by the U.S. Congress in 1980 through the U.S. Environmental Protection Agency, designed to identify and clean up hazardous waste sites and protect citizens from harmful effects of waste sites.

swine flu (H1N1): Caused by a virus that primarily infects pigs, but human infections also occur. H1N1 is a strain that infected people in various outbreaks in 2009 and subsequent years.

synthetic biology: The use of manmade DNA sequences for creating modified cells or organisms.

synthetic genome: See *synthetic biology*.

systems biology: Involves combining genomic data with information about the structure, function, regulation, and interactions of different pathways (such as a metabolic pathway) to provide a more comprehensive understanding of the interactions between different "systems" in a cells (rather than just studying genomes or enzymes for example).

T cell receptors (TCRs): Molecules present on the surface of T lymphocytes (T cells), responsible for recognizing fragments of antigens (foreign materials) as peptides bound to major histocompatibility complex (MHC) molecules; involved in engineering T cells for immunotherapy.

T lymphocytes (T cells): Type of white blood cell (leukocyte); T lymphocytes, also called "T cells" play an essential role in helping the immune system recognize and respond to foreign materials (antigens).

***Taq* DNA polymerase (or *Taq*):** DNA-synthesizing enzyme isolated from Thermus aquaticus, a thermophilic Archae that lives in hot springs; its ability to withstand high temperatures (thermostable) without denaturation makes it valuable for use in PCR experiments.

TATA box: Short nucleotide sequence (TATA) usually located approximately 20 to 30 base pairs "upstream" (in the 5′ direction) of the start site of many eukaryotic genes; part of promoter sequence bound by transcription factors used to stimulate RNA polymerase.

telomere: The end structures of a eukaryotic chromosome; in humans, consists of specific repeating sequences of DNA (TTAGGG).

template strand: After RNA polymerase binds to a promoter, it unwinds a region of the DNA to separate the two strands. Only one of the strands, called the template strand (the opposite strand is called the coding strand), is copied by RNA polymerase.

teratogenic: A substance that creates abnormal developmental effects in a an embryo.

thalidomide: Compound used initially to combat morning sickness in pregnant women; certain chemical forms of thalidomide caused severe birth defects; thalidomide derivatives are still being investigated for their use in treating cancer, HIV, and other diseases.

The Cancer Genome Atlas Project (TCGA): Initiated in 2005 and completed in 2017, TCGA involved a collaboration between U.S. research agencies to catalogue genetic mutations responsible for different types of cancer, using genome sequencing and bioinformatics to improve our ability to diagnose, treat, and prevent cancer through a better understanding of the genetic basis of cancer.

therapeutic cloning: Using a patient's DNA to create (clone) an embryo as a source of stem cells that could be used in therapeutic applications to treat the patient.

thermophiles: Organisms with high optimal growth temperatures. For example, bacteria that live in hot springs are thermophiles.

thermostable enzyme: An enzyme that is capable of withstanding high temperatures and is isolated from theromophiles. For example, Taq DNA polymerase is a thermostable enzyme.

third-generation sequencing (TGS): Newest approach to DNA sequencing technology; involes reading nucleotide sequences in single molecules of DNA; allows for reading long sequences of DNA because TGS does not require breaking DNA into smaller pieces as is often necessary for other sequencing approaches.

Thymine (T): Abbreviated T; pyrimidine base present in DNA nucleotides.

tissue engineering: Designing and growing tissues for use in regenerative medicine applications.

Touch DNA: DNA left from a fingermark.

traits: Inherited features of an organism such as skin color and body shape.

transcription: The synthesis of RNA from DNA, which occurs in the nucleus of eukaryotic cells and the cytoplasm of prokaryotic cells.

transcription factors: DNA-binding proteins that bind promoter regions of a gene and stimulate transcription of a gene by RNA polymerase.

transcriptional regulation: Form of gene-expression regulation that involves controlling the process of transcription by controlling the amount of RNA produced by a cell.

transcriptome: All RNA molecules (coding and noncoding) in one cell or a population of cells.

transfection: Introducing DNA into animal or plant cells.

transfer RNA (tRNA): Small RNA molecules that transport amino acids to a ribosome during protein synthesis. tRNA binds to specific codons in mRNA sequences during translation.

transformation: The process by which bacteria take in DNA from the surroundings. Term also is used to define changes that cause a normal cell to become a cancer cell.

transgene: Gene from one organism introduced into another organism to create a transgenic; term usually applies to genes used to create transgenic animals and plants.

transgenic animals: Animals that contain genes from another source. For instance, human genes for clotting proteins can be introduced into cows for the production of these proteins in their milk.

transgenic organisms: An organism that received a DNA sequence (usually a gene or genes; called a transgene) from another organism or cell; transgene allows for expression of specific traits not normally present in the transgenic organism.

translation: The synthesis of proteins from genetic information in messenger (mRNA) molecules. Translation occurs in the cytoplasm of all cells.

triploids: Three sets of chromosomes (3n); used to describe organisms with three sets of chromosomes.

tuberculosis (TB): Disease caused by the bacterium Mycobacterium tuberculosis, which grows slowly and can exist in a human for several years before the individual develops TB.

two-dimensional electrophoresis: Separation based on charge in two directions.

type I, or insulin-dependent, diabetes mellitus: Disease caused by a lack of the pancreatic hormone insulin, which is required for carbohydrate metabolism. Creates elevated blood sugar levels (hyperglycemia).

ultrafiltration: Separation of particles smaller than 20 μm.

upstream processing: Adjustments in the purification process based on changes in the biologic process, making processing more efficient.

U.S. Department of Agriculture (USDA): Agency created in 1862 that has many functions related to the advancement and regulation of agriculture. Some of those functions include the regulation of plant pests, plants, and veterinary biologics.

U.S. Environmental Protection Agency (EPA): Agency whose primary purpose is to protect human health and to safeguard the natural environment (air, water, and land) by working with other federal agencies in the United States and state and local governments to develop and enforce regulations under existing environmental laws.

U.S. Food and Drug Administration (FDA): A federal agency of the United States Department of Health and Human Services. The FDA is responsible for protecting and promoting public health through the control and supervision of food safety, tobacco products, dietary supplements, prescription and over-the-counter pharmaceutical drugs, vaccines, biopharmaceuticals, blood transfusions, medical devices, electromagnetic radiation emitting devices, cosmetics, animal foods & feed and veterinary products.

United Nations Educational, Scientific and Cultural Organization(UNESCO): A specialized agency of the United Nations that promotes peace and encourage sustainable development through education, sciences, culture, communication, and information.

utilitarian approach: Line of ethical thought that states that actions are moral if the result produces the greatest good for the greatest number of humans; also referred to as consequential ethics because it focuses on results or consequences, not on intentions.

utilitarianism: Principle referred to in ethics that an action is ethical, correct of justified if it provides the greatest benefit for the greatest number of people.

vaccination: The process of administering a vaccine to provide an organism with immunity to an infectious microorganism.

vaccines: A preparation of a microorganism or its components that is used to stimulate the production of antibodies (antibody-mediated immunity) in an organism.

variable number tandem repeats (VNTRs): The recognition that variable numbers of repeated nucleotides can be found in DNA and can be used for identification of individuals.

vectors: DNA (or viruses) that can be used to carry and replicate other pieces of DNA in molecular biology experiments; for example, plasmid DNA, viruses used for gene therapy; also refers to organisms that carry disease.

venture capital (VC): Funding for an enterprise provided by financiers who see promise in the enterprise and expect a substantial financial benefit for the high risk (or expect to take a loss).

Virus Like Particles (VLPs) : Virus coat proteins that can be produced by plants by gene insertion that can prevent plant virus infection.

Western blot analysis: Laboratory technique for separating protein molecules by gel electrophoresis and transferring (blotting) proteins onto a filter paper blot that is usually probed with antibodies to study protein structure and function.

Western blotting: See *Western blot analysis*.

Worked genetic resources: The genetic material that has undergone some amount of research and development.

World Intellectual Property Organization (WIPO): A specialized agency of the United Nations that promotes the protection of intellectual property throughout the world.

World Organisation for Animal Health (OIE): An intergovernmental organization dedicated to sharing and improving knowledge about global animal health, collecting and analyzing veterinary scientific information, and strengthening the structure of animal health systems of its member countries.

World Trade Organization (WTO): An international organization that regulates trade between nations. The WTO provides a framework for negotiating trade agreements and resolving trade-related disputes.

whole-exome sequencing (WES): A technique for sequencing all of the protein-coding regions (exons) in a genome.

whole-genome "shotgun" sequencing: DNA sequencing approaches in which an entire genome can be fragmented and its sequence determined. Bioinformatics methods are then used to assemble the sequenced fragments of a genome to assemble the entire genome sequence.

World Health Organization (WHO): An agency of the United Nations that focuses on issues of public health globally.

xenotransplantation: The transplantation of living cells, tissues or organs from one species to another.

XNAzymes: Catalysts whose genetic sequence includes (instead of DNA or RNA), synthetic nucleic acid analogues, termed Xeno Nucleic Acid (XNA) are used as information carriers. Protein translation can then include the incorporation of non-proteinogenic amino acids into proteins.

yeasts: A unicellular fungus.

yeast artificial chromosomes (YACs): Plasmid vectors grown in yeast cells that can replicate very large pieces of DNA; used to clone pieces of human chromosomes for the Human Genome Project.

yeast two-hybrid system: Laboratory technique involving the use of yeast to join together different proteins (creating a hybrid protein) as a way to study protein function.

Zika virus (ZIKV): A member of the virus family *Flavivirida;* spread by *Aedes* mosquitoes, such as *A. aegypti* and *A. albopictus*.From 2007 to 2016, the virus spread eastward, across the Pacific Ocean to the Americas, leading to the 2015–16 Zika virus epidemic. Developing fetuses in pregnant women are particularly succeptible to the effects of ZIKV which can cause a variety of birth defects including microcephaly (abnormal development of the brain, resulting in a smaller than normal head size).

zinc-finger nucleases (ZFNs): Artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain.

zygote: Diploid cell formed when haploid gametes such as a sperm and egg cell unite.

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