
Prescott's
MICROBIOLOGY
ELEVENTH EDITION
JOANNE WILLEY
KATHLEEN SANDMAN
DOROTHY WOOD
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eleventh edition

Prescott's Microbiology

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DURHAM TECHNICAL COMMUNITY COLLEGE

Mc Graw Hill Education

PRESCOTT'S MICROBIOLOGY, ELEVENTH EDITION

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Brief Contents

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About the Authors

Courtesy of Joanne Willey

Joanne M. Willey has been a professor at Hofstra University on Long Island, New York, since 1993, where she is the Leo A. Guthart Professor of Biomedical Science and Chair of the Department of Science Education at the Donald and Barbara Zucker School of Medicine at Hofstra/Northwell. Dr. Willey received her B.A. in Biology from the University of Pennsylvania, where her interest in microbiology began with work on cyanobacterial growth in eutrophic streams. She earned her Ph.D. in biological oceanography (specializing in marine microbiology) from the Massachusetts Institute of Technology–Woods Hole Oceanographic Institution Joint Program in 1987. She then went to Harvard University, where she spent her postdoctoral fellowship studying the filamentous soil bacterium *Streptomyces coelicolor.* Dr. Willey has coauthored a number of publications that focus on its complex developmental cycle. She is an active member of the American Society for Microbiology (ASM), and served on the editorial board of the journal *Applied and Environmental Microbiology* for nine years and as Chair of the Division of General Microbiology. Dr. Willey taught microbiology to biology majors for 20 years and now teaches microbiology and infectious disease to medical students. She has taught courses in cell biology, marine microbiology, and laboratory techniques in molecular genetics. Dr. Willey lives on the north shore of Long Island and has two grown sons. She is an avid runner and enjoys skiing, hiking, sailing, and reading. She can be reached at joanne.m.willey@hofstra.edu.

Courtesy of Adele Anderson

Kathleen M. Sandman received her B.A. in Biology from La Salle University and her Ph.D. in Cellular and Developmental Biology from Harvard University. She was inspired to a career in science by her older brother's experience as an organic chemist and by the developing technology in recombinant DNA in the 1970s. Her graduate work used a transposable element as a mutagen in *Bacillus subtilis* to study gene expression during endospore formation. She continued in the genetics of Gram-positive bacteria with a postdoctoral year studying *Bacillus thuringiensis* at the University of Cambridge in the United Kingdom. Another postdoctoral opportunity at The Ohio State University provided an introduction to the emerging field of archaeal molecular biology, where Dr. Sandman discovered archaeal histones and continued research in the structural biology of archaeal chromatin for about 20 years. She served the National Science Foundation as a research grant reviewer and panelist for the Life in Extreme Environments program, and has organized conference sessions on archaeal molecular biology and proteins from extremophiles. Dr. Sandman has taught microbiology to hundreds of students, at both the introductory level and in an advanced molecular microbiology laboratory. Dr. Sandman has worked as a consultant in a variety of industries, including industrial microbiology, environmental geomicrobiology, and technical publishing. She lives with her husband in Columbus, Ohio, and has two grown daughters. She enjoys biking, fabric arts, reading, and genealogy, and can be reached at kathleenmsandman@gmail.com.

Courtesy of Dorothy Wood

Dorothy H. Wood has taught microbiology and general biology at Durham Technical Community College in North Carolina since 2004. Dr. Wood received her B.A. in Biology from Rhode Island College where her love of microbes began, nurtured by Dr. Charles Owens. She earned her Ph.D. in Cell and Molecular Pathology from the University of North Carolina at Chapel Hill, focusing on pancreatic damage caused by antimicrobial drugs, and investigated alternative therapies based on receptor binding by novel compounds. After three years as Assistant Professor at NC Central University, Dr. Wood made the move to the NC Community College System to focus her attention on her primary interest of teaching. Throughout her career she has developed several courses, including graduate bacteriology, pathophysiology, and biotechnology. She serves as a visiting scholar at Duke University where she is a mentor for the Preparing Future Faculty program. Dr. Wood is a member of the American Society for Microbiology and the Association of College and University Biology Educators, as well as several local organizations that foster pedagogy. She is a digital faculty consultant for McGraw-Hill and has worked on several textbooks in a variety of disciplines, developing and editing digital content to accompany the texts. Outside of the classroom, Dr. Wood is a fitness professional, leads health and wellness seminars, and has been the treasurer of a nonprofit organization for the past 10 years. She enjoys life in North Carolina with her husband and two grown children and can be reached at woodd@durhamtech.edu.

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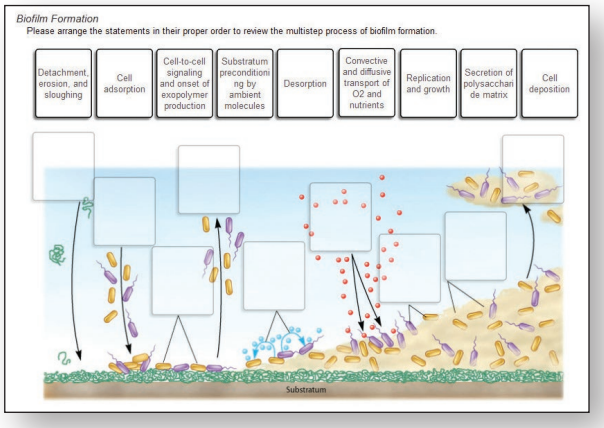

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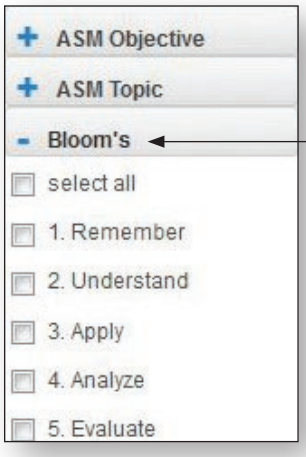

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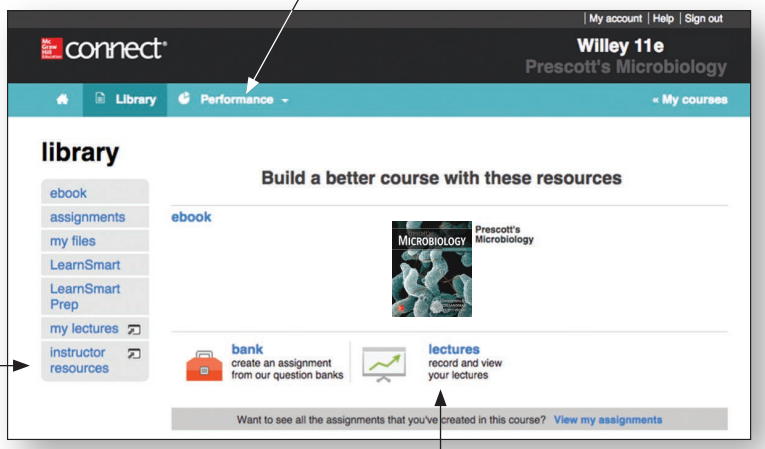

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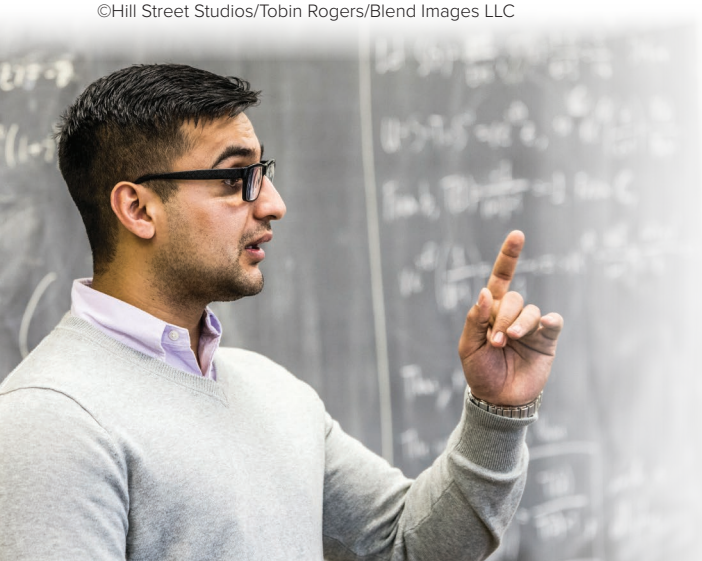

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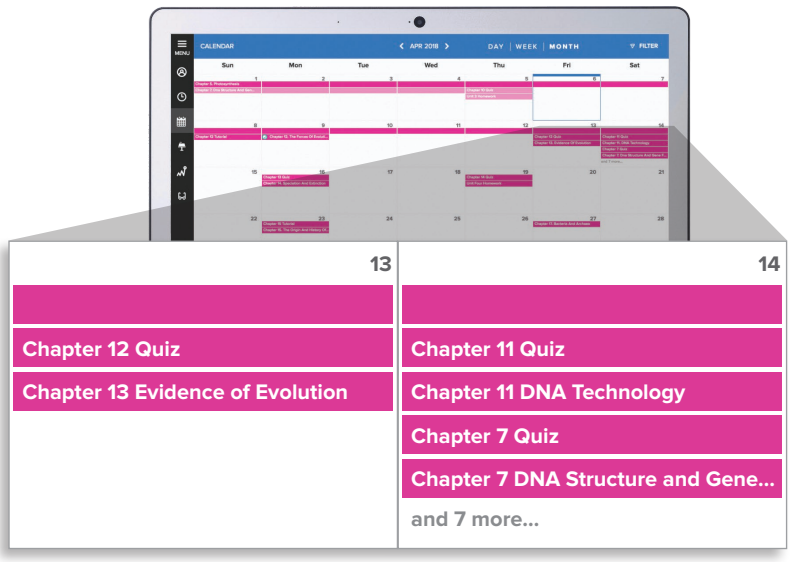

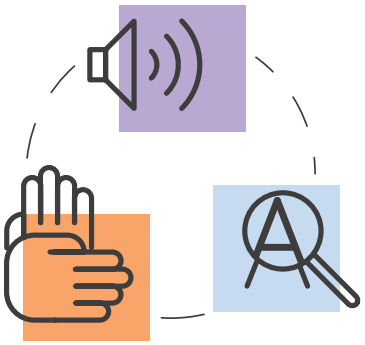

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A Modern Approach to Microbiology

Evolution as a Framework

Introduced immediately in chapter 1 and used as an overarching theme throughout, evolution helps unite microbiological concepts and provides a framework upon which students can build their knowledge.

An Introduction to the Entire Microbial World

Covered in chapters 3–6, separate chapters on the structure and function of bacteria and archaea are followed by the discussion of eukaryotic cells and viruses.

Broad Coverage of Microbial Ecology

The importance and multidisciplinary nature of microbial ecology are demonstrated by content that ranges from global climate change to the human microbiome.

Brief Contents

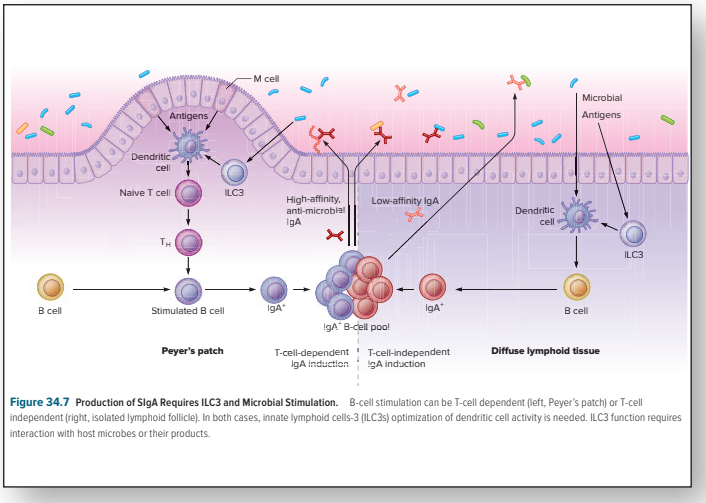
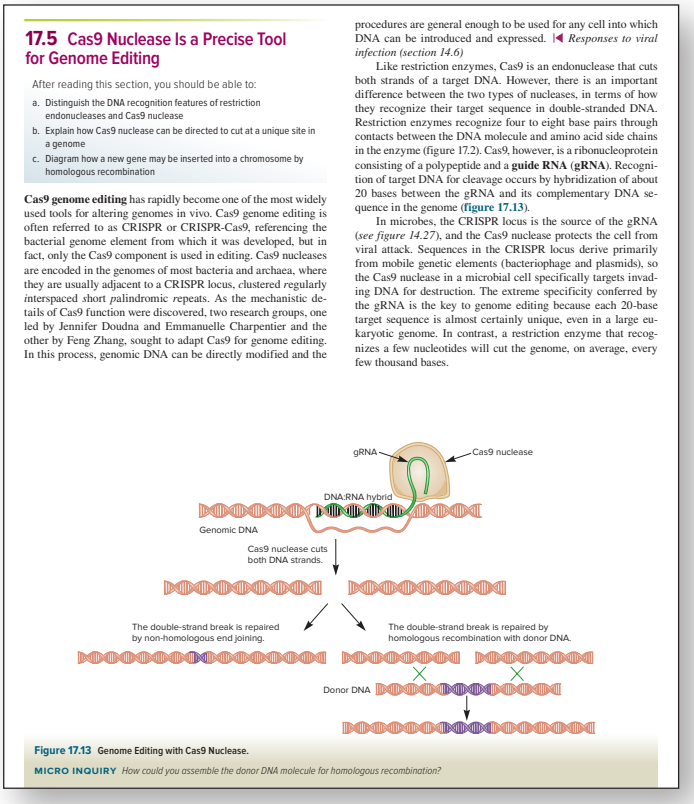


Figure 34.7 Production of SIgA Requires ILC3 and Microbial Stimulation. B-cell stimulation can be T-cell dependent (left, Peyer's patch) or T-cell independent (right, isolated lymphoid follicle). In both cases, innate lymphoid cells-3 (ILC3s) optimization of dendritic cell activity is needed. ILC3 function requires interaction with host microbes or their products.

Molecular Microbiology and Immunology

The eleventh edition includes updates on genetics, biotechnology, genomics and metagenomics, immunology, and the human microbiome. A streamlined discussion of immunity, with enhanced detail between innate and adaptive linkages, helps students grasp the complexity and specificity of immune responses. A new chapter, The Microbe-Human Ecosystem, introduces students to the development and impact of the human microbiome.

A Modern Approach to Microbiology



17.5 Cas9 Nuclease Is a Precise Tool for Genome Editing

After reading this section, you should be able to:

a. Distinguish the DNA recognition features of restriction endonucleases and Cas9 nuclease
b. Explain how Cas9 nuclease can be directed to cut at a unique site in a genome
c. Diagram how a new gene may be inserted into a chromosome by homologous recombination

Cas9 genome editing has rapidly become one of the most widely used tools for altering genomes in vivo. Cas9 genome editing is often referred to as CRISPR or CRISPR-Cas9, referencing the bacterial genome element from which it was developed, but in fact, only the Cas9 component is used in editing. Cas9 nucleases are encoded in the genomes of most bacteria and archaea, where they are usually adjacent to a CRISPR locus, *c*lustered *r*egularly *i*nterspaced *s*hort *p*alindromic *r*epeats. As the mechanistic details of Cas9 function were discovered, two research groups, one led by Jennifer Doudna and Emmanuelle Charpentier and the other by Feng Zhang, sought to adapt Cas9 for genome editing. In this process, genomic DNA can be directly modified and the procedures are general enough to be used for any cell into which DNA can be introduced and expressed. ◀ *Responses to viral infection (section 14.6)*

Like restriction enzymes, Cas9 is an endonuclease that cuts both strands of a target DNA. However, there is an important difference between the two types of nucleases, in terms of how they recognize their target sequence in double-stranded DNA. Restriction enzymes recognize four to eight base pairs through contacts between the DNA molecule and amino acid side chains in the enzyme (figure 17.2). Cas9, however, is a ribonucleoprotein consisting of a polypeptide and a **guide RNA** (**gRNA**). Recognition of target DNA for cleavage occurs by hybridization of about 20 bases between the gRNA and its complementary DNA sequence in the genome (**figure 17.13**).

In microbes, the CRISPR locus is the source of the gRNA (*see figure 14.27*), and the Cas9 nuclease protects the cell from viral attack. Sequences in the CRISPR locus derive primarily from mobile genetic elements (bacteriophage and plasmids), so the Cas9 nuclease in a microbial cell specifically targets invading DNA for destruction. The extreme specificity conferred by the gRNA is the key to genome editing because each 20-base target sequence is almost certainly unique, even in a large eukaryotic genome. In contrast, a restriction enzyme that recognizes a few nucleotides will cut the genome, on average, every few thousand bases.

Figure 17.13 Genome Editing with Cas9 Nuclease.

MICRO INQUIRY *How could you assemble the donor DNA molecule for homologous recombination?*

21st-Century Microbiology

Prescott's Microbiology leads the way with text devoted to global climate change, biofuels, and microbial fuel cells. For more, see chapters 28, 30, 42, and 43.

Metagenomics and the Human Microbiome

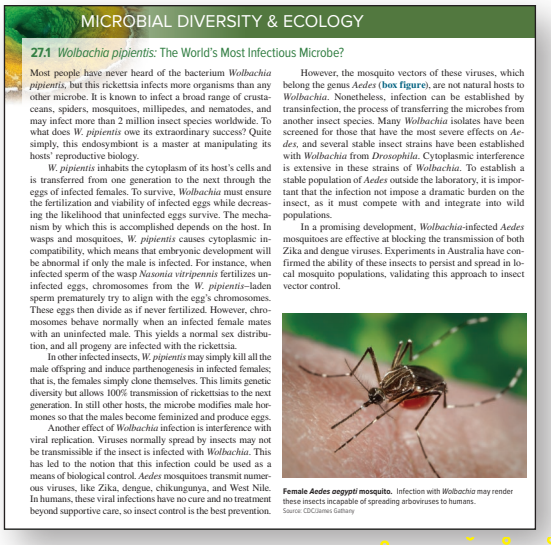
The importance of metagenomics in understanding the role of microbes in all environments and in exploring symbionts of invertebrates is threaded throughout the text. A new chapter, The Microbe-Human Ecosystem, explores the human microbiome.

Laboratory Safety

Reflecting recommendations from the Centers for Disease Control and Prevention, along with the American Society for Microbiology, chapter 37 provides specific guidance for laboratory best practices to help instructors provide safe conditions during the teaching of laboratory exercises.

Special Interest Essays

Organized into four themes—Microbial Diversity & Ecology, Techniques & Applications, Historical Highlights, and Disease—these focused and interesting essays provide additional insight into relevant topics.



MICROBIAL DIVERSITY & ECOLOGY

27.1 *Wolbachia pipientis:* The World's Most Infectious Microbe?

Most people have never heard of the bacterium *Wolbachia pipientis*, but this rickettsia infects more organisms than any other microbe. It is known to infect a broad range of crustaceans, spiders, mosquitoes, millipedes, and nematodes, and may infect more than 2 million insect species worldwide. To what does *W. pipientis* owe its extraordinary success? Quite simply, this endosymbiont is a master at manipulating its hosts' reproductive biology.

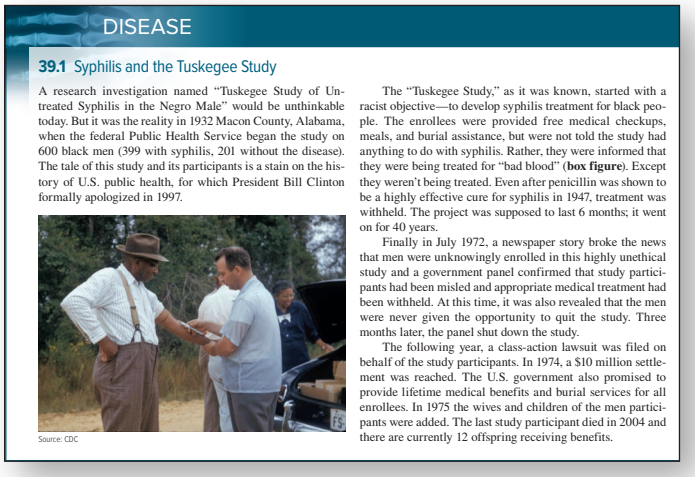
W. pipientis inhabits the cytoplasm of its host's cells and is transferred from one generation to the next through the eggs of infected females. To survive, *Wolbachia* must ensure the fertilization and viability of infected eggs while decreasing the likelihood that uninfected eggs survive. The mechanism by which this is accomplished depends on the host. In wasps and mosquitoes, *W. pipientis* causes cytoplasmic incompatibility, which means that embryonic development will be abnormal if only the male is infected. For instance, when infected sperm of the wasp *Nasonia vitripennis* fertilizes uninfected eggs, chromosomes from the *W. pipientis*–laden sperm prematurely try to align with the egg's chromosomes. These eggs then divide as if never fertilized. However, chromosomes behave normally when an infected female mates with an uninfected male. This yields a normal sex distribution, and all progeny are infected with the rickettsia.

In other infected insects, *W. pipientis* may simply kill all the male offspring and induce parthenogenesis in infected females; that is, the females simply clone themselves. This limits genetic diversity but allows 100% transmission of rickettsias to the next generation. In still other hosts, the microbe modifies male hormones so that the males become feminized and produce eggs.

Another effect of *Wolbachia* infection is interference with viral replication. Viruses normally spread by insects may not be transmissible if the insect is infected with *Wolbachia*. This has led to the notion that this infection could be used as a means of biological control. *Aedes* mosquitoes transmit numerous viruses, like Zika, dengue, chikungunya, and West Nile. In humans, these viral infections have no cure and no treatment beyond supportive care, so insect control is the best prevention. However, the mosquito vectors of these viruses, which belong the genus *Aedes* (**box figure**), are not natural hosts to *Wolbachia*. Nonetheless, infection can be established by transinfection, the process of transferring the microbes from another insect species. Many *Wolbachia* isolates have been screened for those that have the most severe effects on *Aedes*, and several stable insect strains have been established with *Wolbachia* from *Drosophila*. Cytoplasmic interference is extensive in these strains of *Wolbachia*. To establish a stable population of *Aedes* outside the laboratory, it is important that the infection not impose a dramatic burden on the insect, as it must compete with and integrate into wild populations.

In a promising development, *Wolbachia*-infected *Aedes* mosquitoes are effective at blocking the transmission of both Zika and dengue viruses. Experiments in Australia have confirmed the ability of these insects to persist and spread in local mosquito populations, validating this approach to insect vector control.

Female *Aedes aegypti* mosquito. Infection with *Wolbachia* may render these insects incapable of spreading arboviruses to humans.
Source: CDC/James Gathany



DISEASE

39.1 Syphilis and the Tuskegee Study

A research investigation named "Tuskegee Study of Untreated Syphilis in the Negro Male" would be unthinkable today. But it was the reality in 1932 Macon County, Alabama, when the federal Public Health Service began the study on 600 black men (399 with syphilis, 201 without the disease). The tale of this study and its participants is a stain on the history of U.S. public health, for which President Bill Clinton formally apologized in 1997.

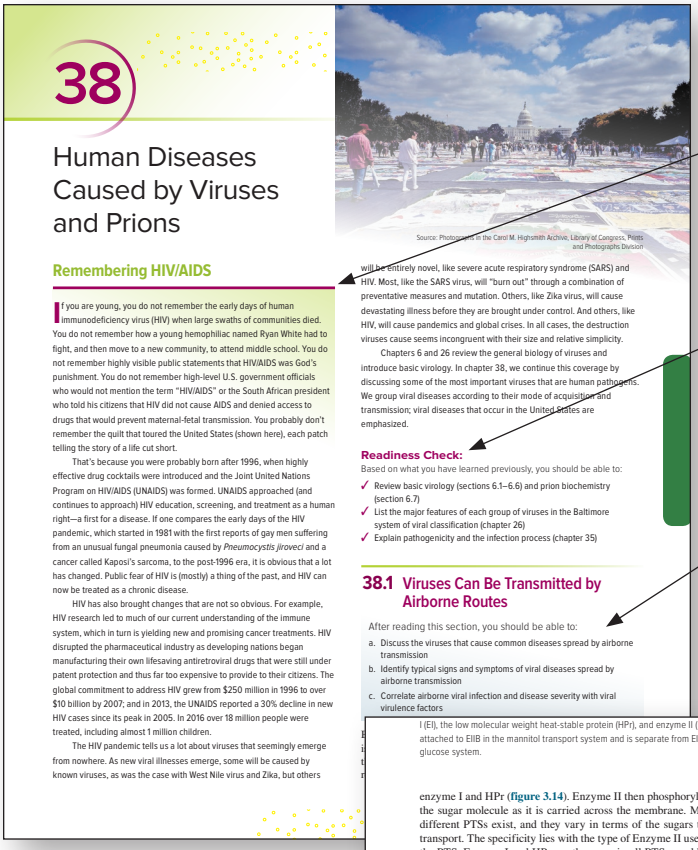
Source: CDC

The "Tuskegee Study," as it was known, started with a racist objective—to develop syphilis treatment for black people. The enrollees were provided free medical checkups, meals, and burial assistance, but were not told the study had anything to do with syphilis. Rather, they were informed that they were being treated for "bad blood" (**box figure**). Except they weren't being treated. Even after penicillin was shown to be a highly effective cure for syphilis in 1947, treatment was withheld. The project was supposed to last 6 months; it went on for 40 years.

Finally in July 1972, a newspaper story broke the news that men were unknowingly enrolled in this highly unethical study and a government panel confirmed that study participants had been misled and appropriate medical treatment had been withheld. At this time, it was also revealed that the men were never given the opportunity to quit the study. Three months later, the panel shut down the study.

The following year, a class-action lawsuit was filed on behalf of the study participants. In 1974, a $10 million settlement was reached. The U.S. government also promised to provide lifetime medical benefits and burial services for all enrollees. In 1975 the wives and children of the men participants were added. The last study participant died in 2004 and there are currently 12 offspring receiving benefits.

Student-Friendly Organization



38

Human Diseases Caused by Viruses and Prions

Remembering HIV/AIDS

If you are young, you do not remember the early days of human immunodeficiency virus (HIV) when large swaths of communities died. You do not remember how a young hemophiliac named Ryan White had to fight, and then move to a new community, to attend middle school. You do not remember highly visible public statements that HIV/AIDS was God's punishment. You do not remember high-level U.S. government officials who would not mention the term "HIV/AIDS" or the South African president who told his citizens that HIV did not cause AIDS and denied access to drugs that would prevent maternal-fetal transmission. You probably don't remember the quilt that toured the United States (shown here), each patch telling the story of a life cut short.

That's because you were probably born after 1996, when highly effective drug cocktails were introduced and the Joint United Nations Program on HIV/AIDS (UNAIDS) was formed. UNAIDS approached (and continues to approach) HIV education, screening, and treatment as a human right—a first for a disease. If one compares the early days of the HIV pandemic, which started in 1981 with the first reports of gay men suffering from an unusual fungal pneumonia caused by *Pneumocystis jirovecí* and a cancer called Kaposi's sarcoma, to the post-1996 era, it is obvious that a lot has changed. Public fear of HIV is (mostly) a thing of the past, and HIV can now be treated as a chronic disease.

HIV has also brought changes that are not so obvious. For example, HIV research led to much of our current understanding of the immune system, which in turn is yielding new and promising cancer treatments. HIV disrupted the pharmaceutical industry as developing nations began manufacturing their own lifesaving antiretroviral drugs that were still under patent protection and thus far too expensive to provide to their citizens. The global commitment to address HIV grew from $250 million in 1996 to over $10 billion by 2007; and in 2013, the UNAIDS reported a 30% decline in new HIV cases since its peak in 2005. In 2016 over 18 million people were treated, including almost 1 million children.

The HIV pandemic tells us a lot about viruses that seemingly emerge from nowhere. As new viral illnesses emerge, some will be caused by known viruses, as was the case with West Nile virus and Zika, but others will be entirely novel, like severe acute respiratory syndrome (SARS) and HIV. Most, like the SARS virus, will "burn out" through a combination of preventative measures and mutation. Others, like Zika virus, will cause devastating illness before they are brought under control. And others, like HIV, will cause pandemics and global crises. In all cases, the destruction viruses cause seems incongruent with their size and relative simplicity.

Chapters 6 and 26 review the general biology of viruses and introduce basic virology. In chapter 38, we continue this coverage by discussing some of the most important viruses that are human pathogens. We group viral diseases according to their mode of acquisition and transmission; viral diseases that occur in the United States are emphasized.

Source: Photograph is the Carol M. Highsmith Archive, Library of Congress, Prints and Photographs Division

Readiness Check:

Based on what you have learned previously, you should be able to:

- ✓ Review basic virology (sections 6.1–6.6) and prion biochemistry (section 6.7)
- ✓ List the major features of each group of viruses in the Baltimore system of viral classification (chapter 26)
- ✓ Explain pathogenicity and the infection process (chapter 35)

38.1 Viruses Can Be Transmitted by Airborne Routes

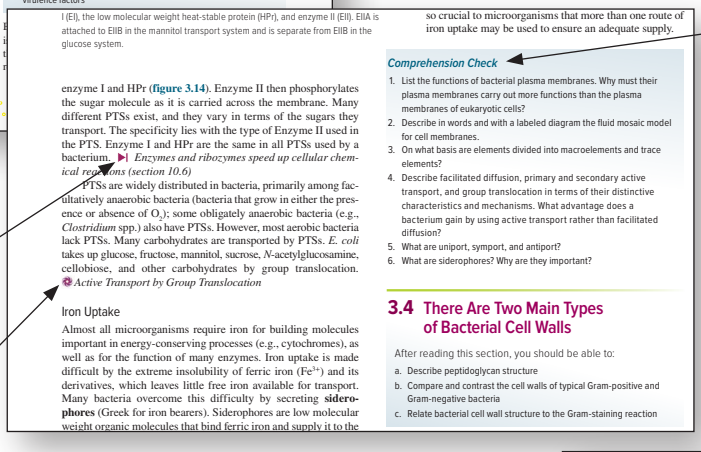
After reading this section, you should be able to:

a. Discuss the viruses that cause common diseases spread by airborne transmission
b. Identify typical signs and symptoms of viral diseases spread by airborne transmission
c. Correlate airborne viral infection and disease severity with viral virulence factors

Micro Focus—Each chapter begins with a real-life story illustrating the relevance of the content covered in the upcoming text.

Readiness Check—The introduction to each chapter includes a skills checklist that defines the prior knowledge students need to understand the material that follows.

Learning Outcomes—Every section in each chapter begins with a list of content-based activities students should be able to perform after reading.



I (EI), the low molecular weight heat-stable protein (HPr), and enzyme II (EII). EIIA is attached to EIIB in the mannitol transport system and is separate from EIIB in the glucose system.

enzyme I and HPr (figure 3.14). Enzyme II then phosphorylates the sugar molecule as it is carried across the membrane. Many different PTSs exist, and they vary in terms of the sugars they transport. The specificity lies with the type of Enzyme II used in the PTS. Enzyme I and HPr are the same in all PTSs used by a bacterium. ▶| *Enzymes and ribozymes speed up cellular chemical reactions (section 10.6)*

PTSs are widely distributed in bacteria, primarily among facultatively anaerobic bacteria (bacteria that grow in either the presence or absence of O_2); some obligately anaerobic bacteria (e.g., *Clostridium* spp.) also have PTSs. However, most aerobic bacteria lack PTSs. Many carbohydrates are transported by PTSs. *E. coli* takes up glucose, fructose, mannitol, sucrose, *N*-acetylglucosamine, cellobiose, and other carbohydrates by group translocation. ❁ *Active Transport by Group Translocation*

Iron Uptake

Almost all microorganisms require iron for building molecules important in energy-conserving processes (e.g., cytochromes), as well as for the function of many enzymes. Iron uptake is made difficult by the extreme insolubility of ferric iron (Fe^{3+}) and its derivatives, which leaves little free iron available for transport. Many bacteria overcome this difficulty by secreting **siderophores** (Greek for iron bearers). Siderophores are low molecular weight organic molecules that bind ferric iron and supply it to the so crucial to microorganisms that more than one route of iron uptake may be used to ensure an adequate supply.

Comprehension Check

1. List the functions of bacterial plasma membranes. Why must their plasma membranes carry out more functions than the plasma membranes of eukaryotic cells?
2. Describe in words and with a labeled diagram the fluid mosaic model for cell membranes.
3. On what basis are elements divided into macroelements and trace elements?
4. Describe facilitated diffusion, primary and secondary active transport, and group translocation in terms of their distinctive characteristics and mechanisms. What advantage does a bacterium gain by using active transport rather than facilitated diffusion?
5. What are uniport, symport, and antiport?
6. What are siderophores? Why are they important?

3.4 There Are Two Main Types of Bacterial Cell Walls

After reading this section, you should be able to:

a. Describe peptidoglycan structure
b. Compare and contrast the cell walls of typical Gram-positive and Gram-negative bacteria
c. Relate bacterial cell wall structure to the Gram-staining reaction

Comprehension Check—Questions within the narrative of each chapter help students master section concepts before moving on to other topics.

Cross-Referenced Notes—In-text references refer students to other parts of the book to review.

Animation Icon—This symbol indicates that material presented in the text is accompanied by an animation within Instructor Resources in Connect. Create a file attachment assignment in Connect to have your students view the animation, or post it to your Learning Management System for students.

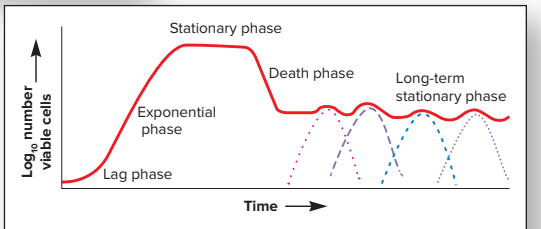


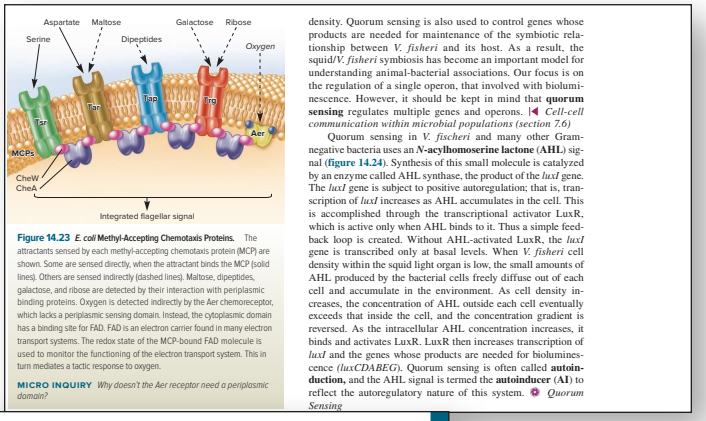
Figure 7.10 **Microbial Growth Curve in a Closed System.** The five phases of the growth curve are identified. The dotted lines shown during the long-term stationary phase represent successive waves of genetic variants that evolve during this phase of the growth curve.

MICRO INQUIRY *Identify the regions of the growth curve in which (1) nutrients are rapidly declining and (2) wastes accumulate.*

Micro Inquiry—Selected figures in every chapter contain probing questions, adding another assessment opportunity for the student.

Student-Friendly Organization

Vivid Instructional Art—Three-dimensional renditions and bright, attractive colors enhance learning.



density. Quorum sensing is also used to control genes whose products are needed for maintenance of the symbiotic relationship between *V. fisheri* and its host. As a result, the squid/*V. fisheri* symbiosis has become an important model for understanding animal-bacterial associations. Our focus is on the regulation of a single operon, that involved with bioluminescence. However, it should be kept in mind that **quorum sensing** regulates multiple genes and operons. |◀ *Cell-cell communication within microbial populations (section 7.6)*

Quorum sensing in *V. fischeri* and many other Gram-negative bacteria uses an ***N*-acylhomoserine lactone** (**AHL**) signal (**figure 14.24**). Synthesis of this small molecule is catalyzed by an enzyme called AHL synthase, the product of the *luxI* gene. The *luxI* gene is subject to positive autoregulation; that is, transcription of *luxI* increases as AHL accumulates in the cell. This is accomplished through the transcriptional activator LuxR, which is active only when AHL binds to it. Thus a simple feedback loop is created. Without AHL-activated LuxR, the *luxI* gene is transcribed only at basal levels. When *V. fisheri* cell density within the squid light organ is low, the small amounts of AHL produced by the bacterial cells freely diffuse out of each cell and accumulate in the environment. As cell density increases, the concentration of AHL outside each cell eventually exceeds that inside the cell, and the concentration gradient is reversed. As the intracellular AHL concentration increases, it binds and activates LuxR. LuxR then increases transcription of *luxI* and the genes whose products are needed for bioluminescence (*luxCDABEG*). Quorum sensing is often called **autoinduction,** and the AHL signal is termed the **autoinducer** (**AI**) to reflect the autoregulatory nature of this system. ● *Quorum Sensing*

Figure 14.23 ***E. coli*** **Methyl-Accepting Chemotaxis Proteins.** The attractants sensed by each methyl-accepting chemotaxis protein (MCP) are shown. Some are sensed directly, when the attractant binds the MCP (solid lines). Others are sensed indirectly (dashed lines). Maltose, dipeptides, galactose, and ribose are detected by their interaction with periplasmic binding proteins. Oxygen is detected indirectly by the Aer chemoreceptor, which lacks a periplasmic sensing domain. Instead, the cytoplasmic domain has a binding site for FAD. FAD is an electron carrier found in many electron transport systems. The redox state of the MCP-bound FAD molecule is used to monitor the functioning of the electron transport system. This in turn mediates a tactic response to oxygen.

MICRO INQUIRY *Why doesn't the Aer receptor need a periplasmic domain?*

Annotated Figures—All key metabolic pathways and molecular processes are annotated, so each step is clearly illustrated and explained.

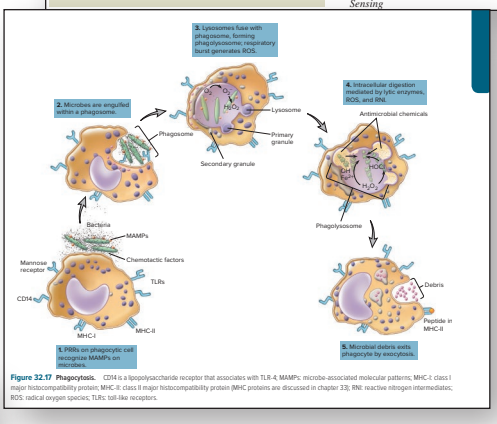


Figure 32.17 **Phagocytosis.** CD14 is a lipopolysaccharide receptor that associates with TLR-4; MAMPs: microbe-associated molecular patterns; MHC-I: class I major histocompatibility protein; MHC-II: class II major histocompatibility protein (MHC proteins are discussed in chapter 33); RNI: reactive nitrogen intermediates; ROS: radical oxygen species; TLRs: toll-like receptors.

Key Concepts

2.1 Lenses Create Images by Bending Light

- A light ray moving from air to glass or vice versa is bent in a process known as refraction (**figure 2.1**).
- Lenses focus light rays at a focal point and magnify images (**figure 2.2**).

2.2 There Are Several Types of Light Microscopes

- In a compound microscope such as the bright-field microscope, the primary image is an enlarged image formed by the objective lens. The primary image is further enlarged by the ocular lens to yield the final image (**figure 2.3**).
- Microscope resolution increases as the wavelength of radiation used to illuminate the specimen decreases and as the numerical aperture increases. The maximum resolution of a light microscope is about 0.2 µm (**figure 2.4**).
- The dark-field microscope uses only refracted light to form an image, and objects appear light against a black background (**figure 2.6**).
- The phase-contrast microscope converts variations in the refractive index into changes in light intensity and thus makes colorless, unstained, live cells visible (**figures 2.8–2.10**).
- The differential interference contrast microscope uses two beams of light to create high-contrast images of live specimens (**figure 2.11**).
- The fluorescence microscope illuminates a fluorochrome-labeled specimen and forms an image from its fluorescence (**figures 2.12–2.14**).
- The confocal microscope is used to study thick, complex specimens. It creates an image by using only the light emanating from the plane of focus, while blocking out light from above and below the plane of focus (**figures 2.15** and **2.16**).

2.3 Staining Specimens Helps to Visualize and Identify Microbes

- Specimens are often fixed and stained before viewing them in the bright-field microscope. There are two fixation methods: heat fixation and chemical fixation.
- Most dyes are either positively charged basic dyes or negatively charged acidic dyes that bind to ionized parts of cells.
- In simple staining, a single dye is used to stain microorganisms (**figure 2.17**).
- Differential staining procedures such as Gram and acid-fast staining distinguish between microbial groups by staining them differently (**figures 2.18** and **2.19*a,b***). Other differential staining techniques are specific for particular structures such as bacterial capsules and flagella (**figure 2.19*c,d***).

2.4 Electron Microscopes Use Beams of Electrons to Create Highly Magnified Images

- The transmission electron microscope (TEM) uses magnetic lenses to form an image from electrons that have passed through a very thin section of a specimen (**figure 2.22**). Resolution is high because the wavelength of a beam of electrons is very short.
- Specimens for TEM are usually prepared by methods that increase contrast. Specimens can be stained by treatment with solutions of heavy metals such as osmium tetroxide, uranium, and lead. They can also be prepared for TEM by negative staining, shadowing with metal, or freeze-etching (**figures 2.24** and **2.25**).
- The scanning electron microscope is used to study external surface features of microorganisms (**figures 2.26** and **2.27**).
- Electron cryotomography freezes specimens rapidly, keeps them frozen while being examined, and creates images from a series of directions that are combined and processed to form a three-dimensional reconstruction of the object (**figure 2.28**).

2.5 Scanning Probe Microscopy Can Visualize Molecules and Atoms

- Scanning probe microscopes reach very high magnifications that allow scientists to observe biological molecules (**figures 2.29** and **2.31**).
- Scanning tunneling microscopy enables the visualization of molecular surfaces using electron interaction between the probe and the specimen, whereas atomic force microscopy can scan the surface of molecules that do not conduct electricity well (**figure 2.30**).

Active Learning

1. You have prepared a specimen for light microscopy, stained it using the Gram staining procedure, but failed to see anything when you looked through your light microscope. Discuss the things that you may have done incorrectly.
2. In a journal article, find an example of a light micrograph, a scanning or transmission electron micrograph, or a confocal image. Discuss why the figure was included in the article and why that particular type of microscopy was the method of choice for the research. What other figures would you like to see used in this study? Outline the steps that the investigators would take to obtain such photographs or figures.

Key Concepts—At the end of each chapter, organized by numbered headings, this feature distills the content to its essential components with cross-references to figures and tables.

Active Learning—Includes questions taken from current literature; designed to stimulate analytical problem-solving skills.

List of Content Changes

Each chapter has been thoroughly reviewed.

Part One

Chapter 1—Evolution is the driving force of all biological systems; this is made clear by introducing essential concepts of microbial evolution first. Advances in the discipline of microbiology and the increasing contributions of genomics and metagenomics are discussed.

Chapter 2—Microscopy was and is critical to the study of microorganisms and this chapter considers the most commonly used methods, including expanded coverage of phase-contrast microscopy.

Chapter 3—Coverage of bacterial cellular structure and function. New material includes a discussion of membrane microdomains, and the effect of macromolecular crowding in the cytoplasm.

Chapter 4—Discussion of archaea has been updated to include recent discoveries, including expanded taxonomy, polyploidy, and the role of nucleoid-associated proteins. Comparisons to bacteria are made throughout the chapter.

Chapter 5—An introduction to eukaryotic cell structure and function, with emphasis on eukaryotic microbes. More detailed information on protist and fungal cells is presented in chapters 24 (Protists) and 25 (*Fungi*), which also focus on the diversity of these microbes. The current understanding of the evolution of mitochondria and mitochondria-like organelles is considered. Comparisons between bacteria, archaea, and eukaryotes are included throughout the chapter.

Chapter 6—This chapter surveys essential morphological, physiological, and genetic elements of viruses as well as viroids, satellites, and prions. Images and descriptions of archaeal viruses have been incorporated. This chapter completes our four-chapter introduction to microbial life.

Part Two

Chapter 7—Discussion of the growth of microbes has been updated to include new information about chromosome partitioning and the archaeal cell cycle.

Chapter 8—A new chapter-opening story and updated tables reflect the challenges associated with controlling prions. A new Microbial Diversity & Ecology box describes the conditions required in NASA spacecraft assembly facilities.

Chapter 9—Content focuses on the mechanism of action of each class of antimicrobial agents and introduces mechanisms of drug resistance.

Part Three

Chapter 10—This introduction to metabolism includes a section outlining the nature of biochemical pathways. The concept of metabolic flux is presented by discussing the interconnected biochemical pathways used by cells.

Chapter 11—An introduction to metabolic diversity and nutritional types is followed by an exploration of the energy-conserving process of each nutritional type. An introduction to flavin-based electron bifurcation has been added.

Chapter 12—New comparison of pathways used to synthesize lipids in bacteria and archaea.

Part Four

Chapter 13—A revised section now covers posttranslational modifications, protein folding, and secretion systems. Membrane vesicles are introduced.

Chapter 14—The regulation of bacterial cellular processes, with updated coverage of regulation by messengers like c-di-GMP. A new section on responses to viral infection includes a discussion of restriction-modification and CRISPR.

Chapter 15—Recent developments in archaeal replication, gene regulation, and protein secretion have been included.

Chapter 16—Covers mutation, repair, and recombination in the context of processes that introduce genetic variation into populations. Updated coverage of integrative conjugative elements and mobilizable genomic islands.

Chapter 17—This chapter has been completely reorganized to update the content on gene cloning and heterologous gene expression. Cas9 genome engineering methodologies are described.

Chapter 18—Next-generation nucleotide sequencing and single-cell genome sequencing are covered in the context of metagenomics as it relates to the microbial ecology of natural systems, including the human microbiome.

Part Five

Chapter 19—This overview of microbial evolution has been updated to include whole genome comparison and related computational techniques in determining relatedness.

Chapter 20—The discussion of archaeal taxonomy has been revised and updated to reflect the new diversity uncovered by metagenomics. The methanogenesis discussion has been updated to include the mechanism of flavin-based electron bifurcation.

Chapter 21—In addition to the ecology and physiology of photosynthetic bacteria, the recently described *Planctomycetes,*

List of Content Changes

Verrucomicrobia, Chlamydia (PVC) superphylum is introduced with an updated review of each of these genera. New information about the *Deinococcus* radiation response is included.

Chapter 22—This chapter's coverage includes a discussion of the proteobacterial origin of mitochondria.

Chapter 23—This overview of Gram-positive bacteria includes firmicutes and actinobacteria. The discussion of the evolutionary aspects of diderm firmicutes is expanded.

Chapter 24—This chapter introduces protist morphology and diversity, with an emphasis on physiological adaptation and ecology.

Chapter 25—Fungal diversity is presented within a phylogenetic framework. Morphology, ecology, and reproductive strategies are stressed.

Chapter 26—Updated discussion of the molecular mechanisms in the bacteriophage T4 life cycle.

Chapter 27—Important model systems for the exploration of microbial symbioses are presented. Updated discussion of *Wolbachia*-infected insects.

Part Six

Chapter 28—The description of each nutrient cycle is accompanied by a "student-friendly" figure that distinguishes between reductive and oxidative reactions. Updated coverage of the role of biogeochemical cycling in global climate change.

Chapter 29—This chapter continues to emphasize culture-based techniques as the "gold standard" and reviews culture-independent approaches such as mass spectrometry in the identification of microbial taxa as well as metatranscriptomics and metaproteomics in the study of community activity.

Chapter 30—Updated discussion of the role of marine microbes in the global carbon budget as well as an update on subsurface microbes.

Chapter 31—New coverage of the microbial ecology of the phyllosphere, rhizoplane, and rhizosphere. Expanded discussion of fungal plant pathogens.

Part Seven

Chapter 32—Streamlined and updated, this chapter on innate host resistance provides in-depth coverage of physical and chemical components of the nonspecific host response, followed by an overview of cells, tissues, and organs of the immune system. The chapter concludes with an overview of the molecular mechanisms that drive phagocytosis and inflammation.

Chapter 33—Updated to enhance linkages between innate and adaptive immune activities. Discussions integrate concepts of cell biology, physiology, and genetics to present the immune system as a unified response having various components. Implications of dysfunctional immune actions are also discussed.

Chapter 34—This new chapter introduces the establishment of a human microbiome as a developmental process from infancy through adulthood. The importance of the microbiome to host homeostasis is emphasized by discussion of its role in metabolism, immune function, and the gut-brain axis as well as an introduction to the consequences of dysbiosis.

Chapter 35—This chapter has been reorganized to delineate the development of disease from microbial transmission to host cell damage. Emphasis is placed on the overlap between microbial molecules that facilitate survival and those that act as virulence factors. This chapter is placed after the immunology chapters to stress that the host-parasite relationship is dynamic, with adaptations and responses offered by both host and parasite.

Part Eight

Chapter 36—This chapter presents the development of modern epidemiology as an investigative science, emphasizing its role in preventative medicine. The latest epidemiological data from the Centers for Disease Control and Prevention are reported.

Chapter 37—This chapter has been updated to reflect the technological advances in the modern clinical laboratory. Emphasis is on modern diagnostic testing to identify infectious disease.

Chapter 38—Updated and expanded coverage includes viral pathogenesis, common viral infections, and prion-mediated diseases.

Chapter 39—Updated coverage of bacterial organisms and the ways in which they commonly lead to human disease.

Chapter 40—Updated and expanded coverage of fungal and protozoal diseases.

Part Nine

Chapter 41—The essentials of both food safety and microbial processes involved in food production have been updated.

Chapter 42—Includes updated coverage of biofuel production (first introduced in chapter 21) and an introduction to synthetic biology.

Chapter 43—This chapter complements our 21st-century approach to microbiology by emphasizing the importance of clean water and the power of microbial environmental remediation.

Lab Tools for Your Success

LearnSmart® Prep is an adaptive learning tool that prepares students for college-level work in Microbiology. LearnSmart Prep individually identifies concepts the student does not fully understand and provides learning resources to teach essential concepts so he or she enters the classroom prepared. Data-driven reports highlight areas where students are struggling, helping to accurately identify weak areas.

Acknowledgements

In the preparation of each edition, we are guided by the collective wisdom of reviewers who are expert microbiologists and excellent teachers. They represent experience in community colleges, liberal arts colleges, comprehensive institutions, and research universities. We have followed their recommendations, while remaining true to our overriding goal of writing readable, student-centered content. Each feature incorporated into this edition has been carefully considered in terms of how it may be used to support student learning in both the traditional and the flipped learning environment.

Also in this edition, we are very excited to incorporate real student data points and input, derived from thousands of our LearnSmart users, to help guide our revision. With this information, we were able to hone both book and digital content.

The authors wish to extend their gratitude to our team at McGraw-Hill Education, including Marija Magner, Darlene Schueller, Valerie Kramer, Laura Bies, Matt Backhaus, Tammy Juran, and Beth Cray. Finally, we thank our spouses and children, who provided support and tolerated our absences (mental, if not physical) while we completed this demanding project.

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eleventh edition

Prescott's Microbiology

1

The Evolution of Microorganisms and Microbiology

The Microbial Universe

If you have ever gazed at the night sky on a cloudless evening in a region far from light pollution, you have probably been amazed and perhaps a little humbled by the vast number of stars. It's hard to estimate just how many stars are out there; best estimates start with our own Milky Way Galaxy, which has roughly 100 billion (1×10^{11}) stars. Astronomers figure there is something like 10 trillion (1×10^{13}) galaxies in the universe. Assuming all galaxies are roughly the size of the Milky Way, you wind up with a number around 1×10^{24} stars in the universe—not the most accurate number, but nonetheless daunting.

If you turn your gaze back to Earth, however, you will find even more awesome abundance. For example, if you stacked all of the 1×10^{31} viruses on Earth one on top of the other, they would stretch about 100 million light-years. That's about 43 times further away than the Andromeda Galaxy. And next time you take a dip in the sea, consider that there are at least a hundred thousand (1×10^{5}) more bacteria in the ocean (about 1.3×10^{29}) than stars in the universe. Or perhaps you prefer staying on land where a teaspoon of soil has about a billion (1×10^{9}) microorganisms.

What are all these microorganisms doing? The short answer is, making life for the rest of us possible. How? Starting about 2.4 billion years ago, bacteria called cyanobacteria were the first organisms to release oxygen in abundance into the atmosphere. This has been dubbed the "great oxidation event," and it set the stage for oxygen-consuming organisms (like us) to evolve. Microorganisms have another starring role in the evolution of life because only bacteria can fix nitrogen—that is, take gaseous nitrogen and convert it to organic nitrogen used by plants, animals, and other microbes. Finally, can you imagine what life on Earth would look like if dead organic material were not degraded? Probably best not to. Much better to think of foods like beer, wine, chocolate, cheese, and yogurt—all microbial products.

But of course not all microorganisms make life possible, or even easier. Each year about 16 million (1.6×10^{7}) people die from infectious disease; and many of these deaths are preventable by either vaccination or antibiotic treatments. Ironically most vaccines and antibiotics are also microbial products. Although we know the most about disease-causing microorganisms, because less than 1% of all microorganisms cause disease, there is a lot left to learn about microbes. In fact, like the number of stars, the number of microbial species (including bacteria, viruses, fungi, and protists) is actively debated. What is certain is that microbes are important for the life of the planet and its plants and animals.

Our goal in this chapter is to introduce you to this amazing world of microorganisms and to outline the history of their evolution and discovery. Microbiology is a biological science, and as such, much of what you will learn in this text is similar to what you have learned in high school and college biology classes that focus on large organisms. But microbes have unique properties, so microbiology has unique approaches to understanding them. These too will be introduced. But before you delve into this chapter, check to see if you have the background needed to get the most from it.

Readiness Check:

Based on what you have learned previously, you should be able to:

- ✓ List the features of eukaryotic cells that distinguish them from other cell types
- ✓ Understand the basic structure of the macromolecules, nucleic acids, proteins, carbohydrates, and lipids (*see appendix I*)

1.1 Members of the Microbial World

After reading this section, you should be able to:

a. Define the term *microbiology*
b. Explain Carl Woese's contributions in establishing the three-domain system for classifying cellular life
c. Determine the type of microbe (bacterium, fungus, etc.) when given a description of a newly discovered one
d. Provide an example of the importance to humans of each of the major types of microbes

Microorganisms are defined as those organisms too small to be seen clearly by the unaided eye (**figure 1.1**). They are generally 1 millimeter or less in diameter. Although small size is an important characteristic of microbes, it is not sufficient to define them.

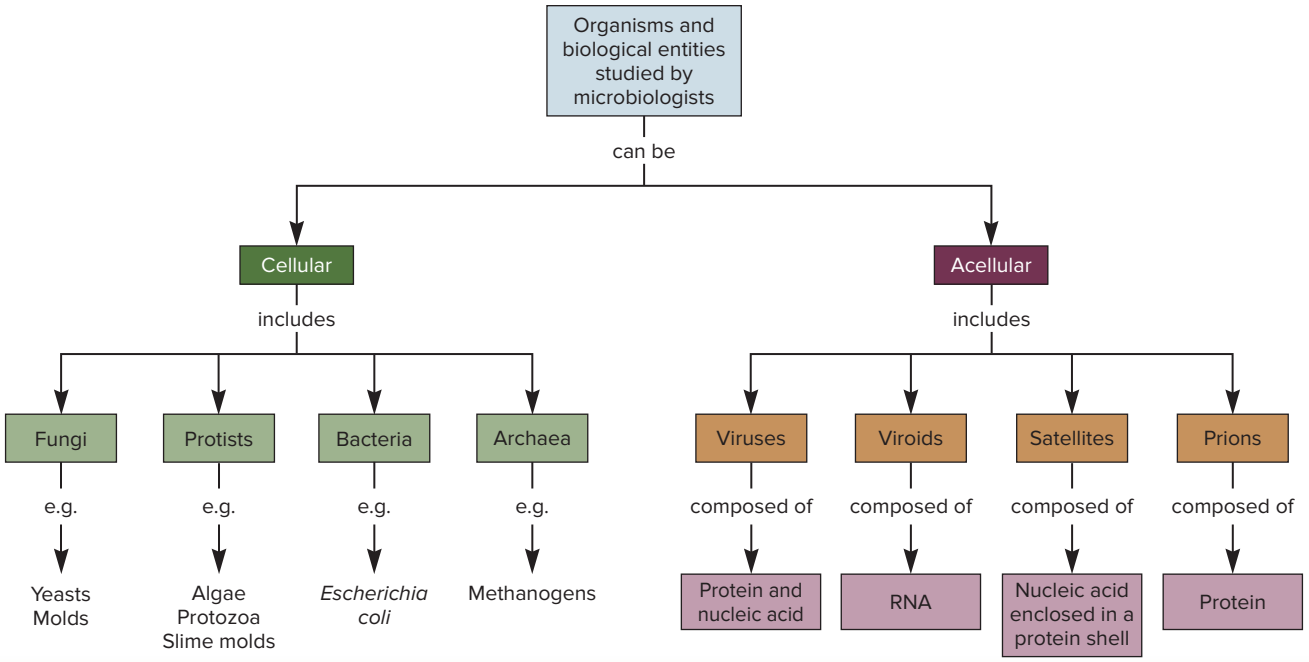


Figure 1.1 **Concept Map Showing the Types of Biological Entities Studied by Microbiologists.**

MICRO INQUIRY *How would you alter this concept map so that it also distinguishes cellular organisms from each other?*

Some microbes, such as bread molds and filamentous photosynthetic microbes (e.g., "pond scum"), are actually visible without microscopes. These macroscopic microbes often consist of small aggregations of cells. Some macroscopic microorganisms are multicellular. They are distinguished from other multicellular life forms such as plants and animals by their lack of highly differentiated tissues. Most unicellular microbes are microscopic. In summary, cellular microbes are usually smaller than 1 millimeter in diameter, often unicellular and, if multicellular, lack differentiated tissues.

In addition to microorganisms, microbiologists study a variety of acellular biological entities (figure 1.1). These include viruses and subviral agents. The terms "microorganism" and "microbe" are sometimes applied to these acellular agents as well.

The diversity of microorganisms has always presented a challenge to microbial taxonomists. Early descriptions of cellular microbes as either plants or animals were too simple. For instance, some microbes are motile like animals but also have cell walls and are photosynthetic like plants. An important breakthrough in microbial taxonomy arose from studies of their cellular architecture, when it was discovered that cells exhibited one of two possible "floor plans." Cells that came to be called **prokaryotic cells** (Greek *pro,* before; *karyon,* nut or kernel) have an open floor plan. That is, their contents are not divided into compartments ("rooms") by membranes. Only **eukaryotic cells** (Greek *eu,* true) have a nucleus and other membrane-bound organelles (e.g., mitochondria, chloroplasts) that separate some cellular materials and processes from others.

These observations eventually led to the development of a classification scheme that divided organisms into five kingdoms: *Monera, Protista, Fungi, Animalia,* and *Plantae.* Microorganisms (except for viruses and other acellular infectious agents, which have their own classification system) were placed in the first three kingdoms. In this scheme, all organisms with prokaryotic cell structure were placed in *Monera*. The five-kingdom system was an important development in microbial taxonomy, but it is no longer accepted by microbiologists. This is because "prokaryotes" are too diverse to be grouped together in a single kingdom. Furthermore, it is currently argued that the term *prokaryote* is not meaningful and should be abandoned. ▶| *Use of the term "prokaryote" is controversial (section 3.1)*

Great progress has been made in three areas that profoundly affect microbial classification. First, much has been learned about the detailed structure of microbial cells from the use of electron microscopy. Second, microbiologists have determined the biochemical and physiological characteristics of many different microorganisms. Third, the sequences of nucleic acids and proteins from a wide variety of organisms have been compared. The comparison of ribosomal RNA (rRNA) nucleic acid sequences, begun by Carl Woese (1928–2012) in the 1970s, was instrumental in demonstrating that there are two very different groups of organisms with prokaryotic cell architecture: *Bacteria* and *Archaea*. Later studies based on rRNA comparisons showed that *Protista* is not a cohesive taxonomic unit (i.e., taxon) and that it should be divided into three or more kingdoms. These studies and others led many taxonomists to reject the five-kingdom system in favor of one that divides cellular organisms

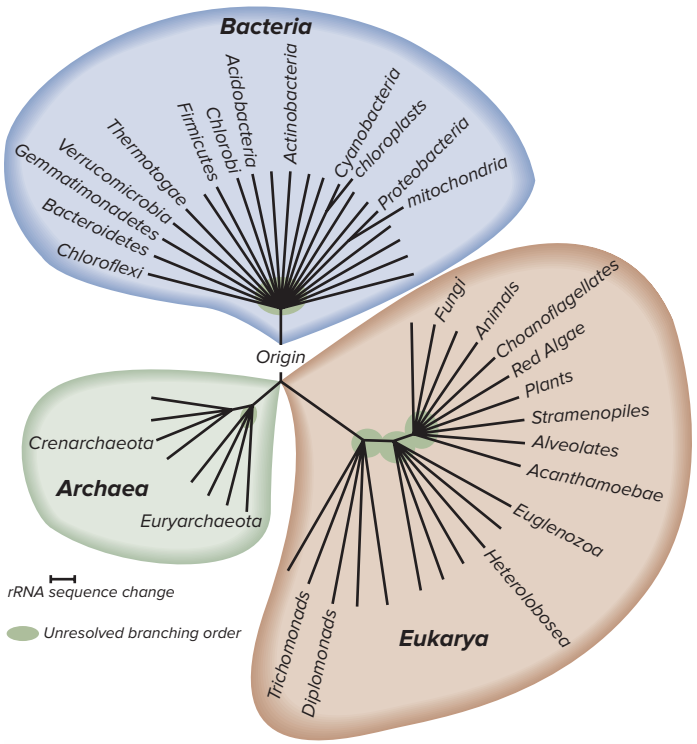


Figure 1.2 Universal Phylogenetic Tree. These evolutionary relationships are based on rRNA sequence comparisons. Only representative lineages have been identified.

MICRO INQUIRY *How many of the taxa listed in the figure include microbes?*

into three domains: *Bacteria*, *Archaea*, and *Eukarya* (all eukaryotic organisms) (**figure 1.2**). ▶| *Nucleic acids (appendix I); Proteins (appendix I)*

Members of domain ***Bacteria*** are usually single-celled organisms.[1] Most have cell walls that contain the structural molecule peptidoglycan. Bacteria are abundant in soil, water, and air, including sites that have extreme temperatures, pH, or salinity. Bacteria are also major inhabitants of our bodies, forming the human **microbiome.** Indeed, more microbial cells are found in and on the human body than there are human cells. These microbes begin to colonize humans shortly after birth. As the microbes establish themselves, they contribute to the development of the body's immune system. Those microbes that inhabit the large intestine help the body digest food and produce vitamins. In these and many other ways, the human microbiome helps maintain our health and well-being. ▶| *Overview of bacterial cell wall structure (section 3.4); The microbe-human ecosystem (chapter 34)*

Unfortunately some bacteria cause disease, and some of these diseases have had a huge impact on human history. In 1347 the plague (Black Death), a disease carried by bacteria living in fleas, struck Europe with brutal force, killing one-third of the population within 4 years. Over the next 80 years, the disease struck repeatedly, eventually wiping out roughly half of the European population. The plague's effect was so great that most historians believe it changed European culture and prepared the way for the Renaissance.

Members of domain ***Archaea*** are distinguished from bacteria by many features, most notably their distinctive rRNA sequences, lack of peptidoglycan in their cell walls, and unique membrane lipids. Some have unusual metabolic characteristics, such as the ability to generate methane (natural) gas. Many archaea are found in extreme environments, including those with high temperatures (thermophiles) and high concentrations of salt (extreme halophiles). Although some archaea are members of a community of microbes involved in gum disease in humans, their role in causing disease has not been clearly established.

Domain ***Eukarya*** includes plants, animals, and microorganisms classified as protists or fungi. **Protists** are generally unicellular but larger than most bacteria and archaea. They have traditionally been divided into protozoa and algae. However, these terms lack taxonomic value because protists, algae, and protozoa do not form three groups, each with a single evolutionary history. Nonetheless, for convenience, we use these terms here.

The major types of protists are algae, protozoa, slime molds, and water molds. **Algae** are photosynthetic. They, together with cyanobacteria, produce about 50% of the planet's oxygen and are the foundation of aquatic food chains. **Protozoa** are usually motile and many free-living protozoa function as the principal hunters and grazers of the microbial world. They obtain nutrients by ingesting organic matter and other microbes. They can be found in many different environments, and some are normal inhabitants of the intestinal tracts of animals, where they aid in digestion of complex materials such as cellulose. A few cause disease in humans and other animals. **Slime molds** are protists that behave like protozoa in one stage of their life cycle but like fungi in another. In the protozoan phase, they hunt for and engulf food particles, consuming decaying vegetation and other microbes. **Water molds** are protists that grow on the surface of freshwater and moist soil. They feed on decaying plant material. Some water molds have produced devastating plant infections, including the Great Potato Famine of 1846–1847 in Ireland, which led to the mass exodus of Irish to the United States and other countries. Although slime and water molds are protists, they were once thought to be fungi, thus the confusing nomenclature. ▶| *Protists (chapter 24)*

Fungi are a diverse group of microorganisms that range from unicellular forms (yeasts) to molds and mushrooms. Molds and mushrooms are multicellular fungi that form thin, threadlike structures called hyphae. They absorb all their nutrients from their environment. Because of their metabolic capabilities, many fungi play beneficial roles, including making bread dough rise, producing antibiotics, and decomposing dead organisms. Some fungi associate with plant roots to form mycorrhizae. Mycorrhizal fungi transfer nutrients to the roots, improving growth of the plants, especially in poor soils. Other fungi cause plant diseases (e.g., rusts, powdery mildews, and smuts) and diseases in humans and other animals. ▶| Fungi *(chapter 25)*

1 In this text, the term *bacteria* (s., *bacterium*) is used to refer to those microbes belonging to domain *Bacteria,* and the term *archaea* (s., *archaeon*) is used to refer to those that belong to domain *Archaea.* In some publications, the term *bacteria* is used to refer to all cells having prokaryotic cell structure. That is not the case in this text.

The microbial world also includes numerous acellular infectious agents. **Viruses** are acellular entities that must invade a host cell to multiply. The simplest virus particles (also called virions) are composed only of proteins and a nucleic acid, and can be extremely small (the smallest is 10,000 times smaller than a typical bacterium). However, their small size belies their power. They cause many animal and plant diseases and have caused epidemics that have shaped human history. Viral diseases include smallpox, rabies, influenza, AIDS, the common cold, and some cancers. Viruses also play important roles in aquatic environments, where they play a critical role in shaping aquatic microbial communities. **Viroids** are infectious agents composed only of ribonucleic acid (RNA). They cause numerous plant diseases. **Satellites** are composed of a nucleic acid enclosed in a protein shell. They must coinfect a host cell with virus, called a helper virus, to complete their life cycle. Viroids, on the other hand, cause only plant diseases while satellites and their helper viruses cause both plant and animal diseases. Finally, **prions,** infectious agents composed only of protein, are responsible for causing a variety of spongiform encephalopathies such as scrapie and "mad cow disease." ▶| *Viruses and other acellular infectious agents (chapter 6)*

Comprehension Check

1. How did the methods used to classify microbes change, particularly in the last half of the twentieth century? What was the result of these technological advances?
2. Identify one characteristic for each of these types of microbes that distinguishes it from the other types: bacteria, archaea, protists, fungi, viruses, viroids, satellites, and prions.

1.2 Microbes Have Evolved and Diversified for Billions of Years

After reading this section, you should be able to:

a. Propose a timeline of the origin and history of microbial life and integrate supporting evidence into it
b. Design a set of experiments that could be used to place a newly discovered cellular microbe on a phylogenetic tree based on small subunit (SSU) rRNA sequences
c. Compare and contrast the definitions of plant and animal species, microbial species, and microbial strains

A review of figure 1.2 reminds us that in terms of the number of taxa, microbes are the dominant organisms on Earth. How has microbial life been able to radiate to such an astonishing level of diversity? To answer this question, we must consider microbial evolution. The field of microbial evolution, like any other scientific endeavor, is based on the formulation of hypotheses, the gathering and analysis of data, and the reformation of hypotheses based on newly acquired evidence. That is to say, the study of microbial evolution is based on the scientific method. To be sure, it is difficult to amass evidence when considering events that occurred millions, and often billions, of years ago, but the application of molecular methods has revealed a living record of life's ancient history. This section describes the outcome of this scientific research.

Theories of the Origin of Life Depend Primarily on Indirect Evidence

Dating meteorites through the use of radioisotopes places our planet at an estimated 4.5 to 4.6 billion years old. However, conditions on Earth for the first 100 million years or so were far too harsh to sustain any type of life. Eventually bombardment by meteorites decreased, water appeared on the planet in liquid form, and gases were released by geological activity to form Earth's atmosphere. These conditions were amenable to the origin of the first life forms. But how did this occur, and what did these life forms look like?

To find evidence of life and to develop hypotheses about its origin and subsequent evolution, scientists must be able to define life. Although even very young children can examine an object and correctly determine whether it is living or not, defining life succinctly has proven elusive for scientists. Thus most definitions of life consist of a set of attributes. The attributes of particular importance to paleobiologists are an orderly structure, the ability to obtain and use energy (i.e., metabolism), and the ability to reproduce. Just as NASA scientists are using the characteristics of microbes on Earth today to search for life elsewhere, so too are scientists examining **extant organisms,** those organisms present today, to explore the origin of life. Some extant organisms have structures and molecules that represent "relics" of ancient life forms. These can provide scientists with ideas about the type of evidence to seek when testing hypotheses.

The best direct evidence for the nature of primitive life would be a fossil record. There have been reports of microbial fossil discoveries since 1977 (**figure 1.3**). These have always met with skepticism because finding them involves preparing thin slices of ancient rocks and examining the slices for objects that look like cells. Unfortunately some things that look like cells can be formed by geological forces that occurred as the rock was formed. The result is that the fossil record for microbes is sparse and always open to reinterpretation. Despite these problems most scientists agree that life was present on Earth about 3.5–3.7 billion years ago (**figure 1.4**). To reach this conclusion, biologists rely primarily on indirect evidence. Among the indirect evidence used are molecular fossils. These are chemicals found in rock or sediment that are chemically related to molecules found in cells. For instance, the presence in a rock of molecules called hopanes is an indication that when the rock was formed, bacteria were present. This conclusion is reached because hopanes are formed from hopanoids, which are found in the plasma membranes of extant bacteria. As you can see, no single piece of evidence can

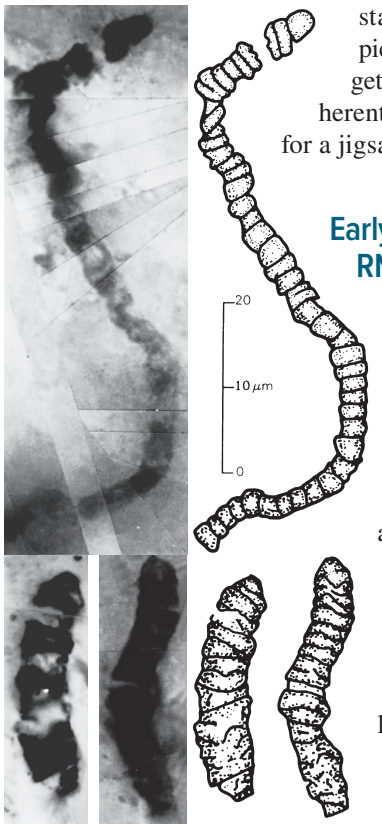


Figure 1.3 Possible Microfossils Found in the Archaeon Apex Chert of Australia. Chert is a type of granular sedimentary rock rich in silica. These structures were discovered in 1977. Because of their similarity to filamentous cyanobacteria they were proposed to be microfossils. In 2011 scientists reported that similar structures from the same chert were not biological in origin. They used spectrometry and microscopy techniques not available in 1977 to show that the structures were fractures in the rock filled with quartz and hematite. Scientists are still debating whether or not these truly are microfossils.

stand alone. Instead many pieces of evidence are put together in an attempt to get a coherent picture to emerge, much as for a jigsaw puzzle.

Early Life Was Probably RNA Based

The origin of life rests on a single question: How did early cells arise? At a minimum, modern cells consist of a plasma membrane enclosing water in which numerous chemicals are dissolved and subcellular structures float. It seems likely that the first self-replicating entity was much simpler than even the most primitive modern living cells. Before there was life, most evidence suggests that Earth was a very different place: hot and anoxic, with an atmosphere rich in water vapor, carbon dioxide, and nitrogen. In the oceans, hydrogen, methane, and carboxylic acids were formed by geological and chemical processes. Areas near hydrothermal vents or in shallow pools may have provided the conditions that allowed chemicals to react with one another, randomly "testing" the usefulness of the reaction and the stability of its products. Some reactions released energy and would eventually become the basis of modern cellular metabolism. Other reactions generated molecules that functioned as catalysts, some aggregated with other molecules to form the predecessors of modern cell structures, and others were able to replicate and act as units of hereditary information.

In modern cells, three different molecules fulfill the roles of catalysts, structural molecules, and hereditary molecules (**figure 1.5**). Proteins have two major roles in modern cells: catalytic and structural. Catalytic proteins are **enzymes** and structural proteins serve a myriad of functions such as transport, attachment, and motility. DNA stores hereditary information that is replicated and passed on to the next generation. RNA is involved in converting the information stored in DNA into protein. Any hypothesis about the origin of life must account for the evolution of these molecules, but the very nature of their relationships to each other in modern cells complicates attempts to imagine how they evolved. As demonstrated in figure 1.5, proteins can do cellular work, but their synthesis involves other proteins and RNA, and uses information stored in DNA. DNA can't do cellular work and proteins are needed for its replication. RNA synthesis requires both DNA as the template and proteins as the catalysts for the reaction.

Based on these considerations, it is hypothesized that at some time in the evolution of life, there must have been a single molecule that could do both cellular work and replicate. This idea was supported in 1981 when Thomas Cech discovered a catalytic RNA molecule in a protist (*Tetrahymena* sp.) that could cut out an internal section of itself and splice the remaining sections back together. Since then, other catalytic RNA molecules have been discovered, including an RNA found in ribosomes that is responsible for forming peptide bonds—the bonds that hold together amino acids, the building blocks of proteins. Catalytic RNA molecules are now called **ribozymes.**

The discovery of ribozymes suggested that RNA at some time was capable of storing, copying, and expressing genetic information, as well as catalyzing other chemical reactions. In 1986 Nobel laureate Walter Gilbert coined the term **RNA world** to describe this precellular stage in the evolution of life. However, for this precellular RNA-based stage to proceed to the evolution of cellular life forms, a lipid membrane must have formed around the RNA (**figure 1.6**). This important evolutionary step is easier to imagine than other events in the origin of cellular life forms because lipids, major structural components of the membranes of modern organisms, spontaneously form liposomes—vesicles bounded by a lipid bilayer. ▶| *Lipids (appendix I)*

Jack Szostak, also a Noble laureate, is a leader in exploring how RNA-containing cells, so-called protocells, may have formed. When his group created liposomes using simpler fatty acids than those found in membranes today, the liposomes were leaky. These leaky liposomes allowed single RNA nucleotides to move into the liposome, but prevented large RNA chains from moving out. Furthermore, researchers could prod the liposomes into growing and dividing. Dr. Szostak's group has also been able to create conditions in which an RNA molecule could serve as a template for synthesis of a complementary RNA strand. Thus their experiments may have recapitulated the early steps of the evolution of cells. As seen in figure 1.6, several other processes would need to occur to reach the level of complexity found in extant cells.

Apart from its ability to perform catalytic activities, the function of RNA suggests its ancient origin. Consider that much

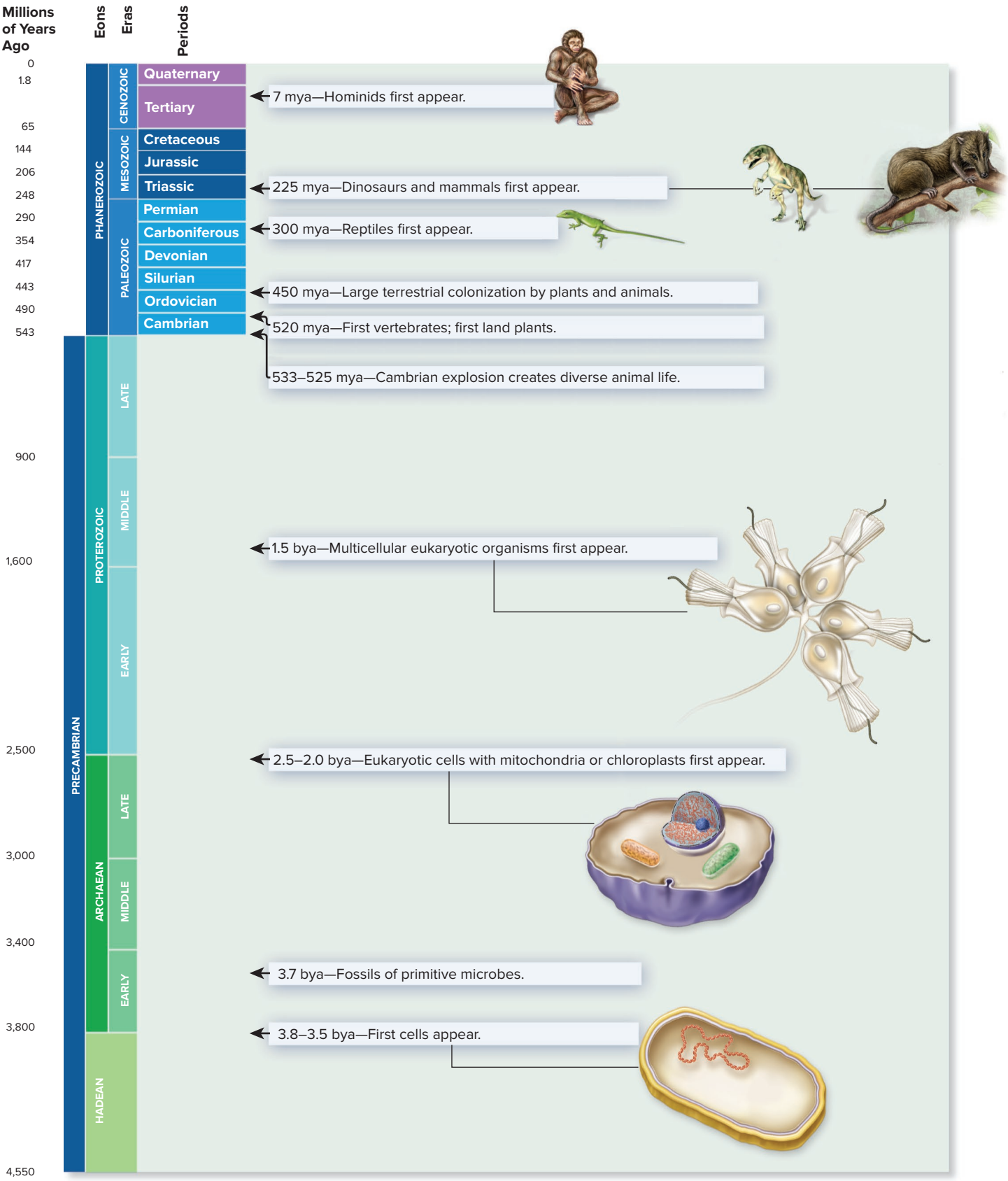


Figure 1.4 **An Overview of the History of Life on Earth.** mya = million years ago; bya = billion years ago.

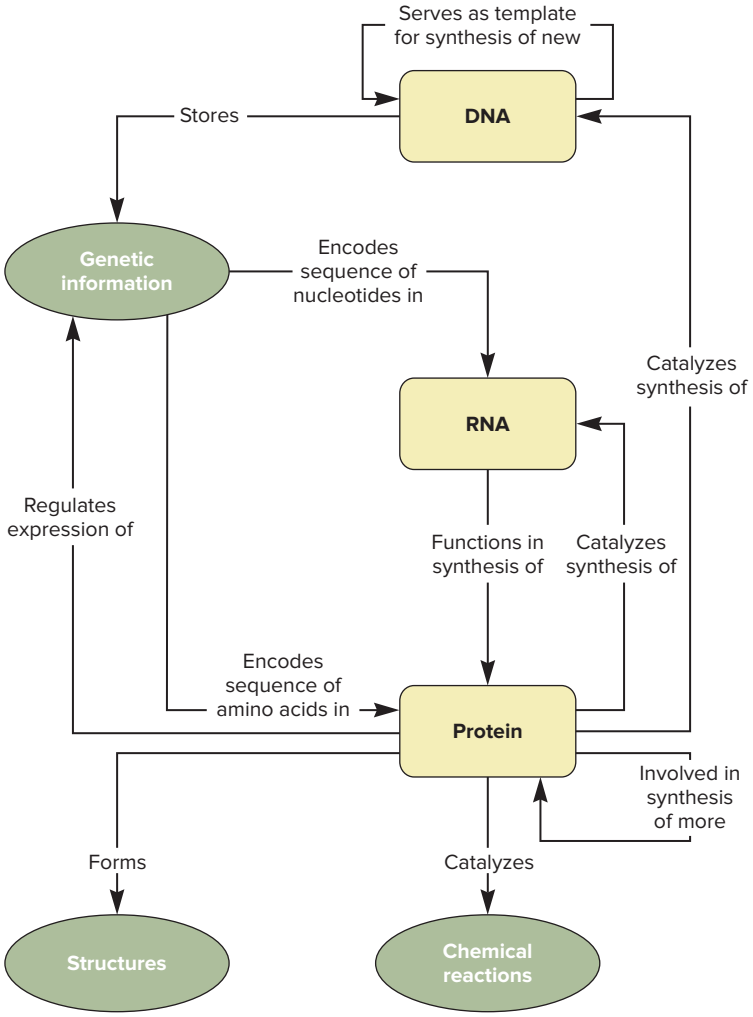


Figure 1.5 **Functions of DNA, RNA, and Protein, and Their Relationships to Each Other in Extant Cells.**

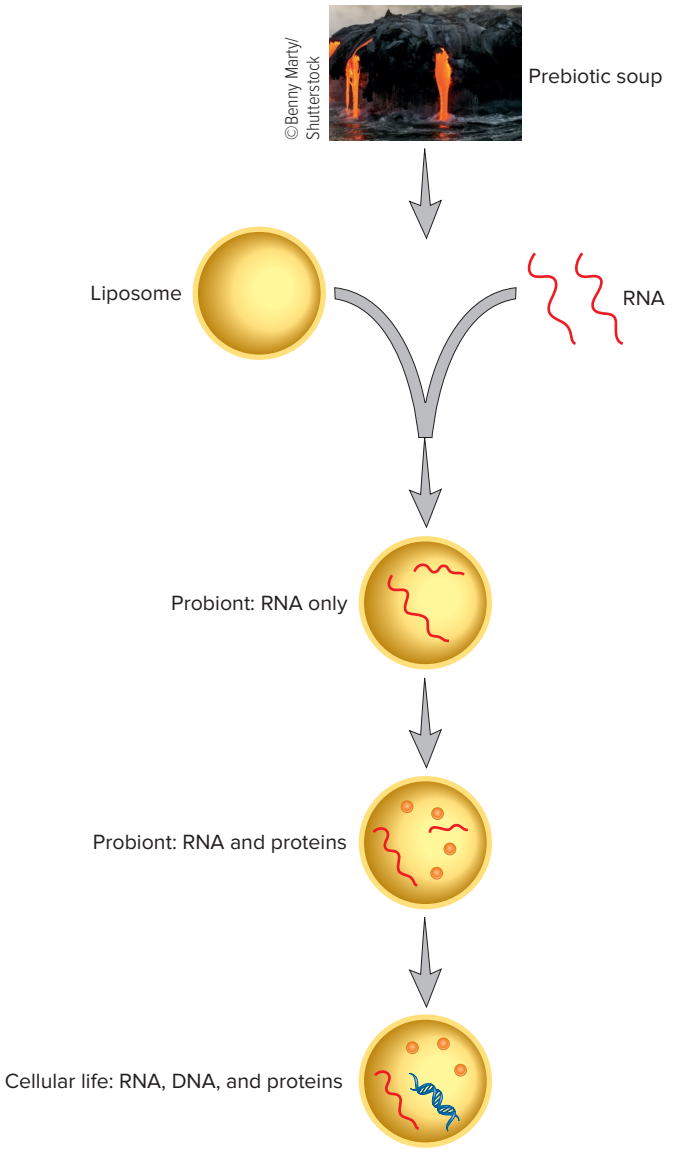
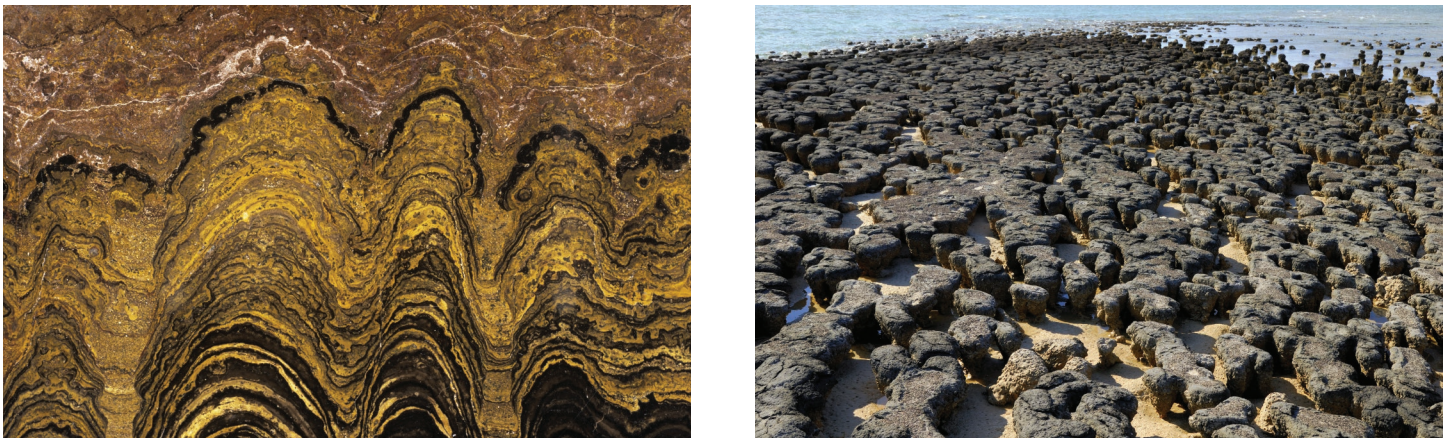


Figure 1.6 **The RNA World Hypothesis for the Origin of Life.**

MICRO INQUIRY *Why are the probionts pictured above not considered cellular life?*

of the cellular pool of RNA in modern cells exists in the ribosome, a structure that consists largely of rRNA and uses messenger RNA (mRNA) and transfer RNA (tRNA) to construct proteins. Also rRNA itself catalyzes peptide bond formation during protein synthesis. Thus RNA seems to be well poised for its importance in the development of proteins. Because RNA and DNA are structurally similar, RNA could have given rise to double-stranded DNA. It is suggested that once DNA evolved, it became the storage facility for genetic information because it provided a more chemically stable structure. Two other pieces of evidence support the RNA world hypothesis: the fact that the energy currency of cells, ATP, is a ribonucleotide and the discovery that RNA can regulate gene expression. So it would seem that proteins, DNA, and cellular energy can be traced back to RNA. ▶| *ATP: the major energy currency of cells (section 10.2); Riboswitches (section 14.3); Translational riboswitches (section 14.4)*

Despite evidence supporting the RNA world hypothesis, it is not without problems, and many argue against it. Another area of research also fraught with considerable debate is the evolution of metabolism, in particular the evolution of energy-conserving metabolic processes. The early Earth was a hot environment that lacked oxygen. Thus the cells that arose there must have been able to use the available energy sources under these harsh conditions. Today there are heat-loving archaea capable of using inorganic molecules such as FeS as a source of energy. Some suggest that this interesting metabolic capability is a remnant of the first form of energy metabolism. Another metabolic strategy, oxygen-releasing photosynthesis (oxygenic photosynthesis), appears to have evolved perhaps as early as 2.7 billion years ago. Fossils of cyanobacteria-like cells found in rocks dating to that time



(a) (b)

Figure 1.7 Stromatolites. (a) Section of a fossilized stromatolite. Evolutionary biologists think the layers of material were formed when mats of cyanobacteria, layered one on top of the other, became mineralized. (b) Modern stromatolites from Western Australia. Each stromatolite is a rocklike structure, typically 1 m in diameter, containing layers of cyanobacteria.

support this hypothesis, as does the discovery of ancient stromatolites (**figure 1.7*a***). Stromatolites are layered rocks formed by the incorporation of mineral sediments into layers of microorganisms growing in thick mats on surfaces (figure 1.7*b*). The appearance of cyanobacteria-like cells was an important step in the evolution of life on Earth. The oxygen they released is thought to have altered Earth's atmosphere to its current oxygen-rich state, allowing the evolution of additional energy-capturing strategies such as aerobic respiration, the oxygen-consuming metabolic process that is used by many microbes and animals.

Evolution of the Three Domains of Life

As noted in section 1.1, rRNA comparisons were an important breakthrough in the classification of microbes; this analysis also provides insights into the evolutionary history of all life. What began with the examination of rRNA from relatively few organisms has been expanded by the work of many others, including Norman Pace. Dr. Pace has developed a **universal phylogenetic tree** (figure 1.2) based on comparisons of small subunit rRNA molecules (SSU rRNA), the rRNA found in the small subunit of the ribosome. Here we examine how these comparisons are made and what the universal phylogenetic tree tells us. ▶| *Bacterial ribosomes (section 3.6); Microbial taxonomy and phylogeny are largely based on molecular characterization (section 19.3)*

Comparing SSU rRNA Molecules

The details of phylogenetic tree construction are discussed in chapter 19. However, the general concept is not difficult to understand. In one approach, the sequences of nucleotides in the genes that encode SSU rRNAs from diverse organisms are aligned, and pair-wise comparisons of the sequences are made. For each pair of SSU rRNA gene sequences, the number of differences in the nucleotide sequences is counted (**figure 1.8**). This value serves as a measure of the evolutionary distance between the organisms; the more differences counted, the greater the evolutionary distance. The evolutionary distances from many comparisons are used by sophisticated computer programs to construct the tree. The tip of each branch in the tree represents one of the organisms used in the comparison. The distance from the tip of one branch to the tip of another is the evolutionary distance between the two organisms.

Two things should be kept in mind when examining phylogenetic trees developed in this way. The first is that they are molecular trees, not organismal trees. In other words, they represent, as accurately as possible, the evolutionary history of a molecule and the gene that encodes it. Second, the distance between branch tips is a measure of relatedness, not of time. If the distance along the lines is very long, then the two organisms are more evolutionarily diverged (i.e., less related). However, we do not know when they diverged from each other. This concept is analogous to a printed map that accurately shows the distance between two cities but because of many factors (traffic, road conditions, etc.) cannot show the time needed to travel that distance.

LUCA

What does the universal phylogenetic tree tell us about the evolution of life? At the center of the tree is a line labeled "Origin" (figure 1.2). This is where data indicate the *l*ast *u*niversal *c*ommon *a*ncestor (LUCA) to all three domains should be placed. LUCA is on the bacterial branch, which means that *Archaea* and *Eukarya* evolved independently, separate from *Bacteria*. Thus the universal phylogenetic tree presents a picture in which all

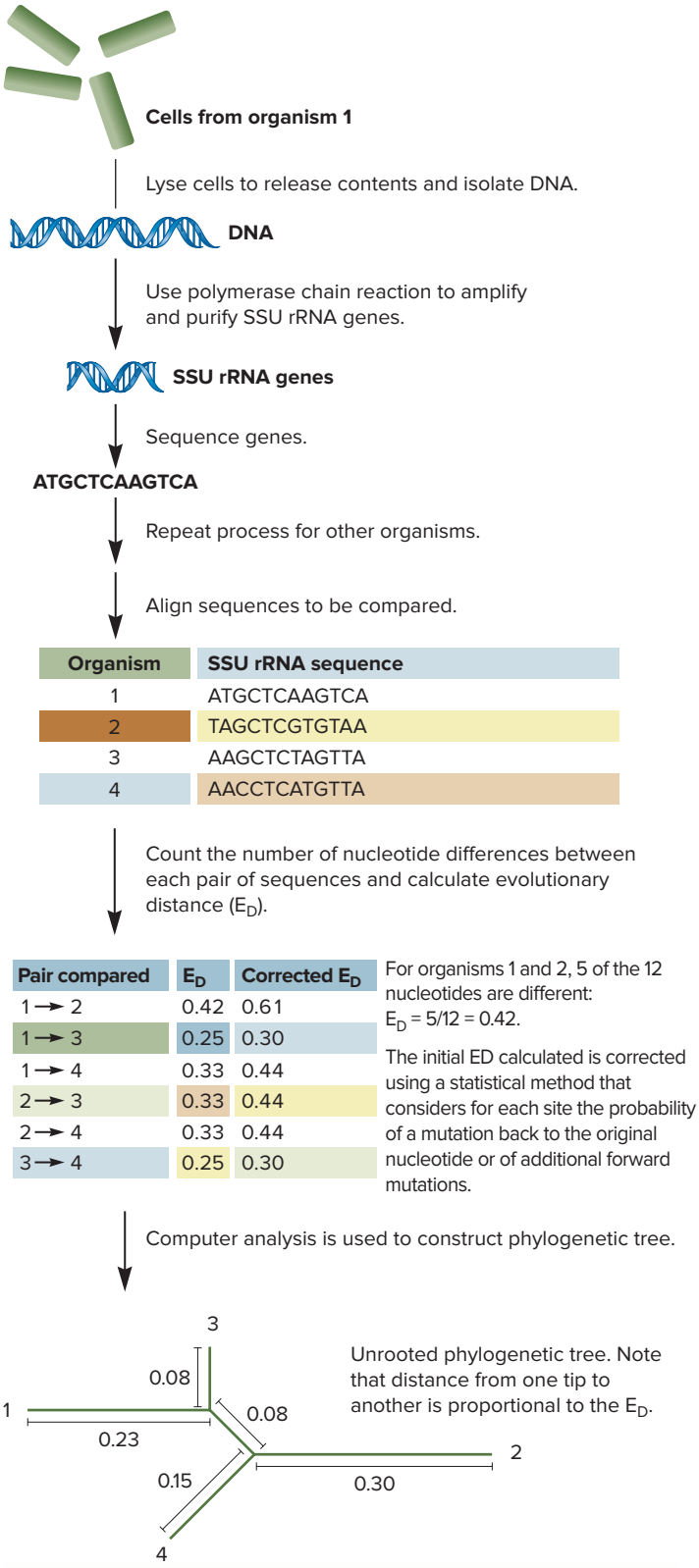


Figure 1.8 **The Construction of Phylogenetic Trees Using a Distance Method. The polymerase chain reaction is described in chapter 17.**

MICRO INQUIRY *Why does the branch length indicate amount of evolutionary change but not the time it took for that change to occur?*

life, regardless of eventual domain, arose from a single common ancestor. One can envision the universal tree of life as a real tree that grows from a single seed.

The evolutionary relationship of *Archaea* and *Eukarya* is still the matter of considerable debate. According to the universal phylogenetic tree we show here, *Archaea* and *Eukarya* shared common ancestry but diverged and became separate domains. Other versions suggest that *Eukarya* evolved out of *Archaea*. The close evolutionary relationship of these two forms of life is still evident in the manner in which they process genetic information. For instance, certain protein subunits of archaeal and eukaryotic RNA polymerases, the enzymes that catalyze RNA synthesis, resemble each other to the exclusion of those of bacteria. However, archaea have other features that are most similar to their counterparts in bacteria (e.g., mechanisms for conserving energy). This has further complicated and fueled the debate. The evolution of the nucleus and endoplasmic reticulum is also at the center of many controversies. However, hypotheses regarding the evolution of other membrane-bound organelles are more widely accepted and are considered next.

Mitochondria, Mitochondria-Like Organelles, and Chloroplasts Evolved from Endosymbionts

The **endosymbiotic hypothesis** is generally accepted as the origin of several eukaryotic organelles, including mitochondria, chloroplasts, and hydrogenosomes. **Endosymbiosis** is an interaction between two organisms in which one organism lives inside the other. The original endosymbiotic hypothesis proposed that over time a bacterial endosymbiont of an ancestral cell in the eukaryotic lineage lost its ability to live independently, becoming either a mitochondrion, if the intracellular bacterium used aerobic respiration, or a chloroplast, if the endosymbiont was a photosynthetic bacterium (*see figure 19.7*).

Although the mechanism by which the endosymbiotic relationship was established is unknown, there is considerable evidence to support the hypothesis. Mitochondria and chloroplasts contain DNA and ribosomes; both are similar to bacterial DNA and ribosomes. Peptidoglycan, the unique bacterial cell wall molecule, has even been found between the two membranes that enclose the chloroplasts of some algae. Indeed, inspection of figure 1.2 shows that both organelles belong to the bacterial lineage based on SSU rRNA analysis. More specifically, mitochondria are most closely related to bacteria called proteobacteria. The chloroplasts of plants and green algae are thought to have descended from an ancestor of the cyanobacterial genus *Prochloron*, which contains species that live within marine invertebrates. ▶| *Phylum* Cyanobacteria: *oxygenic photosynthetic bacteria (section 21.4); The proteobacterial origin of mitochondria (section 22.1)*

Recently the endosymbiotic hypothesis for mitochondria has been modified by the **hydrogen hypothesis.** This asserts that the endosymbiont was an anaerobic bacterium that produced H_2 and CO_2 as end products of its metabolism. Over time,