

GLOBAL  
EDITION



# Brock Biology of Microorganisms

FIFTEENTH EDITION

Madigan • Bender • Buckley • Sattley • Stahl



Pearson

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# Where cutting edge science meets state of the art learning.

The Fifteenth Edition of the world-renowned *Brock Biology of Microorganisms* introduces today's students to cutting edge microbiology research and ensures core concept mastery, enhanced by **MasteringMicrobiology**®.

## Microbial Growth and Its Control

**microbiologynow**

### Picking Apart a Microbial Consortium

In nature, certain metabolic processes are carried out by microbes that team up to get the job done, a cozy arrangement called a consortium. Such is the case with the oxidation of methane ( $\text{CH}_4$ ) linked to the reduction of sulfate ( $\text{SO}_4^{2-}$ ) in anoxic marine sediments. The overall reaction ( $\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$ ) is exergonic and the small amount of energy released is shared between two distinct microbes. The methane oxidizer in the consortium is a species of *Archaea* nicknamed ANME (for anaerobic methanotroph, blue in photo), and its sulfate-reducing partner is a species of *Bacteria* (brown in photo). The consortium is thought to play a key role in the carbon cycle as a major methane sink, and thus a detailed picture of how it works is important to our understanding of the global carbon economy, climate change, and marine biogeochemistry.

Researchers have tried for years to separate the consortium into its components but always found that methane oxidation required both organisms. However, some researchers hypothesized that it might be possible to replace the sulfate reducer with an artificial electron acceptor and that this might unlock the consortium and allow the methanotroph to grow in pure culture. Using an electron acceptor called AQDS, the scientists discovered that they could turn off sulfate reduction in the consortium while maintaining  $\text{CH}_4$  oxidation. During this process, the methanotroph used electrons from  $\text{CH}_4$  to reduce AQDS rather than passing them on to its sulfate-reducing partner. Several other electron acceptors known to support anaerobic respiration also sustained methane oxidation, giving hope that ANME may eventually be obtained in pure culture.

The ability to grow a microbe in pure culture is the "gold standard" for the study of its physiology, biochemistry, regulation, and several other aspects of its biology. In the case of the ANME-sulfate reducer consortium, several physiologies were active at once, and resolving these many reactions proved to be a major scientific challenge. However, if further work shows that ANME can be removed from the consortium and grown in pure culture, detailed aspects of its biology can be studied that were not possible when the organism was tightly coupled to its partner in the consortium (photo).

**Source:** Scheller, S., H. Yu, G.L. Chadwick, S.E. McGlynn, and V.J. Orphan. 2016. Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction. *Science* 351: 703–706.



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**NEW & REVISED! Microbiology**  
Now chapter opening features highlight cutting edge, engaging research that is important to how we understand microbiology today. Paired assessments in **MasteringMicrobiology** engage students in the course material and foster deep concept mastery.

## Microbial Infection and Pathogenesis

**microbiologynow**

### The Microbial Community That Thrives on Your Teeth

Few people have such superb oral hygiene that they lack dental plaque, the microbial biofilm that forms on and between teeth and along or near the gumline. If not removed regularly, dental plaque invades and leads to dental caries (cavities), the result of which is destruction of tooth enamel and dentin break down from the onslaught of bacterial activities. Dental plaque and dental caries develop from the natural tendency of oral bacteria such as *Streptococcus mutans* and its close relative *S. sobrinus* to attach firmly to the teeth and gums and ferment sucrose (table sugar) to lactic acid, which attacks the teeth and slowly rots them away.

Until recently, dental plaque was thought to consist largely of the aforementioned streptococci. Both species could easily be isolated from dental plaque and both light and electron microscopy typically showed large numbers of cocci in chains, a hallmark of the genus *Streptococcus*. But a recent molecular ecology study of the microbial diversity of dental plaque revealed that this material is composed of more than just streptococci and develops in a precisely structured way.

The photo here is a light micrograph of a section through human dental plaque stained by fluorescence in situ hybridization (FISH). Different oligonucleotides, each specific for a different major phylum of *Bacteria* and containing a distinct fluorescent dye, were allowed to hybridize to the ribosomal RNA in the cells and then observed by fluorescence microscopy. Surprisingly, instead of seeing primarily streptococci, the researchers saw a diverse and highly organized microbial community. The micrograph shows streptococci (stained green) located primarily at the periphery of the plaque beyond several other bacteria that combine to form a scaffold emerging from the tooth surface. These include *Corynebacterium* (purple), *Capnocytophaga* (red), *Fusobacterium* (yellow), *Leptotrichia* (blue-green), and *Haemophilus* (orange), among others. A major conclusion that emerged from this study was that the scaffolding microbes likely function to position the streptococci out into the oral cavity where sucrose should be more available.

New views of old problems often reveal surprising results. In the case of dental plaque, FISH technology has revealed a whole new microbial world in a habitat previously thought to be dominated by only two species of well-characterized bacteria.

**Source:** Mark Welch, J.L., et al. 2016. Biogeography of a human oral microbiome at the micron scale. *Proc. Natl. Acad. Sci. (USA)* 113: doi: 10.1073/pnas.1522149113.

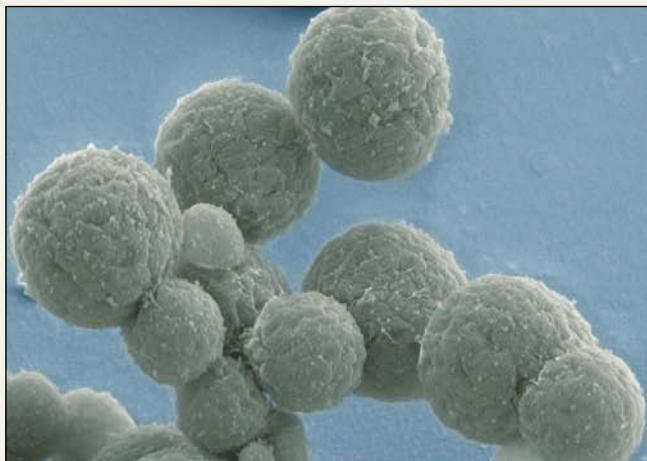


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# Microbiology today and tomorrow.

Genomics, and the various “omics” it has spawned, support content throughout the Fifteenth Edition ensuring that today’s students understand the transformation that biology, and specifically microbiology, has undergone – and preparing them for the fast paced nature of the science.



## microbiologynow

### Creation of a New Life Form: Design of a Minimal Cell

A cell's genome is its blueprint for life. However, what is the bare minimum number of genes needed to sustain a free-living cell? This is a question that microbiologists at the J. Craig Venter Institute (JCVI) have attempted to answer ever since they sequenced the genomes of several *Mycoplasma* species in the 1990s. Because *Mycoplasma* species are parasitic bacteria, their genomes are already reduced in size and hence provide an excellent foundation for creating a "minimal cell." However, little did the scientists at JCVI suspect that it would take 20 years to satisfy their scientific curiosity!

Instead of beginning by genetically manipulating a *Mycoplasma* species, microbiologists at JCVI wanted to have more control. To begin unraveling the genetic requirements for life, they first generated a synthetic self-replicating *Mycoplasma* (described in this chapter). The genome of this pioneering synthetic life form was synthesized from scratch based on its known genome sequence. The synthetic cell did not possess a "designer genome," or even a minimal one; it simply contained its own genome but one completely constructed in the laboratory. This breakthrough in synthetic biology provided the technology needed for microbiologists to create designer genomes.

Using comparative genomics and prior knowledge about specific gene sequences, microbiologists at JCVI continued their work by designing and synthesizing several minimal genomes that they hypothesized would sustain life. To their dismay, none of these resulted in a viable cell. So instead, they generated modules of DNA corresponding to a *Mycoplasma* genome and sewed different combinations together to form synthetic genomes. Once viable cells were obtained from transplanting these genomes, nonessential genes from the smallest genome were identified by transposon mutagenesis. After removing these unnecessary genes, a synthetic minimal cell coined JCVI-syn3.0 was created (see photo). This autonomous life form possesses a 531-kilobase genome encoding 473 genes; JCVI-syn3.0 thus contains a genome smaller than any other free-living cell.

While this work showcases the amazing advancements in synthetic biology and the potential for creating designer cells with novel functions, a surprising mystery surrounds this minimal cell: The roles for almost a third of JCVI-syn3.0's genes remain unknown, highlighting how much we still need to learn about the genetic foundation of a living cell.

**Source:** Hutchison, C. A. 3rd, et al. 2016. Design and synthesis of a minimal bacterial genome. *Science* 351(6280): aad253. Photo provided by Clyde Hutchison and J. Craig Venter, JCVI and Thomas Deerinck and Mark Ellisman, NCMIR.

## EXPLORE THE MICROBIAL WORLD

### TINY CELLS

**V**iruses are very small microbes and range in diameter from as small as 20 nm to almost 750 nm. Although no cells exist that are as small as most viruses, the recent discovery of ultra-small bacterial cells,<sup>1,2</sup> has pushed the boundaries of what even the most advanced microbiologists feel must be very close to the minimum size.

Microbiologists collected groundwater, which flows through Earth's deep subsurface, from a Colorado (USA) aquifer (Figure 1) and ran it through a membrane filter whose pores were only 0.2 µm in diameter. The liquid was then passed through the filter and subjected to microbiological analysis. Surprisingly, the filters with 0.2-µm pores have been found to contain many tiny bacterial cells from solutions to generate "sterile solutions," prokaryotic cells were present in the groundwater filtrate. In fact, a diverse array of bacterial species were found, revealing that the groundwater was inhabited by a microbial community of tiny cells! These investigators have come to call "ultramicrobacteria."

Cryo-electron microscopy, in which a specimen is examined at extremely cold temperatures to prevent damage from the environment that can alter a cell's morphology, showed that groundwater ultramicrobacteria are considerably smaller than most cells under 0.2 µm in diameter (Figure 2). The volume of these cells was calculated to be about 1/100 that of a cell of the bacterium *Escherichia coli* (the rod-shaped cell used to represent the small cell could fit into one E. coli cell 50 times). The tiny cells contained about 50 ribosomes, which is also about 1/100 the number present in a rapidly growing (100-min generation time) cell of *E. coli*. The very small size of the

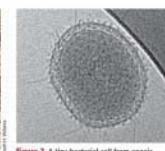


Figure 1 Sampling the anoxic groundwater aquifer that parallels the Colorado River near Rifle, Colorado.

Figure 2 A tiny bacterial cell from anoxic groundwater that passes through a filter with 0.2-µm pores. The cell is not quite 0.2 µm in diameter.

ultramicrobacteria ultramicrobacteria gives them an enormous surface-to-volume ratio, and it is hypothesized that this advantage benefits them in extracting resources from their nutrient-poor environment.

Although we do not yet know exactly how small a microbial cell can be, microbiologists are continuing to push the boundaries.

## EXPLORE THE MICROBIAL WORLD

### THE GUT-BRAIN WORLD

**I**n addition to the gut-microbe and host-brain axis, there is another axis that connects the gut-brain and central nervous system.

We know that the gut microbiome influences brain health and perhaps such inflammatory responses as depression and anxiety. However, gut bacteria is also associated with a general sense of well-being and the gut-brain connection may be the germinal disorder that can emerge as the first life-threatening condition in children. It is in social interaction and communication that the gut-brain connection is actually formed.

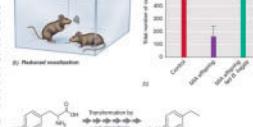
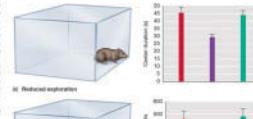


Figure 3. Deficiencies in gut microbiota reduce oxygen consumption and locomotor activity.

Figure 4. Tyrosine chloroformate lyase converts tyrosine to 4-chlorophenylaldehyde.

These studies offer an example of the importance of the gut microbiota-host connection in maintaining the health of the gut microbiota and the host.

These findings are important because the science that has it only at the early stages of associating the gut microbiota with the brain.

Yuan, X., L. M. Hsu, and D. W. Bernstein. 2013. Microbiome influences gut-brain axis via metabolites. *Nature Reviews Microbiology* 11: 289–296.

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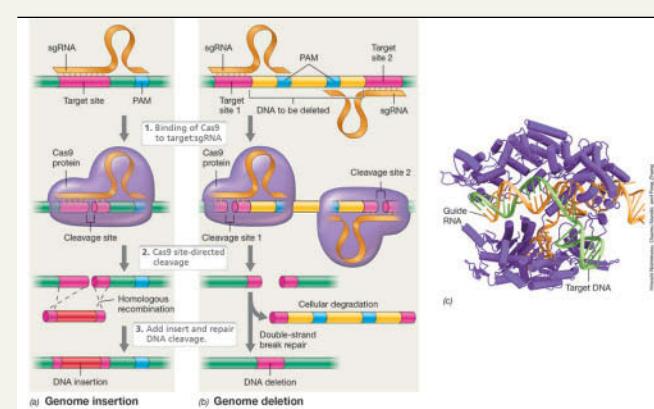
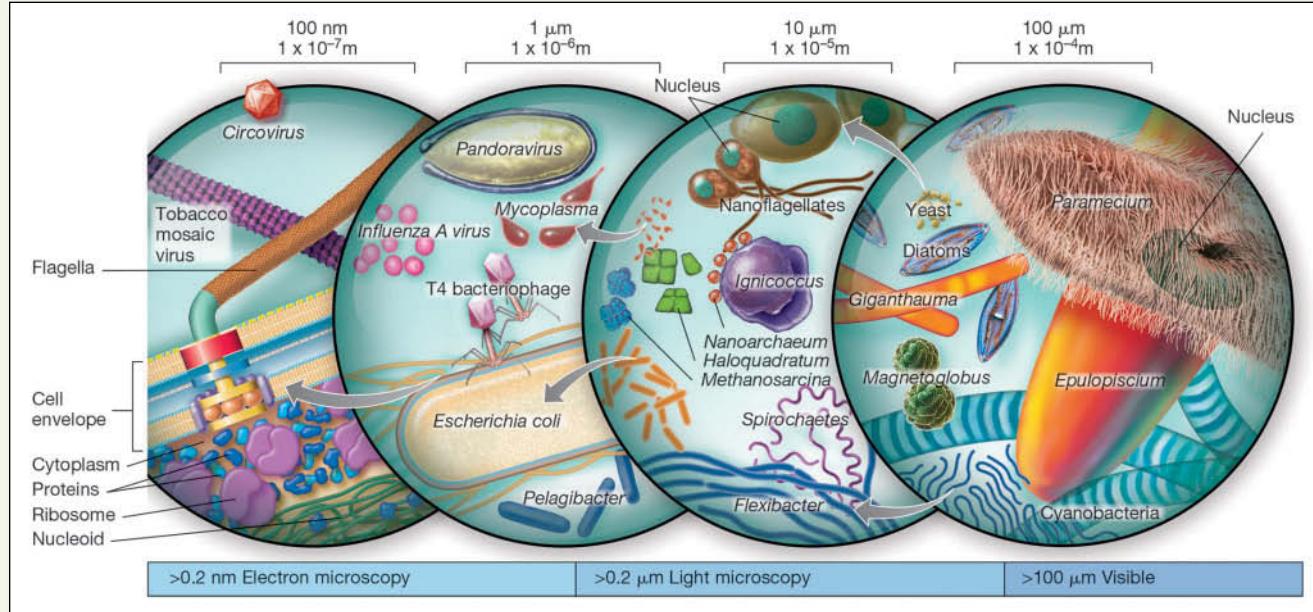


Figure 12.36 CRISPR/Cas9 genome editing. sgRNA represents the synthetic guide RNA, while PAM represents a protospacer adjacent motif. Note that each genome target site must possess a PAM sequence for DNA cleavage to occur. (a) Insertion of foreign DNA into a targeted site of the genome. An sgRNA is synthesized to bind to a single target site on the genome through complementary base pairing of the sgRNA to the DNA target site. Foreign DNA ends homologous to the cleavage site can be incorporated into the cut site through homologous recombination. This results in a genomic insertion. (b) Deletion of a genomic region. Two separate target sites flanking the DNA to be deleted are selected. After the design, addition, and binding of sgRNAs corresponding to these regions, Cas9

utilizes the Cas9 protein to cleave the genome at the target site. Foreign DNA ends homologous to the cleavage site can be incorporated into the cut site through homologous recombination. This results in a genomic deletion. (c) Crystal structure of the *Streptococcus pyogenes* Cas9 protein. The target DNA is shown in green and the sgRNA in orange.

# Authoritative. Accurate. Accessible.

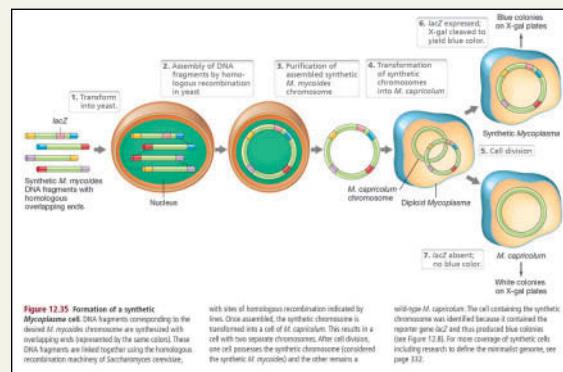
The Fifteenth Edition continues its legacy of authoritative, accessible writing; beautiful and clear art; and student-focused pedagogy, engaging learners in the science.



Student focused pedagogy informs the organization and design of each chapter feature

**TABLE 9.6 Some omics terminology**

<b>DNA</b>	<b>Genome</b> the total complement of genetic information of a cell or a virus <b>Metagenome</b> the total genetic complement of all the cells present in a particular environment <b>Epigenome</b> the total number of possible epigenetic changes <b>Methylome</b> the total number of methylated sites on the DNA (whether epigenetic or not) <b>Mobilome</b> the total number of mobile genetic elements in a cell
<b>RNA</b>	<b>Transcriptome</b> the total RNA produced in an organism under a specific set of conditions
<b>Protein</b>	<b>Proteome</b> the total set of proteins encoded by a genome; sometimes also used in place of <i>translatome</i> <b>Translatome</b> the total set of proteins present under specified conditions <b>Interactome</b> the total set of interactions between proteins (or other macromolecules) <b>Secretome</b> the total set of proteins secreted by a cell
<b>Metabolites</b>	<b>Metabolome</b> the total complement of small molecules and metabolic intermediates <b>Glycome</b> the total complement of sugars and other carbohydrates
<b>Organisms</b>	<b>Microbiome</b> the total complement of microorganisms in an environment (including those associated with a higher organism) <b>Virome</b> the total complement of viruses in an environment <b>Mycobiome</b> the total complement of fungi in a natural environment



**Chapter Review**

**I - Fundamentals of Host Defense**

26.1 Innate immunity is an inherent protective response to infection characterized in part by recognition and elimination of common pathogens, primarily through the activity of phagocytic cells. Innate immunity is the acquired ability of the immune system to eliminate most foreign pathogens from the body via lymphocyte-mediated responses, including the production of antibodies that bind foreign antigens on pathogens or their products.

26.2 Two different types of phagocytes. How do T cells and B cells differ in their functions? From where in the human body do all of these cells originate and which regions of the body do they patrol for signs of infection?

26.3 The human body possesses numerous protective defenses against infectious agents. Natural host resistance to infection includes physical barriers to infection posed by the skin and mucosa, as well as chemical barriers to infection including acidic secretions, defensins, and lysozyme. The specificity of pathogens for particular tissues limits which hosts and tissues might be susceptible to infection.

26.4 How does the human normal microbiota play a role in preventing disease?

**II - Cells and Organs of the Immune System**

26.5 Cells involved in innate and adaptive immunity originate from hematopoietic stem cells in bone marrow. The blood and lymph systems circulate cells and proteins that are important components of the immune response. Diverse leukocytes participate in immune responses in all parts of the body.

26.6 Innate recognition of common pathogens occurs through pathogen-associated molecular patterns (PAMPs). Phagocytes recognize PAMPs through preformed pattern recognition receptors. The resulting signal transduction and intracellular process stimulates phagocytes to destroy the pathogen through a signal transduction mechanism that induces phagocytosis of the infectious agent.

26.7 Identify some PAMPs that are recognized by PRRs. Which cells express PRRs? How do PRRs associate with PAMPs to promote innate immunity?

26.8 Phagocytosis is the engulfing of infectious particles by phagocytes. Engulfed pathogens are bathed in toxic enzymes and acids that break down the pathogen and degrading them. However, some pathogens have developed various defense mechanisms to avoid or inhibit phagocytes, including secretion of leukocidins, the presence of a capsule, and biosynthesis of carotenoid pigments, which combat oxidative stress.

26.9 Explain how phagocytes kill microorganisms, with

# Continuous Learning Before, During, and After Class

MasteringMicrobiology improves results by engaging students before, during, and after class.

## BEFORE CLASS

Reading Questions, art-based activities and MCAT Prep, along with Quantitative Questions, prepare students for in-depth class discussion.

Alphaproteobacteria can be distinguished by which of the following characteristics?

- They are the smallest class of *Proteobacteria*.
- All the organisms are anaerobic.
- All the organisms are gram-negative.
- All the organisms are copiotrophs.

[Submit](#) [Hints](#) [My Answers](#) [Give Up](#) [Review Part](#)

Identify the steps that lead to a mutation, and also correctly identify which type of mutation is indicated after these steps have occurred. Drag the appropriate labels to their respective targets.

The diagram illustrates the process of gene expression and the types of mutations that can occur. It starts with a DNA sequence: 5' ... TAC ... ATG ... 5'. This undergoes transcription to mRNA (5' ... UAC ... ATG ...). From there, it undergoes translation to a protein. Mutations can occur at various stages:

- Normal DNA replication:** leads to a **Wild type** protein (Tyrrosine codon, ATG).
- Misense mutation:** leads to an **Incomplete protein** (Stop codon, UAG).
- Silent mutation:** leads to a **Normal protein** (Tyrrosine codon, UAU).
- Nonsense mutation:** leads to a **Faulty protein** (Asparagine codon, AAC).
- Transcription of light green strand:** leads to a **Wild type** protein (Tyrrosine codon, UAC).

[Submit](#) [My Answers](#) [Give Up](#)

## Microbial Symbioses with Humans

### microbiology now

#### Frozen in Time: The Iceman Microbiome

Humans and their microbial associates—collectively called the *human microbiome*—have coevolved for millennia. As we will see in this chapter, the human microbiome influences a person's health, disease, and predisposition to disease. Among the most interesting of these symbiotic bacteria is *Helicobacter pylori*, known to have developed a close relationship with humans in the distant past and to have coevolved with humans. *H. pylori* colonizes the stomachs of about half the human race. Although this bacterium generally does not cause overt disease, it is a major risk factor for the development of ulcers and stomach cancer. Moreover, because *H. pylori* is transmitted primarily by contact within families, the distribution of genetic variants of this bacterium may yield clues to past human migrations.

Understanding the *H. pylori* genome has been complicated by the ability of different strains of this bacterium to recombine their genetic information. Because the DNA of various strains has mixed over long periods, the reconstruction of population movement inferred from genome sequences of modern *H. pylori* strains is incomplete. One of the biggest unanswered questions was the origin of strains now common among modern Europeans, which appear to be hybrids of strains originating in Asia and Africa. Until recently, the sequence data did not point to a reliable time or place in which this mingling of human populations occurred—an important period of human migration that was estimated to have occurred 10,000–50,000 years ago.

This estimate has now been greatly refined following the remarkable discovery of a well-preserved 5300-year-old European Copper Age mummy frozen in the Italian Alps. Using the newest methods for DNA sequencing, it was possible to reconstruct the genome of *H. pylori* preserved in the stomach of the "Iceman" (see photo). The corpse discovered when melting ice revealed that the *H. pylori* genome sequence turned out to be an almost pure representative of the Asian population, which means this *H. pylori* strain was present in Europe before hybridization of African and Asian strains produced the modern *H. pylori*. Thus, by employing historical biogeography, we now know that period of human migration was much more recent than thought.

F. et al. 2016. The 5300-year-old *Helicobacter pylori* genome of the Iceman. *Nature* 537: 162–165.

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23 Microbial Interactions with Humans | Microbiology Now: The Fungal Microbiome of the Skin

Item Type: Coaching Activities | Difficulty: 3 | Time: 1 hr | Contact the Publisher

Part C - Living microenvironments: pH, temperature, and immune response

In the study described in the introductory passage, researchers were interested in knowing whether different species of organisms were more common in different microenvironments. For the examples below, what word best fits to describe the particular microenvironment mentioned?

Drag and drop the words on the left to complete the sentences on the right.

Lower Higher

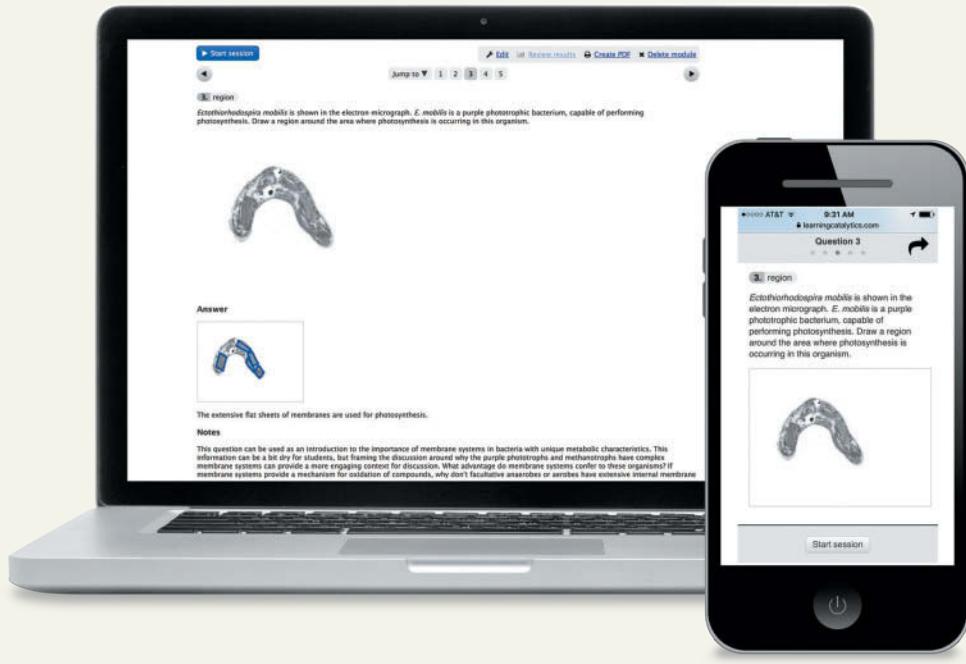
1. The likelihood of colonization by skin pathogens is \_\_\_\_\_ when the normal microbiota are present.
2. In general, \_\_\_\_\_ temperature in specific body regions under normal (healthy) circumstances is associated with lower microbial diversity.
3. The occurrence of colonization by pathogens is \_\_\_\_\_ in mucous membranes than in cutaneous skin.
4. In general, \_\_\_\_\_ immune levels are associated with lower microbial diversity.
5. The occurrence of opportunistic infections is \_\_\_\_\_ in immunocompetent patients.
6. Gastrointestinal bacteria have a \_\_\_\_\_ diversity of microbes than is found on the skin.
7. Bacteria in the oral cavity thrive in \_\_\_\_\_ pH than stomach bacteria.
8. *H. pylori*, bacteria that live in the stomach, thrive in \_\_\_\_\_ pH than skin bacteria.

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Instructors can further encourage students to apply microbiology principles to today's research by assigning MicrobiologyNow Coaching Activities.

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## DURING CLASS



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A wide variety of interactive coaching activities as well as high-level assessments can be assigned after class to continue student learning and concept mastery.

MicroFlix Activity: Immunology -- Cell-Mediated Immunity

Can you put the steps of cell-mediated immunity in order and label the cells and molecules involved? To review cell-mediated immunity, watch this MicroFlix animation: [T Cells and Cellular Immunity](#).

Part A - Correctly sort the steps involved in cell-mediated immunity

Put the steps involved in cell-mediated immunity in order.

In the lymph nodes, cytotoxic T cells encounter dendritic cells displaying epitope on MHC-I. The Tc cell is activated.

The active cytotoxic T cell (CTL) leaves the lymph node "looking" for infected host cells displaying the same epitope on their MHC-I. The CTL uses its surface receptors to recognize the infected cell.

The CTL secretes specialized molecules to penetrate the infected host cell causing programmed cell death.

1 ? → 2 ? → 3 ?

Submit Hints My Answers Give Up Review Part reset help

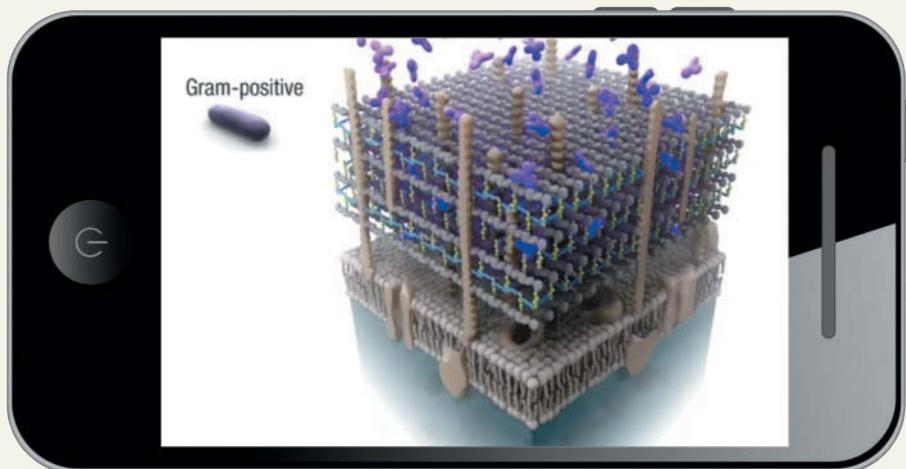
# Visualize Microbiology

## AFTER CLASS

### NEW! Interactive

**Microbiology** is a dynamic suite of interactive tutorials and animations that teach key concepts in microbiology, including Operons; Biofilms and Quorum Sensing; Aerobic Respiration in Bacteria; Complement; and more. Students actively engage with each topic via a case study and learn by manipulating variables, predicting outcomes, and answering formative and summative assessment questions.

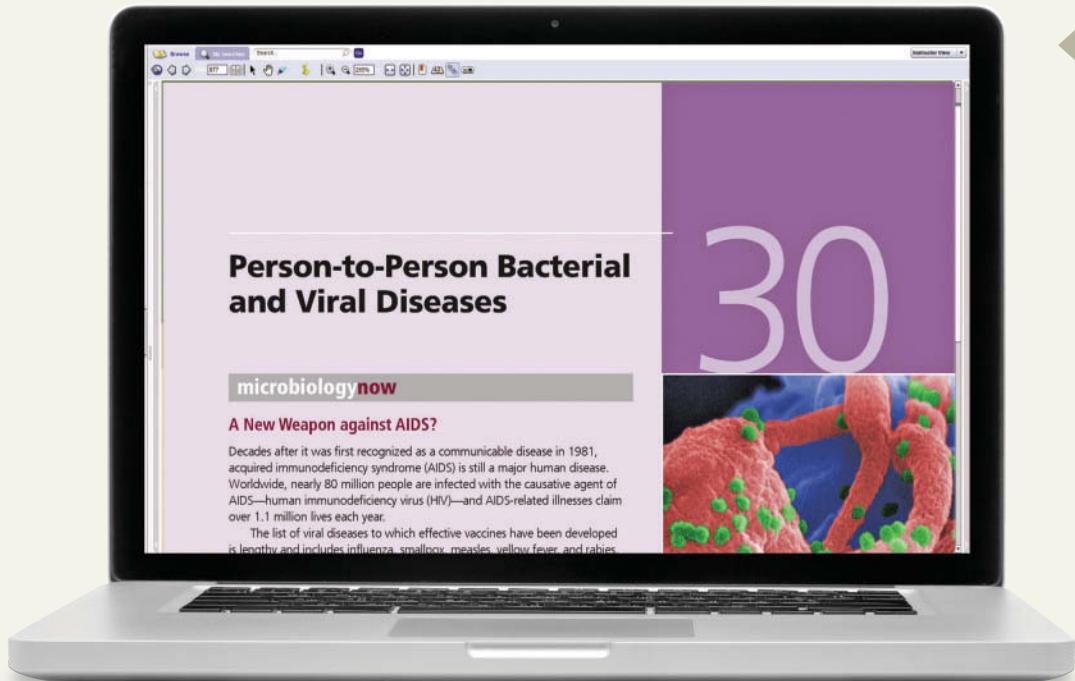
The image shows a screenshot of the Interactive Microbiology software interface. At the top, there's a navigation bar with icons for search, refresh, and user profile, followed by the title "Interactive Microbiology" and the subtitle "Biofilms and Quorum Sensing". On the right side of the title are links for "GLOSSARY" and "CREDITS". Below the title is a vertical list of "Learning goals" numbered 1 through 8. The first few items include "Case Study Introduction", "Biofilm Formation", and "Activity: Biofilm Formation". To the right of the list is a photograph of a young child lying in a hospital bed, connected to medical equipment. Below the photo is a 3D animation of red, rod-shaped bacteria swimming in a green environment, with small blue dots representing quorum sensing molecules. At the bottom of the interface is a video player window showing a cross-section of a biofilm structure with various colored bacteria and organic matter, with a play button and a progress bar.



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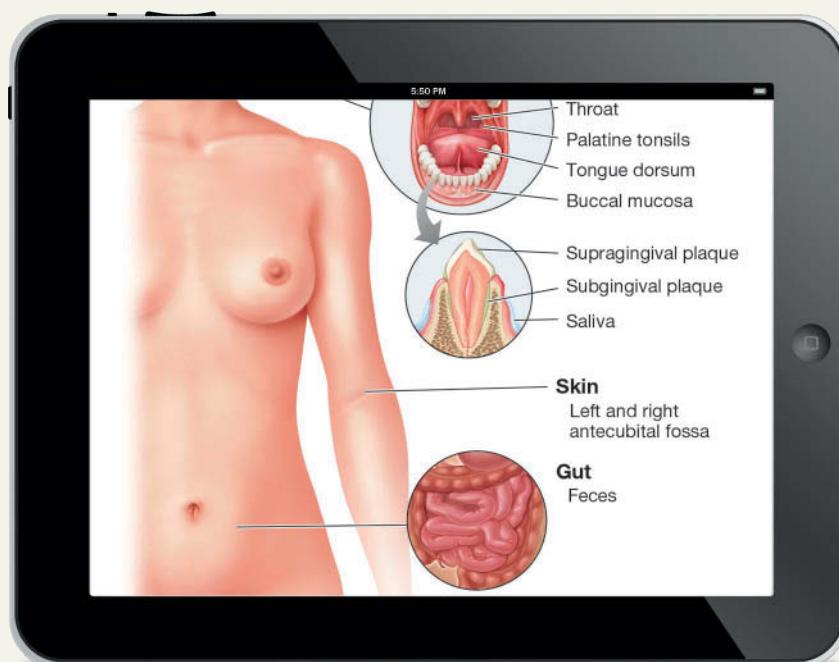
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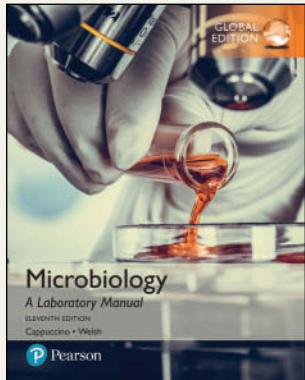


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# BROCK BIOLOGY OF MICROORGANISMS

FIFTEENTH EDITION  
GLOBAL EDITION

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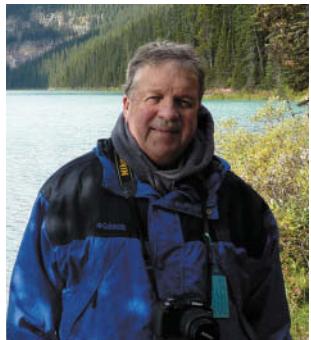
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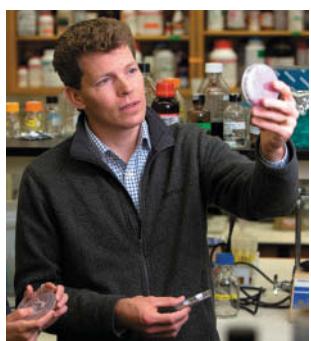
# About the Authors



**Michael T. Madigan** received his B.S. in Biology and Education from Wisconsin State University-Stevens Point (1971) and his M.S. (1974) and Ph.D. (1976) in Bacteriology from the University of Wisconsin-Madison in the laboratory of Thomas Brock. Following a postdoc at Indiana University with Howard Gest, Mike moved to Southern Illinois University Carbondale, where he taught courses in introductory microbiology and bacterial diversity as a professor of microbiology for 33 years. In 1988 Mike was selected as the Outstanding Teacher in the College of Science and in 1993, the Outstanding Researcher. In 2001 he received the SIUC Outstanding Scholar Award. In 2003 he received the Carski Award for Distinguished Undergraduate Teaching from the American Society for Microbiology, and he is an elected Fellow of the American Academy of Microbiology. Mike's research is focused on bacteria that inhabit extreme environments, and for the past 20 years his emphasis has been Antarctic microbiology. Mike has co-edited a major treatise on phototrophic bacteria and served for 10 years as chief editor of the journal *Archives of Microbiology*. He currently serves on the editorial board of the journals *Environmental Microbiology* and *Antonie van Leeuwenhoek*. Mike's other interests include forestry, swimming, reading, and caring for his dogs and horses. He lives on a quiet lake with his wife, Nancy, three dogs (Kato, Nut, and Merry), and three horses (Eddie, Gwen, and Georgie).



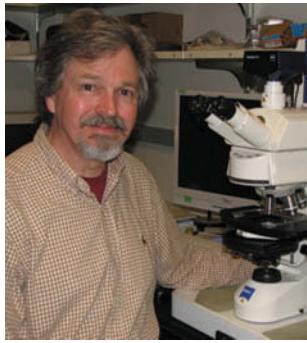
**Kelly S. Bender** received her B.S. in Biology from Southeast Missouri State University (1999) and her Ph.D. (2003) in Molecular Biology, Microbiology, and Biochemistry from Southern Illinois University Carbondale. Her dissertation research focused on the genetics of perchlorate-reducing bacteria. During her postdoctoral fellowship, Kelly worked on the genetic regulation of sulfate-reducing bacteria in the laboratory of Judy Wall at the University of Missouri-Columbia. She also completed a transatlantic biotechnology fellowship at Uppsala University in Sweden researching regulatory small RNAs in bacteria. In 2006, Kelly returned to her alma mater, Southern Illinois University Carbondale, as an Assistant Professor in the Department of Microbiology and in 2012 was tenured and promoted to Associate Professor. Her lab studies a range of topics including regulation in sulfate-reducing bacteria and the microbial community dynamics of sites impacted by acid mine drainage. Kelly teaches courses in introductory microbiology and microbial diversity, has served on numerous federal grant review panels, and is an active member of the American Society for Microbiology (ASM). Her other interests include spending time with her daughter, Violet, and husband, Dick.



**Daniel H. Buckley** is a Professor at Cornell University in the School of Integrative Plant Science. He earned his B.S. in Microbiology (1994) at the University of Rochester and his Ph.D. in Microbiology (2000) at Michigan State University. His graduate research focused on the ecology of soil microbial communities and was conducted in the laboratory of Thomas M. Schmidt in affiliation with the Center for Microbial Ecology. Dan's postdoctoral research examined linkages between microbial diversity and biogeochemistry in marine microbial mats and stromatolites and was conducted in the laboratory of Pieter T. Visscher at the University of Connecticut. Dan joined the Cornell faculty in 2003. His research program investigates the ecology and evolution of microbial communities in soils with a focus on the causes and consequences of microbial diversity. He has taught both introductory and advanced courses in microbiology, microbial diversity, and microbial genomics. He received a National Science Foundation Faculty Early Career Development (CAREER) award in 2005 for excellence in integrating research and education. He has served as Director of the Graduate Field of Soil and Crop Sciences at Cornell and Co-Director of the Microbial Diversity summer course of the Marine Biological Laboratory in Woods Hole, Massachusetts. He currently serves on the editorial boards of *Applied and Environmental Microbiology* and *Environmental Microbiology*. Dan lives in Ithaca, New York, with his wife, Merry, and sons, Finn and Colin.



**W. Matthew Sattley** received his B.A. in Biology in 1998 from Blackburn College (Illinois) and his Ph.D. (2006) in Molecular Biology, Microbiology, and Biochemistry from Southern Illinois University Carbondale. His graduate studies focused on the microbiology of sulfur cycling and other biogeochemical processes in permanently ice-covered lakes of Antarctica. In his postdoctoral research at Washington University in Saint Louis, he studied the physiology and genomics of anoxygenic phototrophic bacteria in Robert Blankenship's laboratory. Matt then accepted a faculty appointment to the Department of Biology at MidAmerica Nazarene University (Kansas), where he supervised undergraduate research and taught courses in microbiology, environmental science, and cell biology. In 2010, Matt transitioned to the Division of Natural Sciences at Indiana Wesleyan University, where he is a Professor of Biology and Director of the Hodson Summer Research Institute, a faculty-led summer research program for undergraduate students in the Natural Sciences. His research group investigates the ecology, diversity, and genomics of bacteria that inhabit extreme environments. Matt is a member of the American Society for Microbiology (including its Indiana Branch) and the Indiana Academy of Science, and he currently serves as an expert reviewer for the undergraduate microbiology research journal *Fine Focus*. Matt lives in Marion, Indiana, with his wife, Ann, and sons, Josiah and Samuel. Outside of teaching and research, Matt enjoys playing drums, reading, motorcycling, and talking baseball and cars with his boys.



**David A. Stahl** received his B.S. degree in Microbiology from the University of Washington, Seattle, and completed graduate studies in microbial phylogeny and evolution with Carl Woese in the Department of Microbiology at the University of Illinois at Urbana-Champaign. Subsequent work as a postdoctoral fellow with Norman Pace, then at the National Jewish Hospital in Colorado, involved early applications of 16S rRNA-based sequence analysis to the study of natural microbial communities. In 1984 Dave joined the faculty at the University of Illinois with appointments in Veterinary Medicine, Microbiology, and Civil Engineering. In 1994 he moved to the Department of Civil Engineering at Northwestern University, and in 2000 returned to the University of Washington as professor in the Departments of Civil and Environmental Engineering and Microbiology. Dave is known for his work in microbial evolution, ecology, and systematics, and received the 1999 Bergey Award and the 2006 ASM Procter & Gamble Award in Applied and Environmental Microbiology. Dave is an elected fellow of the American Academy of Microbiology and a member of the National Academy of Engineering. His main research interests surround the biogeochemistry of nitrogen and sulfur and the microbial communities that sustain the associated nutrient cycles. His laboratory was first to culture ammonia-oxidizing *Archaea*, a group believed to be the key mediators of this process in the nitrogen cycle. Dave has taught several courses in environmental microbiology, was one of the founding editors of the journal *Environmental Microbiology*, and has served on many advisory committees. Outside the lab, Dave enjoys hiking, bicycling, spending time with family, reading a good science fiction book, and—with his wife, Lin—renovating an old farmhouse on Bainbridge Island.

# Dedications

## **Michael T. Madigan**

dedicates this edition to students who have drawn inspiration from his textbook to make some aspect of microbiology their life's work.

## **Kelly S. Bender**

dedicates this book to the memory of her grandmother, Alberta, whose biggest regret in life was not being able to attend school past the fifth grade.

## **Daniel H. Buckley**

dedicates this book to the memory of his mother, Judy, who taught me to see joy and wonder, even in the smallest of things.

## **W. Matthew Sattley**

dedicates this book to his amazing wife, Ann, for her endless support and understanding.

## **David A. Stahl**

dedicates this book to his wife, Lin. My love, and one that helps me keep the important things in perspective.

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# Preface

Welcome to an exciting new edition of *Brock Biology of Microorganisms* (BBOM). This Fifteenth Edition is the strongest yet and presents microbiology in the context of the excitement this science generates today. For three generations, students and instructors have relied on the accuracy, authority, consistency, and up-to-date presentation of BBOM to learn or teach the principles of modern microbiology. Both students and instructors will benefit from the Fifteenth Edition in at least four major ways: (1) from the use of cutting-edge research to illustrate basic concepts; (2) from the seamless integration of molecular and ecological microbiology with evolution, diversity, the immune system, and infectious diseases; (3) from the visually stunning art program and spectacular photos; and (4) from the wide assortment of teaching and learning tools that accompany the book itself.

Veteran authors Madigan, Bender, Buckley, and Stahl welcome new coauthor Matt Sattley to the Fifteenth Edition. Matt, a professor at Indiana Wesleyan University, teaches both general microbiology and health professions microbiology and did a great job of reorganizing and refreshing our coverage of immunology and related areas. With an extremely strong author team that employs experts in each of our major areas of emphasis, we sincerely feel that BBOM 15e is the best learning resource available in microbiology today.

## What's New in the 15th Edition?

The Fifteenth Edition guides students through the six major themes of microbiology as outlined by the American Society for Microbiology Conference on Undergraduate Education (ASMCUE): Evolution, Cell Structure and Function, Metabolic Pathways, Information Flow and Genetics, Microbial Systems, and the Impact of Microorganisms. With enhanced and revised artwork complemented with over 90 new color photos, BBOM 15e presents microbiology as the visual science it is. Thirty-three new MicrobiologyNow chapter-opening vignettes were composed for this edition, each designed to introduce a chapter's theme through a recent discovery published in the microbiology literature. Several new Explore the Microbial World features were also developed for this edition, each designed to give students a feel for exciting special topics in microbiology and to fuel their scientific curiosity.

Genomics, and all of the various “omics” it has spawned, support content in every chapter of BBOM 15e, reflecting how the omics revolution has transformed all of biology, especially microbiology. Mastering the principles of the dynamic field of microbiology today requires an understanding of the supportive molecular biology. Hence, we have constructed BBOM 15e in a way that provides both the foundation for the science and the science itself. The result is a robust and modern treatment of microbiology that now includes exciting new chapters devoted to microbial systems

biology, synthetic biology, the human microbiome, and the molecular biology of microbial growth.

To strengthen the learning experience, each section summary in the chapter review is followed immediately by a review question to better link concept review with concept mastery. BBOM 15e is supported by MasteringMicrobiology™, Pearson's online homework, tutorial, and assessment system that assists students in pacing their learning and keeps instructors current on class performance. MasteringMicrobiology includes chapter-specific reading quizzes, MicrobiologyNow, Clinical Case and MicroCareer coaching activities, animation quizzes, MCAT Prep questions, and many additional study and assessment tools, including tutorials and assessments for the microbiology lab. Collectively, the content and presentation of BBOM 15e, coupled with the powerful learning tools of MasteringMicrobiology, create an unparalleled educational experience in microbiology.

## Revision Highlights

### Chapter 1

- The book begins with a revised and reorganized kickoff chapter that weaves introductory concepts in microbiology within an historical narrative. Foundational aspects of microbiology are now presented in the context of the major discoveries that have expanded our knowledge of the microbial world.
- Some highlights: introducing the principles of microscopy in a historical context; a new section on molecular biology and the importance of microbes in understanding the unity of life; the contributions of Carl Woese and the use of rRNA sequences to develop the universal tree of life; an introduction to the viral world; spectacular new summary art that explores the diversity of microbial life across a wide range of spatial scales.

### Chapter 2

- Microbial cell structure and function are key pillars of microbiology, and this newly reworked and streamlined chapter offers a thorough introduction to comparative cell structure and provides the instructor with all of the tools necessary for effective classroom presentations. Coverage of nutrient transport systems has been moved to Chapter 3 to better present this topic in its proper context.
- Some highlights: a new Explore the Microbial World entitled “Tiny Cells”; unique attachment structures of *Archaea*; new coverage of archaella.

### Chapter 3

- The essential features of microbial metabolism necessary for understanding how microbes transform energy are laid out in a

logical sequence and at just the right level for introductory students. With the material on membrane transport now located here, the uptake of nutrients is highlighted as the initial step of any metabolic process.

- Some highlights: new coverage of the macromolecular composition of a cell; a more complete picture of energy transformation and the importance of free energy change; coverage of the citric acid cycle prior to (rather than following) discussion of the proton motive force.

## Chapter 4

- Chapter 4 has been reorganized to provide the streamlined view of molecular biology necessary for both supporting and understanding virtually all aspects of microbiology today.
- Some highlights: new coverage of coupled transcription and translation in *Bacteria* and *Archaea*; new material on the assembly of cofactor-containing enzymes; stronger coverage of types I–VI secretion systems in gram-negative bacteria; updated art throughout.

## Chapter 5

- Unit 2 is all about growth and begins with the Chapter 5 presentation of the essential principles of microbial growth and cultivation. Coverage of microbial growth control balances this chapter with a practical view of how microbial growth can be suppressed for both health and aesthetic reasons.
- Some highlights: new material on budding cell division and on biofilms; reworked chemostat coverage better explains continuous culture and its connection to basic growth principles; new coverage on how the environment affects growth previews the extensive coverage of microbial ecology and environmental microbiology later in the book.

## Chapter 6

- This chapter on microbial regulation includes broad coverage of the classic forms of regulation but has been streamlined by moving the regulation of cell differentiation and biofilm formation to Chapter 7; this allowed for enhanced coverage of hot new areas in metabolic regulation such as regulation by anti-sigma factors and transcriptional regulation in *Archaea*.
- Some highlights: new coverage of the global phosphate regulon; new coverage of dual-acting transcriptional regulators in *Archaea* and how the stringent response affects the ecology of bacteria as diverse as *Escherichia coli*, *Caulobacter crescentus*, and *Mycobacterium tuberculosis*; updated art throughout.

## Chapter 7

- A new chapter focused on the molecular biology of microbial growth showcases the orchestrated events leading to cell division and surveys the molecular processes targeted by antibiotics. Coverage of peptidoglycan synthesis, developmental stages in various *Bacteria*, and biofilm formation—previously scattered through the book—has been consolidated here to unite their common underlying themes.

- Some highlights: An introduction to the powerful tool of super-resolution microscopy includes several spectacular examples of how this breakthrough in resolution has remolded our view of molecular events in microbial growth; expanded coverage of biofilm formation; new coverage of bacterial persistence, a growing problem in medical microbiology; updated art throughout.

## Chapter 8

- The introductory virology chapter is now included in the microbial growth unit and provides an introduction to the structure, replication, and lifestyles of viruses without overshadowing these important principles with the extensive diversity of the viral world, now covered in Chapter 10.
- Some highlights: discussion of the parallels between bacterial growth and viral replication; expanded coverage of how host cell growth is impacted by viral infection; high-resolution viral images; updated art throughout.

## Chapter 9

- This revolutionary chapter on microbial systems biology kicks off our unit on genomics and genetics by underscoring the importance of microbial genome sequences and the field of functional “omics” to modern microbiology today. The chapter also includes examples of how systems biology can be used to model an organism’s response to its environment.
- Some highlights: how functional and metabolic predictions are gleaned from genomic analyses; expanded coverage of RNA-Seq and metabolomic analyses; coverage of all of the common “omics” and how they relate to one another; new coverage of the systems biology of the important pathogen *Mycobacterium tuberculosis* and other systems biology studies related to human health; metagenomics and metabolomics of human skin; updated and spectacular new art and photos throughout.

## Chapter 10

- Chapter 10, entitled “Viral Genomics, Diversity, and Ecology,” now includes coverage of viral ecology and diversity that was previously in Chapter 8. The many diverse genomes and replication schemes of viruses form the foundation for coverage of the diversity and ecological activities of viruses.
- Some highlights: the viral “immune system” of *Bacteria* and *Archaea*—CRISPR; large viruses and viral evolution; the human virome; beneficial prions; viral host preferences; updated and new art throughout.

## Chapter 11

- Chapter 11, “Genetics of *Bacteria* and *Archaea*,” has been streamlined to focus on the essential concepts of mutation and gene transfer in prokaryotic cells. New high-resolution images have been included to illustrate gene transfer processes.
- Some highlights: new coverage on the utility of transposon mutagenesis; a spectacular photo series illustrating the concept of competence; new coverage on defective bacteriophages as “gene transfer agents”; updated art throughout.

## Chapter 12

- This highly reorganized chapter entitled “Biotechnology and Synthetic Biology” covers the essential tools of biotechnology and discusses commercial products produced by genetically engineered microbes. New coverage presents the remarkable advances in synthetic biology and CRISPR genome editing.
- Some highlights: engineering microbes to produce biofuels; expanded coverage of synthetic pathways and synthetic cells; new coverage of the biocontainment of genetically modified organisms; updated art throughout.

## Chapter 13

- Chapter 13 sets the stage for our unit on evolution and diversity by revealing how nucleic acid sequences have revealed the true diversity of the microbial world. The chapter has also been revised and reorganized to increase the emphasis on the origin and diversification of life and microbial systematics.
- Some highlights: revised text places phylogeny into firm context with microbial systematics; how the tree of life and molecular sequences form the foundation of our understanding of the origin and diversification of the three domains; revised coverage of phylogenetic tree construction and what such trees can tell us about microbial evolution.

## Chapter 14

- Our discussion of microbial metabolism has been revised and reorganized to highlight the modularity of microbial metabolism and to include coverage of newly discovered microbial metabolisms.
- Some highlights: a new section on assimilatory processes of autotrophy and nitrogen fixation; grouping respiratory processes by electron donor, electron acceptor, or one-carbon metabolisms; new art depicting electron flow in oxygenic photosynthesis, sulfur chemolithotrophy, and acetogenesis; discussion of the role of flavin-based electron bifurcation in energy conservation; coverage of the exciting discoveries of intra-aerobic methanotrophy and interspecies electron transfer in anaerobic methane oxidation.

## Chapters 15 and 16

- These chapters, covering functional and phylogenetic diversity of *Bacteria*, respectively, have been updated and streamlined in spots to provide the highly organized view of bacterial diversity that offers instructors the freedom to present this subject in the way that best suits their course needs.
- Some highlights: functional diversity organized by metabolism, unique morphologies, and other special properties shows how functional diversity is often unlinked to phylogenetic diversity; phylogenetic diversity organized around the major phyla of *Bacteria* shows how phylogenetic diversity is often unlinked to metabolic properties.

## Chapter 17

- Chapter 17, entitled “Diversity of *Archaea*,” has been updated to include new coverage of recent discoveries in archaeal diversity

including the fact that *Archaea* are widespread in nature and not just restricted to extreme environments.

- Some highlights: updated coverage of methanogenic *Archaea* to include the extensive diversity characteristic of this group; new coverage of the evolutionary origins and distribution of methanogens within the archaeal domain; the latest story on *Archaea* and the upper temperature limit for life.

## Chapter 18

- Coverage of the microbial eukaryotes has been revised to include significant new advances in our understanding of the phylogeny of *Eukarya*.
- Some highlights: a new phylogenetic tree of *Eukarya*; updated terminology throughout; the “SAR” lineages; the new understanding of fungal diversity that incorporates the *Microsporidia* as a deeply divergent fungal group.

## Chapter 19

- This chapter begins a new unit on ecology and environmental microbiology. The modern tools of the microbial ecologist are described with examples of how each has helped sculpt the science.
- Some highlights: complete coverage of the omics revolution and how it is being exploited to solve complex problems in microbial ecology; Raman microspectroscopy and its use for nondestructive molecular and isotopic analyses of single cells; high-throughput cultivation methods and how they can be used to bring novel microbes into laboratory culture.

## Chapter 20

- The properties and microbial diversity of major microbial ecosystems including soils and both freshwater and marine systems are compared and contrasted in an exciting new way.
- Some highlights: new environmental census data for deep marine sediments reveal the novel *Archaea* and *Bacteria* living thousands of meters below the seafloor; expanded coverage of the links between terrestrial and marine microorganisms and climate change.

## Chapter 21

- Extensive coverage of the major nutrient cycles in nature and the microbes that catalyze them presented in a fashion that allows the cycles to be taught as individual entities or as interrelated metabolic loops.
- Some highlights: new coverage of how humans are affecting the nitrogen and carbon cycles; microbial respiration of solid metal oxides in the iron and manganese cycles including the concept of “microbial wires” that can carry electrons over great distances; how microbes contribute to mercury contamination of aquatic life.

## Chapter 22

- A newly revised chapter on the “built environment” shows how humans create new microbial habitats through construction of buildings, supporting infrastructure, and habitat modification.

- Some highlights: coverage of the effects microbes have on wastewater treatment, mining and acid mine drainage, the corrosion of metals, and the degradation of stone and concrete; the pathogens of most concern in drinking water and how we eliminate them; the major microbes that inhabit our household and work environments.

## Chapter 23

- A chapter devoted to nonhuman microbial symbioses describes the major microbial partners that live in symbiotic or other types of close associations with plants and animals.
- Some highlights: using our knowledge of plant and animal symbioses to develop microbially centered insect pest controls; revealing the common symbiotic mechanism used by certain bacteria and fungi to provide plants with key nutrients.

## Chapter 24

- A new chapter devoted exclusively to the human microbiome kicks off our unit on microbe–human interactions and the immune system by introducing the dramatic advances in our understanding of the microbes that inhabit the human body and their relationship to health and disease.
- Some highlights: extensive coverage of “who lives where (and why)” in and on the human body; how the new understanding of our intimate microbial partners was used to develop novel microbial-based disease therapies; mapping the biogeography of our skin microbiota using new molecular techniques; how gut microbes likely influence both our health and behavior; a new Explore the Microbial World entitled “The Gut–Brain Axis.”

## Chapter 25

- This heavily reworked and more visually appealing chapter is devoted exclusively to microbial infection and pathogenesis. Major topics in the first part include microbial adherence, colonization, invasion and pathogenicity, and virulence and attenuation. The second part is focused on the destructive enzymes and toxins produced by pathogenic bacteria. Microbial and host factors are compared as to how each can tip the balance toward health or disease.
- Some highlights: eight new color photos bring host–microbe relationships into better focus; new coverage of dental caries is supported by a spectacular fluorescent micrograph that reveals the previously hidden diversity of this disease; increased coverage of microbial infection and the compromised host.

## Chapter 26

- Coverage of the immune response has been completely reorganized to provide a fresh take on immune mechanisms. Concepts of innate and adaptive immunity are now organized into separate chapters (26 and 27, respectively) that provide a more teachable format and enhance the student experience. The new organization provides a natural progression to the updated topics in clinical microbiology and immunology presented in Chapter 28.

- Some highlights: extensively revised and reorganized text and vibrant new artwork clearly illustrate the roles of inflammation, fever, and interferons in the innate immune response; stronger, clearer coverage of the complement system, including extensive new artwork, helps clarify its important role in innate immunity.

## Chapter 27

- Fundamental concepts of the adaptive immune response are now reorganized into a dedicated chapter and presented in a thoroughly revised and more streamlined format.
- Some highlights: beautifully enhanced art and new photos more clearly orient students to key concepts including clonal selection and deletion of B cells and T cells, antibody structure, and antigen binding and presentation.

## Chapter 28

- Clear and concise new text now includes automated culture systems, antibody precipitation, and monoclonal antibody production, as well as a reorganized treatment of antimicrobial drugs. Both reimagined and totally new art supported by 20 new color photos brightly illustrate complex topics and enhance the visual experience.
- Some highlights: how a clinical microbiology laboratory actually functions; an exciting new Explore the Microbial World feature on MRSA describes how emerging resistance to antibiotics in *Staphylococcus aureus* has led to high global incidence of what is now a virtually untreatable bacterial pathogen.

## Chapter 29

- A significantly reworked and streamlined discussion of epidemiology kicks off our unit on infectious diseases with a visual presentation of the everyday language of epidemiology and then closely integrates this terminology throughout the chapter. Fewer lengthy tables are presented and visual appeal is greater, while the essential concepts of disease spread and control remain the major themes of the chapter.
- Some highlights: updated and new coverage of emerging infectious diseases and current pandemics, including HIV/AIDS, cholera, and influenza; the key role of the epidemiologist in tracking disease outbreaks and maintaining public health.

## Chapter 30

- This is the first of four chapters on microbial diseases grouped by their modes of transmission; this approach emphasizes the common ecology of these diseases despite differences in etiology. Classical as well as emerging and reemerging bacterial and viral diseases transmitted person to person are the focus of this highly visual chapter.
- Some highlights: several new photos add to the already extensive visual showcase of infectious diseases; new coverage of Ebola describes why this pathogen is so dangerous and the extraordinary precautions healthcare workers must take to prevent infection; new coverage of hepatitis, a widespread disease with serious implications.

## Chapter 31

- Vectorborne microbial diseases are becoming more and more common worldwide and are covered in detail in this visually appealing chapter. From diseases with high mortality, such as rabies and hantavirus syndromes, to those with high incidence and low mortality but significant side effects, such as Lyme and West Nile diseases, all of the major vectorborne infectious diseases found today are consolidated in one place.
- Some highlights: new coverage of Zika and Chikungunya diseases and their relationship to dengue and yellow fevers; updated coverage of Lyme, West Nile, and *Coxiella* (Q fever) infections supported by new color photos.

## Chapter 32

- Food- and waterborne illnesses are still common, even in developed countries. This chapter consolidates these topics to emphasize their “common source” modes of transmission while differentiating the major pathogens seen in each vehicle.

- Some highlights: a clearer distinction between food infections and food poisonings; new coverage of the potentially fatal food-borne infection caused by the intracellular pathogenic bacterium *Listeria*.

## Chapter 33

- Major infectious diseases caused by eukaryotic microbes—fungi, parasites, and pathogenic helminths—are organized into one highly visual chapter. With climate change affecting infectious disease ecology, many of these diseases previously found only in tropical or subtropical countries are now creeping northward.
- Some highlights: new emphasis on the different modes of transmission (food, water, vector) of major eukaryotic pathogens; new coverage of river blindness and trichinosis as common filariases.

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# ASM Recommended Curriculum Guidelines for Undergraduate Microbiology

The American Society for Microbiology (ASM) endorses a concept-based curriculum for undergraduate microbiology, emphasizing skills and concepts that have lasting importance beyond the classroom and laboratory. The ASM (in its *Curriculum Guidelines for Understanding Microbiology Education*) recommends deep understanding of 27 key concepts, 4 scientific thinking competencies, and 7 key skills. These guidelines follow scientific literacy reports and recommendations from the American Association for the Advancement of Science and the Howard Hughes Medical Institute by encouraging an active learning, student-based course. Consider these guiding statements as you progress through this book and master principles, problem solving, and laboratory skills in microbiology.

## ASM Guideline Concepts and Statements

### Evolution: Chapters 1, 9–14, 21, 28, 29

- Cells, organelles (e.g., mitochondria and chloroplasts), and all major metabolic pathways evolved from early prokaryotic cells.
- Mutations and horizontal gene transfer, with the immense variety of microenvironments, have selected for a huge diversity of microorganisms.
- Human impact on the environment influences the evolution of microorganisms (e.g., emerging diseases and the selection of antibiotic resistance).
- Traditional concept of species is not readily applicable to microbes due to asexual reproduction and the frequent occurrence of horizontal gene transfer.
- Evolutionary relatedness of organisms is best reflected in phylogenetic trees.

### Cell Structure and Function: Chapters 1, 2, 8, 10, 14

- Structure and function of microorganisms have been revealed by the use of microscopy (including bright-field, phase contrast, fluorescence, and electron).
- Bacteria have unique cell structures that can be targets for antibiotics, immunity, and phage infection.
- Bacteria* and *Archaea* have specialized structures (e.g., flagella, endospores, and pili) that often confer critical capabilities.
- While microscopic eukaryotes (for example, fungi, protozoa, and algae) carry out some of the same processes as bacteria, many of the cellular properties are fundamentally different.
- Replication cycles of viruses (lytic and lysogenic) differ among viruses and are determined by their unique structures.

### Metabolic Pathways: Chapters 1, 3, 5, 7, 12, 14

- Bacteria* and *Archaea* exhibit extensive, and often unique, metabolic diversity (e.g., nitrogen fixation, methane production, anoxygenic photosynthesis).
- Interactions of microorganisms among themselves and with their environment are determined by their metabolic abilities (e.g., quorum sensing, oxygen consumption, nitrogen transformations).
- Survival and growth of any microorganism in a given environment depends on its metabolic characteristics.
- Growth of microorganisms can be controlled by physical, chemical, mechanical, or biological means.

### Information Flow and Genetics: Chapters 1, 4, 6–9, 11

- Genetic variations can impact microbial functions (e.g., in biofilm formation, pathogenicity, and drug resistance).
- Although the central dogma is universal in all cells, the processes of replication, transcription, and translation differ in *Bacteria*, *Archaea*, and eukaryotes.
- Regulation of gene expression is influenced by external and internal molecular cues and/or signals.
- Synthesis of viral genetic material and proteins is dependent on host cells.
- Cell genomes can be manipulated to alter cell function.

### Microbial Systems: Chapters 1, 9, 14–18, 23–33

- Microorganisms are ubiquitous and live in diverse and dynamic ecosystems.
- Many bacteria in nature live in biofilm communities.
- Microorganisms and their environment interact with and modify each other.
- Microorganisms, cellular and viral, can interact with both human and nonhuman hosts in beneficial, neutral, or detrimental ways.

### Impact of Microorganisms: Chapters 1, 5, 7, 12, 19–22

- Microbes are essential for life as we know it and the processes that support life (e.g., in biogeochemical cycles and plant and/or animal microbiota).
- Microorganisms provide essential models that give us fundamental knowledge about life processes.
- Humans utilize and harness microorganisms and their products.
- Because the true diversity of microbial life is largely unknown, its effects and potential benefits have not been fully explored.

# The Microbial World

# 1

## microbiologynow

### Microorganisms, Our Constant Companions

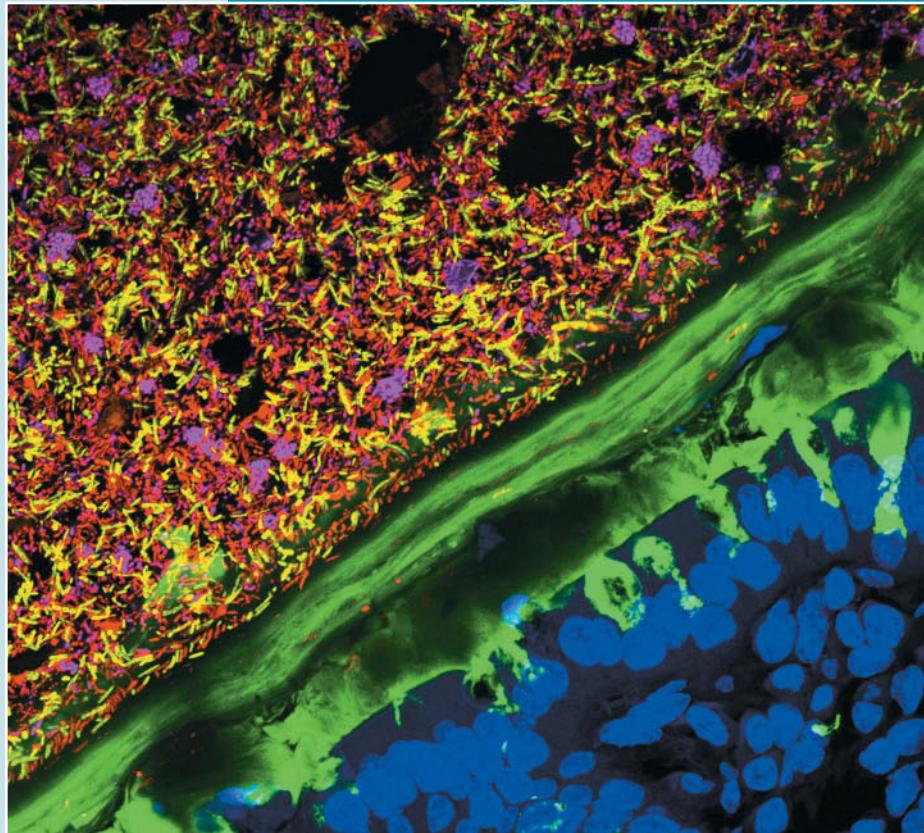
Microorganisms are everywhere, and though small, their activities have tremendous impacts on everything in our biosphere. As you learn more about microbiology you will realize that many of our day-to-day interactions with the world are influenced, for better or worse, by microbial life. Indeed, hundreds of trillions of bacteria are working within your body now to digest your last meal. The total complement of microbial cells in and on your body—your microbiome—contains thousands of species each adapted to grow best in a particular part of your body. For example, your gut microbiome encodes enzymes that help digest your food and synthesize vitamins critical to your health. The composition of your microbiome changes in response to your diet, your genes, your health, and the medicines you take. Our microbiome is absolutely essential to our health and well-being, yet we are only beginning to understand the diverse ways in which we depend upon our gut microorganisms.

Our knowledge of the microbial world is highly dependent on technological developments. Recent advances in microscopic techniques have made it possible to visualize microbes in intimate association with the lining of the gut (see photo). This image, generated by laser scanning confocal microscopy, reveals a cross section from the colon of a mouse and shows the dense and complex microbial community residing within the gut. Mice, which can be raised in a germ-free condition and then inoculated with a human gut microbiome, are used as a model system to explore microbiome function. Fluorescent stains identify the mucus layer (green) and host cell nuclei (blue) of the gut epithelium. *Bacteria* of the phylum *Firmicutes* stain yellow and those of the family *Bacteroidaceae* stain pink; all other bacteria stain red. Changes in diet alter the thickness of the mucus layer and the potential for microbial interactions with the epithelium. Such interactions can change gut function and possibly lead to inflammation.

In the chapters that follow, the exciting science of microbiology will unfold, and you will see that there is still much to learn about the inner workings of the microbial world.



**Source:** Earle, K.A., et al. 2015. Quantitative imaging of gut microbiota spatial organization. *Cell Host & Microbe* 18: 478–488.

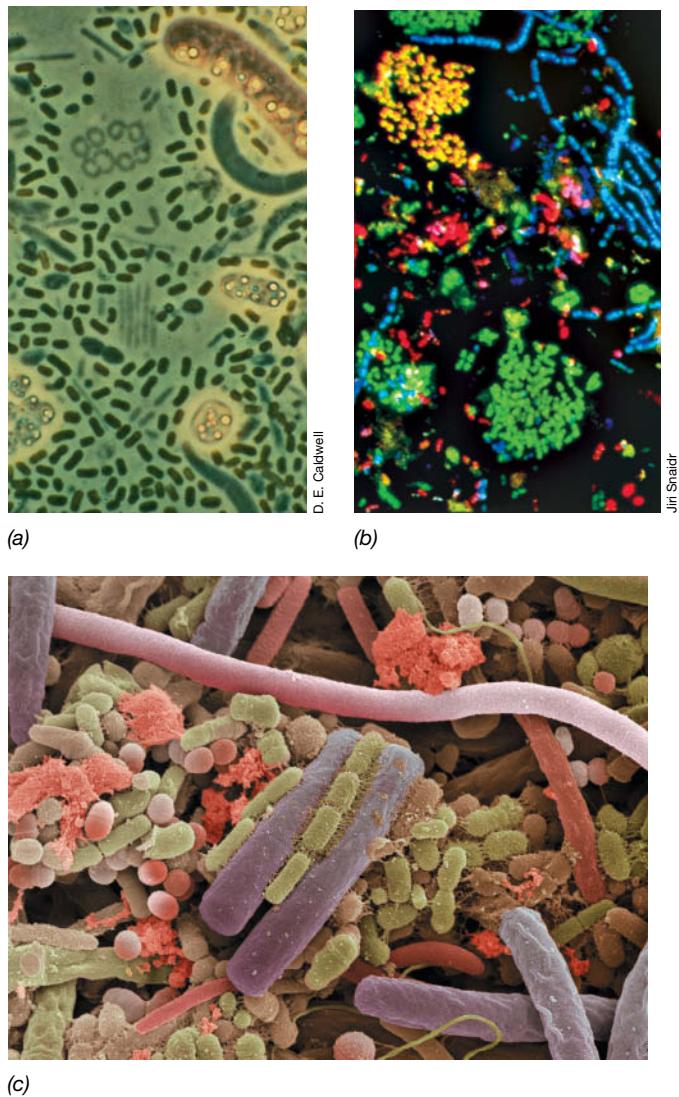


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# I • Exploring the Microbial World

## 1.1 Microorganisms, Tiny Titans of the Earth

**Microorganisms** (also called *microbes*) are life forms too small to be seen by the unaided human eye. These microscopic organisms are diverse in form and function and they inhabit every environment on Earth that supports life. Many microbes are undifferentiated single-celled organisms, but some can form complex structures, and some are even multicellular. Microorganisms typically live in complex **microbial communities** (Figure 1.1), and their activities are regulated by interactions with each other, with



**Figure 1.1** Microbial communities. (a) A bacterial community that developed in the depths of a small Michigan lake, showing cells of various phototrophic bacteria. The bacteria were visualized using phase-contrast microscopy. (b) A bacterial community in a sewage sludge sample. The sample was stained with a series of dyes, each of which stained a specific bacterial group. From *Journal of Bacteriology* 178: 3496–3500, Fig. 2b. © 1996 American Society for Microbiology. (c) Scanning electron micrograph of a microbial community scraped from a human tongue.

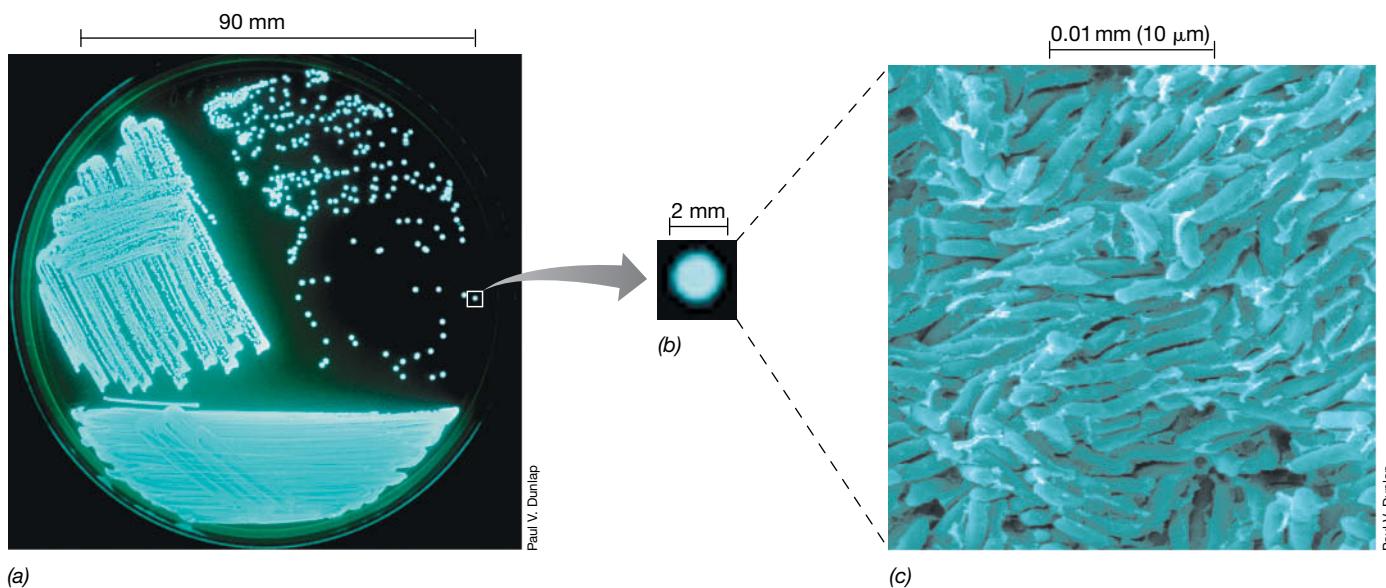
their environments, and with other organisms. The science of microbiology is all about microorganisms, who they are, how they work, and what they do.

Microorganisms were teeming on the land and in the seas for billions of years before the appearance of plants and animals, and their diversity is staggering. Microorganisms represent a major fraction of Earth's biomass, and their activities are essential to sustaining life. Indeed, the very oxygen ( $O_2$ ) we breathe is the result of microbial activities. Plants and animals are immersed in a world of microbes, and their evolution and survival are heavily influenced by microbial activities, by microbial symbioses, and by pathogens—those microbes that cause disease. Microorganisms are woven into the fabric of human life as well, from infectious diseases, to the food we eat, the water we drink, the fertility of our soils, the health of our animals, and even the fuel we put in automobiles. Microbiology is the study of the dominant form of life on Earth, and the effect that microbes have on our planet and all of the living things that call it home.

Microbiologists have many tools for studying microorganisms. Microbiology was born of the microscope, and microscopy is foundational to microbiology. Microbiologists have developed an array of methods for visualizing microorganisms, and these microscopic techniques are essential to microbiology. The cultivation of microorganisms is also foundational to microbiology. A microbial **culture** is a collection of cells that have been grown in or on a nutrient medium. A **medium** (plural, media) is a liquid or solid nutrient mixture that contains all of the nutrients required for a microorganism to grow. In microbiology, we use the word **growth** to refer to the increase in cell number as a result of cell division. A single microbial cell placed on a solid nutrient medium can grow and divide into millions of cells that form a visible **colony** (Figure 1.2). The formation of visible colonies makes it easier to see and grow microorganisms. Comprehension of the microbial basis of disease and microbial biochemical diversity has relied on the ability to grow microorganisms in the laboratory.

The ability to grow microorganisms rapidly under controlled conditions makes them highly useful for experiments that probe the fundamental processes of life. Most discoveries relating to the molecular and biochemical basis of life have been made using microorganisms. The study of molecules and their interactions is essential to defining the workings of microbial cells, and the tools of molecular biology and biochemistry are foundational to microbiology. Molecular biology has also provided a variety of tools to study microorganisms without need for their cultivation in the laboratory. These molecular tools have greatly expanded our knowledge of microbial ecology and diversity. Finally, the tools of genomics and molecular genetics are also cornerstones of modern microbiology and allow microbiologists to study the genetic basis of life, how genes evolve, and how they regulate the activities of cells.

This chapter begins our journey into the microbial world. Here we will begin to discover what microorganisms are, what they do, and how they can be studied. We will also place microbiology in historical context, as a process of scientific discovery.



**Figure 1.2 Microbial cells.** (a) Bioluminescent (light-emitting) colonies of the bacterium *Photobacterium* grown in laboratory culture on a Petri plate. (b) A single colony can contain more than 10 million ( $10^7$ ) individual cells. (c) Scanning electron micrograph of cells of *Photobacterium*.

### MINIQUIZ

- In what ways are microorganisms important to humans?
- Why are microbial cells useful for understanding the basis of life?
- What is a microbial colony and how is one formed?

## 1.2 Structure and Activities of Microbial Cells

Microbial cells are living compartments that interact with their environment and with other cells in dynamic ways. In Chapter 2 we will examine the structure of cells in detail and relate specific structures to specific functions. Here we present a snapshot of microbial structure and activities. We purposely exclude viruses in most of this discussion because although they resemble cells in many ways, viruses are not cells but instead a special category of microorganism. We consider the structure, diversity, and activities of viruses in Section 1.14 and in Chapters 8 and 10.

### Elements of Microbial Structure

All cells have much in common and contain many of the same components (Figure 1.3). All cells have a permeability barrier called the **cytoplasmic membrane** that separates the inside of the cell, the **cytoplasm**, from the outside. The cytoplasm is an aqueous mixture of **macromolecules** (for example proteins, lipids, nucleic acids, and polysaccharides), small organic molecules (mostly the precursors of macromolecules), various inorganic ions, and ribosomes. **Ribosomes** are the structures responsible for protein synthesis and are found in all cells. Some cells have a **cell wall** that lends structural strength to a cell. The cell wall is a relatively permeable structure located outside the cell membrane and is a much stronger layer than the membrane itself. Plant cells

and most microorganisms have cell walls, whereas animal cells typically do not.

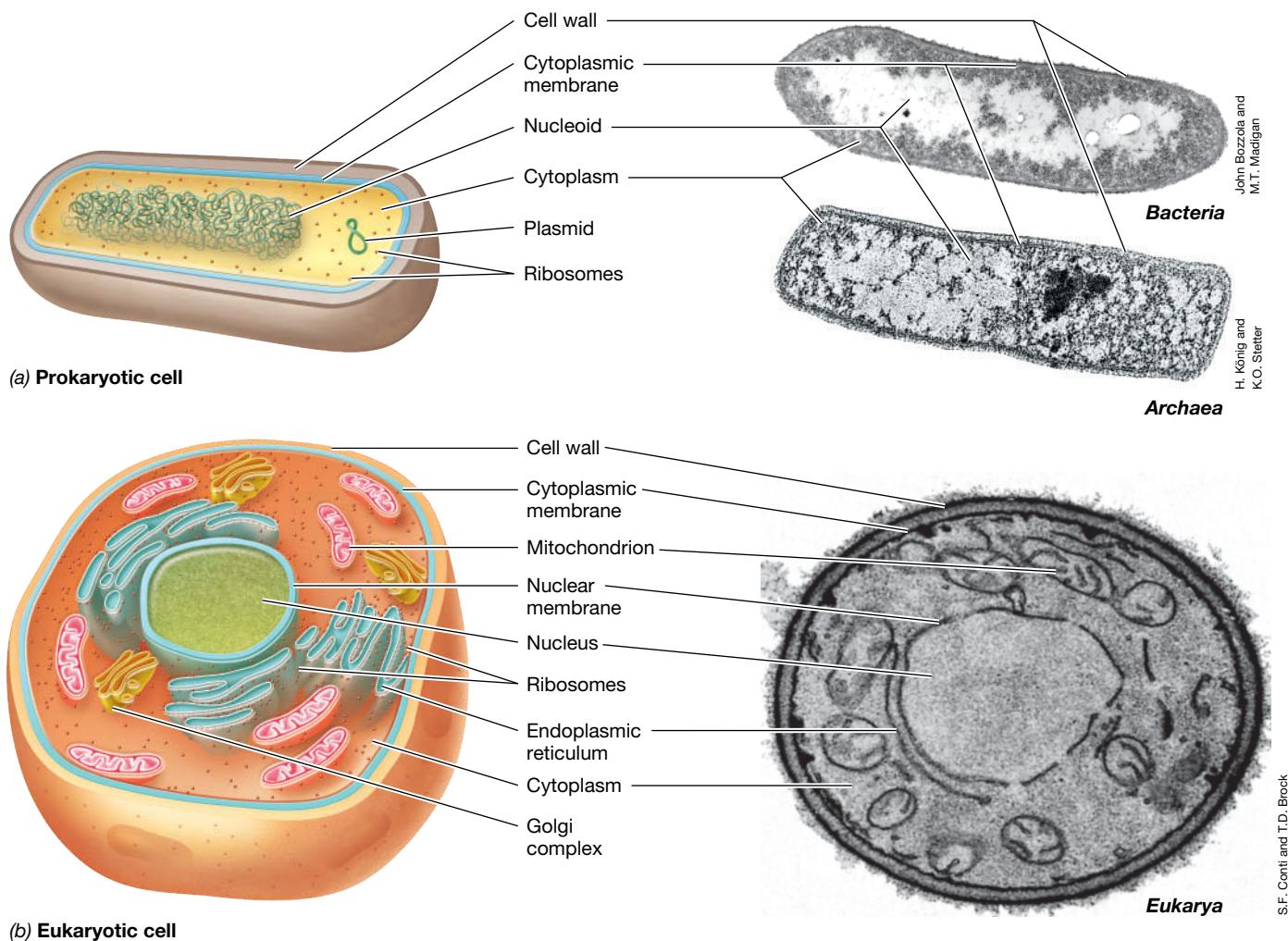
Examination of cell structure reveals there are two major structural classes of cells, called **prokaryotic** cells and **eukaryotic** cells (Figure 1.3). Eukaryotic cells are found in the phylogenetic domain *Eukarya*. This group includes plants and animals as well as diverse microbial eukaryotes such as algae, protozoa, and fungi. Eukaryotic cells contain an assortment of membrane-enclosed cytoplasmic structures called **organelles** (Figure 1.3b). These include, most prominently, the DNA-containing nucleus but also mitochondria and chloroplasts, organelles that specialize in supplying the cell with energy, and various other organelles.

Prokaryotic cells are found in the domains *Bacteria* and *Archaea*. Prokaryotic cells have few internal structures, they lack a nucleus, and they typically lack organelles (Figure 1.3a). The prokaryotic cell structure evolved prior to the evolution of the eukaryotic cell (Section 1.3). While *Archaea* and *Bacteria* both contain exclusively prokaryotic cells, these groups have diverged greatly and we will see later that the *Archaea* actually share many molecular and genetic characteristics with cells of *Eukarya*.

### Genes, Genomes, Nucleus, and Nucleoid

In addition to a cytoplasmic membrane and ribosomes, all cells also possess a DNA **genome**. The genome is the complement of all genes in a cell. A gene is a segment of DNA that encodes a protein or an RNA molecule. The genome is the living blueprint of an organism; the characteristics, activities, and very survival of a cell are governed by its genome.

The genomes of prokaryotic cells and eukaryotic cells are organized differently. In eukaryotes, DNA is present as several linear molecules within the membrane-enclosed **nucleus**. By contrast, the genomes of *Bacteria* and *Archaea* are typically closed circular chromosomes (though a few prokaryotes have linear



**Figure 1.3** Microbial cell structure. (a) (Left) Diagram of a prokaryotic cell. (Right) Electron micrograph of *Helio bacterium modestus* (Bacteria, cell is about 1  $\mu\text{m}$  in diameter) and *Thermoproteus neutrophilus* (Archaea, cell is about 0.5  $\mu\text{m}$  in diameter). (b) (Left) Diagram of a eukaryotic cell. (Right) Electron micrograph of a cell of *Saccharomyces cerevisiae* (Eukarya, cell is about 8  $\mu\text{m}$  in diameter).

chromosomes). The chromosome aggregates within the prokaryotic cell to form the **nucleoid**, a mass visible in the electron microscope (Figure 1.3a). Most prokaryotic cells have only a single chromosome, but many also contain one or more small circles of DNA distinct from that of the chromosome, called **plasmids**. Plasmids typically contain genes that are not essential and confer some special property on the cell (such as a unique metabolism, or antibiotic resistance). The genomes of *Bacteria* and *Archaea* are typically small and compact and most contain between 500 and 10,000 genes encoded by 0.5 to 10 million base pairs. Eukaryotic cells typically have much larger and much less compact genomes than prokaryotic cells. A human cell, for example, contains approximately 3 billion base pairs, which encode about 20,000–25,000 genes.

### Activities of Microbial Cells

What activities do microbial cells carry out? We will see that in nature, microbial cells typically live in groups called microbial communities (Figure 1.1). Figure 1.4 considers some of the ongoing

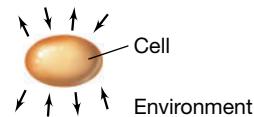
cellular activities within the microbial community. All cells show some form of **metabolism** by taking up nutrients from the environment and transforming them into new cell materials and waste products. During these transformations, energy is conserved to support synthesis of new structures. Production of these new structures culminates in the division of the cell to form two cells. Microbial growth results from successive rounds of cell division.

During metabolism and growth, genes are decoded to form proteins that regulate cellular processes. **Enzymes**, those proteins that have catalytic activity, are required to carry out reactions that supply the energy and precursors necessary for the biosynthesis of all cell components. Enzymes and other proteins are synthesized during *gene expression* in the sequential processes of transcription and translation (Figure 1.4). **Transcription** is the process by which the information on DNA is copied into an RNA molecule, and **translation** is the process whereby the information on an RNA molecule is used by a ribosome to synthesize a protein (Chapter 4). Gene expression and enzyme activity in a microbial cell are coordinated and highly regulated to ensure that the cell remains

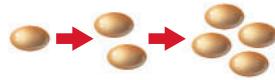
**Properties of all cells:****Metabolism**

Cells take up nutrients, transform them, and expel wastes.

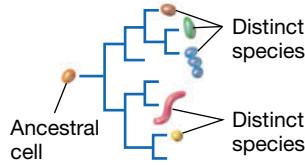
1. **Genetic** (replication, transcription, translation)
2. **Catalytic** (energy, biosyntheses)

**Growth**

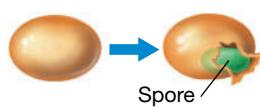
Nutrients from the environment are converted into new cell materials to form new cells.

**Evolution**

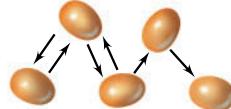
Cells evolve to display new properties. Phylogenetic trees capture evolutionary relationships.

**Properties of some cells****Differentiation**

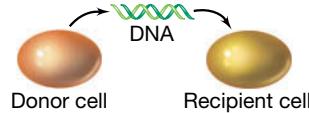
Some cells can form new cell structures such as a spore.

**Communication**

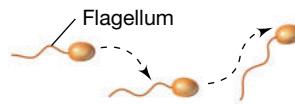
Cells interact with each other by chemical messengers.

**Genetic exchange**

Cells can exchange genes by several mechanisms.

**Motility**

Some cells are capable of self-propulsion.



**Figure 1.4** The properties of microbial cells. Major activities ongoing in cells in the microbial community are depicted.

optimally tuned to its surroundings. Ultimately, microbial growth requires replication of the genome through the process of **DNA replication**, followed by cell division. All cells carry out the processes of transcription, translation, and DNA replication.

Microorganisms have the ability to sense and respond to changes in their local environment. Many microbial cells are capable of **motility**, typically by self-propulsion (Figure 1.4). Motility allows cells to relocate in response to environmental conditions. Some microbial cells undergo **differentiation**, which may result in the formation of modified cells specialized for growth, dispersal, or survival. Cells respond to chemical signals in their environment, including those produced by other cells of either the same or different species, and these signals often trigger new cellular activities. Microbial cells thus exhibit **intercellular communication**; they are “aware” of their neighbors and can respond accordingly. Many prokaryotic cells can also exchange genes with neighboring cells, either of the same species or of a different species, in the process of **horizontal gene transfer**.

**Evolution** (Figure 1.4) results when genes in a population of cells change in sequence and frequency over time, leading to descent with modification. The evolution of microorganisms can

be very rapid relative to the evolution of plants and animals. For example, the indiscriminate use of antibiotics in human and veterinary medicine has selected for the proliferation of antibiotic resistance in pathogenic bacteria. The rapid pace of microbial evolution can be attributed in part to the ability of microorganisms to grow very quickly and to acquire new genes through the process of horizontal gene transfer.

Not all of the processes depicted in Figure 1.4 occur in all cells. Metabolism, growth, and evolution, however, are universal and will be major areas of emphasis throughout this book.

**MINIQUIZ**

- What structures are universal to all type of cells?
- What processes are universal to all types of cells?
- What structures can be used to distinguish between prokaryotic cells and eukaryotic cells?

**1.3 Microorganisms and the Biosphere**

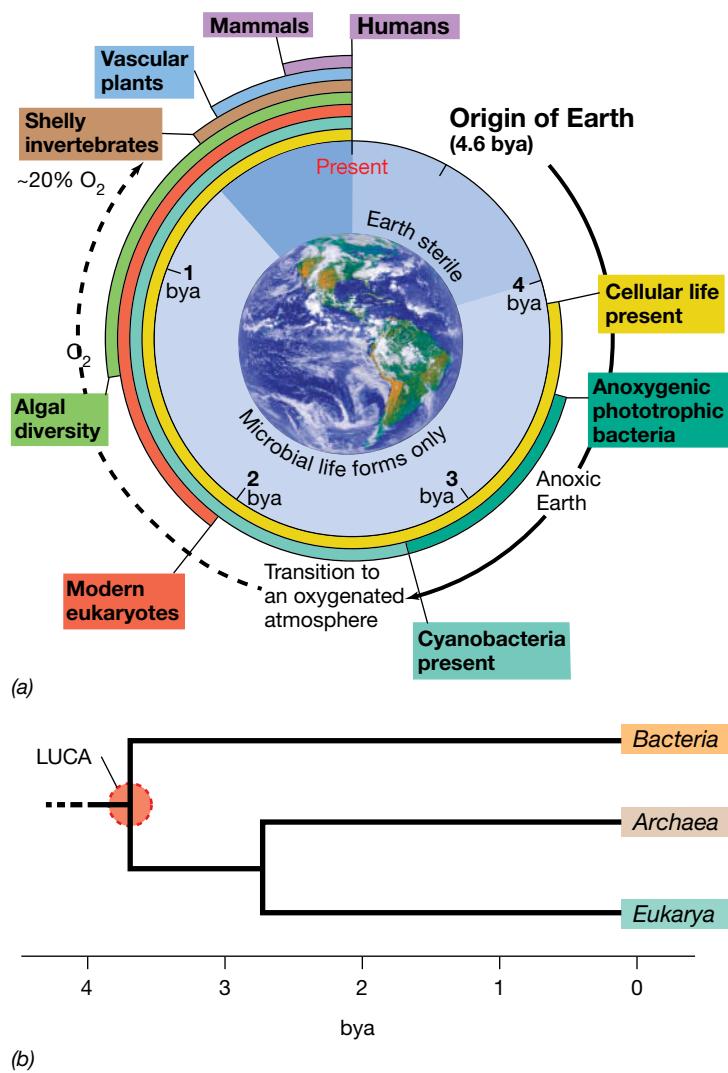
Microbes are the oldest form of life on Earth, and they have evolved to perform critical functions that sustain the biosphere. In this section we will learn how microbes have changed our planet and how they continue to do so.

**A Brief History of Life on Earth**

Earth is about 4.6 billion years old, and microbial cells first appeared between 3.8 and 4.3 billion years ago (Figure 1.5). During the first 2 billion years of Earth’s existence, its atmosphere was anoxic ( $O_2$  was absent), and only nitrogen ( $N_2$ ), carbon dioxide ( $CO_2$ ), and a few other gases were present. Only microorganisms capable of anaerobic metabolisms (that is, metabolisms that do not require  $O_2$ ) could survive under these conditions.

The evolution of phototrophic microorganisms—organisms that harvest energy from sunlight—occurred within 1 billion years of the formation of Earth (Figure 1.5a). The first phototrophs were anoxygenic (non-oxygen-producing), such as the purple sulfur bacteria and green sulfur bacteria we know today (Figure 1.6). *Cyanobacteria* (oxygenic phototrophs) (Figure 1.6f) evolved nearly a billion years later (Figure 1.5a) and began the slow process of oxygenating Earth’s atmosphere. These early phototrophs lived in structures called *microbial mats*, which are still found on Earth today (Figure 1.6a–c). After the oxygenation of Earth’s atmosphere, multicellular life forms eventually evolved, culminating in the plants and animals we know today. But plants and animals have only existed for about half a billion years. The timeline of life on Earth (Figure 1.5a) shows that *80% of life’s history was exclusively microbial*, and thus in many ways, Earth can be considered a microbial planet.

As evolutionary events unfolded, three major lineages of microbial cells—the *Bacteria*, the *Archaea*, and the *Eukarya* (Figure 1.5b)—were distinguished. These three major cell lineages are called **domains**, and all known cellular organisms belong to one of these three domains. All cellular organisms also share certain characteristics and genes. For example, approximately 60 genes are universally present in cells of all domains. Examination of these genes reveals that all three domains have descended from a



**Figure 1.5** A summary of life on Earth through time and origin of the cellular domains. (a) At its origin, Earth was sterile. Cellular life was present on Earth by 3.8 billion years ago (bya). Cyanobacteria began the slow oxygenation of Earth about 3 bya, but current levels of O<sub>2</sub> in the atmosphere were not achieved until 500–800 million years ago. (b) The three domains of cellular organisms are *Bacteria*, *Archaea*, and *Eukarya*. *Archaea* and *Eukarya* diverged long before eukaryotic cells appear in the fossil record. LUCA, last universal common ancestor.

common ancestor, the *last universal common ancestor* (LUCA, Figure 1.5b). Over enormous periods of time, microorganisms derived from these three domains have evolved to fill every suitable environment on Earth.

### Microbial Abundance and Activity in the Biosphere

Microorganisms are present everywhere on Earth that will support life. They constitute a major fraction of global biomass and are key reservoirs of nutrients essential for life. There are an estimated  $2 \times 10^{30}$  microbial cells on Earth. To put this number in context, the universe in all its vast extent is estimated to contain merely  $7 \times 10^{22}$  stars. The total amount of carbon present in all microbial cells is a significant fraction of Earth's biomass (Figure 1.7). Moreover, the total amount of nitrogen and phosphorus (essential nutrients for life) within microbial cells is nearly four

times that in all plant and animal cells combined. Microbes also represent a major fraction of the total DNA in the biosphere (about 31%), and their genetic diversity far exceeds that of plants and animals (see Figure 1.36).

Microbes are even abundant in habitats that are much too harsh for other forms of life, such as volcanic hot springs, glaciers and ice-covered regions, high-salt environments, extremely acidic or alkaline habitats, and deep in the sea or deep in the earth at extremely high pressure. Such microorganisms are called **extremophiles** and their properties define the physiochemical limits to life as we know it (Table 1.1). We will revisit many of these organisms in later chapters and discover the special structural and biochemical properties that allow them to thrive under extreme conditions.

All ecosystems are influenced greatly by microbial activities. The metabolic activities of microorganisms can change the habitats in which they live, both chemically and physically, and these changes can affect other organisms. For example, excess nutrients added to a habitat can cause aerobic (O<sub>2</sub>-consuming) microorganisms to grow rapidly and consume O<sub>2</sub>, rendering the habitat anoxic (O<sub>2</sub>-free). Many human activities release nutrients into the coastal oceans, thereby stimulating excessive microbial growth, which can cause enormous anoxic zones in these waters. These “dead zones” cause massive mortality of fish and shellfish in coastal oceans worldwide, because most aquatic animals require O<sub>2</sub> and die if it is not available. Only by understanding microorganisms and microbiology can we predict and minimize the effects of human activity on the biosphere that sustains us.

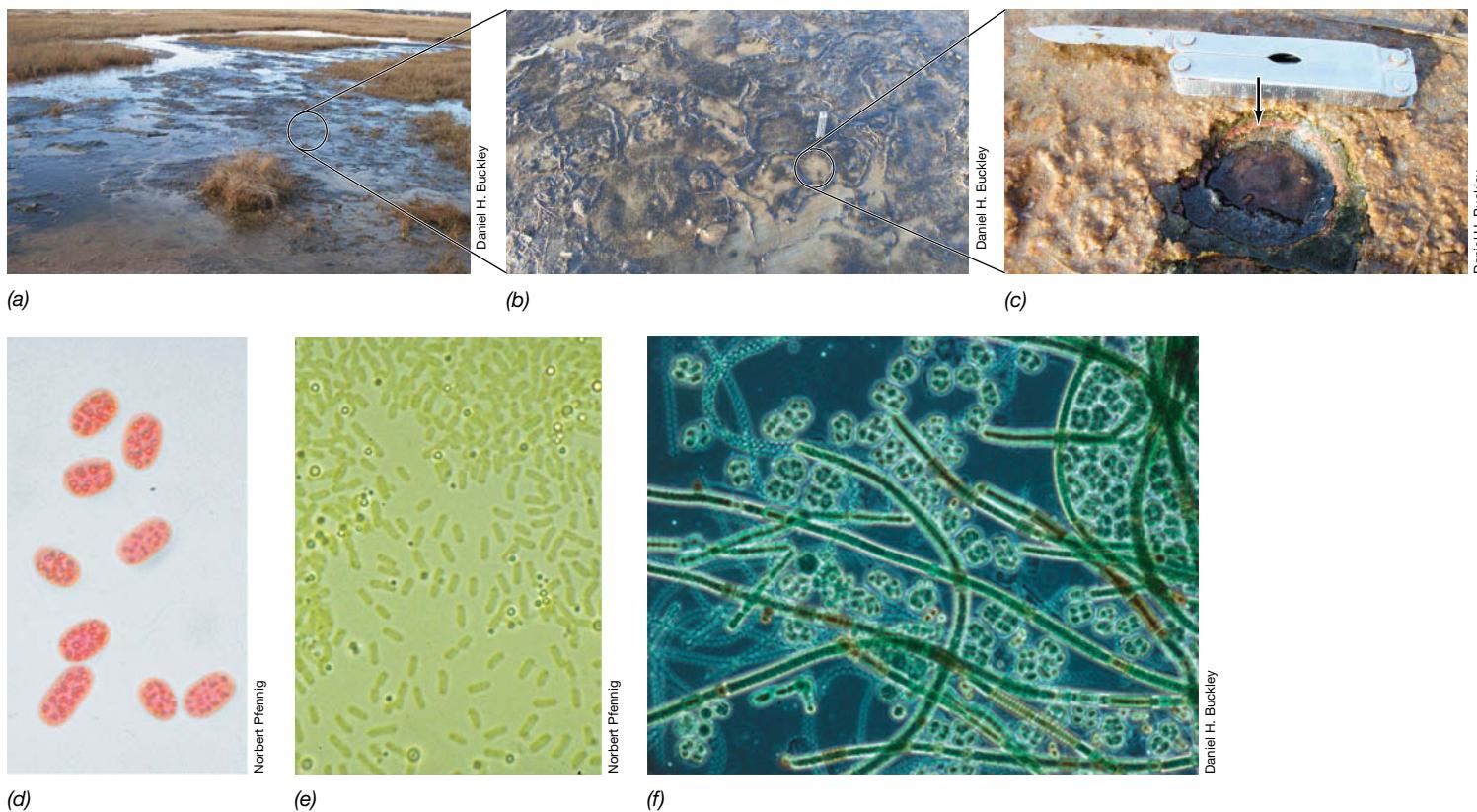
Though diverse habitats are influenced strongly by microorganisms, their contributions are often overlooked because of their small sizes. Within the human body, for example, there are between one and ten microbial cells (mainly of *Bacteria*) for every human cell and more than 200 microbial genes for every human gene. These microbes provide nutritional and other benefits that are essential to human health. In later chapters we will return to a consideration of the ways in which microorganisms affect animals, plants, and the entire global ecosystem. This is the science of **microbial ecology**, perhaps the most exciting subdiscipline of microbiology today. We will see that microbes are important to myriad issues of global importance to humans including climate change, agricultural productivity, and even energy policy.

### MINIQUIZ

- How old is Earth and when did cells first appear on Earth?
- Name the three domains of life.
- Why were cyanobacteria so important in the evolution of life on Earth?

## 1.4 The Impact of Microorganisms on Human Society

Microbiologists have made great strides in discovering how microorganisms function, and application of this knowledge has greatly advanced human health and welfare. Besides understanding



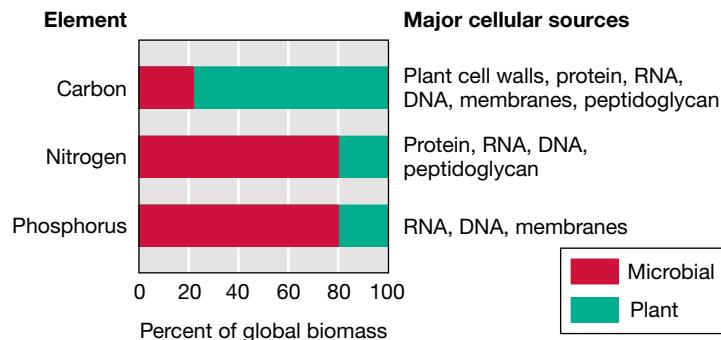
**Figure 1.6** Phototrophic microorganisms.

The earliest phototrophs lived in microbial mats. (a) Photosynthetic microbial mats in the Great Sippewissett Marsh, a salt marsh in Massachusetts, USA. (b) Mats develop a cohesive structure that forms at the sediment surface. (c) A slice through the mat shows colored layers

that form due to the presence of photopigments. Cyanobacteria form the green layer nearest the surface, purple sulfur bacteria form the purple and yellow layers below, and green sulfur bacteria form the bottommost green layer. The scale on the knife is in cm. (d) Purple sulfur bacteria, (e) green sulfur bacteria, and

(f) cyanobacteria imaged by bright-field and phase-contrast microscopy. Purple and green sulfur bacteria are anoxygenic phototrophs that appeared on Earth long before oxygenic phototrophs evolved (see Figure 1.5a).

microorganisms as agents of disease, microbiology has made great advances in understanding the important roles microorganisms play in food and agriculture, and microbiologists have been able to exploit microbial activities to produce valuable human products, generate energy, and clean up the environment.



**Figure 1.7** Contribution of microbial cells to global biomass. Microorganisms comprise a significant fraction of the carbon (C) and a majority of the nitrogen (N) and phosphorus (P) in the biomass of all organisms on Earth. C, N, and P are the macronutrients required in the greatest quantity by living organisms. Animal biomass is a minor fraction (<0.1%) of total global biomass and is not shown.

## Microorganisms as Agents of Disease

The statistics summarized in Figure 1.8 show how microbiologists and clinical medicine have combined to conquer infectious diseases in the past 100 years. At the beginning of the twentieth century, the major causes of human death were infectious diseases caused by bacterial and viral **pathogens**. In those days children and the aged in particular succumbed in large numbers to microbial diseases. Today, however, infectious diseases are much less deadly, at least in developed countries. Control of infectious disease has come from a combination of advances including our increased understanding of disease processes, improved sanitary and public health practices, active vaccine campaigns, and the widespread use of antimicrobial agents, such as antibiotics. As we will see later in this chapter, the development of microbiology as a science can be traced to pioneering studies of infectious disease.

While pathogens and infectious disease remain a major threat to humanity, and combating these harmful organisms remains a major focus of microbiology, most microorganisms are not harmful to humans. In fact, most microorganisms are beneficial, and in many cases are even essential to human welfare and the functioning of the planet. We turn our attention to these microorganisms and microbial activities now.

**TABLE 1.1** Classes and examples of extremophiles<sup>a</sup>

Extreme	Descriptive term	Genus/species	Domain	Habitat	Minimum	Optimum	Maximum
<b>Temperature</b>							
High	Hyperthermophile	<i>Methanopyrus kandleri</i>	Archaea	Undersea hydrothermal vents	90°C	106°C	122°C <sup>b</sup>
Low	Psychrophile	<i>Psychromonas ingrahamii</i>	Bacteria	Sea ice	-12°C <sup>c</sup>	5°C	10°C
<b>pH</b>							
Low	Acidophile	<i>Picrophilus oshimae</i>	Archaea	Acidic hot springs	-0.06	0.7 <sup>d</sup>	4
High	Alkaliphile	<i>Natronobacterium gregoryi</i>	Archaea	Soda lakes	8.5	10 <sup>e</sup>	12
<b>Pressure</b>							
	Barophile (piezophile)	<i>Moritella yayanosii</i>	Bacteria	Deep ocean sediments	500 atm	700 atm <sup>f</sup>	>1000 atm
<b>Salt (NaCl)</b>							
	Halophile	<i>Halobacterium salinarum</i>	Archaea	Salterns	15%	25%	32% (saturation)

<sup>a</sup>The organisms listed are the current “record holders” for growth in laboratory culture at the extreme condition listed.

<sup>b</sup>Anaerobe showing growth at 122°C only under several atmospheres of pressure.

<sup>c</sup>The permafrost bacterium *Planococcus halocryophilus* can grow at -15°C and metabolize at -25°C. However, the organism grows optimally at 25°C and grows up to 37°C and thus is not a true psychrophile.

<sup>d</sup>*P. oshimae* is also a thermophile, growing optimally at 60°C.

<sup>e</sup>*N. gregoryi* is also an extreme halophile, growing optimally at 20% NaCl.

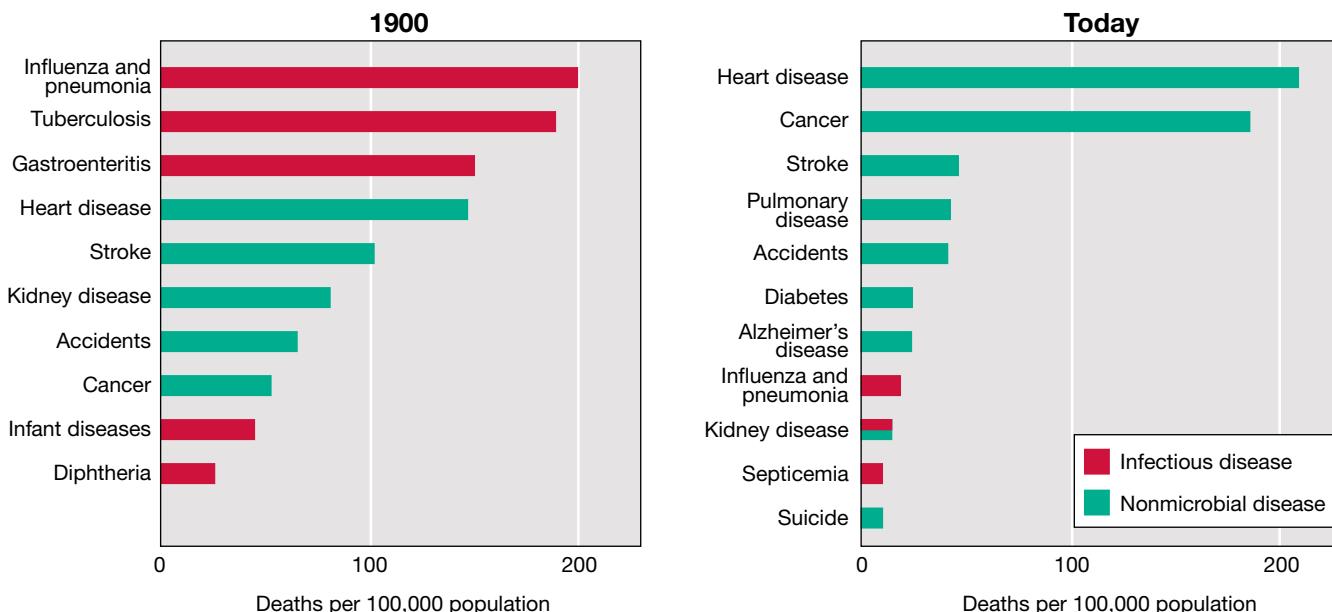
<sup>f</sup>*M. yayanosii* is also a psychrophile, growing optimally near 4°C.

## Microorganisms, Agriculture, and Human Nutrition

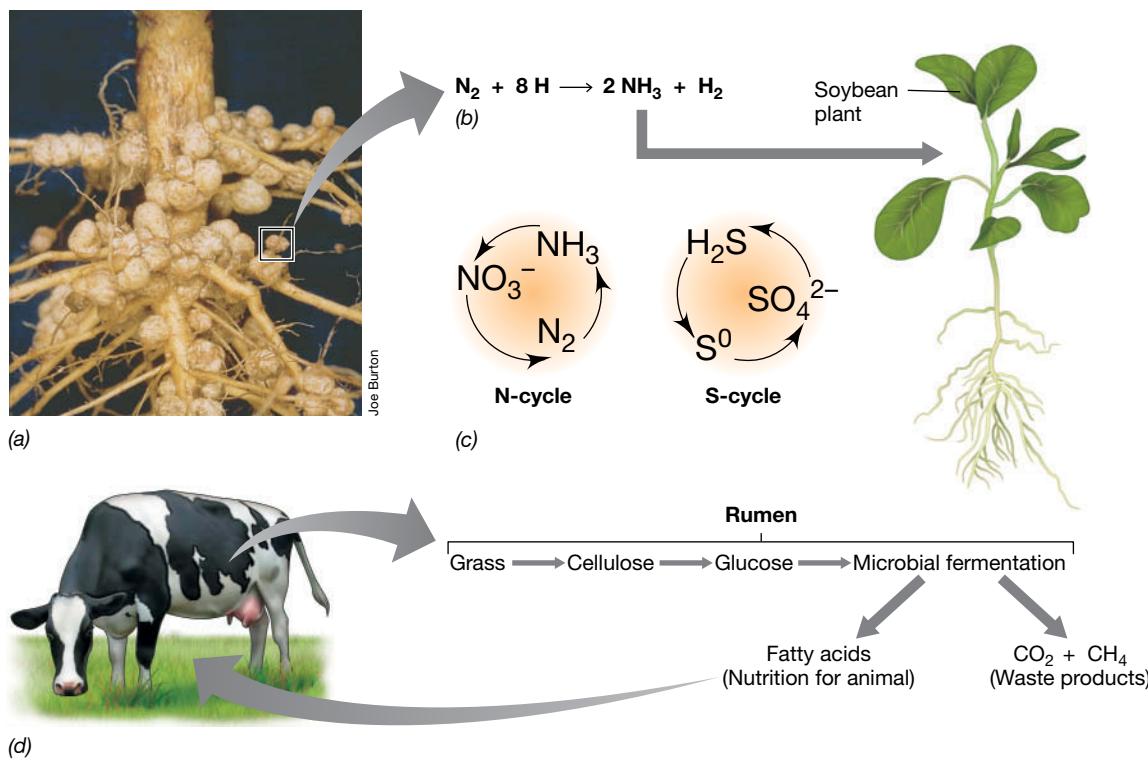
Agriculture benefits from the cycling of key plant nutrients by microorganisms. For example, legumes are a diverse family of plants that include major crop species such as beans, peas, and lentils, among others. Legumes live in close association with bacteria that form structures called *nodules* on their roots. In the nodules, these bacteria convert atmospheric nitrogen ( $N_2$ ) into ammonia ( $NH_3$ ) through the process of *nitrogen fixation*.  $NH_3$  is the major nutrient found in fertilizer and is used as a nitrogen source for plant growth (Figure 1.9). In this way bacteria allow legumes to make their own fertilizer, thereby reducing the need for farmers to apply fertilizers produced industrially. Bacteria

regulate nutrient cycles, such as the nitrogen cycle and the sulfur cycle (Figure 1.9), transforming and recycling nutrients that form the basis of soil fertility.

Also of major agricultural importance are microorganisms that inhabit the *rumen* of ruminant animals, such as cattle and sheep. The rumen is a microbial ecosystem in which microbial communities digest and ferment the polysaccharide cellulose (Figure 1.9d), the major component of plant cell walls. Without these symbiotic microorganisms, ruminants could not thrive on cellulose-rich (but otherwise nutrient-poor) food such as grass and hay. Many domesticated and wild herbivorous mammals—including deer, bison, camels, giraffes, and goats—are also ruminants.



**Figure 1.8** Death rates for the leading causes of death in the United States: 1900 and today. Infectious diseases were the leading causes of death in 1900, whereas today they account for relatively few deaths. Kidney diseases can be caused by microbial infections or systemic sources (diabetes, cancers, toxicities, metabolic diseases, etc.). Data are from the United States National Center for Health Statistics and the Centers for Disease Control and Prevention.



**Figure 1.9 Microorganisms in modern agriculture.** (a, b) Root nodules on this soybean plant contain bacteria that fix molecular nitrogen ( $N_2$ ) for use by the plant. (c) The nitrogen and sulfur cycles, key nutrient cycles in nature. (d) Ruminant animals. Microorganisms in the rumen of the cow convert cellulose from grass into fatty acids that can be used by the animal. The other products are not so desirable, as  $CO_2$  and  $CH_4$  are the major gases that cause global warming.

The human gastrointestinal (GI) tract lacks a rumen, but complex carbohydrates (which can represent 10–30% of food energy) are digested by the **gut microbiome**. The colon, or large intestine (Figure 1.10), follows the stomach and small intestine in the digestive tract, and it contains about  $10^{11}$  microbial cells per gram of colonic contents. Microbial cell numbers are low in the very acidic (pH 2) stomach (about  $10^4$  per gram) but increase to about  $10^8$  per gram near the end of the small intestine (pH 5) and then reach maximal numbers in the colon (pH 7) (Figure 1.10). The colon contains diverse microbial species that assist in the digestion of complex carbohydrates, and that synthesize vitamins and other nutrients essential to host nutrition. The gut microbiome develops from birth, but it can change over time with the human host. The composition of the gut microbiome has major effects on GI function and human health.

### Microorganisms and Food

Microbes are intimately associated with the foods we eat. Microbial growth in food can cause food spoilage and foodborne disease. The manner in which we harvest and store food (e.g., canning, refrigeration, drying, salting, etc.), the ways in which we cook it, and even the spices we use, have all been fundamentally influenced by microbes in order to minimize microbial growth and eliminate harmful organisms. Microbial food safety and prevention of food spoilage is a major focus of the food industry and a major cause of economic loss every year.

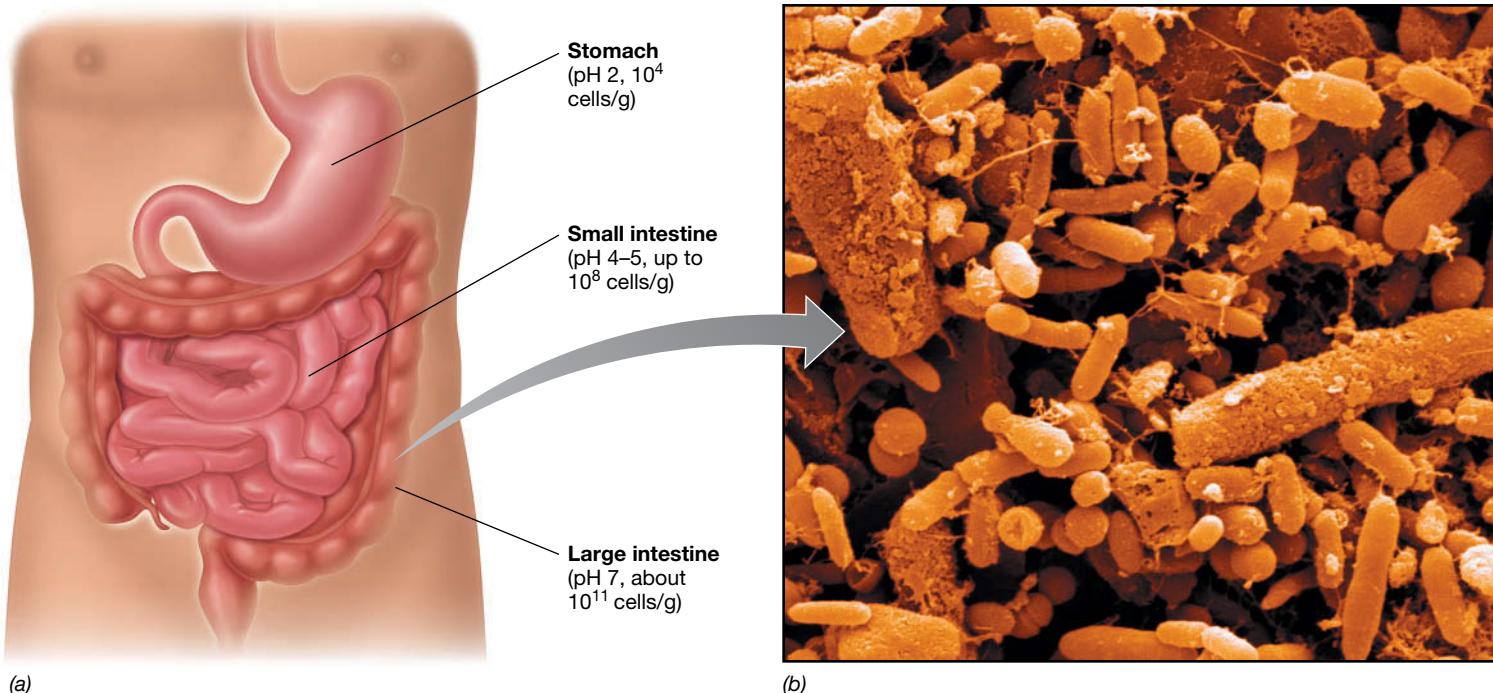
While some microbes can cause foodborne disease and food spoilage, not all microorganisms in foods are harmful. Indeed, beneficial microbes have been used for thousands of years to improve food safety and to preserve foods (Figure 1.11). For example, cheeses, yogurt, and buttermilk are all produced by the microbial fermentation of dairy products to produce acids that improve

shelf life and prevent the growth of foodborne pathogens. Such microbial fermentations are used to produce a variety of foods including sauerkraut, kimchi, pickles, and certain sausages. Even the production of chocolate and coffee rely on microbial fermentation. Moreover, baked goods and alcoholic beverages rely on the fermentative activities of yeast, which generate carbon dioxide ( $CO_2$ ) to raise the dough and alcohol as a key component (Figure 1.11), respectively. Fermentation products affect the flavor and taste of foods, and can prevent spoilage as well as the growth of deleterious organisms.

### Microorganisms and Industry

Microorganisms play important roles in all manner of human industry. Microbes can grow in almost any habitat containing liquid water, including structures made by humans. For example, microbes often grow on submerged surfaces, forming *biofilms*. Biofilms that grow in pipes and drains can cause fouling and blockages in factory settings and pipelines, in sewers, and even in water distribution systems. In addition, biofilms that grow on ships' hulls can cause marked reductions in speed and efficiency. Biofilms can even grow in tanks that store oil and fuel, leading to spoilage of these products. We will learn that biofilms are also of great importance in medicine, as biofilms that form on implanted medical devices (► Figure 5.4a) can cause infections that are extremely difficult to treat.

Microorganisms can be harnessed to produce commercially valuable products. In *industrial microbiology*, naturally occurring microorganisms are grown on a massive scale to make large amounts of products at relatively low cost, such as antibiotics, enzymes, and certain chemicals. By contrast, *biotechnology* employs genetically engineered microorganisms to synthesize products of high value, such as insulin or other human proteins, usually on a small scale.

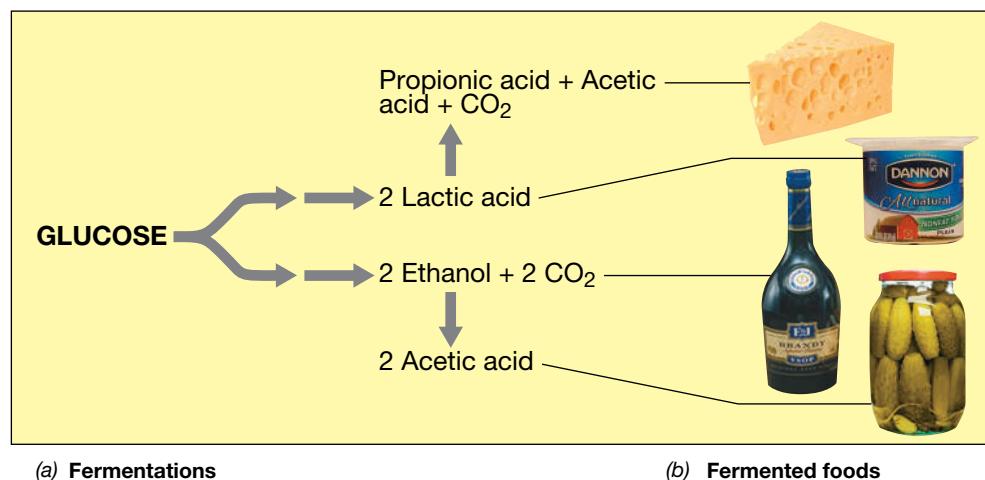


**Figure 1.10** The human gastrointestinal tract. (a) Diagram of the human GI tract showing the major organs. (b) Scanning electron micrograph of microbial cells in the human colon (large intestine). Cell numbers in the colon can reach as high as  $10^{11}$  per gram. As well as high numbers of cells, the microbial diversity in the colon is also quite high.

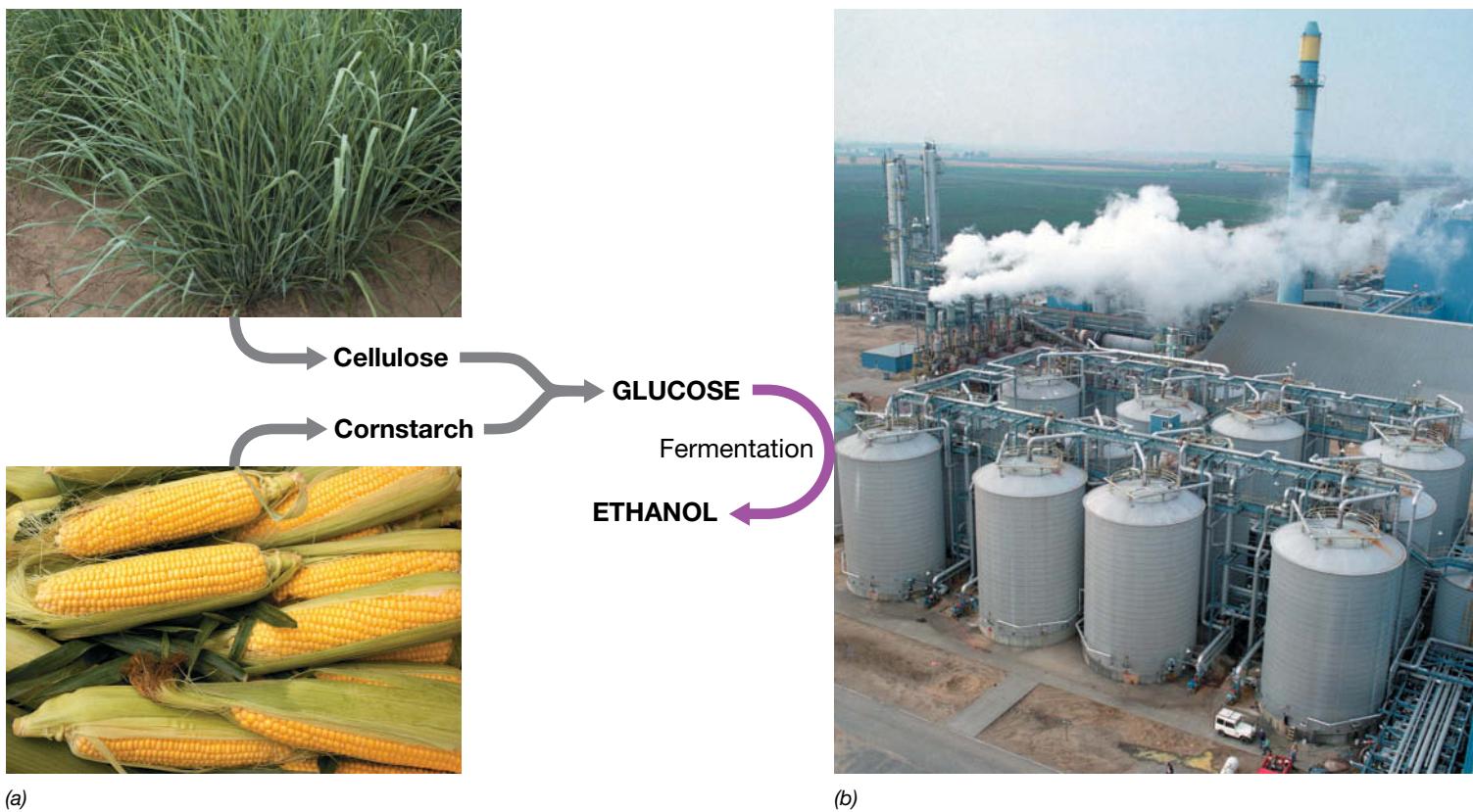
Microorganisms can also be used to produce *biofuels*. For example, natural gas (methane,  $\text{CH}_4$ ) is a product of the anaerobic metabolism of a group of *Archaea* called *methanogens*. Ethyl alcohol (ethanol) is a major fuel supplement, which is produced by the microbial fermentation of glucose obtained from carbon-rich feedstocks such as sugarcane, corn, or rapidly growing grasses (Figure 1.12). Microorganisms can even convert waste materials, such as domestic refuse, animal wastes, and cellulose, into ethanol and methane.

Microorganisms are also used to clean up wastes. Wastewater treatment is essential to sanitation and human health. Wastewater treatment relies on microbes to treat water contaminated with human waste so that it can be reused or returned safely to the environment. Waterborne disease such as cholera and typhoid can proliferate in the absence of proper wastewater treatment. Microbes can also be used to clean up industrial pollution in a process called *bioremediation*. In bioremediation, microorganisms are used to transform spilled oil, solvents, pesticides, heavy metals, and other environmentally toxic pollutants. Bioremediation accelerates the cleanup process either by adding special microorganisms to a polluted environment or by adding nutrients that stimulate indigenous microorganisms to degrade the pollutants. In either case the goal is to accelerate disappearance of the pollutant.

As these examples show, the influence of microorganisms on humans is great and their activities are essential for the functioning of the planet. Or, as the eminent French chemist and early microbiologist Louis Pasteur so aptly put it: “The role of the infinitely small in nature is infinitely large.” Microscopes provide an essential portal through which microbiologists such as Pasteur gazed into the world of microbes. We therefore continue our introduction to the microbial world with an overview of microscopy.



**Figure 1.11** Fermented foods. (a) Major fermentations in various fermented foods. It is the fermentation product (ethanol, or lactic, propionic, or acetic acids) that both preserves the food and renders in it a characteristic flavor. (b) Photo of several fermented foods showing the characteristic fermentation product in each.



**Figure 1.12 Ethanol as a biofuel.** (a) Major crop plants used as feedstocks for biofuel ethanol production. Top: switchgrass, a source of cellulose. Bottom: corn, a source of cornstarch. Both cellulose and starch are composed of glucose, which is fermented to ethanol by yeast. (b) An ethanol plant in the United States. Ethanol produced by fermentation is distilled and then stored in the tanks.

### MINIQUIZ

- How do microbes contribute to the nutrition of animals such as humans and cows?
- Describe several ways in which microorganisms are important in the food and agricultural industries.
- What is wastewater treatment and why is it important?

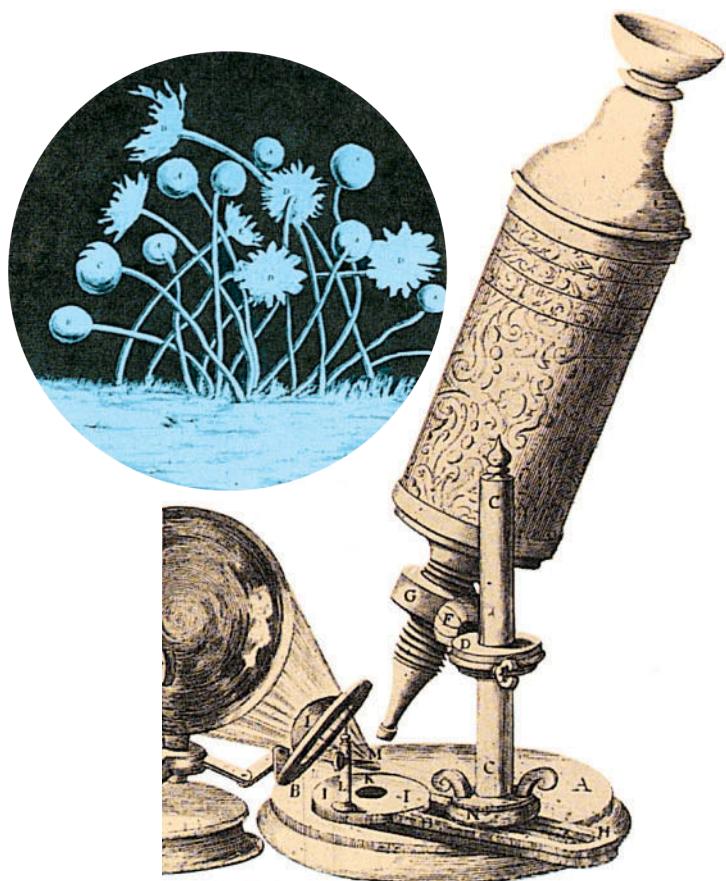
## 1.5 Light Microscopy and the Discovery of Microorganisms

Although the existence of creatures too small to be seen with the naked eye had been suspected for centuries, their discovery had to await invention of the microscope. The English mathematician and natural historian Robert Hooke (1635–1703) was an excellent microscopist. In his famous book *Micrographia* (1665), the first book devoted to microscopic observations, Hooke illustrated many microscopic images including the fruiting structures of molds (Figure 1.13). This was the first known description of microorganisms.

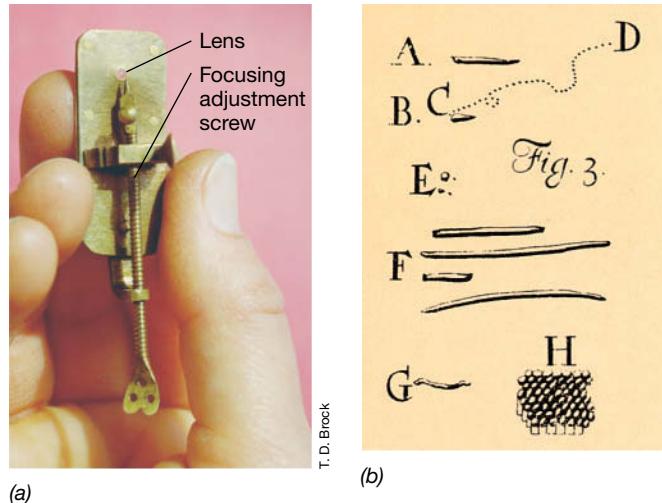
The first person to see bacteria, the smallest microbial cells, was the Dutch draper and amateur microscopist Antoni van Leeuwenhoek (1632–1723). Van Leeuwenhoek constructed extremely simple microscopes containing a single lens to examine various natural substances for microorganisms (Figure 1.14). These microscopes were crude by today's standards, but by careful manipulation and focusing, van Leeuwenhoek was able to see bacteria. He discovered bacteria in 1676 while studying pepper-water infusions, and reported his observations in a series of letters to the prestigious Royal Society of London, which published them in English translation in 1684. Drawings of some of van Leeuwenhoek's "wee animalcules," as he referred to them, are shown in Figure 1.14b, and a photo taken through a van Leeuwenhoek microscope is shown in Figure 1.14c.

## II • Microscopy and the Origins of Microbiology

**H**istorically, the science of microbiology has taken its greatest leaps forward as new tools are developed and old tools improve. The microscope is the microbiologist's oldest and most fundamental tool for studying microorganisms. Indeed, microbiology did not exist before the invention of the microscope. Many forms of microscopy are available and some are extremely powerful. Throughout this text you will see images of microorganisms that were taken through the microscope using a variety of different techniques. So let's take a moment to explore how microscopy can be used to visualize microbial cells, starting at the very beginning with the invention of the microscope.



**Figure 1.13 Robert Hooke and early microscopy.** A drawing of the microscope used by Robert Hooke in 1664. The lens was fitted at the end of an adjustable bellows (G) and light focused on the specimen by a separate lens (1). Inset: Hooke's drawing of a bluish mold he found degrading a leather surface; the round structures contain spores of the mold.



**Figure 1.14 The van Leeuwenhoek microscope.** (a) A replica of Antoni van Leeuwenhoek's microscope. (b) Van Leeuwenhoek's drawings of bacteria, published in 1684. Even from these simple drawings we can recognize several shapes of common bacteria: A, C, F, and G, rods; E, cocci; H, packets of cocci. (c) Photomicrograph of a human blood smear taken through a van Leeuwenhoek microscope. Red blood cells are clearly apparent.

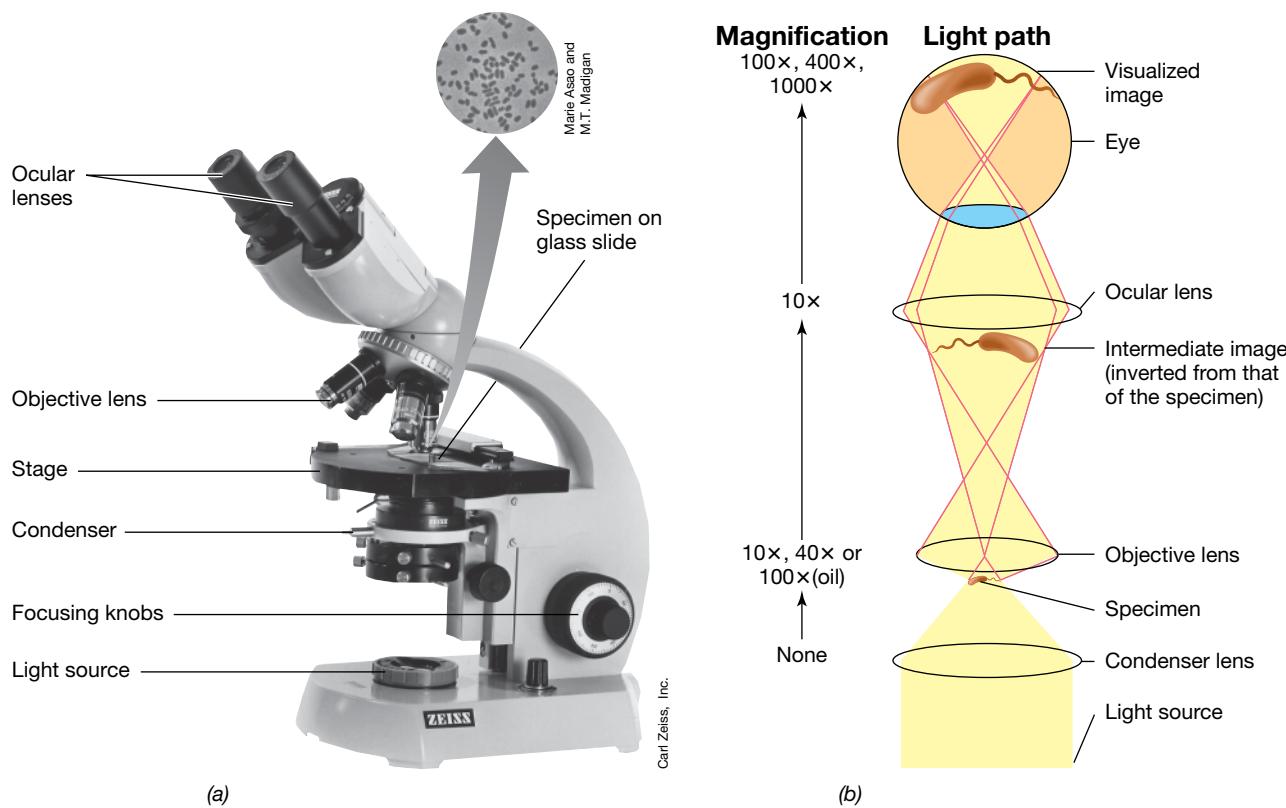
Van Leeuwenhoek's microscope was a *light* microscope, and his design used a simple lens that could magnify an image at least 266 times. In a light microscope the sample is illuminated with visible light. **Magnification** describes the capacity of a microscope to enlarge an image. All microscopes employ lenses that provide magnification. Magnification, however, is not the limiting factor in our ability to see small objects. It is resolution that governs our ability to see the very small. **Resolution** is the ability to distinguish two adjacent objects as distinct and separate. The limit of resolution for a light microscope is about  $0.2\text{ }\mu\text{m}$  ( $\mu\text{m}$  is the abbreviation for micrometer,  $10^{-6}\text{ m}$ ). What this means is that two objects that are closer together than  $0.2\text{ }\mu\text{m}$  cannot be resolved as distinct and separate.

Microscopy has improved considerably since the days of van Leeuwenhoek. Several types of light microscopy are now available, including *bright-field*, *phase-contrast*, *differential interference contrast*, *dark-field*, and *fluorescence*. With the modern compound light microscope, light from a light source is focused on the specimen by the condenser (Figure 1.15), and this light passes through the sample and is collected by the lenses. The modern compound light microscope contains two types of lenses, *objective* and *ocular*, that function in combination to magnify the image. Microscopes used in microbiology have ocular lenses that magnify  $10\text{--}30\times$  and objective lenses that magnify  $10\text{--}100\times$  (Figure 1.15b). The total magnification of a compound light microscope is the *product* of the magnification of its objective and ocular lenses (Figure 1.15b). Magnification of  $1000\times$  is required to resolve objects  $0.2\text{ }\mu\text{m}$  in diameter, which is the limit of resolution for most light microscopes (increasing magnification beyond  $1000\times$  provides little improvement in the resolution of a light microscope).

The limit of resolution for a light microscope is a function of the wavelength of light used and the light-gathering ability of the objective lens, a property known as its *numerical aperture*. There is a correlation between the magnification of a lens and its numerical aperture; lenses with higher magnification typically have higher numerical apertures.

The diameter of the smallest object resolvable by any lens is equal to  $0.5\lambda/\text{numerical aperture}$ , where  $\lambda$  is the wavelength of light used. With objectives that have a very high numerical aperture (such as the  $100\times$  objective), an optical grade oil is placed between the microscope slide and the objective. Lenses on which oil is used are called *oil-immersion* lenses. Immersion oil increases the light-gathering ability of a lens, that is, it increases the amount of light that is collected and viewed by the lens.

In light microscopy, specimens are visualized because of differences in **contrast** that exist between them and their surroundings. In bright-field microscopy, contrast results when cells absorb or scatter light



**Figure 1.15 Microscopy.** (a) A compound light microscope (inset photomicrograph of unstained cells taken through a phase-contrast light microscope). (b) Path of light through a compound light microscope. Figure 1.19 compares cells visualized by bright field with those visualized by phase contrast.

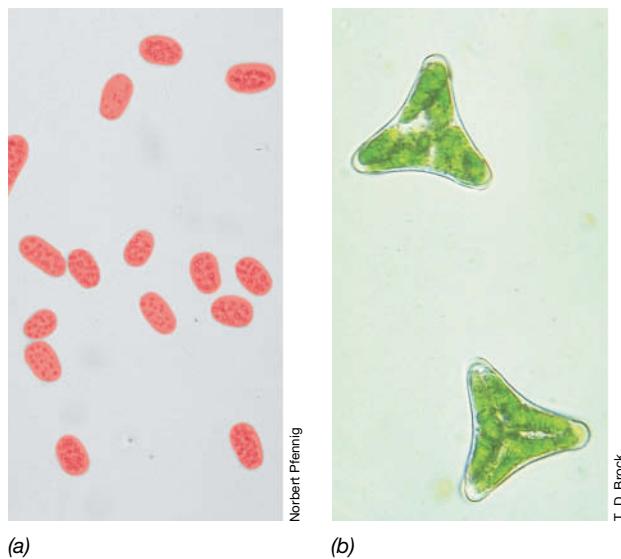
differently from their surroundings. Bacterial cells typically lack contrast, that is, their optical properties are similar to the surrounding medium, and hence they are difficult to see well with the bright-field microscope. Pigmented microorganisms are an exception because the color of the organism adds contrast, thus improving visualization by bright-field optics (Figure 1.16). For cells lacking pigments there are several ways to boost contrast, and we consider these methods in the next section.

### MINIQUIZ

- Define the terms magnification and resolution.
- What is the limit of resolution for a bright-field microscope? What defines this limit?

## 1.6 Improving Contrast in Light Microscopy

Contrast is necessary in light microscopy to distinguish microorganisms from their surroundings. Cells can be stained to improve contrast, and staining is commonly used to visualize bacteria with bright-field microscopy. In addition to staining, other methods of light microscopy have been developed to improve contrast, such as phase contrast, differential interference contrast, dark field, and fluorescence.



**Figure 1.16 Bright-field photomicrographs of pigmented microorganisms.** (a) Purple phototrophic bacteria (*Bacteria*). The bacterial cells are about 5  $\mu\text{m}$  wide. (b) A green alga (eukaryote). The green structures are chloroplasts. The algal cells are about 15  $\mu\text{m}$  wide. Purple bacteria are anoxygenic phototrophs, whereas algae are oxygenic phototrophs. Both groups contain photosynthetic pigments but only oxygenic phototrophs produce  $\text{O}_2$  (Section 1.3 and Figure 1.5a).

## Staining: Increasing Contrast for Bright-Field Microscopy

Dyes can be used to stain cells and increase their contrast so that they can be more easily seen in the bright-field microscope. Each class of dye has an affinity for specific cellular materials. Many dyes used in microbiology are positively charged, and for this reason they are called *basic dyes*. Examples of basic dyes include methylene blue, crystal violet, and safranin. Basic dyes bind strongly to negatively charged cell components, such as nucleic acids and acidic polysaccharides. These dyes also stain the surfaces of cells, because cell surfaces tend to be negatively charged. These properties make basic dyes useful general-purpose stains that nonspecifically stain most bacterial cells.

To perform a simple stain, one begins with dried preparations of cells (Figure 1.17). A clean glass slide containing a dried suspension of cells is flooded for a minute or two with a dilute solution of a basic dye, rinsed several times in water, and blotted dry. Because their cells are so small, it is common to observe dried, stained preparations of bacterial cells with a high-power (oil-immersion) lens.

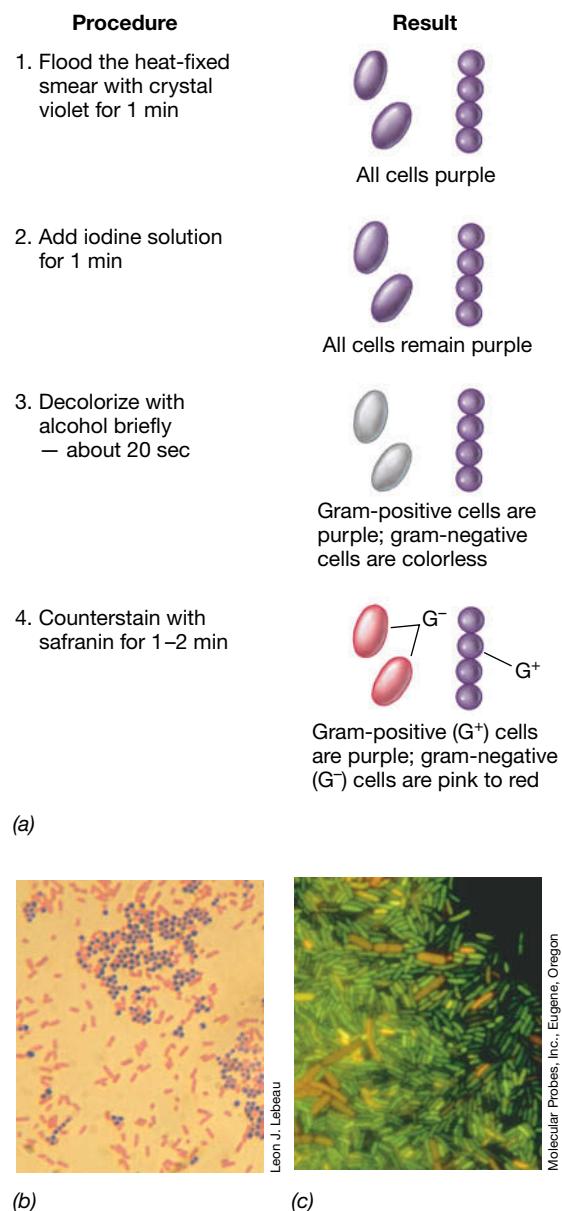
## Differential Stains: The Gram Stain

Stains that render different kinds of cells different colors are called *differential stains*. An important differential-staining procedure used in microbiology is the **Gram stain** (Figure 1.18). On the basis of their reaction in the Gram stain, bacteria can be divided into two



**Figure 1.17 Staining cells for microscopic observation.** Stains improve the contrast between cells and their background. Step 3 center: Same cells as shown in Figure 1.15 inset but stained with a basic dye.

major groups: **gram-positive** and **gram-negative**. After Gram staining, gram-positive bacteria appear purple-violet and gram-negative bacteria appear pink (Figure 1.18b). The color difference in the Gram stain arises because of differences in the cell wall structure of gram-positive and gram-negative cells (► Section 2.4). Staining with a basic dye such as crystal violet renders cells purple in color. Cells are then treated with ethanol, which decolorizes gram-negative cells but not gram-positive cells. Finally, cells are counterstained with a different-colored stain, typically the red stain safranin. As a result, gram-positive and gram-negative cells can be distinguished microscopically by their different colors (Figure 1.18b).



**Figure 1.18 The Gram stain.** (a) Steps in the procedure. (b) Microscopic observation of gram-positive (purple) and gram-negative (pink) bacteria. The organisms are *Staphylococcus aureus* and *Escherichia coli*, respectively. (c) Cells of *Pseudomonas aeruginosa* (gram-negative, green) and *Bacillus cereus* (gram-positive, orange) stained with a one-step fluorescent staining method. This method allows for differentiating gram-positive from gram-negative cells in a single staining step.

The Gram stain is the most common staining procedure used in microbiology, and it is often performed to begin the characterization of a new bacterium. If a fluorescence microscope is available, the Gram stain can be reduced to a one-step procedure; gram-positive and gram-negative cells fluoresce different colors when treated with a special chemical (Figure 1.18c).

### Phase-Contrast and Dark-Field Microscopy

Although staining is widely used in light microscopy, staining often kills cells and can distort their features. Two forms of light microscopy improve image contrast of unstained (and thus live) cells. These are phase-contrast microscopy and dark-field microscopy (Figure 1.19). The phase-contrast microscope in particular is widely used in teaching and research for the observation of living preparations.

Phase-contrast microscopy is based on the principle that cells differ in refractive index (that is, the ability of a material to alter the speed of light) from their surroundings. Light passing through a cell thus differs in phase from light passing through the surrounding liquid. This subtle difference is amplified by a device in the objective lens of the phase-contrast microscope called the *phase ring*, resulting in a dark image on a light background (Figure 1.19b; see also inset to Figure 1.15a). The ring consists of a phase plate that amplifies the variation in phase to produce the higher-contrast image.

In the dark-field microscope, light does not pass through the specimen. Instead, light is directed from the sides of the specimen and only light that is scattered when it hits the specimen can reach the lens. Thus, the specimen appears light on a dark background (Figure 1.19c). Dark-field microscopy often has better resolution than light microscopy, and some objects can be resolved by dark-field that cannot be resolved by bright-field or even by phase-contrast microscopes. Dark-field microscopy is a particularly good way to observe microbial motility, as bundles of flagella (the structures responsible for swimming motility) are often resolvable with this technique.

### Fluorescence Microscopy

The fluorescence microscope visualizes specimens that fluoresce. In fluorescence microscopy, cells are made to fluoresce (to emit light) by illuminating them from above with light of a single color. Filters are used so that only fluorescent light is seen, and thus cells appear to glow in a black background (Figure 1.20).

Cells fluoresce either because they contain naturally fluorescent substances such as chlorophyll (autofluorescence, Figure 1.20b, d) or because they have been stained with a fluorescent dye (Figure 1.20e). DAPI (*4',6-diamidino-2-phenylindole*) is a widely used fluorescent dye that stains cells bright blue because it complexes with the cell's DNA (Figure 1.20e). DAPI can be used to visualize cells in their natural habitats, such as soil, water, food, or a clinical specimen. Fluorescence microscopy using DAPI is widely used in clinical diagnostic microbiology and also in microbial ecology for enumerating bacteria in a natural environment or in a cell suspension (Figure 1.20e).

### MINIQUIZ

- What color will a gram-negative cell be after Gram staining by the conventional method?
- What major advantage does phase-contrast microscopy have over staining?
- How can cells be made to fluoresce?

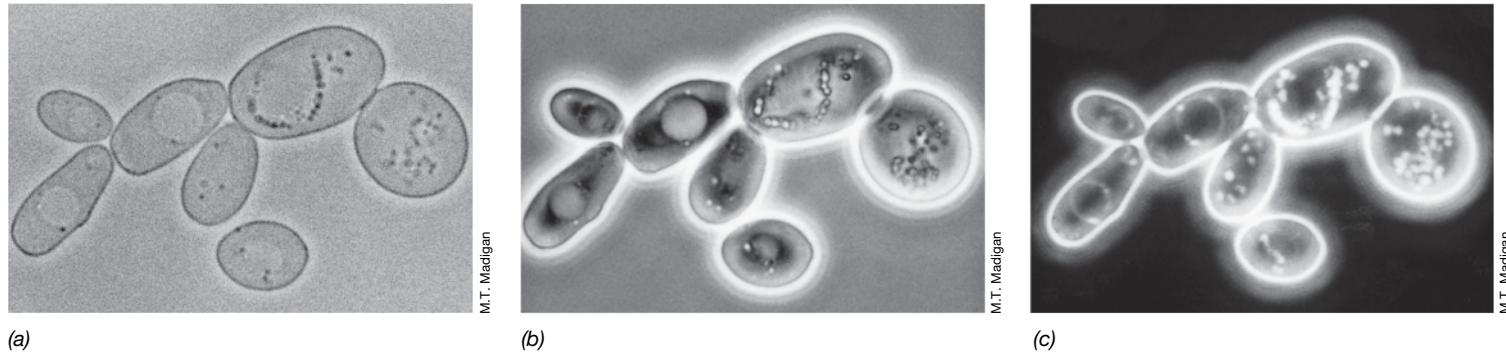
## 1.7 Imaging Cells in Three Dimensions

Thus far we have only considered forms of microscopy in which the rendered images are two-dimensional. Two methods of light microscopy can render a more three-dimensional image, and in this section we explore these forms of microscopy.

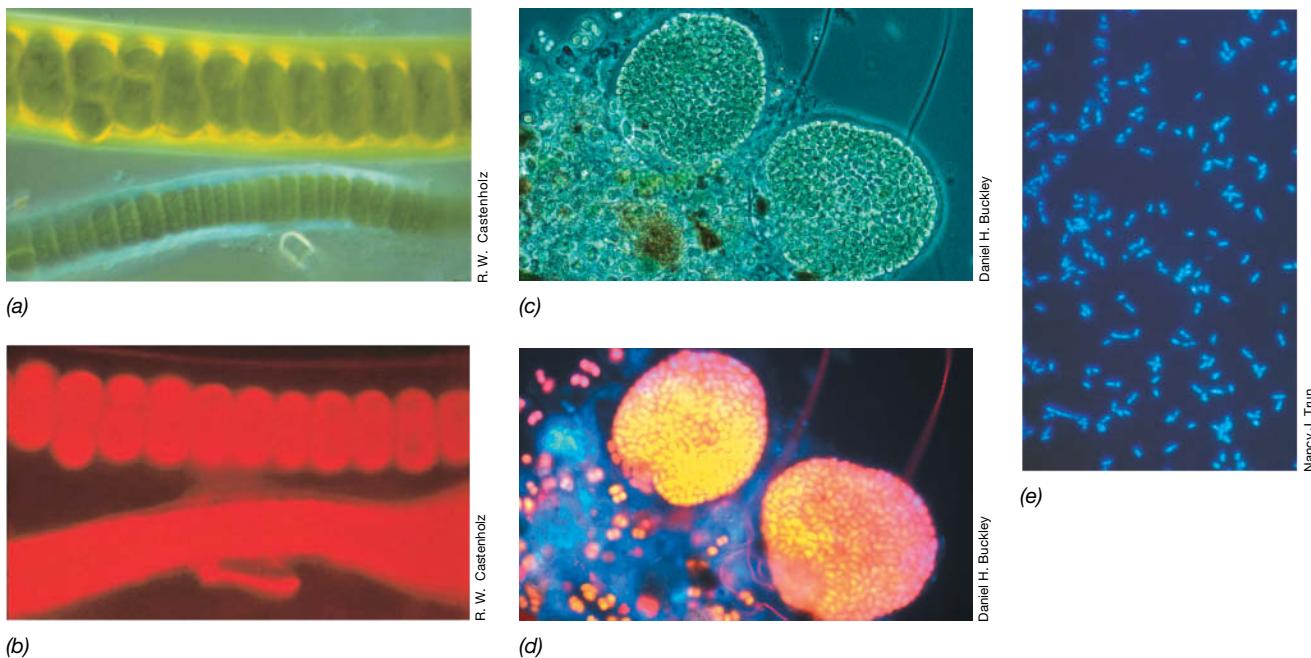
### Differential Interference Contrast Microscopy

Differential interference contrast (DIC) microscopy is a form of light microscopy that employs a polarizer in the condenser to produce polarized light (light in a single plane). The polarized light then passes through a prism that generates two distinct beams. These beams pass through the specimen and enter the objective lens, where they are recombined into one. Because the two beams pass through substances that differ in refractive index, the combined beams are not totally in phase but instead interfere with each other. This optical effect provides a three-dimensional perspective, which enhances subtle differences in cell structure.

Using DIC microscopy, cellular structures such as the nucleus of eukaryotic cells (Figure 1.21), or endospores, vacuoles, and inclusions of bacterial cells, appear more three-dimensional than in



**Figure 1.19 Cells visualized by different types of light microscopy.** The same field of cells of the yeast *Saccharomyces cerevisiae* visualized by (a) bright-field microscopy, (b) phase-contrast microscopy, and (c) dark-field microscopy. Cells average 8–10  $\mu\text{m}$  wide.



**Figure 1.20** Fluorescence microscopy. (a, b, c, d) Cyanobacteria. The same cells are observed by phase-contrast microscopy (a, c) and by fluorescence microscopy (b, d). The cells fluoresce because they

contain chlorophyll *a* and other pigments. The image in *b* was generated using a filter specific for the fluorescence of chlorophyll *a*, while the image in *d* was generated using a permissive filter that shows fluorescence from a

range of pigments that occur naturally in cyanobacteria. (e) Fluorescence photomicrograph of cells of *Escherichia coli* made fluorescent by staining with the fluorescent dye DAPI, which binds to DNA.

other forms of light microscopy. DIC microscopy is typically used on unstained cells as it can reveal internal cell structures that are nearly invisible by bright-field microscopy without the need for staining (compare Figure 1.19*a* with Figure 1.21).

### Confocal Scanning Laser Microscopy

A confocal scanning laser microscope (CSLM) is a computer-controlled microscope that couples a laser to a fluorescence microscope. The laser generates a high-contrast, three-dimensional image and allows the viewer to access several planes of focus in the specimen (Figure 1.22). The laser beam is precisely adjusted such that only a particular layer within a specimen is in perfect focus at

one time. Cells struck by the laser fluoresce to generate the image as in fluorescence microscopy (Section 1.6).

Cells in CSLM preparations can also be stained with fluorescent dyes to make them more distinct (Figure 1.22*a*). The laser then scans up and down through the layers of the sample, generating an image for each layer. A computer assembles the pictures to compose the many layers into a single high-resolution, three-dimensional image. Thus, for a relatively thick specimen such as a bacterial biofilm (Figure 1.22*a*), not only can cells on the surface of the biofilm be observed, as with conventional light microscopy, but cells in the various layers are also observed by adjusting the plane of focus of the laser beam. CSLM is particularly useful when thick specimens need to be examined.



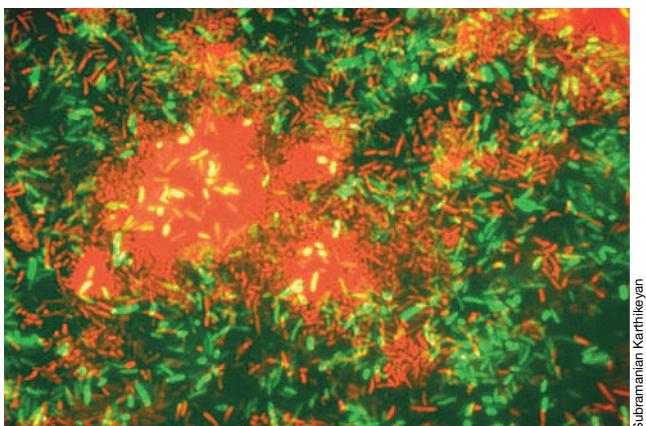
**Figure 1.21** Differential interference contrast microscopy. The yeast cells are about 8  $\mu\text{m}$  wide. Note the clearly visible nucleus and compare to the bright-field image of yeast cells in Figure 1.19*a*.

### MINIQUIZ

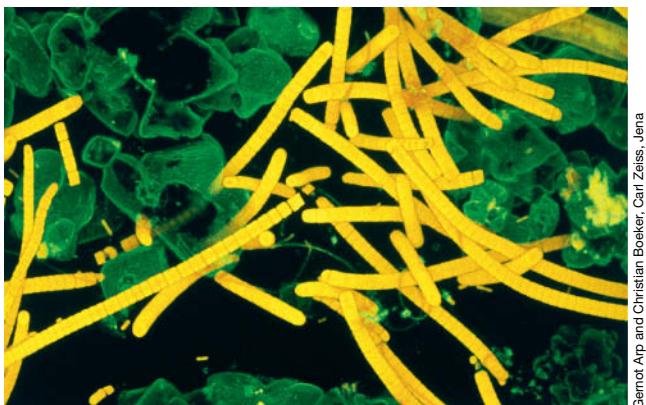
- What structure in eukaryotic cells is more easily seen in DIC than in bright-field microscopy? (Hint: Compare Figures 1.19*a* and 1.21).
- Why is CSLM able to view different layers in a thick preparation while bright-field microscopy cannot?

## 1.8 Probing Cell Structure: Electron Microscopy

Electron microscopes use electrons instead of visible light (photons) to image cells and cell structures. In the electron microscope, electromagnets function as lenses, and the whole system operates in a vacuum (Figure 1.23). Electron microscopes are fitted with



(a)



(b)

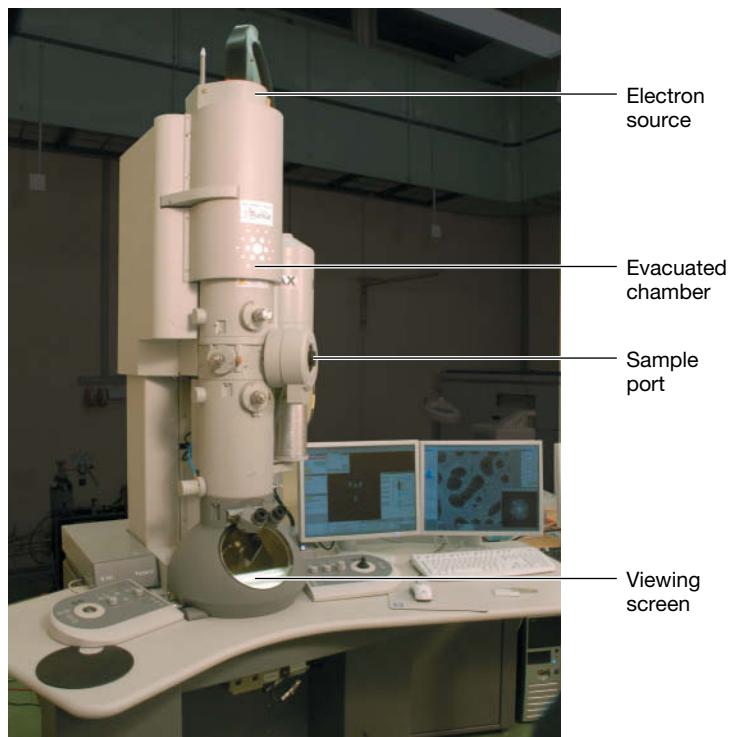
**Figure 1.22 Confocal scanning laser microscopy.** (a) Confocal image of a microbial biofilm community. The green, rod-shaped cells are *Pseudomonas aeruginosa* experimentally introduced into the biofilm. Cells of different colors are present at different depths in the biofilm. (b) Confocal image of a filamentous cyanobacterium growing in a soda lake. Cells are about 5  $\mu\text{m}$  wide.

cameras to allow a photograph, called an *electron micrograph*, to be taken. Two types of electron microscopy are in routine use in microbiology: transmission and scanning.

### Transmission Electron Microscopy

The *transmission electron microscope* (TEM) is used to examine cells and cell structure at very high magnification and resolution. The resolving power of a TEM is much greater than that of the light microscope, even allowing one to view structures at the molecular level (Figure 1.24). This is because the wavelength of electrons is much shorter than the wavelength of visible light, and, as we have learned, wavelength affects resolution (Section 1.5). For example, whereas the resolving power of a light microscope is about 0.2 *micrometer*, the resolving power of a TEM is about 0.2 *nanometer*, a thousandfold improvement. With such powerful resolution, objects as small as individual protein and nucleic acid molecules can be visualized by transmission electron microscopy (Figure 1.24b).

Unlike photons, electrons are very poor at penetrating; even a single cell is too thick to penetrate with an electron beam. Consequently, to view the internal structure of a cell, *thin sections* of the



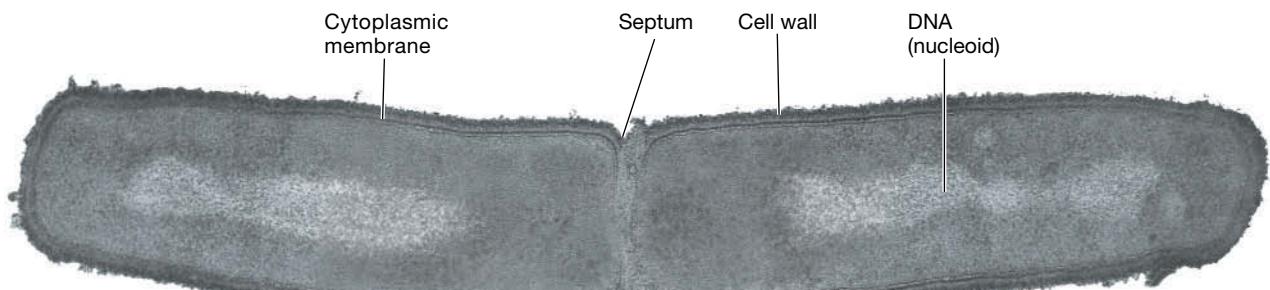
**Figure 1.23 The electron microscope.** This instrument encompasses both transmission and scanning electron microscope functions.

cell are needed, and the sections must be stabilized and stained with various chemicals to make them visible. A single bacterial cell, for instance, is cut into extremely thin (20–60 nm) slices, which are then examined individually by TEM (Figure 1.24a). To obtain sufficient contrast, the sections are treated with stains such as osmic acid, or permanganate, uranium, lanthanum, or lead salts. Because these substances are composed of atoms of high atomic weight, they scatter electrons well and thus improve contrast. If only the *external* features of an organism are to be observed, thin sections are unnecessary. Intact cells or cell components can be observed directly in the TEM by a technique called *negative staining* (Figure 1.24b).

### Scanning Electron Microscopy

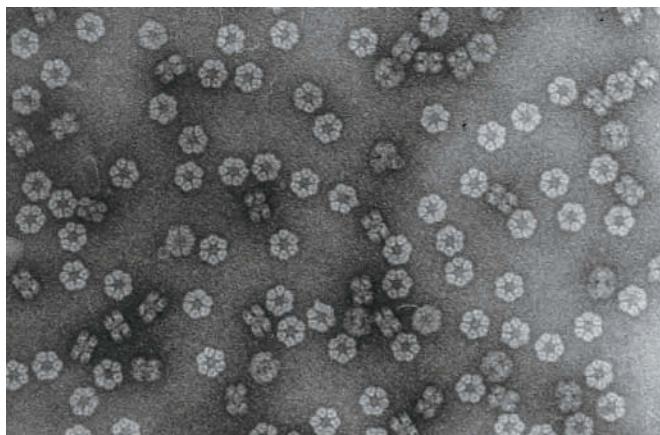
For optimal three-dimensional imaging of cells, a *scanning electron microscope* (SEM) is used. In scanning electron microscopy, the specimen is coated with a thin film of a heavy metal, typically gold. An electron beam then scans back and forth across the specimen. Electrons scattered from the metal coating are collected and projected on a monitor to produce an image (Figure 1.24c). In the SEM, even fairly large specimens can be observed, and the depth of field (the portion of the image that remains in sharp focus) is extremely good. A wide range of magnifications can be obtained with the SEM, from as low as 15 $\times$  up to about 100,000 $\times$ , but only the *surface* of an object is typically visualized.

Electron micrographs taken by either TEM or SEM are black-and-white images. Although the original image contains the maximum amount of scientific information that is available, color is often added to scanning electron micrographs by manipulating

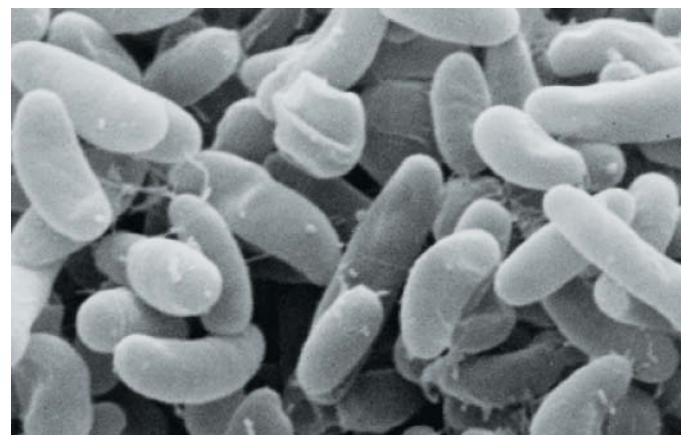


Stanley C. Holt

(a)



(b)



(c)

**Figure 1.24 Electron micrographs.** (a) Micrograph of a thin section of a dividing bacterial cell, taken by transmission electron microscopy (TEM). The cell is about 0.8  $\mu\text{m}$  wide. (b) TEM of negatively stained molecules of hemoglobin. Each hexagonal-shaped molecule is about 25 nanometers (nm) in diameter and consists of two doughnut-shaped rings, a total of 15 nm wide. (c) Scanning electron micrograph (SEM) of bacterial cells. A single cell is about 0.75  $\mu\text{m}$  wide.

them in a computer. However, such false color does not improve resolution of a micrograph. In this book, false color will be used sparingly in electron micrographs so as to present the micrographs in their original scientific context.

### MINIQUIZ

- What is an electron micrograph? Why do electron micrographs have greater resolution than light micrographs?
- What type of electron microscope would be used to view a cluster of cells? What type would be used to observe internal cell structure?

included the development of **aseptic technique**, which is a collection of practices that allow for the preparation and maintenance of **sterile** (that is, without the presence of living organisms) nutrient media and solutions (► Figure 5.12). Aseptic technique is essential for the isolation and maintenance of pure cultures of bacteria. **Pure cultures** are those that contain cells from only a single type of microorganism and are of great value for the study of microorganisms. Finally, **enrichment culture techniques**, which allow for the isolation from nature of microbes having particular metabolic characteristics, facilitate the discovery of diverse microorganisms.

Advances in microbial cultivation are directly responsible for success in fighting infectious disease, the discovery of microbial diversity, and the use of microbes as model systems to discover the fundamental properties of all living cells. Important advances in microbial cultivation occurred in the nineteenth century as microbiologists sought to answer two major questions of that time: (1) Does spontaneous generation occur? (2) What is the nature of infectious disease? Answers to these seminal questions emerged from the work of two giants in the field of microbiology: the French chemist Louis Pasteur and the German physician Robert Koch. We begin with the work of Pasteur.

## III • Microbial Cultivation Expands the Horizon of Microbiology

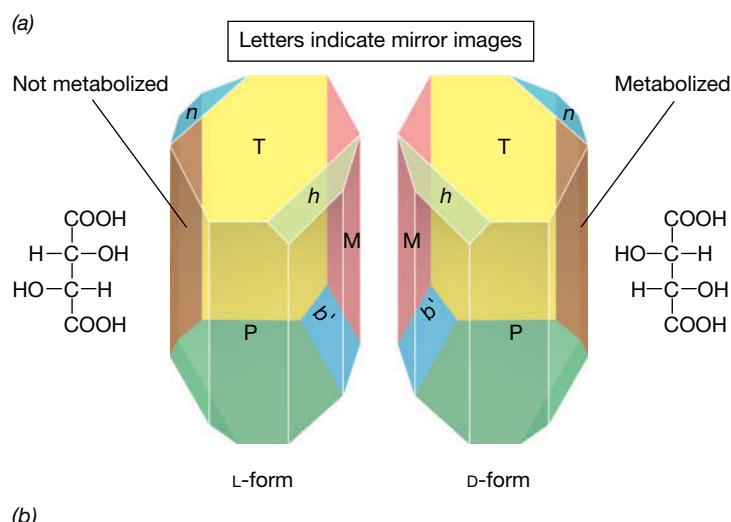
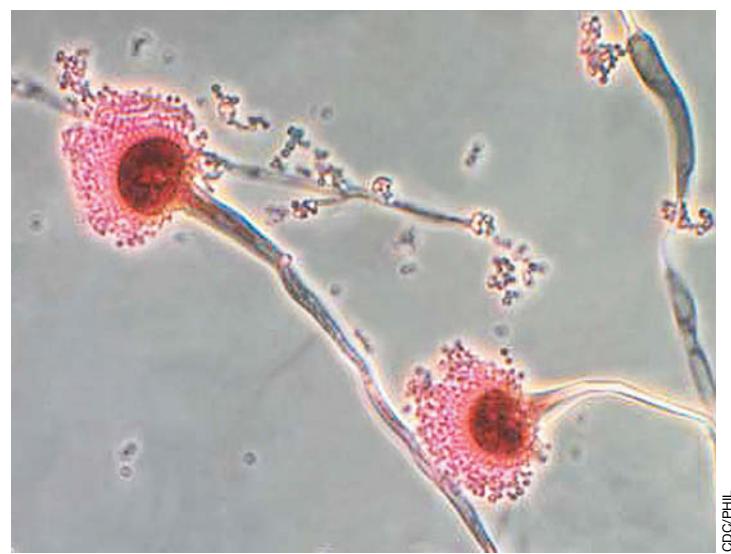
Following the discovery of microorganisms driven by microscopic methods, major discoveries in microbiology were fueled by advances in microbial cultivation. Important advances

## 1.9 Pasteur and Spontaneous Generation

Pasteur was a chemist by training and was one of the first to recognize that many of what were thought to be strictly chemical reactions were actually catalyzed by microorganisms. Pasteur studied the chemistry of crystal formation and he used microscopes to examine crystal structure. His training in chemistry and microscopy prepared him to make a series of foundational discoveries to further the science of microbiology.

### The Microbial Basis of Fermentation

Early in his career, Pasteur studied crystals formed during the production of alcohol. Through careful microscopic observation of tartaric acid crystals formed in wine, he observed two types of crystals that had mirror-image structures (Figure 1.25). He separated these by hand and observed that each type of crystal bent a beam of polarized light in a different direction. In this way he discovered that



**Figure 1.25** Louis Pasteur and optical isomers. (a) Light micrograph of cells of the mold *Aspergillus*. (b) Pasteur's drawings of crystals of tartaric acid. Left-handed L-form crystals bend light to the left, and right-handed D-form crystals bend light to the right. Note that the two crystals are mirror images of one another, a hallmark of optical isomers. Pasteur found that only D-tartrate was metabolized by *Aspergillus*.

chemically identical substances can have *optical isomers*, which have different molecular structures that can influence their properties. Pasteur went on to discover that microorganisms could discriminate between optical isomers. For example, cultures of the mold *Aspergillus* (Figure 1.25a) metabolized exclusively D-tartrate but not its optical isomer, L-tartrate (Figure 1.25b). The fact that a living organism could discriminate between optical isomers led Pasteur to suspect that many reactions that were thought to be purely chemical in nature were actually catalyzed by specific microorganisms.

While a professor of chemistry, Pasteur encountered a local businessman who produced alcohol industrially from beet juice. The businessman was losing money because many of his vats produced, instead of alcohol, a product that smelled like sour milk, which Pasteur determined to be lactic acid. In the mid-nineteenth century the production of alcohol was thought to be solely a chemical process. Pasteur studied the broth with his microscope, but instead of crystals he observed cells. Pasteur observed that the vats that produced alcohol were full of yeast, but the sour vats were full of rod-shaped bacteria. He hypothesized that these were living organisms whose growth produced either alcohol or lactic acid.

Pasteur needed to grow these organisms to prove his hypothesis. He prepared an extract of yeast cells, deducing that this would contain all of the nutrients that yeast need to grow. He then used a porcelain filter to remove all cells from this yeast extract nutrient medium, rendering it sterile. If he introduced living yeast back into this sterile yeast extract medium he could observe their growth and show the production of alcohol, but if he instead introduced the small rods he then observed lactic acid formation. Heating of these cultures eliminated growth *and* the production of either alcohol or lactic acid. In this way he proved that fermentation is carried out by microorganisms and that different microorganisms perform different fermentation reactions.

During his work on fermentation, Pasteur observed that other organisms would often grow in his yeast extract medium. He deduced that these organisms were being introduced from the air. Pasteur's work on fermentation had prepared him to conduct a series of classic experiments on spontaneous generation, experiments that are forever linked to his name and which helped establish microbiology as a modern science.

### Spontaneous Generation

The concept of **spontaneous generation** existed for thousands of years and its basic tenet can be easily grasped. If food or some other perishable material is allowed to stand for some time, it putrefies. When examined microscopically, the putrefied material is teeming with microorganisms. From where do these organisms arise? Prior to Pasteur it was common belief that life arose spontaneously from nonliving materials, that is, by *spontaneous generation*.

Pasteur became a powerful opponent of spontaneous generation. He predicted that microorganisms in putrefying materials were descendants of cells that entered from the air or cells that had been initially present on the decaying materials. Pasteur reasoned that if food were treated in such a way as to destroy all living organisms present—that is, if it were rendered sterile—and if it were kept sterile, it would not putrefy.

Pasteur used heat to kill contaminating microorganisms, and he found that extensive heating of a nutrient solution followed by

sealing kept it from putrefying. Proponents of spontaneous generation criticized these experiments by declaring that “fresh air” was necessary for the phenomenon to occur. In 1864 Pasteur countered this objection simply and brilliantly by constructing a swan-necked flask, now called a *Pasteur flask* (Figure 1.26). In such a flask, nutrient solutions could be heated to boiling and sterilized. After the flask cooled, air could reenter, but the bend in the neck prevented particulate matter (including microorganisms) from entering the nutrient solution and initiating putrefaction. Nutrient solutions in such flasks remained sterile indefinitely. Microbial growth was observed only after particulate matter from the neck of the flask was allowed to enter the liquid in the flask (Figure 1.26c). This experiment settled the spontaneous generation controversy forever.

Pasteur’s work on spontaneous generation demonstrated the importance of sterilization and led to the development of effective sterilization procedures that were eventually standardized and applied widely in microbiology, medicine, and industry. For example, the British physician Joseph Lister (1827–1912) deduced from Pasteur’s discoveries that surgical infections were caused by microorganisms. He implemented a range of techniques designed to kill microorganisms and to prevent microbial infection of surgical patients. Lister is credited with the introduction of aseptic techniques for surgeries (1867), and his methods were adopted worldwide; these greatly improved the survival rate of surgical patients. The food industry also benefited from the work of Pasteur, as his principles were quickly adapted for the preservation of milk and many other foods by heat treatment, which we now call *pasteurization*.

### Other Accomplishments of Pasteur

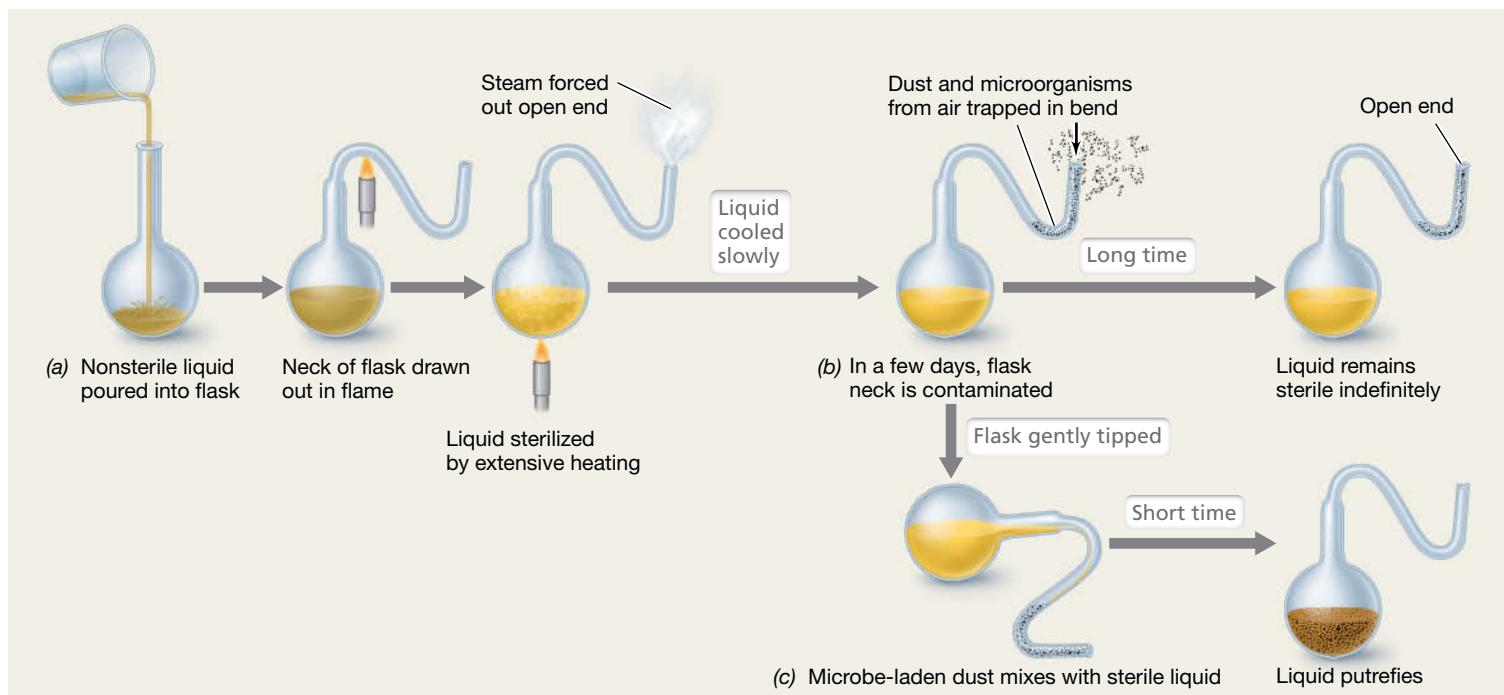
Pasteur went on to many other triumphs in microbiology and medicine. Some highlights include his development of vaccines

for the diseases anthrax, fowl cholera, and rabies. Pasteur’s work on rabies was his most famous success, culminating in July 1885 with the first administration of a rabies vaccine to a human, a young French boy named Joseph Meister who had been bitten by a rabid dog. In those days, a bite from a rabid animal was invariably fatal. News spread quickly of the success of Meister’s vaccination, and of one administered shortly thereafter to a young shepherd boy, Jean-Baptiste Jupille (Figure 1.27a). Within a year several thousand people bitten by rabid animals had traveled to Paris to be treated with Pasteur’s rabies vaccine.

Pasteur’s fame was legendary and led the French government to establish the Pasteur Institute in Paris in 1888 (Figure 1.27b). Originally established as a clinical center for the treatment of rabies and other contagious diseases, the Pasteur Institute today is a major biomedical research center focused on antiserum and vaccine research and production. The medical and veterinary breakthroughs of Pasteur not only were highly significant in their own right but helped solidify the concept of the germ theory of disease, whose principles were being developed at about the same time by a second giant of this era, Robert Koch.

### MINIQUIZ

- Define the term *sterile*. What two methods did Pasteur used to make solutions *sterile*?
- How did Pasteur’s experiments using swan-necked flasks defeat the theory of spontaneous generation?
- Besides ending the controversy over spontaneous generation, what other accomplishments do we credit to Pasteur?



**Figure 1.26** The defeat of spontaneous generation: Pasteur’s swan-necked flask experiment. In (c) the liquid putrefies because microorganisms enter with the dust. The bend in the flask allowed air to enter (a key objection to Pasteur’s sealed flasks) but prevented microorganisms from entering.



(a)



(b)

**Figure 1.27 Louis Pasteur and some symbols of his contributions to microbiology.** (a) A French 5-franc note honoring Pasteur. The shepherd boy Jean-Baptiste Jupille is shown killing a rabid dog that had attacked children. Pasteur's rabies vaccine saved Jupille's life. In France, the franc preceded the euro as a currency. (b) Part of the Pasteur Institute, Paris, France. Today this structure, built for Pasteur by the French government, houses a museum that displays some of the original swan-necked flasks used in his experiments and a chapel containing Pasteur's crypt.

## 1.10 Koch, Infectious Diseases, and Pure Cultures

Proof that some microorganisms can cause disease provided the greatest impetus for the development of microbiology as an independent biological science. As early as the sixteenth century it was suspected that some agent of disease could be transmitted from a diseased person to a healthy person. After microorganisms were discovered, a number of individuals proposed that they caused infectious diseases, but skepticism prevailed and definitive proof was lacking. As early as 1847, the Hungarian physician Ignaz Semmelweis promoted sanitary methods including hand washing as a method for preventing infections. His methods are credited with saving many lives, but he could not prove why these methods worked and his advice was met with scorn by most of the medical community. The work of Pasteur and Lister provided strong evidence that microbes were the cause of infectious disease, but it was not until the work of the German physician Robert Koch

(1843–1910) (Figure 1.28) that the germ theory of infectious disease had direct experimental support.

### The Germ Theory of Disease and Koch's Postulates

In his early work Koch studied anthrax, a disease of cattle and occasionally of humans. Anthrax is caused by an endospore-forming bacterium called *Bacillus anthracis*. By careful microscopy and staining, Koch established that the bacteria were always present in the blood of an animal that was succumbing to the disease. However, Koch reasoned that the mere *association* of the bacterium with the disease was not actual proof of *cause and effect*, and he seized the opportunity to study cause and effect experimentally using anthrax and laboratory animals. The results of this study formed the standard by which infectious diseases have been studied ever since.

Koch used mice as experimental animals. Using appropriate controls, Koch demonstrated that when a small drop of blood from a mouse with anthrax was injected into a healthy mouse, the latter quickly developed anthrax. He took blood from this second animal, injected it into another, and again observed the characteristic disease symptoms. However, Koch carried this experiment a critically important step further. He discovered that the anthrax bacteria could be grown in a nutrient medium *outside the host* and



**Figure 1.28 Robert Koch.** The German physician and microbiologist is credited with founding medical microbiology and formulating his famous postulates.

that even after many transfers in laboratory culture, the bacteria still caused the disease when inoculated into a healthy animal.

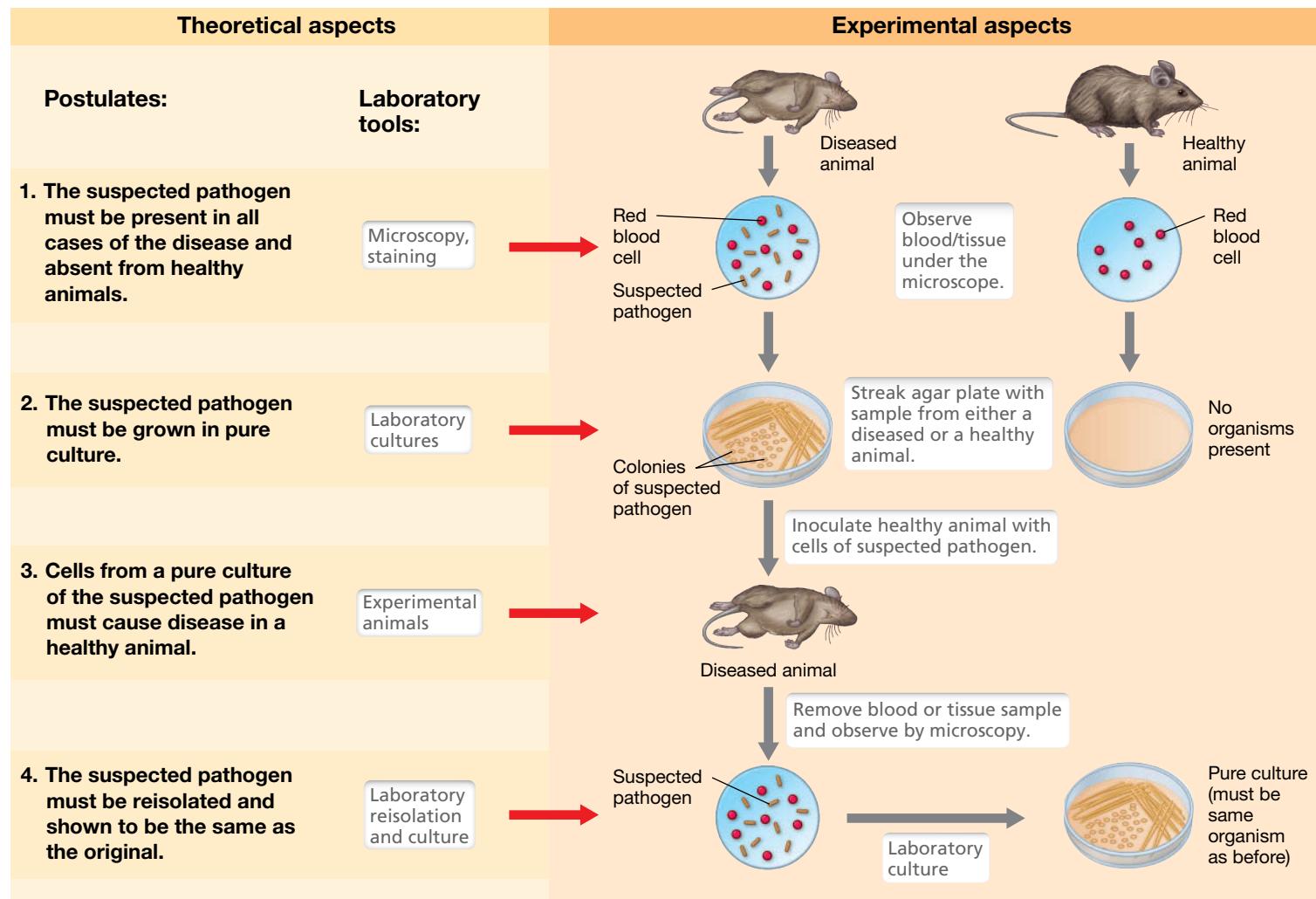
On the basis of these experiments and others on the causative agent of tuberculosis, Koch formulated a set of rigorous criteria, now known as **Koch's postulates**, for definitively linking cause and effect in an infectious disease. Koch's postulates, summarized in **Figure 1.29**, stressed the importance of *laboratory culture* of the putative infectious agent followed by introduction of the suspected agent into virgin animals and recovery of the pathogen from diseased or dead animals. With these postulates as a guide, Koch, his students, and those that followed them discovered the causative agents of most of the important infectious diseases of humans and domestic animals. These discoveries also led to the development of successful treatments for the prevention and cure of many of these diseases, greatly improving the scientific basis of clinical medicine and human health and welfare (Figure 1.8).

## Koch, Pure Cultures, and Microbial Taxonomy

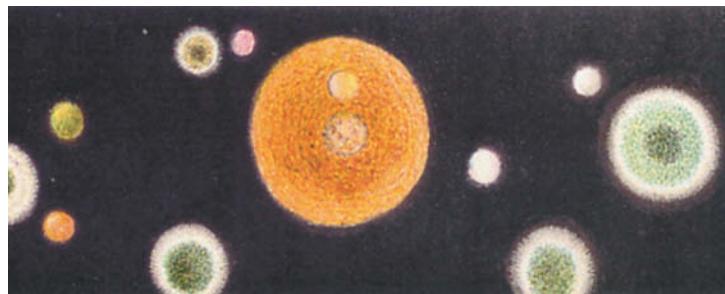
The second of Koch's postulates states that the suspected pathogen must be isolated and grown away from other microorganisms in laboratory culture (Figure 1.29); in microbiology we say that such a culture is *pure*. To accomplish this important goal, Koch and his associates developed several simple but ingenious methods of obtaining and growing bacteria in pure culture, and many of these methods are still used today.

Koch started by using natural surfaces such as a potato slice to obtain pure cultures, but he quickly developed more reliable and reproducible growth media employing liquid nutrient solutions solidified with gelatin, and later with agar, an algal polysaccharide with excellent properties for this purpose. Along with his associate Walther Hesse, Koch observed that when a solid surface was incubated in air, masses of microbial cells called colonies developed, each having a characteristic shape and color (Figure 1.30).

## KOCH'S POSTULATES



**Figure 1.29** Koch's postulates for proving cause and effect in infectious diseases. Note that following isolation of a pure culture of the suspected pathogen, the cultured organism must both initiate the disease and be recovered from the diseased animal. Establishing the correct conditions for growing the pathogen is essential; otherwise it will be missed.



**Figure 1.30** A hand-colored photograph taken by Walther Hesse of colonies formed on agar. The colonies include those of molds and bacteria obtained during Hesse's studies of the microbial content of air in Berlin, Germany, in 1882. From Hesse, W. 1884. "Ueber quantitative Bestimmung der in der Luft enthaltenen Mikroorganismen." *Mittheilungen aus dem Kaiserlichen Gesundheitsamte*. 2: 182–207.

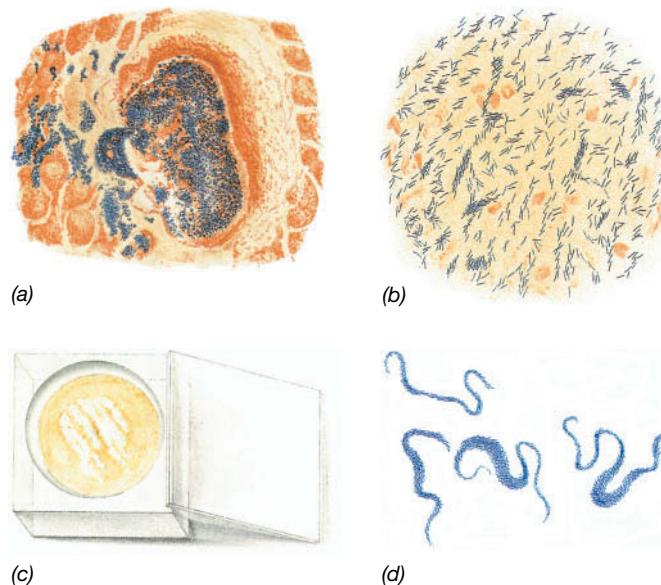
He inferred that each colony had arisen from a single bacterial cell that had grown to yield the mass of cells (see also Figure 1.2). Koch reasoned that each colony harbored a pure culture (a population of identical cells), and he quickly realized that solid media provided an easy way to obtain pure cultures. Richard Petri, another associate of Koch, developed the transparent double-sided "Petri dish" in 1887, and this quickly became the standard tool for obtaining pure cultures.

Koch was keenly aware of the implications his pure culture methods had for classifying microorganisms. He observed that colonies that differed in color and size (Figure 1.30) bred true and that cells from different colonies typically differed in size and shape and often in their nutrient requirements as well. Koch realized that these differences were analogous to the criteria taxonomists had established for the classification of larger organisms, such as plant and animal species, and he suggested that the different types of bacteria should be considered as "species, varieties, forms, or other suitable designation." Such insightful thinking was important for the rapid acceptance of microbiology as a new biological science, rooted as biology was in classification during Koch's era.

### Koch and Tuberculosis

Koch's crowning scientific accomplishment was his discovery of the causative agent of tuberculosis. At the time Koch began this work (1881), one-seventh of all reported human deaths were caused by tuberculosis (Figure 1.8). There was a strong suspicion that tuberculosis was a contagious disease, but the suspected agent had never been seen, either in diseased tissues or in culture. Following his successful studies of anthrax, Koch set out to demonstrate the cause of tuberculosis, and to this end he brought together all of the methods he had so carefully developed in his previous studies with anthrax: microscopy, staining, pure culture isolation, and an animal model system (Figure 1.29).

The bacterium that causes tuberculosis, *Mycobacterium tuberculosis*, is very difficult to stain because *M. tuberculosis* cells contain large amounts of a waxlike lipid in their cell walls. Nevertheless, Koch devised a staining procedure for *M. tuberculosis* cells in lung tissue samples. Using this method, he observed the blue, rod-shaped cells of *M. tuberculosis* in tubercular tissues but not in healthy tissues (Figure 1.31). Obtaining cultures of *M. tuberculosis*



**Figure 1.31** Robert Koch's drawings of *Mycobacterium tuberculosis*.

(a) Section through infected lung tissue showing cells of *M. tuberculosis* (blue).  
(b) *M. tuberculosis* cells in a sputum sample from a tubercular patient.  
(c) Growth of *M. tuberculosis* on a glass plate of coagulated blood serum stored inside a glass box to prevent contamination.  
(d) *M. tuberculosis* cells taken from the plate in c and observed microscopically; cells appear as long, cordlike forms. Original drawings from Koch, R. 1884. "Die Aetiologie der Tuberkulose." *Mittheilungen aus dem Kaiserlichen Gesundheitsamte* 2: 1–88.

was not easy, but eventually Koch succeeded in growing colonies of this organism on a solidified medium containing blood serum. Under the best of conditions, *M. tuberculosis* grows slowly in culture, but Koch's persistence and patience eventually led to pure cultures of this organism from human and animal sources.

From this point Koch used his postulates (Figure 1.29) to obtain definitive proof that the organism he had isolated was the cause of the disease tuberculosis. Guinea pigs can be readily infected with *M. tuberculosis* and eventually succumb to systemic tuberculosis. Koch showed that tuberculous guinea pigs contained masses of *M. tuberculosis* cells in their lungs and that pure cultures obtained from such animals transmitted the disease to healthy animals. In this way, Koch successfully satisfied all four of his postulates, and the cause of tuberculosis was understood. Koch announced his discovery of the cause of tuberculosis in 1882, and for this accomplishment he was awarded the 1905 Nobel Prize for Physiology or Medicine. Koch had many other triumphs in the growing field of infectious diseases, including the discovery of the causative agent of cholera (the bacterium *Vibrio cholerae*) and the development of methods to diagnose infection with *M. tuberculosis* (the tuberculin skin test).

### MINIQUIZ

- How do Koch's postulates ensure that cause and effect of a given disease are clearly differentiated?
- What advantages do solid media offer for the isolation of microorganisms?
- What is a pure culture?

## 1.11 Discovery of Microbial Diversity

As microbiology entered the twentieth century, its initial focus on basic principles, methods, and medical aspects broadened to include studies of the microbial diversity of soil and water and the metabolic processes that microorganisms carried out in these habitats. Major contributors of this era included the Dutchman Martinus Beijerinck and the Russian Sergei Winogradsky.

### Martinus Beijerinck and the Enrichment Culture Technique

Martinus Beijerinck (1851–1931) was a professor at the Delft Polytechnic School in Holland and was originally trained in botany, so he began his career in microbiology studying plants. Beijerinck's greatest contribution to the field of microbiology was his clear formulation of the *enrichment culture technique*.

The media of Pasteur and Koch were rich in nutrients and while they supported the growth of many organisms, they did not select for specific types of organisms. In enrichment culture, microorganisms are isolated by using highly selective media and incubation conditions that favor a particular metabolic group of organisms. For example, Beijerinck devised a precise chemically defined medium to isolate rhizobia and prove that they are responsible for the formation of the root nodules of legumes (Figure 1.9).

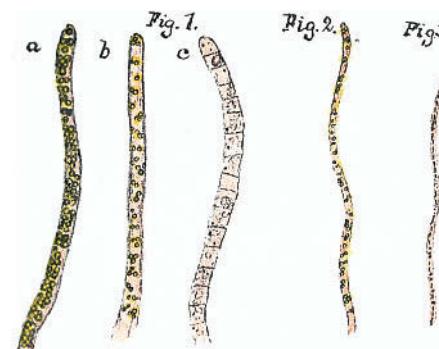
Using the enrichment culture technique, Beijerinck isolated the first pure cultures of many soil and aquatic microorganisms, including sulfate-reducing and sulfur-oxidizing bacteria, lactic acid bacteria, green algae, various anaerobic bacteria, and many others. In addition, in his classic studies of “mosaic disease” of tobacco, Beijerinck used selective filters to show that the infectious agent in this disease (a virus) was smaller than a bacterium and that it somehow became incorporated into cells of the living host plant. In this insightful work, Beijerinck described not only the first virus but also the basic principles of virology, which we expand upon in Chapters 8 and 10.

### Sergei Winogradsky, Chemolithotrophy, and Nitrogen Fixation

Like Beijerinck, Sergei Winogradsky (1856–1953) was interested in the bacterial diversity of soils and waters and was highly successful in isolating several notable bacteria from natural samples. Winogradsky was particularly interested in bacteria that cycle nitrogen and sulfur compounds, such as the nitrifying bacteria and the sulfur bacteria (Figure 1.32). He studied *Beggiatoa*, which are large bacteria commonly observed in marine sediments. He observed that *Beggiatoa* would not grow on the rich nutrient media used by Koch. He designed specific enrichment media to imitate the environment in which *Beggiatoa* lived. He showed that these bacteria catalyze specific chemical transformations in nature and proposed the important concept of **chemolithotrophy**, the oxidation of *inorganic* compounds to yield energy. Winogradsky further showed that these organisms, which he called *lithotrophs* (meaning, literally, “stone eaters”),



(a)



From Winogradsky, S. 1949.  
Microbiologie du Sol.  
Masson, Paris

(b)

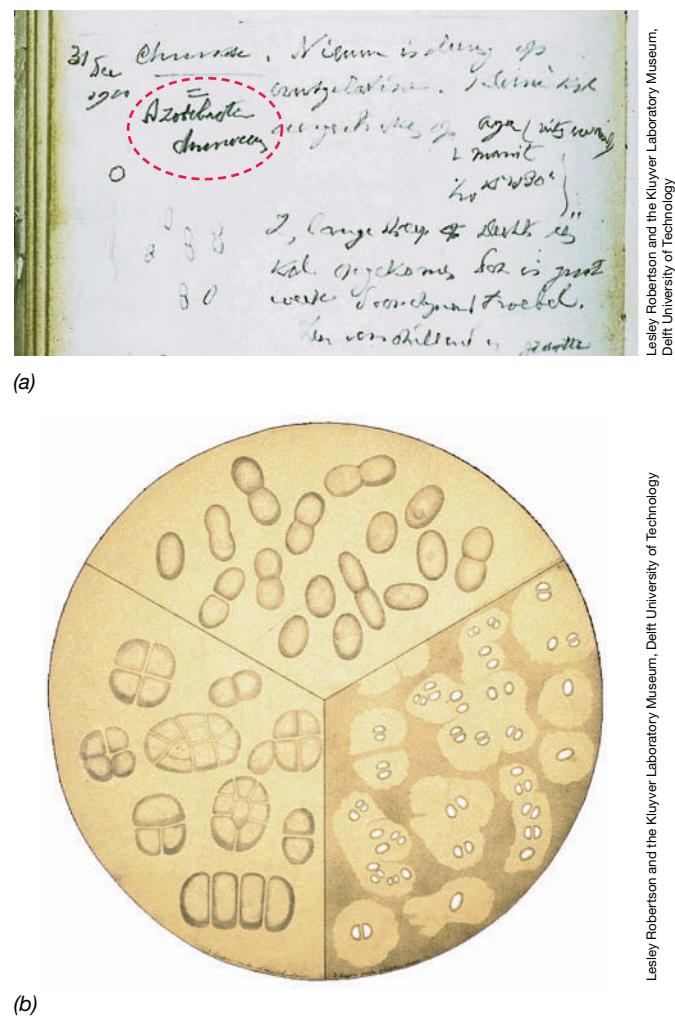
**Figure 1.32 Sulfur bacteria.** The original drawings were made by Sergei Winogradsky in the late 1880s and then copied and hand-colored by his wife Hélène. (a) Purple sulfur phototrophic bacteria. Figures 3 and 4 show cells of *Chromatium okenii* (compare with photomicrographs of *C. okenii* in Figures 1.1a and 1.6d, and 1.16a). (b) *Beggiaota*, a sulfur chemolithotroph (compare with Figure 15.27a).

are widespread in nature. Winogradsky thus revealed that, like photosynthetic organisms, chemolithotrophic bacteria obtain their carbon from CO<sub>2</sub>.

Winogradsky isolated diverse metabolic types of bacteria. Using an enrichment medium that lacked nitrogen, he isolated the anaerobic nitrogen-fixing bacterium *Clostridium pasteurianum*, becoming the first to demonstrate the process of nitrogen fixation. Beijerinck would use a similar technique shortly thereafter to isolate the first aerobic nitrogen-fixing bacterium, *Azotobacter* (Figure 1.33). Winogradsky also isolated the first nitrifying bacteria by using an enrichment medium that contained ammonium salts and CO<sub>2</sub>.

### MINIQUIZ

- What is meant by the term “enrichment culture”?
- What is meant by the term “chemolithotrophy”? In what way are chemolithotrophs like plants?



**Figure 1.33** Martinus Beijerinck and *Azotobacter*. (a) A page from the laboratory notebook of M. Beijerinck dated 31 December 1900 describing the aerobic nitrogen-fixing bacterium *Azotobacter chroococcum* (name circled in red). Compare Beijerinck's drawings of pairs of *A. chroococcum* cells with the photomicrograph of cells of *Azotobacter* in Figure 15.32a. (b) A painting by M. Beijerinck's sister, Henriëtte Beijerinck, showing cells of *A. chroococcum*. Beijerinck used such paintings to illustrate his lectures.

## IV • Molecular Biology and the Unity and Diversity of Life

The development of aseptic technique and methods for the enrichment, isolation, and propagation of bacteria at the end of the nineteenth century gave rise to explosive growth in the pace of microbiological discovery. Moreover, microbiologists realized that the ability to grow bacteria rapidly and in controlled laboratory conditions made them excellent model systems in which to explore the fundamental nature of life.

### 1.12 Molecular Basis of Life

Experiments with bacterial cultures in the twentieth century were critical in describing the foundations of molecular biology, molecular genetics, and biochemistry. Microbiologists came to realize

that while microorganisms were incredibly diverse, all cells operated on similar principles.

### Unity in Biochemistry

Albert Jan Kluyver (1888–1956) was Beijerinck's successor at what was then called the Delft Institute of Technology. Kluyver recognized that though microbial diversity was tremendous, microorganisms used many of the same biochemical pathways and their metabolic processes faced similar thermodynamic constraints. Kluyver promoted the study of comparative biochemistry to identify the unifying features of all cells. He famously proclaimed, “From elephant to butyric acid bacterium—it is all the same!” This was later reformulated by Jacques Monod (1910–1976) into the expression, “What is true for *E. coli* is also true for the elephant,” a statement that proclaimed the importance of working with bacteria to understand the fundamental principles that govern all living things.

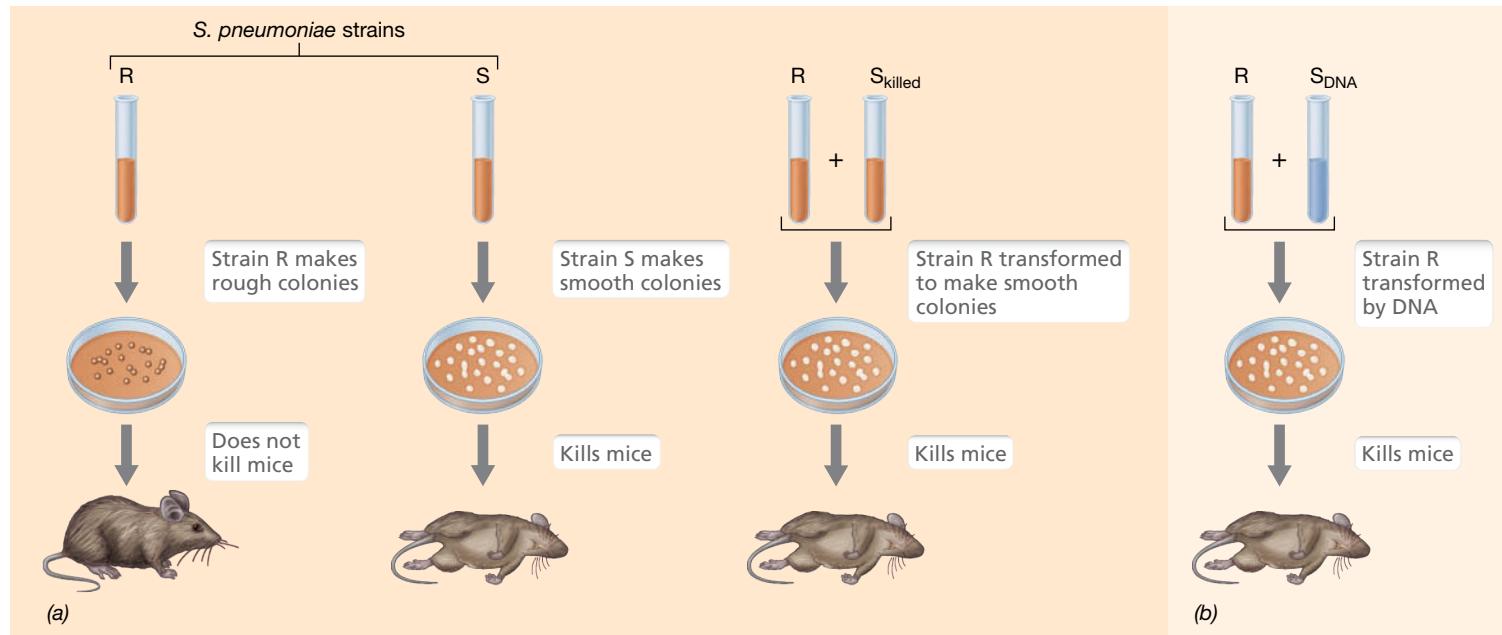
The use of microbes as metabolic model systems led to the discovery that certain macromolecules and biochemical reactions are universal, and that to understand their function in one cell is to understand their function in all cells. These discoveries were of central importance to understanding microbial evolution and none were as important as the discovery of DNA as the molecular basis of heredity, a discovery that is less than 80 years old.

### Cracking the Code of Life

In the early twentieth century, it was clear that some molecule carried the hereditary information from parent to offspring, but the molecular basis of heredity remained a mystery. Most biologists thought that proteins carried this hereditary information. DNA had been discovered but it was thought to be merely a structural molecule, and too simple in its composition to encode cellular functions. The hunt for the molecular basis of heredity began in earnest with an experiment by Frederick Griffith (1879–1941).

Griffith worked with a virulent strain of *Streptococcus pneumoniae*, a cause of bacterial pneumonia in both humans and mice. This strain, strain S, produced a polysaccharide coat (that is, a capsule, [Section 2.7](#)) that caused cells to form smooth colonies and conferred the ability to kill infected mice (**Figure 1.34a**). A related strain, strain R, lacked this polysaccharide and produced “rough” colonies that did not cause disease. However, Griffith observed that strain R could be *transformed* to type S, forming smooth colonies and causing disease, when it was mixed with the dead remains of cells of strain S (**Figure 1.34a**). He reasoned that some molecule that contained genetic information must have been transferred from strain R to strain S in this process, and this experiment showed that genetic transfer could be studied in bacteria.

Later, the Avery–MacLeod–McCarty experiment (1944), named for three scientists at the Rockefeller University, would show that this “transforming principle” is DNA. They treated the dead remains of cells of strain S with chemicals and enzymes that destroyed protein and left behind only DNA. They then repeated Griffith's experiment with the pure DNA of strain S and showed that this DNA was sufficient to cause transformation, causing strain R cells to become S-type cells and virulent (**Figure 1.34b**). They also demonstrated that transformation failed if the DNA



**Figure 1.34** Early evidence that DNA is the molecular basis of heredity. (a) Griffith's experiment showed that bacteria can transfer genetic information. *Streptococcus pneumoniae* strain R makes rough colonies and does not kill mice, but strain S

makes smooth colonies and does kill mice. Heat-killed cells of strain S do not cause disease, but if these killed cells are mixed with cells of strain R, then strain R is “transformed” to the S type and begins to make smooth colonies and kill mice. (b) The Avery–MacLeod–McCarty

experiment showed that DNA contains genetic information. DNA isolated from strain S can transform strain R to cause disease, though the DNA itself does not cause disease. Degraded DNA lacks the ability to transform strain R.

from strain S was degraded. These experiments proved that DNA is the genetic material of cells.

The discovery that DNA is the basis of heredity was followed by intense effort to understand how this molecule stores genetic information. The structure of DNA was ultimately solved by James D. Watson (1928–) and Francis Crick (1916–2004) using X-ray diffraction images of DNA taken by their colleague Rosalind Franklin (1920–1958). They revealed that DNA is composed of a double helix that contains four nitrogenous bases: guanine, cytosine, adenine, and thymine (➡ Section 4.1). Later research would reveal how the genetic code is read from DNA and translated into a protein alphabet, and these principles are covered in Chapter 4. Once again, however, this research to crack the code of life was enabled by a microbial model system, in this case, the bacterium *Escherichia coli* (commonly called *E. coli*).

Not long after the discovery that genetic information is encoded in the sequence of biological molecules, Emile Zuckerkandl (1922–2013) and Linus Pauling (1901–1994) proposed that molecular sequences could be used to reconstruct evolutionary relationships. They recognized that evolution, as described by Darwin, required variation in offspring and that these variations must be caused by changes in molecular sequences. They predicted that these sequence differences occur randomly in a clocklike fashion over time. This led to the conclusion that the evolutionary history of organisms is inscribed in the sequence of molecules such as DNA. Carl Woese seized upon these insights to pursue the ambitious goal of reconstructing the evolutionary history of all cells.

### MINIQUIZ

- Describe the experiments that proved DNA was the transforming principle described by Griffith.
- Why are microbial cells useful tools for basic science?

## 1.13 Woese and the Tree of Life

Evolutionary relationships between microorganisms remained a mystery until it was discovered that certain molecular sequences maintain a record of evolutionary history. Here we will examine how the sequence of **ribosomal RNA (rRNA)** genes, present in all cells, revolutionized the understanding of microbial evolution and made it possible to construct the first universal tree of life.

### Molecular Sequence Data Has Revolutionized Microbial Phylogeny

For over a hundred years, following the 1859 publication of Charles Darwin's *On the Origin of Species*, evolutionary history was studied primarily with the tools of paleontology (through examining fossils) and comparative biology (through comparing the traits of living organisms). These approaches led to progress in understanding the evolution of plants and animals, but they were powerless to explain the evolution of microorganisms. The vast majority of microorganisms do not leave behind fossils, and their morphological and physiological traits provide few clues about their evolutionary history. Moreover, microorganisms do not share any morphological traits with plants and animals; thus it

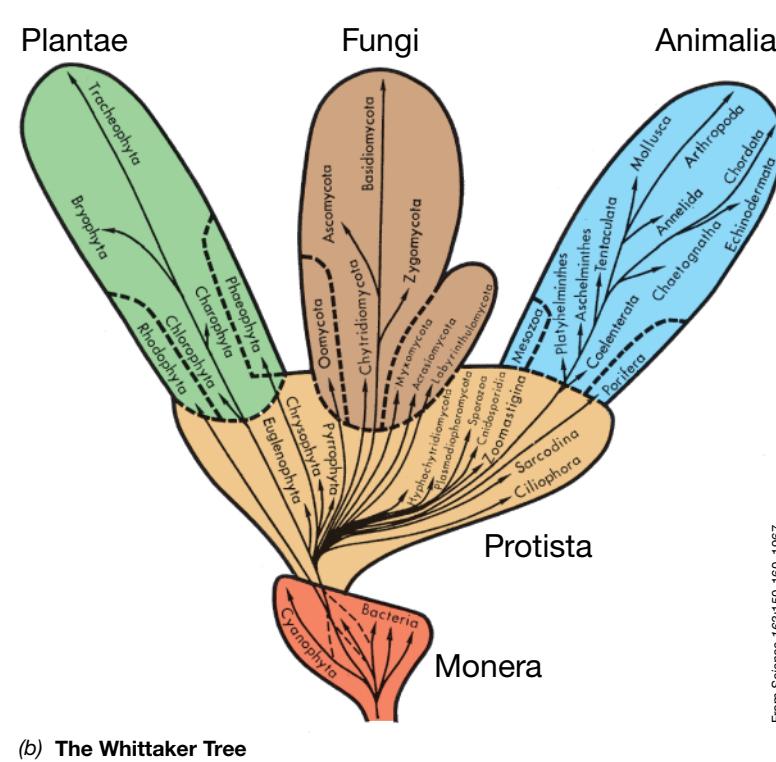
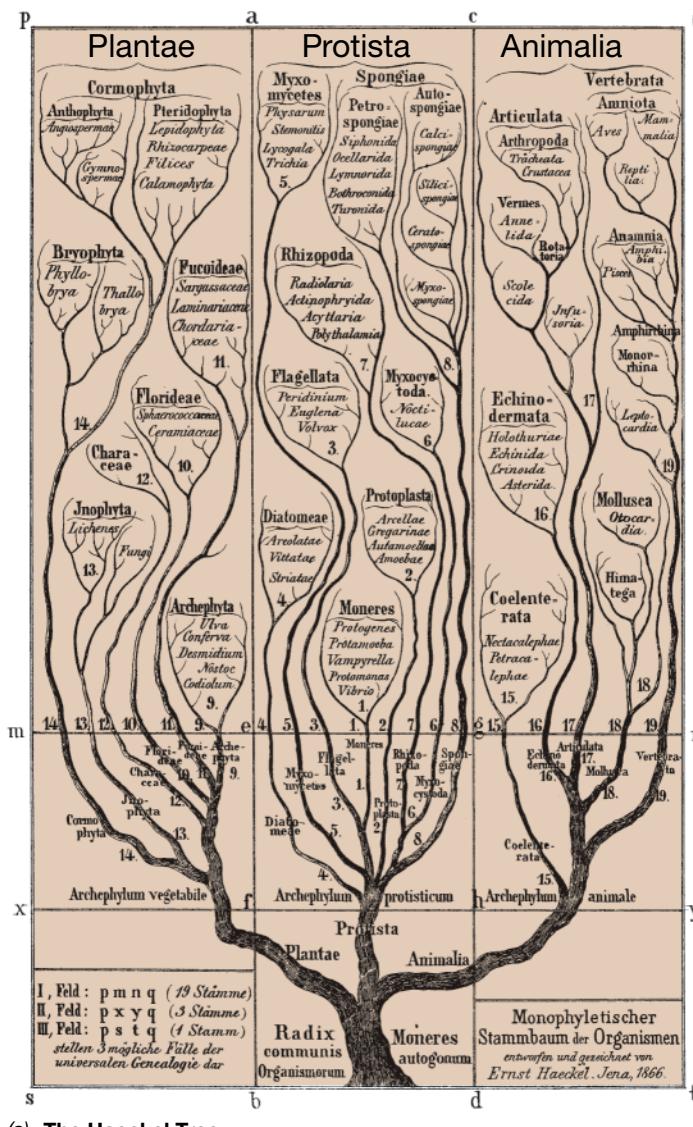
was impossible to create a robust evolutionary framework that included microorganisms.

The first attempt to depict the common evolutionary history of all living cells was published by Ernst Haeckel in 1866 (**Figure 1.35a**). Haeckel correctly suggested that single-cell organisms, which he called *Monera*, were ancestral to other forms of life, but his scheme, which included plants, animals, and protists, did not attempt to resolve evolutionary relationships among microorganisms. The situation was little changed as late as 1967 when Robert Whittaker proposed a five-kingdom classification scheme (Figure 1.35b). Whittaker's scheme distinguished the fungi as a distinct lineage, but it was still largely impossible to resolve evolutionary relationships among most microorganisms. Hence, microbial phylogeny had made little progress since Haeckel's day.

Everything changed after the structure of DNA was discovered and it was recognized that evolutionary history is recorded in DNA sequence. Carl Woese (1928–2012), a professor at the University of Illinois (USA), realized in the 1970s that the sequence of

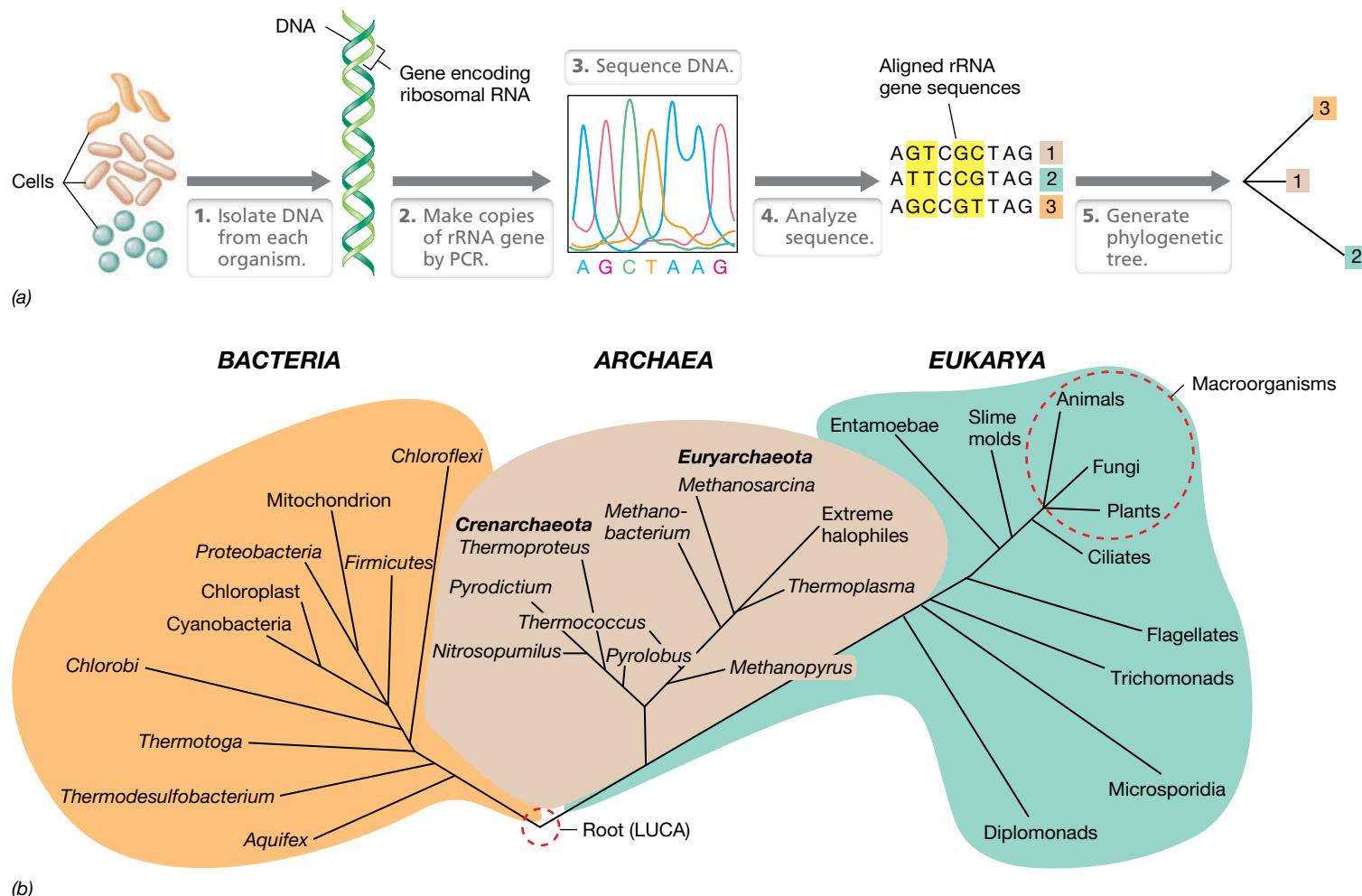
ribosomal RNA (rRNA) molecules and the genes that encode them could be used to infer evolutionary relationships between organisms. Ribosomal RNAs are components of ribosomes, the structures that synthesize new proteins in the process of translation (Section 1.2). Woese recognized that genes encoding rRNAs are excellent candidates for phylogenetic analysis because they are (1) universally distributed, (2) functionally constant, (3) highly conserved (that is, slowly changing), and (4) of adequate length to provide a deep view of evolutionary relationships.

Woese compared the sequences of rRNA molecules from many microorganisms. Among the microbes he examined were methanogens. To his astonishment, he found that the rRNA sequences from methanogens were distinct from those of both *Bacteria* and *Eukarya*, the only two domains recognized at that time. He named this new group of prokaryotic cells the *Archaea* (originally *Archaeabacteria*) and recognized them as the third domain of life alongside the *Bacteria* and the *Eukarya* (**Figure 1.36b**). More importantly, Woese demonstrated that the analysis of rRNA gene sequences could be used to reveal evolutionary relationships



From Science 163:150-160, 1967  
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**Figure 1.35** Early efforts to depict the universal tree of life. (a) Tree of life published in 1866 by Ernst Haeckel in *Generelle Morphologie der Organismen*. (b) Tree of life published by Robert H. Whittaker in 1969. The terms "Monera" and "Moneres" are antiquated terms used to refer to prokaryotic cells. Compare these conceptual trees with the tree generated from rRNA gene sequences in Figure 1.36b.



**Figure 1.36** Evolutionary relationships and the phylogenetic tree of life. (a) The technology behind ribosomal RNA gene phylogenies. 1. DNA is extracted from cells. 2. Copies of the gene encoding rRNA are made by the polymerase chain reaction (PCR; [Section 12.1](#)). 3. The gene is sequenced and the

sequence aligned with sequences from other organisms. A computer algorithm makes pairwise comparisons at each base and generates a phylogenetic tree, 5, that depicts evolutionary relationships. In the example shown, the sequence differences are highlighted in yellow and are as follows: organism 1 versus organism 2, three

differences; 1 versus 3, two differences; 2 versus 3, four differences. Thus organisms 1 and 3 are closer relatives than are 2 and 3 or 1 and 2. (b) The phylogenetic tree of life. The tree shows the three domains of organisms and a few representative groups in each domain.

between *all cells*, providing the first effective tool for the evolutionary classification of microorganisms.

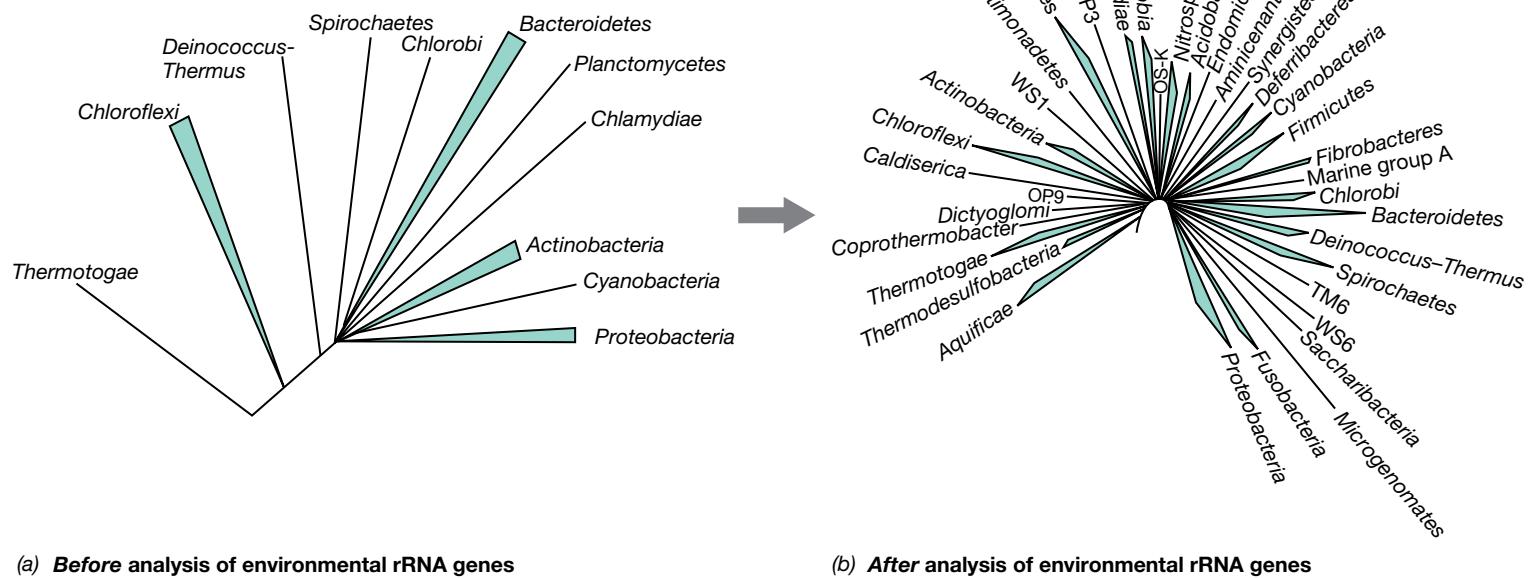
### The Tree of Life Based on rRNA Genes

The universal tree of life based on rRNA gene sequences (Figure 1.36b) is a genealogy of all life on Earth. It is a true **phylogenetic tree**, a diagram that depicts the evolutionary history—the **phylogeny**—of all cells and clearly reveals the three domains. The root of the universal tree represents a point in time when all extant life on Earth shared a common ancestor, the last universal common ancestor, LUCA (Figures 1.5b and 1.36b). From the last universal common ancestor of all cells, evolution proceeded along two paths to form the domains *Bacteria* and *Archaea*. At some later time, the domain *Archaea* diverged to distinguish the *Eukarya* from the *Archaea* (Figures 1.5b and 1.36b). The three domains of cellular life are evolutionarily distinct and yet they share features indicative of their common descent from a universal cellular ancestor.

### Revealing the Extent of Microbial Diversity

The tools Woese developed to build the tree of life were first used to determine the evolutionary history of microorganisms in pure culture (Figure 1.36a). However, Norman Pace (1942–), a professor at the University of Colorado (USA), realized that Woese's approach could be applied to rRNA molecules isolated *directly from the environment* as a way to probe the diversity of microbial communities without first cultivating their component organisms (Chapter 19).

The cultivation-independent methods of rRNA analysis pioneered by Pace greatly improved our picture of microbial diversity (Figure 1.37) and have led to the staggering conclusion that most microorganisms on Earth have yet to be brought into laboratory culture! Furthermore, because the ability of microbiologists to culture the microbial diversity that abounds in nature has lagged behind the ability to detect this diversity, microbiology is now in a position to flesh out the true diversity of microbial life.



### Figure 1.37 Analysis of environmental rRNA genes [e]

(a) In 1987 Carl Woese described 11 phyla of *Bacteria* from analysis of rRNA genes from cultured species. (b) By 1998, analyses of rRNA genes from environmental samples, as described by Norman Pace, had revealed evidence for 36 bacterial phyla. Today there is evidence for more than 80 bacterial phyla.

With an evolutionary framework of the microbial world to guide future research, advances in microbial diversity, both in obtaining cultures and in devising even more powerful methods of assessing diversity, are happening quickly. Besides unveiling the previously hidden concept of three evolutionary domains of life, the contributions of Carl Woese and his associates have given microbiologists the tools they need to understand the scope of microbial diversity at a level similar to biologists' understanding of the diversity of plants and animals.

**MINIQUIZ**

- What kinds of evidence support the three-domain concept of life?
  - What is a phylogenetic tree?
  - List three reasons why rRNA genes are suitable for phylogenetic analyses.

## 1.14 An Introduction to Microbial Life

All cells are unified by the facts that their genetic blueprints are encoded in DNA (Section 1.12) and that evolution is the process by which their blueprints change over time (Section 1.13). We now move on from these fundamental unifying principles to the microbes themselves and take a peek at the diversity of microbial life that evolution has generated.

Microorganisms vary dramatically in size, shape, and structure. And, while much of our focus in this chapter has been on cellular

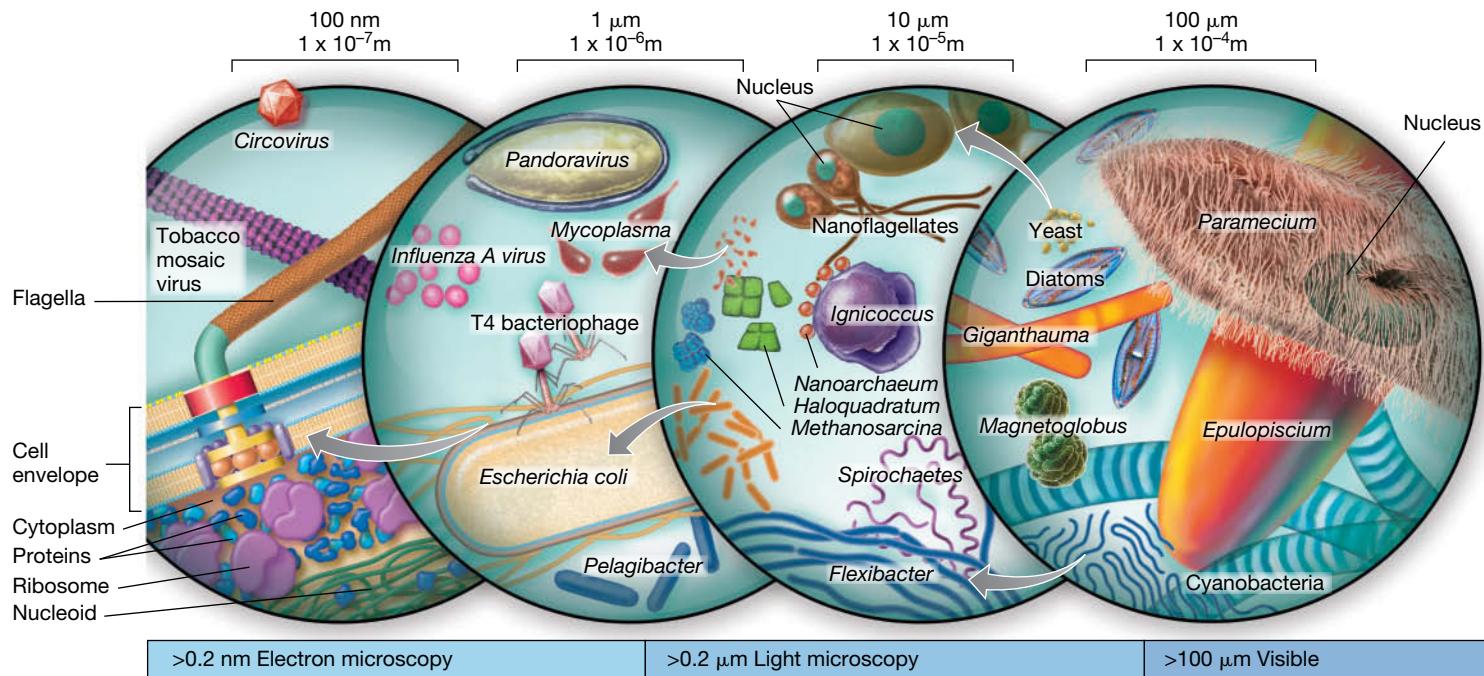
forms of life, not all microbes form cells. In this section we will learn about *Bacteria*, *Archaea*, *Eukarya*, and viruses—the four groups into which all known microorganisms can be classified.

## **Bacteria**

Bacteria have a prokaryotic cell structure (Figure 1.3a). Bacteria are often thought of as undifferentiated single cells with a length that ranges from 1 to 10  $\mu\text{m}$ . While bacteria that fit this description are common, the *Bacteria* are actually tremendously diverse in appearance and function. The smallest bacteria are no more than 0.15–0.2  $\mu\text{m}$  in diameter and the largest can be as much as 700  $\mu\text{m}$  long (Figure 1.38)! Some bacteria can differentiate to form multiple cell types and others are even multicellular (for example, *Magnetoglobus*, Figure 1.38).

Among the *Bacteria*, 30 major phylogenetic lineages (called phyla) have been described, and some key ones are shown in Figures 1.36 and 1.37. Some of these phyla contain thousands of described species while others contain only a few. More than 90% of bacteria in cultivation belong to one of only four phyla: *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*. The analysis of rRNA gene sequences and even entire genome sequences from environmental samples reveals that at least 80 bacterial phyla likely exist.

Although species in some bacterial phyla are characterized by unique phenotypic traits, most bacterial phyla contain a wide diversity of species and show tremendous physiological diversity. The *Proteobacteria* illustrate this concept well as they include organisms with a diverse array of physiological traits including



**Figure 1.38** Microorganisms vary greatly in size and shape. The smallest known microbe is the circovirus (20 nm) and the largest shown here is the bacterium *Epulopiscium* (700  $\mu\text{m}$ ), which represents a 35,000-fold difference in length! Certain protozoa can be even larger than *Epulopiscium* ( $>2$  mm long) and are visible to the unaided eye. Included in the figure are Eukarya: *Paramecium* ( $300 \mu\text{m} \times 85 \mu\text{m}$ ), diatoms

(*Navicula*,  $50 \mu\text{m} \times 12 \mu\text{m}$ ), yeast (*Saccharomyces*, 5  $\mu\text{m}$ ), and nanoflagellates (*Cafeteria*, 2  $\mu\text{m}$ ); Bacteria: *Epulopiscium* ( $700 \mu\text{m} \times 80 \mu\text{m}$ ), cyanobacteria (*Oscillatoria*, 10- $\mu\text{m}$ -diameter multicellular filaments), *Magnetoglobus* (multicellular aggregate, 20  $\mu\text{m}$  diameter), *Spirochaetes* (2–10  $\mu\text{m} \times 0.25 \mu\text{m}$ ), *Flexibacter* (5–100  $\mu\text{m} \times 0.5 \mu\text{m}$  filaments), *Escherichia coli* (2  $\mu\text{m} \times 0.5 \mu\text{m}$ ), *Pelagibacter* (0.4  $\mu\text{m} \times 0.15 \mu\text{m}$ ),

and *Mycoplasma* (0.2  $\mu\text{m}$ ); Archaea: *Giganthauma* (10- $\mu\text{m}$ -diameter multicellular filament), *Ignicoccus* (6  $\mu\text{m}$ ), *Nanoarchaeum* (0.4  $\mu\text{m}$ ), *Haloquadratum* (2  $\mu\text{m}$ ), *Methanoscincina* (2  $\mu\text{m}$  per cell in packet); and viruses: *Pandoravirus* (1  $\mu\text{m} \times 0.4 \mu\text{m}$ ), T4 bacteriophage (200 nm  $\times$  90 nm), *Influenza A virus* (100 nm), *Tobacco mosaic virus* (300 nm  $\times$  20 nm), *Circovirus* (20 nm).

respiration (both with and without oxygen), fermentations of various types, diverse forms of phototrophy, and chemolithotrophic metabolisms using H<sub>2</sub>, sulfur or nitrogen compounds, or even metals (as described in Chapters 14 and 15). Species of *Proteobacteria* also possess a wide range of ecological strategies and can be found in all but the hottest and most salty environments on Earth. It is important to remember that while most phyla of plants and animals originated within the last 600 million years (Figure 1.5a), bacterial phyla are billions of years old and this time has allowed for extensive experimentation and diversification. The diversity of *Bacteria* is discussed in detail in Chapters 15 and 16.

### Archaea

Like *Bacteria*, *Archaea* also have a prokaryotic cell structure (Figure 1.3a). While *Archaea* are quite diverse in their physiology, cultured isolates have less morphological diversity than *Bacteria*, and most described *Archaea* exist as undifferentiated cells that are 1 to 10  $\mu\text{m}$  in length. The domain *Archaea* consists of five well-described phyla: *Euryarchaeota*, *Crenarchaeota*, *Thaumarchaeota*, *Nanoarchaeota*, and *Korarchaeota*. As for the *Bacteria*, many lineages of *Archaea* are known only from rRNA genes or genome sequences recovered from the environment. Analysis of these environmental DNA sequences indicate more than 12 archaeal phyla likely exist.

*Archaea* have historically been associated with extreme environments; the first isolates came from hot, salty, or acidic sites. But not all *Archaea* are extremophiles. *Archaea* are indeed common in

the most extreme environments that support life, such as those associated with volcanic systems, and species of *Archaea* hold many of the records that define the chemical and physical limits of life (Table 1.1). But in addition to these, *Archaea* are found widely in nature. For example, methanogens are common in wetlands and in the guts of animals (including humans). Methanogenic *Archaea* produce methane and have a major impact on the greenhouse gas composition of our atmosphere. In addition, species of *Thaumarchaeota* inhabit soils and oceans worldwide and are important contributors to the global nitrogen cycle (Chapter 17).

*Archaea* are also notable in that this domain lacks any known pathogens or parasites of plants or animals. Most described species of *Archaea* fall within the phyla *Crenarchaeota* and *Euryarchaeota* (Figure 1.36b) while only a handful of species have been described for the *Nanoarchaeota*, *Korarchaeota*, and *Thaumarchaeota*. We discuss *Archaea* in detail in Chapter 17.

### Eukarya

Plants, animals, and fungi are the most well-characterized groups of *Eukarya*. These groups are relatively young in relation to *Bacteria* and *Archaea*, originating during a burst of evolutionary radiation called the Cambrian explosion, which began about 600 million years ago. The first eukaryotes, however, were unicellular microbes. Microbial eukaryotes, which include diverse algae and protozoa, may have first appeared as early as 2 billion years ago, well before the origin of plants, animals, and fungi (Figure 1.5). The major

lineages of *Eukarya* are traditionally called kingdoms instead of phyla. There are at least six kingdoms of *Eukarya*, and this diverse domain contains microorganisms as well as the plants and animals.

Microbial eukaryotes vary dramatically in size, shape, and physiology (Figure 1.38). Among the smallest are the nanoflagellates, which are microbial predators that can be as small as 2  $\mu\text{m}$  long. In addition, *Ostreococcus*, a genus of green algae that contains species that are 0.8  $\mu\text{m}$  in diameter, is smaller than many bacteria. The largest single-celled organisms are eukaryotes, but they are hardly microbial. Xenophyophores are amoeba-like, single-celled organisms that live exclusively in the deep ocean. Exploration of the Mariana Trench has revealed xenophyophores up to 10 cm in length. In addition, plasmodial slime molds consisting of a single cytoplasmic compartment can be up to 30 cm in diameter. Microbial eukaryotes include diverse phototrophic organisms, microbial predators, symbionts and parasites, along with a range of other physiological types. In Chapter 18 we consider microbial eukaryotes in detail.

## Viruses

Viruses are not found on the tree of life. Indeed, it can be argued that they are not truly alive. Viruses are obligate parasites that can only replicate within the cytoplasm of a host cell. Viruses are not cells, and they lack the cytoplasmic membrane, cytoplasm, and ribosomes found in all forms of cellular life. Viruses cannot conserve energy and they do not carry out metabolic processes; instead, they take over the metabolic systems of infected cells and turn them into vessels for producing more viruses. Unlike cells,

which all have genomes composed of double-stranded DNA, viruses have genomes composed of DNA or RNA that can be either double- or single-stranded. Viral genomes are often quite small, with the smallest having only three genes. The small size of most viral genomes means that no genes are conserved among all viruses, or between all viruses and all cells; hence it may be impossible to ever place viruses into the tree of life or build a universal viral phylogenetic tree that includes all viruses.

Viruses are as diverse as the cells they infect, and viruses are known to infect cells from all three domains of life. Viruses are often classified on the basis of structure, genome composition, and host specificity. Viruses that infect bacteria are called *bacteriophages* (or *phages*, for short). Bacteriophages have been used as model systems to explore many aspects of viral biology. While most viruses are considerably smaller than bacterial cells (Figure 1.38), there are also unusually large viruses such as the *Pandoraviruses*, which can be more than 1 micrometer long and have a genome of as many as 2500 genes, larger than that of many bacteria! We will learn more about viruses in Chapters 8 and 10.

## MINIQUIZ

- How are viruses different from *Bacteria*, *Archaea*, and *Eukarya*?
- What four bacterial phyla contain the most well-characterized species?
- What phylum of *Archaea* is common worldwide in soils and in the oceans?

## MasteringMicrobiology®

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## Chapter Review

### I • Exploring the Microbial World

**1.1** Microorganisms are single-celled microscopic organisms that are essential for the well-being and functioning of other life forms and the planet. The tools of microscopy, microbial cultivation, molecular biology, and genomics are cornerstones of modern microbiology.

**Q** **What are bacterial colonies and how are they formed?**

**1.2** Prokaryotic and eukaryotic cells differ in cellular architecture, and an organism's characteristics are defined by its complement of genes—its genome. All cells have a cytoplasmic membrane, a cytoplasm, ribosomes, and a double-stranded DNA genome. All cells carry out activities including metabolism, growth, and evolution.

**Q** **What cellular structures distinguish prokaryotic and eukaryotic cells? What are some differences between a cell wall and a cell membrane? In what types of organisms would you expect to find these structures?**

**1.3** Diverse microbial populations were widespread on Earth for billions of years before plants and animals appeared. Microbes are abundant in the biosphere and their activities greatly affect the chemical and physical properties of their habitats. *Bacteria*, *Archaea*, and *Eukarya* are the major phylogenetic lineages (domains) of cells.

**Q** **Why can Earth, in many ways, be considered a microbial planet? Which event in Earth's history eventually lead to the evolution of multicellular life forms?**

**1.4** Microorganisms can be both beneficial and harmful to humans, although many more microorganisms are beneficial (or even essential) than are harmful. Agriculture, food, energy, and the environment are all affected in major ways by microorganisms.

**Q** **The gut microbiome directly benefits humans by digesting complex carbohydrates and synthesizing vitamins and other nutrients. In what other ways do microorganisms benefit humans?**

## II • Microscopy and the Origins of Microbiology

- 1.5** Microscopes are essential for studying microorganisms. Bright-field microscopy, the most common form of microscopy, employs a microscope with a series of lenses to magnify and resolve the image. The limit of resolution for a light microscope is about 0.2 µm.
- Q** What is the difference between magnification and resolution? Can either increase without the other?
- 1.6** An inherent limitation of bright-field microscopy is the lack of contrast between cells and their surroundings. This problem can be overcome by the use of stains or by alternative forms of light microscopy, such as phase contrast or dark field.
- Q** What is the function of staining in light microscopy? What is the advantage of phase-contrast microscopy over bright-field microscopy?
- 1.7** Differential interference contrast (DIC) microscopy and confocal scanning laser microscopy allow enhanced three-dimensional imaging or imaging through thick specimens.
- Q** How is confocal scanning laser microscopy different from fluorescence microscopy? In what ways are they similar? How does differential interference contrast microscopy differ from bright-field microscopy?
- 1.8** Electron microscopes have far greater resolving power than do light microscopes, the limits of resolution being about 0.2 nm. The two major forms of electron microscopy are transmission, used primarily to observe internal cell structure, and scanning, used to examine the surface of specimens.
- Q** Why does an electron microscope have a higher resolution, or greater resolving power, than a light microscope?

## III • Microbial Cultivation Expands the Horizon of Microbiology

- 1.9** Louis Pasteur devised ingenious experiments proving that living organisms cannot arise spontaneously from nonliving matter. Pasteur introduced many concepts and techniques central to the science of microbiology, including sterilization, and developed a number of key vaccines for humans and other animals.
- Q** Explain the principle behind the Pasteur flask in studies on spontaneous generation. Why were the results of this experiment inconsistent with the theory of spontaneous generation?
- 1.10** Robert Koch developed a set of criteria called Koch's postulates for linking cause and effect in infectious

diseases. Koch also developed the first reliable and reproducible means for obtaining and maintaining microorganisms in pure culture.

**Q** What are Koch's postulates and how did they influence the development of microbiology? Why are Koch's postulates still relevant today?

- 1.11** Martinus Beijerinck and Sergei Winogradsky explored soil and water for microorganisms that carry out important natural processes, such as nutrient cycling and the biodegradation of particular substances. Out of their work came the enrichment culture technique and the concepts of chemolithotrophy and nitrogen fixation.
- Q** What were the major microbiological interests of Martinus Beijerinck and Sergei Winogradsky? It can be said that both men discovered nitrogen fixation. Explain.

## IV • Molecular Biology and the Unity and Diversity of Life

- 1.12** All cells share certain characteristics, and microorganisms are used as model systems to explore the fundamental processes that define life. The discoveries of DNA as the molecular basis of heredity, and of its structure and function, paved the way for progress in molecular genetics, microbial phylogeny, and genomics.
- Q** Describe the experiments that proved DNA to be the molecule at the basis of heredity.
- 1.13** Carl Woese discovered that ribosomal RNA (rRNA) sequences can be used to determine the evolutionary history of microorganisms, and in so doing, he diagrammed the tree of life and discovered the domain *Archaea*. Analysis of rRNA sequences from the environment reveals that microbial diversity is exceptional and that the majority of microorganisms have not yet been cultivated.
- Q** What insights led to the reconstruction of the tree of life? Which domain, *Archaea* or *Eukarya*, is more closely related to *Bacteria*? What evidence is there to justify your answer?
- 1.14** The greatest diversity of microorganisms is found in the *Bacteria*, while many extremophiles are found within the *Archaea*. Microbial eukaryotes can vary tremendously in size, with some species being smaller than bacteria. Viruses are acellular and because of this cannot be placed on the tree of life.
- Q** What features (or lack of features) can be used to distinguish between viruses, *Bacteria*, *Archaea*, and *Eukarya*?

## Application Questions

- Pasteur's experiments on spontaneous generation contributed to the methodology of microbiology, understanding of the origin of life, and techniques for the preservation of food. Explain briefly how Pasteur's experiments affected each of these topics.
- Describe the lines of proof Robert Koch used to definitively associate the bacterium *Mycobacterium tuberculosis* with the disease tuberculosis. How would his proof have been flawed if any of the tools he developed for studying bacterial diseases had not been available for his study of tuberculosis?
- Imagine that all microorganisms suddenly disappeared from Earth. From what you have learned in this chapter, why do you think that animals would eventually disappear from Earth? Why would plants disappear? By contrast, if all higher organisms suddenly disappeared, what in Figure 1.5a tells you that a similar fate would not befall microorganisms?

## Chapter Glossary

**Aseptic technique** the manipulation of sterile instruments or culture media in such a way as to maintain sterility

**Cell wall** a rigid layer present outside the cytoplasmic membrane; it confers structural strength on the cell

**Chemolithotrophy** a form of metabolism in which energy is generated from the oxidation of inorganic compounds

**Colony** a macroscopically visible population of cells growing on solid medium, arising from a single cell

**Contrast** the ability to resolve a cell or structure from its surroundings

**Culture** a collection of microbial cells grown using a nutrient medium

**Cytoplasm** the fluid portion of a cell, enclosed by the cytoplasmic membrane

**Cytoplasmic membrane** a semipermeable barrier that separates the cell interior (cytoplasm) from the environment

**Differentiation** modification of cellular components to form a new structure, such as a spore

**Domain** one of the three main evolutionary lineages of cells: the *Bacteria*, the *Archaea*, and the *Eukarya*

**DNA replication** the process by which information from DNA is copied into a new strand of DNA

**Enrichment culture technique** a method for isolating specific microorganisms from nature using specific culture media and incubation conditions

**Enzyme** a protein (or in some cases an RNA) catalyst that functions to speed up chemical reactions

**Eukaryotic** having a membrane-enclosed nucleus and various other membrane-enclosed organelles; cells of *Eukarya*

**Evolution** a change over time in gene sequence and frequency within a population of organisms, resulting in descent with modification

**Extremophiles** microorganisms that inhabit environments characterized by extremes of temperature, pH, pressure, or salinity

**Genome** an organism's full complement of genes

**Gram-negative** a bacterial cell with a cell wall containing small amounts of peptidoglycan and an outer membrane

**Gram-positive** a bacterial cell whose cell wall consists chiefly of peptidoglycan; it lacks the outer membrane of gram-negative cells

**Gram stain** a differential staining procedure that stains cells either purple (gram-positive cells) or pink (gram-negative cells)

**Growth** in microbiology, an increase in cell number with time

**Gut microbiome** the microbial communities present in the animal gastrointestinal tract

**Horizontal gene transfer** the transfer of genes between cells through a process uncoupled from reproduction

**Intercellular communication** interactions between cells using chemical signals

**Koch's postulates** a set of criteria for proving that a given microorganism causes a given disease

**Macromolecules** a polymer of monomeric units, for example proteins, nucleic acids, polysaccharides, and lipids

**Magnification** the optical enlargement of an image

**Medium (plural, media)** in microbiology, the liquid or solid nutrient mixture(s) used to grow microorganisms

**Metabolism** all biochemical reactions in a cell

**Microbial community** two or more populations of cells that coexist and interact in a habitat

**Microbial ecology** the study of microorganisms in their natural environments

**Microorganism** an organism that is too small to be seen by the unaided human eye

**Motility** the movement of cells by some form of self-propulsion

**Nucleoid** the aggregated mass of DNA that makes up the chromosome(s) of prokaryotic cells

**Nucleus** a membrane-enclosed structure in eukaryotic cells that contains the cell's DNA genome

**Organelle** a bilayer-membrane-enclosed structure such as the mitochondrion, found in eukaryotic cells

**Pathogen** a disease-causing microorganism

**Phylogenetic tree** a diagram that depicts the evolutionary history of organisms

**Phylogeny** the evolutionary history of organisms

**Plasmid** an extrachromosomal genetic element that is not essential for growth

**Prokaryotic** lacking a membrane-enclosed nucleus and other organelles; cells of *Bacteria* or *Archaea*

**Pure culture** a culture containing a single kind of microorganism

**Resolution** the ability to distinguish two objects as distinct and separate when viewed under the microscope

**Ribosomes** a structure composed of RNAs and proteins upon which new proteins are made

**Ribosomal RNA (rRNA)** the types of RNA found in the ribosome

**Spontaneous generation** the hypothesis that living organisms can originate from nonliving matter

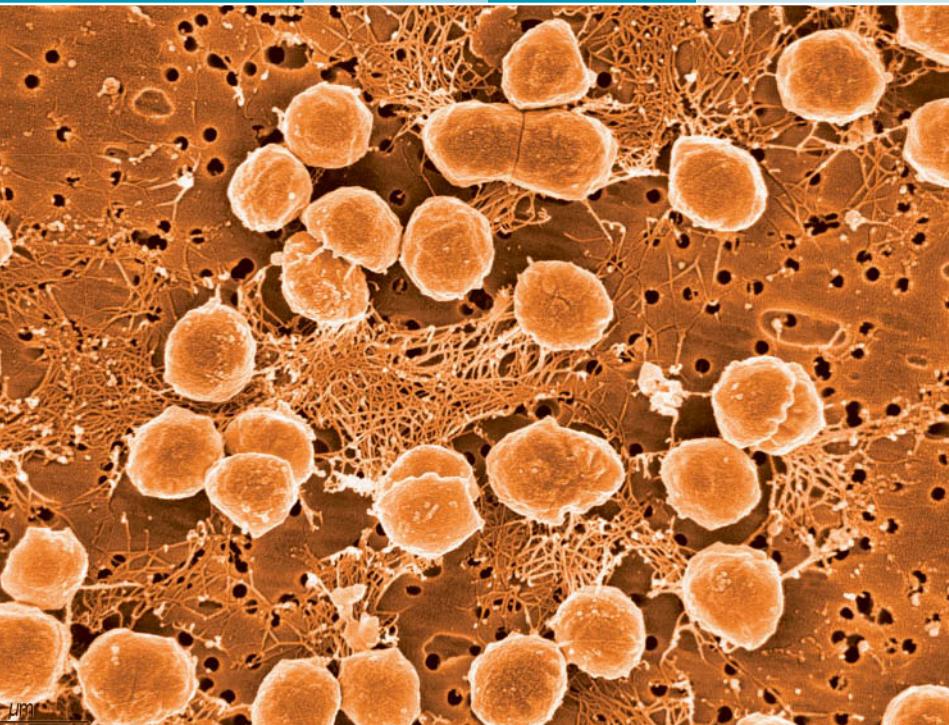
**Sterile** free of all living organisms (cells) and viruses

**Transcription** the synthesis of an RNA molecule complementary to one of the two strands of a double-stranded DNA molecule

**Translation** the synthesis of protein by a ribosome using the genetic information in a messenger RNA as a template

# 2

## Microbial Cell Structure and Function



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### microbiologynow

#### The Archaellum: Motility for the Archaea

Motility is important for microbes because it allows cells to explore new habitats and exploit their resources.

Motility has been studied for over 50 years in the bacterium *Escherichia coli*, and it is with *E. coli* that scientists first discovered that the bacterial flagellum rotates like a propeller and is powered not by ATP but by the proton motive force. Subsequent studies of other motile *Bacteria* indicated that the structure and function of the flagellum was highly conserved.

When *Archaea* were first discovered, it was clear that some species, such as the archaeon *Methanocaldococcus* (see photo), were also motile and that their flagella also functioned by rotating. So it was only natural to assume that archaeal flagella were structurally related to bacterial flagella. But as scientists began to pick apart the archaeal flagellum, they were in for a big surprise.

Studies showed that archaeal flagella—now called *archaella* to distinguish them from flagella—were thinner than their bacterial counterparts and were com-

posed not of one major protein but of several proteins. For example, whereas the flagellar filament (the actual rotating structure) is composed of a single type of protein called *flagellin*, the archaeal filament is composed of at least three proteins, none of which are structurally related to flagellin. Motor proteins in the flagellum and archaellum are also distinct, as is the overall structure of the motor.

Despite these clear differences, genomic studies of motile *Archaea* surprisingly revealed that the archaellum did have a structural counterpart in *Bacteria*—the type IV pilus. In *Bacteria*, type IV pili do not rotate but facilitate “twitching motility” and are often important in attaching pathogenic (disease-causing) bacteria to their host tissues. However, unlike the flagellum, the activities of type IV pili in *Bacteria* are powered by ATP, and ATP also drives rotation of the archaellum.

The archaellum is thus a “rotating type IV pilus” and is a good example of how evolution can modify a single structure to drive different functions. Such discoveries also demonstrate how even firmly entrenched paradigms (such as the structure and function of the bacterial flagellum) can be turned upside down when scientists begin to probe phylogenetically distinct species.

 **Source:** Albers, S-V., and K.F. Jarrell. 2015. The archaellum: How Archaea swim. *Front. Microbiol.* 6: doi: 10.3389/fmicb.2015.00023.

# I • Cells of *Bacteria* and *Archaea*

In the opening chapter, we painted a picture of the microbial world using a broad brush. There we considered in a very general way several key aspects of microbiology essential to a modern understanding of the science. In Chapter 2, we move on to begin a more detailed examination of microbial life, with a focus on cell structure and function.

Microscopic examination of microorganisms immediately reveals their shape and size. A variety of cell shapes pervade the microbial world, and although microscopic by their very nature, microbial cells—both prokaryotic and eukaryotic—come in a variety of sizes. Cell shape can be useful for distinguishing different microbial cells and often has ecological significance. Moreover, the very small size of most microbial cells has a profound effect on their ecology and dictates many aspects of their biology. We begin by considering cell shape and then consider cell size.

## 2.1 Cell Morphology

In microbiology, the term **morphology** means cell shape. Several morphologies are found among *Bacteria* and *Archaea*, and the most common ones are described by terms that are part of the essential lexicon of the microbiologist.

### Major Morphologies of Prokaryotic Cells

Common morphologies of prokaryotic cells are shown in **Figure 2.1**. A cell that is spherical or ovoid in morphology is called a *coccus* (plural, *cocci*). A cylindrically shaped cell is called a *rod* or a *bacillus*. Some cells form curved or loose spiral shapes and are called *spirilla*. The cells of some *Bacteria* and *Archaea* remain together in groups or clusters after cell division, and the arrangements are often characteristic. For instance, some cocci form long chains (for example, the bacterium *Streptococcus*), others occur in

three-dimensional cubes (*Sarcina*), and still others in grapelike clusters (*Staphylococcus*).

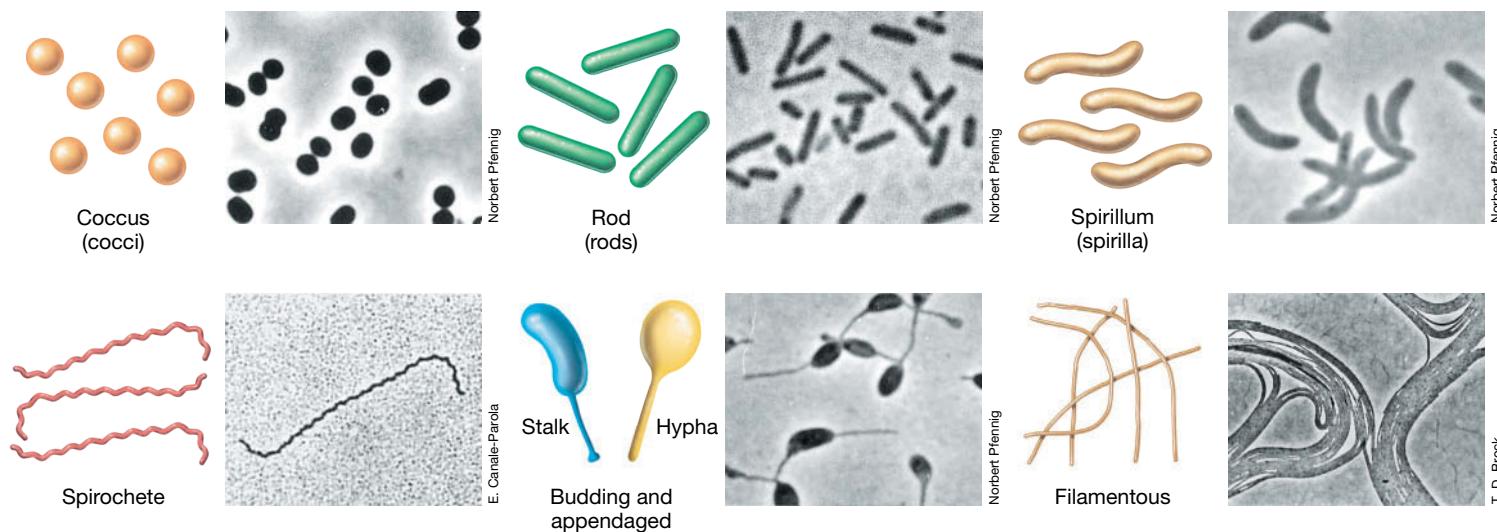
Some morphological groups are immediately recognizable by the unusual shapes of their individual cells. Examples include the spirochetes, which are tightly coiled *Bacteria*; bacteria that form extensions of their cells as long tubes or stalks (appendaged forms); and filamentous bacteria, which form long, thin cells or chains of cells (Figure 2.1).

The cell morphologies described here are representative but certainly not exhaustive; many variations of these morphologies are known. For example, there can be fat rods, thin rods, short rods, and long rods, a rod simply being a cell—roughly in the shape of a cylinder—that is longer in one dimension than in the other. As we will see, there are even square bacteria and star-shaped bacteria! Cell morphologies thus form a continuum, with some shapes, such as rods and cocci, being very common, whereas others, such as spiral, budding, and filamentous shapes, are less common.

### Morphology and Biology

Although cell morphology is easily determined, it is a poor predictor of other properties of a cell. For example, under the microscope many rod-shaped *Archaea* are indistinguishable from rod-shaped *Bacteria*, yet we know they are of different phylogenetic domains (► Section 1.13). With rare exceptions, it is impossible to predict the physiology, ecology, phylogeny, pathogenic (disease-causing) potential, or virtually any other major property of a prokaryotic cell by simply knowing its morphology. Nevertheless, cell morphology is an important characteristic that is always noted when describing a particular species of *Bacteria* or *Archaea*.

Why are the cells of a given species the shape they are? Although we know quite a bit about *how* cell shape is controlled, we know relatively little about *why* a particular cell displays the morphology it does. The morphology of a given microbe is undoubtedly the result of the selective forces that have shaped its evolution to



**Figure 2.1** Cell morphologies. Beside each drawing is a phase-contrast photomicrograph of cells showing that morphology. Coccus (cell diameter in photomicrograph, 1.5  $\mu\text{m}$ ); rod (1  $\mu\text{m}$ ); spirillum (1  $\mu\text{m}$ ); spirochete (0.25  $\mu\text{m}$ ); budding (1.2  $\mu\text{m}$ ); filamentous (0.8  $\mu\text{m}$ ). All photomicrographs are of species of *Bacteria*. Not all of these morphologies are known among the *Archaea*, but cocci, rods, and spirilla are common.

maximize fitness for competitive success in its habitat. Some examples of these might include evolving an optimal cell shape to maximize nutrient uptake for survival in nutrient-limiting environments (small cells and others with high surface-to-volume ratios, such as appendaged cells), evolving a morphology to exploit swimming motility in viscous environments (helical- or spiral-shaped cells), or evolving a morphology that facilitates gliding motility along a surface (filamentous bacteria) (Figure 2.1).

### MINIQUIZ

- How do cocci and rods differ in morphology?
- Using a microscope, could you differentiate a coccus from a spirillum? A pathogen from a nonpathogen?

## 2.2 The Small World

Cells of *Bacteria* and *Archaea* vary in size from as small as about 0.2 micrometer ( $\mu\text{m}$ ) in diameter to those more than 700  $\mu\text{m}$  in diameter (Table 2.1). The vast majority of rod-shaped species that have been cultured are between 0.5 and 4  $\mu\text{m}$  wide and less than 15  $\mu\text{m}$  long. A few very large *Bacteria* are known, such as *Epulopiscium fishelsoni*, whose cells exceed 600  $\mu\text{m}$  (0.6 millimeter) in length (Figure 2.2a; Figure 1.38). This bacterium, phylogenetically related to the endospore-forming bacterium *Clostridium* and found in the gut of the surgeonfish, contains multiple copies of its genome. The many copies are apparently necessary because the volume of an *Epulopiscium* cell is so large (Table 2.1) that a single copy of its genome would be insufficient to support its transcriptional and translational demands.

Cells of the largest known bacterium, the sulfur-oxidizing chemolithotroph *Thiomargarita* (Figure 2.2b), are even larger than those of *Epulopiscium*, about 750  $\mu\text{m}$  in diameter; such cells are just visible to

the naked eye. Why these cells are so large is not well understood, although for sulfur bacteria a large cell size may have evolved for storing inclusions of sulfur (used as an energy source). No species of *Archaea* are known that rival *Epulopiscium* or *Thiomargarita* in cell size, but that may simply be because they remain undiscovered.

It is hypothesized that the upper size limit for prokaryotic cells results from the decreasing ability of larger and larger cells to transport nutrients (their surface-to-volume ratio is very small; see the next subsection). Since the metabolic rate of a cell varies inversely with the square of its size, for very large cells, nutrient uptake would eventually limit metabolism to the point that the cell would no longer be competitive with smaller cells.

Very large cells are uncommon in the prokaryotic world. In contrast to *Thiomargarita* or *Epulopiscium* (Figure 2.2), the dimensions of an average rod-shaped bacterium, such as *Escherichia coli*, for example, are about 1–2  $\mu\text{m}$ ; these dimensions are typical of cells in the prokaryotic world. By contrast, eukaryotic cells can be as small as 2 to more than 600  $\mu\text{m}$  in diameter, although very small microbial eukaryotes (cells less than about 6  $\mu\text{m}$  in diameter) are uncommon. We explore the world of microbial eukaryotes in Chapter 18.

### Surface-to-Volume Ratios, Growth Rates, and Evolution

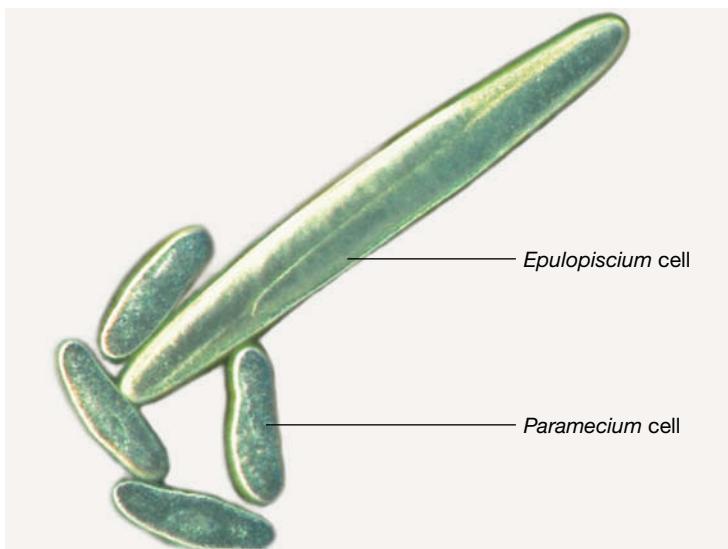
For a cell, there are advantages to being small. Small cells have more surface area relative to cell volume than do large cells and thus have a higher *surface-to-volume ratio*. To understand this, consider a coccus-shaped cell. The volume of a coccus is a function of the cube of its radius ( $V = \frac{4}{3}\pi r^3$ ), whereas its surface area is a function of the square of the radius ( $S = 4\pi r^2$ ). Therefore, the *S/V* ratio of a coccus is  $3/r$  (Figure 2.3). As cell size *increases*, its *S/V* ratio *decreases*. To illustrate this, consider the *S/V* ratio for some of the cells of different sizes listed in Table 2.1: *Pelagibacter ubique*, 22; *E. coli*, 4.5; and *E. fishelsoni* (Figure 2.2a), 0.05. These are all rods

**TABLE 2.1 Cell size and volume of some cells of *Bacteria*, from the largest to the smallest**

Organism	Characteristics	Morphology	Size <sup>a</sup> ( $\mu\text{m}$ ) <sup>3</sup>	Cell volume ( $\mu\text{m}$ ) <sup>3</sup>	Volumes compared to <i>E. coli</i>
<i>Thiomargarita namibiensis</i>	Sulfur chemolithotroph	Cocci in chains	750	200,000,000	100,000,000×
<i>Epulopiscium fishelsoni</i> <sup>a</sup>	Chemoorganotroph	Rods with tapered ends	80 × 600	3,000,000	1,500,000×
<i>Beggiatoa</i> species <sup>a</sup>	Sulfur chemolithotroph	Filaments	50 × 160	1,000,000	500,000×
<i>Achromatium oxaliferum</i>	Sulfur chemolithotroph	Cocci	35 × 95	80,000	40,000×
<i>Lyngbya majuscula</i>	Cyanobacterium	Filaments	8 × 80	40,000	20,000×
<i>Thiovulum majus</i>	Sulfur chemolithotroph	Cocci	18	3,000	1,500×
<i>Staphylothermus marinus</i> <sup>a</sup>	Hyperthermophile	Cocci in irregular clusters	15	1,800	900×
<i>Magnetobacterium bavaricum</i>	Magnetotactic bacterium	Rods	2 × 10	30	15×
<i>Escherichia coli</i>	Chemoorganotroph	Rods	1 × 2	2	1×
<i>Pelagibacter ubique</i> <sup>a</sup>	Marine chemoorganotroph	Rods	0.2 × 0.5	0.014	0.007×
Ultra-small bacteria	Uncultured, from groundwater	Variable	<0.2	0.009	0.0045×
<i>Mycoplasma pneumoniae</i>	Pathogenic bacterium	Pleomorphic <sup>b</sup>	0.2	0.005	0.0025×

<sup>a</sup>Where only one number is given, this is the diameter of spherical cells. The values given are for the largest cell size observed in each species. For example, for *T. namibiensis*, an average cell is only about 200  $\mu\text{m}$  in diameter. But on occasion, giant cells of 750  $\mu\text{m}$  are observed. Likewise, an average cell of *S. marinus* is about 1  $\mu\text{m}$  in diameter. The species of *Beggiatoa* here is unclear and *E. fishelsoni*, *M. bavaricum*, and *P. ubique* are not formally recognized names in taxonomy.

<sup>b</sup>*Mycoplasma* is a bacterium that lacks a cell wall and can thus take on many shapes (*pleomorphic* means “many shapes”).



(a)



(b)

**Figure 2.2 Two very large Bacteria.** (a) *Epulopiscium fishelsoni*. The rod-shaped cell is about 600  $\mu\text{m}$  (0.6 mm) long and 75  $\mu\text{m}$  wide and is shown with four cells of the protist *Paramecium* (a microbial eukaryote), each of which is about 150  $\mu\text{m}$  long. (b) *Thiomargarita namibiensis*, a large sulfur chemolithotroph and currently the largest known of all prokaryotic cells. Cell widths vary from 400 to 750  $\mu\text{m}$ .

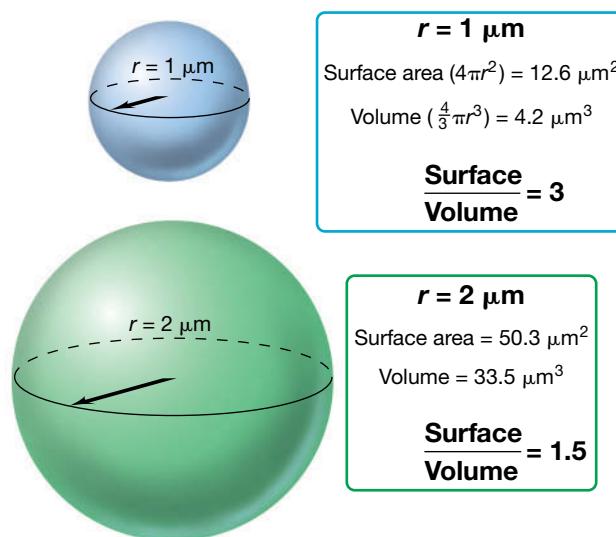
rather than cocci, but if it is assumed that a rod-shaped cell is a perfect cylinder, the same S/V principles that hold for cocci also hold for rods; that is, for rods of a given length, cells with a smaller radius have a greater S/V than do cells with a larger radius.

The S/V ratio of a cell controls many of its properties, including its growth rate and evolution. Because how fast a cell can grow depends in part on the rate at which it can exchange nutrients and waste products with its environment, the higher S/V ratio of small cells supports a faster rate of nutrient and waste exchange per unit of cell volume compared with large cells. As a result, free-living smaller cells tend to grow faster than free-living larger cells, and for a given amount of resources (nutrients available to support

growth), a larger population of small cells than of large cells can be supported. This in turn can affect a cell's evolution.

Each time a cell divides, its chromosome replicates. As DNA is replicated, occasional errors, called *mutations*, occur. Because mutation rates are roughly the same in all cells, large or small, the more chromosome replications that occur, the greater the total number of mutations in the cell population. Mutations are the “raw material” of evolution; the larger the pool of mutations, the greater the evolutionary possibilities. Thus, because prokaryotic cells are quite small and are also genetically haploid (they typically have only one copy of each gene, allowing mutations to be expressed immediately), prokaryotic cells can grow faster and evolve more rapidly than can larger cells.

Because of their typically larger size, not only is the S/V ratio of microbial eukaryotes smaller, the diploid character of the eukaryotic cell (a cell has two copies of each gene) allows for a mutation in one gene to be masked by a second, unmutated gene copy. These fundamental differences in size and genetics between prokaryotic and eukaryotic cells help explain why species of *Bacteria* and *Archaea* adapt rapidly to changing environmental conditions and more easily exploit new habitats than do eukaryotic cells. We illustrate this concept in later chapters when we consider the enormous diversity of cells and metabolisms of *Bacteria* and *Archaea* (Chapters 14–17), the rapidity of prokaryotic evolution (Chapter 13), and the ecological ramifications of microbial activities in nature (Chapters 19–23).



**Figure 2.3 Surface area and volume relationships in cells.** As a cell increases in size, its S/V ratio decreases.

### Lower Limits to Cell Size

From the foregoing, one would predict that smaller and smaller microbes would have greater and greater selective advantages in nature and that as a consequence, only extremely tiny bacterial cells would exist. However, this is not the case, as there are lower limits to cell size and good reasons why there should be.

# EXPLORE THE MICROBIAL WORLD

## TINY CELLS

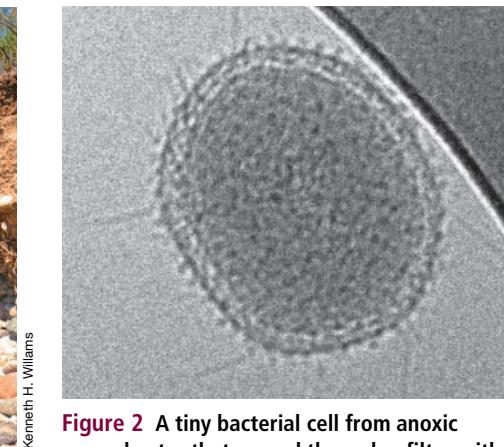
**V**iruses are very small microbes and range in diameter from as small as 20 nm to almost 750 nm. Although no cells exist that are as small as most viruses, the recent discovery of ultra-small bacterial cells<sup>1,2</sup> has pushed the lower limits of cell size to what microbiologists feel must be very close to the minimal value.

Microbiologists collected groundwater, which travels through Earth's deep subsurface, from a Colorado (USA) aquifer (Figure 1) and ran it through a membrane filter whose pores were only 0.2  $\mu\text{m}$  in diameter. The liquid that passed through the filter was then subjected to microbiological analyses. Surprisingly, since filters with 0.2- $\mu\text{m}$  pores have been used for decades to remove bacterial cells from solutions to generate "sterile solutions," prokaryotic cells were present in the groundwater filtrate. In fact, a diverse array of *Bacteria* were present in the filtrate, revealing that the groundwater was inhabited by a microbial community of tiny cells<sup>1</sup> that microbiologists have come to call "ultramicrobacteria."

Cryo-electron microscopy, in which a specimen is examined at extremely cold temperatures without fixation (chemical treatment that can alter a cell's morphology), showed the groundwater ultramicrobacteria to consist primarily of oval-shaped cells about 0.2  $\mu\text{m}$  in diameter (Figure 2). The volume of these cells was calculated to be about 1/100 that of a cell of the bacterium *Escherichia coli* (see Table 2.1) such that nearly 150 of the small cells could fit into one *E. coli* cell! Each of the tiny cells contained about 50 ribosomes, which is also about 1/100 of the number present in a slowly growing (100-min generation time) cell of *E. coli*. The very small size of the



**Figure 1** Sampling the anoxic groundwater aquifer that parallels the Colorado River near Rifle, Colorado.



**Figure 2** A tiny bacterial cell from anoxic groundwater that passed through a filter with 0.2- $\mu\text{m}$  pores. The cell is not quite 0.2  $\mu\text{m}$  in diameter.

Birgit Luef and Jill Banfield

groundwater ultramicrobacteria gives them an enormous surface-to-volume ratio, and it is hypothesized that this advantage benefits them in extracting resources from their nutrient-deficient habitat.

Despite the fact that the tiny groundwater bacteria have yet to be cultured in the laboratory, much is already known about them because their small genomes—less than 1 Mb in size—were obtained and analyzed.<sup>2</sup> From a phylogenetic perspective, the different species detected were distantly related to major phyla of *Bacteria* known from environmental analyses of diverse environments but which have thus far defied laboratory culture. Further analyses showed that genes encoding the enzymes for several core metabolic pathways widely distributed among microorganisms were absent from the genomes of the groundwater ultramicrobacteria. This suggests a

metabolically minimalist lifestyle for these tiny cells and a survival strategy of cross-feeding essential nutrients with neighboring species in their microbial community.

Although we do not yet know exactly how small a microbial cell can be, microbiologists are closing in on this number from environmental analyses such as the Colorado groundwater study. From the same samples that yielded ultra-small *Bacteria* in this study, ultra-small *Archaea* were also detected and found to contain small and highly reduced genomes.<sup>2</sup> Apparently, a large diversity of very small prokaryotic cells occurs in nature, and from the continued study of these tiny cells, more precise values for both the lower limits to cell size and the minimal genomic requirements for life should emerge.

<sup>1</sup> Luef, B., et al. 2015. *Nature Communications*. doi:10.1038/ncomms7372.

<sup>2</sup> Castelle, C.J., et al. 2015. *Current Biology*. 25: 1–12.

If one calculates the volume needed to house the essential components of a free-living cell—proteins, nucleic acids, ribosomes, and so on—cells 0.15–0.2  $\mu\text{m}$  in diameter are probably the lower limit, or very close to it. Many small prokaryotic cells are known and several have been grown in the laboratory. Open ocean water, for example, contains  $10^5$ – $10^6$  prokaryotic cells per milliliter, and these tend to be very small cells, 0.2–0.4  $\mu\text{m}$  in diameter. In addition, populations of bacterial and archaeal cells have been discovered in Earth's deep subsurface that are about 0.2  $\mu\text{m}$  in diameter (see Explore the Microbial World, "Tiny Cells"). Collectively, these cells have been referred to as "ultramicrobacteria" to indicate that

they are considerably smaller than typical bacterial cells. We will see later that the cells of many pathogenic bacteria are also very small.

Interestingly, when the genomes of ultramicrobacteria are unraveled, they are typically found to be highly streamlined and to be missing many genes whose products or functions must be supplied to them by other microbial cells or by the host organisms (plants and animals, for example) that harbor them. In these tiny prokaryotic cells, evolutionary success is apparently linked to a minimalist biochemistry and obligate association with one or more other organisms. In some cases, these associations are so

specific and so tightly linked that the tiny cell (and in some cases, the second organism) cannot survive without its respective partner. We consider the genomes—both large and small—of *Bacteria* and *Archaea* in many chapters of this book and focus exclusively on genomes and related aspects of the systems biology of microbial cells in Chapter 9.

### MINIQUIZ

- What physical property of cells increases as cells become smaller?
- How can the small size and haploid genome of *Bacteria* and *Archaea* accelerate their evolution?
- What are the approximate limits to how small a cell can be? Why should this be so?

## II • The Cell Membrane and Wall

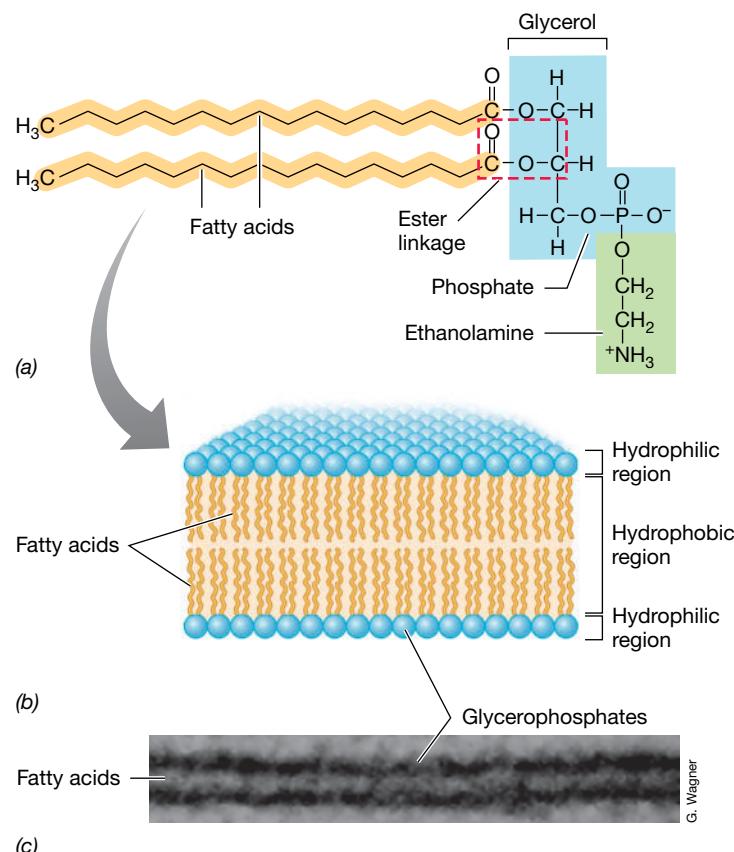
We consider here the structure and function of two of a cell's most essential structures, the *cytoplasmic membrane* and the *cell wall*. The cytoplasmic membrane plays many roles, chief among them as the “gatekeeper” for the entrance and exit of dissolved substances. The cell wall, by contrast, confers structural strength on the cell in order to keep it from bursting due to osmotic pressure.

### 2.3 The Cytoplasmic Membrane

The **cytoplasmic membrane** surrounds the *cytoplasm*—the mixture of macromolecules and small molecules inside the cell—and separates it from the environment. The cytoplasmic membrane is physically rather weak but it is an ideal structure for its major cellular function: selective permeability. In order for a cell to grow, nutrients must be transported inwards and waste products outwards. Both of these events occur across the cytoplasmic membrane. A variety of proteins located in the cytoplasmic membrane facilitate these reactions, and many other membrane proteins play important roles in energy metabolism.

#### The Bacterial Cytoplasmic Membrane

The cytoplasmic membrane of all cells is a phospholipid bilayer containing embedded proteins. Phospholipids are composed of both hydrophobic (water-repelling) and hydrophilic (water-attracting) components (Figure 2.4). In *Bacteria* and *Eukarya*, the hydrophobic component consists of fatty acids and the hydrophilic component of a glycerol molecule containing phosphate and one of several other functional groups (such as sugars, ethanolamine, or choline) bonded to the phosphate. The fatty acids point inward toward each other to form a hydrophobic region, while the hydrophilic portion remains exposed to either the environment or the cytoplasm (Figure 2.4b). That is, the *outer* surface of the cytoplasmic membrane faces the environment while the *inner* surface faces the cytoplasm and interacts with the cytoplasmic milieu. This type of membrane structure is called a *lipid bilayer*, or a *unit membrane* because each phospholipid “leaf” forms half of the unit (see Figure 2.5).



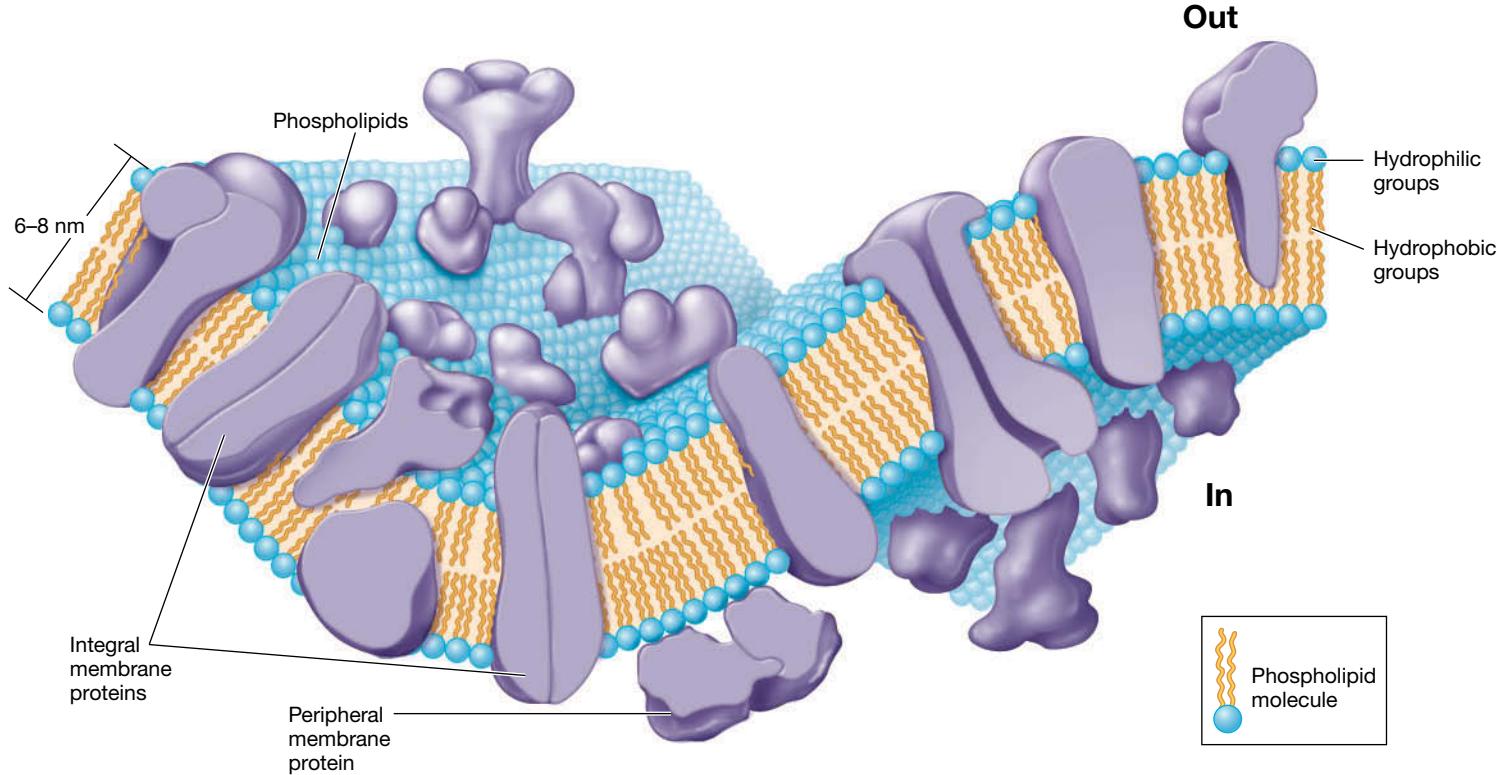
**Figure 2.4 Phospholipid bilayer membrane.** (a) Structure of the phospholipid phosphatidylethanolamine. The side chains are fatty acids and the ester linkage (characteristic of the lipids of *Bacteria* and *Eukarya* but not *Archaea*) is boxed with a red dashed line. (b) General architecture of a bilayer membrane; the blue spheres depict glycerol with phosphate and/or other hydrophilic groups. (c) Transmission electron micrograph of a membrane. The light inner area is the hydrophobic region of the model membrane shown in b. G. Wagner

The cytoplasmic membrane is only 8–10 nanometers wide but can be resolved easily by transmission electron microscopy (Figure 2.4c). In addition, although physically weak, the cytoplasmic membranes of some *Bacteria* are strengthened by sterol-like molecules called *hopanoids*. Sterols are rigid and planar molecules that strengthen the membranes of eukaryotic cells (Section 2.14), many of which lack a cell wall.

A variety of proteins are attached to or integrated into the cytoplasmic membrane; membrane proteins typically have hydrophobic domains that span the membrane and hydrophilic domains that contact the environment or the cytoplasm (Figure 2.5). Proteins significantly embedded in the membrane are called *integral* membrane proteins. By contrast, *peripheral* membrane proteins are more loosely attached. Some peripheral membrane proteins are *lipoproteins*, proteins that contain a hydrophobic lipid tail that anchors the protein into the membrane (Figure 2.5). Peripheral membrane proteins typically interact with integral membrane proteins in important cellular processes such as energy metabolism and transport.

#### Archaeal Membranes

The cytoplasmic membrane of *Archaea* is structurally similar to those of *Bacteria* and *Eukarya*, but the chemistry is somewhat



**Figure 2.5 Structure of the cytoplasmic membrane.** The inner surface (**In**) faces the cytoplasm and the outer surface (**Out**) faces the environment. Phospholipids compose the matrix of the cytoplasmic membrane with proteins embedded (integral) or surface associated (peripheral). The general design of the cytoplasmic membrane is similar in both prokaryotic and eukaryotic cells, although there are chemical differences between different species.

different. In contrast to the lipids of *Bacteria* and *Eukarya* in which ester linkages bond *fatty acids* to glycerol (Figure 2.4), the lipids of *Archaea* contain *ether* bonds between glycerol and a hydrophobic side chain that is not a fatty acid (Figure 2.6). The hydrophobic region of archaeal membranes is formed from repeating units of the five-carbon hydrocarbon *isoprene*, rather than from fatty acids (compare Figures 2.4 and 2.6).

The cytoplasmic membrane of *Archaea* is constructed from either phosphoglycerol diethers, which have C<sub>20</sub> side chains (called a *phytanyl* group), or diphosphoglycerol tetraethers (C<sub>40</sub> side chains, called a *biphytanyl* group) (Figure 2.6). In the tetraether lipid structure, the ends of the inwardly pointing phytanyl groups are covalently linked at their termini to form a *lipid monolayer* (Figure 2.6e) instead of a lipid bilayer (Figure 2.6d) membrane.

Some archaeal lipids contain rings within the hydrocarbon side chains. For example, *crenarchaeol*, a common membrane lipid in cells of *Crenarchaeota* (a major phylum of *Archaea*) contains four C<sub>5</sub> rings and one C<sub>6</sub> ring (Figure 2.6c). These rings affect the chemical properties of the lipids and thus influence membrane function. As in other organisms, the polar head groups in archaeal lipids can be sugars, ethanolamine, or a variety of other molecules. Hopanoids, present in the cytoplasmic membranes of many *Bacteria*, have not been found in the cytoplasmic membranes of *Archaea*.

Despite differences in chemistry between the cytoplasmic membranes of *Archaea* and organisms in the other phylogenetic domains, the fundamental construction of the archaeal cytoplasmic membrane—inner and outer hydrophilic surfaces and a

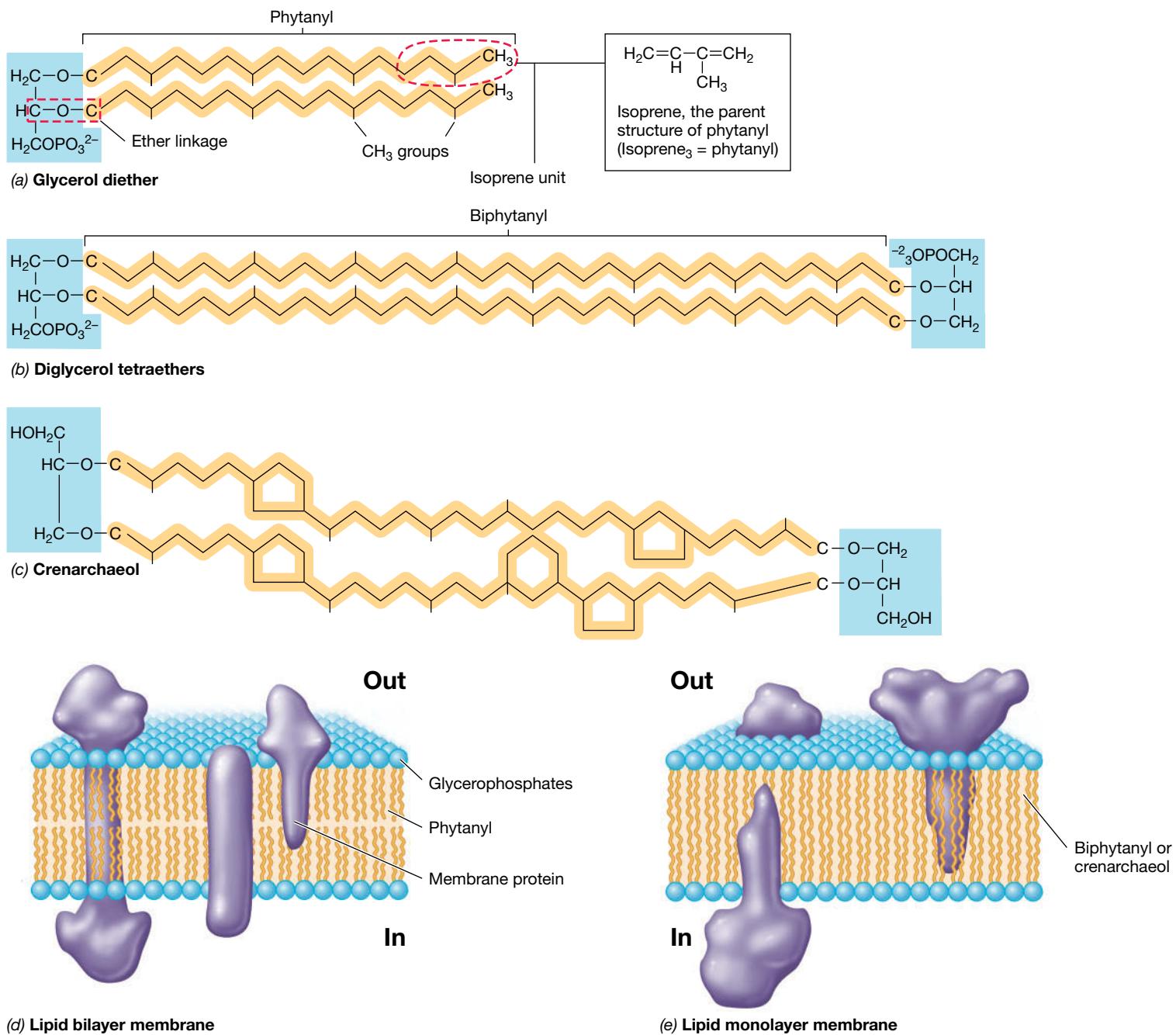
hydrophobic interior—is the same as that of membranes in all cells. Obviously, evolution has selected this fundamental design as the best solution to the major functions of the cytoplasmic membrane, an issue we turn to now.

### Cytoplasmic Membrane Function

The cytoplasmic membrane has at least three major functions (Figure 2.7). First, it is the cell's permeability barrier, preventing the passive leakage of solutes into or out of the cell. Second, the cytoplasmic membrane anchors several proteins that catalyze a suite of key cell functions. And third, the cytoplasmic membrane of *Bacteria* and *Archaea* plays a major role in energy conservation and consumption.

The cytoplasmic membrane is a barrier to the diffusion of most substances, especially polar or charged molecules. Because the cytoplasmic membrane is so impermeable, most substances that enter or leave the cell must be carried in or out by *transport proteins*. These are not simply ferrying proteins but instead function to *accumulate* solutes against the concentration gradient, a process that diffusion alone cannot do (Figure 2.8). Transport, which requires energy, ensures that the cytoplasm has sufficient concentrations of the nutrients it needs to perform biochemical reactions efficiently.

Transport proteins typically display high sensitivity and high specificity. If the concentration of a solute is high enough to saturate the transporter, which often occurs at the very low concentrations of nutrients found in nature, the rate of uptake can be near maximal (Figure 2.8). Some nutrients are transported by a

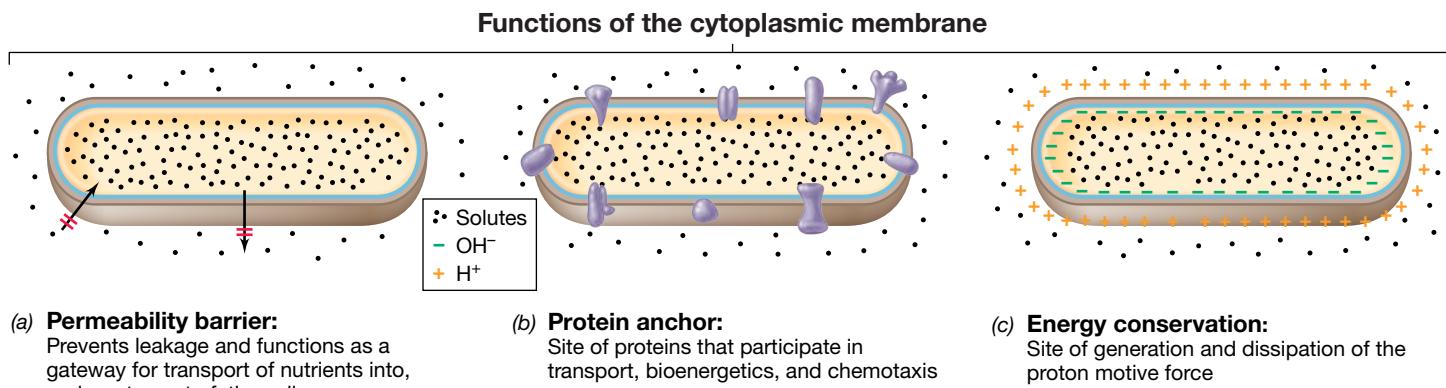


**Figure 2.6 Major lipids of Archaea and the architecture of archaeal membranes.** (a, b) Note that the hydrocarbon of the lipid is bonded to the glycerol by an ether linkage (in dashed red box in a) in both cases. The hydrocarbon is phytanyl ( $\text{C}_{20}$ ) in a and biphytanyl ( $\text{C}_{40}$ ) in b; both are multiples of the parent structure, isoprene (in dashed red oval; detailed structure shown in black box). (c) A major lipid of Thaumarchaeota is crenarchaeol, a lipid containing 5- and 6-carbon rings. (d, e) The membrane structure in Archaea may form a lipid bilayer or a lipid monolayer (or a mix of both).

low-affinity transporter when present at high external concentration and by a separate, typically higher-affinity, transporter for those present at low concentration (Figure 2.8). Moreover, many transport proteins transport only a single kind of molecule while others carry a related class of molecules, such as different sugars or different amino acids. This economizing reduces the need for separate transport proteins for each different sugar or

amino acid. We revisit the important issue of nutrient transport in Section 3.2, where we focus on transport mechanisms.

In addition to its permeability and transport functions, the cytoplasmic membrane of *Bacteria* and *Archaea* is a major site of both energy conservation and consumption. We discuss in Chapter 3 how the cytoplasmic membrane can be energized when protons ( $\text{H}^+$ ) are separated from hydroxyl ions ( $\text{OH}^-$ ) across the membrane

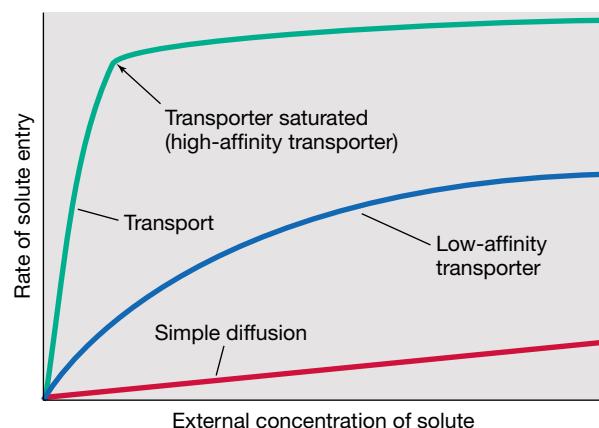


**Figure 2.7** The major functions of the cytoplasmic membrane. Although physically weak, the cytoplasmic membrane controls at least three critically important cellular functions: preventing leakage, anchoring proteins, and conserving energy.

surface (Figure 2.7c). This charge separation creates an energized state of the membrane called the *proton motive force*, analogous to the potential energy present in a charged battery. Dissipation of the proton motive force can be coupled to several energy-requiring reactions, such as transport, cell locomotion, and the biosynthesis of ATP. In eukaryotic microbial cells, although transport across the cytoplasmic membrane is just as necessary as it is in prokaryotic cells, energy conservation takes place in the membrane systems of the cell's key organelles, the mitochondrion (respiration) and chloroplast (photosynthesis).

### MINIQUIZ

- Draw the basic structure of a lipid bilayer and label the hydrophilic and hydrophobic regions. Why is the cytoplasmic membrane a good permeability barrier?
- How are the membrane lipids of *Bacteria* and *Archaea* similar, and how do they differ?
- Describe the major functions of the cytoplasmic membrane.



**Figure 2.8** The importance of transport in membrane function. In transport, the uptake rate shows saturation at relatively low external concentrations. Both high-affinity and low-affinity transport systems are depicted.

## 2.4 Bacterial Cell Walls: Peptidoglycan

The cytoplasm of prokaryotic cells maintains a high concentration of dissolved solutes that creates significant osmotic pressure—about 2 atm (203 kPa); this is about the same as the pressure in an automobile tire. To withstand these pressures and prevent bursting—a process called *cell lysis*—most cells of *Bacteria* and *Archaea* have a layer outside the cytoplasmic membrane called the *cell wall*. Besides protecting against osmotic lysis, cell walls also confer shape and rigidity on the cell.

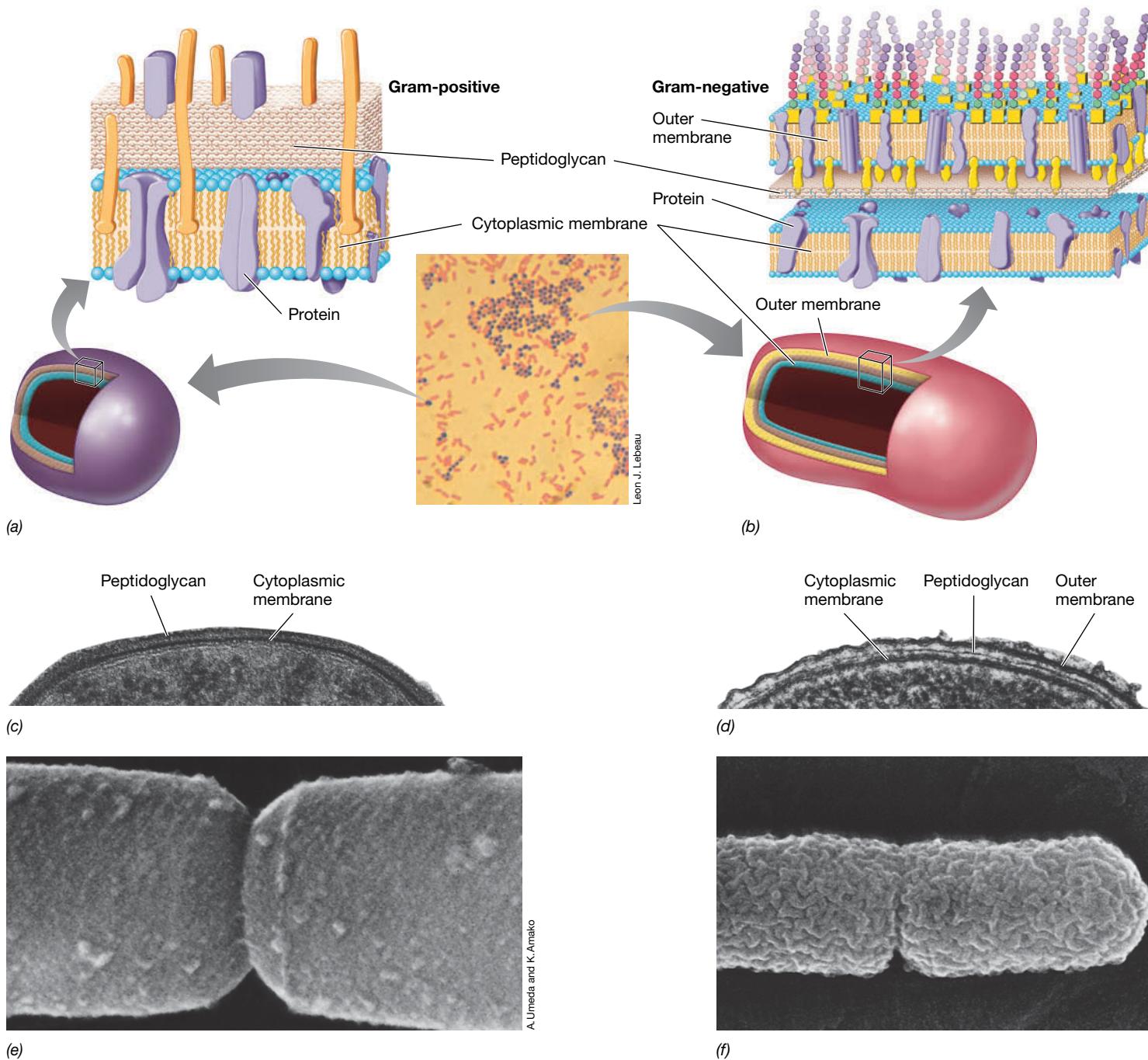
Knowledge of cell wall structure and function is important not only for understanding the biology of microbial cells, but also because certain antibiotics, for example, the penicillins and cephalosporins, target bacterial cell wall synthesis, leaving the cell susceptible to osmotic lysis. Since human cells lack cell walls and are therefore not a target of such antibiotics, these drugs are of obvious benefit for treating bacterial infections.

Cells of *Bacteria* can be divided into two major groups, *gram-positive* and *gram-negative*. The distinction between gram-positive and gram-negative bacteria is based on the Gram stain reaction (► Section 1.6), and differences in cell wall structure play a major role in the reaction. The surface of gram-positive and gram-negative cells as viewed in the electron microscope differs markedly, as shown in Figure 2.9. The gram-negative cell wall, or *cell envelope* as it is also called, consists of at least two layers, whereas the gram-positive cell wall is typically thicker and consists primarily of a single type of molecule.

Our focus in this section is on a key molecule found in the cell walls of both gram-positive and gram-negative *Bacteria*. In Section 2.5, we describe some additional wall components present in gram-negative *Bacteria*, and in Section 2.6, we describe the cell walls of *Archaea*.

### Structure of Peptidoglycan

The walls of cells of *Bacteria* contain a rigid polysaccharide called **peptidoglycan** that confers structural strength on the cell. Peptidoglycan is found in all *Bacteria* that contain a cell wall, but it is not present in the cell walls of *Archaea* or *Eukarya*. Peptidoglycan is composed of alternating repeats of two modified glucose residues

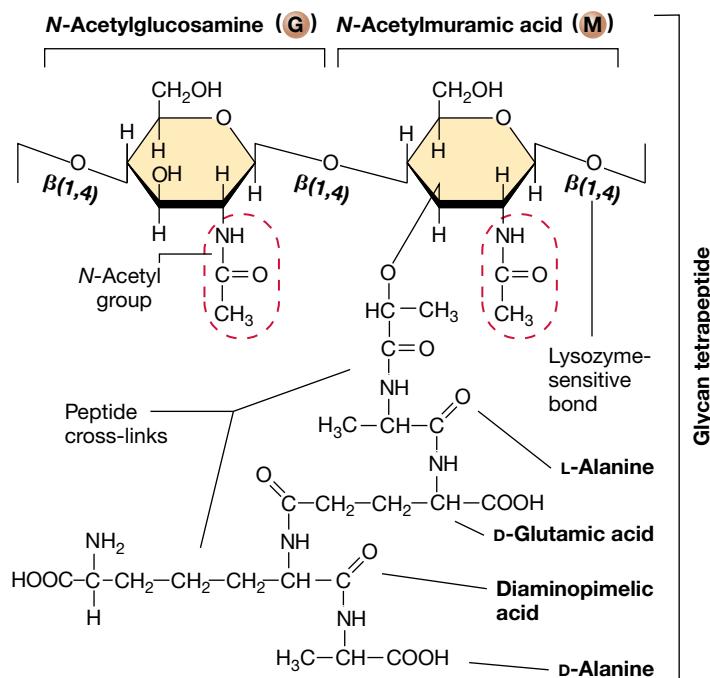


**Figure 2.9 Cell walls of Bacteria.** (a, b) Schematic diagrams of gram-positive and gram-negative cell walls; the Gram stain procedure was described in Section 1.6 and shown in Figure 1.18. The photo of Gram-stained bacteria in the center shows cells of *Staphylococcus aureus* (purple, gram-positive) and *Escherichia coli* (pink, gram-negative). (c, d) Transmission electron micrographs showing the cell wall of a gram-positive bacterium and a gram-negative bacterium, respectively. (e, f) Scanning electron micrographs of gram-positive and gram-negative bacteria, respectively. Note differences in surface texture. Each cell is about 1  $\mu\text{m}$  wide.

called *N-acetylglucosamine* and *N-acetylmuramic acid* along with the amino acids L-alanine, D-alanine, D-glutamic acid, and either L-lysine or diaminopimelic acid (DAP). These constituents are connected in an ordered way to form the *glycan tetrapeptide* (Figure 2.10), and long chains of this basic unit form peptidoglycan.

Strands of peptidoglycan are biosynthesized adjacent to one another to form a sheet surrounding the cell, and the individual

strands are connected by peptide cross-links; this forms a polymer that is strong in both X and Y directions (Figure 2.11). In gram-negative bacteria, the cross-link forms from the amino group of DAP of one glycan strand to the carboxyl group of the terminal D-alanine on the adjacent glycan strand (Figure 2.11a). In gram-positive bacteria, the cross-link often contains a short peptide “interbridge,” the kinds and numbers of amino acids in the



**Figure 2.10** Structure of the repeating unit in peptidoglycan, the glycan tetrapeptide. The structure given is that of *Escherichia coli* and most other gram-negative Bacteria. In some Bacteria, other amino acids are present as cross-linkers.

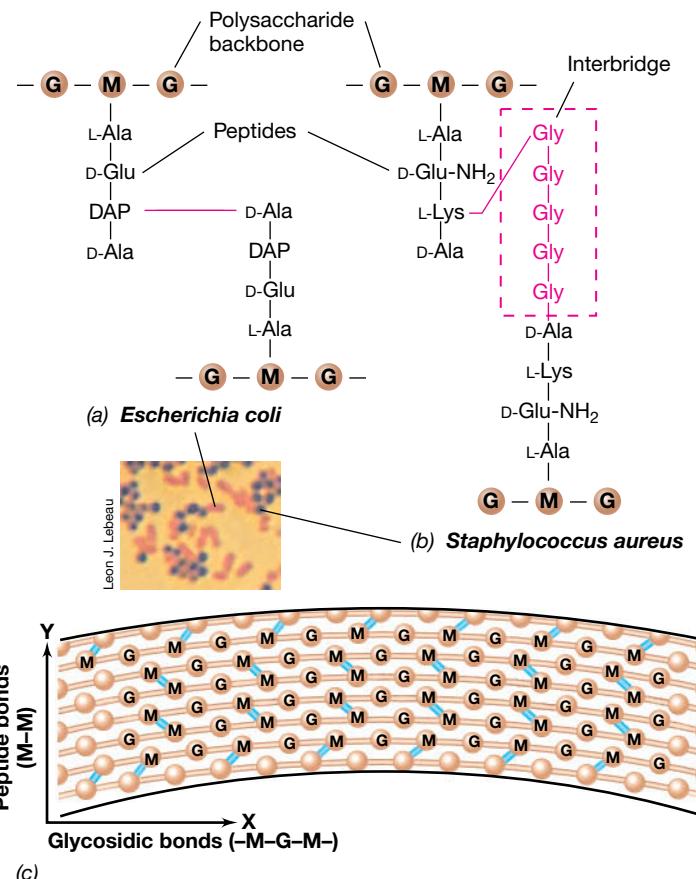
interbridge varying between species. In the gram-positive bacterium *Staphylococcus aureus*, for example, a bacterium whose cell wall chemistry is well understood, the interbridge consists of five glycines (Figure 2.11b). The overall structure of peptidoglycan is shown in Figure 2.11c.

Peptidoglycan can be destroyed by *lysozyme*, an enzyme that cleaves the glycosidic bond between *N*-acetylglucosamine and *N*-acetylmuramic acid (Figure 2.11). This weakens the peptidoglycan and can cause cell lysis. Lysozyme is present in human secretions including tears, saliva, and other bodily fluids, and functions as a major line of defense against bacterial infection. When we consider peptidoglycan biosynthesis in Chapter 7, we will see that the antibiotic penicillin also destroys peptidoglycan, but in a different way than lysozyme does. Whereas lysozyme destroys preexisting peptidoglycan, penicillin blocks a key step in its biosynthesis; this also weakens the molecule and leads to osmotic lysis.

An unusual feature of peptidoglycan is the presence of two amino acids of the D stereoisomer, D-alanine and D-glutamic acid. Teichoic acids, to be described shortly, also contain D-amino acids. By contrast, proteins are always constructed of L-amino acids. All in all, more than 100 chemically distinct peptidoglycans have been described that vary in their peptide cross-links and/or interbridge structures. By contrast, the glycan portion of all peptidoglycans appears to be universal; only alternating repeats of *N*-acetylglucosamine and *N*-acetylmuramic acid in  $\beta$ -1,4 linkage are known (Figures 2.10 and 2.11).

### Overview of the Gram-Positive Cell Wall

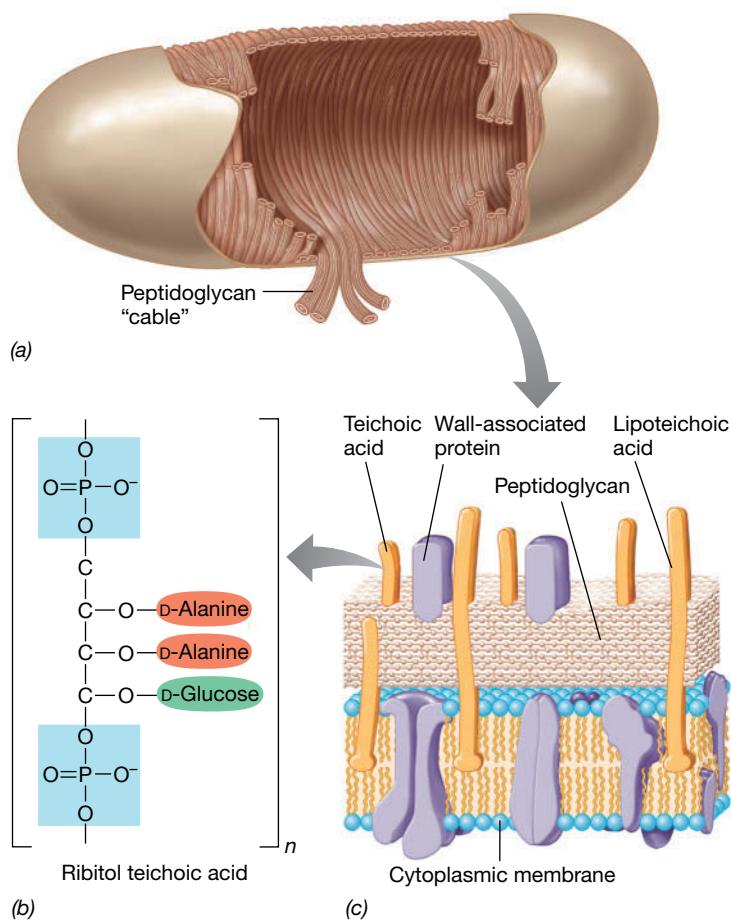
As much as 90% of the cell wall of a gram-positive bacterium can consist of peptidoglycan. Although some bacteria have only a



**Figure 2.11** Peptidoglycan in *Escherichia coli* and *Staphylococcus aureus*.  
(a) No interbridge is present in *E. coli* peptidoglycan nor in that of other gram-negative Bacteria. (b) The glycine interbridge in the gram-positive bacterium *S. aureus*.  
(c) Overall structure of peptidoglycan. G, *N*-acetylglucosamine; M, *N*-acetylmuramic acid. Note how glycosidic bonds confer strength on peptidoglycan in the X direction whereas peptide bonds confer strength in the Y direction.

single layer of peptidoglycan, many gram-positive bacteria form several layers of peptidoglycan stacked one upon another (Figure 2.11c). It is thought that peptidoglycan is synthesized by the cell in the form of “cables” about 50 nm wide, with each cable consisting of several glycan strands (Figure 2.12a). As peptidoglycan is synthesized, the cables themselves become cross-linked to form an even stronger cell wall structure.

In addition to peptidoglycan, many gram-positive bacteria produce acidic molecules called **teichoic acids** embedded in their cell wall. Teichoic acids are composed of glycerol phosphate or ribitol phosphate with attached molecules of glucose or D-alanine (or both). Individual alcohol molecules are then connected through their phosphate groups to form long strands, and these are then covalently linked to peptidoglycan (Figure 2.12b). Teichoic acids also function to bind divalent metal ions, such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , prior to their transport into the cell. Some teichoic acids are covalently bonded to membrane lipids rather than to peptidoglycan, and these are called *lipoteichoic acids*. Figure 2.12c summarizes the structure of the cell wall of gram-positive Bacteria and shows how teichoic acids and lipoteichoic acids are arranged in the overall wall structure.



**Figure 2.12** Structure of the gram-positive bacterial cell wall. (a) Schematic of a gram-positive rod showing the internal architecture of the peptidoglycan "cables." (b) Structure of a ribitol teichoic acid. The teichoic acid is a polymer of the repeating ribitol unit shown here. (c) Summary diagram of the gram-positive bacterial cell wall.

A very few *Bacteria* and *Archaea* lack cell walls altogether. These include in particular the mycoplasmas, pathogenic *Bacteria* related to gram-positive bacteria that cause a variety of infectious diseases of humans and other animals, and *Thermoplasma* and some of its relatives (*Archaea*). Lacking a cell wall, these cells would be expected to contain unusually tough cytoplasmic membranes, and chemical analyses show that they do. For example, most

mycoplasmas contain sterols in their cytoplasmic membranes; these molecules function to add strength and rigidity to the membrane as they do in the cytoplasmic membranes of eukaryotic cells (Section 2.14). *Thermoplasma* membranes contain molecules called *lipoglycans* that serve a similar strengthening function.

### MINIQUIZ

- Why do bacterial cells need cell walls? Do all bacteria have cell walls?
- Why is peptidoglycan such a strong molecule?
- What do the enzyme lysozyme and the antibiotic penicillin have in common?

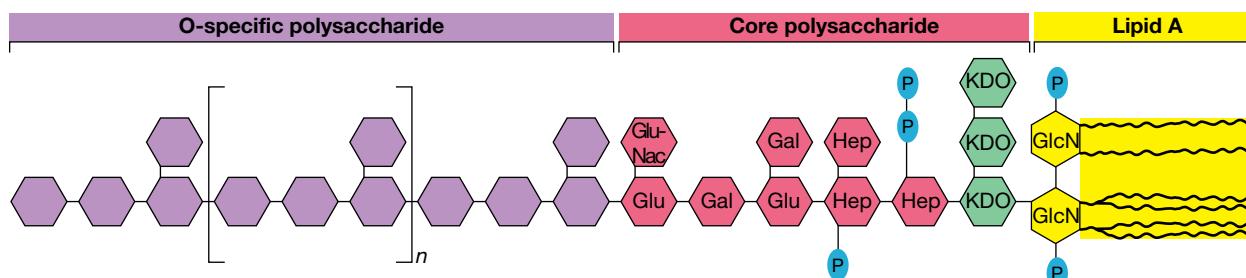
## 2.5 LPS: The Outer Membrane

In gram-negative bacteria, only a small amount of the total cell wall consists of peptidoglycan, as most of the wall is composed of the **outer membrane**. This layer is effectively a second lipid bilayer, but it is not constructed solely of phospholipid and protein, as is the cytoplasmic membrane (Figures 2.4 and 2.5). Instead, the outer membrane also contains polysaccharide, and the lipid and polysaccharide are linked to form a complex. Hence, the outer membrane is often called the **lipopolysaccharide** layer, or simply **LPS** for short.

The outer membrane confers only modest structural strength on the gram-negative cell (peptidoglycan remains the major strengthening agent), but it acts as an effective barrier against many substances such as lipophilic antibiotics and other harmful agents that might otherwise penetrate the cytoplasmic membrane. Indeed, many antibiotics that are clinically useful against gram-positive bacterial pathogens show little to no activity against gram-negative pathogens because of their outer membrane.

### Structure and Activity of LPS

The structure of LPS from several bacteria is known, and there are many variations. As seen in Figure 2.13, the polysaccharide portion of LPS consists of two components, the *core polysaccharide* and the *O-specific polysaccharide*. In *Salmonella* species, where LPS has been well studied, the core polysaccharide consists of ketodeoxyoctonate (KDO), various seven-carbon sugars (heptoses),



**Figure 2.13** Structure of bacterial lipopolysaccharide. The chemistry of lipid A and the polysaccharide components varies among species of gram-negative *Bacteria*, but the major components (lipid A-KDO-core-O-specific) are typically the same. The O-specific polysac-

charide is highly variable among species. KDO, ketodeoxyoctonate; Hep, heptose; Glu, glucose; Gal, galactose; GluNac, N-acetylglucosamine; GlcN, glucosamine; P, phosphate. Glucosamine and the lipid A fatty acids are linked through the amine groups of GlcN.

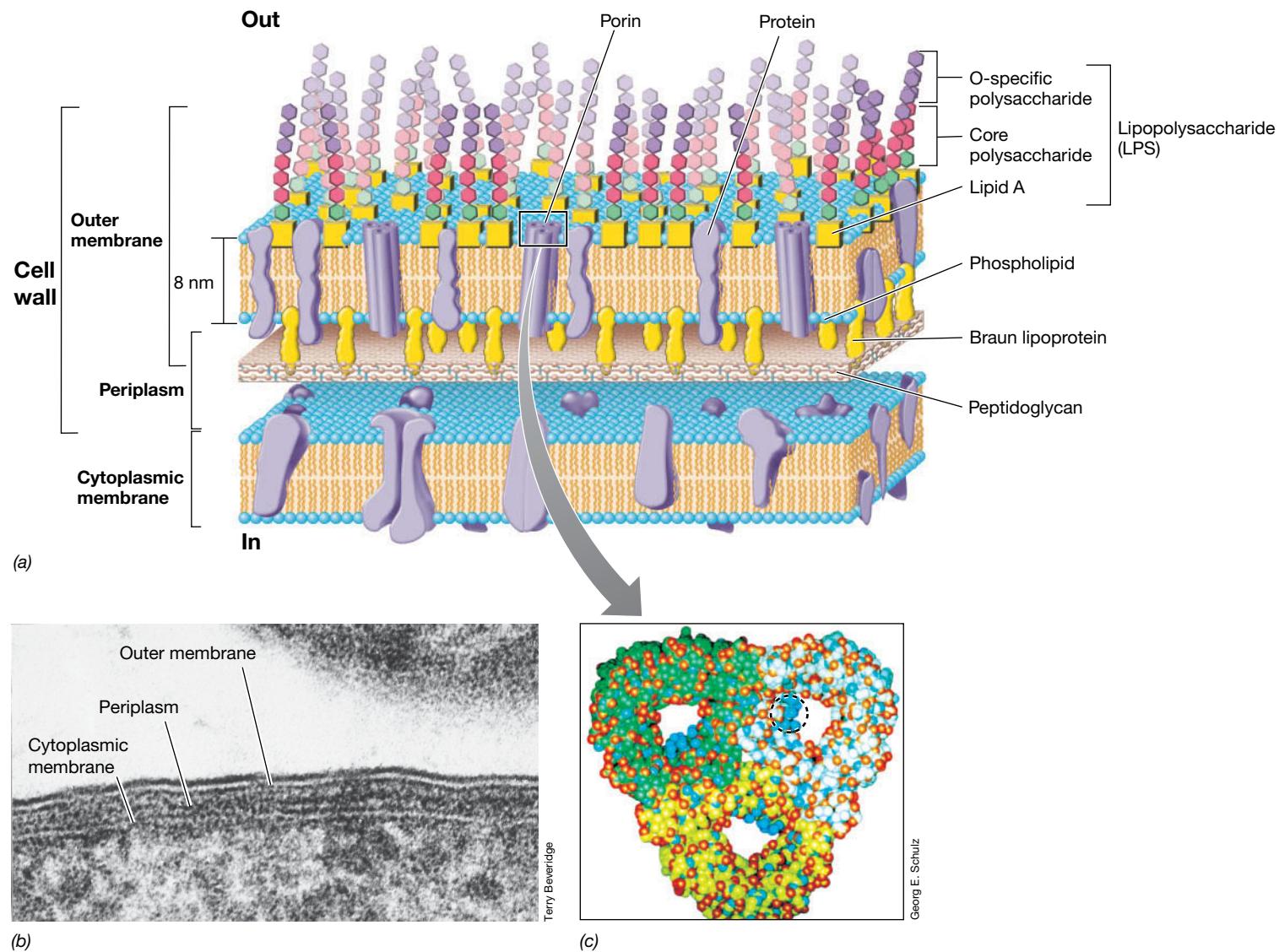
The lipid A portion of LPS can be toxic to animals and comprises the endotoxin complex (► Section 25.7 and Figure 25.18). Compare this figure with Figure 2.14 and follow the LPS components by their color-coding.

glucose, galactose, and *N*-acetylglucosamine. Connected to the core is the O-specific polysaccharide, which typically contains galactose, glucose, rhamnose, and mannose, as well as one or more dideoxyhexoses, such as abequose, colitose, paratose, or tyvelose. These sugars are connected in four- or five-membered sequences, which often are branched. When the sequences repeat, the long O-specific polysaccharide is formed.

The relationship of the LPS layer to the overall gram-negative cell wall is shown in **Figure 2.14**. The lipid portion of the LPS, called *lipid A*, is not a typical glycerol lipid (see Figure 2.4a); instead the fatty acids are bonded through the amine groups from a disaccharide composed of glucosamine phosphate. The disaccharide is attached to the core polysaccharide through KDO (Figure 2.13). Fatty acids commonly found in lipid A include caproic ( $C_6$ ), lauric

( $C_{12}$ ), myristic ( $C_{14}$ ), palmitic ( $C_{16}$ ), and stearic ( $C_{18}$ ) acids. LPS replaces much of the phospholipid in the outer half of the outer membrane, and although the outer membrane is technically a lipid bilayer, its many unique components distinguish it from the cytoplasmic membrane. The outer membrane is anchored to the peptidoglycan layer by the *Braun lipoprotein*, a molecule that spans the gap between the LPS layer and the peptidoglycan layer (in the periplasm, discussed in the next subsection) (Figure 2.14a).

An important biological activity of LPS is its toxicity to animals. Common gram-negative pathogens for humans include species of *Salmonella*, *Shigella*, and *Escherichia*, among many others, and some of the gastrointestinal symptoms these pathogens elicit are due to their toxic outer membrane components. Toxicity is specifically linked to the LPS layer, in particular, to lipid A. The term



**Figure 2.14** The gram-negative bacterial cell wall. (a) Arrangement of lipopolysaccharide, lipid A, phospholipid, porins, and Braun lipoprotein in the outer membrane. See Figure 2.13 for details of the structure of LPS. (b) Transmission electron micrograph of a cell of *Escherichia coli* showing the cytoplasmic membrane and wall. (c) Molecular model of porin proteins. Note the four pores present, one within each of the proteins forming a porin molecule and a smaller central pore (circled) between the porin proteins. The view is perpendicular to the plane of the membrane.

*endotoxin* refers to this toxic component of LPS. Some endotoxins cause violent symptoms in humans, including gas, diarrhea, and vomiting, and the endotoxins produced by *Salmonella* and enteropathogenic strains of *E. coli* transmitted in contaminated foods are classic examples of this. We discuss major gram-negative enteric pathogens in Chapter 32 and endotoxin in Section 25.7.

### The Periplasm and Porins

The outer membrane is impermeable to proteins and other very large molecules. In fact, a major function of the outer membrane is to prevent cellular proteins whose activities must occur outside the cytoplasm from diffusing away from the cell. These extracellular proteins reside in the **periplasm**. This space, located between the outer surface of the cytoplasmic membrane and the inner surface of the outer membrane, spans about 15 nm (Figure 2.14a, b).

The periplasm may contain several different classes of proteins. These include hydrolytic enzymes, which function in the initial degradation of polymeric substances; binding proteins, which begin the process of transporting substrates (► Section 3.2); chemoreceptors, which are proteins that govern the chemotaxis response (Section 2.13); and proteins that construct extracellular structures (such as peptidoglycan and the outer membrane) from precursor molecules secreted through the cytoplasmic membrane. Most periplasmic proteins reach the periplasm by way of a protein-exporting system present in the cytoplasmic membrane (► Sections 4.12 and 4.13).

The outer membrane is relatively permeable to small molecules because of proteins called *porins* that function as channels for the entrance and exit of solutes (Figure 2.14a, c). Several porins are known, including both specific and nonspecific classes. Nonspecific porins form water-filled channels through which virtually any very small hydrophilic substance can pass. By contrast, specific porins contain a binding site for one or a group of structurally related substances. Porins are transmembrane proteins composed of three identical polypeptides. Besides the channel present in each subunit of a porin, the porin subunits aggregate in such a way that a hole about 1 nm in diameter is formed through which very small molecules can travel (Figure 2.14c).

The signature molecule of *Bacteria*—peptidoglycan—is absent from the *Archaea*. Nevertheless, cells of *Archaea* face the same osmotic stresses as do cells of *Bacteria* and thus need to counter these stresses with a cell wall. We consider the cell walls of *Archaea* now and see that the cell wall chemistries of these fascinating microbes occasionally hint at those of *Bacteria* but often confer their structural strength in chemically unique ways.

### MINIQUIZ

- Describe the major chemical components in the outer membrane of gram-negative bacteria.
- What is the function of porins and where are they located in a gram-negative cell wall?
- What component of the gram-negative cell has endotoxin properties?

## 2.6 Archaeal Cell Walls

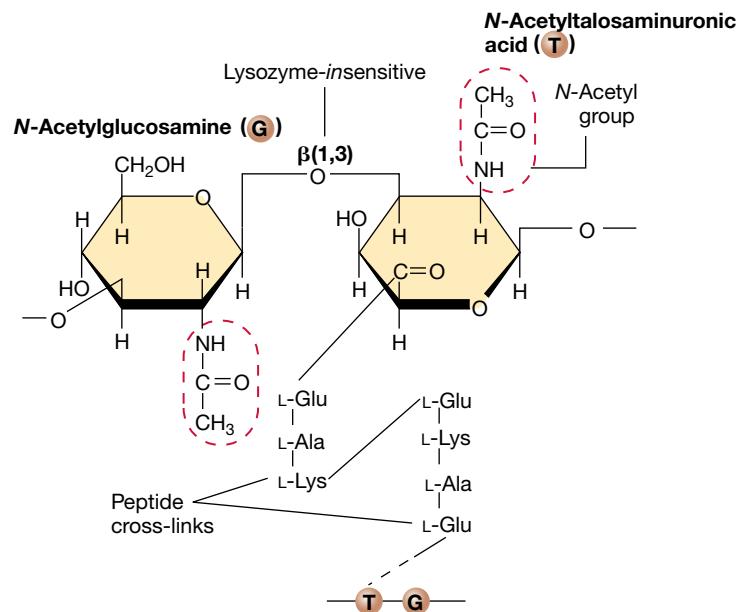
A variety of cell wall structures are found in *Archaea*, including walls containing polysaccharides, proteins, or glycoproteins or some mixture of these macromolecules.

### Pseudomurein and Other Polysaccharide Cell Walls

The cell walls of certain methane-producing *Archaea* (methanogens) contain a molecule that is remarkably similar to peptidoglycan, a polysaccharide called *pseudomurein* (the term “murein” is from the Latin word for wall and was an old term for peptidoglycan) (Figure 2.15). The backbone of pseudomurein is formed from alternating repeats of *N*-acetylglucosamine (also present in peptidoglycan) and *N*-acetyltalosaminuronic acid; the latter replaces the *N*-acetylmuramic acid of peptidoglycan (Section 2.4). Pseudomurein also differs from peptidoglycan in that the glycosidic bonds between the sugar derivatives are  $\beta$ -1,3 instead of  $\beta$ -1,4, and the amino acids are all of the L stereoisomer (Figure 2.15).

Because in many respects they are so similar, it is likely that peptidoglycan and pseudomurein are variants of a cell wall polysaccharide originally present in the common ancestor of *Bacteria* and *Archaea*. However, although they are structurally very similar, they differ sufficiently that pseudomurein is immune from destruction by both lysozyme and penicillin, molecules that destroy peptidoglycan (Section 2.4).

Cell walls of some other *Archaea* lack pseudomurein and instead contain other polysaccharides. For example, *Methanosarcina* species have thick polysaccharide walls composed of polymers of glucose, glucuronic acid, galactosamine uronic acid, and acetate. Extremely halophilic (salt-loving) *Archaea* such as *Halococcus*, which are related to *Methanosarcina*, have similar cell walls that contain large amounts of sulfate. The negative charges on the sulfate ion ( $\text{SO}_4^{2-}$ ) bind the abundant  $\text{Na}^+$  present in the habitats of *Halococcus*.



**Figure 2.15 Pseudomurein.** Structure of pseudomurein, the cell wall polymer of *Methanobacterium* species. Note the similarities and differences between pseudomurein and peptidoglycan (Figures 2.10 and 2.11).

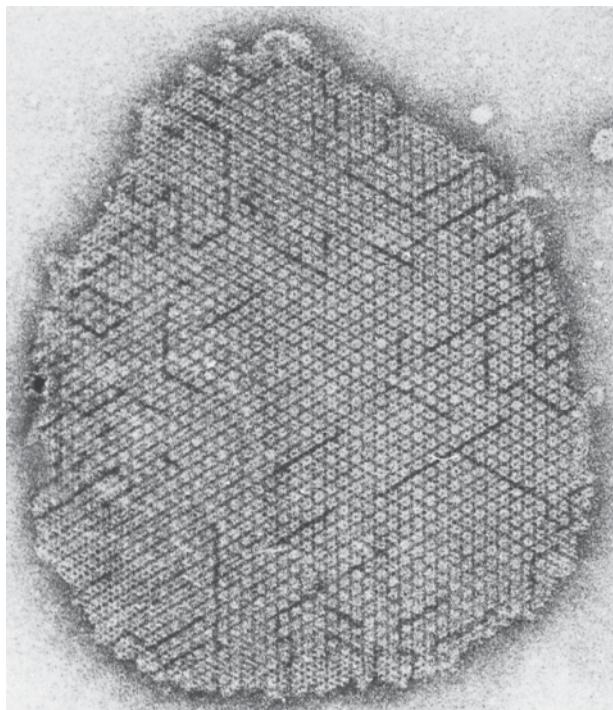
(salt evaporation ponds and saline seas and lakes), and this sulfate–sodium complex helps stabilize the *Halococcus* cell wall.

### S-Layers

The most common type of cell wall in *Archaea* is the paracrystalline surface layer, or **S-layer** as it is called. S-layers consist of interlocking molecules of protein or glycoprotein (Figure 2.16). The paracrystalline structure of S-layers can form various symmetries, including hexagonal, tetragonal, or trimeric, depending upon the number and structure of the subunits of which it is composed. S-layers have been found in representatives of all major lineages of *Archaea* and also in some species of *Bacteria* (Figure 2.16).

The cell walls of some *Archaea*, for example the methanogen *Methanocaldococcus jannaschii*, consist only of an S-layer. Thus, S-layers are sufficiently strong to withstand osmotic pressures without any other wall components. However, in many organisms S-layers are present in addition to other cell wall components, usually polysaccharides. When an S-layer accompanies other wall components, the S-layer is always the *outermost* wall layer; that is, the layer that is in direct contact with the environment.

Besides serving as protection from osmotic lysis, S-layers undoubtedly have other functions. For example, as the interface between the cell and its environment, it is likely that the S-layer functions as a selective sieve, allowing the passage of low-molecular-weight solutes while excluding large molecules or structures (such as viruses or lytic enzymes). The S-layer may also function to retain proteins near the cell surface that must function outside the cytoplasmic membrane, much as the outer membrane (Section 2.5) retains periplasmic proteins and prevents their drifting away in gram-negative *Bacteria*.



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**Figure 2.16 The S-layer.** Transmission electron micrograph of an S-layer fragment showing its paracrystalline nature. Shown is the S-layer from *Aquaspirillum* (*Bacteria*); this S-layer shows hexagonal symmetry common in S-layers of *Archaea*.

### MINIQUIZ

- How does pseudomurein resemble peptidoglycan? How do the two molecules differ?
- What is the structure of an S-layer, and what are its functions?

## III • Cell Surface Structures and Inclusions

In addition to the cytoplasmic membrane and cell wall, cells of *Bacteria* and *Archaea* may have other layers or structures in contact with the environment and often contain one or more types of cellular inclusions. We examine some of these here.

### 2.7 Cell Surface Structures

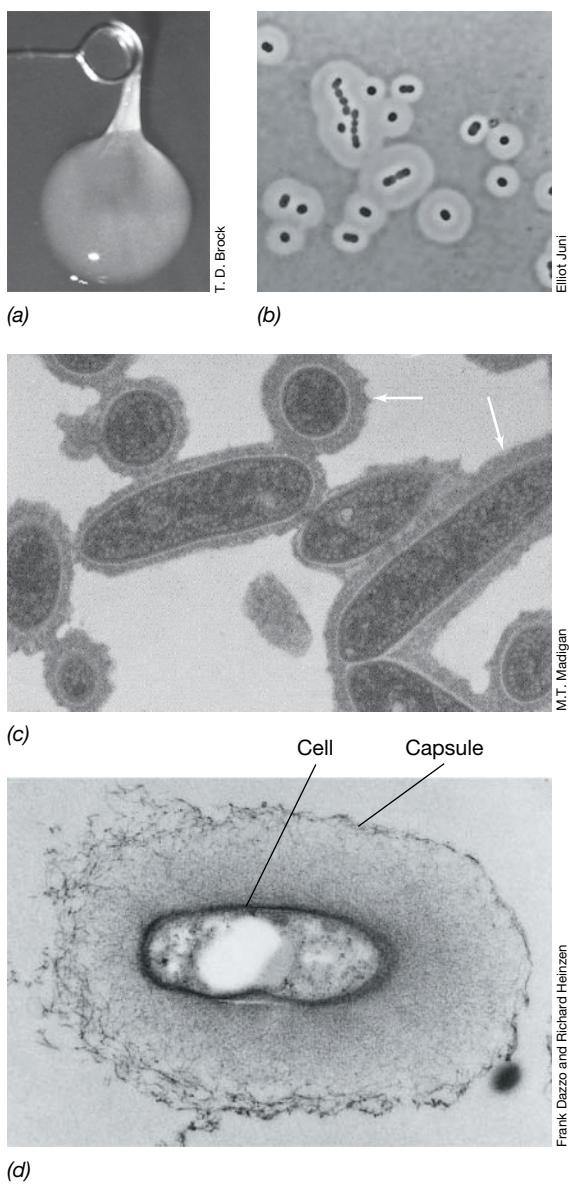
Many *Bacteria* and *Archaea* secrete sticky or slimy materials on their cell surface that consist of either polysaccharide or protein. However, these are not considered part of the cell wall because they do not confer significant structural strength on the cell. The terms “capsule” and “slime layer” are used to describe these layers.

#### Capsules and Slime Layers

The terms capsule and slime layer are often used interchangeably, but the two terms do not refer to the same thing. If the layer is organized in a tight matrix that excludes small particles and is tightly attached, it is called a **capsule**. Capsules are readily visible by light microscopy if cells are treated with India ink, which stains the background but not the capsule, and can also be seen in the electron microscope (Figure 2.17b–d). By contrast, if the layer is more easily deformed and loosely attached, it will not exclude particles and is more difficult to see microscopically. This form is called a *slime layer* and is easily recognized in colonies of slime-forming species such as the lactic acid bacterium *Leuconostoc* (Figure 2.17a).

Outer surface layers have several functions. Surface polysaccharides assist in the attachment of microorganisms to solid surfaces. As we will see later, pathogenic microorganisms that enter the body by specific routes usually do so by first binding specifically to surface components of host tissues; this binding is often facilitated by bacterial cell surface polysaccharides. When the opportunity arises, many bacteria will bind to solid surfaces, often forming a thick layer of cells called a *biofilm*. Extracellular polysaccharides play a key role in the development and maintenance of biofilms as well.

Besides attachment, outer surface layers have other functions. These include acting as virulence factors (molecules that contribute to the pathogenicity of a bacterial pathogen) and preventing dehydration. For example, the causative agent of the diseases anthrax and bacterial pneumonia—*Bacillus anthracis* and *Streptococcus pneumoniae*, respectively—each contain a thick capsule of either protein (*B. anthracis*) or polysaccharide (*S. pneumoniae*). Encapsulated cells of these bacteria avoid destruction by the host’s immune system because the immune cells that would otherwise recognize these pathogens as foreign and destroy them are blocked from doing so by the bacterial capsule. In addition to this role in

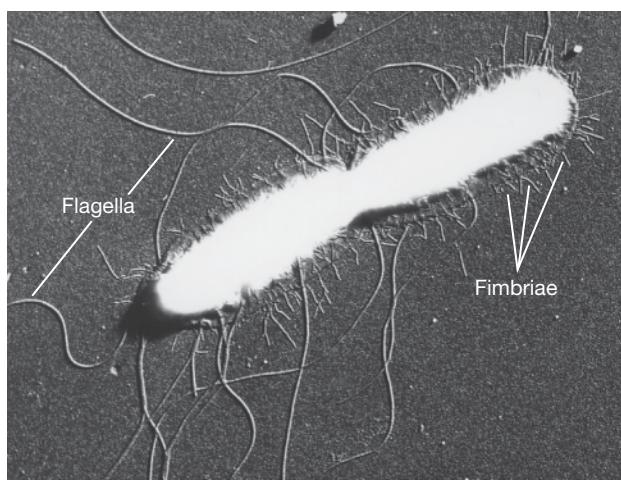


**Figure 2.17 Bacterial capsules and slime formation.** (a) A viscous colony of the bacterium *Leuconostoc mesenteroides* (lifted up by an inoculating loop) contains a thick dextran (glucose polymer) slime layer formed by the cells. (b) Capsules of *Acinetobacter* species observed by phase-contrast microscopy after negative staining with India ink. India ink does not penetrate the capsule and so the capsule appears as a light area surrounding the cell, which appears black. (c) Transmission electron micrograph of a thin section of cells of *Rhodobacter capsulatus* with capsules (arrows) clearly evident; cells are about 0.9  $\mu\text{m}$  wide. (d) Transmission electron micrograph of *Rhizobium trifoli* stained with ruthenium red to reveal the capsule. The cell is about 0.7  $\mu\text{m}$  wide.

disease, outer surface layers of virtually any type bind water and because of this likely protect the cell from desiccation in periods of dryness.

### Fimbriae, Pili, and Hami

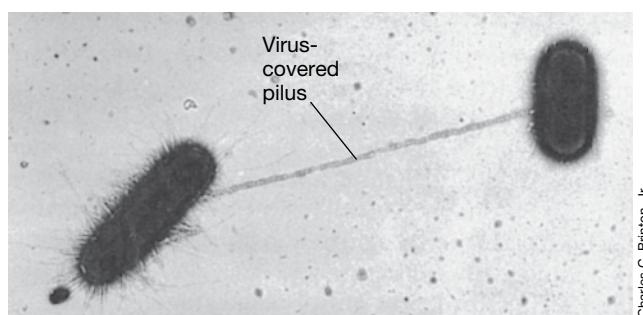
Fimbriae and pili are thin (2–10 nm in diameter) filamentous structures made of protein that extend from the surface of a cell and can have many functions. **Fimbriae** (Figure 2.18) enable cells to stick to surfaces, including animal tissues in the case of patho-



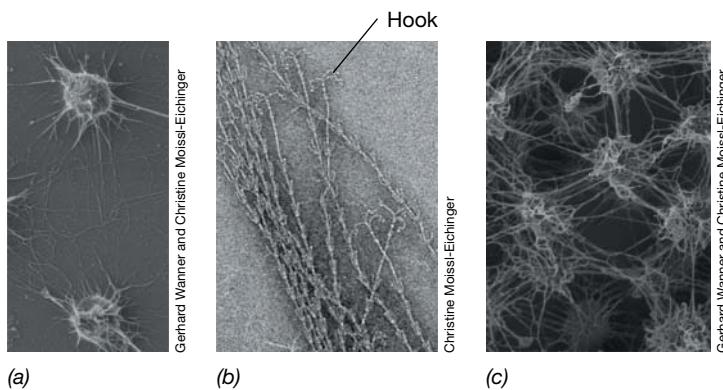
**Figure 2.18 Fimbriae.** Electron micrograph of a dividing cell of *Salmonella enterica* (*typhi*), showing flagella and fimbriae. A single cell is about 0.9  $\mu\text{m}$  wide.

genic bacteria, or to form pellicles (thin sheets of cells on a liquid surface) or biofilms on solid surfaces. **Pili** are similar to fimbriae, but are typically longer and only one or a few pili are present on the surface of a cell. All gram-negative bacteria produce pili of one sort or another, and many gram-positive bacteria also contain these structures. Because pili can be receptors for certain types of viruses, they can be easily seen under the electron microscope when they become coated with virus particles (Figure 2.19).

Many classes of pili are known, distinguished by their structure and function. Two very important functions of pili include facilitating genetic exchange between cells in a process called *conjugation* (conjugative or sex pili) and enabling the adhesion of pathogens to specific host tissues that they subsequently invade (type IV and other pili). *Type IV pili* not only facilitate specific adhesion but also support an unusual form of cell movement called *twitching motility* in certain bacterial species. On rod-shaped cells that move by twitching, type IV pili are present only at the poles. Twitching motility is a type of gliding motility, movement along a solid surface (Section 2.12). In twitching motility, extension of pili followed by their retraction drags the cell along a solid surface, and ATP supplies the energy necessary for this movement. The motility of certain species of *Pseudomonas* and *Moraxella* are the best-known examples of twitching motility.



**Figure 2.19 Pili.** The pilus on an *Escherichia coli* cell that is undergoing conjugation (a form of genetic transfer, Chapter 11) with a second cell is better resolved because viruses have adhered to it. The cells are about 0.8  $\mu\text{m}$  wide.



**Figure 2.20 Unique attachment structures in the SM1 group of Archaea: Hami.** (a) Cells of SM1 Archaea showing the pili-like surface structures called hami. (b) Transmission electron micrograph of isolated hami. A hamus “grappling hook” (labeled “Hook” in the micrograph) is about 60 nm in diameter. (c) A biofilm of SM1 cells showing the network of hami connecting individual cells.

Type IV pili have also been implicated as key colonization factors for certain human pathogens, including the gram-negative pathogens *Vibrio cholerae* (cholera) and *Neisseria gonorrhoeae* (gonorrhea) and the gram-positive pathogen *Streptococcus pyogenes* (strep throat and scarlet fever). The twitching motility of these organisms assists them in locating specific sites for attachment to initiate the disease process. Type IV pili also mediate genetic transfer by the process of transformation in some bacteria, which, along with conjugation and transduction, are the three known means of horizontal gene transfer in *Bacteria* and *Archaea* (Chapter 11). Type IV pili are also widespread in the *Archaea*, functioning in surface adhesion and cell aggregation events that lead to biofilm formation.

An unusual group of *Archaea*, the SM1 group, forms a unique attachment structure called a *hamus* (plural, *hami*) that resembles a tiny grappling hook (Figure 2.20a, b). The SM1 group inhabits anoxic groundwater in Earth’s deep subsurface, and *hami* function to affix cells to a surface to form a networked biofilm (Figure 2.20c). *Hami* structurally resemble type IV pili except for their barbed terminus, which functions to attach cells both to surfaces and to each other (Figure 2.20c). The biofilms formed by SM1 *Archaea* are likely an ecological strategy that allows these microbes to more efficiently trap the scarce nutrients present in their deep subsurface habitat. Although cells of the SM1 group are not as small as the groundwater ultramicrobacterial cells described earlier (see Explore the Microbial World, “Tiny Cells”), they are less than 1  $\mu\text{m}$  in diameter and live in a similar nutrient-limiting habitat. Thus, their *hami* probably play an important role in preventing cells from being washed away in groundwater flowage.

### MINIQUIZ

- Could a bacterial cell dispense with a cell wall if it had a capsule? Why or why not?
- How do fimbriae differ from pili, both structurally and functionally?
- How can type IV pili facilitate pathogenesis? What are *hami*?

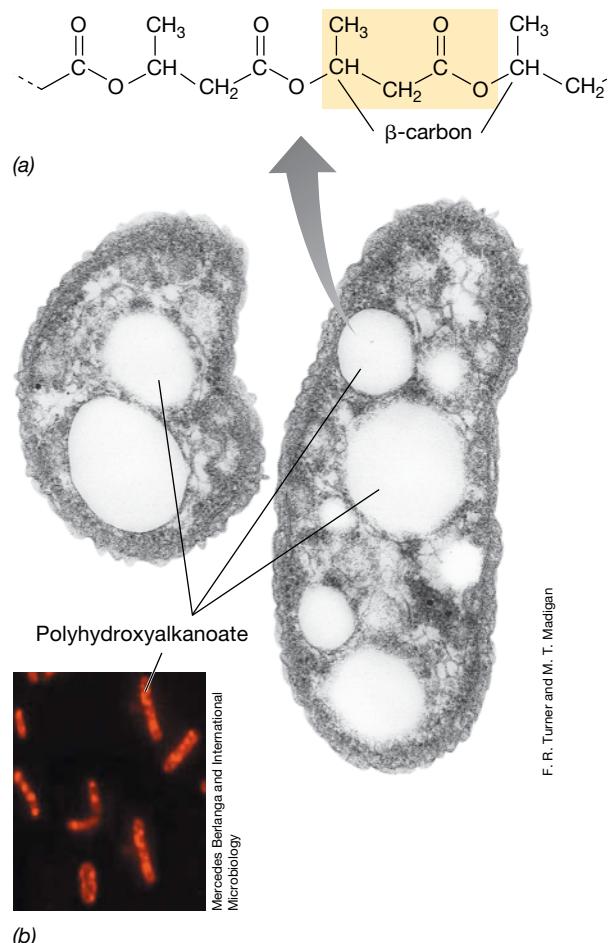
## 2.8 Cell Inclusions

Prokaryotic cells often contain inclusions of one sort or another. Inclusions function as energy reserves and/or carbon reservoirs or have special functions. Inclusions can often be seen in cells with the light microscope and are enclosed by a thin membrane that partitions off the inclusion in the cytoplasm. Storing carbon or other substances in an insoluble form is advantageous because it reduces the osmotic stress that the cell would encounter should the same amount be dissolved in the cytoplasm.

### Carbon Storage Polymers

One of the most common inclusion bodies in prokaryotic organisms is **poly- $\beta$ -hydroxybutyric acid (PHB)**, a lipid that is formed from  $\beta$ -hydroxybutyric acid units. The monomers of PHB polymerize by ester linkage and then the polymer aggregates into granules; the granules can be seen by either light or electron microscopy (Figure 2.21).

The monomer in the polymer is usually hydroxybutyrate ( $\text{C}_4$ ) but can vary in length from as short as  $\text{C}_3$  to as long as  $\text{C}_{18}$ . Thus,



**Figure 2.21 Poly- $\beta$ -hydroxyalkanoates (PHAs).** (a) Chemistry of poly- $\beta$ -hydroxybutyrate, a common PHA. A monomeric unit is shown in color. Other PHAs are made by substituting longer-chain hydrocarbons for the  $-\text{CH}_3$  group on the  $\beta$ -carbon. (b) Electron micrograph of a thin section of cells of a bacterium containing granules of PHB. Color photo: Nile red-stained cells of a PHA-containing bacterium.

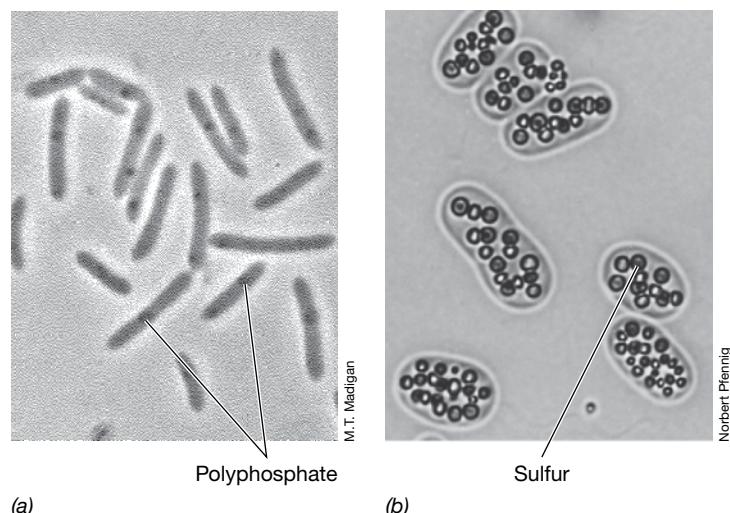
the more generic term poly- $\beta$ -hydroxyalkanoate (PHA) is often used to describe this class of carbon- and energy-storage polymers. PHAs are synthesized by cells when there is an excess of carbon and are broken down as carbon or energy sources when conditions warrant. Many *Bacteria* produce PHAs, as do several extremely halophilic species of *Archaea*.

Another storage inclusion is *glycogen*, which is a polymer of glucose; like PHA, glycogen is a reservoir of both carbon and energy and is produced when carbon is in excess. Glycogen resembles starch, the major storage reserve of plants, but differs slightly from starch in the manner in which the glucose units are linked together.

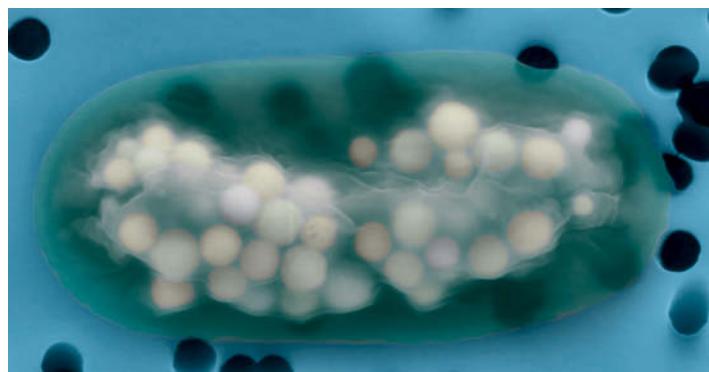
### Polyphosphate, Sulfur, and Carbonate Minerals

Many prokaryotic and eukaryotic microbes accumulate inorganic phosphate ( $\text{PO}_4^{3-}$ ) in the form of *polyphosphate* granules (Figure 2.22a). These granules are formed when phosphate is in excess and can be drawn upon as a source of phosphate for nucleic acid and phospholipid biosynthesis when phosphate is limiting. In addition, in some organisms, polyphosphate can be broken down to synthesize the energy-rich compound ATP from ADP.

Many gram-negative *Bacteria* and *Archaea* oxidize reduced sulfur compounds, such as hydrogen sulfide ( $\text{H}_2\text{S}$ ); these organisms are the “sulfur bacteria,” discovered by the great Russian microbiologist Sergei Winogradsky (Section 1.11). The oxidation of sulfide generates electrons for use in energy metabolism (chemolithotrophy) or  $\text{CO}_2$  fixation (autotrophy). In either case, *elemental sulfur* ( $\text{S}^0$ ) from the oxidation of sulfide may accumulate in the cell in microscopically visible granules (Figure 2.22b). This sulfur remains as long as the source of reduced sulfur from which it was derived is still present. However, as the reduced sulfur source becomes limiting, the  $\text{S}^0$  in the granules is oxidized to sulfate ( $\text{SO}_4^{2-}$ ), and the granules slowly disappear. Interestingly, although sulfur globules appear to reside in the cytoplasm (Figure 2.22b), they are actually



**Figure 2.22** Polyphosphate and sulfur storage products. (a) Phase-contrast photomicrograph of cells of *Heliobacterium modesticaldum* showing polyphosphate as dark granules; a cell is about 1  $\mu\text{m}$  wide. (b) Bright-field photomicrograph of cells of the purple sulfur bacterium *Isochromatium budei*. The periplasmic inclusions are sulfur globules formed from the oxidation of hydrogen sulfide ( $\text{H}_2\text{S}$ ). A cell is about 4  $\mu\text{m}$  wide.



**Figure 2.23** Biomineralization by a cyanobacterium. Electron micrograph of a cell of the cyanobacterium *Gloeomargarita* containing granules of the mineral benstonite  $[(\text{Ba}, \text{Sr})_6(\text{Ca}, \text{Mn})_6\text{Mg}(\text{CO}_3)_{13}]$ . A cell is about 2  $\mu\text{m}$  wide.

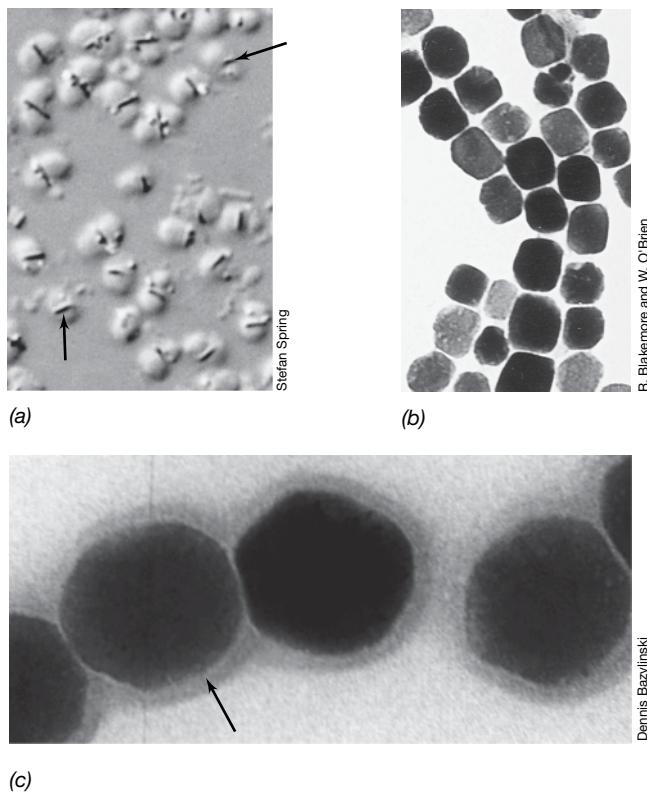
present in the periplasm (Section 2.5). In these cells the periplasm expands outward to accommodate the growing globules as  $\text{H}_2\text{S}$  is oxidized to  $\text{S}^0$  and then contracts inward as  $\text{S}^0$  is oxidized to  $\text{SO}_4^{2-}$  (Section 14.9).

Filamentous cyanobacteria have long been known to form carbonate minerals on the external surface of their cells. However, some cyanobacteria also form carbonate minerals *inside* the cell, as cell inclusions. For example, the unicellular cyanobacterium *Gloeomargarita* forms intracellular granules of benstonite, a carbonate mineral that contains barium, strontium, and magnesium (Figure 2.23). The microbiological process of forming minerals is called *biomineralization*. It is unclear why benstonite is formed by *Gloeomargarita*, although it might function as ballast to maintain cells of this cyanobacterium in their habitat, deep in an alkaline lake. Alternatively (or in addition), the mineral could be a way to sequester carbonate (a source of  $\text{CO}_2$ ) to support autotrophic growth. The biomineralization of several different minerals is catalyzed by various bacteria, but only in the case of *Gloeomargarita* and magnetosomes (to be discussed next) do we see the process yield actual intracellular inclusions.

### Magnetic Storage Inclusions: Magnetosomes

Some bacteria can orient themselves within a magnetic field because they contain **magnetosomes**. These structures are biomineralized particles of the magnetic iron oxides magnetite  $[\text{Fe}(\text{II})\text{Fe}(\text{III})_2\text{O}_4]$  or greigite  $[\text{Fe}(\text{II})\text{Fe}(\text{III})_2\text{S}_4]$  (Figure 2.24). Magnetosomes impart a magnetic dipole on a cell, allowing it to orient itself in a magnetic field. This allows the cell to undergo *magnetotaxis*, the process of migrating along Earth’s magnetic field lines. Magnetosomes have been found in several aquatic organisms that grow best at low  $\text{O}_2$  concentrations or are anaerobic. It has thus been hypothesized that one function of magnetosomes may be to guide these aquatic cells downward (the direction of Earth’s magnetic field) toward the sediments where  $\text{O}_2$  is low or absent.

Magnetosome synthesis begins with insertion of magnetosome-specific proteins into the cytoplasmic membrane followed by invagination of the membrane to form a vesicle. The vesicle is then filled with iron—primarily iron in the Fe(II) oxidation state—and biomineralization proceeds through the



**Figure 2.24** Magnetotactic bacteria and magnetosomes. (a) Differential interference contrast micrograph of coccoid magnetotactic bacteria; note chains of magnetosomes (arrows). A cell is 2.2  $\mu\text{m}$  wide. (b) Magnetosomes isolated from the magnetotactic bacterium *Magnetospirillum magnetotacticum*; each particle is about 50 nm wide. (c) Transmission electron micrograph of magnetosomes from an unnamed magnetic coccus. The arrow points to the membrane that surrounds each magnetosome. A single magnetosome is about 90 nm wide.

activities of the magnetosome proteins, which includes an iron oxidase that generates the Fe(III) needed to form the magnetic minerals. The morphology of magnetosomes varies and appears to be species-specific; several morphologies are possible, but square, rectangular, or spike-shaped magnetosomes are most common.

### MINIQUIZ

- Under what nutritional conditions would you expect PHAs or glycogen to be produced?
- Why would it be impossible for gram-positive bacteria to store sulfur as gram-negative sulfur-oxidizing chemolithotrophs can?
- How are magnetosomes and the *Gloeo margarita* inclusions similar and how do they differ?

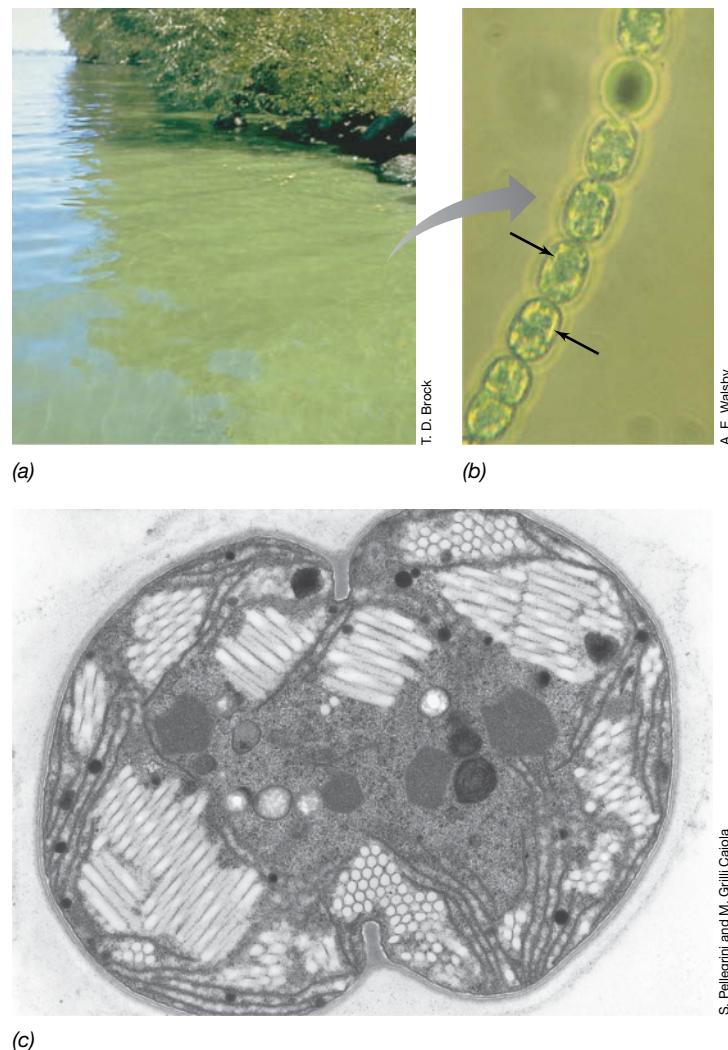
## 2.9 Gas Vesicles

Some *Bacteria* and *Archaea* are *planktonic*, meaning that they inhabit the water column of lakes and the oceans. Most planktonic organisms move up and down with changes in currents, but some can float because they contain **gas vesicles**, structures that confer buoyancy and allow the cells to position themselves in regions of the water column that best suit their metabolism.

The most dramatic examples of gas-vesiculate microbes are cyanobacteria that form massive accumulations called *blooms* in lakes or other bodies of water (Figure 2.25a). Cyanobacteria are oxygenic phototrophic bacteria (see Section 14.4). Gas-vesiculate cells rise to the surface of the lake and are blown by winds into dense masses. Several other primarily aquatic *Bacteria* and *Archaea* have gas vesicles, but the structures are not found in microbial eukaryotes.

### Gas Vesicle Structure

Gas vesicles are conical-shaped structures made of protein; they are hollow yet rigid and of variable length and diameter (Figure 2.25b, c and see Figure 2.26a). Gas vesicles in different species vary in length from about 300 to more than 1000 nm and in width from 45 to 120 nm, but the vesicles of a given species are of constant size. Gas vesicles may number from a few to hundreds per cell and are impermeable to water and solutes but permeable to gases. The presence of gas vesicles in cells can be detected

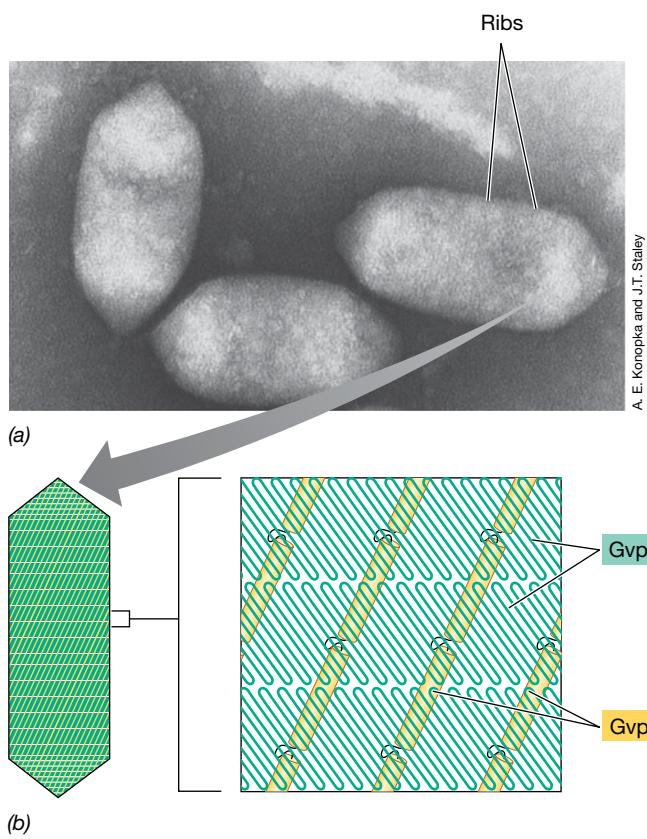


**Figure 2.25** Buoyant cyanobacteria and their gas vesicles. (a) Flotation of a bloom of gas-vesiculate cyanobacteria in a freshwater lake. (b) Phase-contrast photomicrograph of *Anabaena*. Clusters of gas vesicles form phase-bright gas vacuoles (arrows). (c) Transmission electron micrograph of *Microcystis*. Gas vesicles are arranged in bundles, here seen in both longitudinal and cross-section. Both cells are about 5  $\mu\text{m}$  wide.

either by light microscopy, where clusters of vesicles, called *gas vacuoles*, appear as irregular bright inclusions (Figure 2.25b), or by transmission electron microscopy of cell thin sections (Figure 2.25c).

Gas vesicles are composed of two distinct proteins (Figure 2.26b). The major gas vesicle protein, called *GvpA*, forms the watertight vesicle shell and is a small, hydrophobic, and very rigid protein; multiple copies of *GvpA* align to form the parallel “ribs” of the vesicle. The rigidity is essential for the structure to resist the pressures exerted on it from outside. A minor protein, called *GvpC*, functions to strengthen the shell of the gas vesicle by cross-linking and binding the ribs at an angle to group several *GvpA* molecules together (Figure 2.26b).

The composition and pressure of the gas inside a gas vesicle is that in which the organism is suspended. Because an inflated gas vesicle has a density only one-tenth that of the cell proper, inflated gas vesicles combine to decrease a cell’s density and thereby increase its buoyancy. If and when vesicles are collapsed, buoyancy is lost. Phototrophic bacteria (Chapter 14) in particular can benefit from gas vesicles because they allow cells to adjust their vertical position in a water column to sink or rise to regions where conditions (for example, light intensity) are optimal for photosynthesis.



**Figure 2.26** Gas vesicle architecture. (a) Transmission electron micrograph of gas vesicles purified from the bacterium *Ancylobacter aquaticus* and examined in negatively stained preparations. A single vesicle is about 100 nm in diameter. (b) Model of how gas vesicle proteins *GvpA* and *GvpC* interact to form a watertight but gas-permeable structure. *GvpA*, a rigid  $\beta$ -sheet, makes up the rib, and *GvpC*, an  $\alpha$ -helix structure, is the cross-link. (See Figure 4.30b, c for the structures of a  $\beta$ -sheet and an  $\alpha$ -helix.)

### MINIQUIZ

- What gas is present in a gas vesicle? Why might a photosynthetic cell benefit from controlling its buoyancy?
- How are the two proteins that make up the gas vesicle, *GvpA* and *GvpC*, arranged to form such a water-impermeable structure?

## 2.10 Endospores

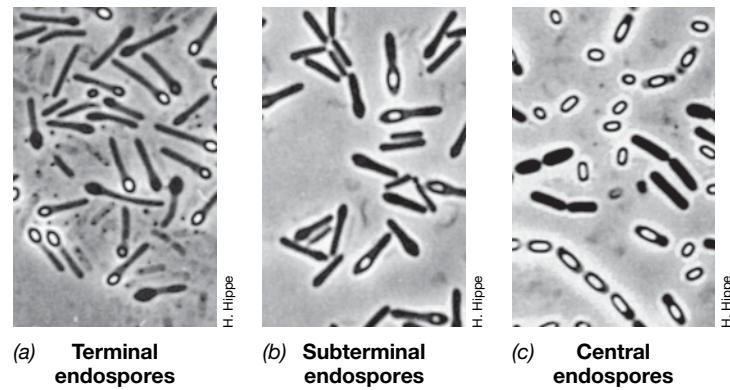
Certain species of *Bacteria* produce structures called **endospores** (Figure 2.27) during a process called *endosporulation* (or just *sporulation* for short). Endospores (the prefix *endo-* means “within”) are highly differentiated cells that are extremely resistant to heat, harsh chemicals, and radiation. Endospores function as survival structures and enable the organism to endure unfavorable growth conditions, including but not limited to extremes of temperature, drying, or nutrient depletion. Endospores can thus be thought of as the dormant stage of a bacterial life cycle: vegetative cell  $\rightarrow$  endospore  $\rightarrow$  vegetative cell. Endospores are easily dispersed by wind, water, or through the animal gut, and hence endospore-forming bacteria are widely distributed in nature.

The endospore-forming bacteria *Bacillus* and *Clostridium* are common in soil and the best-studied representatives. Some endospore-forming bacteria are serious pathogens of humans and other animals, the endospore stage being an effective way of surviving outside the host or until conditions within the host can support disease. Botulism, tetanus, and several foodborne bacterial infections are caused by species of endospore-forming bacteria.

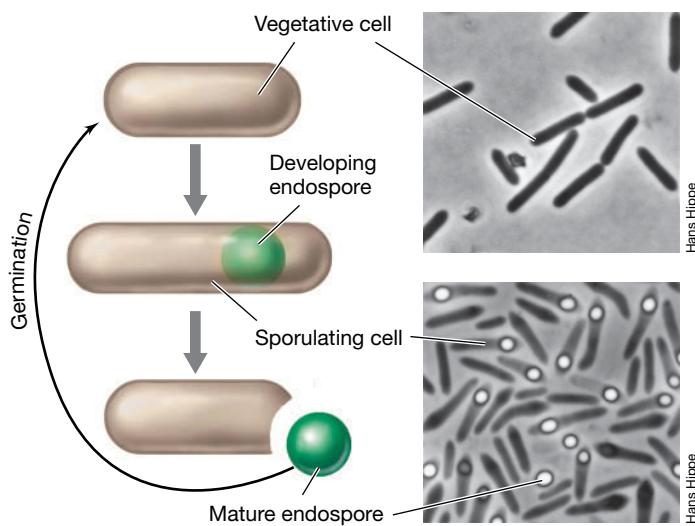
### Endospore Formation and Germination

During endospore formation, a vegetative cell is converted into a nongrowing, heat-resistant, and light-refractive structure (Figure 2.28). Cells do not sporulate when they are actively growing but only when growth ceases owing to the exhaustion of an essential nutrient. Thus, cells of *Bacillus*, a typical endospore-forming bacterium, cease vegetative growth and begin sporulation when, for example, a key nutrient such as carbon or nitrogen becomes limiting (► Section 7.6).

An endospore can remain dormant for years but can convert back to a vegetative cell rapidly. This process occurs in three



**Figure 2.27** The bacterial endospore. Phase-contrast photomicrographs showing different intracellular locations of endospores in different species of bacteria. Endospores appear bright by phase-contrast microscopy.

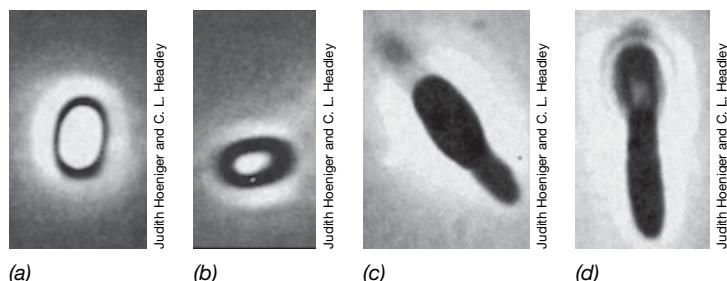


**Figure 2.28** The life cycle of an endospore-forming bacterium. The phase-contrast photomicrographs are of cells of *Clostridium pascui*. A cell is about 0.8  $\mu\text{m}$  wide.

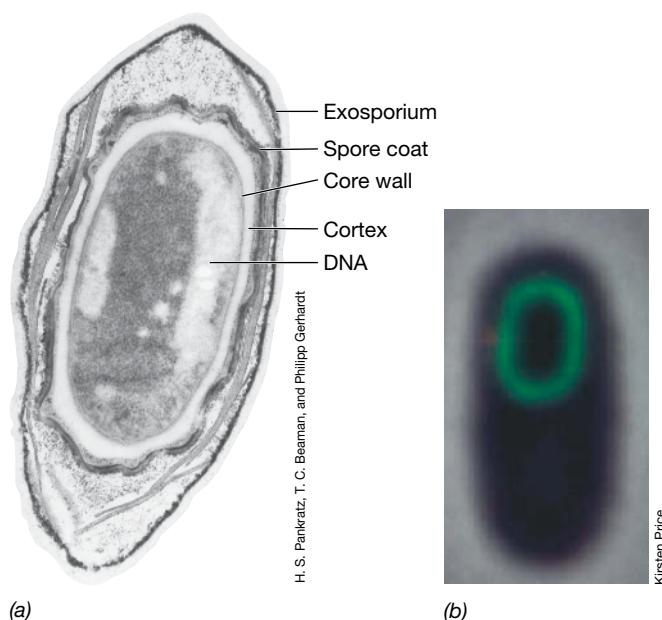
steps: *activation*, *germination*, and *outgrowth* (Figure 2.29). Activation occurs when endospores are heated for several minutes at an elevated but sublethal temperature. Activated endospores are then conditioned to germinate when supplied with certain nutrients, such as certain amino acids. Germination, typically a rapid process (occurring in a matter of minutes), is signaled by the loss of refractivity of the endospore (Figure 2.29b) and loss of resistance to heat and chemicals. The final stage, outgrowth (Figure 2.29c, d), involves visible swelling due to water uptake and synthesis of RNA, proteins, and DNA. The vegetative cell emerges from the broken endospore and begins to grow, remaining in vegetative growth until environmental signals once again trigger sporulation.

### Endospore Structure and Features

Endospores are visible by light microscopy as strongly refractile structures (Figures 2.27 and 2.29a). Endospores are impermeable to most dyes, so occasionally they are seen as unstained regions within cells that have been stained with basic dyes such as methylene blue. To stain endospores, special stains and procedures must be used. In the classical endospore-staining protocol, the stain malachite green is used and is infused into the spore with steam.



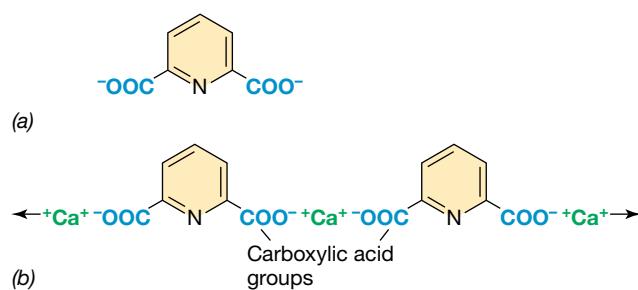
**Figure 2.29** Endospore germination in *Bacillus*. Conversion of an endospore into a vegetative cell. The series of phase-contrast photomicrographs shows the sequence of events starting from (a) a highly refractile endospore. (b) Activation: Refractivity is diminishing. (c, d) Outgrowth: The new vegetative cell is emerging.



**Figure 2.30** Structure of the bacterial endospore. (a) Transmission electron micrograph of a thin section through an endospore of *Bacillus megaterium*. (b) Fluorescent photomicrograph of a cell of *Bacillus subtilis* undergoing sporulation. The green color is a dye that specifically stains a sporulation protein in the spore coat.

The structure of the endospore as seen with the electron microscope differs distinctly from that of the vegetative cell (Figure 2.30). The endospore contains many layers absent from the vegetative cell. The outermost layer is the *exosporium*, a thin protein covering. Moving inward there are several *spore coats*, composed of layers of spore-specific proteins (Figure 2.30b). Below the spore coat is the *cortex*, which consists of loosely cross-linked peptidoglycan, and inside the cortex is the *core*, which contains the core wall, cytoplasmic membrane, cytoplasm, nucleoid, ribosomes, and other cellular essentials. Thus, the endospore differs structurally from the vegetative cell primarily in the kinds of structures found outside the core wall.

One substance found in endospores but not in vegetative cells is **dipicolinic acid** (Figure 2.31a), which accumulates in the core. Endospores also contain large amounts of calcium ( $\text{Ca}^{2+}$ ), most of which is complexed with dipicolinic acid (Figure 2.31b). The calcium-dipicolinic acid (DPA) complex forms about 10% of the dry weight of the endospore and functions to bind free water within the endospore, helping to dehydrate the developing



**Figure 2.31** Dipicolinic acid (DPA). (a) Structure of DPA. (b) How  $\text{Ca}^{2+}$  cross-links DPA molecules to form a complex.

endospore. In addition, the DPA complex inserts between bases in DNA, which helps stabilize DNA against heat denaturation.

The core of the endospore differs significantly from the cytoplasm of the vegetative cell that produced it. The endospore core contains less than one quarter of the water found in the vegetative cell, and thus the consistency of the core cytoplasm is that of a gel. Dehydration of the core greatly increases the heat resistance of macromolecules within the spore. Some bacterial endospores survive heating to temperatures as high as 150°C, although 121°C, the standard for microbiological sterilization (121°C is autoclave temperature, [Section 5.15](#)), kills the endospores of most species. Dehydration has also been shown to provide endospores with resistance to toxic chemicals, such as hydrogen peroxide ( $H_2O_2$ ), and causes enzymes in the core to become inactive. In addition to the low water content of the endospore, the pH of the core is about one unit lower than that of the vegetative cell cytoplasm.

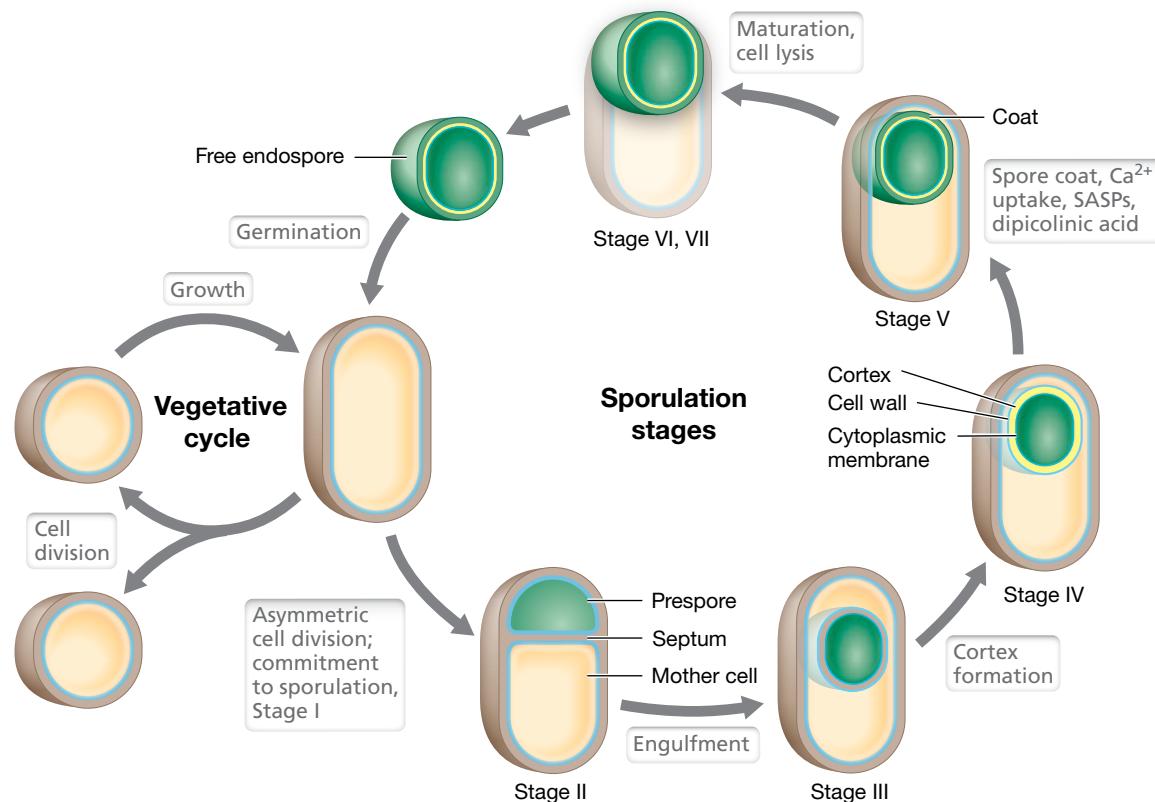
The endospore core contains high levels of *small acid-soluble spore proteins* (SASPs). These proteins are only made during the sporulation process and have at least two functions. SASPs bind tightly to DNA in the core and protect it from potential damage from ultraviolet radiation, desiccation, and dry heat. Ultraviolet resistance is conferred when SASPs alter the molecular structure of DNA from the normal “B” form to the (more compact) “A” form. A-form DNA better resists pyrimidine dimer formation by UV radiation, which can cause mutations ([Section 11.4](#)), and resists the denaturing effects of dry heat. In addition, SASPs function as a carbon and energy source for the outgrowth of a new vegetative cell from the endospore during germination.

**TABLE 2.2 Differences between endospores and vegetative cells**

Characteristic	Vegetative cell	Endospore
Microscopic appearance	Nonrefractile	Refractile
Calcium content	Low	High
Dipicolinic acid	Absent	Present
Enzymatic activity	High	Low
Respiration rate	High	Low or absent
Macromolecular synthesis	Present	Absent
Heat resistance	Low	High
Radiation resistance	Low	High
Resistance to chemicals	Low	High
Lysozyme	Sensitive	Resistant
Water content	High, 80–90%	Low, 10–25% in core
Small acid-soluble spore proteins	Absent	Present

### The Sporulation Cycle

Sporulation is a form of cellular differentiation ([Figure 1.4](#)), and many genetically directed changes in the cell occur during the conversion from vegetative growth to sporulation ([Table 2.2](#)). The structural changes in sporulating cells of *Bacillus* are shown in [Figure 2.32](#). Sporulation can be divided into several stages. In



**Figure 2.32 Stages in endospore formation.** The stages are defined from genetic and microscopic analyses of sporulation in *Bacillus subtilis*, the model organism for studies of sporulation. SASPs, small acid-soluble proteins.

*Bacillus subtilis*, which has been studied in detail, the conversion of a vegetative cell into an endospore takes about 8 hours and begins with asymmetric cell division (Figure 2.32). Note how key events such as asymmetric cell division, cortex formation, and SASP production take place in a defined sequence and at specific times in the sporulation cycle (Figure 2.32). Genetic studies of mutants of *Bacillus subtilis*, each blocked at one of the stages of endosporulation, indicate that more than 200 spore-specific genes exist.

Endosporulation requires differential protein synthesis. This occurs by the sequential activation of several families of endospore-specific genes and the turning off of many vegetative cell functions. The proteins encoded by sporulation-specific genes catalyze the series of events leading from the moist, metabolizing, vegetative cell to the relatively dry, metabolically inert, but extremely resistant endospore (Table 2.2). In Section 7.6 we examine some of the molecular events that take place during the endosporulation process.

### Diversity and Phylogenetic Aspects of Endospore Formation

Nearly 20 genera of *Bacteria* form endospores, although the process has only been studied in detail in a few species. Nevertheless, most of the major events described here, such as the formation of DPA complexes and the production of endospore-specific SASPs, seem universal. From a phylogenetic perspective, the capacity to produce endospores is limited to a particular lineage of the gram-positive bacteria. Despite this, the physiologies of endospore-forming bacteria are highly diverse and include anaerobes, aerobes, phototrophs, and chemolithotrophs. In light of this physiological diversity, the actual triggers for endospore formation may vary with different species and could include signals other than simple nutrient starvation, the major trigger for endospore formation in *Bacillus*. No *Archaea* have been shown to form endospores, suggesting that the capacity to produce endospores evolved sometime after *Bacteria* and *Archaea* diverged about 3.5 billion years ago.

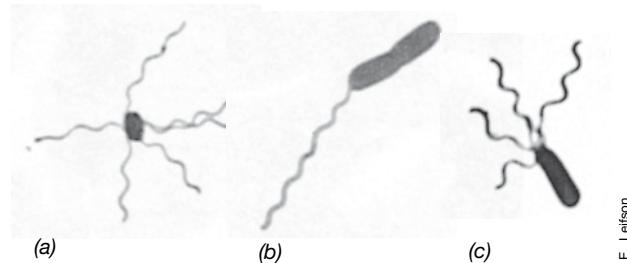
#### MINIQUIZ

- What is dipicolinic acid and the DPA complex, and where is it found?
- What are SASPs and what is their function?
- What is formed when an endospore germinates?

## IV • Cell Locomotion

We finish our survey of prokaryotic structure and function by examining cell locomotion. Many microbial cells can move under their own power. Motility allows cells to reach different parts of their environment, and in nature, a new location may offer additional resources for a cell and spell the difference between life and death.

We examine here the two major types of prokaryotic cell movement, *swimming* and *gliding*. We then consider how motile cells are able to move in a directed fashion toward or away from particular stimuli (phenomena called *taxis*) and present examples of these simple behavioral responses.



E. Leifson

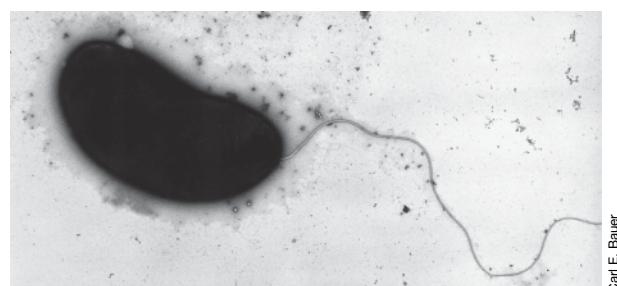
**Figure 2.33 Bacterial flagella.** Classic light photomicrographs taken by Einar Leifson of bacteria containing different arrangements of flagella. Cells are stained with the Leifson flagella stain. (a) Peritrichous. (b) Polar. (c) Lophotrichous.

### 2.11 Flagella, Archaella, and Swimming Motility

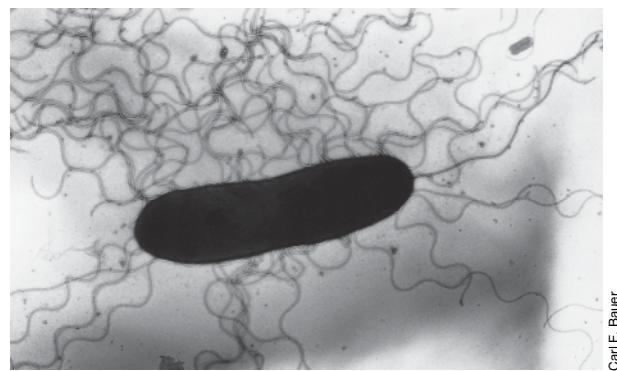
Many *Bacteria* are motile by swimming due to a structure called the **flagellum** (plural, flagella) (Figure 2.33); an analogous structure called the **archaellum** is present in many *Archaea*. Flagella and archaella are tiny rotating machines that function to push or pull the cell through a liquid.

#### Flagella and Flagellation

Bacterial flagella are long, thin appendages (15–20 nm wide, depending on the species) free at one end and anchored into the



(a)



(b)

Carl E. Bauer

Carl E. Bauer

**Figure 2.34 Bacterial flagella as observed by negative staining in the transmission electron microscope.** (a) A single polar flagellum. (b) Peritrichous flagella. Both micrographs are of cells of the phototrophic bacterium *Rhodospirillum centenum*, which are about 1.5  $\mu\text{m}$  wide. Cells of *R. centenum* are normally polarly flagellated but under certain growth conditions form peritrichous flagella. See Figure 2.44b for a photo of colonies of *R. centenum* cells that move toward an increasing gradient of light (phototaxis).

cell at the other end. Flagella can be stained and observed by light microscopy (Figure 2.33) or electron microscopy (Figure 2.34).

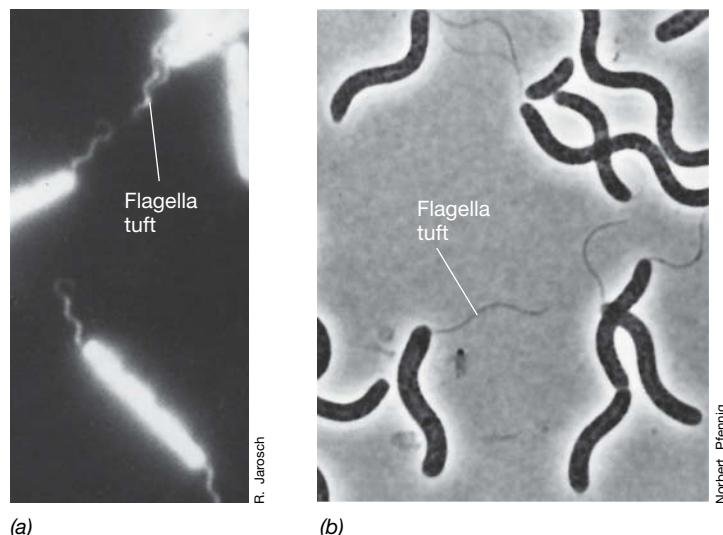
Flagella can be anchored to a cell in different locations. In **polar flagellation**, the flagella are attached at one or both ends of a cell (Figure 2.33b). Occasionally a group of flagella (called a *tuft*) may arise at one end of the cell, a type of polar flagellation called *lophotrichous* (Figure 2.33c). Tufts of flagella can sometimes be seen in large unstained cells by dark-field or phase-contrast microscopy (Figure 2.35). When a tuft of flagella emerges from both poles of the cell, flagellation is called *amphitrichous*. In **peritrichous flagellation** (Figures 2.33a and 2.34b), flagella are inserted around the cell surface.

Flagella do not rotate at a constant speed but increase or decrease their rotational speed in relation to the strength of the proton motive force. Flagella can rotate at up to 1000 revolutions per second to support a swimming speed of up to 60 cell-lengths/sec. The fastest known land animal, the cheetah, can move at about 25 body-lengths/sec. Thus, a bacterium swimming at 60 cell-lengths/sec is actually moving over twice as fast—relative to its size—as the fastest animal!

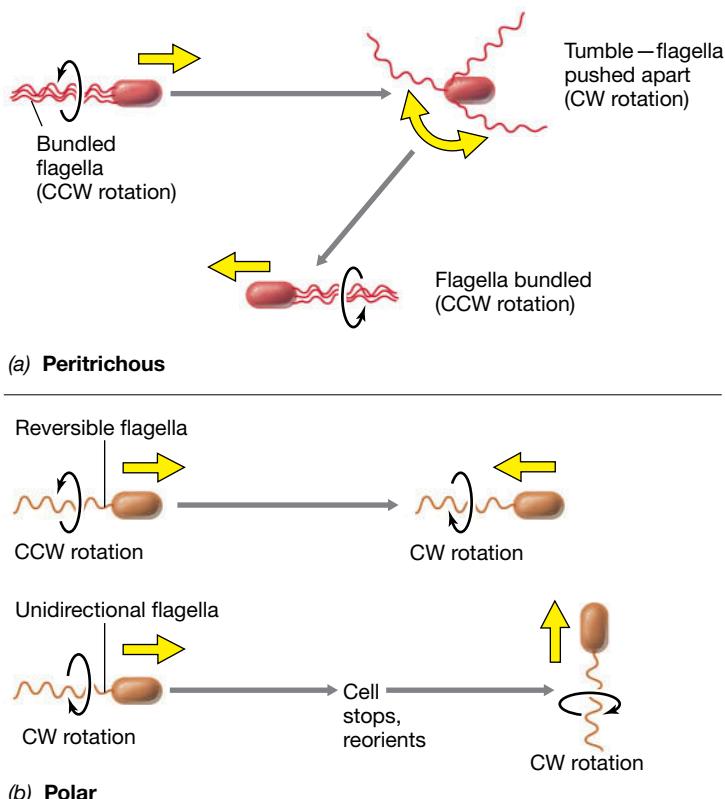
The swimming motions of polarly and lophotrichously flagellated organisms differ from those of peritrichously flagellated organisms, and these can be distinguished microscopically (Figure 2.36). Peritrichously flagellated organisms typically move slowly in a straight line. By contrast, polarly flagellated organisms move more rapidly, often spinning around and seemingly dashing from place to place. The different behavior of flagella on polar and peritrichous organisms, including differences in reversibility of the flagellum, is illustrated in Figure 2.36.

## Flagella Structure and Activity

Flagella are not straight structures but are helical. The main part of the flagellum, called the *filament*, is composed of many copies of a



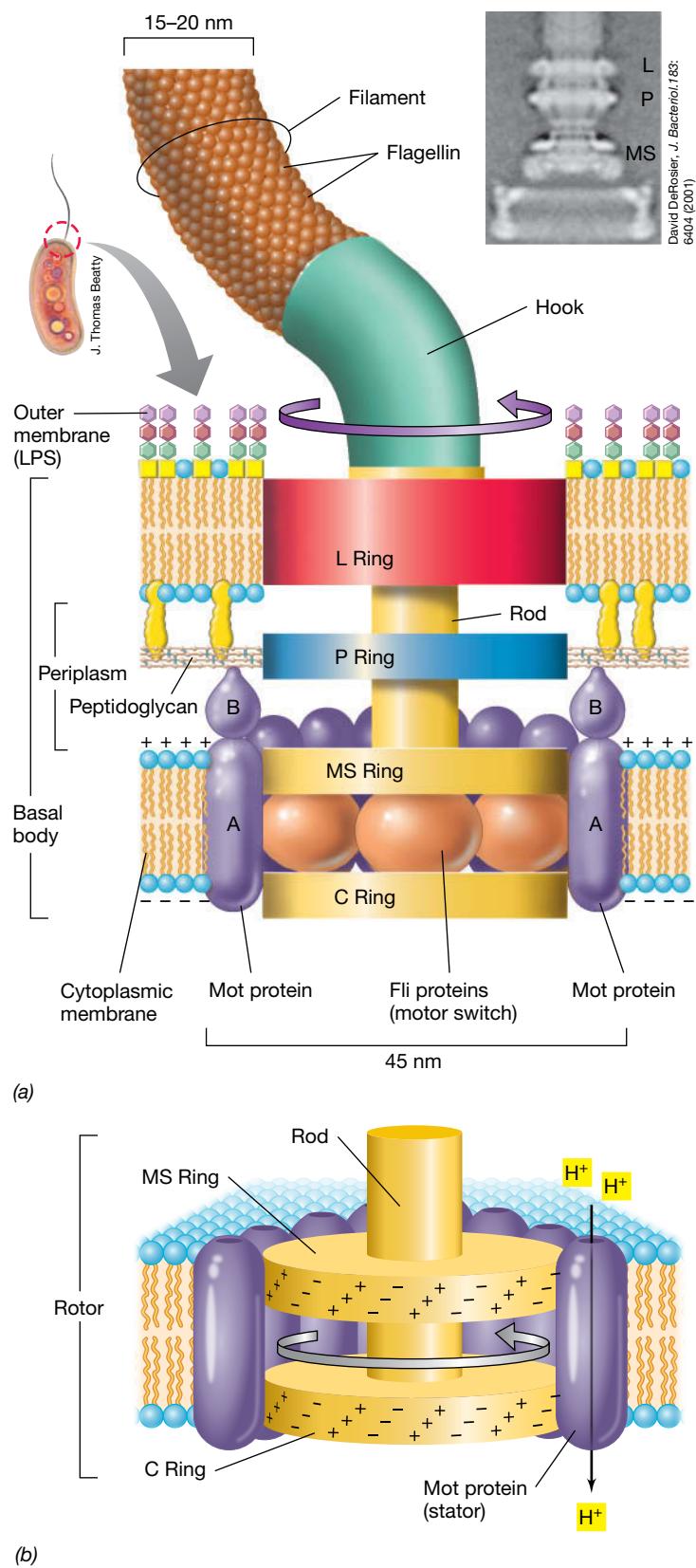
**Figure 2.35** Bacterial flagella observed in living cells. (a) Dark-field photomicrograph of a group of large rod-shaped bacteria with flagellar tufts at each pole (a condition called *amphitrichous flagellation*). A single cell is about 2  $\mu\text{m}$  wide. (b) Phase-contrast photomicrograph of cells of the large phototrophic purple bacterium *Rhodospirillum photometricum* with a tuft of lophotrichous flagella that emanate from one of the poles. A cell measures about  $4 \times 25 \mu\text{m}$ .



**Figure 2.36** Movement in peritrichously and polarly flagellated prokaryotic cells. (a) Peritrichous: Forward motion is imparted by all flagella forming into a bundle and rotating counterclockwise (CCW). Clockwise (CW) rotation causes the bundle to break apart and the cell to tumble. A return to counterclockwise rotation leads the cell off in a new direction. (b) Polar: Cells change direction by reversing flagellar rotation (thus pulling instead of pushing the cell) or, with unidirectional flagella, by stopping periodically to reorient and then moving forward by clockwise rotation of its flagella. The yellow arrows show the direction the cell is traveling.

protein called *flagellin*. The amino acid sequence of flagellin is highly conserved in *Bacteria*, suggesting that flagellar motility evolved early and has deep roots within this domain. In addition to the filament, a flagellum consists of several other components. A wider region at the base of the filament called the *hook* consists of a single type of protein and connects the filament to the flagellum motor in the base (Figure 2.37).

The flagellum motor is a reversible rotating machine composed of several proteins and is anchored in the cytoplasmic membrane and cell wall. The motor consists of a central rod that passes through a series of rings. In gram-negative bacteria, an outer ring, called the *L ring*, is anchored in the outer membrane (Section 2.5). A second ring, called the *P ring*, is anchored in the peptidoglycan layer. A third set of rings, called the *MS* and *C rings*, are located within the cytoplasmic membrane and the cytoplasm, respectively (Figure 2.37a). In gram-positive bacteria, which lack an outer membrane, only the inner pair of rings is present. Surrounding the inner ring and anchored in the cytoplasmic membrane and peptidoglycan are a series of proteins called *Mot proteins*. Another set of proteins, called *Fli proteins* (Figure 2.37a), function as the motor switch, reversing the direction of rotation of the flagella in response to intracellular signals.



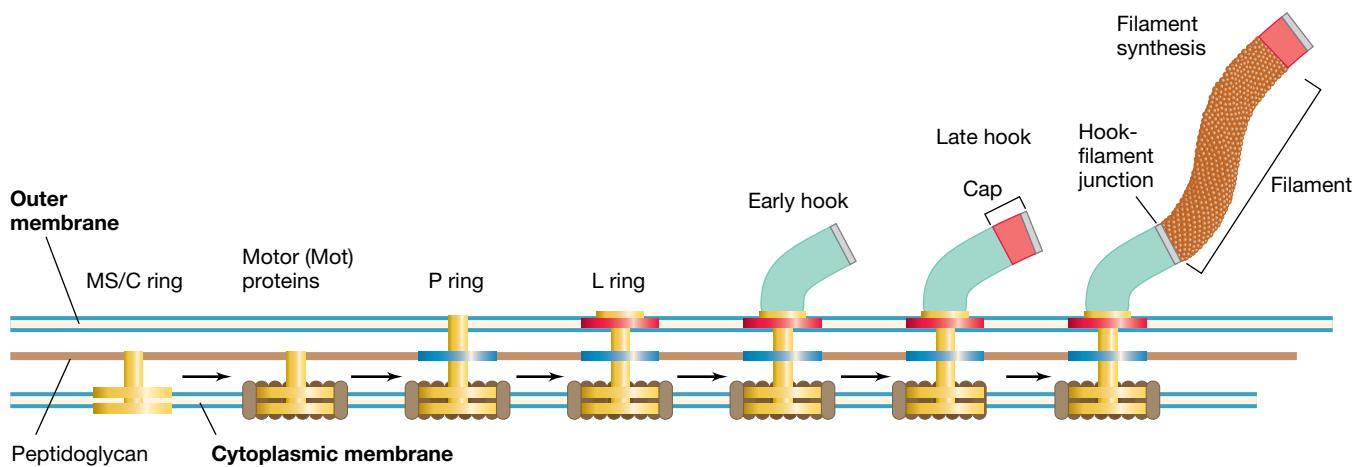
The flagellum motor contains two main components: the *rotor* and the *stator*. The rotor consists of the central rod and the L, P, C, and MS rings. Collectively, these structures make up the flagellar **basal body** (Figure 2.37). The stator consists of the Mot proteins that surround the rotor and function to generate torque. Rotation of the flagellum occurs at the expense of the proton motive force (Section 2.3), and it is thought that rotation is imparted to the flagellum by a type of “proton turbine” process. In this model, proton translocation through the Mot complex drives rotation of the flagellum, with about 1200 protons being translocated per each rotation of the flagellum (Figure 2.37b). Protons flowing through the Mot proteins exert electrostatic forces on helically arranged charges on the rotor proteins. Alternating attractions between positive and negative charges on the rotor as protons flow through the Mot proteins then cause the entire basal body to rotate. Rotational speed of the flagellum is set by the proton flow rate through the Mot proteins, which is a function of the intensity of the proton motive force.

### Flagellar Synthesis

Several genes encode the motility apparatus of *Bacteria*. In *Escherichia* and *Salmonella* species, in which motility studies have been extensive, over 50 genes are linked to motility in one way or another. These genes encode the structural proteins of the flagellum and motor apparatus, of course, but also encode proteins that export the structural proteins through the cytoplasmic membrane to the outside of the cell and proteins that regulate the synthesis of new flagella.

A flagellar filament grows not from its base, as does an animal hair, but from its tip. The MS ring is synthesized first and inserted into the cytoplasmic membrane. Then other anchoring proteins are synthesized along with the hook before the filament forms (Figure 2.38). Flagellin molecules synthesized in the cytoplasm pass up through a 3-nm channel inside the filament and add on at the terminus to form the mature flagellum. A protein “cap” is present at the end of the growing flagellum. Cap proteins assist flagellin molecules that have diffused through the filament channel to assemble in the proper fashion at the flagellum terminus (Figure 2.38). Approximately 20,000 flagellin protein molecules are needed to make one filament. The flagellum grows more or less continuously until it reaches its final length. Broken flagella still rotate and can be repaired with new flagellin units passed through the filament channel to replace the lost ones.

**Figure 2.37** Structure and function of the flagellum in gram-negative *Bacteria*. (a) Structure. The L ring is embedded in the LPS and the P ring in peptidoglycan. The MS ring is embedded in the cytoplasmic membrane and the C ring in the cytoplasm. A narrow channel exists in the rod and filament through which flagellin molecules diffuse to reach the site of flagellar synthesis. The Mot proteins function as the flagellar motor, whereas the Fli proteins function as the motor switch. The flagellar motor rotates the filament to propel the cell through the medium. Inset photos: Top left, a cell of the purple sulfur bacterium *Chromatium* containing a tuft of polar flagella; Top right, transmission electron micrograph of a flagellar basal body from *Salmonella enterica* with the various rings labeled. (b) Function. A “proton turbine” model explains rotation of the flagellum. Protons, flowing through the Mot proteins, exert forces on charges present on the C and MS rings, thereby spinning the rotor.



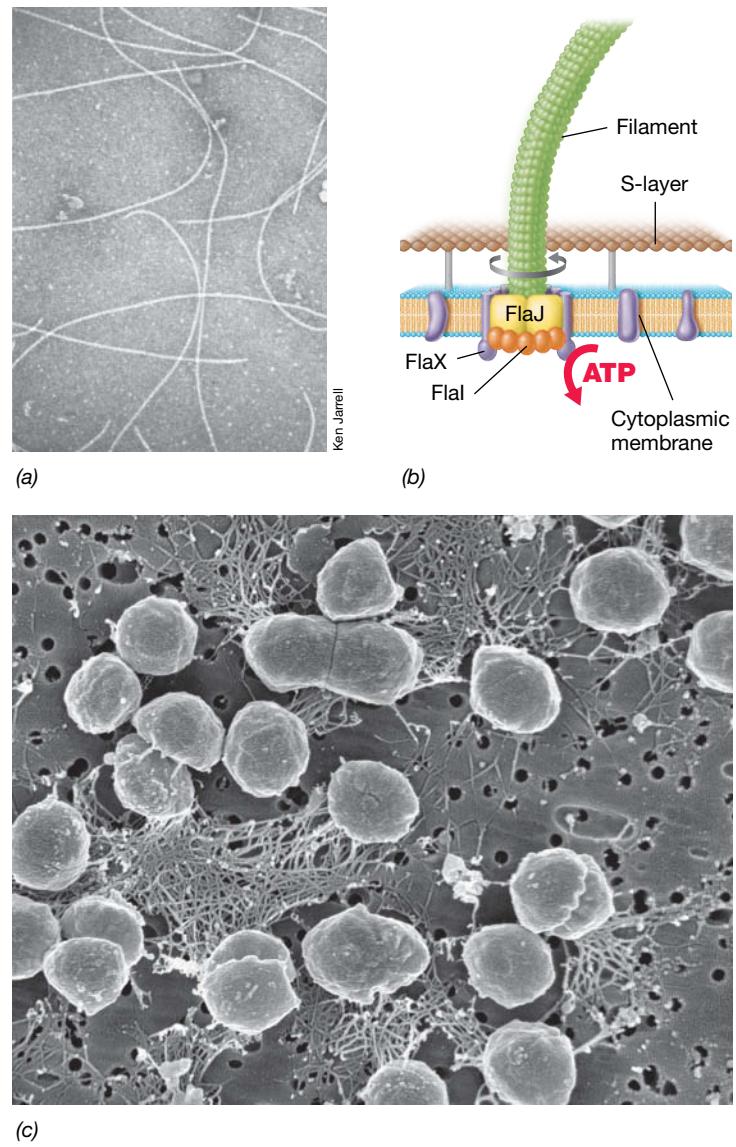
**Figure 2.38 Flagella biosynthesis.** Synthesis begins with assembly of MS and C rings in the cytoplasmic membrane, followed by the other rings, the hook, and the cap. Flagellin protein flows through the hook to form the filament and is guided into position by cap proteins.

## Archaea

As in *Bacteria*, swimming motility is widespread among species of *Archaea* due to rotation of their flagella analog, the archaellum (see also page 70). These structures are roughly half the diameter of flagella, measuring about 10–13 nm in width (Figure 2.39a), and impart movement to the cell by rotating, as do flagella. However, unlike *Bacteria*, in which a single type of protein makes up the filament, several different filament proteins are known in *Archaea*, and the genes that encode them bear little sequence homology to genes that encode bacterial flagellin. Depending on the archaeal species, 7–12 genes encode the major proteins that make up the archaellum. Archaelle have been particularly well studied in the salt-loving archaeon *Halobacterium*, the heat- and acid-loving archaeon *Sulfolobus*, and the methane-producing archaeon *Methanocaldococcus*.

Studies of swimming cells of *Halobacterium* show that they swim at speeds only about one-tenth that of cells of *Escherichia coli*. This could be due to the smaller diameter of the archaellum compared to the flagellum, as this would be expected to reduce the torque of the structure significantly. However, this hypothesis has been questioned since the discovery that some *Archaea* swim incredibly fast. For example, cells of *Methanocaldococcus* (Figure 2.39c) swim nearly 50 times faster than cells of *Halobacterium* and 10 times faster than cells of *Escherichia coli* (*Bacteria*). In fact, *Methanocaldococcus* swims at nearly 500 cell lengths per second, which makes it the fastest organism on Earth! Thus, the net torque or rotational speeds of archaelle from different species of *Archaea* can obviously vary significantly.

The overall structure of the archaellum bears a strong resemblance to that of type IV pili (Figure 2.39b), and it is clear that the archaellum is structurally related to these appendages (Section 2.7).



**Figure 2.39 Archaea.** (a) Transmission electron micrograph of archaelle isolated from the methanogen *Methanococcus maripaludis*. A single archaellum is about 12 nm wide. (b) Depiction of an archaellum embedded in the archaeal cell wall and cytoplasmic membrane. ATP (rather than the proton motive force, see Figure 2.37b) drives archaellum rotation. (c) Scanning electron micrograph of cells of *Methanocaldococcus jannaschii* containing abundant archaelle.

In fact, the archaellum can be considered a rotating type IV pilus capable of both clockwise and counterclockwise rotation. Moreover, in contrast to the flagellum, whose energy requirement is met by dissipation of the proton motive force (Figure 2.37b), rotation of the archaellum is driven by the hydrolysis of ATP. Thus, although flagella and archaella are functionally similar—rotating filaments that drive cell propulsion—their flagellar motors are powered in fundamentally different ways. This suggests that swimming motility evolved separately in *Bacteria* and *Archaea* as these domains diverged some 3.5 billion years ago.

### MINIQUIZ

- Cells of *Salmonella* are peritrichously flagellated, those of *Pseudomonas* polarly flagellated, and those of *Spirillum* lophotrichously flagellated. Using a sketch, show how each organism would appear in a flagella stain.
- Compare flagella and archaella in terms of their structure, function, and energy source.

## 2.12 Gliding Motility

Some bacteria are motile but lack flagella. Most of these nonswimming yet still motile cells move by *gliding*. Unlike flagellar motility, in which cells stop and then start off in a different direction, gliding motility is a slower and smoother form of movement and typically occurs along the long axis of the cell.

### Diversity of Gliding Motility

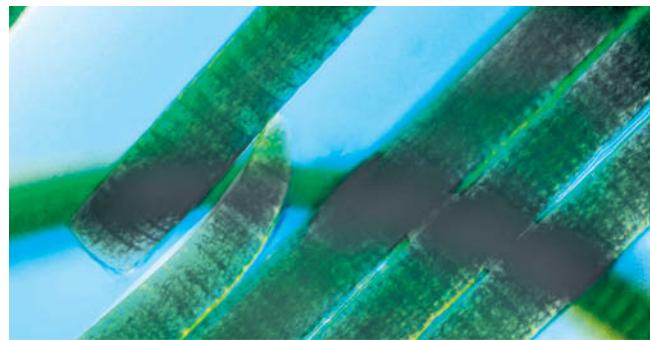
Gliding motility is widely distributed among *Bacteria* but has been well studied in only a few groups. The gliding movement itself—up to 10  $\mu\text{m/sec}$  in some gliding bacteria—is considerably slower than propulsion by flagella but still offers the cell a means of moving about its habitat.

Gliding bacteria are typically filamentous or rod-shaped in morphology, and the gliding process requires that the cells be in contact with a solid surface (Figure 2.40). The morphology of colonies of a typical gliding bacterium is distinctive because cells glide out and move away from the center of the colony (Figure 2.40c). Perhaps the best-known gliding bacteria are the filamentous cyanobacteria (Figure 2.40a, b), certain gram-negative bacteria such as *Myxococcus* and other myxobacteria, and species of *Cytophaga* and *Flavobacterium* (Figure 2.40c, d). No gliding *Archaea* are known.

### Mechanisms of Gliding Motility

More than one mechanism drives gliding motility. Cyanobacteria glide by secreting a polysaccharide slime from pores onto the outer surface of the cell. The slime contacts both the cell surface and the solid surface against which the cell moves. As the excreted slime adheres to the surface, the cell slides along. The nonphotosynthetic gliding bacterium *Cytophaga* also glides at the expense of slime excretion, rotating along its long axis as it does.

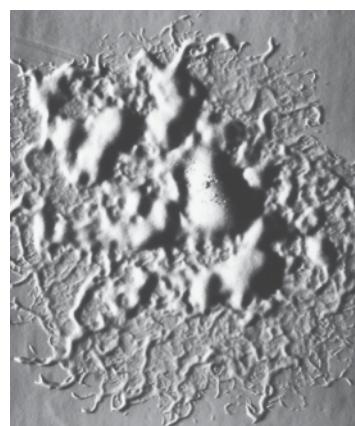
Cells capable of “twitching motility” also display a form of gliding motility using a mechanism by which repeated extension and retraction of type IV pili (Section 2.7) drag the cell along a surface. The gliding myxobacterium *Myxococcus xanthus* has two forms of gliding motility. One form is driven by type IV pili, whereas the



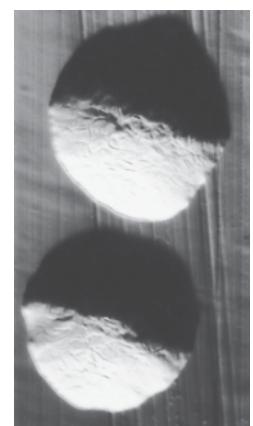
Richard W. Castenholz



Richard W. Castenholz



Mark J. McBride

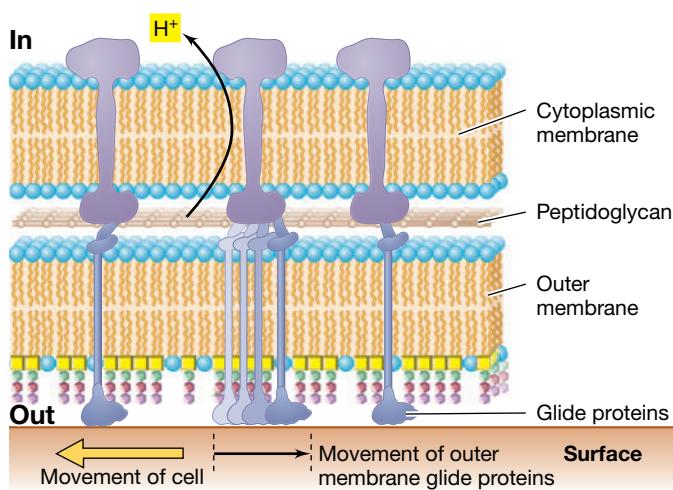


Mark J. McBride

**Figure 2.40** Gliding bacteria. (a, b) The large filamentous cyanobacterium *Oscillatoria* has cells about 35  $\mu\text{m}$  wide. (b) *Oscillatoria* filaments gliding on an agar surface. (c) Masses of the bacterium *Flavobacterium johnsoniae* gliding away from the center of the colony (the colony is about 2.7 mm wide). (d) Nongliding mutant strain of *F. johnsoniae* showing typical colony morphology of nongliding bacteria (the colonies are 0.7–1 mm in diameter). See also Figure 2.41.

other is distinct from either the type IV pili or the slime extrusion methods. In this form of *M. xanthus* motility, a protein adhesion complex is formed at one pole of the rod-shaped cell and remains at a fixed position on the surface as the cell glides forward. This means that the adhesion complex moves in the direction opposite that of the cell, presumably fueled by some sort of cytoplasmic motility engine.

Neither slime extrusion nor twitching is the mechanism of gliding in other gliding bacteria. In the genus *Flavobacterium* (Figure 2.40c and Figure 2.41), for example, no slime is excreted and



**Figure 2.41** Gliding motility in *Flavobacterium johnsoniae*. Tracks (yellow) exist in the peptidoglycan that connect cytoplasmic proteins to outer membrane glide proteins and propel the glide proteins along the solid surface. Note that the glide proteins and the cell proper move in opposite directions.

cells lack type IV pili. Instead of using one of these gliding mechanisms, the movement of proteins on the *Flavobacterium* cell surface supports gliding motility in this organism. Specific motility proteins anchored in the cytoplasmic and outer membranes are thought to propel cells of *Flavobacterium* forward by a ratcheting mechanism (Figure 2.41). Movement of gliding-specific proteins in the cytoplasmic membrane is driven by energy from the proton motive force, and this motion is then transmitted to complementary glide proteins in the outer membrane. Movement of the outer membrane proteins against the solid surface then pulls the cell forward (Figure 2.41).

Like other forms of motility, gliding motility has ecological relevance. Gliding allows a cell to exploit new resources and to

interact with other cells. Myxobacteria, such as *Myxococcus xanthus*, have a very social and cooperative lifestyle, and gliding motility may play an important role in the intimate cell-to-cell interactions necessary to complete their life cycle (► Section 15.17).

### MINIQUIZ

- How does gliding motility differ from swimming motility in both mechanism and requirements?
- Contrast the mechanism of gliding motility in a filamentous cyanobacterium and in *Flavobacterium*.

## 2.13 Chemotaxis and Other Taxes

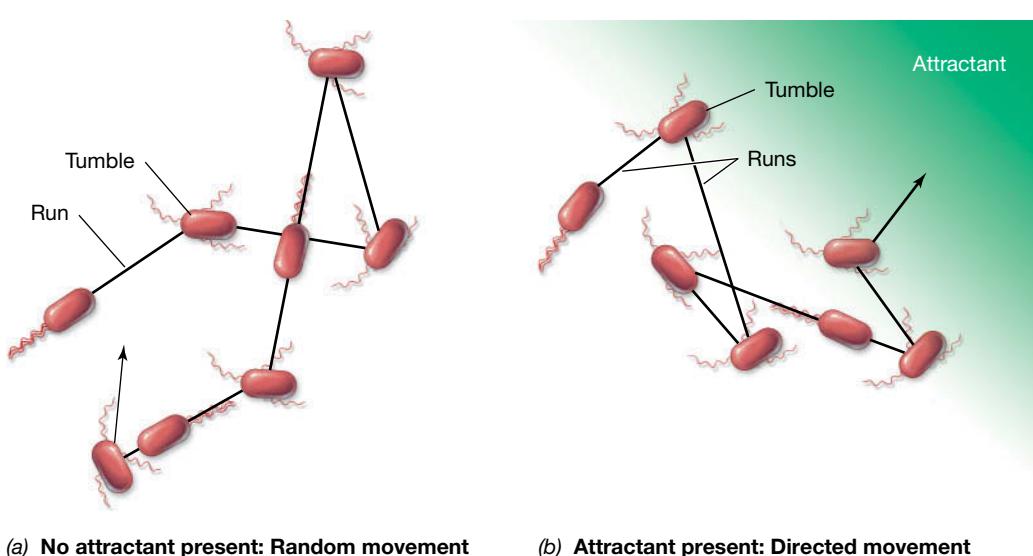
Cells of *Bacteria* and *Archaea* often encounter gradients of physical or chemical agents in nature and have evolved means to respond to these gradients by moving either toward or away from the agent. Such a directed movement is called a *taxis* (plural, *taxes*).

**Chemotaxis**, a response to chemicals, and **phototaxis**, a response to light, are two well-studied taxes. The ability of a cell to move toward or away from various stimuli has ecological significance in that the directed movement may enhance a cell's access to resources or allow it to avoid harmful substances that could damage or kill it.

Chemotaxis has been well studied in swimming bacteria, and much is known at the genetic level about how the chemical state of a cell's environment is communicated to the flagellum. Our discussion here will thus deal solely with swimming bacteria. However, some gliding bacteria are also chemotactic, and there are phototactic movements in filamentous cyanobacteria (Figure 2.40a, b). In addition, many swimming species of *Archaea* are also chemotactic, and several of the proteins that control chemotaxis in *Bacteria* have homologs in these *Archaea*. Here we discuss microbial taxes in a general way. In Section 6.7 we examine the molecular mechanism of chemotaxis and its genetics and regulation in *Escherichia coli* as a general model for the control of taxes in both *Bacteria* and *Archaea*.

### Chemotaxis in Peritrichously Flagellated Bacteria

Much research on chemotaxis has been done with the peritrichously flagellated bacterium *E. coli*. To understand how chemotaxis affects the behavior of *E. coli*, consider the situation in which a cell encounters a gradient of some chemical in its environment (Figure 2.42). In the absence of the gradient, cells move in a random fashion that includes *runs*, in which the cell is swimming forward in a smooth fashion, and *tumbles*, when the cell stops and jiggles about. During forward movement in a run,



**Figure 2.42** Chemotaxis in a peritrichously flagellated bacterium. (a) In the absence of a chemical attractant, the cell swims randomly in runs, changing direction during tumbles. (b) In the presence of an attractant, runs become biased, and the cell moves up the gradient of the attractant. The attractant gradient is depicted in green, with the highest concentration where the color is most intense.

the flagellar motor rotates counterclockwise. When flagella rotate clockwise, the bundle of flagella pushes apart, forward motion ceases, and the cells tumble (Figure 2.42).

Following a tumble, the direction of the next run is random. Thus, by means of runs and tumbles, the cell moves about its environment in a random fashion. However, if a gradient of a chemical attractant is present, these random movements become biased. If the organism senses that it is moving toward higher concentrations of the attractant, runs become longer and tumbles are less frequent. The result of this behavioral response is that the organism moves up the concentration gradient of the attractant (Figure 2.42b). If the organism senses a repellent, the same mechanism applies, although in this case it is the *decrease* in concentration of the repellent (rather than the *increase* in concentration of an attractant) that promotes runs.

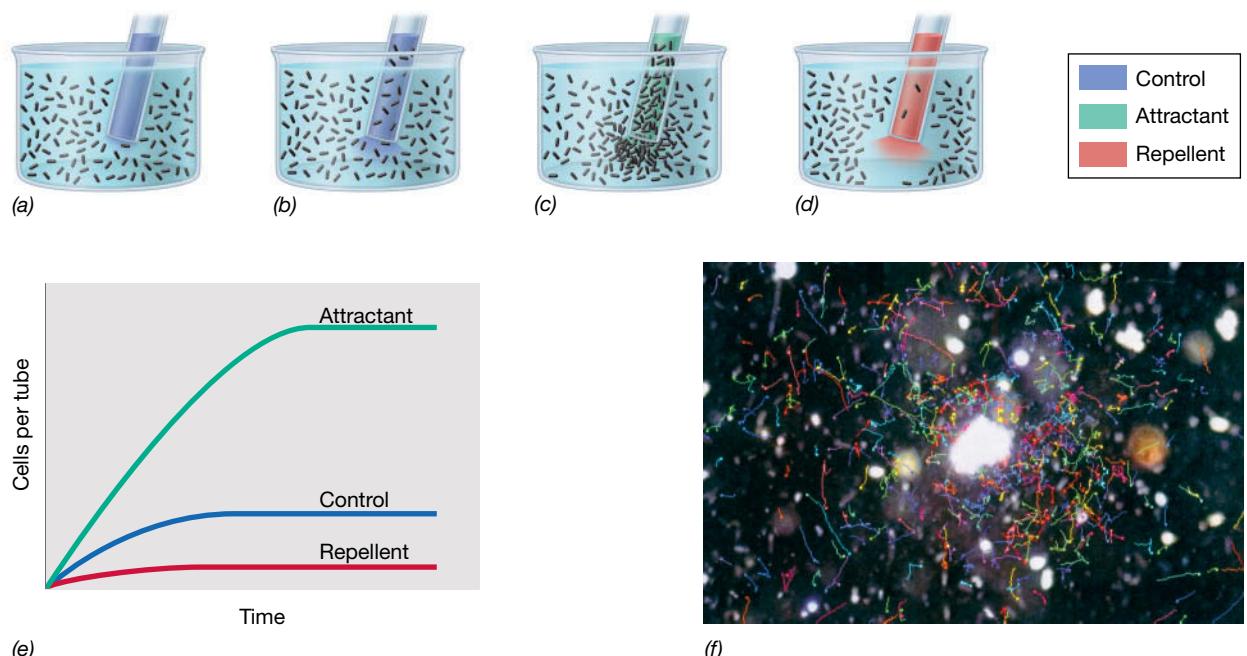
How are chemical gradients sensed? Prokaryotic cells are too small to sense a gradient of a chemical along the length of a single cell. Instead, while moving, cells “monitor” their environment by sampling chemicals periodically and comparing the concentration of a particular chemical with that sensed a few moments before. Bacterial cells thus respond to *temporal* rather than *spatial* differences in the concentration of a chemical as they swim. Sensory information is fed through an elaborate cascade of proteins that eventually affect the direction of rotation of the flagellar motor. The attractants and repellents are sensed by a series of membrane proteins called *chemoreceptors*. These sensory proteins

bind the chemicals and begin the process of sensory transduction to the flagellum (► Section 6.7). Chemotaxis can thus be considered a type of *sensory response system*, analogous to sensory responses in the nervous system of animals.

### Chemotaxis in Polarly Flagellated Bacteria

Chemotaxis in polarly flagellated cells is similar but not identical to that in peritrichously flagellated cells such as *E. coli*. Many polarly flagellated bacteria, such as *Pseudomonas* species, can fully reverse the direction of rotation of their flagella. In so doing, they do not tumble but immediately reverse their direction of movement (Figure 2.36b). However, in the phototrophic bacterium *Rhodobacter*, cells of which have only a single flagellum that can rotate in just one direction, rotation of the flagellum stops periodically. When the flagellum stops rotating, the cell becomes reoriented by Brownian motion (Figure 2.36b). Then as the flagellum begins to rotate again, the cell moves off in a new direction.

Despite this seemingly random activity, cells of *Rhodobacter* are strongly chemotactic to various organic compounds and also to oxygen and light. *Rhodobacter* cannot reverse its flagellar motor and tumble as *E. coli* can, but cells do maintain runs as long as they sense an increasing concentration of attractant. If the cells sense a decreasing concentration of attractant, movement ceases. By such starting and stopping, a cell eventually finds the path of increasing attractant and maintains a run until either its chemoreceptors are saturated or it senses a decrease in the level of attractant.



**Figure 2.43** Measuring chemotaxis using a capillary tube assay. (a) Insertion of the capillary into a bacterial suspension. As the capillary is inserted, a gradient of the chemical begins to form. (b) Control capillary contains a salt solution that is neither an attractant nor a repellent. Cell concentration inside the capillary becomes

the same as that outside. (c) Accumulation of bacteria in a capillary containing an attractant. (d) Repulsion of bacteria by a repellent. (e) Time course showing cell numbers in capillaries containing various chemicals. (f) Tracks of motile bacteria in seawater swarming around an algal cell (large white spot, center) photographed with

a tracking video camera system attached to a microscope. The bacterial cells are showing positive aerotaxis by moving toward the oxygen-producing algal cell. The alga is about 60  $\mu\text{m}$  in diameter. The proteins that participate in chemotaxis and the mechanisms by which chemotaxis is regulated, are discussed in detail in Section 6.7.

## Measuring Chemotaxis

Bacterial chemotaxis can be demonstrated and quantified by immersing a small glass capillary tube containing an attractant into a suspension of motile bacteria that does not contain the attractant. From the tip of the capillary, a gradient forms into the surrounding medium, with the concentration of chemical gradually decreasing with distance from the tip (Figure 2.43). When an attractant is present, chemotactic bacteria will move toward it, forming a swarm around the open tip (Figure 2.43c) with many of the bacteria swimming into the capillary itself. Of course, because of random movements some chemotactic bacteria will swim into the capillary even if it contains a solution of the same composition as the medium (control solution, Figure 2.43b). However, when an attractant is present, the number of cells within the capillary will be many times higher than external cell numbers. If the capillary is removed after a time period and the cells within it are counted and compared with that of the control, attractants can easily be identified (Figure 2.43e).

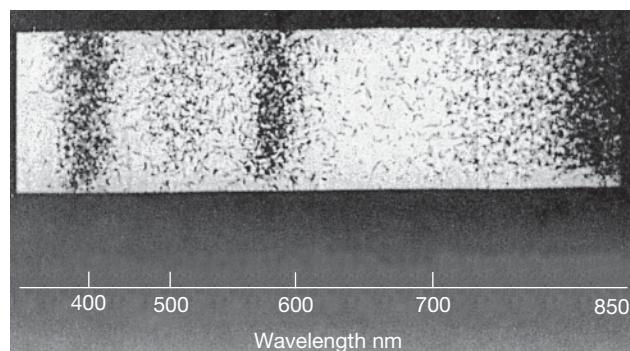
If the inserted capillary contains a repellent, just the opposite occurs; the cells sense an increasing gradient of repellent and the appropriate chemoreceptors affect flagellar rotation to gradually move the cells away from the repellent. In this case, the number of bacteria within the capillary will be fewer than in the control (Figure 2.43d). Using this simple capillary method, it is possible to screen chemicals to see if they are attractants or repellents for a given bacterium.

Chemotaxis can also be observed microscopically. Using a video camera that captures the position of bacterial cells with time and shows the motility tracks of each cell, it is possible to see the chemotactic movements of cells (Figure 2.43f). This method has been used to study chemotaxis of mixtures of microbes in natural environments. In nature it is thought that the major chemotactic agents for bacteria are nutrients excreted from larger microbial cells or from live or dead macroorganisms. Algae, for example, produce both organic compounds and oxygen ( $O_2$ , from photosynthesis) that can trigger chemotactic movements of bacteria toward the algal cell (Figure 2.43f).

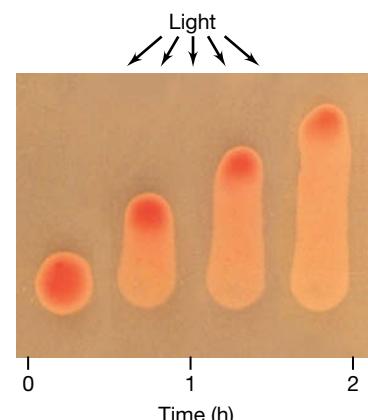
## Phototaxis and Other Taxes

Many phototrophic microorganisms can move toward light, a process called *phototaxis*. Phototaxis allows a phototrophic organism to position itself most efficiently to receive light for photosynthesis. This can be shown if a light spectrum is spread across a microscope slide on which there are motile phototrophic purple bacteria. On such a slide the bacteria accumulate at wavelengths at which their photosynthetic pigments absorb (Figure 2.44a). These pigments include, in particular, bacteriochlorophylls and carotenoids (Chapter 14).

Two different light-mediated taxes are observed in phototrophic bacteria. One, called *scotophobotaxis*, can be observed only microscopically and occurs when a phototrophic bacterium happens to swim outside the illuminated field of view of the microscope into darkness. Entering darkness negatively affects photosynthesis and thus the energy state of the cell and signals the cell to tumble, reverse direction, and once again swim in a run, thus reentering the light. Scotophobotaxis is presumably a mechanism by which phototrophic purple bacteria avoid entering darkened habitats when they are moving about in illuminated ones, and this likely improves their competitive success.



(a)



(b)

**Figure 2.44** Phototaxis of phototrophic bacteria. (a) Scotophobic accumulation of the phototrophic purple bacterium *Thiospirillum jenense* at wavelengths of light at which its pigments absorb. A light spectrum was displayed on a microscope slide containing a dense suspension of the bacteria; after a period of time, the bacteria had accumulated selectively and the photomicrograph was taken. The wavelengths at which the bacteria accumulated are those at which the photosynthetic pigment bacteriochlorophyll *a* absorbs (compare with [Figure 14.2b](#)). (b) Phototaxis of an entire colony of the purple phototrophic bacterium *Rhodospirillum centenum*. These strongly phototactic cells move in unison toward the light source at the top. See Figure 2.34 for electron micrographs of flagellated *R. centenum* cells.

Phototaxis differs from scotophobotaxis in that cells move up a light gradient from lower to higher intensities. Phototaxis is analogous to chemotaxis except that the attractant is light instead of a chemical. In some phototactic organisms, such as the highly motile phototrophic purple bacterium *Rhodospirillum centenum* (Figure 2.34), *entire colonies* of cells show phototaxis and move in unison toward the light (Figure 2.44b).

Several components of the regulatory system that govern chemotaxis also control phototaxis. A *photoreceptor*, a protein that functions similarly to a chemoreceptor but senses a gradient of light instead of chemicals, is the initial sensor in the phototaxis response. The photoreceptor then interacts with the same cytoplasmic proteins that control flagellar rotation in chemotaxis, maintaining the cell in a run if it is swimming toward an increasing intensity of light. Section 6.7 describes the activities of these proteins in more detail.

Other bacterial taxes, such as movement toward or away from oxygen (*aerotaxis*, see Figure 2.43f) or toward or away from conditions of high ionic strength (*osmotaxis*), are known among various

swimming bacteria. In some gliding cyanobacteria, *hydrotaxis* (movement toward water), has also been observed. Hydrotaxis allows gliding cyanobacteria that inhabit dry environments, such as desert soils, to glide toward a gradient of increasing hydration.

### MINIQUIZ

- Define the word chemotaxis. How does chemotaxis differ from aerotaxis?
- What causes a run versus a tumble?
- How can chemotaxis be measured quantitatively?
- How does scotophobotaxis differ from phototaxis?

## V • Eukaryotic Microbial Cells

**C**ompared with prokaryotic cells, microbial eukaryotes typically have structurally more complex and much larger cells. We complete our study of microbial cell structure and function with a consideration of structure/function issues in microbial eukaryotes, common models for the study of eukaryotic biology. Microbial eukaryotes include the fungi, the algae, and the

protozoa and other protists. We cover the diversity of microbial eukaryotes in Chapter 18.

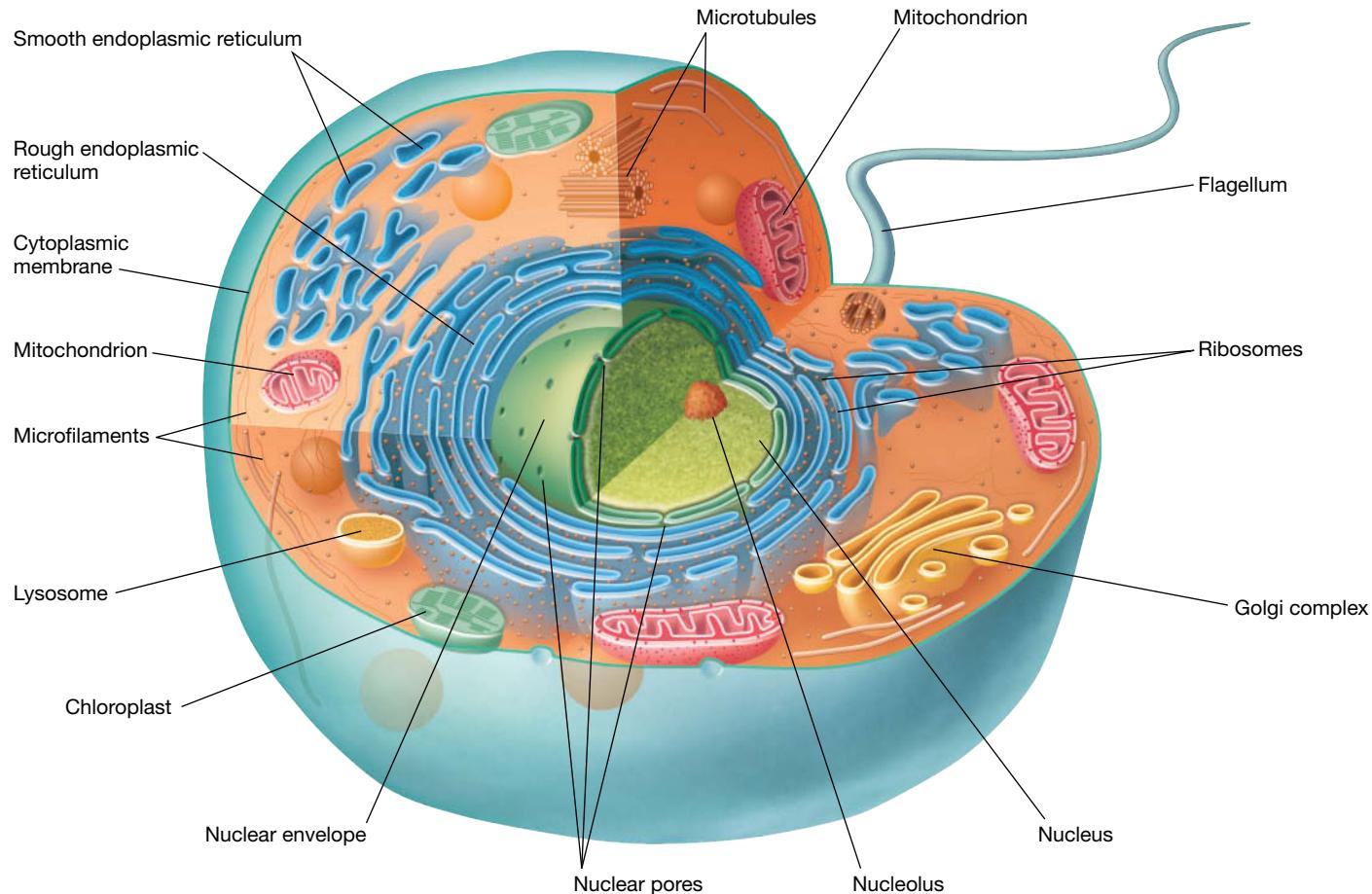
### 2.14 The Nucleus and Cell Division

Eukaryotic cells vary in the complement of organelles they contain, but a unit membrane-enclosed nucleus is universal and a hallmark of the eukaryotic cell. Mitochondria are nearly universal among eukaryotic cells, while pigmented chloroplasts are found only in phototrophic cells. Other structures include the Golgi complex, lysosomes, endoplasmic reticula, and microtubules and microfilaments (Figure 2.45). Some microbial eukaryotes have flagella or cilia—structures that confer motility—and a cell wall is present in many, such as the fungi and algae.

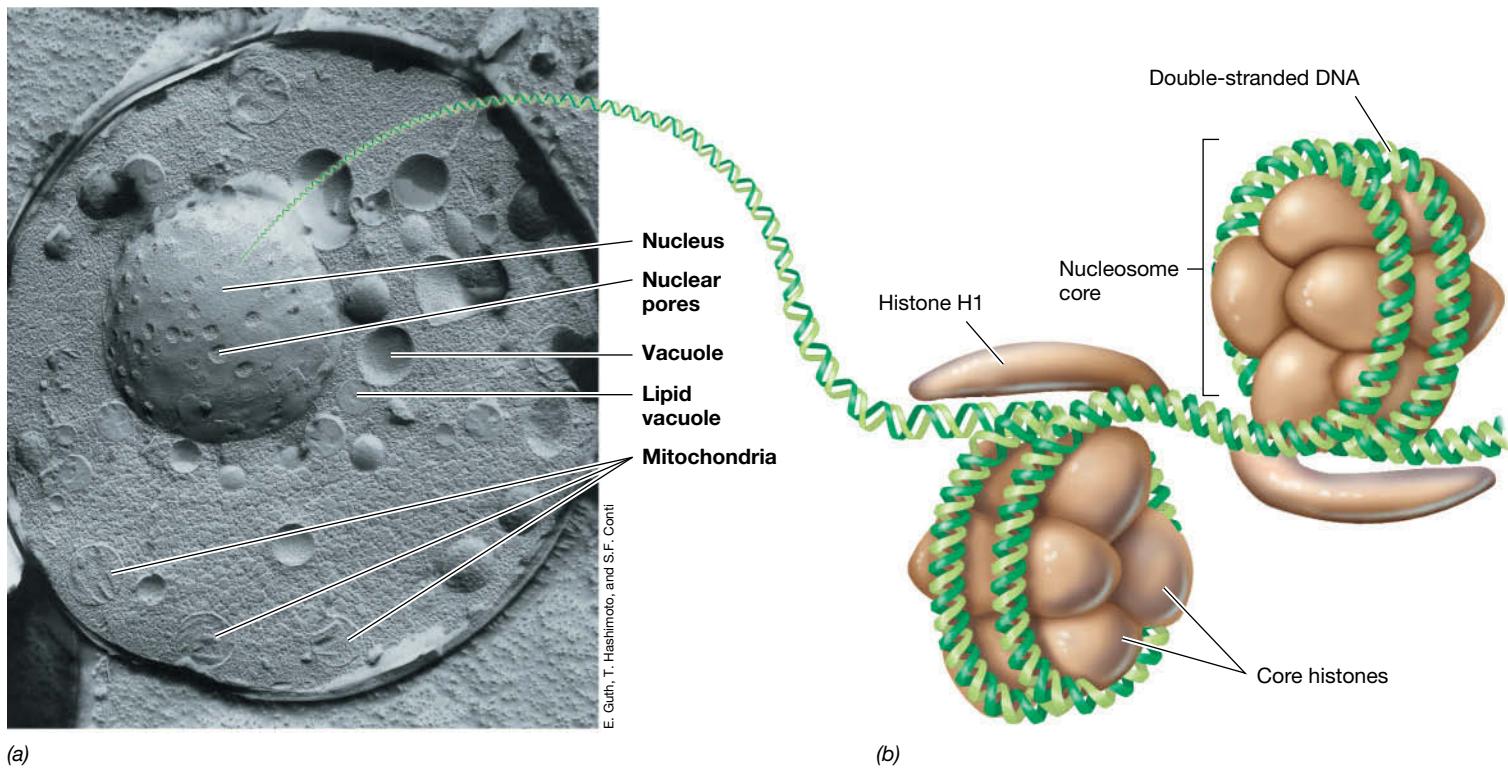
Eukaryotic cell membranes contain **sterols**. These molecules, absent from all but a few prokaryotic cells, lend structural strength to the eukaryotic cell, something especially important to those eukaryotes that lack a cell wall, such as the protozoa or animal cells.

#### The Nucleus

The **nucleus** contains the chromosomes of the eukaryotic cell. DNA within the nucleus is wound around basic (positively charged) proteins called **histones**, which tightly pack the negatively



**Figure 2.45 Cutaway schematic of a microbial eukaryote.** Although all eukaryotic cells contain a nucleus, not all organelles and other structures shown are present in all microbial eukaryotes. Not shown is the cell wall, found in fungi, algae, plants, and a few protists.



**Figure 2.46** The nucleus and DNA packaging in eukaryotes. (a) Electron micrograph of a yeast cell prepared in such a way as to reveal a surface view of the nucleus. The cell is about 8  $\mu\text{m}$  wide. (b) Packaging of DNA around histone proteins to form a nucleosome. Nucleosomes are arranged along the DNA strand like beads on a string and aggregate to form chromosomes during the process of mitosis (see Figure 2.47).

charged DNA to form nucleosomes (Figure 2.46) and from them, chromosomes. The nucleus is enclosed by a pair of membranes, each with its own function, separated by a space. The innermost membrane is a simple sac while the outermost membrane is in many places continuous with the endoplasmic reticulum. The inner and outer nuclear membranes specialize in interactions with the nucleoplasm and the cytoplasm, respectively. The nuclear membranes contain pores (Figures 2.45 and 2.46a), formed from holes where the inner and outer membranes are joined. The pores allow transport proteins to import and export other proteins and nucleic acids into and out of the nucleus, a process called *nuclear transport*.

Within the nucleus is found the *nucleolus* (Figure 2.45), the site of ribosomal RNA (rRNA) synthesis. The nucleolus is rich in RNA, and ribosomal proteins synthesized in the cytoplasm are transported into the nucleolus and combine with rRNA to form the small and large subunits of eukaryotic ribosomes. These are then exported to the cytoplasm, where they associate to form the intact ribosome and function in protein synthesis.

### Cell Division

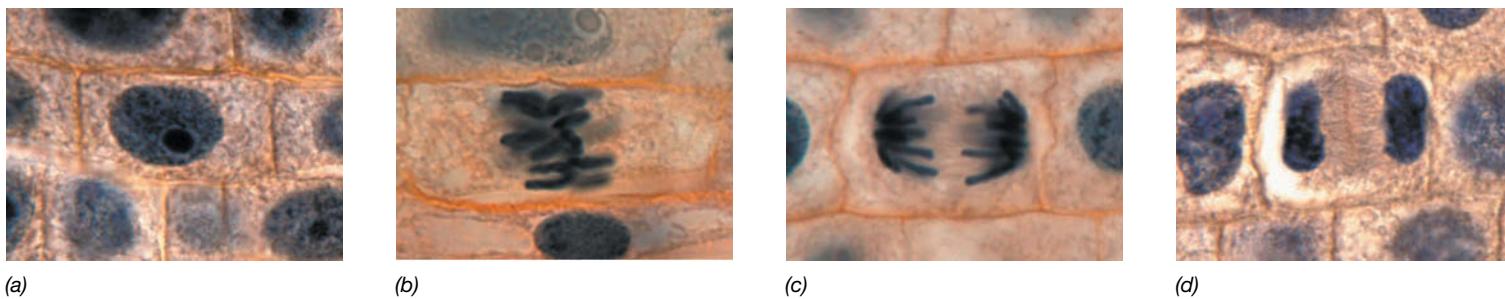
Eukaryotic cells divide by a process in which the chromosomes are replicated, the nucleus is disassembled, the chromosomes are segregated into two sets, and a nucleus is reassembled in each daughter cell (Figure 2.47). Whereas prokaryotic cells are genetically haploid, many microbial eukaryotes can exist in either of

two genetic states: haploid or diploid. *Diploid* cells have two copies of each chromosome whereas *haploid* cells have only one. For example, the brewer's yeast *Saccharomyces cerevisiae* can exist in the haploid state (16 chromosomes) as well as in the diploid state (32 chromosomes). However, regardless of its genetic state, during cell division the chromosome number is first doubled and later halved to give each daughter cell its correct complement of chromosomes. This is the process of **mitosis**, unique to eukaryotic cells. During mitosis, the chromosomes condense, divide, and are separated into two sets, one for each daughter cell (Figure 2.47).

In contrast to mitosis, **meiosis** converts a diploid cell into several haploid cells. Meiosis consists of two successive cell divisions. In the first meiotic division, homologous chromosomes segregate into separate cells, changing the genetic state from diploid to haploid. The second meiotic division is essentially the same as mitosis, as the two haploid cells divide to form a total of four haploid cells called *gametes*. In higher organisms these are the eggs and sperm; in eukaryotic microorganisms, they are spores or related reproductive structures.

### MINIQUIZ

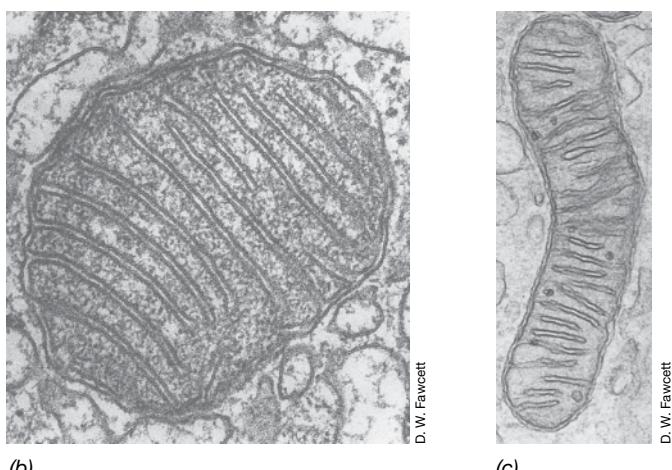
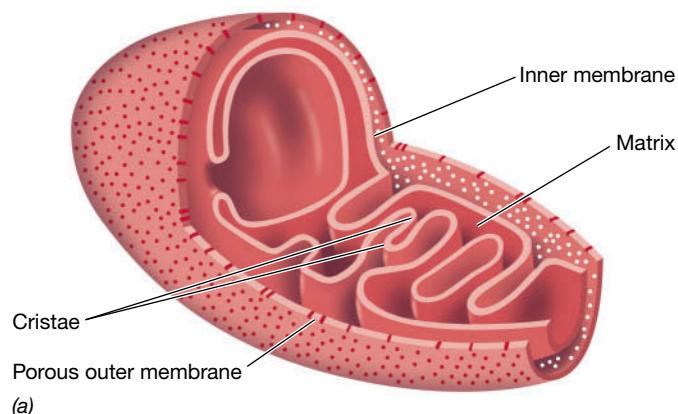
- How is DNA arranged in the chromosomes of eukaryotes?
- What are histones and what do they do?
- What are the major differences between mitosis and meiosis?



**Figure 2.47** Light micrograph of eukaryotic cells undergoing mitosis. (a) Interphase, distinct chromosomes are not apparent. (b) Metaphase. Homologous chromosomes are lining up along the cell center. (c) Anaphase. Homologous chromosomes are pulling apart. (d) Telophase. Chromosomes have separated into the newly forming daughter cells.

## 2.15 Mitochondria, Hydrogenosomes, and Chloroplasts

Organelles that specialize in energy metabolism in eukaryotes include the mitochondrion or hydrogenosome, and in phototrophic eukaryotes, the chloroplast. These organelles have evolutionary roots within the *Bacteria* and provide ATP to the eukaryotic cell from either the oxidation of organic compounds or from light.



**Figure 2.48** Structure of the mitochondrion. (a) Diagram showing the overall structure of the mitochondrion; note the inner and outer membranes. (b, c) Transmission electron micrographs of mitochondria from rat tissue showing the variability in morphology; note the cristae.

### Mitochondria

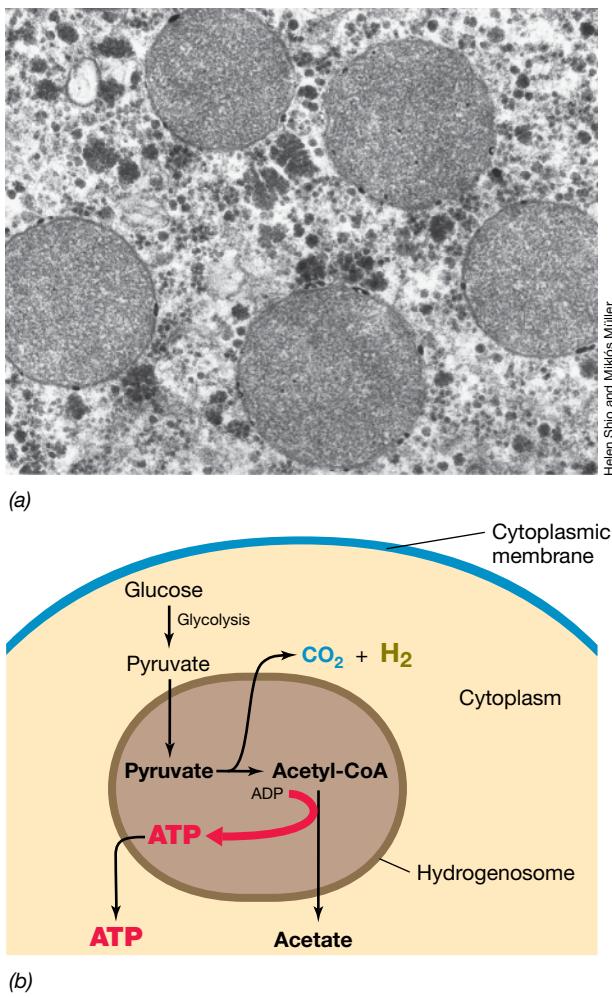
In aerobic eukaryotic cells, respiration occurs in the mitochondrion. **Mitochondria** are of bacterial dimensions and can take on many shapes (Figure 2.48). The number of mitochondria per cell depends somewhat on the cell type and size. A yeast cell may have only a few mitochondria per cell, whereas an animal cell may have over a thousand. The mitochondrion is enclosed by a double membrane system. Like the nuclear membrane, the outermost mitochondrial membrane is relatively permeable and contains pores that allow the passage of small molecules. The innermost membrane is less permeable and its structure more closely resembles that of the cytoplasmic membrane of *Bacteria*.

Mitochondria also contain folded internal membranes called **cristae**. These membranes, formed by invagination of the inner membrane, contain the enzymes needed for respiration and ATP production. Cristae also contain transport proteins that regulate the passage of key molecules such as ATP into and out of the *matrix*, the innermost compartment of the mitochondrion (Figure 2.48a). The matrix contains enzymes for the oxidation of organic compounds, in particular, enzymes of the citric acid cycle, the major pathway for the combustion of organic compounds to  $\text{CO}_2$  (► Section 3.9).

### Hydrogenosomes

Some eukaryotic microorganisms are killed by  $\text{O}_2$  and, like many *Bacteria* and *Archaea*, live an anaerobic lifestyle. Such cells lack mitochondria and some of them contain structures called **hydrogenosomes** (Figure 2.49). Although similar in size to mitochondria, hydrogenosomes lack citric acid cycle enzymes and also lack cristae. Microbial eukaryotes that contain hydrogenosomes carry out a strictly fermentative metabolism. Examples include the human parasite *Trichomonas* (► Sections 18.3 and 33.4) and various protists that inhabit the rumen of ruminant animals (► Section 23.13) or anoxic muds and lake sediments.

The major biochemical reaction in the hydrogenosome is the oxidation of pyruvate to  $\text{H}_2$ ,  $\text{CO}_2$ , and acetate (Figure 2.49b). Some anaerobic eukaryotes have  $\text{H}_2$ -consuming, methane-producing *Archaea* in their cytoplasm. These *methanogens* consume the  $\text{H}_2$  and  $\text{CO}_2$  produced by the hydrogenosome and combine them to form methane ( $\text{CH}_4$ ). Because hydrogenosomes are anoxic and cannot respire, they cannot oxidize the acetate produced from



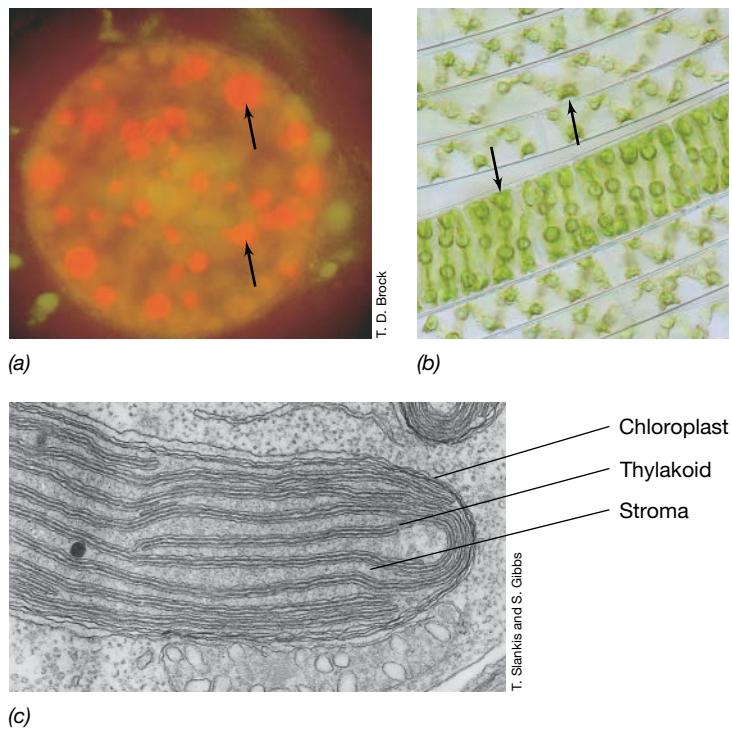
**Figure 2.49** The hydrogenosome. (a) Electron micrograph of a thin section through a cell of the anaerobic protist *Trichomonas vaginalis* showing five hydrogenosomes in cross section. Compare their internal structure with that of mitochondria in Figure 2.48. (b) Biochemistry of the hydrogenosome. Pyruvate is taken up by the hydrogenosome, and  $\text{H}_2$ ,  $\text{CO}_2$ , acetate, and ATP are produced.

pyruvate oxidation as mitochondria do. Acetate is therefore excreted from the hydrogenosome into the cytoplasm of the host cell (Figure 2.49b).

### Chloroplasts

**Chloroplasts** are the chlorophyll-containing organelles of phototrophic microbial eukaryotes such as the algae and function to carry out photosynthesis. Chloroplasts are relatively large and readily visible with the light microscope (Figure 2.50), and their number per cell varies among species.

Like mitochondria, chloroplasts have a permeable outer membrane and a much less-permeable inner membrane. The innermost membrane surrounds the **stroma**, analogous to the matrix of the mitochondrion (Figure 2.50c). The stroma contains the enzyme *ribulose bisphosphate carboxylase* (RubisCO), the key enzyme of the *Calvin cycle*, the series of biosynthetic reactions by which phototrophs convert  $\text{CO}_2$  to organic compounds (► Section 14.5). The permeability of the outermost chloroplast membrane allows glucose and ATP produced during



**Figure 2.50** Chloroplasts of a diatom and a green alga cell. (a) Fluorescence photomicrograph of a diatom shows chlorophyll fluorescence; arrows, chloroplasts. The cell is about 40  $\mu\text{m}$  wide. (b) Phase-contrast photomicrograph of the filamentous green alga *Spirogyra* showing the characteristic spiral-shaped chloroplasts (arrows) of this phototroph. A cell is about 20  $\mu\text{m}$  wide. (c) Transmission electron micrograph showing a chloroplast of a diatom; note the thylakoids.

photosynthesis to diffuse into the cell cytoplasm where they are used in biosynthesis.

Chlorophyll and all other components needed for ATP synthesis in chloroplasts are located in a series of flattened membrane discs called **thylakoids** (Figure 2.50c). Like the cytoplasmic membrane, the thylakoid membrane is highly impermeable and its major function is to form a proton motive force (Figure 2.7c) that results in ATP synthesis.

### Organelles and Endosymbiosis

On the basis of their relative autonomy, size, and morphological resemblance to bacteria, it was hypothesized over 100 years ago that mitochondria and chloroplasts were descendants of respiratory and phototrophic bacterial cells, respectively. By associating with nonphototrophic eukaryal hosts, the latter gained a new form of energy metabolism while the symbiotic bacterial cells received a stable and supportive growth environment inside the host. Over time, these originally free-living symbionts became an intimate part of the eukaryotic cell. This idea of symbiotic bacteria as the ancestors of the mitochondrion, hydrogenosome, and chloroplast is called the **endosymbiotic hypothesis** (► Sections 13.4 and 18.1) and is now well accepted in biology.

Several lines of evidence support the endosymbiotic hypothesis. These include in particular the fact that mitochondria, hydrogenosomes, and chloroplasts contain their own genomes and

ribosomes. The genomes are arranged in a circular fashion as for bacterial chromosomes (☞ Section 9.3), and the sequence of genes that encode ribosomal RNA (☞ Figure 1.36) in organelles clearly points to their bacterial origin. Thus, the eukaryotic cell is a genetic chimera containing genes from two domains of life: the host cell (*Eukarya*) and the endosymbiont (*Bacteria*).

### MINIQUIZ

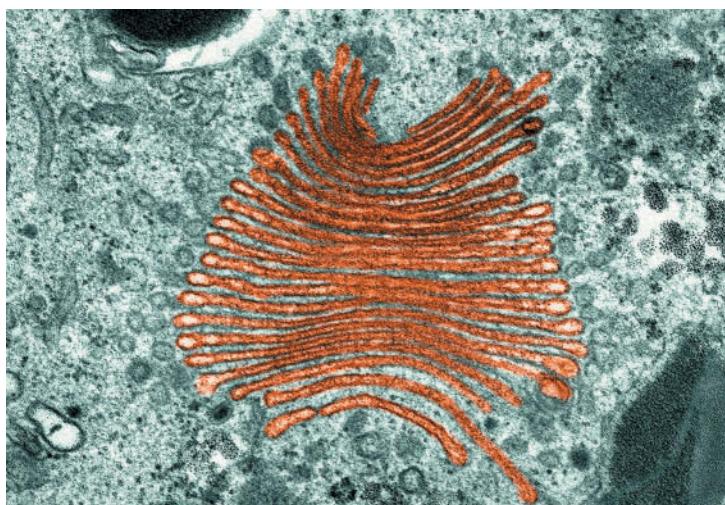
- What key reactions occur in the mitochondrion and in the chloroplast, and what key product is made in each?
- Compare and contrast pyruvate metabolism in the mitochondrion and the hydrogenosome.
- What is the endosymbiotic hypothesis and what evidence is there to support it?

## 2.16 Other Eukaryotic Cell Structures

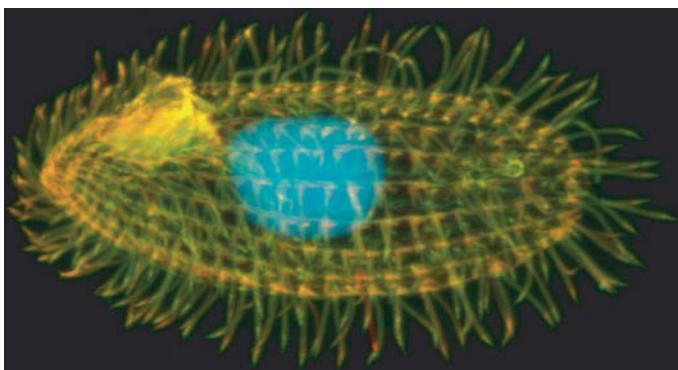
Besides the nucleus and the mitochondrion (or hydrogenosome), and chloroplasts in phototrophic cells, other cytoplasmic structures are present in microbial eukaryotes. These include the endoplasmic reticulum, the Golgi complex, lysosomes, a variety of tubular structures, and structures responsible for motility. However, unlike mitochondria and chloroplasts, these structures lack DNA and are not of endosymbiotic origin. Cell walls are also present in certain microbial eukaryotes and function as they do in prokaryotic cells to provide shape and protect the cell from osmotic lysis. The exact structure of the cell wall varies with the organism, but various polysaccharides and proteins are commonly observed.

### Endoplasmic Reticulum, the Golgi Complex, and Lysosomes

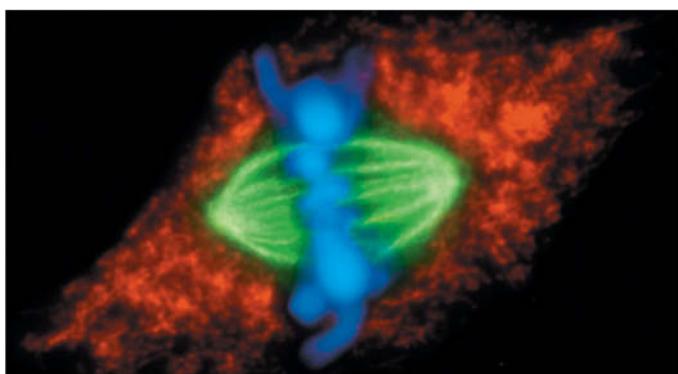
The endoplasmic reticulum (ER) is a network of membranes continuous with the nuclear membrane. Two types of endoplasmic reticulum exist: *rough* ER, which contains attached ribosomes,



**Figure 2.51 The Golgi complex.** Transmission electron micrograph of a portion of a eukaryotic cell showing the Golgi complex (colored in gold). Note the multiple folded membranes of the Golgi complex (membrane stacks are 0.5–1.0 µm in diameter).



(a)



(b)



(c)

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**Figure 2.52 Tubulin and microfilaments.** (a) Fluorescence photomicrograph of a cell of *Tetrahymena* with red- and green-labeled antitubulin antibodies (the two combine to give yellow) and with DAPI, which stains DNA (blue, nucleus). A cell is about 10 µm wide. (b) An animal cell showing the role of tubulin (green) in separating chromosomes (blue) during metaphase of mitosis (cytoplasmic proteins stain red). (c) Electron microscopic image of the cellular slime mold *Dictyostelium discoideum* showing the network of actin microfilaments that along with microtubules functions as the cell cytoskeleton. Microfilaments are about 7 nm in diameter. *D. discoideum* has been used for decades as an experimental model system for eukaryotic cellular development and cell-to-cell cooperation (☞ Figures 18.17 and 18.18).