eleventh edition

Prescott's Microbiology

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PRESCOTT'S MICROBIOLOGY, ELEVENTH EDITION

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About the Authors

Courtesy of Joanne Willey

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

Courtesy of Adele Anderson

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

Courtesy of Dorothy Wood

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

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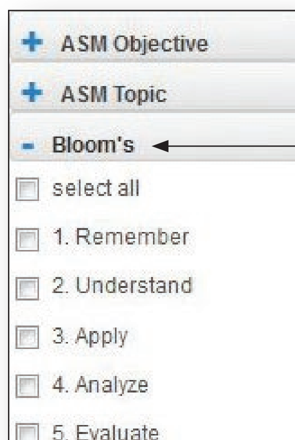

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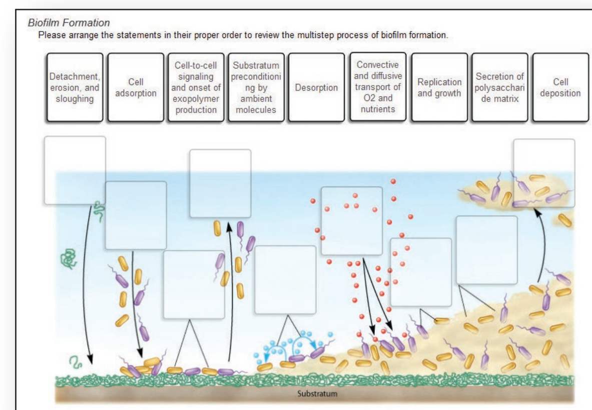

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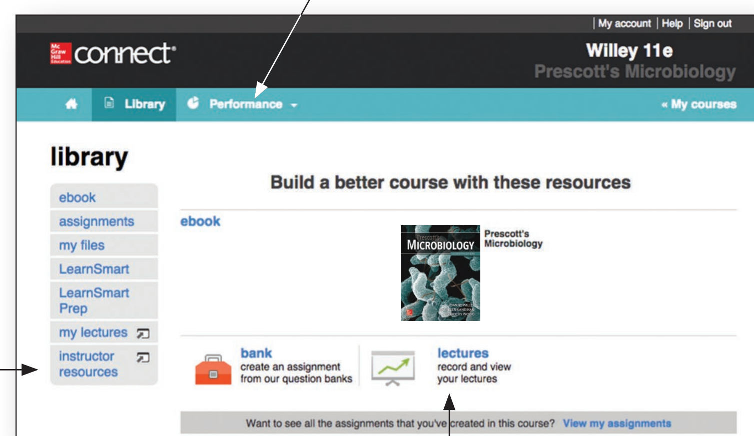

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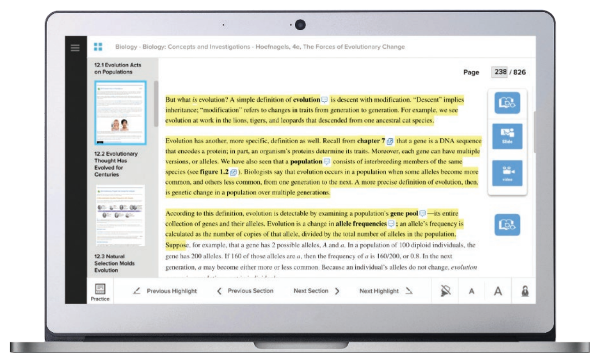

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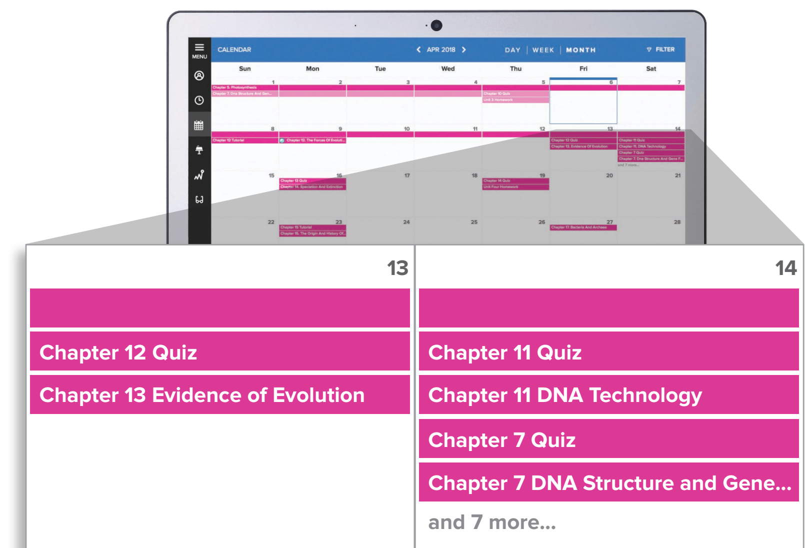

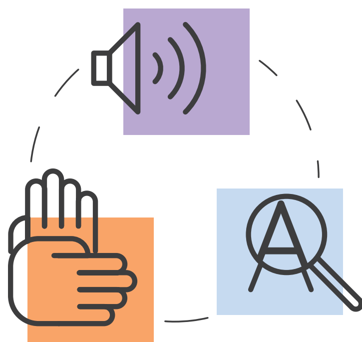

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A Modern Approach to Microbiology

Evolution as a Framework

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

An Introduction to the Entire Microbial World

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

Broad Coverage of Microbial Ecology

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

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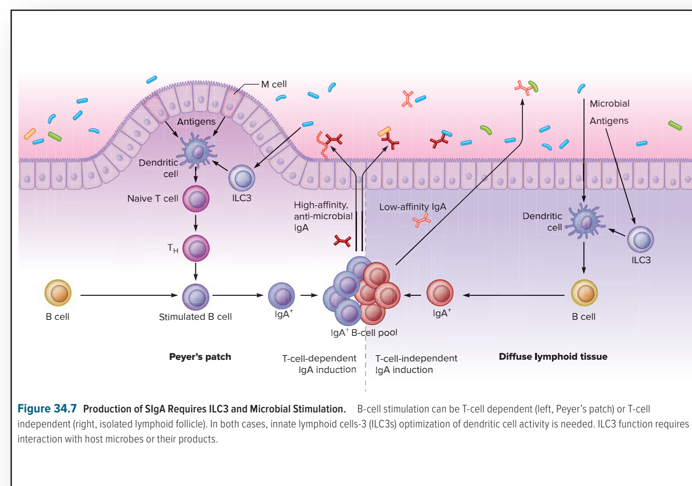

Figure 34.7 Production of SIgA Requires ILC3 and Microbial Stimulation. B-cell stimulation can be T-cell dependent (left, Peyer's patch) or T-cell independent (right, isolated lymphoid follicle). In both cases, innate lymphoid cells-3 (ILC3s) optimization of dendritic cell activity is needed. ILC3 function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

Molecular Microbiology and Immunology

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

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A Modern Approach to Microbiology

17.5 Cas9 Nuclease Is a Precise Tool for Genome Editing

After reading this section, you should be able to:

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

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

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

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

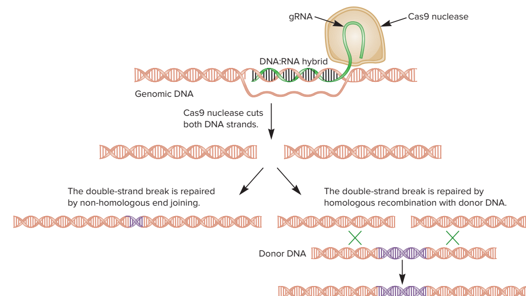


Figure 17.13 Genome Editing with Cas9 Nuclease.

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

21st-Century Microbiology

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

Metagenomics and the Human Microbiome

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

Laboratory Safety

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

Special Interest Essays

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

MICROBIAL DIVERSITY & ECOLOGY

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

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

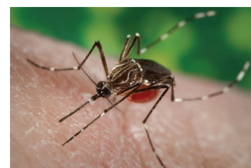
W. pipientis inhabits the cytoplasm of its host's cells and is transferred from one generation to the next through the eggs of infected females. To survive, *Wolbachia* must ensure the fertilization and viability of infected eggs while decreasing the likelihood that uninfected eggs survive. The mechanism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

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

Another effect of *Wolbachia* infection is interference with viral replication. Viruses normally spread by insects may not be transmissible if the insect is infected with *Wolbachia*. This has led to the notion that this infection could be used as a means of biological control. *Aedes* mosquitoes transmit numerous viruses, like Zika, dengue, chikungunya, and West Nile. In humans, these viral infections have no cure and no treatment beyond supportive care, so insect control is the best prevention.

However, the mosquito vectors of these viruses, which belong the genus *Aedes* (**box figure**), are not natural hosts to *Wolbachia*. Nonetheless, infection can be established by transinfection, the process of transferring the microbes from another insect species. Many *Wolbachia* isolates have been screened for those that have the most severe effects on *Aedes,* and several stable insect strains have been established with *Wolbachia* from *Drosophila*. Cytoplasmic interference is extensive in these strains of *Wolbachia*. To establish a stable population of *Aedes* outside the laboratory, it is important that the infection not impose a dramatic burden on the insect, as it must compete with and integrate into wild populations.

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



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

DISEASE

39.1 Syphilis and the Tuskegee Study

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

Source: CDC

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

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

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

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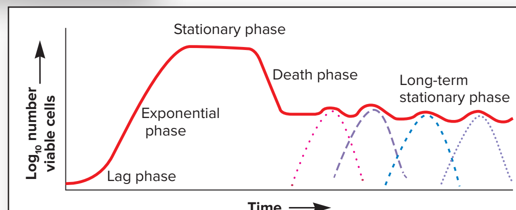

Figure 7.10 Microbial Growth Curve in a Closed System. The five phases of the growth curve are identified. The dotted lines shown during the long-term stationary phase represent successive waves of genetic variants that evolve during this phase of the growth curve.

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

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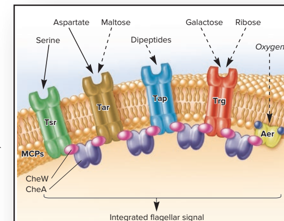

Figure 14.23 *E. coli* **Methyl-Accepting Chemotaxis Proteins.** The attractants sensed by each methyl-accepting chemotaxis protein (MCP) are shown. Some are sensed directly, when the attractant binds the MCP (solid lines). Others are sensed indirectly (dashed lines). Maltose, dipeptides, galactose,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

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

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

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

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

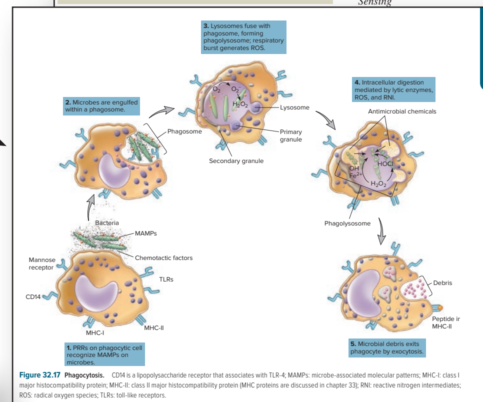

Figure 32.17 **Phagocytosis.** CD14 is a lipopolysaccharide receptor that associates with TLR-4; MAMPs: microbe-associated molecular patterns; MHC-I: class I major histocompatibility protein; MHC-II: class II major histocompatibility protein (MHC proteins are discussed in chapter 33); RNI: reactive nitrogen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

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

Key Concepts

2.1 Lenses Create Images by Bending Light

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

2.2 There Are Several Types of Light Microscopes

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

2.3 Staining Specimens Helps to Visualize and Identify Microbes

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

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

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

2.5 Scanning Probe Microscopy Can Visualize Molecules and Atoms

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

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

Active Learning

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

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List of Content Changes

Each chapter has been thoroughly reviewed.

Part One

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

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

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

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

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

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

Part Two

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

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

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

Part Three

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

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

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

Part Four

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

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

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

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

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

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

Part Five

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

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

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

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List of Content Changes

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

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

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

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

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

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

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

Part Six

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

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

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

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

Part Seven

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

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

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

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

Part Eight

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

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

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

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

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

Part Nine

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

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

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

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Lab Tools for Your Success

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

Acknowledgements

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

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

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

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Prescott's Microbiology

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3

Bacterial Cell Structure

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Hooking Up

Each year over 100 million people around the world become infected with *Neisseria gonorrhoeae*, the bacterium that causes gonorrhea. This troubling statistic is made even more disturbing by the increasing resistance of the bacterium to the antibiotics used to treat the disease. In men, infection is usually readily detected, but for women, infection is often asymptomatic and can lead to serious consequences such as pelvic inflammatory disease (PID) and sterility. These concerns have led scientists to consider methods for preventing infection. One method is to block transmission. Unfortunately, relatively little is known about the transmission process except that it occurs during sexual intercourse and that numerous hairlike structures (called pili) covering the surface of the bacterium play a role in establishing infection. The bacterium uses pili for a type of movement called twitching motility and to adhere to surfaces such as the sperm and epithelial cells of its human host. It has long been thought that by attaching to sperm cells the bacterium could hitch a ride during sexual intercourse. This explained transmission between partners. However, it did not clarify how transmission from women to men occurs.

It turns out that exposure of *N. gonorrhoeae* to seminal fluid increases its twitching motility and enhances formation of small clumps of bacteria. In addition, seminal fluid proteins appear to alter the morphology and function of pili. In particular these proteins cause bundles of pili to separate into single filaments, enhancing the interaction of bacterial cells with each other and with host surfaces. These changes evolved to promote infection of host epithelial cells and increase the likelihood of transmission during sexual intercourse from females to their male partner.

As this story illustrates, even small, seemingly simple organisms such as bacteria can exhibit complex behaviors. To understand these amazing microbes, we must first examine their cell structure and begin to relate it to the functions they carry out. As we consider bacterial cell structure, it is important to remember that only about 1% of bacterial species have been cultured. Of the cultivated species, only a few have been studied in great detail. From this small sample, many generalizations are made, and it is presumed that most other bacteria are like the well-studied model organisms. However, part of the wonder and fun of science is that nature is full of surprises. As the biology of more and more bacteria is explored, our understanding of them may change in interesting and exciting ways.

Readiness Check:

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

- ✓ Describe the application of small subunit (SSU) rRNA analysis to the establishment of the three domain classification system proposed by Carl Woese (section 1.2)
- ✓ Identify the following structures or regions of a plant or animal cell and describe their functions: cell wall, plasma membrane, cytoplasm, mitochondria, chloroplasts, and ribosomes
- ✓ Define and give examples of essential nutrients; describe how they are used by cells

3.1 Use of the Term "Prokaryote" Is Controversial

After reading this section, you should be able to:

a. List the characteristics originally used to describe prokaryotic cells
b. Debate the "prokaryote" controversy using current evidence about bacterial cells

Bacteria and archaea have long been lumped together and referred to as prokaryotes. Although the term was first introduced early in the twentieth century, the concept of a prokaryote was not fully outlined until 1962, when R. Stanier and C. B. van Niel described prokaryotes in terms of what they lacked in comparison to eukaryotic cells. For instance, Stanier and van Niel pointed out that prokaryotes lack a membrane-bound nucleus, a cytoskeleton, membrane-bound organelles, and internal membranous structures such as the endoplasmic reticulum and Golgi apparatus. Since the 1960s, biochemical, genetic, and genomic analyses have shown that *Bacteria* and *Archaea* are distinct taxa. Because of these discoveries, Norman Pace proposed in 2006 that the term *prokaryote* should be abandoned and most microbiologists are in agreement.

This controversy illustrates that microbiology is an exciting, dynamic, and rapidly changing field of study. Throughout this text, we avoid the term *prokaryote* and are as explicit as

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possible about which characteristics are associated with members of *Bacteria*, which with members of *Archaea*, and which with members of both taxa.

3.2 Bacteria Are Diverse but Share Some Common Features

After reading this section, you should be able to:

a. Distinguish a typical bacterial cell from a typical plant or animal cell in terms of cell shapes and arrangements, size, and cell structures
b. Discuss the factors that determine the size and shape of a bacterial cell

Much of this chapter is devoted to a discussion of individual cell components. Therefore a preliminary overview of the features common to many bacterial cells is in order. We begin by considering overall cell morphology and then move to cell structures.

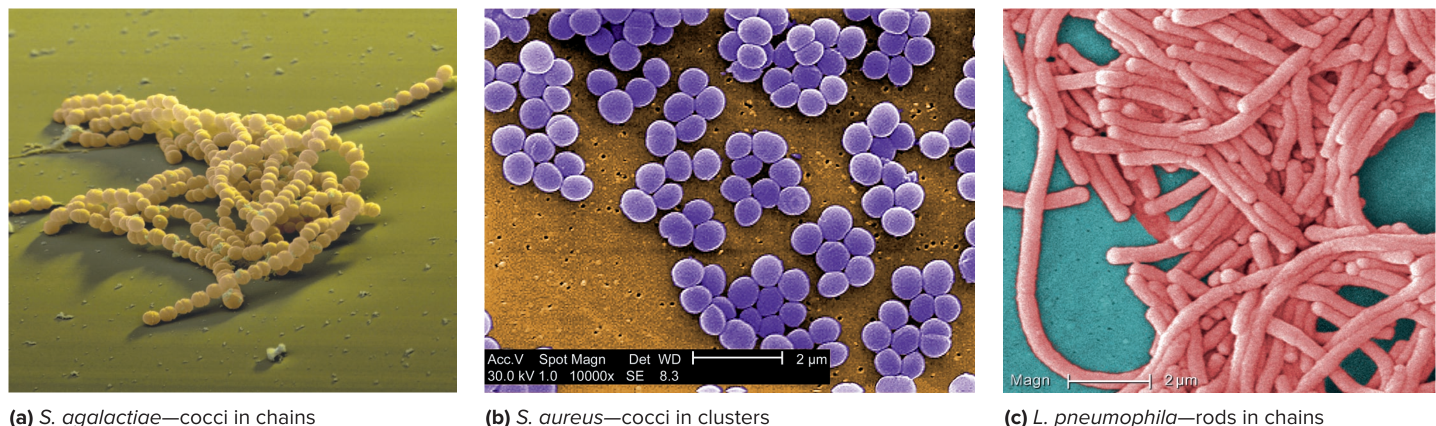
Shape, Arrangement, and Size

It might be expected that bacterial cells, being small and relatively simple, would be uniform in shape and size. This is not the case, as the microbial world offers considerable variety in terms of morphology. However, the two most common shapes are cocci and rods (**figure 3.1**). **Cocci** (s., **coccus**) are roughly spherical cells. They can exist singly or can be associated in characteristic arrangements that can be useful in their identification. **Diplococci** (s., **diplococcus**) arise when cocci divide and remain together to form pairs. Long chains of cocci result when cells adhere after repeated divisions in one plane; this pattern is seen in the genera *Streptococcus, Enterococcus,* and *Lactococcus* (figure 3.1*a*). Members of the genus *Staphylococcus* divide in random planes to generate irregular, grapelike clusters (figure 3.1*b*). Divisions in two or three planes can produce symmetrical groupings of cocci. Bacteria in the genus *Micrococcus* often divide in two planes to form square groups of four cells called tetrads. In the genus *Sarcina,* cocci divide in three planes, producing cubical packets of eight cells.

Legionella pneumophila is an example of a bacterium with a **rod** shape (figure 3.1*c*). Rods, sometimes called **bacilli** (s., **bacillus**), differ considerably in their length-to-width ratio, the coccobacilli being so short and wide that they resemble cocci. The shape of the rod's end often varies between species and may be flat, rounded, football-shaped, or bifurcated. Although many rods occur singly, some remain together after division to form pairs or chains (e.g., *Bacillus megaterium* is found in long chains).

There are several less common cell shapes and arrangements. **Vibrios** are comma-shaped (**figure 3.2*a***). **Spirilla** are rigid, spiral-shaped cells (figure 3.2*b*). Many have tufts of flagella at one or both ends. **Spirochetes** are flexible, spiral-shaped bacteria that have a unique, internal flagellar arrangement (figure 3.2*c*). These bacteria are distinctive in other ways, and all belong to a single phylum, *Spirochaetes.* Some bacteria form stalks (e.g., *Caulobacter crescentus*) (figure 3.2*d*). Other bacteria are **pleomorphic,** being variable in shape and lacking a single, characteristic form. ▶| *Phylum* Spirochaetes *(section 21.6);* Caulobacteraceae *and* Hyphomicrobiaceae *bacteria reproduce in unusual ways (section 22.1); Order* Vibrionales *includes aquatic bioluminescent bacteria and pathogens (section 22.3)*

Some bacteria can be thought of as multicellular. Many actinobacteria form long filaments called hyphae. The hyphae form a network called a **mycelium** (figure 3.2*e*), and in this sense, they are similar to eukaryotic filamentous fungi. Many cyanobacteria, a group of photosynthetic bacteria, are also filamentous. Being filamentous allows some degree of



(a) *S. agalactiae*—cocci in chains

(b) *S. aureus*—cocci in clusters

(c) *L. pneumophila*—rods in chains

Figure 3.1 Cocci and Rods Are the Most Common Bacterial Shapes. These images are color-enhanced scanning electron micrographs. (a) *Streptococcus agalactiae,* the cause of group B streptococcal infections (×4,800). (b) *Staphylococcus aureus.* (c) *Legionella pneumophila,* the cause of Legionnaires' disease.

(*a*) ©Science Source; (*b*, *c*) Source: CDC/Janice Haney Carr.

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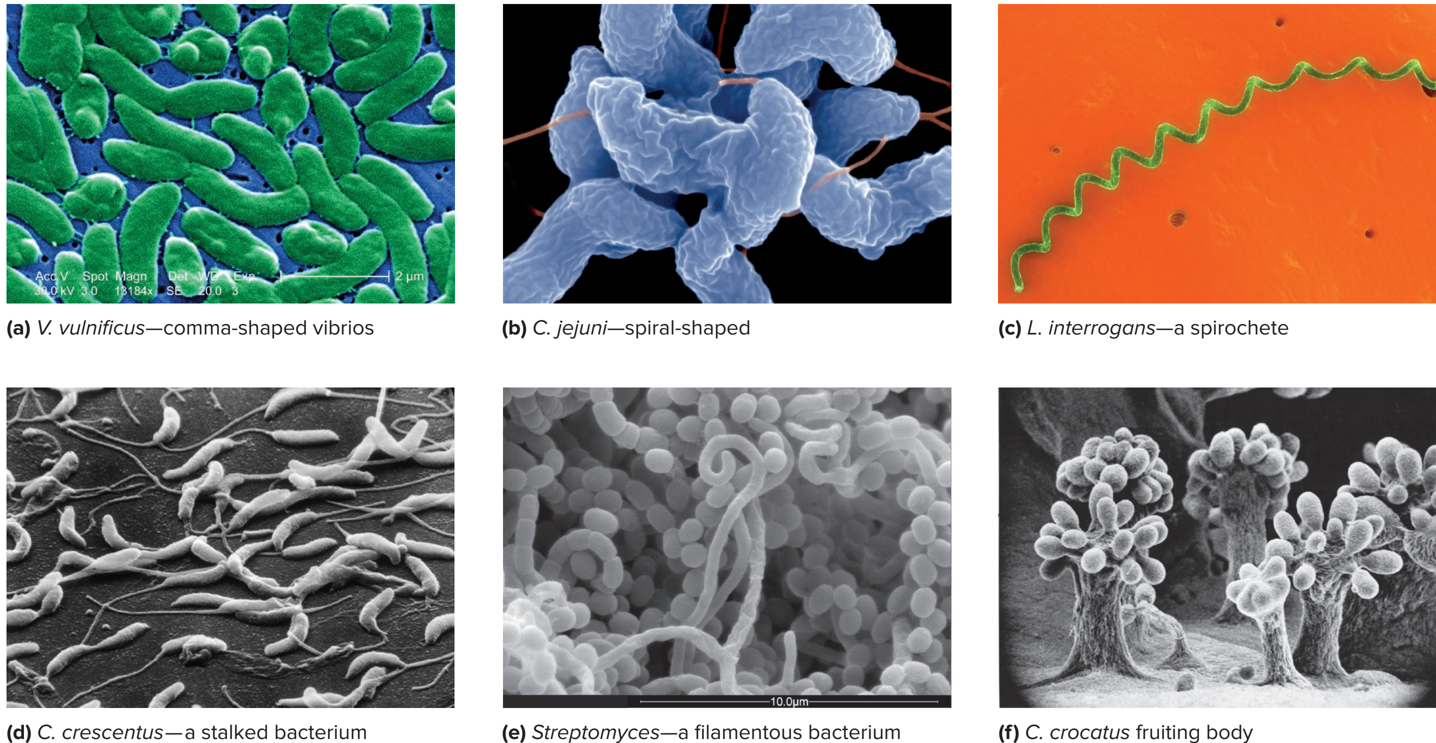

(a) *V. vulnificus*—comma-shaped vibrios

(b) *C. jejuni*—spiral-shaped

(c) *L. interrogans*—a spirochete

(d) *C. crescentus*—a stalked bacterium

(e) *Streptomyces*—a filamentous bacterium

(f) *C. crocatus* fruiting body

Figure 3.2 Other Cell Shapes and Aggregations. (a) *Vibrio vulnificus,* scanning electron micrograph (SEM, X13,184). (b) *Campylobacter jejuni,* SEM. (c) *Leptospira interrogans,* the spirochete that causes the disease leptospirosis. (d) *Caulobacter crescentus,* SEM. (e) *Streptomyces* sp., SEM. (f) Fruiting body of the myxobacterium *Chondromyces crocatus.* The fruiting body is composed of thousands of cells.

(*a*) ©Media for Medical/Getty Images; (*b*) Source: Photo by DeWood, digital colorization by Stephen Ausmus/USDA-ARS; (*c*) ©Sebastian Kaulitzki/Getty Images; (*d*) ©Biology Pics/Science Source; (*e*) ©Dr. Amy Gehring; (*f*) ©Yoav Levy/DIOMEDIA

differentiation among cells in the filament. For instance, some filamentous cyanobacteria form specialized cells within the filament, heterocysts, that carry out nitrogen fixation *(see figure 21.10c).* Myxobacteria are of particular note. These bacteria sometimes aggregate to form complex structures called fruiting bodies (figure 3.2*f*). *Order Streptomycetales: an important source of antibiotics (section 23.1); Phylum* Cyanobacteria: *oxygenic photosynthetic bacteria (section 21.4); Order* Myxococcales: *bacteria with morphological complexity and multicellularity (section 22.4)*

Escherichia coli is an excellent representative of an average-sized bacterium. This rod-shaped bacterium is 1.1 to 1.5 µm wide by 2.0 to 6.0 µm long. However, the size range of bacterial cells extends far beyond this average (**figure 3.3**). Near the small end of the size continuum are members of the genus *Mycoplasma* (0.3 µm in diameter). At the other end of the continuum are bacteria such as some spirochetes, which can reach 500 µm in length, and the cyanobacterium *Oscillatoria,* which is about 7 µm in diameter (the same diameter as a red blood cell). Some bacteria are huge by "bacterial standards." For instance, *Epulopiscium fishelsoni* grows as large as 600 by 80 µm, a little smaller than a printed hyphen and clearly larger than the well-known eukaryote *Paramecium* (**figure 3.4**). An even larger bacterium, *Thiomargarita*

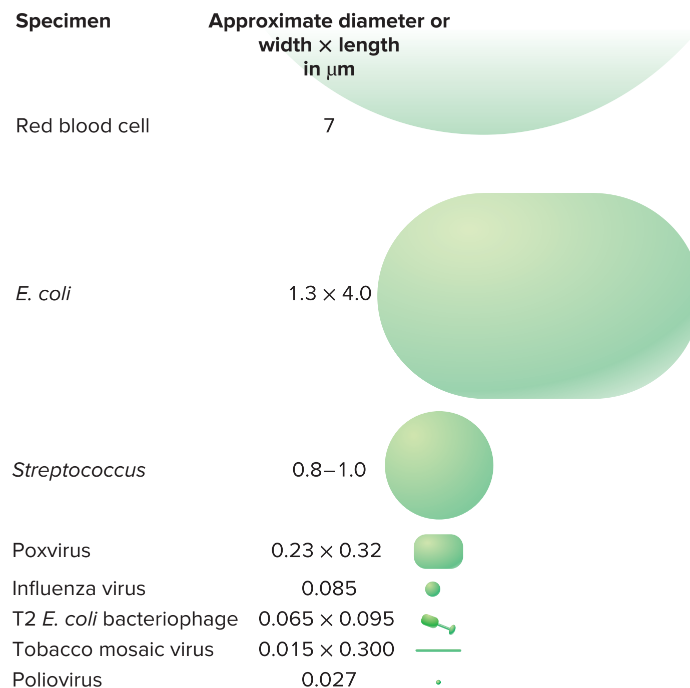

Figure 3.3 Sizes of Bacteria Relative to a Red Blood Cell and Viruses. The larger viruses are comparable in size to the smaller bacteria.

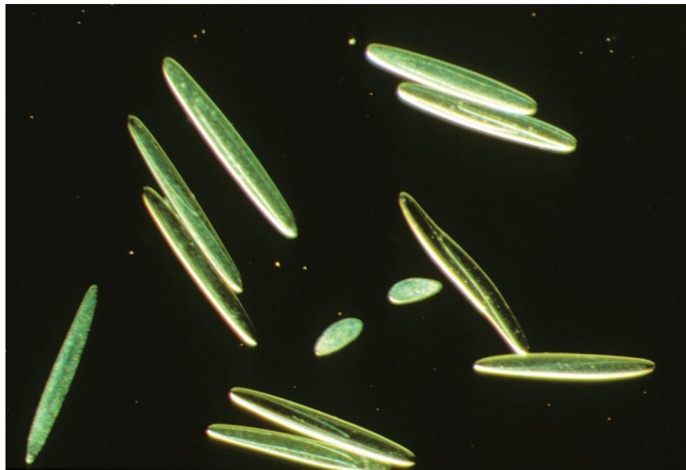

Figure 3.4 A Giant Bacterium. This phase-contrast micrograph shows *Epulopiscium fishelsoni* dwarfing the paramecia, which are protozoa. *E. fishelsoni* cells are about 530 µm long.
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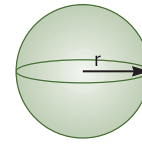


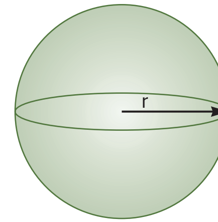


Figure 3.5 The Surface-to-Volume Ratio Is an Important Determinant of Cell Size. Surface area is calculated by the formula $4\pi r^2$. Volume is calculated by the formula $4/3\pi r^3$. Shape also affects the S/V ratio; rods with the same volume as a coccus have a greater S/V ratio.

namibiensis, lives in ocean sediment (*see figure 22.20*). Thus a few bacteria are much larger than the average eukaryotic cell (typical plant and animal cells are around 10 to 50 µm in diameter).

The variety of sizes and shapes exhibited by bacteria raises a fundamental question: What causes a bacterial species to have a particular size and shape? Although far from being answered, recent discoveries have fueled a renewed interest in this question, and it is clear that size and shape determination are related and have been selected for during the evolutionary history of each bacterial species. For many years it was thought that microbes had to be small to increase the surface area-to-volume ratio (S/V ratio; **figure 3.5**). As this ratio increases, the uptake of nutrients and the diffusion of these and other molecules within the cell become more efficient, which in turn facilitates a rapid growth rate. Shape affects the S/V ratio. A rod with the same volume as a coccus has a higher S/V ratio than does the coccus. This means that a rod can have greater nutrient flux across its plasma membrane. However, the discovery of *E. fishelsoni* demonstrates that bacteria can be very large. For bacteria to be large, they must have other characteristics that maximize their S/V ratio, or their size must be beneficial in some way. For instance, *E. fishelsoni* has a highly convoluted plasma membrane, which increases its S/V ratio. In addition, large cells are less likely to be eaten by predatory protists. Cells that are filamentous, have stalks, or are oddly shaped are also less susceptible to predation.

Cell Organization

Structures often observed in bacterial cells are summarized and illustrated in **table 3.1** and **figure 3.6**. Note that no single bacterium possesses all of these structures at all times. Some are found only in certain cells in certain conditions or in certain phases of the life cycle.

There are several common features of bacterial cell structure. Bacterial cells are surrounded by several layers, which are collectively called the cell envelope. The most common cell envelope layers are the plasma membrane, cell wall, and capsule or slime layer. The innermost layer of the cell envelope is the plasma membrane, which surrounds the cytoplasm. Most bacteria have a chemically complex cell wall, which covers the plasma membrane. Many bacteria surround the cell wall with a capsule or slime layer. Because most bacteria do not contain internal, membrane-bound organelles, their interior appears morphologically simple. The genetic material is localized in a discrete region called the nucleoid and is not separated from the surrounding cytoplasm by membranes. Ribosomes and larger masses called inclusions are scattered about the cytoplasm. Finally, many bacteria use flagella for locomotion. In the remaining sections of this chapter, we describe these major structures observed in bacterial cells in more detail.

Comprehension Check

1. Why is the term *prokaryote* considered an inadequate descriptor by some microbiologists?
2. What characteristic shapes can bacteria assume? Describe the ways in which bacterial cells cluster together.
3. What advantages might a bacterial species that forms multicellular arrangements (e.g., clusters or chains) have that are not afforded unicellular bacteria?
4. What is the relevance of the surface area-to-volume ratio?

Table 3.1	Common Bacterial Structures and Their Functions
Plasma membrane	Selectively permeable barrier, mechanical boundary of cell, nutrient and waste transport, location of many metabolic processes (respiration, photosynthesis), detection of environmental cues for chemotaxis
Gas vacuole	An inclusion that provides buoyancy for floating in aquatic environments
Ribosomes	Protein synthesis
Inclusions	Storage of carbon, phosphate, and other substances; site of chemical reactions (microcompartments); movement
Nucleoid	Localization of genetic material (DNA)
Periplasmic space	In typical Gram-negative bacteria, contains hydrolytic enzymes and binding proteins for nutrient processing and uptake; in typical Gram-positive bacteria, may be smaller or absent
Cell wall	Protection from osmotic stress, helps maintain cell shape
Capsules and slime layers	Resistance to phagocytosis, adherence to surfaces
Fimbriae and pili	Attachment to surfaces, bacterial conjugation and transformation, twitching
Flagella	Swimming and swarming motility
Endospore	Survival under harsh environmental conditions

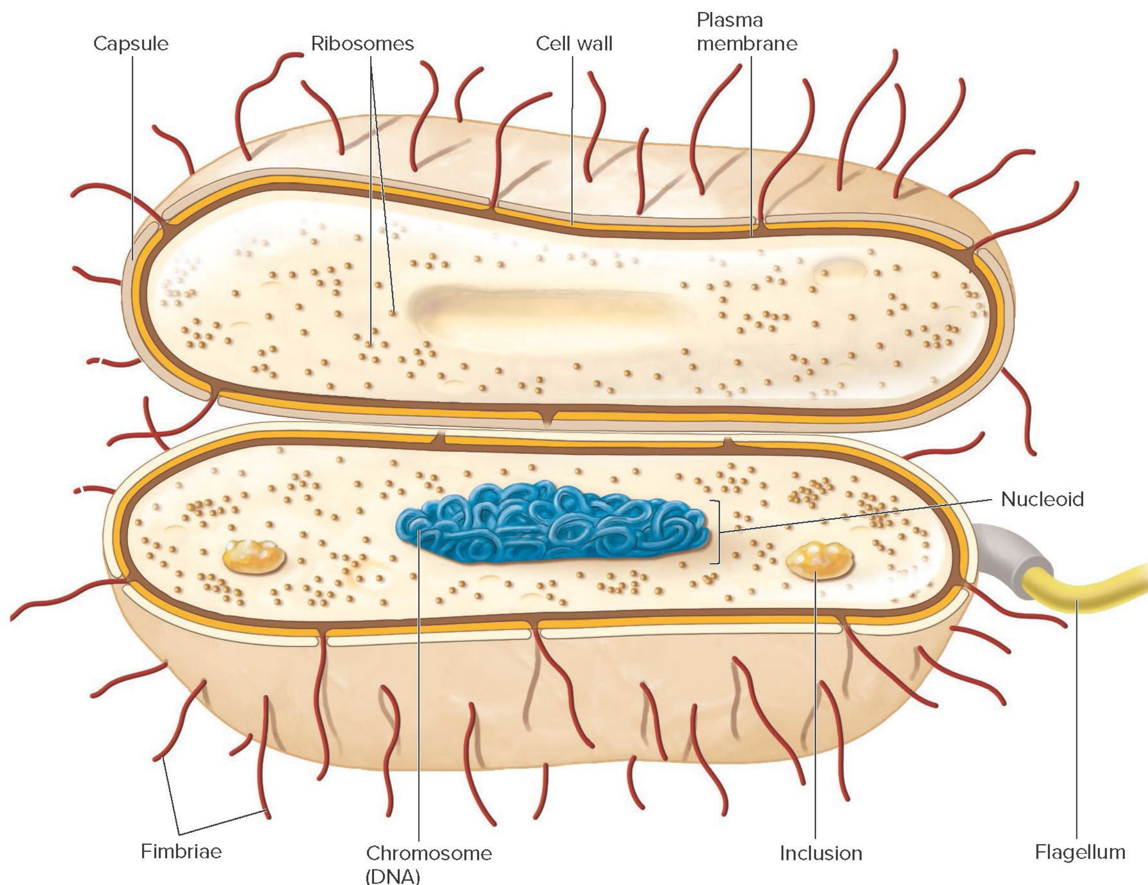


Figure 3.6 Structure of a Bacterial Cell.

3.3 Bacterial Plasma Membranes Control What Enters and Leaves the Cell

After reading this section, you should be able to:

a. Describe the fluid mosaic model of membrane structure and identify the types of lipids typically found in bacterial membranes
b. Distinguish macroelements (macronutrients) from trace elements (micronutrients) and provide examples of each
c. Provide examples of growth factors needed by some microorganisms
d. Compare and contrast passive diffusion, facilitated diffusion, active transport, and group translocation, and provide examples of each
e. Discuss the challenge of iron uptake and describe how bacteria overcome this difficulty

The **cell envelope** is defined as the plasma membrane and all the surrounding layers external to it. The cell envelopes of many bacteria consist of the plasma membrane, cell wall, and at least one additional layer (e.g., capsule or slime layer). Of all these layers, the **plasma membrane** is the most important because it encompasses the cytoplasm and defines the cell. If it is removed or compromised, the cell's contents spill into the environment and the cell dies. Furthermore, despite being the innermost layer of the cell envelope, the plasma membrane is responsible for much of the cell's relationship with the outside world. Thus we begin our consideration of bacterial cell structure by describing the plasma membrane.

First, let's consider what cells do to survive. Cells must interact in a selective fashion with their environment, acquire nutrients, and eliminate waste. They also have to maintain their interior in a constant, highly organized state in the face of external changes. Plasma membranes are an absolute requirement for all living organisms because they are involved in carrying out these cellular tasks.

A primary role of all plasma membranes is that they are selectively permeable barriers: They allow particular ions and molecules to pass either into or out of the cell, while preventing the movement of others. Thus the plasma membrane prevents the loss of essential components through leakage while allowing the movement of other molecules. Bacterial plasma membranes play additional critical roles. They are the location of several crucial metabolic processes: respiration, photosynthesis, and the synthesis of lipids and cell wall constituents.

In addition to the plasma membrane, some bacteria have extensive intracytoplasmic membrane systems. These internal membranes and the plasma membrane share a basic design. However, they can differ significantly in the lipids and proteins they contain. To understand these chemical differences and the many functions of the plasma membrane and other membranes, it is necessary to become familiar with membrane structure.

Fluid Mosaic Model of Membrane Structure

The most widely accepted model for membrane structure is the **fluid mosaic model** of Singer and Nicholson, which proposes that membranes are lipid bilayers within which proteins float (**figure 3.7**). Bacterial membranes have roughly equal amounts of lipids and proteins. Cell membranes are very thin structures, about 2 to 3 nm thick, that look like two dark lines on either side of a light interior when imaged by TEM. This characteristic appearance is evidence that the membrane is composed of two sheets of lipid molecules arranged end-to-end (figure 3.7). Cleavage of membranes by freeze-etching, a technique that reveals fine detail, exposes the proteins lying within the membrane lipid bilayer. ◀ *Electron microscopes use beams of electrons to create highly magnified images (section 2.4); Scanning probe microscopy can visualize molecules and atoms (section 2.5)*

The chemical nature of membrane lipids is critical to their ability to form bilayers. Most membrane-associated lipids (e.g., the phospholipids shown in figure 3.7) are **amphipathic:** They are structurally asymmetric, with polar and nonpolar ends

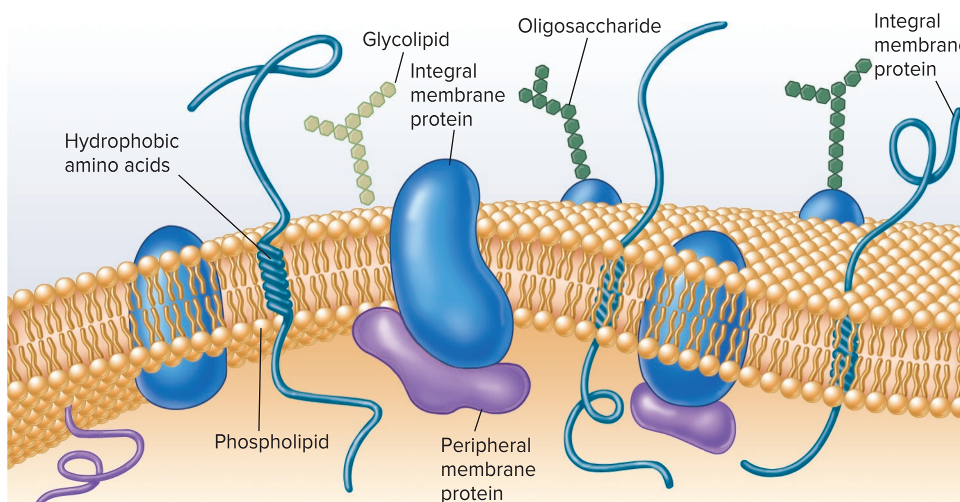


Figure 3.7 The Fluid Mosaic Model of Bacterial Membrane Structure. This diagram shows the integral membrane proteins (blue) floating in a lipid bilayer. Peripheral membrane proteins (purple) are associated loosely with the inner membrane surface and/or integral membrane proteins. Small tan spheres represent the hydrophilic ends of membrane phospholipids, and wiggly tails are the hydrophobic fatty acid chains. Phospholipids are drawn much larger than their actual size. Oligosaccharides (chains of carbohydrates) protrude into the environment and may be attached to proteins or to membrane phospholipids (glycolipids).

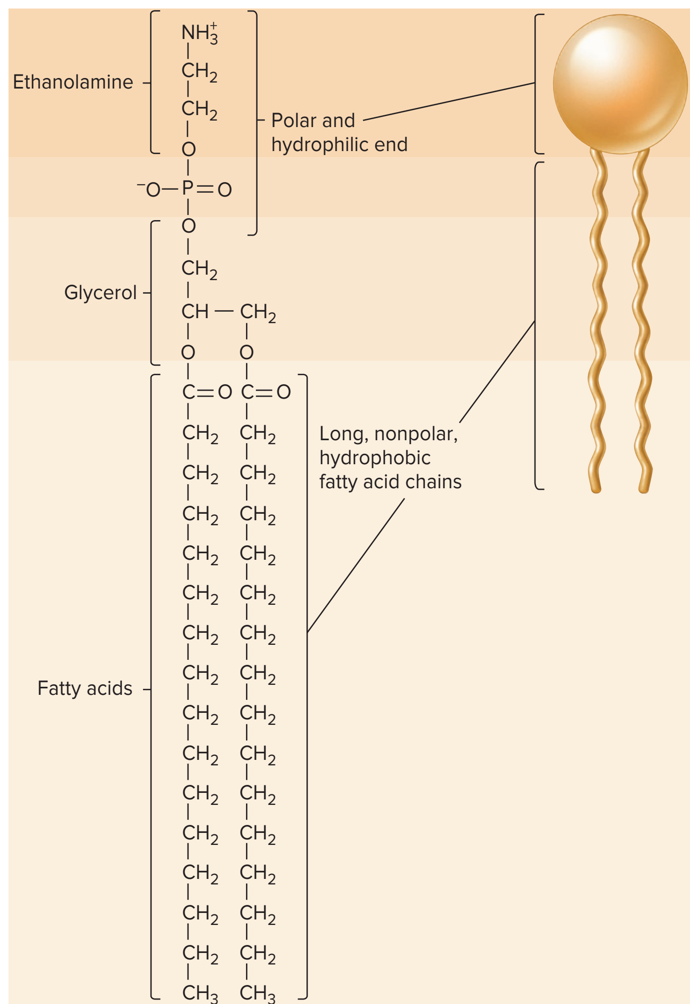


Figure 3.8 **The Structure of a Phospholipid.** Phosphatidylethanolamine, a phospholipid often found in bacterial membranes.

(**figure 3.8**). The polar ends interact with water and are **hydrophilic;** the nonpolar **hydrophobic** ends are insoluble in water and tend to associate with one another. In aqueous environments, amphipathic lipids can interact to form a bilayer. The outer surfaces of the bilayer are hydrophilic, whereas hydrophobic ends are buried in the interior away from the surrounding water (figure 3.7). ▶| *Lipids (appendix I)*

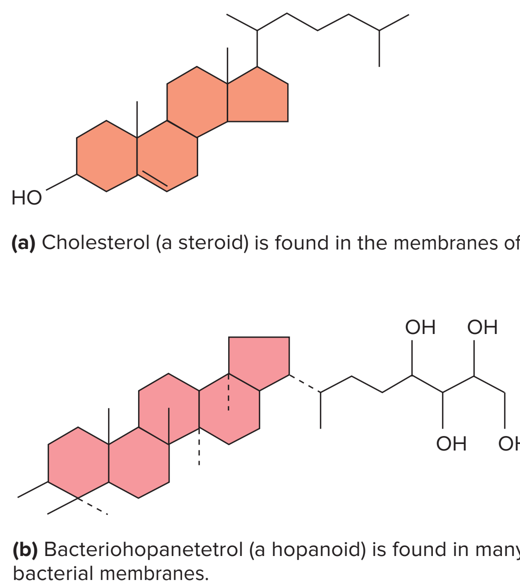
Two types of membrane proteins have been identified based on their ability to be separated from the membrane. **Peripheral membrane proteins** are loosely connected to the membrane and can be easily removed (figure 3.7). They are soluble in aqueous solutions and make up about 20 to 30% of total membrane protein. The remaining proteins are **integral membrane proteins.** These are not easily extracted from membranes and are insoluble in aqueous solutions when freed of lipids. Integral membrane proteins, like membrane lipids, are amphipathic; their hydrophobic regions are buried in membrane lipids while the hydrophilic portions project from the membrane surface (figure 3.7).

Integral membrane proteins carry out some of the most important functions of the membrane. Many are transport proteins used to move materials either into or out of the cell. Others are involved in energy-conserving processes, such as the proteins found in electron transport chains. Those integral membrane proteins with regions exposed to the outside of the cell enable the cell to interact with its environment. ▶| *Proteins (appendix I)*

Bacterial Plasma Membranes Are Dynamic

Bacterial membranes are lipid bilayers and many of their amphipathic lipids are phospholipids (figure 3.8). The plasma membrane is dynamic: The lipid composition varies with environmental temperature in such a way that the membrane remains fluid during growth. For example, bacteria growing at lower temperatures have more unsaturated fatty acids in their membrane phospholipids; that is, there are one or more double covalent bonds in the long hydrocarbon chains. At higher temperatures, their phospholipids have more saturated fatty acids—those in which the carbon atoms are connected only with single covalent bonds. ▶| *Environmental factors affect microbial growth (section 7.5)*

Although most aspects of the fluid mosaic model are well supported by experimentation, the suggestion that membrane lipids and integral proteins are homogeneously distributed has been challenged by the discovery of **microdomains.** These regions of the membrane are formed by phospholipids in conjunction with other lipids like farnesol, hopanoids, and carotenoids (*see figure 11.31*). **Hopanoids** are similar in structure to cholesterol found in eukaryotic membranes (**figure 3.9**), and their rigid planar structure makes them more hydrophobic than phospholipids. The specific lipids found in microdomains vary among organisms, but they all regulate membrane rigidity and mark microdomain boundaries.



(a) Cholesterol (a steroid) is found in the membranes of eukaryotes.

(b) Bacteriohopanetetrol (a hopanoid) is found in many bacterial membranes.

Figure 3.9 **Membrane Steroids and Hopanoids.**

The integral membrane proteins in microdomains differ from those in the bulk membrane, and are organized by proteins called **flotillins.** Flotillins are themselves integral membrane proteins, and they function to assemble large protein complexes like secretion systems for transporting molecules out of the cell and complexes that transmit signals from the environment to molecules in the cytoplasm.

Bacteria Use Many Mechanisms to Bring Nutrients into the Cell

All plasma membranes function as barriers. Yet they must also allow movement of nutrients into the cell. If a microbe does not obtain nutrients from its environment, it will quickly exhaust its supply of amino acids, nucleotides, and other molecules needed to survive. In addition, if a microbe is to thrive and reproduce, it must have a source of energy. The energy source is used to generate the cell's major energy currency: the high-energy molecule ATP. Clearly, obtaining energy and nutrient sources is one of the most important jobs an organism has, and it is primarily a function of the bacterial plasma membrane. Here we discuss nutrient uptake, but first let's define some terms used to describe the nutrients needed by cells.

Microbiologists refer to carbon, oxygen, hydrogen, nitrogen, sulfur, and phosphorus as **macroelements** or macronutrients because they are required in relatively large amounts. They are found in organic molecules such as proteins, lipids, nucleic acids, and carbohydrates. Other macroelements are potassium, calcium, magnesium, and iron. They exist as cations and generally are associated with and contribute to the activity and stability of molecules and cell structures such as enzymes and ribosomes. Thus they are important in many cellular processes, including protein synthesis and energy conservation. ▶| *Enzymes and ribozymes speed up cellular chemical reactions (section 10.6); Translation in bacteria (section 13.7); Electron transport chains: sets of sequential redox reactions (section 10.4)*

Other elements are required in small amounts—amounts so small that in the lab they are often obtained as contaminants in water, glassware, and growth media. Likewise in nature, they are ubiquitous and usually present in adequate amounts to support the growth of microbes. Microbiologists call these elements **micronutrients** or **trace elements.** The micronutrients—manganese, zinc, cobalt, molybdenum, nickel, and copper—are needed by most cells. Micronutrients are part of certain enzymes, and they aid in catalysis of reactions and maintenance of protein structure.

Some microbes are able to synthesize all the organic molecules they need from macroelements. However, some microbes are unable to synthesize certain molecules needed for survival. These molecules are called **growth factors,** and they must be obtained from the environment. There are three types of growth factors: amino acids, purines and pyrimidines, and vitamins.

What are the common features of nutrient uptake by bacteria? Bacteria can only take in dissolved molecules. Uptake mechanisms are specific; that is, the necessary substances, and not others, are acquired. It does a cell no good to take in a substance that it cannot use. Bacteria are able to transport nutrients into the cell even when the concentration of a nutrient inside the cell is higher than the concentration outside. Thus they are able to move nutrients up a concentration gradient. This is important because bacteria often live in nutrient-poor habitats. In view of the enormous variety of nutrients and the complexity of the task, it is not surprising that bacteria use several different transport mechanisms: passive diffusion, facilitated diffusion, primary and secondary active transport, and group translocation.

Passive Diffusion

Passive diffusion, often called diffusion or simple diffusion, is the process by which molecules move from a region of higher concentration to one of lower concentration; that is, the molecules move down the concentration gradient. The rate of passive diffusion depends on the size of the concentration gradient between a cell's exterior and its interior (**figure 3.10**). A large concentration gradient is required for adequate nutrient uptake by passive diffusion (i.e., the external nutrient concentration must be high while the internal concentration is low). The rate of diffusion decreases as more nutrient accumulates in the cell. This occurs if the nutrient is not used immediately upon entry.

Most substances cannot freely diffuse into a cell. However, water and some gases, including O_2 and CO_2, easily cross the

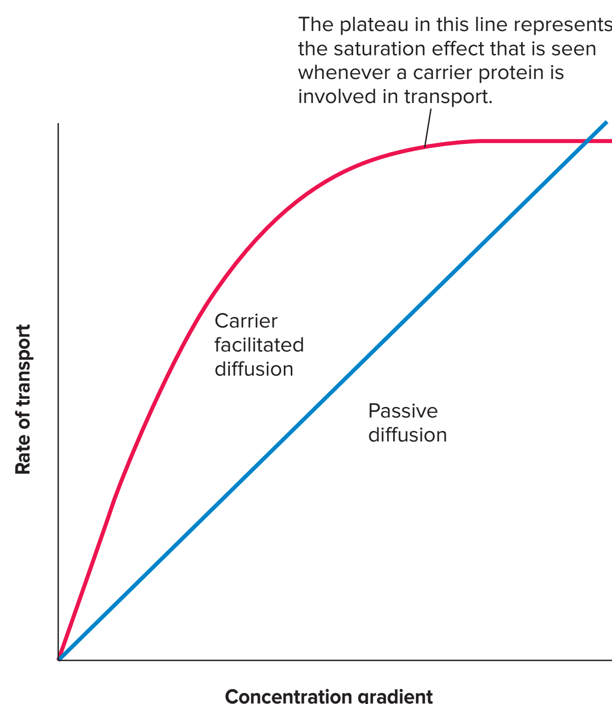


Figure 3.10 Passive and Facilitated Diffusion. The rate of diffusion depends on the size of the solute's concentration gradient (the ratio of the extracellular concentration to the intracellular concentration). This example of facilitated diffusion involves a carrier protein that can be saturated. Sometimes facilitated diffusion is mediated by a channel. Channels often do not exhibit a saturation effect.

plasma membrane by passive diffusion. H_2O also moves across membranes by passive diffusion. Larger molecules, ions, and polar substances must enter the cell by other mechanisms, all of which involve specialized proteins that are referred to as transport proteins. *How Diffusion Works*

Facilitated Diffusion

During **facilitated diffusion,** substances move across the plasma membrane with the assistance of transport proteins that are either channels or carriers. Channels, as their name indicates, are proteins that form pores in membranes through which substances can pass; they are often involved in facilitated diffusion. Channels show some specificity for the substances that pass through them, but this is considerably less than that shown by carriers, which are far more substrate specific. The rate of facilitated diffusion increases with the concentration gradient much more rapidly and at lower concentrations of the diffusing molecule than that of passive diffusion (figure 3.10). When the transporter is a carrier, the diffusion rate reaches a plateau above a specific gradient value because the carrier protein is saturated; that is, it is transporting as many solute molecules as possible. The resulting curve resembles an enzyme-substrate curve (*see figure 10.16)* and is different from the linear response seen with passive diffusion. An example of channel-mediated facilitated diffusion is that involving aquaporins (*see figure 2.31*), which transport water. Aquaporins are members of the major intrinsic protein (MIP) family of proteins. MIPs facilitate diffusion of small polar molecules, and they are observed in all organisms.

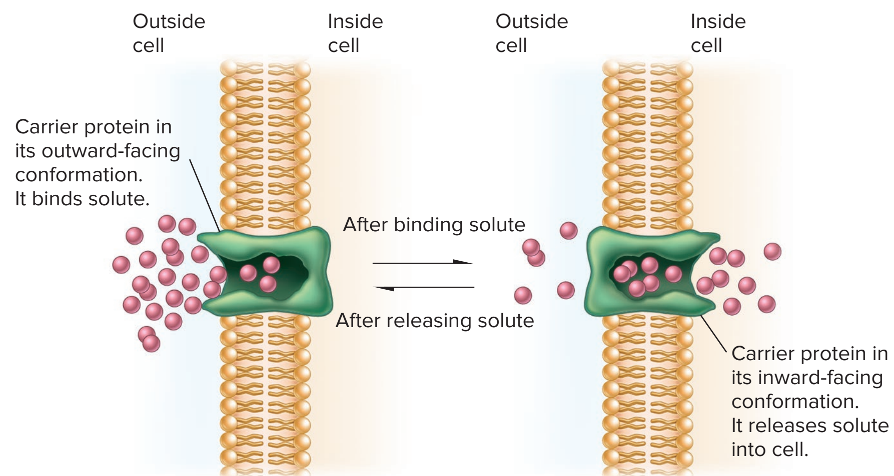


Figure 3.11 A Model of Facilitated Diffusion. Because there is no energy input, molecules continue to enter only as long as their concentration is greater on the outside.

Although facilitated diffusion relies on transport proteins, it is truly diffusion because a concentration gradient spanning the membrane drives the movement of molecules, and no energy is used. If the concentration gradient disappears, net inward movement ceases. The gradient can be maintained by converting the transported nutrient to another compound, as occurs when a nutrient is metabolized.

Considerable work has been done on the mechanism of carrier-mediated facilitated diffusion. When the solute molecule binds to the outside of the carrier, it changes conformation and releases the molecule on the cell interior (**figure 3.11**). The carrier subsequently changes back to its original shape and is ready to pick up another molecule. The net effect is that a hydrophilic molecule can enter the cell in response to its concentration gradient.

Facilitated diffusion has been documented in some bacteria but it does not seem to be the major uptake mechanism for these microbes. Recall that many bacteria live in environments where nutrient concentrations are low, and facilitated diffusion cannot concentrate nutrients inside cells. Therefore energy-dependent transport mechanisms capable of concentrating nutrients are significantly more important uptake mechanisms for bacterial cells.

Primary and Secondary Active Transport

Active transport is the transport of solute molecules to higher concentrations (i.e., against a concentration gradient) with the input of metabolic energy. Three types of active transport are observed in bacteria: primary active transport, secondary active transport, and group translocation. They differ in terms of the energy used to drive transport and whether or not the transported molecule is modified as it enters.

Active transport resembles facilitated diffusion in that it involves carrier proteins. Recall that carrier proteins bind particular solutes with great specificity. Active transport is also characterized by the carrier saturation effect at high solute concentrations (figure 3.10). Nevertheless, active transport differs from facilitated diffusion because it uses metabolic energy and can concentrate substances within the cell.

Primary active transport is mediated by carriers called primary active transporters. They use energy provided by ATP hydrolysis to move substances against a concentration gradient without modifying them. Primary active transporters are **uniporters;** that is, they move a single molecule across the membrane (**figure 3.12**). ***A*****TP-*****b*****inding *****c*****assette transporters** (**ABC transporters**) are important primary active transporters. Our focus here is on those ABC transporters that are used for import of substances. Other ABC transporters are used for export of substances, in particular proteins; these exporters are described in chapter 13. *Protein maturation and secretion (section 13.8)*

Most ABC transporters consist of two hydrophobic membrane-spanning regions (domains) with two ATP-binding

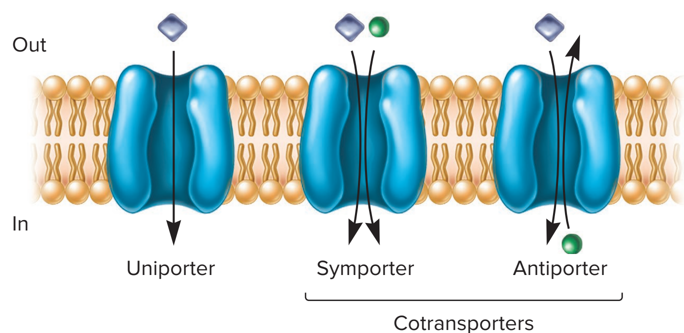


Figure 3.12 Carrier Proteins Can Be Uniporters or Cotransporters. Uniporters move a single substance into the cell. Cotransporters simultaneously move two substances across the membrane. When both substances move in the same direction, the carrier is a symporter. When the two substances move in opposite directions, the carrier is an antiporter.

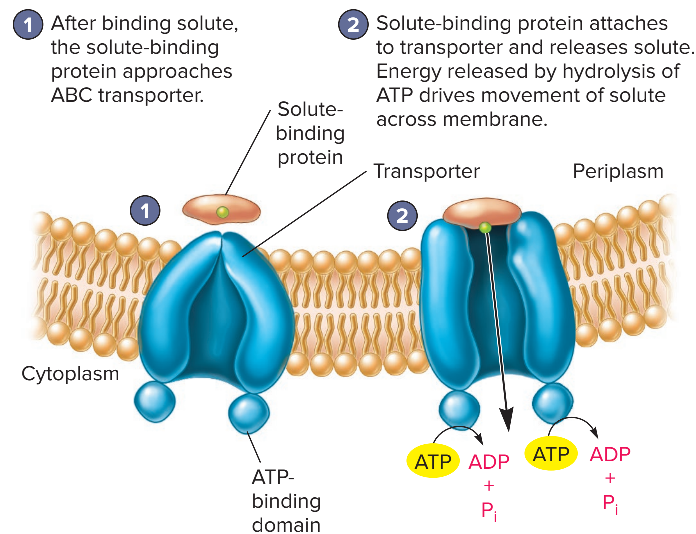


Figure 3.13 ABC Transporter Function. Shown here is a transporter that works with a solute-binding protein free in the periplasm. Other solute-binding proteins are associated with the plasma membrane, always associated with the transporter, or even fused to the transporter.

domains facing the cytoplasm (**figure 3.13**). The membrane-spanning domains form a pore in the membrane, and the ATP-binding domains bind and hydrolyze ATP to drive uptake. Most ABC transporters employ solute-binding proteins to deliver the molecule to be transported to the transporter.

Secondary active transport couples the potential energy of ion gradients to transport of substances without modifying them. Secondary active transporters are cotransporters (figure 3.12). They move two substances simultaneously: the ion whose gradient powers transport and the substance being moved across the membrane. When the ion and other substance both move in the same direction, it is called **symport.** When they move in opposite directions, it is called **antiport.** *Cotransport (Symport and Antiport)*

The ion gradients used by secondary active transporters arise primarily in three ways. The first results from bacterial metabolic activity. During energy-conserving processes, electron transport generates a proton gradient in which protons are at a higher concentration outside the cell than inside. The proton gradient is used to do cellular work, including secondary active transport. Some bacteria use the second method, in which an enzyme called a V-type ATPase hydrolyzes ATP and uses the energy released to create either a proton gradient or a sodium gradient across the plasma membrane. Finally, a proton gradient can be used to create another ion gradient such as a sodium gradient. This is accomplished by an antiporter that brings protons in as sodium ions are moved out of the cell. The sodium gradient can then be used to drive uptake of nutrients by a symport mechanism. ▶| *Electron transport and oxidative phosphorylation (step 3) generate the most ATP (section 11.6)*

The lactose permease of *E. coli* is a well-studied symport secondary active transporter. It is a single protein that transports a lactose molecule inward as a proton simultaneously enters the cell. The proton is moving down a proton gradient, and the energy released drives solute transport. X-ray diffraction studies show that the carrier protein exists in outward- and inward-facing conformations. When lactose and a proton bind to separate sites on the outward-facing conformation, the protein changes to its inward-facing conformation, and the sugar and proton are released into the cytoplasm.

Bacteria often have more than one transport system for a nutrient, as can be seen with *E. coli.* This bacterium has at least five transport systems for the sugar galactose, three systems each for the amino acids glutamate and leucine, and two potassium transport complexes. When several transport systems exist for the same substance, the systems differ in such properties as their energy source, their affinity for the solute transported, and the nature of their regulation. This diversity gives the bacterium an added competitive advantage in a variable environment.

Group Translocation

The distinguishing characteristic of **group translocation** is that a molecule is chemically modified as it is brought into the cell. The best-known group translocation system is the **phosphoenolpyruvate: sugar phosphotransferase system** (**PTS**), which is observed in many bacteria. The PTS transports a variety of sugars while phosphorylating them, using phosphoenolpyruvate (PEP) as the phosphate donor. PEP is a high-energy molecule that can be used to synthesize ATP, the cell's energy currency. However, when it is used in PTS reactions, the energy present in PEP is used to energize sugar uptake rather than ATP synthesis. ▶| *ATP: The major energy currency of cells (section 10.2)*

The transfer of phosphate from PEP to the incoming molecule involves several proteins and is an example of a **phosphorelay system.** In *E. coli* and *Salmonella,* the PTS consists of two enzymes and a low molecular weight heat-stable protein (HPr). A phosphate is transferred from PEP to enzyme II with the aid of

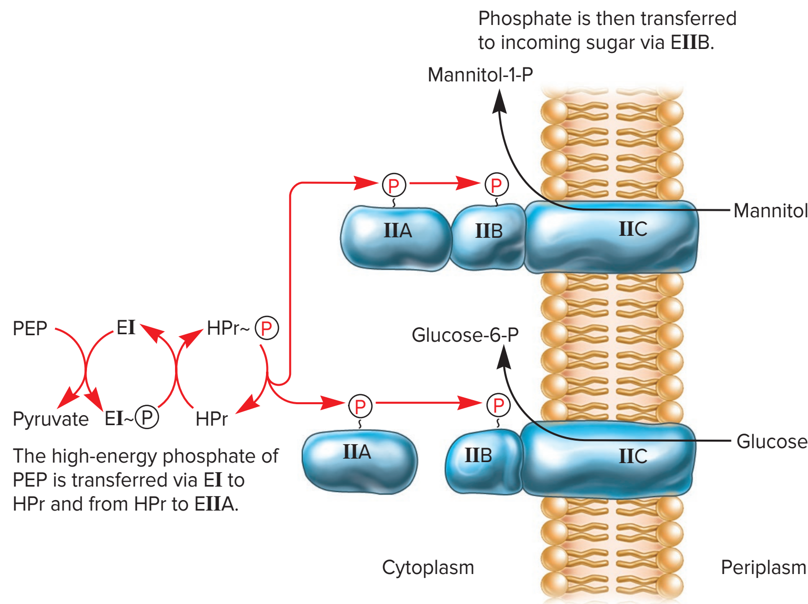


Figure 3.14 Group Translocation: Bacterial PTS Transport. Two examples of the phosphoenolpyruvate: sugar phosphotransferase system (PTS) are illustrated. The following components are involved in the system: phosphoenolpyruvate (PEP), enzyme I (EI), the low molecular weight heat-stable protein (HPr), and enzyme II (EII). EIIA is attached to EIIB in the mannitol transport system and is separate from EIIB in the glucose system.

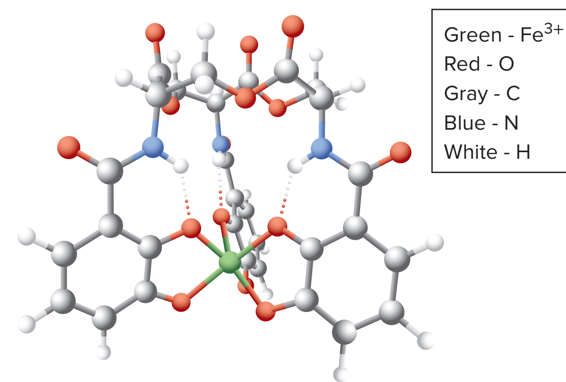


Figure 3.15 Enterobactin: A Siderophore Produced by *E. coli*. Ball-and-stick model of enterobactin complexed with Fe^{3+}.

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

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

Iron Uptake

Almost all microorganisms require iron for building molecules important in energy-conserving processes (e.g., cytochromes), as well as for the function of many enzymes. Iron uptake is made difficult by the extreme insolubility of ferric iron (Fe^{3+}) and its derivatives, which leaves little free iron available for transport. Many bacteria overcome this difficulty by secreting **siderophores** (Greek for iron bearers). Siderophores are low molecular weight organic molecules that bind ferric iron and supply it to the cell (**figure 3.15**). ▶| *Electron transport chains: sets of sequential redox reactions (section 10.4)*

Microorganisms secrete siderophores when iron is scarce in the medium. Once the iron-siderophore complex has reached the cell surface, it binds to a siderophore-receptor protein. Then either the iron is released to enter the cell directly or the whole iron-siderophore complex is transported inside by an ABC transporter. Iron is so crucial to microorganisms that more than one route of iron uptake may be used to ensure an adequate supply.

Comprehension Check

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

3.4 There Are Two Main Types of Bacterial Cell Walls

After reading this section, you should be able to:

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

The **cell wall** is the layer that lies just outside the plasma membrane. It is one of the most important structures for several reasons:

It helps maintain cell shape and protect the cell from osmotic lysis; it can protect the cell from toxic substances; and in pathogens, it can contribute to pathogenicity. Cell walls are so important that most bacteria have them. Those that lack them have other features that fulfill cell wall function. Bacterial cell wall synthesis is an important target for many antibiotics. ▶| *Antibacterial drugs (section 9.4)*

Overview of Bacterial Cell Wall Structure

After Christian Gram developed the Gram stain in 1884, it soon became evident that most bacteria could be divided into two major groups based on their response to the Gram-staining procedure. Gram-positive bacteria stained purple, whereas Gram-negative bacteria were pink or red. The true structural difference between these two groups did not become clear until the advent of the transmission electron microscope. Here we describe the long-held models of Gram-positive and Gram-negative cell walls developed from these studies. More recent studies of diverse groups of bacteria have shown that these models do not hold true for all bacteria (**Microbial Diversity & Ecology 3.1**). Because of ongoing discussions related to these new studies, we will refer to bacteria that fit the models as being typical Gram-positive or typical Gram-negative bacteria. |◀ *Differential staining (section 2.3)*

The cell walls of *Bacillus subtilis* and many other typical Gram-positive bacteria consist of a single, 20- to 80-nm-thick homogeneous layer of **peptidoglycan (murein)** lying outside the plasma membrane (**figure 3.16**). In contrast, the cell walls of *E. coli* and many other typical Gram-negative bacteria have two distinct layers: a 2- to 7-nm-thick peptidoglycan layer covered by a 7- to 8-nm-thick **outer membrane.**

One important feature seen in typical Gram-negative bacteria is a space between the plasma membrane and the outer membrane. It also is sometimes observed between the plasma membrane and cell wall in typical Gram-positive bacteria. This space is called the **periplasmic space.** The substance that occupies the periplasmic space is the **periplasm.**

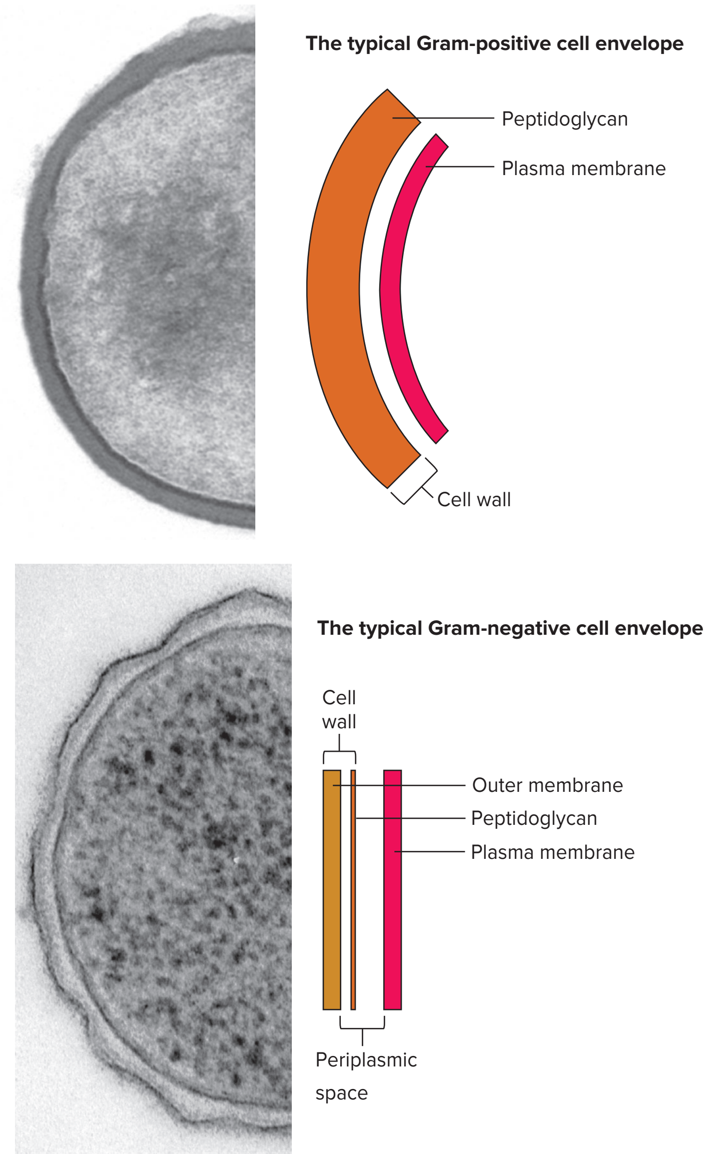


Figure 3.16 Cell Envelopes of Typical Gram-Positive and Gram-Negative Bacteria. Cell envelopes consist of the plasma membrane and any layers (e.g., cell wall) exterior to it. For simplicity, we show only the plasma membrane and cell wall. *Staphylococcus aureus* (top) has a typical Gram-positive cell wall that consists primarily of peptidoglycan. *Myxococcus xanthus* (bottom) has a typical Gram-negative cell wall consisting of a thin layer of peptidoglycan, an outer membrane, and the periplasmic space.

Peptidoglycan Structure

The feature common to nearly all bacterial cell walls is the presence of peptidoglycan, which forms an enormous meshlike structure often referred to as the peptidoglycan sacculus. Peptidoglycan is composed of many identical subunits. Each subunit within the sacculus contains two sugar derivatives, *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM), and several different amino acids. The amino acids form a short peptide, sometimes called the stem peptide, consisting of four alternating D- and L-amino acids; the peptide is connected to the carboxyl group of NAM (**figure 3.17**). Three of the amino acids are not found in proteins: D-glutamic acid, D-alanine, and *meso*-diaminopimelic acid. The presence of D-amino acids in the stem peptide protects against degradation by most peptidases, which recognize only the L-isomers of amino acid residues. The peptidoglycan subunit of many bacteria is shown in figure 3.17. ▶| *Carbohydrates (appendix I); Proteins (appendix I); Proteins are polymers of amino acids (section 13.2)*

The peptidoglycan sacculus is formed by linking the sugars of the peptidoglycan subunits together to form a strand; the strands are then cross-linked to each other by covalent bonds formed between the stem peptides extending from each strand. As seen in **figure 3.18**, the backbone of each strand is composed of alternating NAG and NAM residues. The strand is helical, and the stem peptides extend out from the backbone in different directions. There are two types of cross-links: direct and indirect

MICROBIAL DIVERSITY & ECOLOGY

3.1 Gram Positive and Gram Negative or Monoderms and Diderms?

The importance of the Gram stain in the history of microbiology cannot be overstated. The Gram stain reaction was for many years one of the critical pieces of information used by bacterial taxonomists to construct taxa, and it is still useful in identifying bacteria in clinical settings. The initial studies done to differentiate bacteria that stained Gram positive from those that stain Gram negative were done using model organisms such as *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative). At the time, it was thought that all other bacteria would have similar cell wall structures. However, the long-held models of Gram-positive and Gram-negative cell walls do not hold true for all bacteria. Iain Sutcliffe has proposed that microbiologists stop referring to bacteria as either Gram positive or Gram negative. He suggests that instead we should more precisely describe bacterial cell envelope architectures by focusing on the observation that some bacteria have envelopes with a single membrane—the plasma membrane as seen in typical Gram-positive bacteria—while others have envelopes with two membranes—the plasma membrane and an outer membrane as seen in typical Gram-negative bacteria. He proposed calling the former monoderms and the latter diderms.

But why make this change? Sutcliffe begins by pointing out that some bacteria staining Gram positive are actually diderms and some staining Gram negative are actually monoderms. By referring to Gram-positive-staining diderms as Gram-positive bacteria, it is too easy to mislead scientists and many a budding microbiologist into thinking that the bacterium has a typical Gram-positive envelope. He also argues that by relating cell envelope architecture to the phylogenies of various bacterial taxa, we may gain insight into the evolution of these architectures. He notes that the phyla *Firmicutes* and *Actinobacteria* are composed almost completely of monoderm bacteria, whereas almost all other bacterial phyla consist of diderms.

There are interesting exceptions to the relationship of phylogeny and cell envelope structure. For instance, members of the genus *Mycobacterium* (e.g., *M. tuberculosis*) belong to the predominantly monoderm phylum *Actinobacteria*. Mycobacteria have cell walls that consist of peptidoglycan and an outer membrane. The outer membrane is composed of mycolic acids rather than the phospholipids and lipopolysaccharides (LPSs) found in the typical Gram-negative cells' outer membrane. ▶| *Order* Corynebacteriales *includes important human pathogens (section 23.1)*

Members of the genus *Deinococcus* are another interesting exception. These bacteria stain Gram positive but are diderms. Their cell envelopes consist of the plasma membrane, what appears to be a typical Gram-negative cell wall, and an S-layer. Their outer membrane is distinctive because it lacks LPS. Deinococci are not unique in this respect, however. It is now known that members of several taxa have outer membranes that lack LPS.

Source: Sutcliffe, I. C. 2010. A phylum level perspective on bacterial cell envelope architecture. Trends Microbiol. *18(10):464–70.*

via a peptide interbridge. A direct cross-link is characterized by connecting the carboxyl group of an amino acid in one stem peptide to the amino group of an amino acid in another stem peptide. For instance, many bacteria cross-link the strands by connecting the carboxyl group of the D-alanine at position 4 of the stem peptide directly to the amino group of diaminopimelic acid (position 3) of the other peptidoglycan strand's stem peptide (the position 5 D-alanine is removed as the cross-link is formed). Bacteria that have indirect linkage use a **peptide interbridge** (also called an interpeptide bridge), a short chain of amino acids that links the stem peptide of one peptidoglycan strand to that of another (**figure 3.19**). Cross-linking results in one dense, interconnected network of peptidoglycan strands ▶| *Synthesis of peptidoglycan occurs in the cytoplasm, at the plasma membrane, and in the periplasmic space (section 12.4)*

The peptidoglycan sacculus is strong but elastic. It is able to stretch and contract in response to osmotic pressure. This is due to the rigidity of the backbone coupled with the flexibility of the cross-links. Peptidoglycan sacculi are also rather porous, allowing globular proteins having a molecular weight as large as 50,000 to pass through, depending on whether the sacculus is relaxed or stretched; thus only extremely large proteins are unable to pass through peptidoglycan.

Variants of peptidoglycan are found, particularly among typical Gram-positive bacteria. For example, some substitute the diamino acid lysine for *meso*-diaminopimelic acid (**figure 3.20**) and cross-link chains via interpeptide bridges. These interpeptide bridges can vary considerably (**figure 3.21**). Peptidoglycan can also vary in terms of the length of the peptidoglycan strands and the amount of cross-linking. Bacteria that stain Gram positive tend to have much more cross-linking, whereas those that stain Gram negative have considerably less.

Typical Gram-Positive Cell Walls Consist Primarily of Peptidoglycan

Most cultured bacteria that stain Gram positive belong to the phyla *Firmicutes* and *Actinobacteria*, and most of these bacteria have

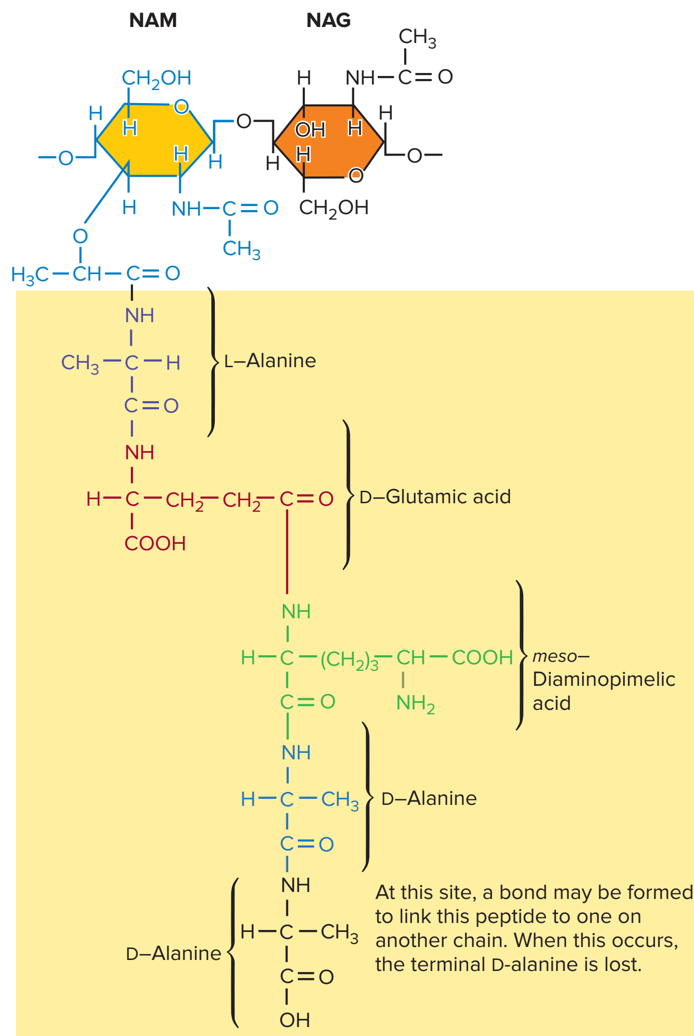


Figure 3.17 Peptidoglycan Subunit Composition. Shown is the peptidoglycan subunit of *E. coli,* many other typical Gram-negative bacteria, and many typical Gram-positive bacteria. This illustration shows the subunit before it has been inserted into the existing peptidoglycan polymer. NAG is *N*-acetylglucosamine. NAM is *N*-acetylmuramic acid. The stem peptide is composed of alternating D- and L-amino acids; it terminates with two D-alanines. The amino acids are shown in different colors for clarity.

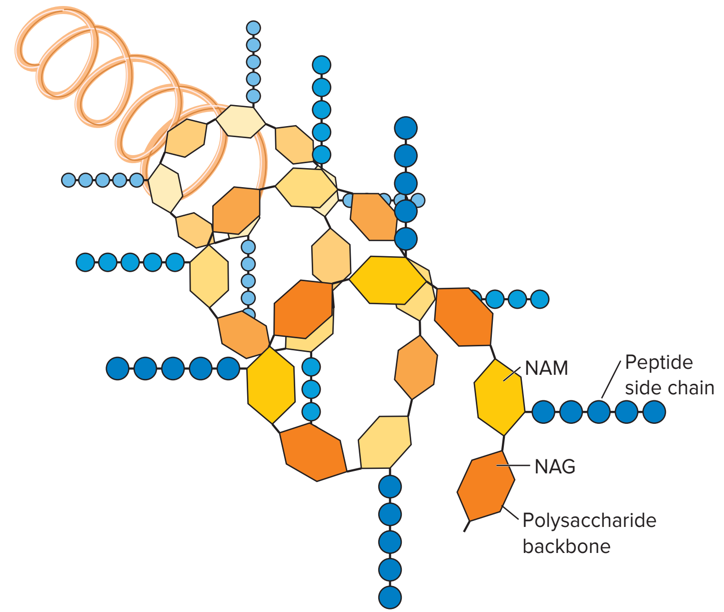


Figure 3.18 A Helical Peptidoglycan Strand. Because of the strand's helical nature, the stem peptides project out in different directions from the NAM-NAG backbone. Here the stem peptides are shown projecting out at 90-degree angles. Some studies suggest that the angle is actually 120 degrees.

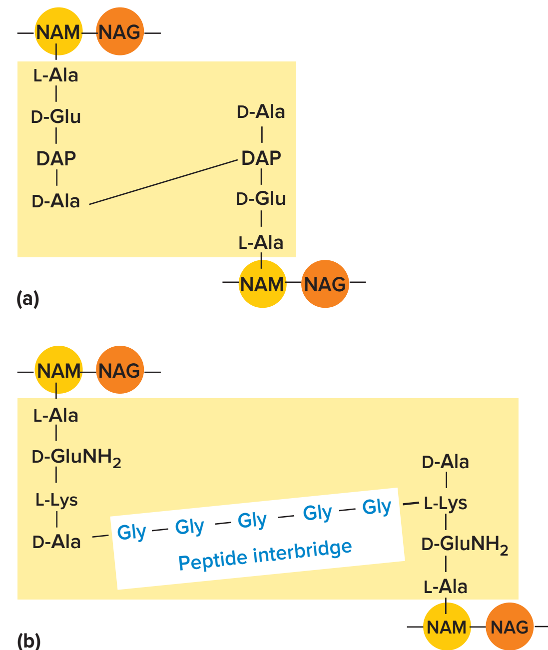


Figure 3.19 Peptidoglycan Cross-Links Can Be Direct or Indirect via a Peptide Interbridge. (a) *E. coli* peptidoglycan with direct cross-linking, typical of many Gram-negative bacteria. (b) *Staphylococcus aureus* peptidoglycan with an interbridge. *S. aureus* stains Gram positive. Gly is glycine. D-GluNH$_2$ is D-glutamic acid with an NH$_2$ group attached to the α carbon (the carbon next to the carboxyl group).

thick cell walls composed of peptidoglycan and large amounts of other polymers such as teichoic acids (**figure 3.22**). **Teichoic acids** are polymers of glycerol or ribitol joined by phosphate groups (**figure 3.23**). Some teichoic acids are covalently linked to peptidoglycan and are referred to as wall teichoic acids. Others are covalently connected to the plasma membrane; they are called lipoteichoic acids. Wall teichoic acids extend beyond the surface of the peptidoglycan. They are negatively charged and help give the cell wall its negative charge. Teichoic acids are not present in other bacteria.

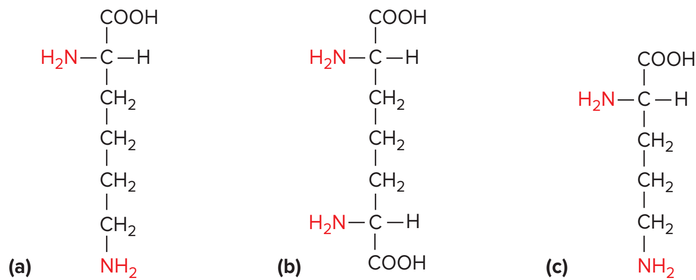


Figure 3.20 Diamino Acids Present in Peptidoglycan. (a) L-lysine, (b) *meso*-diaminopimelic acid, (c) D-ornithine.

Teichoic acids have several important functions. They help create and maintain the structure of the cell envelope by anchoring the wall to the plasma membrane. They are important during cell division, and they protect the cell from harmful substances in the environment (e.g., antibiotics and host defense molecules). In addition, they function in ion uptake and are involved in binding pathogenic species to host tissues, thus initiating the infectious disease process.

The periplasmic space lies between the plasma membrane and the cell wall and is so narrow that it is often not visible by electron microscopy. The periplasm has relatively few proteins; this is probably because the peptidoglycan sacculus is so porous that many proteins translocated across the plasma membrane pass through the sacculus. Some secreted proteins are enzymes called **exoenzymes.** Exoenzymes often serve to degrade polymers such as proteins and polysaccharides that would otherwise be too large for transport across the plasma membrane; the degradation products, the monomer building blocks, are then taken up by the cell. Those proteins that remain in the periplasmic space are usually attached to the plasma membrane.

Not all secreted proteins pass through the peptidoglycan sacculus; some become bound to it instead. These proteins are involved in interactions of the cell with its environment. Some are noncovalently bound to teichoic acids or other cell wall polymers, while others are covalently attached to the peptidoglycan. Membrane-bound enzymes called sortases catalyze the formation of covalent bonds that join these proteins to the peptidoglycan. *Protein maturation and secretion (section 13.8)*

Typical Gram-Negative Cell Walls Include Additional Layers Besides Peptidoglycan

As just noted, most cultured bacteria that stain Gram positive belong to the phyla *Firmicutes* and *Actinobacteria*. With a few exceptions, bacteria belonging to the remaining phyla stain Gram negative (Microbial Diversity & Ecology 3.1). Even a brief inspection of figure 3.16 shows that typical Gram-negative cell walls are more complex than typical Gram-positive walls. One of the most striking differences is the paucity of peptidoglycan. The peptidoglycan layer is very thin (2 to 7 nm, depending on the bacterium) and sits within the periplasmic space.

The periplasmic space is much larger than that of a typical Gram-positive cell, ranging from about 30 to 70 nm wide (**figure 3.24**). Some studies indicate that it may constitute about 20 to 40% of the total cell volume. The periplasmic space is home to a variety of proteins. Some periplasmic proteins participate in nutrient acquisition—for example, hydrolytic enzymes and transport proteins. Some periplasmic proteins are involved in energy conservation. For instance, some bacteria have electron transport proteins in their periplasm (e.g., denitrifying bacteria, which convert nitrate to nitrogen gas). Other periplasmic proteins are involved in peptidoglycan synthesis and modification of toxic compounds that could harm the cell. *Anaerobic respiration uses the same three steps as aerobic respiration (section 11.7); Nitrogen cycle (section 28.1)*

The outer membrane lies outside the thin peptidoglycan layer. It is linked to the cell by Braun's lipoprotein, the most abundant protein in the outer membrane (figure 3.24). This small

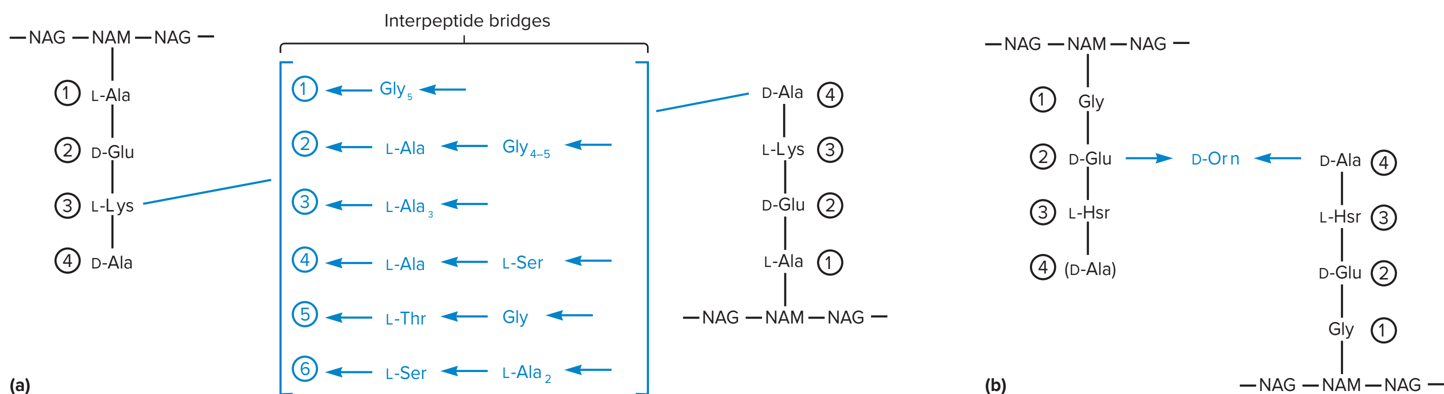


Figure 3.21 Examples of Peptidoglycan Cross-Links. Most variation in peptidoglycan structure occurs in the composition of the stem peptide and the method by which peptidoglycan strands are cross-linked. (a) Examples of interpeptide bridges that link D-alanine in position 4 with L-lysine in position 3. The bracket contains six typical bridges: (1) *Staphylococcus aureus,* (2) *S. epidermidis,* (3) *Micrococcus roseus* and *Streptococcus thermophilus,* (4) *Lactobacillus viridescens,* (5) *Streptococcus salivarius,* and (6) *Leuconostoc cremoris*. The arrows indicate the polarity of peptide bonds running in the C to N direction. (b) An interpeptide bridge observed in *Corynebacterium poinsettiae.* The bridge extends between positions 2 and 4 and consists of the D-diamino acid ornithine (figure 3.20*c*). Note that L-homoserine (L-Hsr) is in position 3 rather than *meso*-diaminopimelic acid or L-lysine.

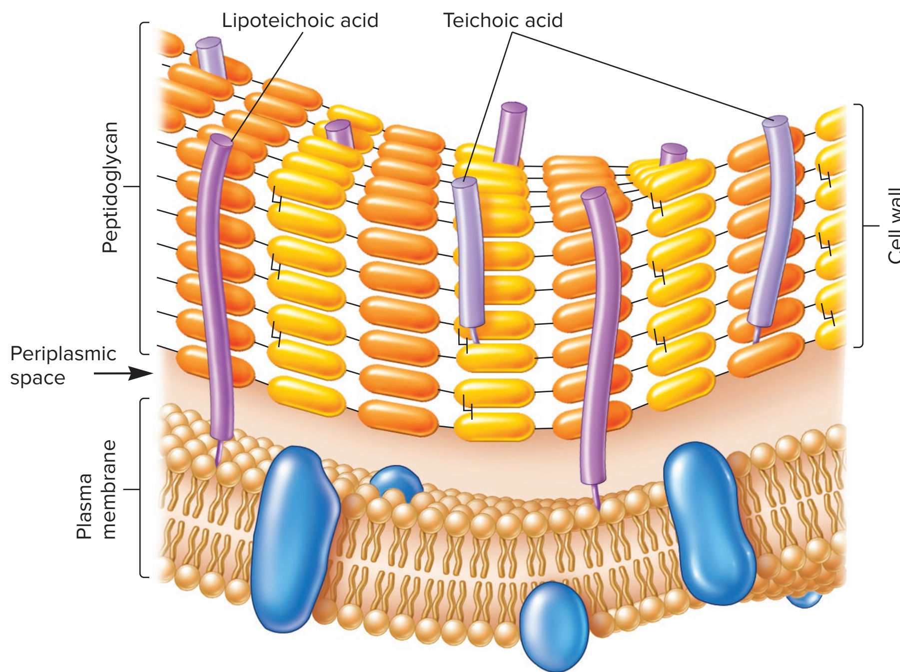


Figure 3.22 Typical Gram-Positive Cell Wall. This component of the cell envelope lies just outside the plasma membrane.

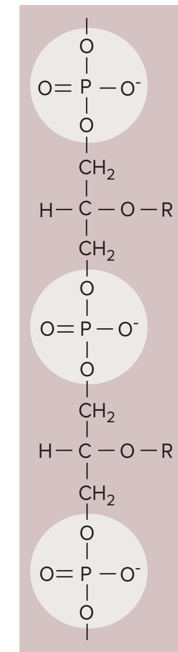


Figure 3.23 Teichoic Acid Structure. The segment of a teichoic acid made of phosphate, glycerol, and a side chain, R. R may represent D-alanine, glucose, or other molecules.

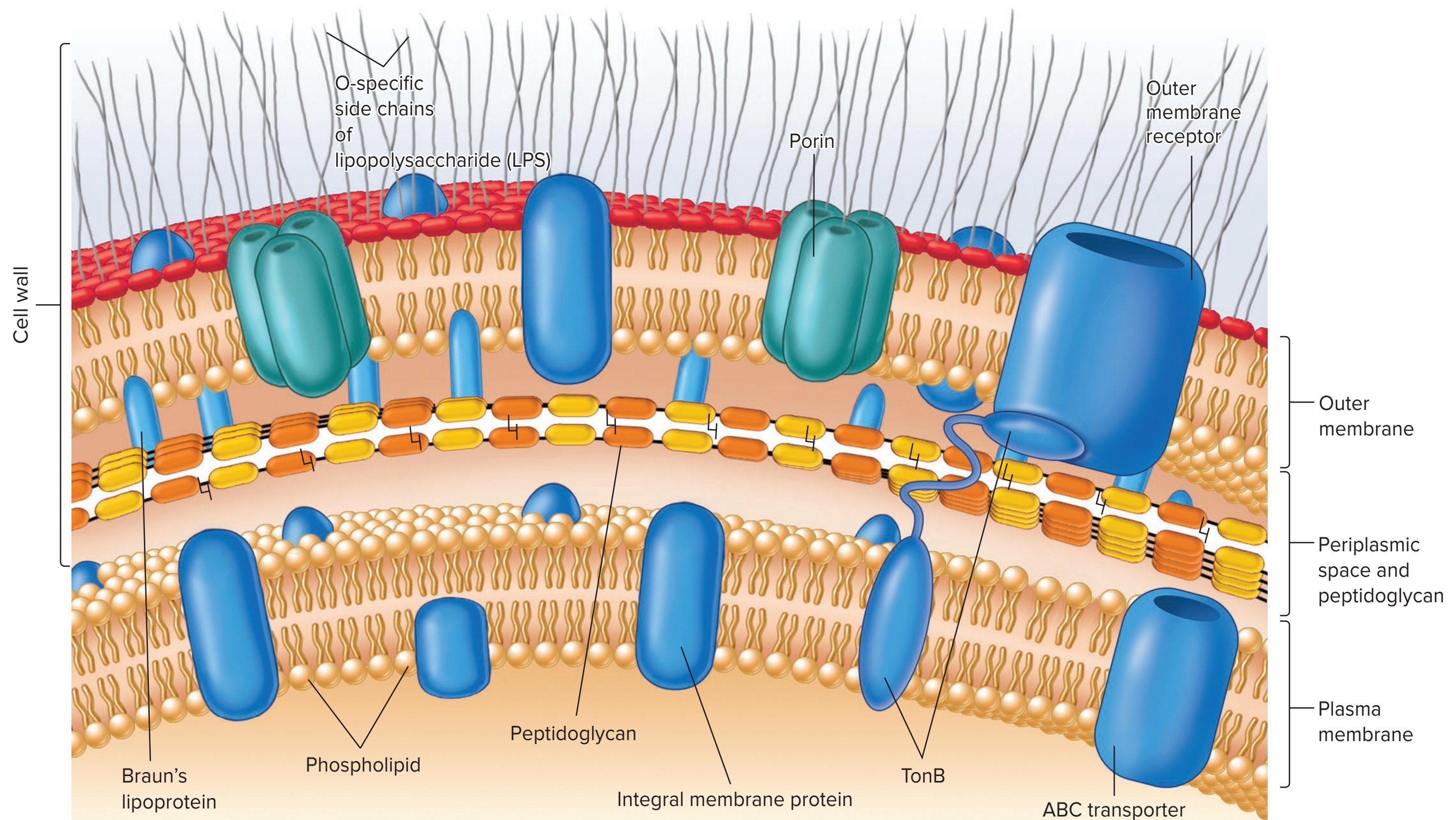


Figure 3.24 Typical Gram-Negative Cell Wall. Notice that these bacteria are bounded by two membranes, the plasma membrane and the outer membrane of the cell wall.

MICRO INQUIRY *How does the outer membrane of the cell wall differ from the plasma membrane?*

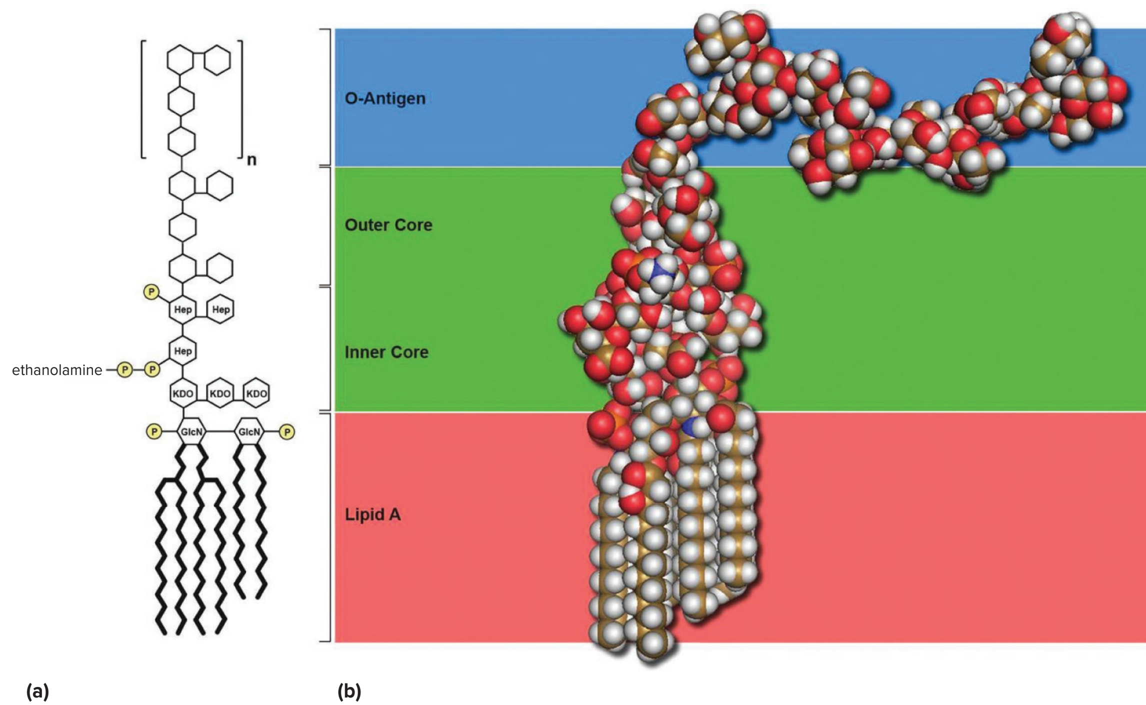


Figure 3.25 Lipopolysaccharide Structure. (a) A simplified diagram of LPS. Abbreviations: GlcN, glucosamine; Hep, heptulose; KDO, 2-keto-3-deoxyoctonate; P, phosphate. Lipid A is buried in the outer membrane. (b) Molecular model of a lipopolysaccharide. The lipid A and core polysaccharide are straight; the O side chain is bent at an angle in this model.

lipoprotein is covalently joined to both the outer membrane and the peptide chain of peptidoglycan.

In contrast to the symmetry of the plasma membrane, the most distinctive structural feature of the outer membrane is the difference between the inner leaflet (facing the periplasm) and the outer leaflet (facing the environment). This external layer of the Gram-negative cell is comprised of **lipopolysaccharides** (**LPSs**). These large, complex molecules contain both lipid and carbohydrate, and consist of three parts: (1) lipid A, (2) the core polysaccharide, and (3) the O side chain. The LPS from *Salmonella* spp. has been studied most, and its general structure is described here (**figure 3.25**). **Lipid A** contains two glucosamine sugar derivatives, each with fatty acids and phosphate attached. The fatty acids of lipid A are embedded in the outer membrane, while the remainder of the LPS molecule projects from the surface. The **core polysaccharide** is joined to lipid A and is constructed of 10 sugars, many of them unusual in structure. The **O side chain** or **O antigen** is a polysaccharide chain extending outward from the core. It has several peculiar sugars and varies in composition between bacterial strains.

LPS has many important functions. (1) It contributes to the negative charge on the bacterial surface because the core polysaccharide usually contains charged sugars and phosphate (figure 3.25). (2) It helps stabilize outer membrane structure because lipid A is a major constituent of the exterior leaflet of the outer membrane. (3) It helps create a permeability barrier. The geometry of LPS (figure 3.25*b*) and interactions between neighboring LPS molecules are thought to restrict the entry of bile salts, antibiotics, detergents, and other toxic substances that might kill or injure the bacterium. (4) LPS helps protect pathogenic bacteria from host defenses. The O side chain of LPS is also called the O antigen because it elicits an immune response by an infected host. This response involves the production of antibodies that bind the strain-specific form of LPS that elicited the response. For example, microbiologists refer to specific strains of Gram-negative bacteria using the O antigen, such as *E. coli* O157; here the O side chain is the antigenic type, number 157. Unfortunately, many bacteria can rapidly change the antigenic nature of their O side chains, thus thwarting host defenses. (5) Importantly, the lipid A portion of LPS can act as a toxin and is called endotoxin. If LPS or lipid A enters the bloodstream, a form of septic shock develops for which there is no direct treatment. ▶| *Endotoxins (section 35.4); Antibodies bind specific 3-D antigens (section 33.7)*

As the outermost layer of the cell envelope, the LPS presents an effective barrier for the cell. Within the LPS, the negatively charged phosphate groups on the core polysaccharide interact with calcium ions to stabilize and tightly pack the LPS molecules. This results in an impermeable barrier that excludes small molecules, including many antibiotics and toxins. Transport across the outer membrane must therefore be regulated by integral membrane proteins, including one class of proteins termed **porins.** Most porin proteins cluster together to form a trimer in the outer membrane (figure 3.24 and **figure 3.26**). Each porin protein spans the outer membrane and is more or less

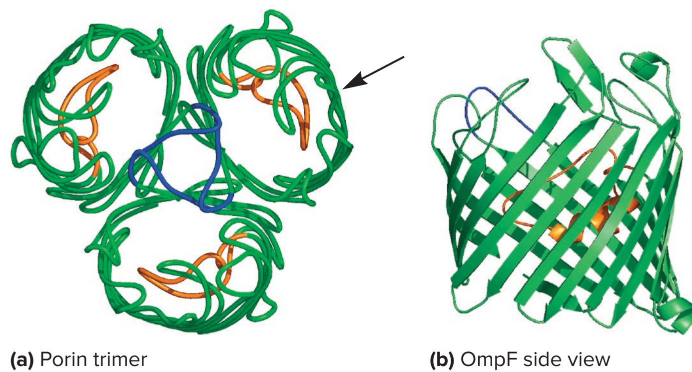


Figure 3.26 Porin Proteins. Two views of the OmpF porin of *E. coli*. (a) Structure of a trimeric porin observed when looking down at the outer surface of the outer membrane (i.e., top view). The center of each porin monomer is a water-filled channel. Each OmpF monomer can be divided into three parts: the green barrel forms the channel, the blue segment interacts with other porin proteins to help form the trimer, and the orange loop narrows the channel and dictates the chemical selectivity of the porin. The arrow indicates the area of a porin molecule viewed from the side in panel (b). Side view of a porin monomer showing the β-barrel structure characteristic of porin proteins.

MICRO INQUIRY *Are these transporter proteins categorized as channels or carriers?*

tube-shaped; its narrow, water-filled channel allows passage of nutrients and hydrophilic molecules smaller than about 600 daltons. Larger molecules such as vitamin B_{12}, carbohydrates, and iron complexes are too large for porins and cross the outer membrane through the action of specific outer membrane receptors (figure 3.24). The receptor-solute complex in the outer membrane interacts with the TonB complex of proteins spanning the periplasm. TonB transfers the energy for the ligand transport from the plasma membrane across the periplasm to the receptor. Upon entering the periplasm, a periplasmic transport protein shuttles the ligand to an ABC-transporter in the plasma membrane for transfer to the cytoplasm.

Mechanism of Gram Staining

The difference between typical Gram-positive bacteria and typical Gram-negative bacteria is due to the physical nature of their cell walls. If the cell wall is removed, typical Gram-positive bacteria stain Gram negative. Furthermore, bacteria that never make cell walls, such as mycoplasmas, also stain Gram negative. ◀ *Differential staining (section 2.3)*

During the Gram-staining procedure, bacteria are first stained with crystal violet, a dye with a positive charge that is attracted to the bacterial cell's net negative charge. They are next treated with iodine, a mordant that interacts with the crystal violet, forming an insoluble complex and thus promoting dye retention. When bacteria are then treated with ethanol in the decolorization step, the pores of the thick peptidoglycan found in the cell walls of typical Gram-positive bacteria appear to shrink, causing the peptidoglycan to act as a permeability barrier that prevents loss of crystal violet. Thus the dye-iodine complex is retained during the decolorization step and the bacteria remain purple, even after the addition of a second dye. In contrast, the peptidoglycan in typical Gram-negative cell walls is very thin, not as highly cross-linked, and has larger pores. Alcohol treatment also may extract enough lipid from the outer membrane to increase the cell wall's porosity further. For these reasons, alcohol more readily removes the crystal violet–iodine complex, decolorizing the cells. The counterstain safranin, also a dye with a net negative charge, easily stains the decolorized cells so that they appear red or pink.

Cell Walls and Osmotic Protection

Microbes have several mechanisms for responding to changes in osmotic pressure. Osmotic stress arises when the concentration of solutes inside the cell differs from that outside, and the responses work to equalize the solute concentrations. However, in certain situations, osmotic pressure can exceed the cell's ability to acclimate. In these cases, additional protection is provided by the cell wall. When cells are in hypotonic solutions—in which the solute concentration is less than that in the cytoplasm—water diffuses into the cell to dilute the solutes, causing it to swell. Without the peptidoglycan layer of the cell wall, the pressure on the plasma membrane would become so great that the membrane would be disrupted and the cell would burst—a process called **lysis.** Conversely, in hypertonic solutions, water flows out and the cytoplasm shrivels up—a process called **plasmolysis.**

The protective nature of peptidoglycan is most clearly demonstrated when bacterial cells are treated with lysozyme or penicillin. The enzyme **lysozyme** attacks peptidoglycan by hydrolyzing the bond that connects *N*-acetylmuramic acid with *N*-acetylglucosamine (figure 3.17; *see also figure 32.4*). Penicillin works by a different mechanism. It inhibits the enzyme transpeptidase, which is responsible for making the cross-links between peptidoglycan chains. If bacteria are treated with either of these substances while in a hypotonic solution, they lyse. However, if they are in an isotonic solution, they can survive and grow normally. Treatment of typical Gram-positive bacteria with lysozyme or penicillin results in the complete loss of the cell wall, and the cell becomes a protoplast. When typical Gram-negative bacteria are exposed to lysozyme or penicillin, the peptidoglycan sacculus is destroyed, but the outer membrane remains. These cells are called **spheroplasts.** Both protoplasts and spheroplasts are osmotically sensitive. If they are transferred to a hypotonic solution, they lyse due to uncontrolled water influx (**figure 3.27**). ▶ *Antibacterial drugs (section 9.4)*

Bacteria That Lack Cell Walls

Most bacteria are defined by their cell wall—they are either Gram positive or Gram negative. A few bacteria, most notably a group called the mycoplasmas, are defined by their lack of a cell wall. Without a cell wall, they are pleomorphic and osmotically sensitive

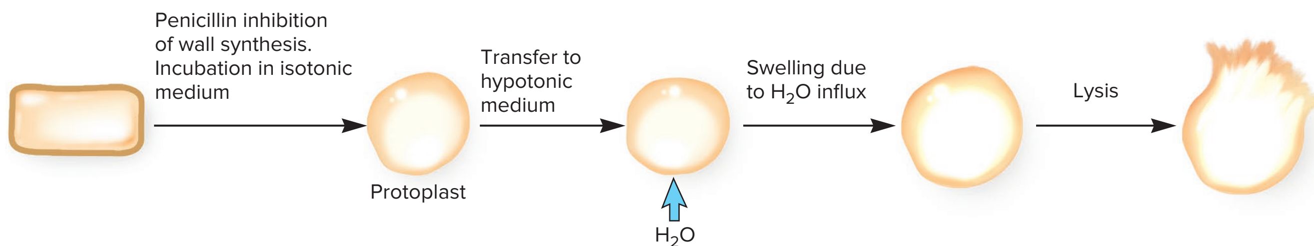


Figure 3.27 Protoplast Formation and Lysis. Protoplast formation induced by incubation with penicillin in an isotonic medium. Transfer to a hypotonic medium will result in lysis.

(see figure 21.3). Despite this, they often grow in dilute media or terrestrial environments because their plasma membranes are more resistant to osmotic pressure than those of walled bacteria. The precise reason for this is not clear, although the presence of sterols in the membranes of many species may provide added strength.

Comprehension Check

1. Describe in detail the composition and structure of peptidoglycan. Why does peptidoglycan contain the unusual D-isomers of alanine and glutamic acid rather than the L-isomers observed in proteins?
2. Draw a Venn diagram to classify the major molecules that make up typical Gram-positive and Gram-negative cell walls and those common to both types of cell wall. How do these molecules contribute to the functions of the cell wall?
3. When protoplasts and spheroplasts are made, the shape of the cell becomes spherical regardless of the original cell shape. Why does this occur?
4. The cell walls of most members of the phyla *Firmicutes* and *Actinobacteria* lack porins. Why is this the case?
5. What two mechanisms allow the passage of nutrients across the outer membrane of typical Gram-negative bacteria?

3.5 The Cell Envelope Often Includes Layers Outside the Cell Wall

After reading this section, you should be able to:

a. List the structures found in all the layers of bacterial cell envelopes
b. Identify the functions and major component molecules in cell envelope structures

Figures 3.22 and 3.24 show the bacterial envelope as consisting solely of the plasma membrane and cell wall. However, many bacteria have another layer in their cell envelopes that lies outside the cell wall. This layer is given different names depending on its makeup and how it is organized.

Capsules and Slime Layers

Capsules are layers that are well organized and not easily washed off (**figure 3.28**). They are most often composed of polysaccharides, but some are constructed of other materials. For example,

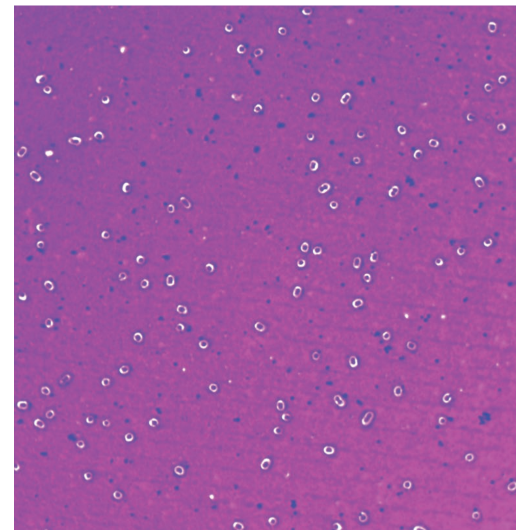


Figure 3.28 Bacterial Capsules. Capsule stain (a negative stain) of *Klebsiella pneumoniae*, bright-field light microscopy. (X1,000)
©McGraw-Hill Education/Lisa Burgess photographer

Bacillus anthracis (the anthrax bacterium) has a proteinaceous capsule composed of poly-D-glutamic acid. Capsules are clearly visible in the light microscope when negative stains or specific capsule stains are employed; they also can be studied with the electron microscope.

Capsules are not required for growth and reproduction in laboratory cultures. However, they confer several advantages when bacteria grow in their normal habitats. They help pathogenic bacteria resist phagocytosis by host phagocytes. *Streptococcus pneumoniae*, which causes ear infections, pneumonia, and other diseases, provides a dramatic example. When it lacks a capsule, it is phagocytosed easily and does not cause disease. On the other hand, the capsulated variant commonly causes disease. Capsules can also protect against desiccation because they contain a great deal of water. They exclude viruses and most hydrophobic toxic materials such as detergents.

A **slime layer** is a zone of diffuse, unorganized material that is removed easily. It is usually composed of polysaccharides but is not as easily observed by light microscopy. Gliding bacteria often produce slime, which in some cases has been shown to facilitate motility (section 3.8).

A bacterial **glycocalyx** is a layer consisting of a network of polysaccharides extending from the surface of the cell. The term can encompass both capsules and slime layers because they usually are composed of polysaccharides. The glycocalyx aids in attachment to solid surfaces, including tissue surfaces in plant and animal hosts. ▶| *Pathogenicity islands encode virulence factors (section 35.4)*

S-Layers

Many bacteria have an ordered covering called an **S-layer** on their surface. The S-layer has a pattern something like floor tiles and is composed of protein or glycoprotein (**figure 3.29**). In typical Gram-negative bacteria, the S-layer adheres noncovalently to the outer membrane; it is associated with the peptidoglycan surface of typical Gram-positive cell walls.

S-layers are of considerable interest not only for their biological roles but also in the growing field of nanotechnology. Their biological roles include protecting the cell against ion and pH fluctuations, osmotic stress, enzymes, or predatory bacteria. The S-layer also helps maintain the shape and envelope rigidity of some cells, and it can promote cell adhesion to surfaces. Finally, the S-layer seems to protect some bacterial pathogens against host defenses, thus contributing to their virulence. The potential use of S-layers in nanotechnology is due to the ability of S-layer proteins to self-assemble; that is, S-layer proteins contain the information required to spontaneously associate and form the S-layer without the aid of any additional enzymes or other factors. Thus S-layer proteins could be used as building blocks for the creation of technologies such as drug-delivery systems and novel detection systems for toxic chemicals or bioterrorism agents.

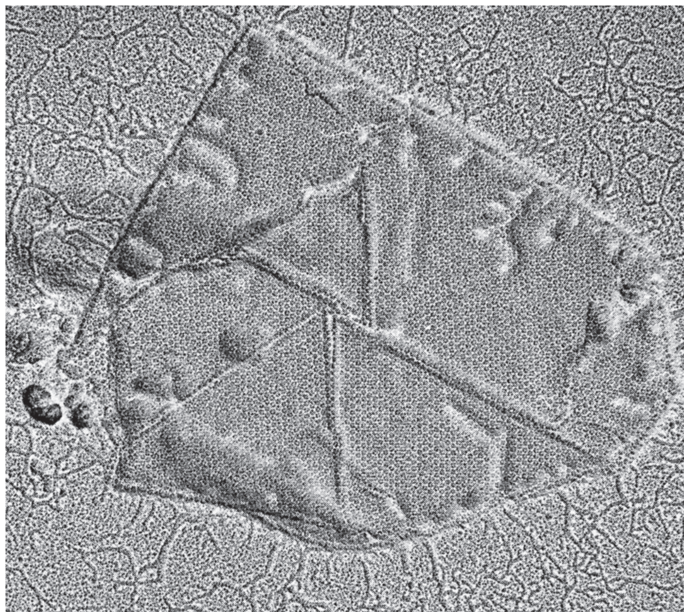


Figure 3.29 The S-Layer. An electron micrograph of the S-layer of the bacterium *Deinococcus radiodurans* after shadowing.
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Comprehension Check

1. What is the difference between a capsule and a slime layer? Why does the term *glycocalyx* usually encompass both?
2. S-layers and some capsules are composed of proteins. How does an S-layer differ from a proteinaceous capsule?

3.6 The Bacterial Cytoplasm Is More Complex than Once Thought

After reading this section, you should be able to:

a. Describe the function of three types of bacterial cytoskeletal proteins and compare their structure with those of eukaryotes
b. Compare and contrast storage inclusions and microcompartments, citing specific examples
c. List the composition of bacterial ribosomes and their spatial organization within the cell
d. Differentiate the structure and function of bacterial chromosomes and plasmids

The plasma membrane and everything within is called the **protoplast.** The **cytoplasm** is the material bounded by the plasma membrane; thus the cytoplasm is a major part of the protoplast. The cytoplasm is further divided into the liquid component, called the **cytosol;** those structures such as inclusions, ribosomes, and plasmids that float in the cytosol; and the many molecules dissolved in the cytosol. The high concentration of these macromolecules and their precursors and metabolites creates a phenomenon known as macromolecular crowding. Abundant solutes result in less available space around each molecule, a familiar experience to anyone who travels around a city at rush hour. The bacterial cytoplasm is estimated to be about 10 times more viscous than water, and physical and chemical processes like diffusion are affected.

For many years, bacterial cells were thought of as bags of biochemicals, but the exciting discovery of cytoskeletal proteins in bacterial cells has forever changed that view. Although the bacterial cytoskeleton is less complex than that of eukaryotes, it clearly helps organize the cytoplasm. In this section, we consider the bacterial cytoskeleton and then a variety of other structures in the cytoplasm.

Bacterial Cytoskeleton

Eukaryotes possess three major cytoskeletal elements: actin filaments, microtubules, and intermediate filaments. Actin filaments are made from actin, and microtubules are made from tubulin. Intermediate filaments are composed of a mixture of one or more members of different classes of proteins. Homologues of all three types of eukaryotic proteins have been identified in bacteria (**table 3.2**). Bacterial cytoskeletal proteins are structurally similar to their eukaryotic counterparts and carry out similar functions: They participate in cell division, localize proteins to certain sites in the cell, and determine cell shape. In addition, some bacterial cytoskeletal proteins appear to be unique. Thus it is likely that the evolution of the cytoskeleton was an early event in the history of

Table 3.2 Some Bacterial Cytoskeletal Proteins

Type	Function	Comments
Tubulin Homologues		
FtsZ	Cell division	Widely observed in bacteria and archaea
TubZ	Bacteriophage replication	Observed in multiple bacteriophages
Actin Homologues		
FtsA	Cell division	Attaches the FtsZ ring to the cell envelope
MamK	Positioning magnetosomes	Observed only in magnetotactic species
MreB/Mbl	Helps determine cell shape; may be involved in chromosome segregation, localizing proteins, motility, and establishing cell polarity	Observed in most rod-shaped bacteria
ParM	Plasmid segregation	Plasmid encoded
Intermediate Filament Homologues		
CreS (crescentin)	Induces curvature in curved rods	*Caulobacter crescentus*
Unique Bacterial Cytoskeletal Proteins		
MinD	Prevents polymerization of FtsZ at cell poles	Observed in many rod-shaped bacteria
ParA	Segregates chromosomes and plasmids, helps localize chemotaxis proteins and type IV pili to one pole of certain rod-shaped bacteria	Observed in many species, including *Vibrio cholerae, C. crescentus,* and *Thermus thermophilus*
Bactofilin	Scaffold for protein and chromosome positioning	Widely observed in bacteria

life on Earth. ▶| *The eukaryotic cytoplasm contains a cytoskeleton and organelles (section 5.3); Bacterial cell cycles can be divided into three phases (section 7.2)*

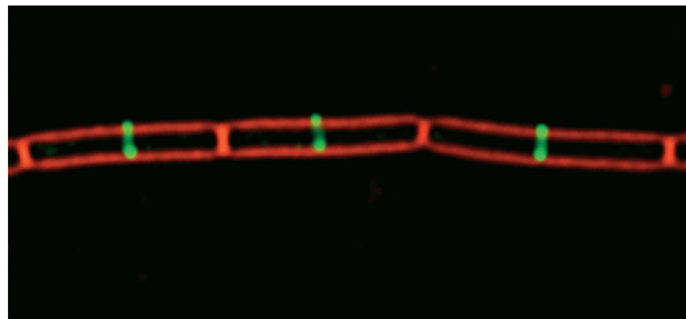
The **cytoskeletons** of *Escherichia coli, Bacillus subtilis,* and *Caulobacter crescentus* are the most important bacterial model systems and are the focus of our discussion. *E. coli* is a Gram-negative rod that has been extensively studied and can be easily manipulated. *B. subtilis* is a Gram-positive rod found in soil. It is an endospore-forming bacterium, making it a good model for cellular differentiation (section 3.9). Both were models for cell wall structure as described in section 3.4. *C. crescentus* is a curved rod found in aquatic habitats. It is interesting in part because it exhibits a complex life cycle that includes two different stages: a motile swarmer cell and a sessile, stalked cell that attaches to surfaces by a holdfast (*see figure 22.8*). ▶| Caulobacteraceae *and* Hyphomicrobiaceae *reproduce in unusual ways* (*section 22.1*)

The best studied bacterial cytoskeletal proteins are FtsZ, MreB, and CreS (also known as crescentin). FtsZ was one of the first bacterial cytoskeletal proteins identified and has since been found in most bacteria as well as mitochondria and chloroplasts. FtsZ is a homologue of the eukaryotic protein tubulin. It forms a ring at the center of a dividing cell and is required for the formation of the septum that will separate the daughter cells (**figure 3.30*a***). MreB and its relative Mbl are actin homologues. Their major function is to determine cell shape in rod-shaped cells so it is not found in cocci. MreB and Mbl determine cell shape by properly positioning the machinery needed for peptidoglycan synthesis (figure 3.30*b*,*c*). CreS (crescentin) was discovered in *C. crescentus* and is responsible for its curved shape. CreS is a homologue of lamin and keratin, two intermediate filament proteins. ▶| *Cellular growth and determination of cell shape (section 7.2)*

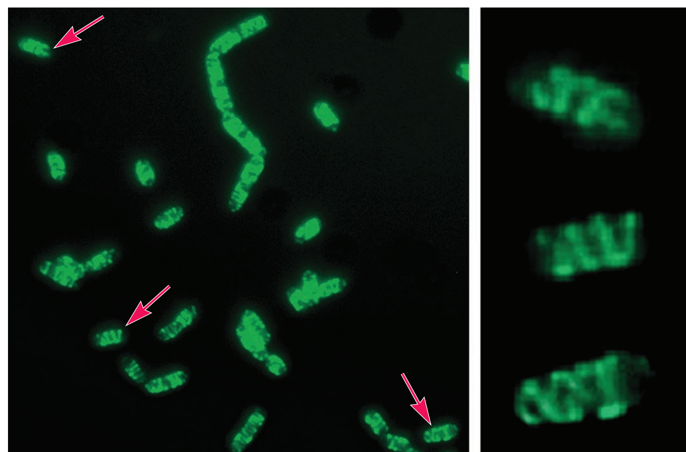
The movement of newly replicated DNA to daughter cells occurs in eukaryotes by DNA attachment to the mitotic spindle. Although it is not a general phenomenon in bacteria, there are a few examples of cytoskeletal filaments contributing to chromosome segregation *(see figure 7.4)*. Certain plasmids, in particular, rely on partition (Par) proteins to ensure equal distribution in daughter cells following cell division. ▶| *Chromosome replication and partitioning (section 7.2)*

Intracytoplasmic Membranes

Although members of *Bacteria* do not contain complex membranous organelles like mitochondria or chloroplasts, internal membranous structures are observed in some bacteria (**figure 3.31**). These can be extensive and complex in photosynthetic bacteria and in bacteria with very high respiratory activity, such as nitrifying bacteria. The internal membranes of the photosynthetic cyanobacteria are called thylakoids and are analogous to the thylakoids of chloroplasts. ▶| *Photosynthetic bacteria are diverse (section 21.4); Nitrifying*



(a) FtsZ



(b) Mbl (c) Mbl

Figure 3.30 The Bacterial Cytoskeleton. (a) FtsZ protein in a chain of *Bacillus subtilis* cells; FtsZ-green fluorescent (GFP) fusion protein viewed by fluorescence microscopy. (b) The MreB-like cytoskeletal protein (Mbl) of *B. subtilis;* Mbl-GFP in live cells was examined by fluorescence microscopy. With this method, Mbl seems to form helices (arrows). More sensitive methods show that Mbl forms patches that move perpendicular to the long axis of the cell. (c) Three of the cells from (b) are shown at a higher magnification.

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bacteria oxidize ammonium or nitrite to gain energy and electrons (section 22.1); Mitochondria, related organelles, and chloroplasts are involved in energy conservation (section 5.6)

The internal membranous structures observed in bacteria may be aggregates of spherical vesicles, flattened vesicles, or tubular membranes (*see figure 22.3*). They are often connected to the plasma membrane and arise from it by invagination. However, these internal membranes differ from the plasma membrane by being enriched for proteins and other molecules involved in energy conservation. For instance, the thylakoids of cyanobacteria contain the chlorophyll and photosynthetic reaction centers responsible for converting light energy into ATP. Thus the function of internal membranes may be to provide a larger membrane surface for greater metabolic activity.

Inclusions

Inclusions are common in all cells. They are formed by the aggregation of substances that may be either organic or inorganic. Inclusions

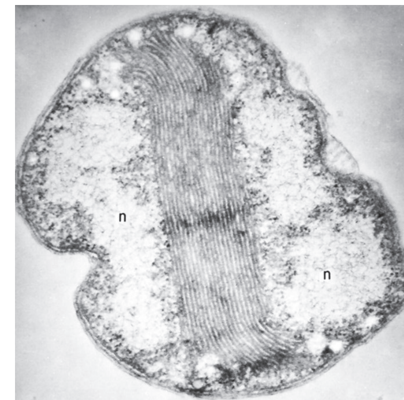

Figure 3.31 Internal Bacterial Membranes. The nitrifying bacterium *Nitrosococcus oceani* has parallel membranes traversing the whole cell. Note the nucleoid (n) with fibrillar structure.

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can take the form of granules, crystals, or globules; some are amorphous. Some inclusions lie free in the cytoplasm. Other inclusions are enclosed by a shell that is single-layered and may consist of proteins or of both proteins and phospholipids. Some inclusions are surrounded by invaginations of the plasma membrane. Many inclusions are used for storage (e.g., of carbon compounds, inorganic substances, and energy) or to reduce osmotic pressure by tying up molecules in particulate form. The quantity of inclusions used for storage varies with the nutritional status of the cell. Some inclusions are so distinctive that they are increasingly being referred to as microcompartments. A brief description of several important inclusions follows.

Storage Inclusions

Many storage inclusions form when one nutrient is in ready supply but another nutrient is not. Others store end products of metabolic processes. In some cases, these end products are used by the microbe when it is in different environmental conditions. The most common storage inclusions are glycogen inclusions, polyhydroxyalkonate granules, sulfur globules, and polyphosphate granules. Some storage inclusions are observed only in certain organisms, such as the cyanophycin granules in cyanobacteria. ▶| *Phylum* Cyanobacteria: *oxygenic photosynthetic bacteria (section 21.4)*

Carbon is often stored as polyhydroxyalkonate (PHA) granules, also termed carbonosomes. Several types of PHA granules have been identified, but the most common contain **poly-β-hydroxybutyrate** (**PHB**) (**figure 3.32**). The structure of PHB inclusions has been well studied, and PHB granules are surrounded by a single-layered shell composed of proteins. Much of the interest in PHB and other PHA granules is due to their industrial use in making biodegradeable plastics. ▶| *Biopolymers (section 42.1)*

Polyphosphate granules and sulfur globules are inorganic inclusions observed in many organisms. **Polyphosphate granules** store the phosphate needed for synthesis of important cell constituents such as nucleic acids. In some cells, they act as an energy reserve, and polyphosphate also can serve as an energy source in some reactions, when the bond linking the final phosphate in the polyphosphate chain is hydrolyzed. Sulfur globules

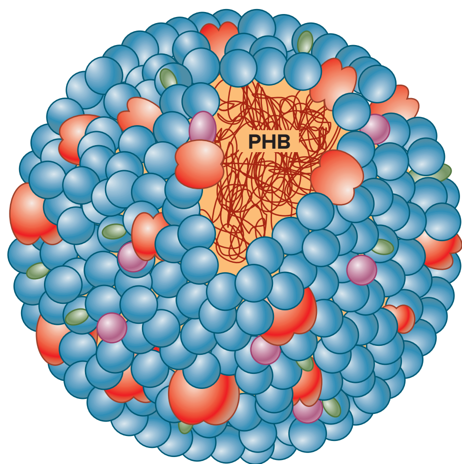
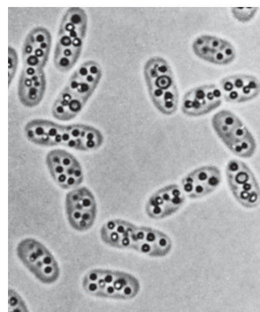
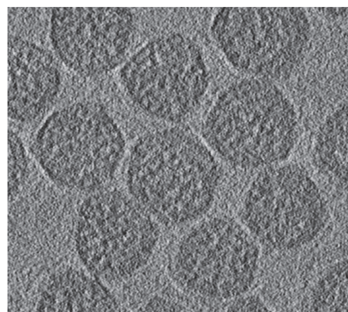


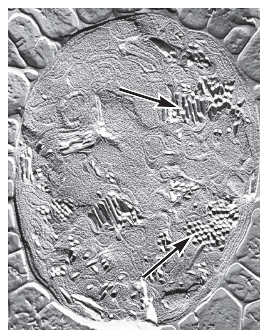
Figure 3.32 PHB Inclusions in Bacteria. PHB is a polymer of β-hydroxybutyrate molecules joined by ester bonds between the carboxyl and hydroxyl groups of adjacent molecules. PHB inclusions are around 0.2 to 0.7 µm in diameter. PHB (red lines) is enclosed by a shell composed of several different proteins, including the PHB-synthesizing enzyme (red) and the PHB-degrading enzyme (green).



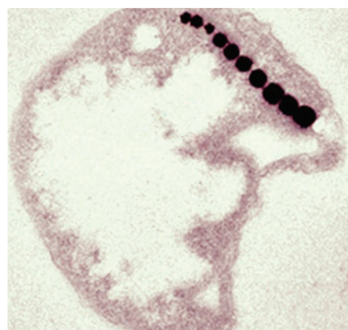
(a) Sulfur globules



(b) Carboxysomes



(c) Gas vacuoles



(d) Magnetosomes

Figure 3.33 Bacterial Cell Inclusions. (a) *Chromatium vinosum,* a purple sulfur bacterium, with intracellular sulfur globules. (b) Carboxysomes in the bacterium *Halothiobacillus neapolitanus.* Each carboxysome is approximately 100 nm in diameter. (c) A freeze-fracture preparation of *Anabaena flosaquae* showing gas vesicles and gas vacuoles. Both longitudinal and cross-section views of gas vesicles are indicated by arrows. (d) *Magnetococcus marinum* cells are roughly spherical with a single chain of magnetite crystals.

(*a*) ©Bryant, N. Pfenning and J.G. Holt (Eds), Bergey's Manual of Systematic Bacteriology, Vol. 3. © 1989 Williams and Wilkins Co., Baltimore; (*b*) ©Dr. Jessup Shively; (*c*) ©Daniel Branton/Harvard University; (*d*) ©Dennis Bazylinski

are formed by bacteria that use reduced sulfur-containing compounds as a source of electrons during their energy-conserving metabolic processes (**figure 3.33*a***). For example, some photosynthetic bacteria use hydrogen sulfide (rather than water) as an electron donor and accumulate the resulting sulfur either externally or internally. ▶| *Light reactions in anoxygenic photosynthesis (section 11.12); Purple sulfur bacteria perform anoxygenic photosynthesis (section 22.3)*

Microcompartments

Some bacterial inclusions serve functions other than simply storing substances for later use. These inclusions, called microcompartments, share several characteristics. They are relatively large polyhedrons formed by one or more different proteins. Enclosed within the protein shell are one or more enzymes. Microcompartments include the ethanolamine utilization (Eut) microcompartment, the propandiol utilization (Pdu) microcompartment, and carboxysomes. We focus here on carboxysomes as they are the best studied.

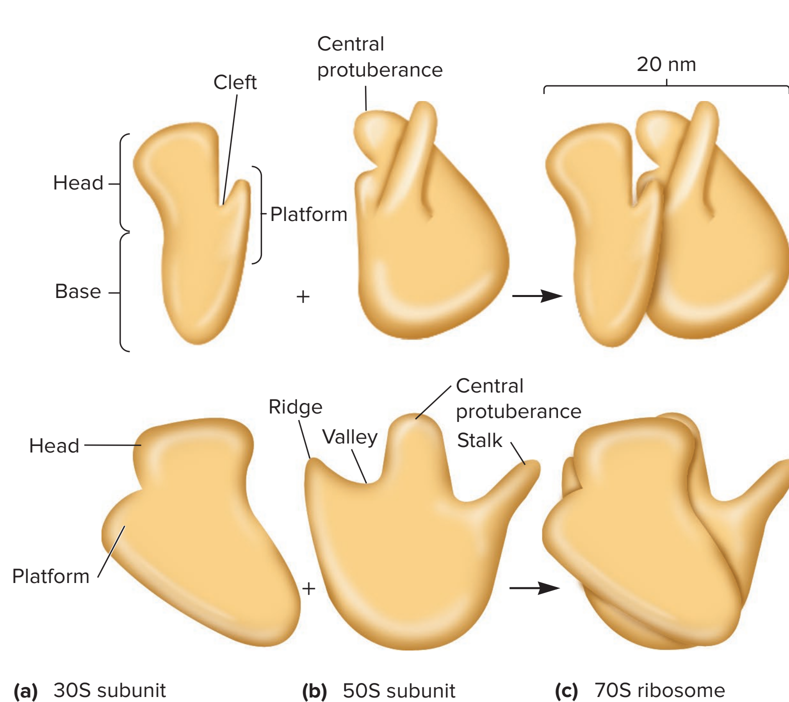
Carboxysomes are present in many cyanobacteria and other CO_2-fixing bacteria (figure 3.33*b*). Their polyhedral coat is composed of three different proteins and is about 100 nm in diameter. Associated with the shell is the enzyme carbonic anhydrase that converts carbonic acid and bicarbonate into CO_2. Recall that biological membranes allow the free diffusion of CO_2. However, the carboxysome shell prevents CO_2 from escaping so it can accumulate. Enclosed within the polyhedron is the enzyme ribulose-1, 5-bisphosphate carboxylase/oxygenase (RubisCO). RubisCO is the critical enzyme for CO_2 fixation, the process of converting CO_2 into sugar. Thus the carboxysome serves as a site for CO_2 fixation. As such, it is critical that carboxysomes be distributed to both daughter cells during cell division. ParA, a cytoskeletal protein (table 3.2), helps ensure appropriate segregation of carboxysomes. ▶| *CO_2 fixation: reduction and assimilation of CO_2 carbon (section 12.3)*

Other Inclusions

Inclusions can be used for functions other than storage or as microcompartments. Two of the most remarkable inclusions are gas vacuoles and magnetosomes. Both are involved in bacterial movement.

The **gas vacuole** provides buoyancy to some aquatic bacteria, many of which are photosynthetic. Gas vacuoles are aggregates of enormous numbers of small, hollow, cylindrical structures called **gas vesicles** (figure 3.33*c*). Gas vesicle walls are composed of many copies of a single small protein. These protein subunits assemble to form a rigid cylinder that is impermeable to water but freely permeable to atmospheric gases. Cells with gas vacuoles can regulate their buoyancy to float at the depth necessary for proper light intensity, oxygen concentration, and nutrient levels. They descend by simply collapsing vesicles and float upward when new ones are constructed.

Aquatic magnetotactic bacteria use **magnetosomes** to orient themselves in Earth's magnetic field. Northern Hemisphere bacteria use their magnetosome chain to determine northward and downward directions, and swim down to nutrient-rich sediments



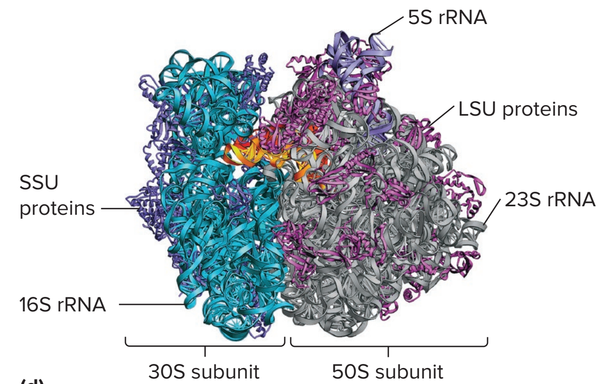


Figure 3.34 Bacterial Ribosomes. (a–c) Schematic representation of the two subunits and the complete 70S ribosome of *E. coli.* (d) Molecular structure of the 70S ribosome of *Thermus thermophilus.* The 50S subunit (LSU) includes 23S rRNA (gray) and 5S rRNA (lavender), while 16S rRNA (turquoise) is found in the 30S subunit (SSU). A molecule of tRNA (gold) is shown in the A site. To generate this structural model, crystals of purified bacterial ribosomes were prepared and exposed to X rays, and the resulting diffraction pattern analyzed.

or locate the optimum depth in freshwater and marine habitats. Magnetotactic bacteria in the Southern Hemisphere generally orient southward and downward, with the same result. Magnetosomes are intracellular chains of magnetite (Fe_3O_4) or greigite (Fe_3S_4) particles (figure 3.33*d*). They are around 35 to 125 nm in diameter and enclosed within invaginations of the plasma membrane. The invaginations contain distinctive proteins that are not found elsewhere in the plasma membrane. For the cell to move properly within a magnetic field, magnetosomes must be arranged in a chain. A cytoskeletal protein called MamK is responsible for establishing a framework upon which the chain can form (table 3.2; *see figure 22.14*).

Bacterial Ribosomes

Ribosomes are the site of protein synthesis, and large numbers (10,000 to 20,000) are found in nearly all cells. The cytoplasm of rapidly growing bacterial cells is often packed with ribosomes, and additional ribosomes may be loosely attached to the plasma membrane. The cytoplasmic ribosomes synthesize proteins destined to remain within the cell, whereas plasma membrane–associated ribosomes make proteins that will reside in the cell envelope or be transported to the outside.

Translation, the process of protein synthesis, is amazingly complex and is discussed in detail in chapter 13. This complexity is evidenced in part by the structure of ribosomes, which are made of dozens of proteins and several ribonucleic acid (RNA) molecules. The ribosomes of all three domains of life share similarities. However, there are important differences. Here we focus strictly on bacterial ribosomes. We compare bacterial and archaeal ribosomes in chapter 4 and compare all three types of ribosomes in chapter 5.

Bacterial ribosomes are called 70S ribosomes and are constructed of a 50S and a 30S subunit (**figure 3.34**). The S in these values stands for **Svedberg unit.** This is the unit of the sedimentation coefficient, a measure of sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged, the greater its Svedberg value. The sedimentation coefficient is a function of a particle's molecular weight, volume, and shape. Heavier and more compact particles normally have larger Svedberg numbers.

Bacterial ribosomes are composed primarily of ribosomal RNA (rRNA) molecules. The small subunit contains 16S rRNA, whereas the large subunit consists of 23S and 5S rRNA molecules. Approximately 55 proteins make up the rest of the mass of the ribosome: 21 in the small subunit, and 34 in the large subunit.

Nucleoid

The **nucleoid** is an ellipsoidal region that contains the cell's chromosome and numerous proteins (**figure 3.35**). Although the nucleoid is not separated from the cytoplasm by a membrane, it is a distinct region of the cell, as observed by microscopy. Ribosomes are excluded from the nucleoid. The chromosome itself has an orientation, and particular regions occupy discrete locations within the nucleoid.

The chromosomes of most bacteria are a circle of double-stranded **deoxyribonucleic acid** (**DNA**), but some bacteria have a linear chromosome. Most bacteria have a single chromosome, but some bacteria, such as *Vibrio cholerae* and *Borrelia burgdorferi* (causative agents of cholera and Lyme disease, respectively), have more than one chromosome. Many bacteria carry a single copy of their chromosome (monoploid), but others are

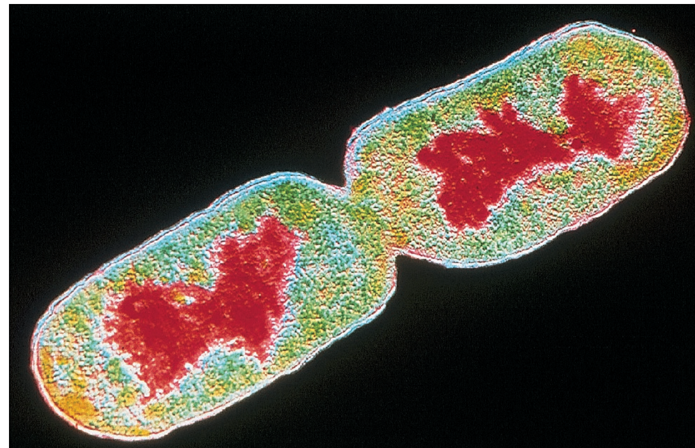


Figure 3.35 ***E. coli*** **Nucleoids and Chromosomes.** Bacterial chromosomes are located in the nucleoid, an area in the cytoplasm. (a) A color-enhanced transmission electron micrograph of a thin section of a dividing *E. coli* cell. The red areas are the nucleoids present in the two daughter cells. (b) Chromosome released from a gently lysed *E. coli* cell. Note how tightly packaged the DNA must be to fit inside the cell.

(*a*) ©CNRI/SPL/Science Source; (*b*) ©Dr. Gopal Murti/SPL/Science Source

known to be polyploid, often with more than 10 copies per cell. In *Deinococcus radiodurans,* this is believed to be necessary to allow the repair of damaged DNA under adverse environmental conditions, and the giant bacterium *Epulopiscium fishelsoni* (figure 3.4) appears to require tens of thousands of chromosome copies.

Bacterial chromosomes are longer than the length of the cell. Therefore, an important and unanswered question is how these microbes manage to fit their chromosomes into the relatively small space occupied by the nucleoid. For instance, *E. coli*'s circular chromosome measures approximately 1,400 µm, or about 230–700 times longer than the cell (figure 3.35*b*). Thus the chromosome must be organized and packaged in a manner that decreases its overall size yet retains easy accessibility to the genetic information. Physical factors like macromolecular crowding and supercoiling contribute to nucleoid organization. Supercoiling produces a dense, central core of DNA with loops of DNA extending out from the core. Besides physical factors, architectural proteins also contribute to nucleoid structure. Nucleoid-associated proteins (NAPs) are small, abundant DNA binding proteins that cause the chromosome to bend and fold. For example, the HU protein, a NAP first identified in *E. coli,* is widely distributed in bacteria, and is abundant enough to cover about 10% of the chromosome. NAPs have multiple functions: They form bridges between one section of the chromosome and another and they participate in the many processes that occur on the chromosome. Cells have several different NAPs, but HU is the only one found in almost all bacteria. NAPs are particularly important during cell division, when they further compact the chromosomes. This extra level of packing is important for proper segregation of daughter chromosomes during cell division. ▶| *DNA is a polymer of deoxyribonucleotides (section 13.2)*

Plasmids

In addition to the genetic material present in the nucleoid, many bacteria contain extrachromosomal DNA molecules called plasmids. The genome of an organism includes all DNA, including plasmids. Indeed, most of the bacterial genomes sequenced thus far include plasmids. In some cases, numerous different plasmids within a single species have been identified. For instance, *B. burgdorferi* carries 12 linear and 9 circular plasmids. Plasmids play many important roles in the lives of the organisms that have them. They also have proved invaluable to microbiologists and molecular geneticists in constructing and transferring new genetic combinations and in cloning genes, as described in chapter 17.

Plasmids are double-stranded DNA molecules that can exist independently of the chromosome. Both circular and linear plasmids have been documented, but most known plasmids are circular. Plasmids have relatively few genes, generally less than 30. Their genetic information is not essential to the bacterium, and cells that lack them usually function normally. However, many plasmids carry genes that confer a selective advantage to the bacterium in certain environments.

Plasmids use the cell's DNA-synthesizing machinery to replicate, but their replication is not linked to any particular stage of the cell cycle. Thus regulation of plasmid and chromosomal replication are independent. However, some plasmids are able to integrate into the chromosome. Such plasmids are called **episomes** and when integrated are replicated as part of the chromosome. Plasmids are inherited stably during cell division, but they are not always equally apportioned into daughter cells and sometimes are lost. The loss of a plasmid is called **curing.** It can occur spontaneously or be induced by treatments that inhibit plasmid replication but not host cell reproduction. Some commonly used curing treatments are acridine mutagens, ultraviolet and ionizing

Table 3.3 Major Types of Bacterial Plasmids

Type	Function	Example	Size (kbp)	Hosts	Phenotypic Features[1]
Conjugative Plasmids[2]	Transfer of DNA from one cell to another	F factor	95–100	*E. coli, Salmonella, Citrobacter*	Sex pilus, conjugation
R Plasmids	Carry antibiotic-resistance genes	RP4	54	*Pseudomonas* and many other Gram-negative bacteria	Sex pilus, conjugation, resistance to Amp, Km, Nm, Tet
Col Plasmids	Produce bacteriocins, substances that destroy closely related species	ColE1	9	*E. coli*	Colicin E1 production
Virulence Plasmids	Carry virulence genes	Ti	200	*Agrobacterium tumefaciens*	Tumor induction in plants

1 Abbreviations used for antibiotics: Amp, ampicillin; Km, kanamycin; Nm, neomycin; Tet, tetracycline.
2 Many R plasmids and others are also conjugative.

radiation, thymine starvation, antibiotics, and growth above optimal temperatures.

Plasmids may be classified in terms of their mode of existence, spread, and function. A brief summary of the types of bacterial plasmids and their properties is given in **table 3.3**. These various types of plasmids can also differ in terms of the number of copies found within the cell. Single-copy plasmids produce only one copy per host cell. Multicopy plasmids may be present at concentrations of 100 or more per cell.

Comprehension Check

1. Briefly describe the nature and function of the cytoplasm, and the regions and structures within it. How is the cytosol different from the cytoplasm?
2. List the most common kinds of inclusions. How are they similar to eukaryotic organelles such as mitochondria and chloroplasts? How do they differ?
3. How do plasmids differ from chromosomes? What is an episome?
4. Explain the importance of each of the following plasmids: conjugative plasmid, R plasmid, Col plasmid, and virulence plasmid.

3.7 Many Bacteria Have External Structures Used for Attachment and Motility

After reading this section, you should be able to:

a. Distinguish pili (fimbriae) and flagella
b. Illustrate the various patterns of flagella distribution

Bacteria must constantly respond to their changing environments. Sometimes it is advantageous to attach to a surface. Other times it is better to move toward or away from something in the environment. Many bacteria have structures that extend beyond the cell envelope and are involved in either attachment to surfaces or motility. In addition, these external structures can function in protection and horizontal gene transfer. Several are discussed in this section.

Bacterial Pili and Fimbriae

Many bacteria have fine, hairlike appendages that are thinner and typically shorter than flagella. These are called **fimbriae** (s., **fimbria**) or **pili** (s., **pilus**). The terms are synonymous, although certain structures are historically called pilus (e.g., sex pilus), while others are called fimbriae. We will use the terms interchangeably, except in those instances. A cell may be covered with up to 1,000 fimbriae, but they are only visible in an electron microscope due to their small size (**figure 3.36**). They are slender tubes composed of helically arranged protein subunits and are about 3 to 10 nm in diameter and up to several

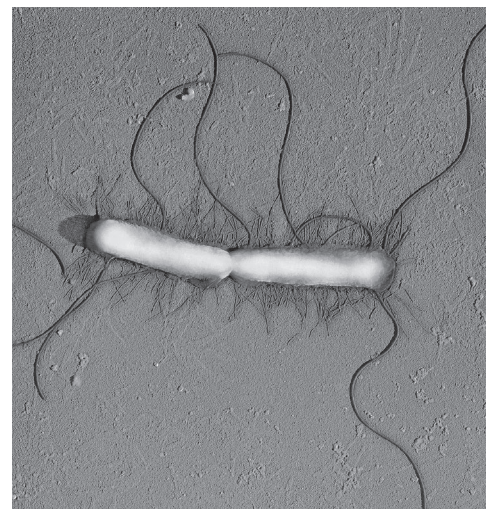


Figure 3.36 Flagella and Fimbriae. The long flagella and numerous shorter fimbriae are evident in this SEM of the bacterium *Proteus vulgaris*.
©Thomas Deerinck, NCMIR/Science Source

micrometers long. Pili grow by adding protein subunits to their base. Several different types have been identified and most function to attach cells to solid surfaces such as rocks in streams and host tissues. Type IV pili are involved in motility (section 3.8) and two gene transfer mechanisms: bacterial transformation and bacterial conjugation. ▶| *Bacterial conjugation requires cell–cell contact (section 16.6); Bacterial transformation is the uptake of free DNA from the environment (section 16.7)*

Many bacteria have up to 10 **sex pili** (s., **sex pilus**) per cell. These hairlike structures differ from other pili in the following ways. Sex pili often are larger than other pili (around 9 to 10 nm in diameter). They are genetically determined by conjugative plasmids and are required for conjugation. Some bacterial viruses attach specifically to sex pili at the start of their infection cycle.

Bacterial Flagella

Many motile bacteria move by means of **flagella** (s., **flagellum**), threadlike locomotor appendages extending outward from the plasma membrane and cell wall. Although the main function of flagella is motility, they can have other roles. They can be involved in attachment to surfaces, and in some bacteria, they are virulence factors, that is, they contribute to the ability of the bacterium to cause disease.

Bacterial flagella are slender, rigid structures about 20 nm across and up to 20 μm long. Flagella are so thin they cannot be observed directly with a light microscope but must be stained with techniques designed to increase their thickness. The detailed structure of a flagellum can only be seen in the electron microscope.

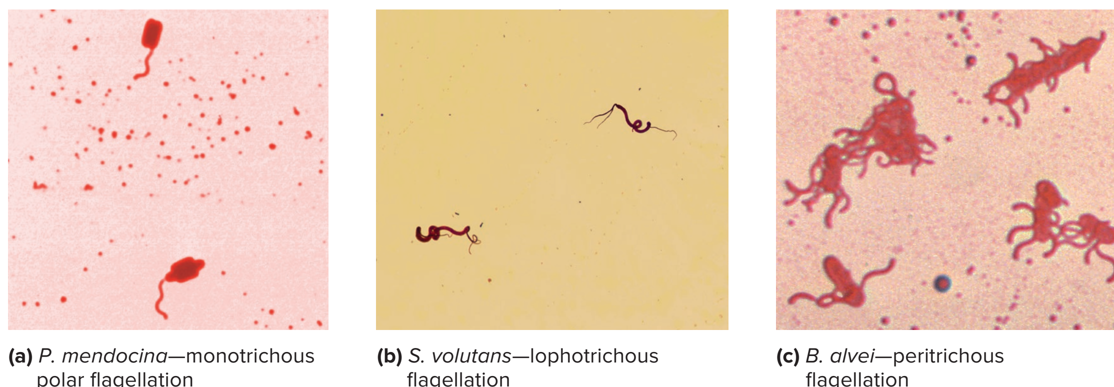
Bacterial species often differ in their patterns of flagella distribution, and these patterns are useful in identifying bacteria. **Monotrichous** bacteria (Greek *trikhos,* hair) have one flagellum; if it is located at an end, it is said to be a **polar flagellum** (**figure 3.37*a***). **Amphitrichous** bacteria (Greek *amphi*, on both sides) have a single flagellum at each pole. In contrast, **lophotrichous** bacteria (Greek *lopho*, crest or tuft) have a cluster of flagella at one or both ends (figure 3.37*b*). Flagella are spread evenly over the whole surface of **peritrichous** (Greek *peri*, around) bacteria (figure 3.37*c*).

Transmission electron microscope studies have shown that the bacterial flagellum is composed of three parts (**figure 3.38**). (1) The **filament** is the longest and most obvious portion. It extends from the cell surface to the tip. (2) The **basal body** is embedded in the cell envelope; and (3) a short, curved segment, the **hook,** links the filament to its basal body and acts as a flexible coupling.

The filament is a hollow, rigid cylinder constructed of subunits of the protein **flagellin,** which ranges in molecular mass from 30,000 to 60,000 daltons, depending on the bacterial species. The filament ends with a capping protein. Some bacteria have sheaths surrounding their flagella. For example, *Vibrio cholerae* flagella have lipopolysaccharide sheaths.

The hook and basal body are quite different from the filament (figure 3.38). Slightly wider than the filament, the hook is made of different protein subunits. The basal body is the most complex part of a flagellum. The basal bodies of *E. coli* and most other typical Gram-negative bacteria have four rings: L, P, MS, and C, which are connected to a central rod (figure 3.38*a*). The L, P, and MS rings are embedded in the cell envelope, and the C ring is on the cytoplasmic side of the MS ring. Typical Gram-positive bacteria have only two rings: an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan (figure 3.38*b*).

The synthesis of bacterial flagella is complex and involves at least 20 to 30 genes. Besides the gene for flagellin, 10 or more genes code for hook and basal body proteins; other genes are concerned with control of flagellar construction or function. Many components of the flagellum lie outside the cell envelope and must be transported out of the cell for assembly. Interestingly, the basal body is a specialized version of the type III protein secretion system observed in typical Gram-negative bacteria. Type III secretion systems have a needlelike structure through which proteins are secreted. In the flagellar type III secretion system, the filament replaces



(a) *P. mendocina*—monotrichous polar flagellation

(b) *S. volutans*—lophotrichous flagellation

(c) *B. alvei*—peritrichous flagellation

Figure 3.37 Flagellar Distribution. Examples of various patterns of flagellation as seen in the light microscope. (a) Flagella stain of *Pseudomonas mendocina*. (b) *Spirillum volutans* (×1,000). Only the tuft of flagella at one end is clearly visible. (c) *Bacillus alvei*.

(*a*, *c*) Source: CDC/Dr. William A. Clark; (*b*) ©McGraw-Hill Education/James Redfearn, photographer,

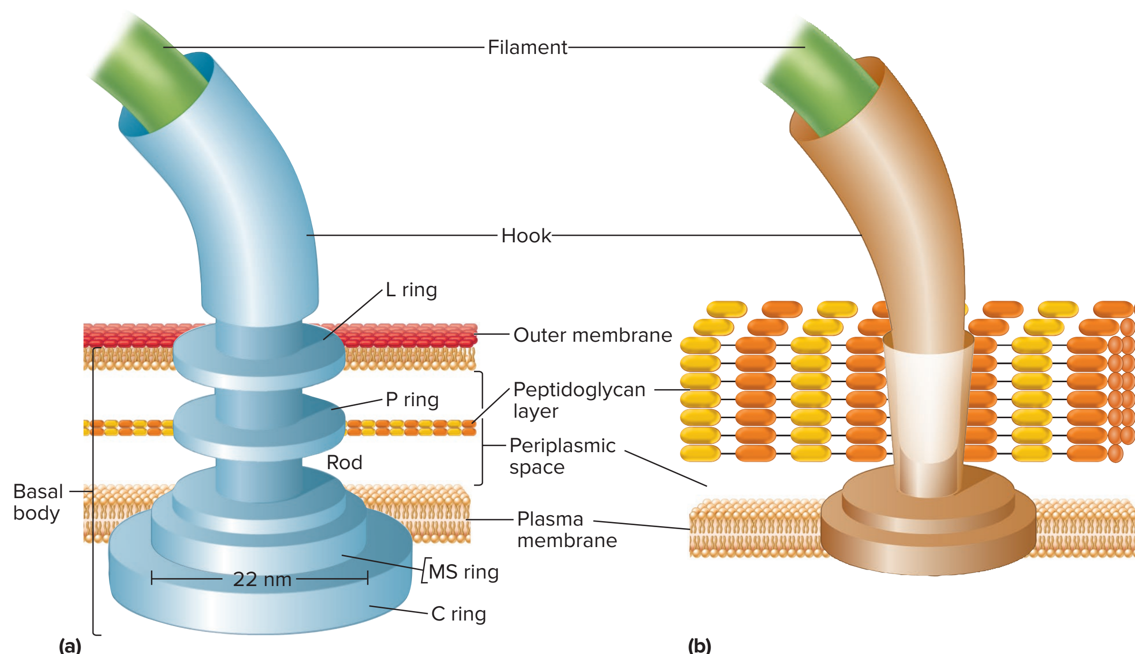


Figure 3.38 The Ultrastructure of Bacterial Flagella. Flagellar basal bodies and hooks in (a) typical Gram-negative and (b) typical Gram-positive bacteria.

the needle. Individual flagellin subunits are transported through the hollow filament. When the subunits reach the tip, they spontaneously aggregate under the direction of a protein called the filament cap; thus the filament grows at its tip rather than at the base (**figure 3.39**). Filament synthesis, like S-layer formation, is an example of **self-assembly.** ▶| *Protein maturation and secretion (section 13.8)*

Comprehension Check

1. What are the functions of fimbriae (pili) and sex pili?
2. What terms are used to describe the different flagella distribution patterns observed among bacteria?
3. What is self-assembly? Why does it make sense that the filament of a flagellum is assembled in this way?

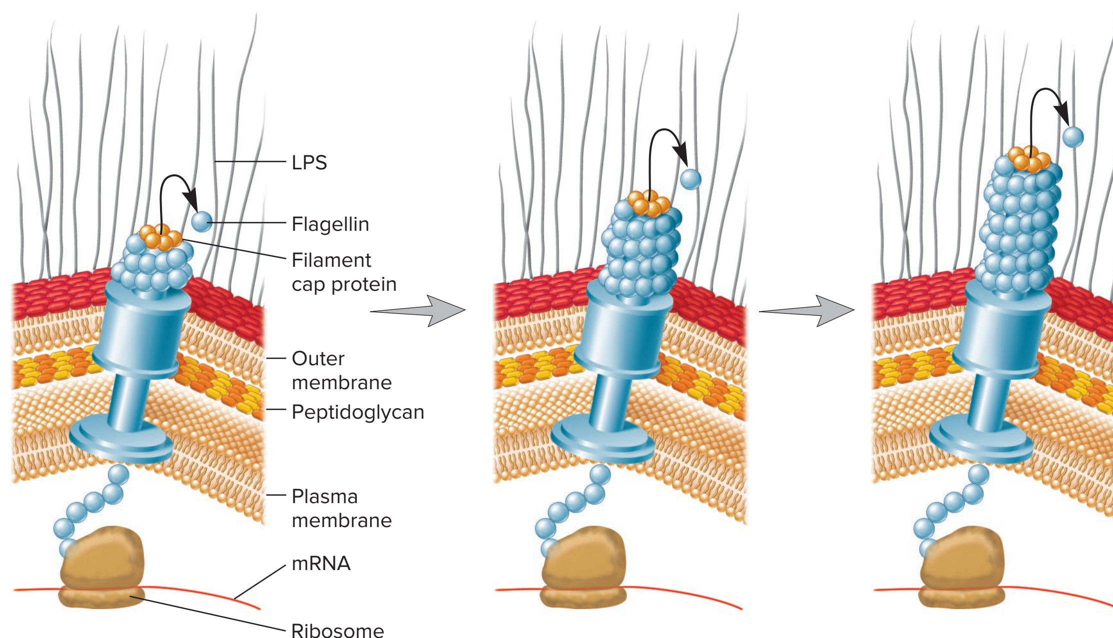


Figure 3.39 Flagellar Filaments Grow at the Tip. Flagellin subunits travel through the flagellar core and attach to the growing tip. Their attachment is directed by the filament cap protein.

MICRO INQUIRY *How does flagellum growth compare to pilus growth?*

3.8 Bacteria Move in Response to Environmental Conditions

After reading this section, you should be able to:

a. Compare and contrast flagellar swimming motility, swarming, spirochete flagellar motility, and twitching and gliding motility
b. State the source of energy that powers flagellar motility
c. Explain why bacterial chemotaxis is referred to as a "biased random walk"

As we note in section 3.7, several structures extending beyond bacterial cell envelopes contribute to motility. Five major methods of movement have been observed: swimming movement conferred by flagella, flagella-mediated swarming, corkscrew movement of spirochetes, twitching motility associated with type IV pili, and gliding motility.

Motile bacteria do not move aimlessly. Rather, motility is used to move toward nutrients such as sugars and amino acids and away from many harmful substances and bacterial waste products. Movement toward chemical attractants and away from repellents is known as chemotaxis. Motile bacteria also can move in response to environmental cues such as temperature (thermotaxis), light (phototaxis), oxygen (aerotaxis), osmotic pressure (osmotaxis), and gravity.

Flagellar Movement

Swimming

The filament of a bacterial flagellum is in the shape of a rigid helix, and the cell moves when this helix rotates like a propeller on a boat. The flagellar motor can rotate very rapidly. The *E. coli* motor rotates 270 revolutions per second (rps); *Vibrio alginolyticus* averages 1,100 rps. For many bacteria in an aquatic environment, flagellar rotation results in two types of movement: a smooth swimming movement often called a **run,** which actually moves the cell from one spot to another, and a **tumble,** which serves to reorient the cell. As we shall see in our discussion of chemotaxis, alternating between runs and tumbles is important for responding to environmental conditions. Often, the direction of flagellar rotation determines whether a run or a tumble occurs. For example, many bacteria with monotrichous, polar flagella use a counterclockwise rotation for a run (**figure 3.40**). When rotation is reversed, the cell tumbles. Many peritrichously flagellated bacteria operate in a somewhat similar way. To move forward in a run, the flagella rotate counterclockwise. As they do so, the flagella bend at their hooks to form a rotating bundle that propels the cell forward. Clockwise rotation of the flagella disrupts the bundle and the cell tumbles.

Not all bacteria use runs and tumbles for swimming motility. For instance, *Rhodobacter sphaeroides* cells alternate between rotating their single flagellum in one direction (run) and no rotation, a so-called run-stop motility. When the cells are stopped, molecules in the environment bombard the cells and cause them to make small changes in orientation. When they resume a run, they move in a new direction. Another type of swimming motility is seen with the monotrichous bacterium *Vibrio alginolyticus*. It uses a run-reverse-flick pattern. It swims forward (run) when the flagellum rotates in one direction. When rotation reverses, the cell moves backward (reverse). Just as the rotation switches again for a run, the flagellum flicks, causing the cell to change its orientation and move in a new direction.

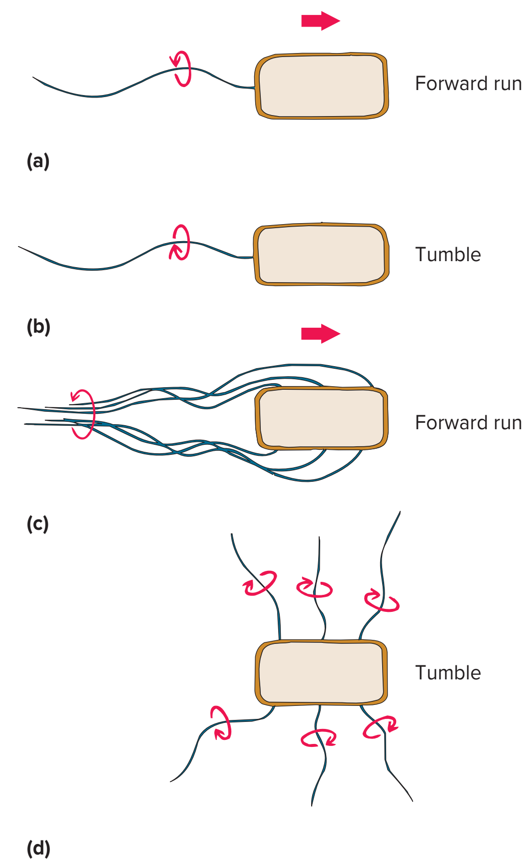
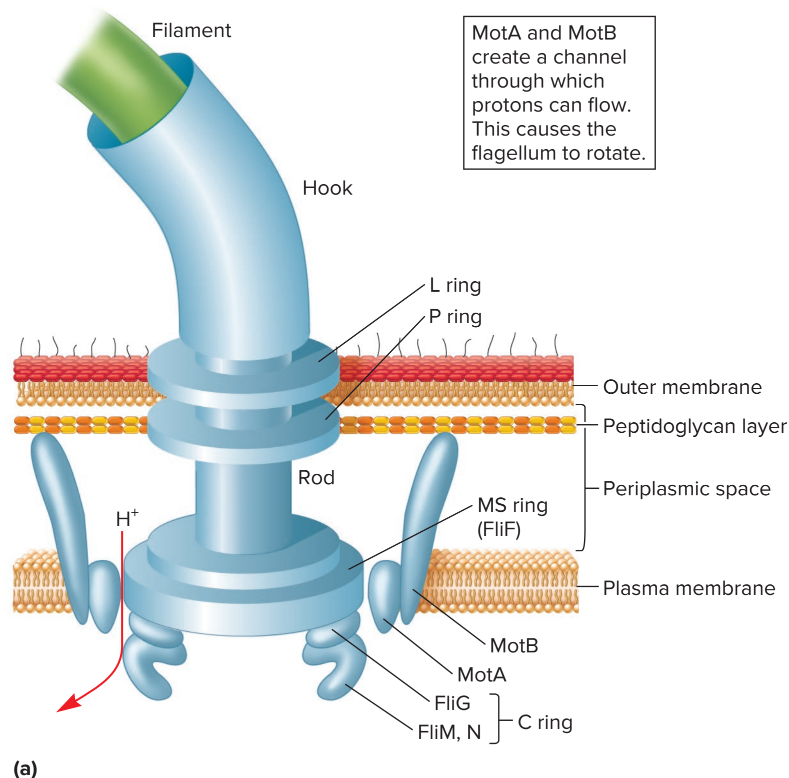


Figure 3.40 The Direction of Flagellar Rotation Often Determines the Way a Bacterium Moves. Parts (a) and (b) describe the motion of bacteria with monotrichous, polar flagella. Parts (c) and (d) illustrate the movements of bacteria with peritrichous flagella.

The motor that drives flagellar rotation is located at the base of the flagellum. Torque generated by the motor is transmitted to the hook and filament. The motor is composed of two components: the rotor (the moving parts) and the stator (the stationary components). It is thought to function like an electrical motor, where the rotor turns in the center of a ring of electromagnets, the stator. In typical Gram-negative bacteria, the rotor is composed of the central rod and all four rings (**figure 3.41**). The C ring protein, FliG, is particularly important because it interacts with the stator. The stator is composed of the proteins MotA and MotB, which form a channel through the plasma membrane. MotB also anchors MotA to cell wall peptidoglycan.



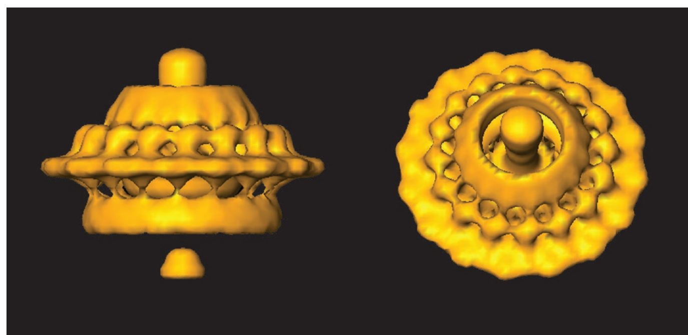


Figure 3.41 Mechanism of Flagellar Movement. (a) This diagram of a flagellum shows some of the more important components and the flow of protons that drives rotation. Some of the many flagellar proteins are labeled. (b) A three-dimensional electron cryotomographic reconstruction of the flagellar motor.

(b)©Gavin Murphy/Nature/Science Source

MICRO INQUIRY *Would this flagellum be found in a typical Gram-negative or Gram-positive bacterium?*

As with all motors, the flagellar motor must have a power source that allows it to generate torque and cause flagellar rotation. The power used by most flagellar motors is a difference in charge and pH across the plasma membrane. This difference is called the proton motive force (PMF). PMF is largely created by the metabolic activities of organisms, as described in chapter 11. One important metabolic process is the transfer of electrons from an electron donor to a terminal electron acceptor via a chain of electron carriers called the electron transport chain (ETC). In bacterial cells, most components of the ETC are located in the plasma membrane. As electrons are transported down the ETC, protons are transported from the cytoplasm to the outside of the cell. Because there are more protons outside the cell than inside, the outside has more positively charged ions (the protons) and a lower pH. PMF is a type of potential energy that can be used to do work: mechanical work, as in the case of flagellar rotation; transport work, the movement of materials into or out of the cell (section 3.3); or chemical work such as the synthesis of ATP, the cell's major energy currency.

How can PMF be used to power the flagellar motor? The channels created by the MotA and MotB proteins allow protons to move across the plasma membrane from the outside to the inside (figure 3.41). Thus the protons move down the charge and pH gradient. This movement releases energy that is used to rotate the flagellum. In essence, the entry of a proton into the channel is like the entry of a person into a revolving door. The "power" of the proton generates torque, rather like a person pushing the revolving door. Indeed, the speed of flagellar rotation is proportional to the magnitude of the PMF.

The flagellum is a very effective swimming device. From the bacterium's point of view, swimming is quite a difficult task because the surrounding water seems as viscous as molasses. The cell must bore through the water with its corkscrew-shaped flagella, and if flagellar rotation ceases, it stops almost instantly. Despite such environmental resistance to movement, bacteria can swim from 10 to 100 μm per second. This is equivalent to traveling from 2 to over 150 cell lengths per second. In contrast, an exceptionally fast human might be able to run around 5 to 6 body lengths per second.

Swarming

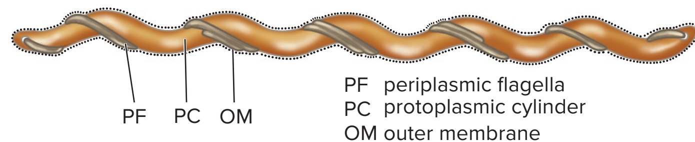
An increasing number of bacterial species has been found to exhibit an interesting type of motility called swarming. This motility occurs on moist surfaces and is a type of group behavior in which cells move in unison across the surface. Most bacteria that swarm have peritrichous flagella. Many also produce and secrete molecules that help them move across the substrate. When bacteria that swarm are cultured in the laboratory on appropriate solid media, they produce characteristic colony morphologies (**figure 3.42**).

Spirochete Motility

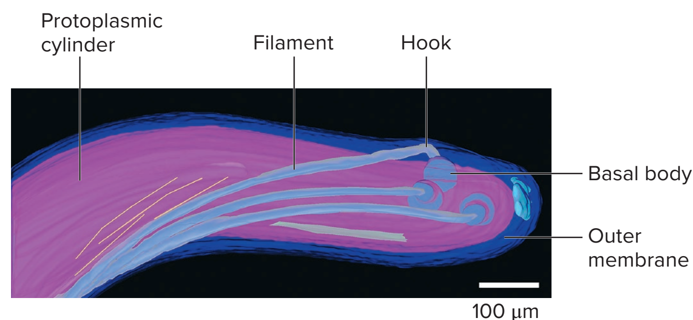
Spirochetes have flagella that work in a distinctive manner. In many spirochetes, multiple flagella arise from each end of the cell and wind around the cell (**figure 3.43**). The flagella do not extend outside the cell wall but rather remain in the periplasmic space and are covered by the outer membrane. They are called periplasmic flagella and rotate like the external flagella of other

Figure 3.42 Swarming Bacteria Often Produce Distinctive Patterns on a Solid Growth Medium. These bacterial cells swarmed from the center of the plate and produced a branching pattern called dendrites.
©Dr. Daniel Kearns

bacteria, causing the corkscrew-shaped outer membrane to rotate and move the cell through the surrounding medium, even very viscous liquids. Flagellar rotation may also flex or bend the cell and account for the creeping or crawling movement observed when spirochetes are in contact with a solid surface. ▶| *Phylum* Spirochaetes *(section 21.6)*



(a)



(b)

Figure 3.43 Spirochete Flagella. (a) Numerous flagella arise from each end of the spirochete. These intertwine and wind around the cell, usually overlapping in the middle. (b) Electron cryotomographic image of the spirochete *Treponema denticola* showing three flagella arising from the tip of the cell.
©Jacques Izard/Harvard School of Dental Medicine

Twitching and Gliding Motility

Twitching and gliding motility occur when cells are on a solid surface. However, unlike swarming, neither involves flagella. Gliding motility varies greatly in rate (from 2 to over 600 μm per minute) and in the nature of the motion. Although first observed over 100 years ago, the mechanism by which many bacteria glide remains a mystery. We describe the gliding motility of *Flavobacterium* spp. and *Mycoplasma* spp. in detail in chapter 21. ▶| *Phylum* Bacteroidetes *includes important gut microbiota (section 21.7); Class* Mollicutes, *Phylum* Tenericutes: *bacteria that lack cell walls (section 21.3)*

Here we focus on *Myxococcus xanthus* because it exhibits both twitching and gliding motility. Its twitching motility is called social (S) motility because it occurs when large groups of cells move together in a coordinated fashion. **Twitching motility** is characterized by short, intermittent, jerky motions of up to several micrometers in length and is normally seen on very moist surfaces. Type IV pili alternately extend and retract to move cells during twitching motility. The extended pilus contacts the surface at a point some distance from the cell body. When the pilus retracts, the cell is pulled forward. ATP hydrolysis powers the extension/retraction process.

In contrast to the jerky movement of twitching motility, **gliding motility** is smooth. The gliding motility exhibited by *Myxococcus xanthus* is called adventurous (A) motility; it is observed when single cells move independently. In *M. xanthus,* proteins similar to MotA and MotB of the flagellar motor function as the motors for gliding motility and these are also powered by PMF. They are located in the plasma membrane (like flagellar motors). Additional motility proteins are located in the periplasm and the outer membrane where they move independently. It is only when the full set of protein complexes aligns across the entire cell envelope that they can exert the mechanical force to propel the cell. Adhesin proteins on the cell surface are compressed and slide on the substrate, pushing the body of the cell forward. ▶| *Order* Myxococcales: *bacteria with morphological complexity and multicellularity (section 22.4)*

Chemotaxis

Imagine a motile bacterium in an ocean. Depending on where it is in the water column, light, oxygen, and nutrient levels will vary. These also vary over time due to the activities of all the organisms present. To position itself in the most beneficial location in the water column, this bacterium needs to sense changes in the environment and move accordingly. The movement toward or away from attractants or repellents is called taxis. As noted earlier, bacteria exhibit taxes to a variety of stimuli, including light and oxygen. The movement of cells toward chemical attractants or away from chemical repellents (**chemotaxis**) is the best-studied type of taxis. Chemotaxis is readily observed in Petri dish cultures. If bacteria are placed in the center of a dish of semisolid agar containing an attractant, the bacteria will exhaust the local supply of the nutrient and swim outward following the attractant gradient they have created. The result is an expanding ring of bacteria. When a disk of repellent is placed in a Petri dish

of semisolid agar and bacteria, the bacteria will swim away from the repellent, creating a clear zone around the disk.

Attractants and repellents are detected by **chemoreceptors,** proteins that bind chemicals and transmit signals to other components of the chemosensing system. Chemosensing systems, located in the plasma membrane, are sensitive and allow the cell to respond to low levels of attractants (about 10^{-8} M for some sugars). Some receptors also participate in the initial stages of sugar transport into the cell.

The chemotactic behavior of *E. coli* has been extensively studied and is our focus here. Its movements can be followed using a tracking microscope, a microscope with a moving stage that automatically keeps an individual bacterium in view. In the absence of a chemical gradient, *E. coli* cells move randomly, switching back and forth between runs and tumbles. During a run, the bacterium swims in a straight or slightly curved line. After a few seconds, the bacterium stops and tumbles. The tumble randomly reorients the cell so that it is facing in a different direction. Therefore when it begins the next run, it usually goes in a different direction (**figure 3.44*a***). In contrast, when *E. coli* is exposed to an attractant, it tumbles less frequently (or has longer runs) when traveling toward the attractant. Although the tumbles can still orient the bacterium away from the attractant, over time the cell gets closer and closer to the attractant (figure 3.44*b*). The opposite response occurs with a repellent. Tumbling frequency decreases (the run time lengthens) when the bacterium moves away from the repellent.

E. coli must have some mechanism for sensing that it is getting closer to the attractant (or moving away from the repellent).

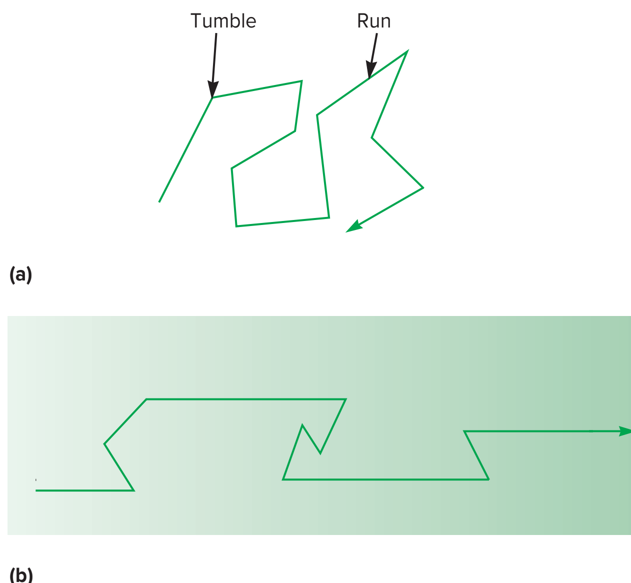


Figure 3.44 Tumbles and Runs Are Used to Direct the Movement of Many Bacteria Toward a Chemical Attractant. (a) Random movement of a bacterium in the absence of a concentration gradient. Tumbling frequency is fairly constant. (b) Movement in an attractant gradient. Tumbling frequency is reduced when the bacterium is moving up the gradient. Therefore runs in the direction of increasing attractant are longer.

The behavior of the bacterium is shaped by temporal changes in chemical concentration. The cell is able to compare the current concentration with the concentration a few seconds earlier. If the concentration of the attractant is increasing, tumbling is suppressed. Likewise, *E. coli* moves away from a repellent because it senses that the concentration of the repellent is decreasing. The bacterium's chemoreceptors play a critical role in this process. The molecular events that enable *E. coli* cells to sense a chemical gradient and respond appropriately are presented in chapter 14.

Comprehension Check

1. Describe the way many flagella operate to move a bacterium.
2. How does swimming differ from swarming?
3. Explain in a general way how bacteria move toward substances such as nutrients and away from toxic materials.
4. Suggest why chemotaxis is sometimes called a "biased random walk."

3.9 Bacterial Endospores Are a Survival Strategy

After reading this section, you should be able to:

a. Describe the structure of a bacterial endospore
b. Explain why bacterial endospores are of particular concern to the food industry and why endospore-forming bacteria are important model organisms
c. Describe in general terms the process of sporulation
d. Describe those properties of endospores that are thought to contribute to its resistance to environmental stresses
e. Describe the three stages that transform an endospore into an active vegetative cell

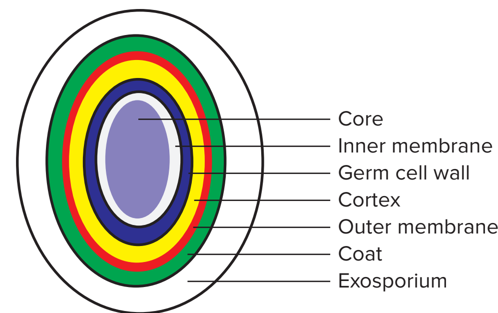
Endospores, dormant cells formed within a so-called mother cell, are fascinating bacterial structures only produced by certain members of the genera *Bacillus* and *Clostridium* (rods), and *Sporosarcina* (cocci) within the phylum *Firmicutes*. It is important to distinguish bacterial endospores from spores formed by fungi and plants. Endospore formation is not a reproductive strategy as only a single endospore is produced by a bacterial cell. There are several reasons why endospores have long held the interest of microbiologists. The primary reason is that endospores are extraordinarily resistant to environmental stresses such as heat, ultraviolet radiation, gamma radiation, chemical disinfectants, and desiccation. In fact, some endospores have remained viable for around 100,000 years. Another important reason is that several species of endospore-forming bacteria are dangerous pathogens. For example, *Clostridium botulinum* causes botulism, a food-borne disease that results from ingestion of botulinum toxin, the deadliest toxin known. The extreme heat resistance of *C. botulinum*'s endospores is a major concern of the food industry. Other medically important endospore-forming bacteria include *B. anthracis* (causes anthrax), *C. tetani* (causes tetanus), and *C. perfringens* (causes gas gangrene and food poisoning). Endospores also are of considerable interest to scientists

because of their complex structures and the equally complex process that generates them. Over the decades, microbiologists have asked many questions about endospores and endospore formation. (1) How are endospores structurally different from vegetative cells? (2) What makes them so resistant to harsh environmental conditions? (3) What triggers endospore formation? (4) What are the steps in endospore formation and how is the process regulated? (5) How do endospores convert back to vegetative cells? In this section, we address these questions. We save the consideration of regulating endospore formation for chapter 14. ▶| *Sporulation in* B. subtilis *(section 14.6)*

Endospore Structure and Resistance

Electron microscopy has been an important tool for dissecting endospore structure. Electron micrographs show that the endospore consists of a core surrounded by several layers that vary significantly in composition. The core has normal cell structures such as ribosomes and a nucleoid but has very low water content (**figure 3.45**). The core is surrounded by an inner membrane, which is in turn covered by the core wall (also called the germ cell wall, as it contains the peptidoglycan that will form the wall of the vegetative cell that grows out of the endospore following germination). Next is the cortex, which may occupy as much as half the endospore volume. It is made of peptidoglycan that is less cross-linked than that in vegetative cells. The cortex is surrounded by a phospholipid bilayer called the outer membrane. Outside of the outer membrane is the coat, a complex structure composed of several layers. It is composed of more than 70 different proteins, which are highly cross-linked to each other. Finally, many endospore-forming bacteria produce endospores enclosed by a thin covering called the exosporium, which is made up of glycoproteins.

The ability of the endospore to survive heat, radiation, and damaging chemicals requires that its enzymes and DNA be protected. The various layers of the endospore contribute to this resistance in several ways. The coat protects the endospore from chemicals and various lytic enzymes such as lysozyme. The inner membrane is extremely impermeable to various chemicals, including those that cause DNA damage. The core has very low water content, high amounts of dipicolinic acid complexed with calcium ions (Ca-DPA), and a slightly lower pH, all of which contribute to the endospore's resistance to harsh conditions. The low water content seems to be especially important for endospore resistance. Evidence exists that the water content of the core is low enough to prevent rotation of enzymes and other proteins present. However, it is not low enough to prevent protein denaturation. It has been suggested that the immobilization prevents them from interacting with each other and becoming entangled. Thus, even though they may become denatured, they are able to refold to their proper active structure as the endospore germinates. The endospore's DNA is protected by two main mechanisms. Ca-DPA complexes are inserted between the nitrogenous bases of DNA, which helps stabilize it. In addition, small, acid-soluble DNA-binding proteins (SASPs) saturate endospore DNA. SASPs alter the three-dimensional structure of DNA in the endospore core, converting it from the common B form to A form. The A form DNA is less susceptible to damage by ultraviolet (UV) light, which accounts for the ability of endospores to tolerate UV exposure. ▶| *DNA is a polymer of deoxyribonucleotides (section 13.2)*



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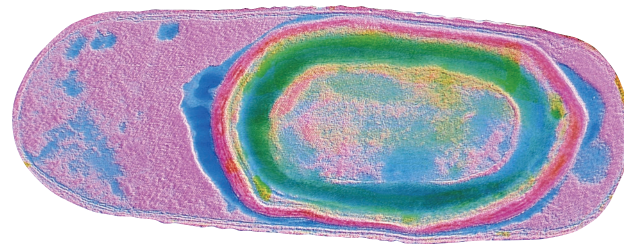

(b)

Figure 3.45 Bacterial Endospores. (a) A schematic showing the various layers of an endospore. They are not drawn to scale. (b) A colorized cross section of a *Bacillus subtilis* cell undergoing sporulation. The oval in the center is an endospore that is almost mature; when it reaches maturity, the mother cell will lyse to release it.

(*b*) ©CNRI/Science Source

Sporulation: Making Endospores

Endospore-forming bacteria are common in soil, where they must be able to withstand fluctuating levels of nutrients. **Sporulation** normally commences when growth slows due to nutrient limitation. Thus it is a survival mechanism that allows the bacterium to produce a dormant cell that can survive until nutrients are again available and vegetative growth can resume. These bacteria cycle between two states: vegetative growth and survival as an endospore. Vegetative growth is the normal, continuous cycle of growth and division. By contrast, sporulation is a complex process that occurs in a highly organized fashion over several hours. The mature endospore occupies a characteristic location in the mother cell (referred to as the sporangium), depending on the species of bacteria.

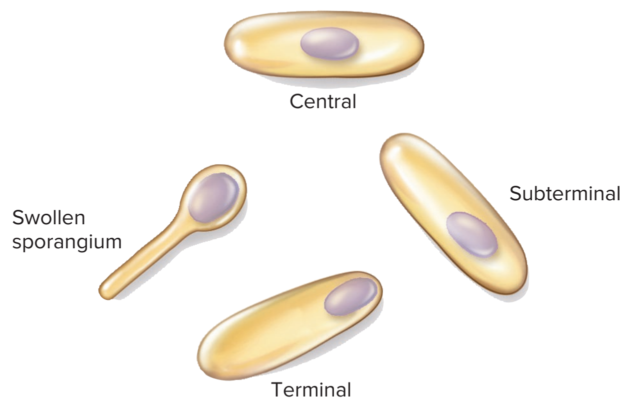

Figure 3.46 Examples of Endospore Location and Size.

Endospores may be centrally located, close to one end (subterminal), or terminal (**figure 3.46**). Sometimes an endospore is so large that it swells the sporangium. One of the best-studied endospore formers is *Bacillus subtilis*, which is an important model organism.

Sporulation may be divided into seven stages (**figure 3.47**). The cell's DNA is replicated (stage I), followed by an inward folding of the cell membrane to enclose part of the DNA and produce the forespore septum (stage II). The mother cell membrane continues to grow and engulfs the immature endospore in a second membrane (stage III). Next, cortex is laid down in the space between the two membranes, and both calcium and dipicolinic acid are accumulated (stage IV). Protein coats are formed around the cortex (stage V), and maturation of the endospore occurs (stage VI). Finally, lytic enzymes destroy the sporangium, releasing the endospore (stage VII). Sporulation requires about 8 to 10 hours. *Bacterial Endospore Formation*

Endospore to Vegetative Cell

The transformation of dormant endospores into active vegetative cells is almost as complex as sporulation. It occurs in three stages: (1) activation, (2) germination, and (3) outgrowth. Activation is a process that prepares endospores for germination and can result from treatments such as heating. This is followed by **germination,** the breaking of the endospore's dormant state. It begins when proteins called germinant receptors, located in the inner membrane and the cortex, detect small molecules such as sugars and amino acids. Upon detection of these molecules by the germinant receptors, a series of events occur. These activated receptors trigger the release of the Ca-DPA complexes, breakdown of the peptidoglycan in the cortex, and water uptake. Eventually

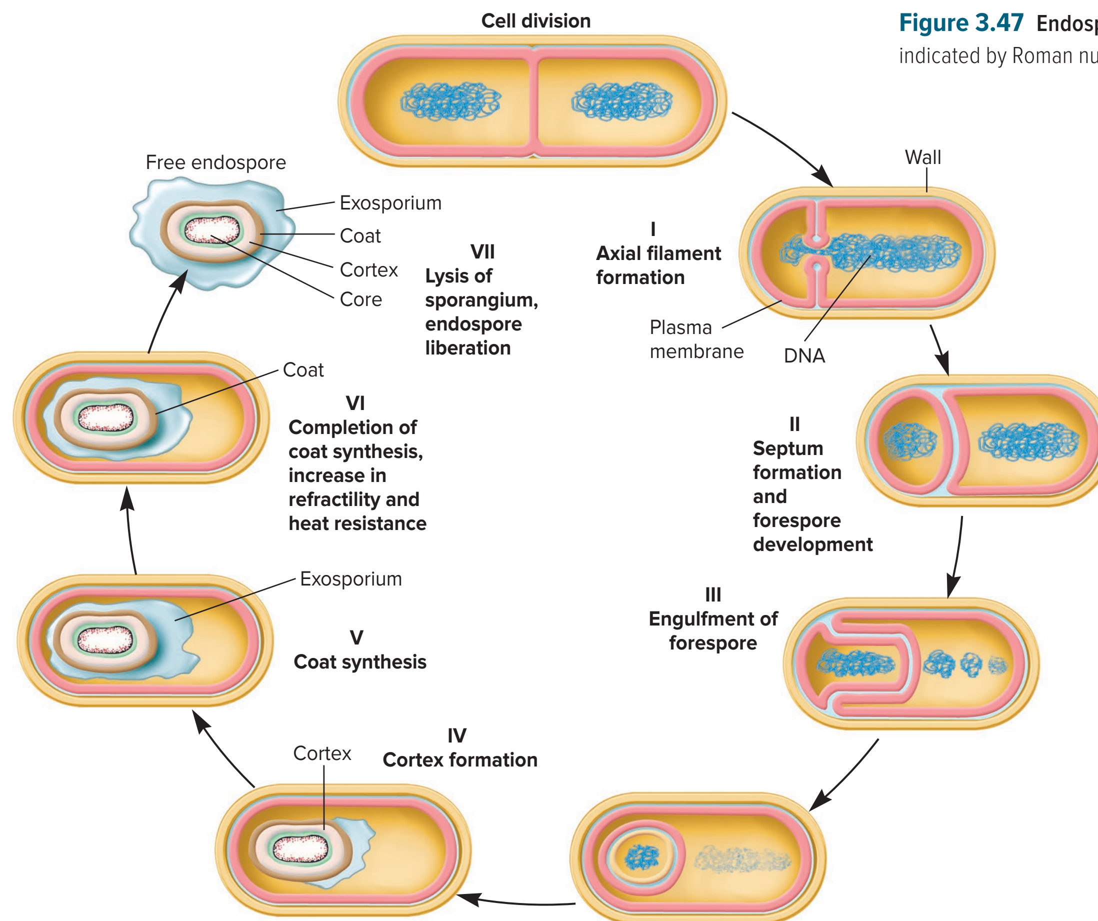

Figure 3.47 Endospore Formation. The stages are indicated by Roman numerals.

water levels inside the germinating endospore reach those characteristic of vegetative cells and enzymes in the core become active. This allows the endospore to begin synthesizing various molecules needed to initiate endospore outgrowth and return to a vegetative state. Because germination of some species' endospores results in disease or toxin production, inhibiting germination by blocking germinant receptors is an active area of research.

Comprehension Check

1. Describe the structure of the bacterial endospore using a labeled diagram.
2. Briefly describe endospore formation and germination. What is the importance of the endospore?
3. What features of the endospore contribute to its resistance to harsh conditions?

Key Concepts

3.1 Use of the Term "Prokaryote" Is Controversial

- Bacteria and archaea are related and share a common cell architecture. However, they have distinct characteristics and evolutionary lineages.

3.2 Bacteria Are Diverse but Share Some Common Features

- Rods (bacilli) and cocci (spheres) are common bacterial shapes. In addition, bacteria may be comma-shaped (vibrio), spiral (spirillum and spirochetes), or filamentous; they may form buds and stalks; or they may have no characteristic shape (pleomorphic) (**figures 3.1 and 3.2**).
- Some cells remain together after division to form pairs, chains, and clusters of various sizes and shapes.
- Frequently observed bacterial cell structures include a cell wall, plasma membrane, cytoplasm, nucleoid, fimbriae, inclusions, capsule, ribosomes, and flagella (**figure 3.6**). **Table 3.1** summarizes the major functions of these structures.

3.3 Bacterial Plasma Membranes Control What Enters and Leaves the Cell

- The cell envelope consists of the plasma membrane and all external coverings, including the cell wall and other layers (e.g., capsules).
- The bacterial plasma membrane fulfills many roles, including acting as a semipermeable barrier, carrying out respiration and photosynthesis, and detecting and responding to chemicals in the environment.
- The fluid mosaic model proposes that cell membranes are lipid bilayers in which integral membrane proteins are buried. Peripheral membrane proteins are loosely associated with the membrane (**figure 3.7**).
- Bacterial membranes are bilayers composed of phospholipids constructed of fatty acids connected to glycerol by ester linkages (**figure 3.8**). Bacterial membranes include microdomains characterized by an altered lipid composition, in which large protein complexes are assembled by flotillins.
- Microorganisms require nutrients, materials that are used in energy conservation and biosynthesis. Macronutrients are needed in relatively large quantities. Micronutrients (trace elements) are used in very small amounts.
- In passive diffusion, a substance moves through the membrane on its own. Movement is down a concentration gradient and does not require an input of energy. Only a few substances enter bacteria by passive diffusion.
- Transport proteins are divided into two major types: channels and carriers. Channels create a pore through which a substance moves. Carriers are so called because they carry the molecule across the membrane.
- In facilitated diffusion, a transport protein (either a channel or a carrier) helps move substances in the direction of decreasing concentration; no metabolic energy is required (**figure 3.11**). Facilitated diffusion is not an important uptake mechanism for bacterial cells.
- Active transport systems use metabolic energy and carrier proteins to concentrate substances by transporting them against a gradient. ATP is used as an energy source by ABC transporters, a type of primary active transporter (**figure 3.13**). ABC transporters are uniporters (one substance is transported). Gradients of ions drive solute uptake in secondary active transport systems. Secondary active transporters are either symporters (two substances are transported in the same direction) or antiporters (two substances are transported in opposite directions) (**figure 3.12**).
- Group translocation is a type of active transport in which bacterial cells transport organic molecules while modifying them (**figure 3.14**).
- The secretion of siderophores, which bind ferric iron, enables the accumulation of iron within bacterial cells (**figure 3.15**).

3.4 There Are Two Main Types of Bacterial Cell Walls

- The vast majority of bacteria have a cell wall outside the plasma membrane to help maintain their shape and protect them from osmotic stress.
- Bacterial walls are chemically complex and usually contain peptidoglycan (**figures 3.16–3.21**). Typical Gram-positive walls have thick, homogeneous layers of peptidoglycan and teichoic acids (**figures 3.22** and **3.23**). Typical Gram-negative bacteria have a thin peptidoglycan

layer surrounded by a complex outer membrane containing lipopolysaccharides (LPSs) and other components (**figures 3.24** and **3.25**). The lipid A portion of LPS is also called endotoxin. Its release into the human body can lead to septic shock.
- The mechanism of the Gram stain is thought to depend on the thickness of peptidoglycan. The thick peptidoglycan of typical Gram-positive bacteria binds crystal violet tightly, preventing its loss during the ethanol wash.

3.5 The Cell Envelope Often Includes Layers Outside the Cell Wall

- Capsules, slime layers, and glycocalyces are layers of material lying outside the cell wall. They can protect cells from certain environmental conditions, allow cells to attach to surfaces, and protect pathogenic bacteria from host defenses (**figure 3.28**).
- S-layers are the external-most layer in some bacteria. They are composed of proteins or glycoprotein and have a characteristic geometric shape (**figure 3.29**).

3.6 The Bacterial Cytoplasm Is More Complex than Once Thought

- The bacterial cytoplasm is a highly concentrated and crowded solution of biochemicals.
- The bacterial cytoplasm contains proteins that are similar in structure and function to the cytoskeletal proteins observed in eukaryotes (**figure 3.30** and **table 3.2**).
- Some bacteria have simple internal membrane systems containing photosynthetic and respiratory machinery (**figure 3.31**).
- Inclusions are observed in all cells (**figure 3.33**). Most are used for storage (e.g., PHB inclusions and polyphosphate granules), but some are used for other purposes (e.g., magnetosomes and gas vacuoles). Microcompartments such as carboxysomes contain enzymes that catalyze important reactions (e.g., CO_2 fixation).
- Bacterial ribosomes are 70S in size. They are composed of numerous proteins and several rRNA molecules (**figure 3.34**).
- The genetic material of bacterial cells is located in an area within the cytoplasm called the nucleoid. The nucleoid is not usually enclosed by a membrane (**figure 3.35**). The chromosome usually consists of a double-stranded, covalently closed, circular DNA molecule.
- Plasmids are extrachromosomal DNA molecules found in many bacteria. Some are episomes—plasmids that exist freely in the cytoplasm or can be integrated into the chromosome. Although plasmids are not required for survival in most conditions, they can encode traits that confer selective advantage in some environments. Many types of plasmids have been identified (**table 3.3**).

3.7 Many Bacteria Have External Structures Used for Attachment and Motility

- Many bacteria have hairlike appendages called fimbriae or pili. Fimbriae function primarily in attachment to surfaces, but type IV pili are involved in twitching motility. Sex pili participate in the transfer of DNA from one bacterium to another (**figure 3.36**).
- Many bacteria are motile, often by means of threadlike, locomotory organelles called flagella. Bacterial species differ in the number and distribution of their flagella (**figure 3.37**). Each bacterial flagellum is composed of a filament, hook, and basal body (**figure 3.38**).

3.8 Bacteria Move in Response to Environmental Conditions

- Several types of bacterial motility have been observed: swimming by flagella, swarming by flagella, spirochete motility, twitching motility, and gliding motility.
- The bacterial flagellar filament is a rigid helix that rotates like a propeller to push the bacterium through water (**figures 3.40** and **3.41**). When many bacteria swim, they alternate between two types of movement: runs and tumbles.
- Some bacterial species move as a group in a behavior called swarming. Swarming is mediated by flagella and occurs on moist surfaces.
- Spirochete motility is brought about by flagella that are wound around the cell and remain within the periplasmic space. When they rotate, the outer membrane of the spirochete is thought to rotate, thus moving the cell (**figure 3.43**).
- *Myxococcus* spp. exhibit both twitching and gliding motility. Twitching motility is a jerky movement brought about by type IV pili, whereas gliding motility is smooth.
- Motile cells can respond to gradients of attractants and repellents, a phenomenon known as chemotaxis. *E. coli* and many other peritrichously flagellated bacteria accomplish movement toward an attractant by increasing the length of time spent moving toward the attractant and shortening the time spent tumbling (**figure 3.44**). Similarly, they increase their run time when they move away from a repellent.

3.9 Bacterial Endospores Are a Survival Strategy

- Some bacteria survive adverse environmental conditions by forming endospores, dormant structures resistant to heat, desiccation, and many chemicals (**figure 3.45**).
- Both endospore formation and germination are complex processes made in response to certain environmental signals (**figure 3.47**).

Active Learning

1. Propose a model for the assembly of a flagellum in a typical Gram-positive cell envelope.
2. The peptidoglycan of bacteria has been compared with the chain mail worn beneath a medieval knight's suit of armor. It provides both protection and flexibility. Describe other structures in biology that have an analogous function. How are they replaced or modified to accommodate the growth of the inhabitant?
3. Why might a microbe have more than one uptake system for certain substrates?
4. Design a demonstration to illustrate the cell wall's role in protecting against lysis.
5. What would you expect to observe if you were able to "transplant" CreS into a rod-shaped bacterium such as *Bacillus subtilis*?
6. Develop a hypothesis to explain why gas vacuoles are bounded by proteins rather than a lipid bilayer membrane.
7. Support or refute the statement that the periplasm is an organelle because it is a membrane-bound compartment of the bacterial cell.
8. Calculate the surface area-to-volume ratio of an ultrasmall bacterium, a coccus of 0.2 µm diameter, and compare it to the ratios calculated in figure 3.5.
9. *Kingella kingae* colonizes the pharynx in young children. It has three distinct surface components that may be responsible for attachment to host cells: a type IV pilus (T4P) that confers twitching motility, a protein adhesin, and a polysaccharide capsule on its outer membrane. To study attachment, four variants were created: one lacking the T4P, one with a T4P unable to twitch, one lacking the adhesin, and one lacking the capsule. Why do you think the researchers created the strain with the disabled T4P?

   Dimensions of these components were measured: The T4P was several microns in length, the adhesin extended about 110 nm from the surface, and the capsule was 700 nm deep. Micrographs of *Kingella* cells adhered to host cells show that the capsule was compressed significantly when the bacteria were in contact with a host cell. Sketch these structures to help visualize the cell surface. The most important determinants in adherence were a functional, twitching T4P and the adhesin. Considering the geometry of the cell surface, how might these two components collaborate in attachment of the bacterium?

   Read the original paper: Kern, B. K., et al. 2017. Defining the mechanical determinants of *Kingella kingae* adherence to host cells. *J. Bacteriol.* 199(23):e00314-17.