
Jawetz, Melnick & Adelberg's
MEDICAL
MICROBIOLOGY
Stefan Riedel
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28th Edition
Mc Graw Hill
LANGE

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Jawetz, Melnick, & Adelberg's Medical Microbiology

Twenty-Eighth Edition

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ISBN: 978-1-26-001203-3
MHID: 1-26-001203-4

The material in this eBook also appears in the print version of this title: ISBN: 978-1-26-001202-6,
MHID: 1-26-001202-6.

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Preface

As all the prior editions of this textbook before, the twenty-eighth edition of *Jawetz, Melnick, & Adelberg's Medical Microbiology* remains true to the goals of the first edition published in 1954, which is to "to provide a brief, accurate and up-to-date presentation of those aspects of medical microbiology that are of particular significance to the fields of clinical infections and chemotherapy."

For the twenty-seventh edition, under the authorship of Dr. Karen Carroll, all chapters had been extensively revised, reflecting the tremendous expansion of medical knowledge afforded by molecular mechanisms and diagnostics, advances in our understanding of microbial pathogenesis, and the discovery of novel pathogens. While Dr. Carroll decided to step down as an author and contributor for this new edition, the remaining authors would like to express their gratitude for her leadership and contributions to the previous, greatly expanded edition. For the 28th edition, Chapter 47, "Principles of Diagnostic Medical Microbiology," and Chapter 48, "Cases and Clinical Correlations," were again updated to reflect the continued expansion in laboratory diagnostics as well as new antimicrobial therapies in the treatment of infectious diseases. Chapter 48 was specifically updated to reflect clinically important and currently emerging infectious disease cases.

New to this edition are Peter Hotez, MD, PhD, Rojelio Mejia, MD, and Stefan Riedel, MD, PhD, D(ABMM). Dr. Hotez is the Dean of the National School of Tropical Medicine at Baylor College of Medicine in Houston, TX, and is a Professor of Pediatrics, Molecular Virology and Microbiology; he brings extensive expertise in parasitology. Dr. Mejia is an Assistant Professor in the Department of Pediatrics, Section of Tropical Medicine, at the National School of Tropical Medicine, Baylor College of Medicine in Houston, TX. Dr. Riedel is the Associate Medical Director of the Clinical Microbiology Laboratories at Beth Israel Deaconess Medical Center in Boston, MA, and holds the academic rank of Associate Professor of Pathology at Harvard Medical School. Following Dr. Carroll's departure as an author and contributor to this textbook, Dr. Riedel assumed the role as Editor-in-Chief for this revised, 28th edition of the textbook.

The authors hope that the changes to this current edition will continue to be helpful to the student of microbiology and infectious diseases.

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SECTION I FUNDAMENTALS OF MICROBIOLOGY

CHAPTER 1

The Science of Microbiology

INTRODUCTION

Microbiology is the study of microorganisms, a large and diverse group of microscopic organisms that exist as single cells or cell clusters; it also includes viruses, which are microscopic but not cellular. Microorganisms have a tremendous impact on all life and the physical and chemical makeup of our planet. They are responsible for cycling the chemical elements essential for life, including carbon, nitrogen, sulfur, hydrogen, and oxygen; more photosynthesis is carried out by microorganisms than by green plants. Furthermore, there are 100 million times as many bacteria in the oceans (13×10^{28}) as there are stars in the known universe. The rate of viral infections in the oceans is about 1×10^{23} infections per second, and these infections remove 20–40% of all bacterial cells each day. It has been estimated that 5×10^{30} microbial cells exist on earth; excluding cellulose, these cells constitute about 90% of the biomass of the entire biosphere. Humans also have an intimate relationship with microorganisms; 50–60% of the cells in our bodies are microbes (see Chapter 10). The bacteria present in the average human gut weigh about 1 kg, and a human adult will excrete his or her own weight in fecal bacteria each year. The number of genes contained within this gut flora outnumber that contained within our genome by 150-fold; even in our own genome, 8% of the DNA is derived from remnants of viral genomes.

BIOLOGIC PRINCIPLES ILLUSTRATED BY MICROBIOLOGY

Nowhere is **biologic diversity** demonstrated more dramatically than by microorganisms, cells, or viruses that are not directly visible to the unaided eye. In form and function, be it biochemical property or genetic mechanism, analysis of microorganisms takes us to the limits of biologic understanding. Thus, the need for **originality**—one test of the merit of a scientific **hypothesis**—can be fully met in microbiology. A useful hypothesis should provide a basis for **generalization**, and microbial diversity provides an arena in which this challenge is ever present.

Prediction, the practical outgrowth of science, is a product created by a blend of technique and theory. **Biochemistry**, **molecular biology**, and **genetics** provide the tools required for analysis of microorganisms. **Microbiology**, in turn, extends the horizons of these scientific disciplines. A biologist might describe such an exchange as **mutualism**, that is, one that benefits all contributing parties. Lichens are an example of microbial mutualism. Lichens consist of a fungus and phototropic partner, either an alga (a eukaryote) or a cyanobacterium (a prokaryote) (Figure 1-1). The phototropic component is the primary producer, and the fungus provides the phototroph with an anchor and protection from the elements. In biology, mutualism is called **symbiosis**, a continuing association of different organisms. If the exchange operates primarily to the benefit of one party, the association is described as **parasitism**, a relationship in which a **host** provides the primary benefit to the parasite. Isolation and characterization of a parasite—such as a pathogenic bacterium or virus—often require effective mimicry in the laboratory of the growth environment provided by host cells. This demand sometimes represents a major challenge to investigators.

The terms *mutualism*, *symbiosis*, and *parasitism* relate to the science of **ecology**, and the principles of environmental biology are implicit in microbiology. Microorganisms are the products of **evolution**, the biologic consequence of **natural**

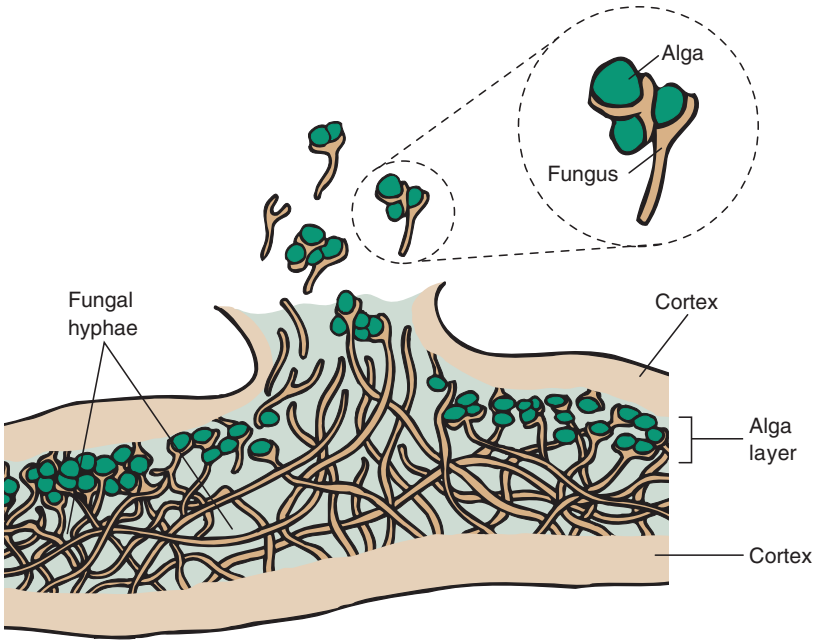

FIGURE 1-1 Diagram of a lichen, consisting of cells of a phototroph, either an alga or a cyanobacterium, entwined within the hyphae of the fungal partner. (Reproduced with permission from Nester EW, Anderson DG, Roberts CE, et al: *Microbiology: A Human Perspective*, 6th ed. McGraw-Hill, 2009, p. 293. © McGraw-Hill Education.)

selection operating on a vast array of genetically diverse organisms. It is useful to keep the complexity of natural history in mind before generalizing about microorganisms, the most heterogeneous subset of all living creatures.

A major biologic division separates the eukaryotes, organisms containing a membrane-bound nucleus from prokaryotes, organisms in which DNA is not physically separated from the cytoplasm. As described in this chapter and in Chapter 2, further major distinctions can be made between eukaryotes and prokaryotes. Eukaryotes, for example, are distinguished by their relatively large size and by the presence of specialized membrane-bound organelles such as mitochondria.

As described more fully later in this chapter, eukaryotic microorganisms—or, phylogenetically speaking, the Eukarya—are unified by their distinct cell structure and phylogenetic history. Among the groups of eukaryotic microorganisms are the **algae**, the **protozoa**, the **fungi**, and the **slime molds**. A class of microorganisms that share characteristics common to both prokaryotes and eukaryotes are the archaebacteria and are described in Chapter 3.

VIRUSES

The unique properties of viruses set them apart from living creatures. Viruses lack many of the attributes of cells, including the ability to self-replicate. Only when it infects a cell does a virus acquire the key attribute of a living system—reproduction. Viruses are known to infect a wide variety of plant and animal hosts as well as protists, fungi, and bacteria. However, most viruses are restricted to infecting specific types of cells of only one host species, a property known as "**tropism**". Recently, viruses called **virophages** have been discovered that infect other viruses. Host–virus interactions tend to be highly specific, and the biologic range of viruses mirrors the diversity of potential host cells. Further diversity of viruses is exhibited by their broad array of strategies for replication and survival.

Viral particles are generally small (eg, adenovirus has a diameter of 90 nm) and consist of a nucleic acid molecule, either DNA or RNA, enclosed in a protein coat, or capsid (sometimes itself surrounded by an envelope of lipids, proteins, and carbohydrates). Proteins—frequently glycoproteins—comprising the capsid and/or making up part of the lipid envelope (e.g., HIV gp120) determine the specificity of interaction of a virus with its host cell. The capsid protects the nucleic acid cargo. The surface proteins, whether they are externally exposed on the capsid or associated with the envelope facilitates attachment and penetration of the host cell by the virus. Once inside the cell, viral nucleic acid redirects the host's enzymatic machinery to functions associated with replication and assembly of the virus. In some cases, genetic information from the virus can be incorporated as DNA into a host chromosome (a **provirus**). In other instances, the viral genetic information can serve as a basis for cellular manufacture and release of copies of the virus. This process calls for replication of the viral nucleic acid and production of specific viral proteins. **Maturation** consists of assembling newly synthesized nucleic acid and protein subunits into mature

viral particles, which are then liberated into the extracellular environment. Some very small viruses require the assistance of another virus in the host cell for their replication. The delta agent, also known as hepatitis D virus (HDV), has a RNA genome that is too small to code for even a single capsid protein (the only HDV-encoded protein is delta antigen) and needs help from hepatitis B virus for packaging and transmission.

Some viruses are large and complex. For example, Mimivirus, a DNA virus infecting *Acanthamoeba*, a free-living soil ameba, has a diameter of 400–500 nm and a genome that encodes 979 proteins, including the first four aminoacyl tRNA synthetases ever found outside of cellular organisms. This virus also encodes enzymes for polysaccharide biosynthesis, a process typically performed by the infected cell. An even larger marine virus has recently been discovered (*Megavirus*); its genome (1,259,197-bp) encodes 1120 putative proteins and is larger than that of some bacteria (see Table 7-1). Because of their large size, these viruses resemble bacteria when observed in stained preparations by light microscopy; however, they do not undergo cell division or contain ribosomes.

Several transmissible plant diseases are caused by **viroids**—small, single-stranded, covalently closed circular RNA molecules existing as highly base-paired rod-like structures. They range in size from 246 to 375 nucleotides in length. The extracellular form of the viroid is naked RNA—there is no capsid of any kind. The RNA molecule contains no protein-encoding genes, and the viroid is therefore totally dependent on host functions for its replication. Viroid RNA is replicated by the DNA-dependent RNA polymerase of the plant host; preemption of this enzyme may contribute to viroid pathogenicity.

The RNAs of viroids have been shown to contain inverted repeated base sequences (also known as insertion sequences) at their 3′ and 5′ ends, a characteristic of transposable elements (see Chapter 7) and retroviruses. Thus, it is likely that they have evolved from transposable elements or retroviruses by the deletion of internal sequences.

The general properties of animal viruses pathogenic for humans are described in Chapter 29. Bacterial viruses, known as bacterial phages, are described in Chapter 7.

PRIONS

A number of remarkable discoveries in the past three decades have led to the molecular and genetic characterization of the transmissible agent causing **scrapie**, a degenerative central nervous system disease of sheep. Studies have identified a specific protein in preparations from scrapie-infected brains of sheep that can reproduce the symptoms of scrapie in previously uninfected sheep (Figure 1-2). Attempts to identify additional components, such as nucleic acid, have been unsuccessful. To distinguish this agent from viruses and viroids, the term *prion* was introduced to emphasize its proteinaceous and infectious nature. The

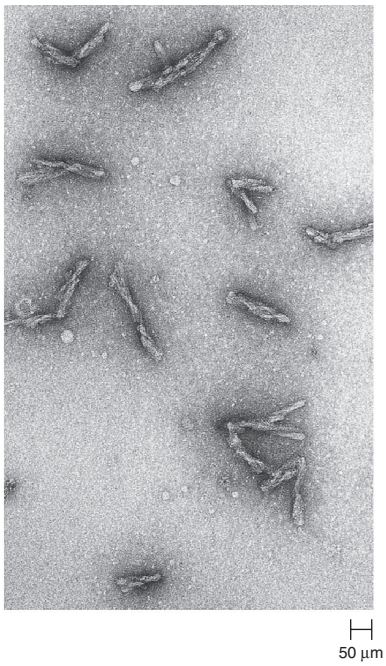

FIGURE 1-2 Prion. Prions isolated from the brain of a scrapie-infected hamster. This neurodegenerative disease is caused by a prion. (Reproduced with permission from Stanley B. Prusiner.)

protein that **prions** are made of (PrP) is found throughout the body, even in healthy people and in animals, and is encoded by the host's chromosomal DNA. The normal form of the prion protein is called PrP^{c}. PrP^{c} is a sialoglycoprotein with a molecular mass of 35,000–36,000 Da and a mainly α-helical secondary structure that is sensitive to proteases and soluble in detergent. Several topological forms exist: one cell surface form anchored by a glycolipid, and two transmembrane forms. The disease scrapie manifests itself when a conformational change occurs in the prion protein, changing it from its normal or cellular form PrP^{c} to the infectious disease-causing isoform, PrP^{Sc} (Figure 1-3); this in turn alters the way the proteins interconnect. The exact three-dimensional structure of PrP^{Sc} is unknown; however, it has a higher proportion of β-sheet structures in place of the normal α-helix structures. Aggregations of PrP^{Sc} form highly structured **amyloid** fibers, which accumulate to form plaques. It is unclear if these aggregates are the cause of the cell damage or are simply a side effect of the underlying disease process. One model of prion replication suggests that PrP^{c} exists only as fibrils, and that the fibril ends bind PrP^{c} and convert it to PrP^{Sc}.

There are several prion diseases of importance (Table 1-1 and see Chapter 42). Kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia affect humans. Bovine spongiform encephalopathy (BSE), which is thought to result from the ingestion of feeds and bone meal prepared from rendered sheep offal, has been responsible for the deaths of more than 184,000 cattle in Great Britain since its discovery in 1985. A new variant

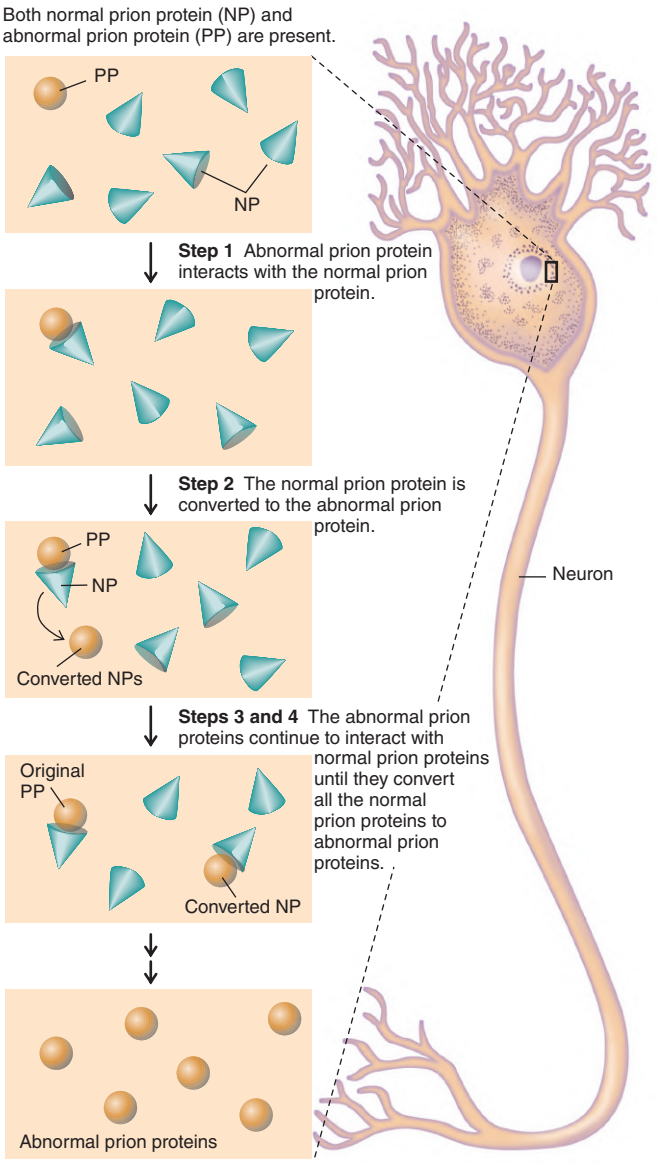

FIGURE 1-3 Proposed mechanism by which prions replicate. The normal and abnormal prion proteins differ in their tertiary structure. (Reproduced with permission from Nester EW, Anderson DG, Roberts CE, et al: *Microbiology: A Human Perspective*, 6th ed. McGraw-Hill, 2009, p. 342. © McGraw-Hill Education.)

of CJD (vCJD) has been associated with human ingestion of prion-infected beef in the United Kingdom and in France. A common feature of all of these diseases is the conversion of a host-encoded sialoglycoprotein to a protease-resistant form as a consequence of infection. Recently, an **α-synuclein** prion was discovered that caused a neurodegenerative disease called multiple system atrophy in humans.

Human prion diseases are unique in that they manifest as sporadic, genetic, and infectious diseases. The study of prion biology is an important emerging area of biomedical investigation, and much remains to be learned.

The general features of the nonliving members of the microbial world are given in Table 1-2.

PROKARYOTES

The primary distinguishing characteristics of the prokaryotes are their relatively small size, usually on the order of 1 μm in diameter, and the absence of a nuclear membrane. The DNA of almost all bacteria is a circle which if extended linearly would be about 1 mM; this is the prokaryotic chromosome. Bacteria are **haploid** (if multiple copies of the chromosome are present they are all the same). Most prokaryotes have only a single large chromosome that is organized into a structure known as a **nucleoid**. The chromosomal DNA must be folded more than 1000-fold just to fit within the confines of a prokaryotic cell. Substantial evidence suggests that the folding may be orderly and may bring specified regions of the DNA into proximity. The **nucleoid** can be visualized by electron microscopy as well as by light microscopy after treatment of the cell to make the nucleoid visible. Thus, it would be a mistake to conclude that subcellular differentiation, clearly demarcated by membranes in eukaryotes, is lacking in prokaryotes. Indeed, some prokaryotes form membrane-bound subcellular structures with specialized function such as the chromatophores of photosynthetic bacteria (see Chapter 2).

Prokaryotic Diversity

The small size and haploid organization of the prokaryotic chromosome limits the amount of genetic information it can contain. Recent data based on genome sequencing indicate that the number of genes within a prokaryote may vary from 468 in *Mycoplasma genitalium* to 7825 in *Streptomyces coelicolor*, and many of these genes must be dedicated to essential functions such as energy generation, macromolecular synthesis, and cellular replication. Any one prokaryote carries relatively few genes that allow physiologic accommodation of the organism to its environment. The range of potential prokaryotic environments is unimaginably broad, and it follows that the prokaryotic group encompasses a heterogeneous range of specialists, each adapted to a rather narrowly circumscribed niche.

The range of prokaryotic niches is illustrated by consideration of strategies used for generation of metabolic energy. Light from the sun is the chief source of energy for life. Some prokaryotes such as the purple bacteria convert light energy to metabolic energy in the absence of oxygen production. Other prokaryotes, exemplified by the blue-green bacteria (**Cyanobacteria**), produce oxygen that can provide energy through respiration in the absence of light. **Aerobic organisms** depend on respiration with oxygen for their energy. Some **anaerobic organisms** can use electron acceptors other than oxygen in respiration. Many anaerobes carry out **fermentations** in which energy is derived by metabolic rearrangement of chemical growth substrates. The tremendous chemical range of potential growth substrates for aerobic or anaerobic growth is mirrored in the diversity of prokaryotes that have adapted to their utilization.

TABLE 1-1 Common Human and Animal Prion Diseases

Type	Name	Etiology
Human prion diseases		
Acquired	Variant Creutzfeldt-Jakob disease[a]	Associated with ingestion or inoculation of prion-infected material
	Kuru	
	Iatrogenic Creutzfeldt-Jakob disease[b]	
Sporadic	Creutzfeldt-Jakob disease	Source of infection unknown
Familial	Gerstmann-Sträussler-Scheinker	Associated with specific mutations within the gene encoding PrP
	Fatal familial insomnia	
	Creutzfeldt-Jakob disease	
Animal prion diseases		
Cattle	Bovine spongiform encephalopathy	Exposure to prion-contaminated meat and bone meal
Sheep	Scrapie	Ingestion of scrapie-contaminated material
Deer, elk	Chronic wasting disease	Ingestion of prion-contaminated material
Mink	Transmissible mink encephalopathy	Source of infection unknown
Cats	Feline spongiform encephalopathy[a]	Exposure to prion-contaminated meat and bone meal

PrP, prion protein.

[a]Associated with exposure to bovine spongiform encephalopathy-contaminated materials.

[b]Associated with prion-contaminated biologic materials, such as dura mater grafts, corneal transplants, and cadaver-derived human growth hormone, or by prion-contaminated surgical instruments.

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Prokaryotic Communities

A useful survival strategy for specialists is to enter into **consortia**, arrangements in which the physiologic characteristics of different organisms contribute to survival of the group as a whole. If the organisms within a physically interconnected community are directly derived from a single cell, the community is a **clone** that may contain up to 10^8 or greater cells. The biology of such a community differs substantially from that of a single cell. For example, the high cell number virtually ensures the presence within the clone of at least one cell carrying a variant of any gene on the chromosome. Thus, genetic variability—the wellspring of the evolutionary process called natural selection—is ensured within a clone. The high number of cells within clones is also likely to provide physiologic protection to at least some members of the group. Extracellular polysaccharides, for example, may afford protection against potentially lethal agents such as antibiotics or heavy metal ions. Large amounts of polysaccharides produced by the high number of cells within a clone may allow cells within the interior to survive exposure to a lethal agent at a concentration that might kill single cells.

TABLE 1-2 Distinguishing Characteristics of Viruses, Viroids, and Prions

Viruses	Viroids	Prions
Obligate intracellular agents	Obligate intracellular agents	Abnormal form of a cellular protein
Consist of either DNA or RNA surrounded by a protein coat	Consist only of RNA; no protein coat	Consist only of protein; no DNA or RNA

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Many bacteria exploit a cell–cell communication mechanism called **quorum sensing** to regulate the transcription of genes involved in diverse physiologic processes, including bioluminescence, plasmid conjugal transfer, and the production of virulence determinants. Quorum sensing depends on the production of one or more diffusible signal molecules (eg, acetylated homoserine lactone [AHL]) termed **autoinducers** or **pheromones** that enable a bacterium to monitor its own cell population density (Figure 1-4). The cooperative activities leading to **biofilm** formation are controlled by quorum sensing. It is an example of multicellular behavior in prokaryotes.

Another distinguishing characteristic of prokaryotes is their capacity to exchange small packets of genetic information. This information may be carried on **plasmids**, small and specialized genetic elements that are capable of replication within at least one prokaryotic cell line. In some cases, plasmids may be transferred from one cell to another and thus may carry sets of specialized genetic information through a population. Some plasmids exhibit a **broad host range** that allows them to convey sets of genes to diverse organisms. Of particular concern

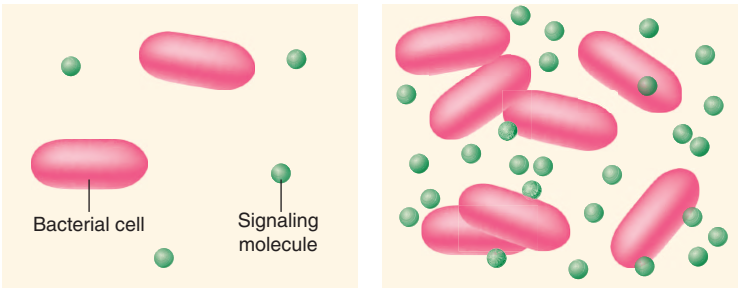

FIGURE 1-4 Quorum sensing. (Reproduced with permission from Nester EW, Anderson DG, Roberts CE, et al: *Microbiology: A Human Perspective*, 6th ed. McGraw-Hill, 2009, p. 181. © McGraw-Hill Education.)

are **drug resistance plasmids** that may render diverse bacteria resistant to antibiotic treatment (Chapter 7).

The survival strategy of a single prokaryotic cell line may lead to a range of interactions with other organisms. These may include **symbiotic** relationships illustrated by complex nutritional exchanges among organisms within the human gut. These exchanges benefit both the microorganisms and their human host. **Parasitic** interactions can be quite deleterious to the host. Advanced symbiosis or parasitism can lead to loss of functions that may not allow growth of the symbiont or parasite independent of its host.

The **mycoplasmas**, for example, are parasitic prokaryotes that have lost the ability to form a cell wall. Adaptation of these organisms to their parasitic environment has resulted in incorporation of a substantial quantity of cholesterol into their cell membranes. Cholesterol, not found in other prokaryotes, is assimilated from the metabolic environment provided by the host. Loss of function is exemplified also by obligate intracellular parasites, the **chlamydiae** and **rickettsiae**. These bacteria are extremely small (0.2–0.5 μm in diameter) and depend on the host cell for many essential metabolites and coenzymes. This loss of function is reflected by the presence of a smaller genome with fewer genes (see Table 7-1).

The most widely distributed examples of bacterial symbionts appear to be chloroplasts and mitochondria, the energy-yielding organelles of eukaryotes. Evidence points to the conclusion that ancestors of these chloroplasts and mitochondria were **endosymbionts**, essentially "domesticated bacteria" that established symbiosis within the cell membrane of the ancestral eukaryotic host. The presence of multiple copies of these organelles may have contributed to the relatively large size of eukaryotic cells and to their capacity for specialization, a trait ultimately reflected in the evolution of differentiated multicellular organisms.

Classification of the Prokaryotes

An understanding of any group of organisms requires their **classification**. An appropriate classification system allows a scientist to choose characteristics that allow swift and accurate categorization of a newly encountered organism. This categorical organization allows prediction of many additional traits shared by other members of the category. In a hospital setting, successful classification of a pathogenic organism may provide the most direct route to its elimination. Classification may also provide a broad understanding of relationships among different organisms, and such information may have great practical value. For example, elimination of a pathogenic organism will be relatively long-lasting if its habitat is occupied by a nonpathogenic variant.

The principles of prokaryotic classification are discussed in Chapter 3. At the outset, it should be recognized that any prokaryotic characteristic might serve as a potential criterion for classification. However, not all criteria are equally effective in grouping organisms. Possession of DNA, for example, is a useless criterion for distinguishing organisms because all cells contain DNA. The presence of a broad host range plasmid is not a useful criterion because such plasmids may be found in diverse hosts and need not be present all of the time. Useful criteria may be structural, physiologic, biochemical, or genetic. **Spores**—specialized cell structures that may allow survival in extreme environments—are useful structural criteria for classification because well-characterized subsets of bacteria form spores. Some bacterial groups can be effectively subdivided based upon their ability to ferment specified carbohydrates. Such criteria may be ineffective when applied to other bacterial groups that may lack any fermentative capability. A biochemical test, the **Gram-stain**, is an effective criterion for classification because response to the stain reflects fundamental differences in the bacterial cell envelope that divide most bacteria into two major groups.

Genetic criteria are increasingly used in bacterial classification, and many of these advances are made possible by the development of DNA-based technologies. It is now possible to design DNA probe or DNA amplification assays (eg, polymerase chain reaction [PCR] assays) that swiftly identify organisms carrying specified genetic regions with

common ancestry. Comparison of DNA sequences for some genes has led to the elucidation of **phylogenetic relationships** among prokaryotes. Ancestral cell lines can be traced, and organisms can be grouped based on their evolutionary affinities. These investigations have led to some striking conclusions. For example, comparison of cytochrome c sequences suggests that all eukaryotes, including humans, arose from one of three different groups of purple photosynthetic bacteria. This conclusion in part explains the evolutionary origin of eukaryotes, but it does not fully take into account the generally accepted view that the eukaryotic cell was derived from the evolutionary merger of different prokaryotic cell lines.

Bacteria and Archaebacteria: The Major Subdivisions Within the Prokaryotes

A major success in molecular phylogeny has been the demonstration that prokaryotes fall into two major groups. Most investigations have been directed to one group, the bacteria. The other group, the archaebacteria, has received relatively little attention until recently, partly because many of its representatives are difficult to study in the laboratory. Some archaebacteria, for example, are killed by contact with oxygen, and others grow at temperatures exceeding that of boiling water. Before molecular evidence became available, the major subgroupings of archaebacteria had seemed disparate. The methanogens carry out an anaerobic respiration that gives rise to methane, the halophiles demand extremely high salt concentrations for growth, and the thermoacidophiles require high temperature and acidity for growth. It has now been established that these prokaryotes share biochemical traits such as cell wall or membrane components that set the group entirely apart from all other living organisms. An intriguing trait shared by archaebacteria and eukaryotes is the presence of **introns** within genes. The function of introns—segments of DNA that interrupts informational DNA within genes—is not established. What is known is that introns represent a fundamental characteristic shared by the DNA of archaebacteria and eukaryotes. This common trait has led to the suggestion that—just as mitochondria and chloroplasts appear to be evolutionary derivatives of the bacteria—the eukaryotic nucleus may have arisen from an archaebacterial ancestor.

PROTISTS

The "true nucleus" of eukaryotes (from Gr *karyon*, "nucleus") is only one of their distinguishing features. The membrane-bound organelles, the microtubules, and the microfilaments of eukaryotes form a complex intracellular structure unlike that found in prokaryotes. The organelles responsible for the motility of eukaryotic cells are flagella or cilia—complex multistranded structures that do not resemble the flagella of prokaryotes. Gene expression in eukaryotes takes place through a series of events achieving physiologic integration of the nucleus with the endoplasmic reticulum, a structure that has no counterpart in prokaryotes. Eukaryotes are set apart by the organization of their cellular DNA in chromosomes separated by a distinctive mitotic apparatus during cell division.

In general, genetic transfer among eukaryotes depends on fusion of **haploid gametes** to form a **diploid** cell containing a full set of genes derived from each gamete. The life cycle of many eukaryotes is almost entirely in the diploid state, a form not encountered in prokaryotes. Fusion of gametes to form reproductive progeny is a highly specific event and establishes the basis for eukaryotic **species**. This term can be applied only metaphorically to the prokaryotes, which exchange fragments of DNA through recombination. Currently, the term *protist* is used informally as a catch-all term for unicellular eukaryotic microorganisms. Because protists as a whole are **paraphyletic,** newer classification systems often split up traditional subdivisions or groups based on morphological or biochemical characteristics.

Traditionally, microbial eukaryotes—**protists**—are placed in one of the four following major groups: algae, protozoa, fungi, and slime molds. These traditional subdivisions, largely based on superficial commonalities, have been largely replaced by classification schemes based on **phylogenetics**. Molecular methods used by modern taxonomists have been used to generate data supporting the redistribution of some members of these groups into diverse and sometimes distantly related phyla. For example, the **water molds** are now considered to be closely related to photosynthetic organisms such as brown algae and diatoms.

Algae

The term *algae* has long been used to denote all organisms that produce O_2 as a product of photosynthesis. One former subgroup of these organisms—the blue-green algae, or cyanobacteria—are prokaryotic and no longer are termed algae. This classification is reserved exclusively for a large diverse group of photosynthetic eukaryotic organisms. Formerly, all algae were thought to contain chlorophyll in the photosynthetic membrane of their chloroplast, a subcellular organelle that is similar in structure to cyanobacteria. Modern taxonomic approaches have recognized that some algae lack chlorophyll and have a free-living heterotrophic or parasitic life style. Many algal species are unicellular microorganisms. Other algae may form extremely large multicellular structures. Kelps of brown algae sometimes are several hundred meters in length. Several algae produce toxins that are poisonous to humans and other animals. Dinoflagellates, a unicellular alga, are responsible for algal blooms, or **red tides**, in the ocean (Figure 1-5). Red tides caused by the dinoflagellate *Gonyaulax* species are serious because this organism produces potent neurotoxins such as

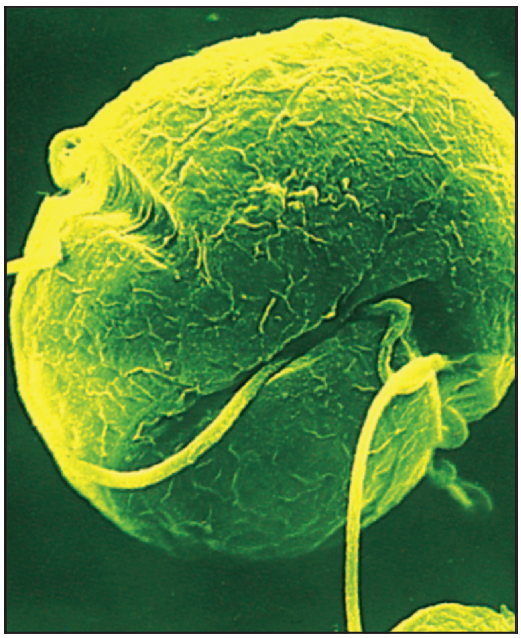

FIGURE 1-5 The dinoflagellate *Gymnodinium* scanning electron micrograph (4000×). (Reproduced with permission from Dr. David Phillips/Visuals Unlimited.)

saxitoxin and **gonyautoxins**, which accumulate in shellfish (eg, clams, mussels, scallops, and oysters) that feed on this organism. Ingestion of these shellfish by humans results in symptoms of **paralytic shellfish poisoning** and can lead to death. Some algae (eg, *Prototheca* and *Helicosporidium*) are parasites of metazoans or plants. **Protothecosis** is a disease of dogs, cats, cattle, and rarely humans caused by a type of algae, *Prototheca*, that lacks chlorophyll. The two most common species are *P. wickerhamii* and *P. zopfii*; most human cases, which are associated with a defective immune system, are caused by *P. wickerhamii*.

Protozoa

Protozoa is an informal term for single-celled nonphotosynthetic eukaryotes that are either free-living or parasitic. Protozoa are abundant in aqueous environments and soil. They range in size from as little as 1μm to several millimeters, or more. All protozoa are **heterotrophic**, deriving nutrients from other organisms, either by ingesting them whole or by consuming their organic tissue or waste products. Some protozoans take in food by **phagocytosis**, engulfing organic particles with **pseudopodia** (eg, amoeba), or taking in food through a mouth-like aperture called a **cytostome.** Other protozoans absorb dissolved nutrients through their cell membranes, a process called **osmotrophy**.

Historically, the major groups of protozoa included: **flagellates**, motile cells possessing whip-like organelles of locomotion; **amoebae**, cells that move by extending pseudopodia; and **ciliates**, cells possessing large numbers of short hair-like organelles of motility. Intermediate forms are known that have flagella at one stage in their life cycle and pseudopodia at another stage. A fourth major group of protozoa, the sporozoa, are strict parasites that are usually nonmotile; most of these reproduce sexually and asexually in alternate generations by means of spores. Recent taxonomic studies have shown that only the ciliates are **monophyletic**, that is, a distinct lineage of organisms sharing common ancestry. The other classes of protozoa are all **polyphyletic** groups made up of organisms that, despite similarities in appearance (eg, flagellates) or way of life (eg, endoparasitic), are not necessarily closely related to one another. Protozoan parasites of humans are discussed in Chapter 46.

Fungi

The fungi are nonphotosynthetic protists that may or may not grow as a mass of branching, interlacing filaments ("hyphae") known as a **mycelium**. If a fungus grows simply as a single cell it is called a **yeast**. If mycelial growth occurs, it is called a **mold**. Most fungi of medical importance grow dimorphically, that is, they exist as a mold at room temperature but as a yeast at body temperature. Remarkably, the largest known contiguous fungal mycelium covered an area of 2400 acres (9.7 km^2) at a site in eastern Oregon. Although the hyphae exhibit cross walls, the cross walls are perforated and allow free passage of nuclei and cytoplasm. The entire organism is thus a **coenocyte** (a multinucleated mass of continuous cytoplasm) confined within a series of branching tubes. These tubes, made of polysaccharides such as chitin, are homologous with cell walls.

The fungi probably represent an evolutionary offshoot of the protozoa; they are unrelated to the actinomycetes, mycelial bacteria that they superficially resemble. The major subdivisions (phyla) of fungi are Chytridiomycota, Zygomycota (the zygomycetes), Ascomycota (the ascomycetes), Basidiomycota (the basidiomycetes), and the "deuteromycetes" (or imperfect fungi). The evolution of the ascomycetes from the phycomycetes is seen in a transitional group, whose members form a zygote but then transform this directly into an ascus. The basidiomycetes are believed to have evolved in turn from the ascomycetes. The classification of fungi and their medical significance are discussed further in Chapter 45.

Slime Molds

These organisms are characterized by the presence, as a stage in their life cycle, of an ameboid multinucleate mass of cytoplasm called a **plasmodium**. The plasmodium of a slime mold is analogous to the mycelium of a true fungus. Both are coenocytic. Whereas in the latter, cytoplasmic flow is confined to the branching network of chitinous tubes, in the former, the cytoplasm can flow in all directions. This flow causes the plasmodium to migrate in the direction of its food source, frequently bacteria. In response to a chemical signal, 3′, 5′-cyclic AMP, the plasmodium, which reaches

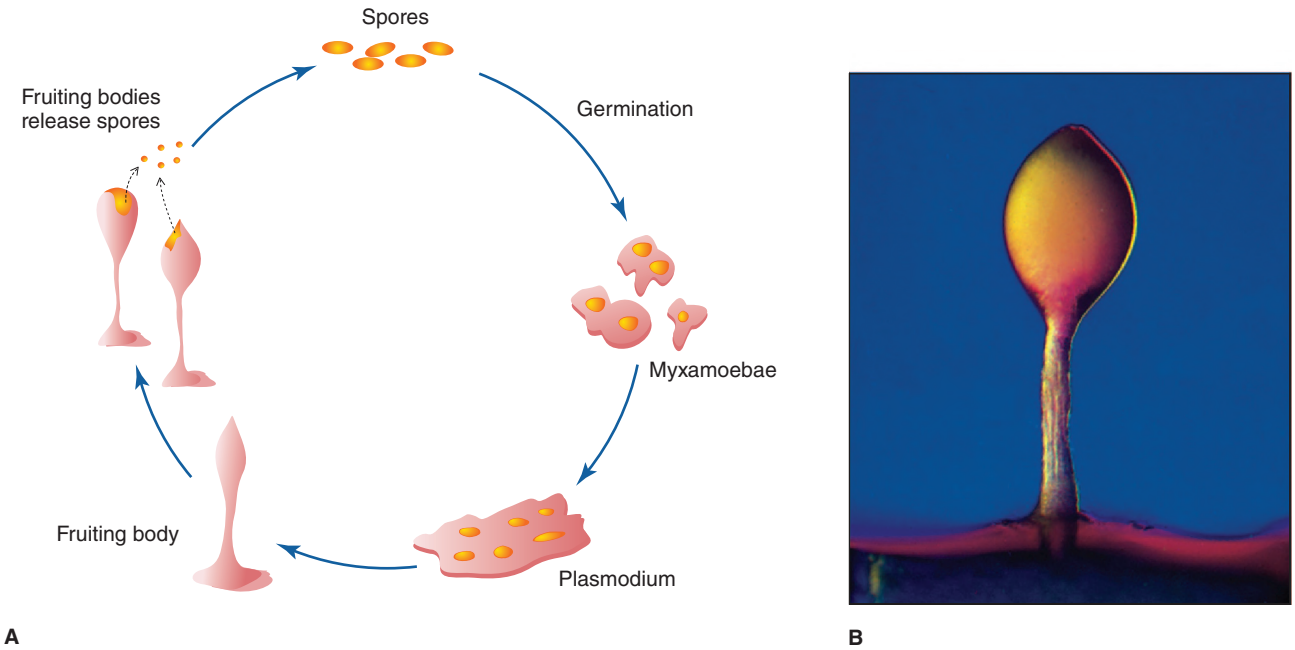

FIGURE 1-6 Slime molds. **A:** Life cycle of an acellular slime mold. **B:** Fruiting body of a cellular slime mold. (Reproduced with permission from Carolina Biological Supply/DIOMEDIA.)

macroscopic size, differentiates into a stalked body that can produce individual motile cells. These cells, flagellated or ameboid, initiate a new round in the life cycle of the slime mold (Figure 1-6). The cycle frequently is initiated by sexual fusion of single cells.

The growth of slime molds depends on nutrients provided by bacterial or, in some cases, plant cells. Reproduction of the slime molds via plasmodia can depend on intercellular recognition and fusion of cells from the same species. The life cycle of the slime molds illustrates a central theme of this chapter—the interdependency of living forms. Full understanding of any microorganism requires both knowledge of the other organisms with which it coevolved and an appreciation of the range of physiologic responses that may contribute to survival.

CHAPTER SUMMARY

- Microorganisms are a large and diverse group of organisms existing as single cells or clusters; they also include viruses, which are microscopic but not cellular.
- A virus consists of a nucleic acid molecule, either DNA or RNA, enclosed in a protein coat, or capsid, sometimes enclosed by an envelope composed of lipids, proteins, and carbohydrates.
- A prion is an infectious protein, which is capable of causing chronic neurologic diseases.
- Prokaryotes consist of bacteria and archaebacteria.
- Prokaryotes are haploid.
- Microbial eukaryotes, or protists, are members of four major groups: algae, protozoa, fungi, and slime molds.
- Eukaryotes have a true nucleus and are diploid.

REVIEW QUESTIONS

1. Which one of the following terms characterizes the interaction between herpes simplex virus and a human?
   (A) Parasitism
   (B) Symbiosis
   (C) Endosymbiosis
   (D) Endoparasitism
   (E) Consortia
2. Which one of the following agents lacks nucleic acid?
   (A) Bacteria
   (B) Viruses
   (C) Viroids
   (D) Prions
   (E) Protozoa
3. Which one of the following is a prokaryote?
   (A) Bacteria
   (B) Algae
   (C) Protozoa
   (D) Fungi
   (E) Slime molds
4. Which one of the following agents simultaneously contains both DNA and RNA?
   (A) Bacteria
   (B) Viruses
   (C) Viroids
   (D) Prions
   (E) Plasmids
5. Which of the following cannot be infected by viruses?
   (A) Bacteria
   (B) Protozoa
   (C) Human cells
   (D) Viruses
   (E) None of the above

6. Viruses, bacteria, and protists are uniquely characterized by their respective size. True or false?
   (A) True
   (B) False
7. Quorum sensing in prokaryotes involves
   (A) Cell–cell communication
   (B) Production of molecules such as AHL
   (C) An example of multicellular behavior
   (D) Regulation of genes involved in diverse physiologic processes
   (E) All of the above
8. A 16-year-old female patient presented to her family physician with a complaint of an abnormal vaginal discharge and pruritus (itching). The patient denied having sexual activity and recently completed a course of doxycycline for the treatment of her acne. An examination of a Gram-stained vaginal smear revealed the presence of Gram-positive oval cells about 4–8 μm in diameter. Her vaginitis is caused by which of the following agents?
   (A) Bacterium
   (B) Virus
   (C) Protozoa
   (D) Fungus
   (E) Prion
9. A 65-year-old man develops dementia, progressive over several months, along with ataxia and somnolence. An electroencephalographic pattern shows paroxysms with high voltages and slow waves, suggestive of CJD. By which of the following agents is this disease caused?
   (A) Bacterium
   (B) Virus
   (C) Viroid
   (D) Prion
   (E) Plasmid
10. Twenty minutes after ingesting a raw clam, a 35-year-old man experiences paresthesias of the mouth and extremities, headache, and ataxia. These symptoms are the result of a neurotoxin produced by algae called
    (A) Amoeba
    (B) Blue-green algae
    (C) Dinoflagellates
    (D) Kelp
    (E) None of the above

Answers

1. A
2. D
3. A
4. A
5. E
6. B
7. E
8. D
9. D
10. C

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CHAPTER 2

Cell Structure

This chapter discusses the basic structure and function of the components that make up eukaryotic and prokaryotic cells. It begins with a discussion of the microscope. Historically, the microscope first revealed the presence of bacteria and later the secrets of cell structure. Today, it remains a powerful tool in cell biology.

OPTICAL METHODS

The Light Microscope

The resolving power of the light microscope under ideal conditions is about half the wavelength of the light being used. (**Resolving power** is the distance that must separate two point sources of light if they are to be seen as two distinct images.) With yellow light of a wavelength of 0.4 μm, the smallest separable diameters are thus about 0.2 μm (ie, one-third the width of a typical prokaryotic cell). The useful magnification of a microscope is the magnification that makes visible the smallest resolvable particles. Several types of light microscopes, which are commonly used in microbiology, are discussed as follows.

A. Bright-Field Microscope

The bright-field microscope is the most commonly used in microbiology courses and consists of two series of lenses (**objective** and **ocular lens**), which function together to resolve the image. These microscopes generally employ a 100-power objective lens with a 10-power ocular lens, thus magnifying the specimen 1000 times. Particles 0.2 μm in diameter are therefore magnified to about 0.2 mm and so become clearly visible. Further magnification would give no greater resolution of detail and would reduce the visible area (**field**).

With this microscope, specimens are rendered visible because of the differences in **contrast** between them and the surrounding medium. Many bacteria are difficult to see well because of their lack of contrast with the surrounding medium. Dyes (stains) can be used to stain cells or their organelles and increase their contrast so that they can be more easily seen in the bright-field microscope.

B. Phase-Contrast Microscope

The phase-contrast microscope was developed to improve contrast differences between cells and the surrounding medium, making it possible to see living cells without staining them; with bright-field microscopes, killed and stained preparations must be used. The phase-contrast microscope takes advantage of the fact that light waves passing through transparent objects, such as cells, emerge in different phases depending on the properties of the materials through which they pass. This effect is amplified by a special ring in the objective lens of a phase-contrast microscope, leading to the formation of a dark image on a light background (Figure 2-1).

C. Dark-Field Microscope

The dark-field microscope is a light microscope in which the lighting system has been modified to reach the specimen from the sides only. This is accomplished through the use of a special condenser that both blocks direct light rays and deflects light off a mirror on the side of the condenser at an oblique angle. This creates a "dark field" that contrasts against the highlighted edge of the specimens and results when the oblique rays are reflected from the edge of the specimen upward into the objective of the microscope. Resolution by dark-field microscopy is quite high. Thus, this technique has been particularly useful for observing organisms such as *Treponema pallidum*, a spirochete that is smaller than 0.2 μm in diameter and therefore cannot be observed with a bright-field or phase-contrast microscope (Figure 2-2A).

D. Fluorescence Microscope

The fluorescence microscope is used to visualize specimens that **fluoresce**, which is the ability to absorb short wavelengths of light (ultraviolet) and give off light at a longer wavelength (visible). Some organisms fluoresce naturally because of the presence within the cells of naturally fluorescent substances such as chlorophyll. Those that do not naturally fluoresce may be stained with a group of fluorescent dyes called **fluorochromes**. Fluorescence microscopy is widely used in clinical diagnostic microbiology. For example, the fluorochrome auramine O, which glows yellow when exposed to ultraviolet light, is strongly absorbed by the cell envelope of

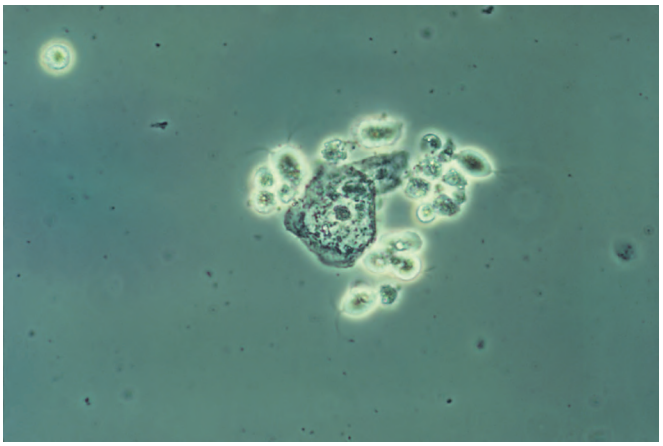


FIGURE 2-1 Using the phase contrast illumination technique, this photomicrograph of a wet mount of a vaginal discharge specimen revealed the presence of the flagellated protozoan, *Trichomonas vaginalis*. (Courtesy of Centers for Disease Control and Prevention, Public Health Image Library, ID# 5238.)

Mycobacterium tuberculosis, the bacterium that causes tuberculosis. When the dye is applied to a specimen suspected of containing *M. tuberculosis* and exposed to ultraviolet light, the bacterium can be detected by the appearance of bright yellow organisms against a dark background.

The principal use of fluorescence microscopy is a diagnostic technique called the **fluorescent-antibody (FA) technique** or **immunofluorescence**. In this technique, specific antibodies (eg, antibodies to *Legionella pneumophila*) are chemically labeled with a fluorochrome such as **fluorescein isothiocyanate (FITC)**. These fluorescent antibodies are then added to a microscope slide containing a clinical specimen. If the specimen contains *L. pneumophila*, the fluorescent antibodies will bind to antigens on the surface of the bacterium, causing it to fluoresce when exposed to ultraviolet light (Figure 2-2B).

E. Differential Interference Contrast Microscope

Differential interference contrast (DIC) microscopes employ a polarizer to produce polarized light. The polarized light beam passes through a prism that generates two distinct beams; these beams pass through the specimen and enter the objective lens, where they are recombined into a single beam. Because of slight differences in refractive index of the substances each beam passed through, the combined beams are not totally in phase but instead create an interference effect, which intensifies subtle differences in cell structure. Structures, such as spores, vacuoles, and granules, appear three dimensional. DIC microscopy is particularly useful for observing unstained cells because of its ability to generate images that reveal internal cell structures that are less apparent by bright-field techniques.

The Electron Microscope

The high resolving power of electron microscopes has enabled scientists to observe the detailed structures of prokaryotic

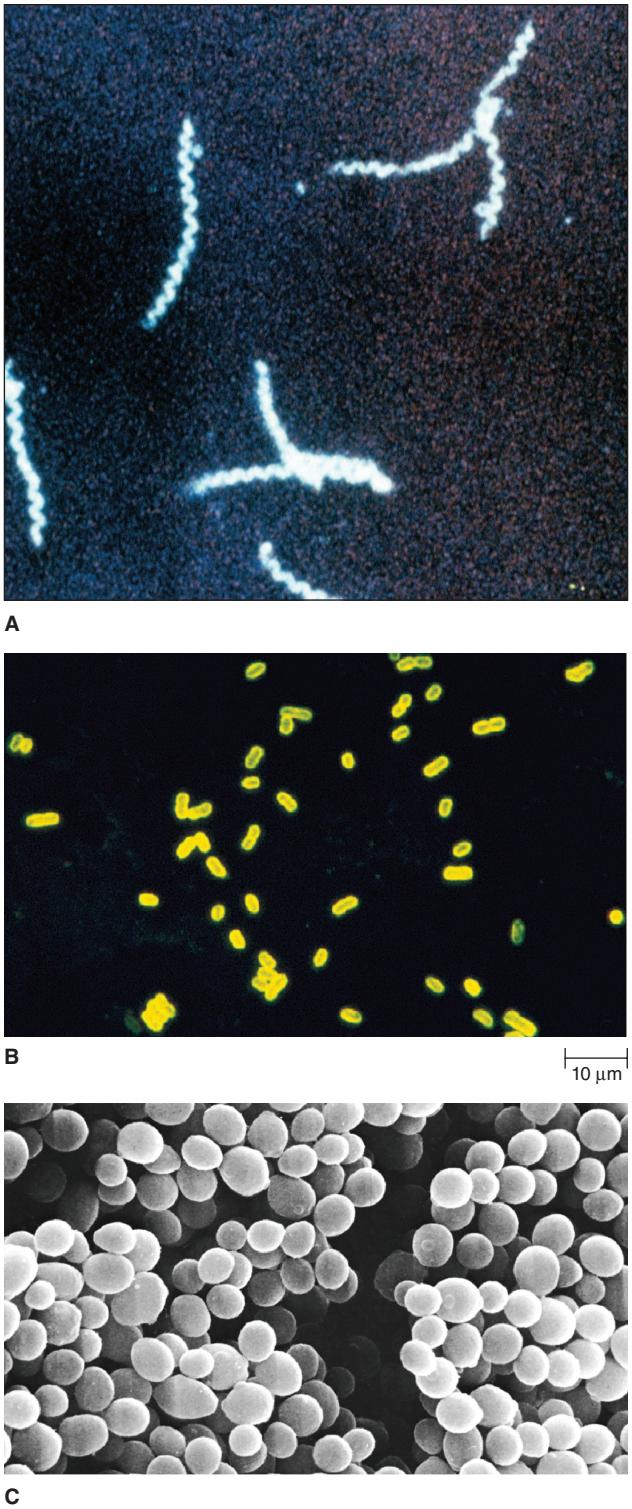


FIGURE 2-2 **A:** Positive dark-field examination. Treponemes are recognizable by their characteristic corkscrew shape and deliberate forward and backward movement with rotation about the longitudinal axis. (Reproduced with permission. © Charles Stratton/Visuals Unlimited.) **B:** Fluorescence photomicrograph. A rod-shaped bacterium tagged with a fluorescent marker. (© Evans Roberts.) **C:** Scanning electron microscope of bacteria—*Staphylococcus aureus* (32,000×). (Reproduced with permission from David M. Phillips/Photo Researchers, Inc.)

and eukaryotic cells. The superior resolution of the electron microscope is because electrons have a much shorter wavelength than the photons of white light.

There are two types of electron microscopes in general use: The **transmission electron microscope (TEM)**, which has many features in common with the light microscope; and the **scanning electron microscope (SEM)**. The TEM was the first to be developed and uses a beam of electrons projected from an electron gun and directed or focused by an electromagnetic condenser lens onto a thin specimen. As the electrons strike the specimen, they are differentially scattered by the number and mass of atoms in the specimen; some electrons pass through the specimen and are gathered and focused by an electromagnetic objective lens, which presents an image of the specimen to the projector lens system for further enlargement. The image is visualized by allowing it to impinge on a screen that fluoresces when struck with the electrons. The image can be recorded on photographic film. TEM can resolve particles 0.001 µm apart. Thus, viruses with diameters of 0.01–0.2 µm are easily resolved by TEM.

The SEM generally has a lower resolving power than the TEM; however, it is particularly useful for providing three-dimensional images of the surface of microscopic objects. Electrons are focused by means of lenses into a very fine point. The interaction of electrons with the specimen results in the release of different forms of radiation (eg, secondary electrons) from the surface of the material, which can be captured by an appropriate detector, amplified, and then imaged on a television screen (Figure 2-2C).

An important technique in electron microscopy is the use of "shadowing." This involves depositing a thin layer of heavy metal (eg, platinum) on the specimen by placing it in the path of a beam of metal ions in a vacuum. The beam is directed at a low angle to the specimen so that it acquires a "shadow" in the form of an uncoated area on the other side. When an electron beam is then passed through the coated preparation in the electron microscope and a positive print is made from the "negative" image, a three-dimensional effect is achieved (eg, see Figure 2-24).

Other important techniques in electron microscopy include the use of ultrathin sections of embedded material, a method of freeze-drying specimens that prevents the distortion caused by conventional drying procedures, and the use of negative staining with an electron-dense material such as phosphotungstic acid or uranyl salts (eg, see Figure 42-1). Without these heavy metal salts, there would not be enough contrast to detect the details of the specimen.

Confocal Scanning Laser Microscope

The **confocal scanning laser microscope (CSLM)** couples a laser light source to a light microscope. In confocal scanning laser microscopy, a laser beam is bounced off a mirror that directs the beam through a scanning device. Then the laser beam is directed through a pinhole that precisely adjusts the plane of focus of the beam to a given vertical layer within the specimen. By precisely illuminating only a single plane of the specimen, illumination intensity drops off rapidly above and below the plane of focus, and stray light from other planes of focus are minimized. Thus, in a relatively thick specimen, various layers can be observed by adjusting the plane of focus of the laser beam.

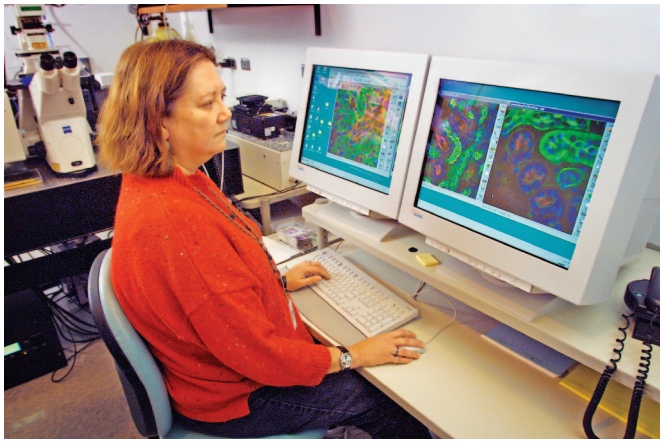


FIGURE 2-3 Using laser light, CDC laboratory scientists sometimes work with a confocal microscope when studying various pathogens. (Courtesy of James Gathany, Centers for Disease Control and Prevention, Public Health Image Library, ID# 1960.)

Cells are often stained with fluorescent dyes to make them more visible. Alternatively, false color images can be generated by adjusting the microscope in such a way as to make different layers take on different colors. The CSLM is equipped with computer software to assemble digital images for subsequent image processing. Thus, images obtained from different layers can be stored and then digitally overlaid to reconstruct a three-dimensional image of the entire specimen (Figure 2-3).

Scanning Probe Microscopes

A new class of microscopes, called **scanning probe microscopes**, measures surface features by moving a sharp probe over the object's surface. The **scanning tunneling microscope** and the **atomic force microscope** are the examples of this new class of microscopes, which enable scientists to view atoms or molecules on the surface of a specimen. For example, interactions between proteins of the bacterium *Escherichia coli* can be studied with the atomic force microscope (Figure 2-4).

EUKARYOTIC CELL STRUCTURE

The Nucleus

The **nucleus** contains the cell's genome. It is bounded by a membrane, which is composed of two lipid bilayer membranes: the inner and the outer membrane. The inner membrane is usually a simple sac, but the outermost membrane is, in many places, continuous with the endoplasmic

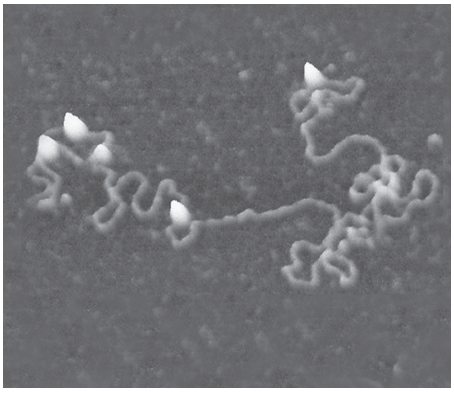


FIGURE 2-4 Atomic force microscopy. Micrograph of a fragment of DNA. The bright peaks are enzymes attached to the DNA. (Reproduced with permission from Torunn Berg, Photo Researchers, Inc.)

reticulum (ER). The **nuclear membrane** exhibits selective permeability because of pores, which consist of a complex of several proteins whose function is to import substances into and export substances out of the nucleus. The chromosomes of eukaryotic cells contain linear DNA macromolecules arranged as a double helix. They are only visible with a light microscope when the cell is undergoing division and the DNA is in a highly condensed form; at other times, the chromosomes are not condensed and appear as in Figure 2-5. Eukaryotic DNA macromolecules are associated with basic proteins called **histones** that bind to the DNA by ionic interactions.

A structure often visible within the nucleus is the **nucleolus**, an area rich in RNA that is the site of ribosomal RNA synthesis (see Figure 2-5). Ribosomal proteins synthesized in the cytoplasm are transported into the nucleolus and combine with ribosomal RNA to form the small and large subunits of the eukaryotic ribosome. These are then exported to the cytoplasm, where they associate to form an intact ribosome that can function in protein synthesis.

Cytoplasmic Structures

The cytoplasm of eukaryotic cells is characterized by the presence of an ER, vacuoles, self-reproducing plastids, and an

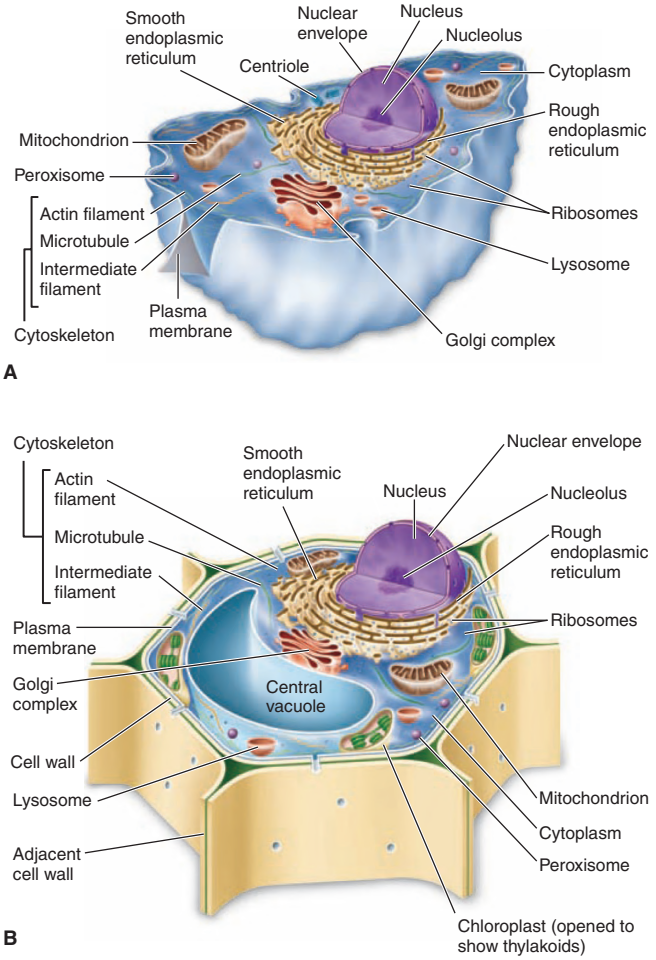


FIGURE 2-5 Eukaryotic cells. **A:** Diagrammatic representation of an animal cell. **B:** Diagrammatic representation of a plant cell. **C:** Micrograph of an animal cell shows several membrane-bound structures, including mitochondria and a nucleus. (Fig. 2-3(A) and (B) Reproduced with permission from Nester EW, Anderson DG, Roberts CE, et al: *Microbiology: A Human Perspective*, 6th ed. McGraw-Hill, 2009. © McGraw-Hill Education. Fig. 2-3(C) Reproduced with permission from Thomas Fritsche, MD, PhD.)

elaborate cytoskeleton composed of microtubules, microfilaments, and intermediate filaments.

The **endoplasmic reticulum (ER)** is a network of membrane-bound channels continuous with the nuclear membrane. Two types of ER are recognized: **rough**, to which 80S ribosomes are attached; and **smooth**, which does not have attached ribosomes (see Figure 2-5). Rough ER is a major producer of glycoproteins as well as new membrane material that is transported throughout the cell; smooth ER participates in the synthesis of lipids and in some aspects of carbohydrate metabolism. The **Golgi complex** consists of a stack of membranes that function in concert with the ER to chemically modify and sort products of the ER into those destined to be secreted and those that function in other membranous structures of the cell.

The plastids include **mitochondria** and **chloroplasts**. Several lines of evidence suggest that mitochondria and chloroplasts arose from the engulfment of a prokaryotic cell by a larger cell **(endosymbiosis)**. Current hypotheses, making use of mitochondrial genome and proteome data, suggest that the mitochondrial ancestor was most closely related to Alphaproteobacteria and that chloroplasts are related to nitrogen-fixing cyanobacteria. Mitochondria are of prokaryotic size (Figure 2-5), and its membrane, which lacks sterols, is much less rigid than the eukaryotic cell's cytoplasmic membrane, which does contain sterols. Mitochondria contain two sets of membranes. The outermost membrane is rather permeable, having numerous minute channels that allow passage of ions and small molecules (eg, adenosine triphosphate [ATP]). Invagination of the outer membrane forms a system of inner folded membranes called **cristae**. The cristae are the sites of enzymes involved in respiration and ATP production. Cristae also contain specific transport proteins that regulate passage of metabolites into and out of the mitochondrial **matrix**. The matrix contains a number of enzymes, particularly those of the citric acid cycle. Chloroplasts are the photosynthetic cell organelles that can convert the energy of sunlight into chemical energy through photosynthesis. Chlorophyll and all other components needed for photosynthesis are located in a series of flattened membrane discs called **thylakoids**. The size, shape, and number of chloroplasts per cell vary markedly; in contrast to mitochondria, chloroplasts are generally much larger than prokaryotes. Mitochondria and chloroplasts contain their own DNA, which exists in a covalently closed circular form and codes for some (not all) of their constituent proteins and transfer RNAs. Mitochondria and chloroplasts also contain 70S ribosomes, the same as those of prokaryotes.

Eukaryotic microorganisms that were previously thought to lack mitochondria (**amitochondriate eukaryotes**) have been recently shown to contain some mitochondrial remnants either through the maintenance of membrane-enclosed respiratory organelles called **hydrogenosomes**, **mitosomes**, or nuclear genes of mitochondrial origin. There are two types of amitochondriate eukaryotes: type II (eg, *Trichomonas vaginalis*) harbors a hydrogenosome, while type I (eg, *Giardia lamblia*) lacks organelles involved in core energy metabolism. Some amitochondrial parasites (eg, *Entamoeba histolytica*) are intermediate and appear to be evolving from a type II to type I. Some hydrogenosomes have been identified that contain DNA and ribosomes. The hydrogenosome, although similar in size to mitochondria, lacks cristae and the enzymes of the tricarboxylic acid cycle. Pyruvate is taken up by the hydrogenosome, and H_2, CO_2, acetate, and ATP are produced. The mitosome has only recently been discovered and named, and its function has not been well characterized.

Lysosomes are membrane-enclosed vesicles that contain various digestive enzymes that the cell uses to digest macromolecules such as proteins, fats, and polysaccharides. The lysosome allows these enzymes to be partitioned away from the cytoplasm proper, where they could destroy key cellular macromolecules if not contained. After the hydrolysis of macromolecules in the lysosome, the resulting monomers pass from the lysosome into the cytoplasm, where they serve as nutrients.

The **peroxisome** is a membrane-enclosed structure whose function is to produce H_2O_2 from the reduction of O_2 by various hydrogen donors. The H_2O_2 produced in the peroxisome is subsequently degraded to H_2O and O_2 by the enzyme **catalase**. Peroxisomes are believed to be of evolutionary origin unrelated to mitochondria.

The **cytoskeleton** is a three-dimensional structure that fills the cytoplasm. Eukaryotic cells contain three main kinds of cytoskeletal filaments: **microfilaments**, **intermediate filaments**, and **microtubules**. Each cytoskeletal filament type is formed by polymerization of a distinct type of protein subunit and has its own shape and intracellular distribution. Microfilaments are about 7 nm in diameter and are polymers composed of the protein **actin**. These fibers form scaffolds throughout the cell, defining and maintaining the shape of the cell. Microfilaments can also carry out intracellular transport/trafficking, and cellular movements, including gliding, contraction, and cytokinesis.

Microtubules are hollow cylinders about 23 nm in diameter (lumen is approximately 15 nm in diameter) most commonly comprising 13 protofilaments that, in turn, are polymers of alpha and beta **tubulin**. Microtubules assist microfilaments in maintaining cell structure, form the spindle fibers for separating chromosomes during mitosis, and play an important role in cell motility. Intermediate filaments are composed of various proteins (eg, **keratin**, **lamin**, and **desmin**) depending on the type of cell in which they are found. They are normally 8–12 nm in diameter and provide tensile strength for the cell. They are most commonly known as the support system or "scaffolding" for the cell and nucleus. All filaments react with **accessory proteins** (eg, Rho and dynein) that regulate and link the filaments to other cell components and each other.

Surface Layers

The cytoplasm is enclosed within a plasma membrane composed of protein and phospholipid similar to the prokaryotic cell membrane illustrated later (see Figure 2-13). Most animal cells have no other surface layers; however, plant cells have an outer cell wall composed of cellulose (Figure 2-5B). Many

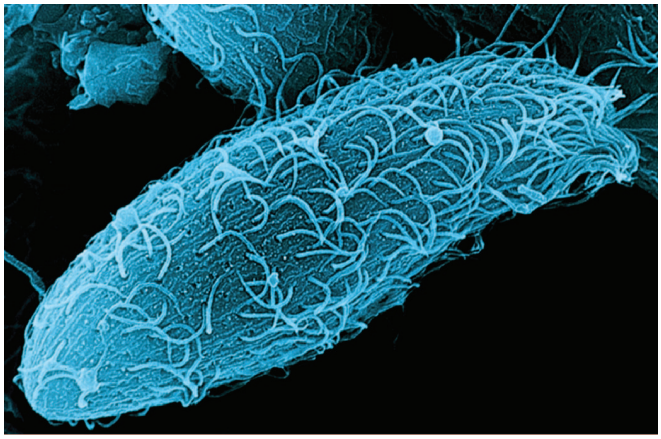


FIGURE 2-6 A paramecium moves with the aid of cilia on the cell surface. (© Manfred Kage.)

eukaryotic microorganisms also have an outer **cell wall**, which may be composed of a polysaccharide such as cellulose or chitin or may be inorganic (eg, the silica wall of diatoms).

Motility Organelles

Many eukaryotic microorganisms have organelles called **flagella** (eg, *T. vaginalis*) or **cilia** (eg, *Paramecium*) that move with a wavelike motion to propel the cell through water. Eukaryotic flagella emanate from the polar region of the cell, and cilia, which are shorter than flagella, surround the cell (Figure 2-6).

Both the flagella and the cilia of eukaryotic cells have the same basic structure and biochemical composition. Both consist of a series of microtubules, hollow protein cylinders composed of a protein called **tubulin** surrounded by a membrane. The arrangement of the microtubules is commonly referred to as the "9 + 2 arrangement" because it consists of nine doublets of microtubules surrounding two single central microtubules (Figure 2-7). Each doublet is connected to another by the protein **dynein**. The dynein arms attached to the microtubule function as the molecular motors.

PROKARYOTIC CELL STRUCTURE

The prokaryotic cell is simpler than the eukaryotic cell at every level, with one exception: The cell envelope is more complex.

The Nucleoid

Prokaryotes have no true nuclei; instead they package their DNA in a structure known as the **nucleoid**. The negatively charged DNA is at least partially neutralized by small polyamines and magnesium ions. Nucleoid-associated proteins exist in bacteria and are distinct from histones in eukaryotic chromatin.

Electron micrographs of a typical prokaryotic cell reveal the absence of a nuclear membrane and a mitotic apparatus. The exception to this rule is the planctomycetes, a divergent group of aquatic bacteria, which have a nucleoid surrounded by a nuclear envelope consisting of two membranes.

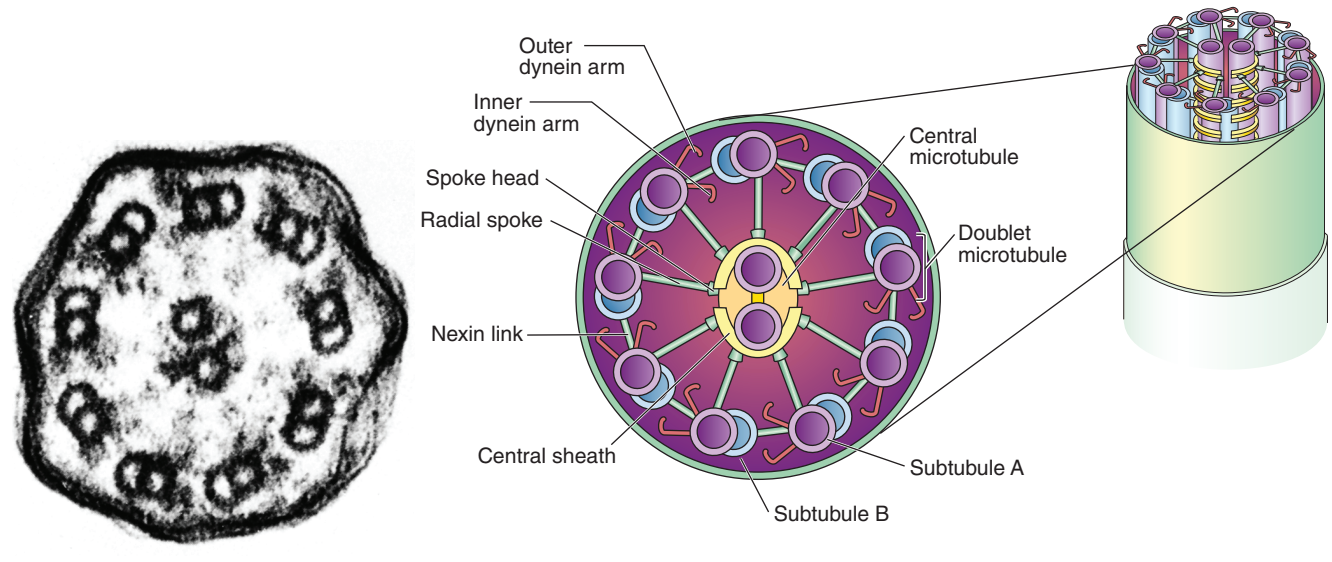
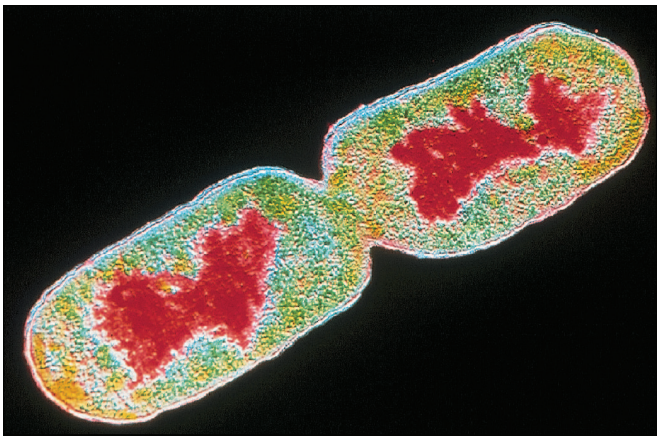


FIGURE 2-7 Cilia and flagella structure. **A:** An electron micrograph of a cilium cross section. Note the two central microtubles surrounded by nine microtubule doublets (160,000×). (Reproduced with permission. © Kallista Images/Visuals Unlimited, Inc.) **B:** A diagram of cilia and flagella structure. (Reproduced with permission from Willey JM, Sherwood LM, Woolverton CJ: *Prescott, Harley, and Klein's Microbiology*, 7th ed. McGraw-Hill; 2008. © McGraw-Hill Education.)



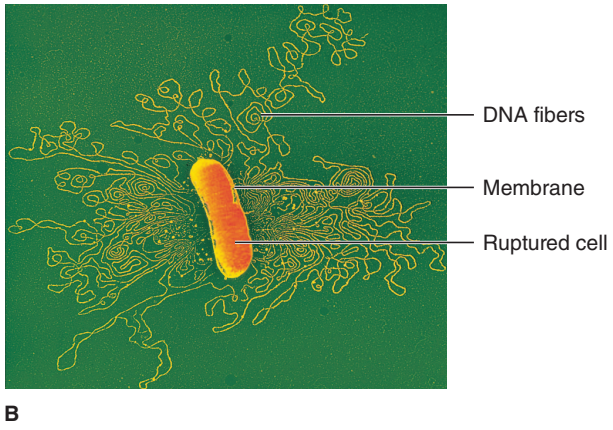


FIGURE 2-8 The nucleoid. **A:** Color-enhanced transmission electron micrograph of *E. coli* with the DNA shown in *red*. (Reproduced with permission. © CNRI/SPL/Photo Researchers, Inc.) **B:** Chromosome released from a gently lysed cell of *E. coli*. Note how tightly packaged the DNA must be inside the bacterium. (Reproduced with permission. © Dr. Gopal Murti/SPL/Photo Researchers Inc.)

The distinction between prokaryotes and eukaryotes that still holds is that prokaryotes have no eukaryotic-type mitotic apparatus. The nuclear region is filled with DNA fibrils (Figure 2-8). The nucleoid of most bacterial cells consists of a single continuous circular molecule ranging in size from 0.58 to almost 10 million base pairs. However, a few bacteria have been shown to have two, three, or even four dissimilar chromosomes. For example, *Vibrio cholerae* and *Brucella melitensis* have two dissimilar chromosomes. There are exceptions to this rule of circularity because some prokaryotes (eg, *Borrelia burgdorferi* and *Streptomyces coelicolor*) have been shown to have a linear chromosome.

In bacteria, the number of nucleoids, and therefore the number of chromosomes, depends on the growth conditions. Rapidly growing bacteria have more nucleoids per cell than slowly growing ones; however, when multiple copies are present, they are all the same (ie, prokaryotic cells are **haploid**).

Cytoplasmic Structures

Prokaryotic cells lack autonomous plastids, such as mitochondria and chloroplasts; the electron transport enzymes are localized instead in the cytoplasmic membrane. The photosynthetic pigments (carotenoids, bacteriochlorophyll) of photosynthetic bacteria are contained in intracytoplasmic membrane systems of various morphologies. Membrane vesicles **(chromatophores)** or lamellae are commonly observed membrane types. Some photosynthetic bacteria have specialized nonunit membrane-enclosed structures called **chlorosomes**. In some cyanobacteria (formerly known as blue-green algae), the photosynthetic membranes often form multilayered structures known as **thylakoids** (Figure 2-9). The major accessory pigments used for light harvesting are the phycobilins found on the outer surface of the thylakoid membranes.

Bacteria often store reserve materials in the form of insoluble granules, which appear as refractile bodies in the cytoplasm when viewed by phase-contrast microscopy. These so-called **inclusion bodies** almost always function in the storage of energy or as a reservoir of structural building blocks. Most cellular inclusions are bounded by a thin nonunit membrane consisting of lipid, which serves to separate the inclusion from the cytoplasm proper. One of the most common inclusion bodies consists of **poly-β-hydroxybutyric acid (PHB)**, a lipid-like compound consisting of chains of

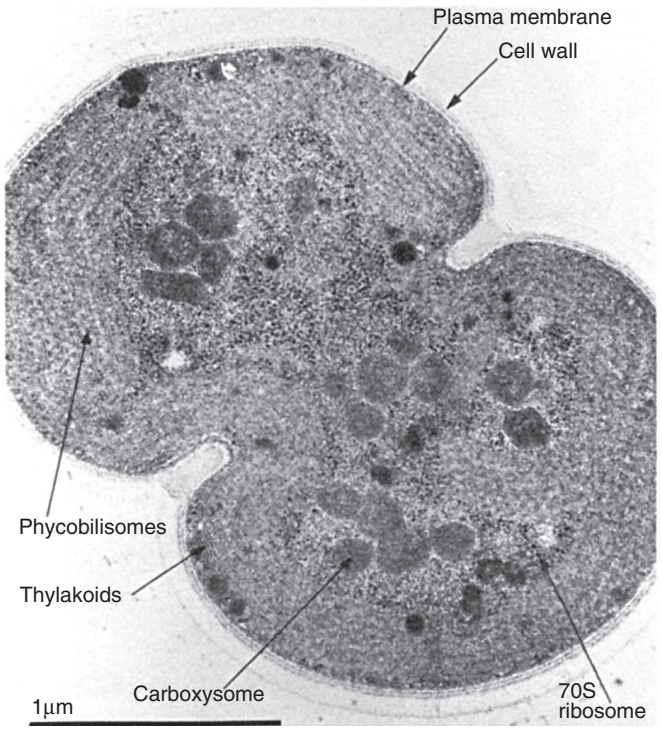
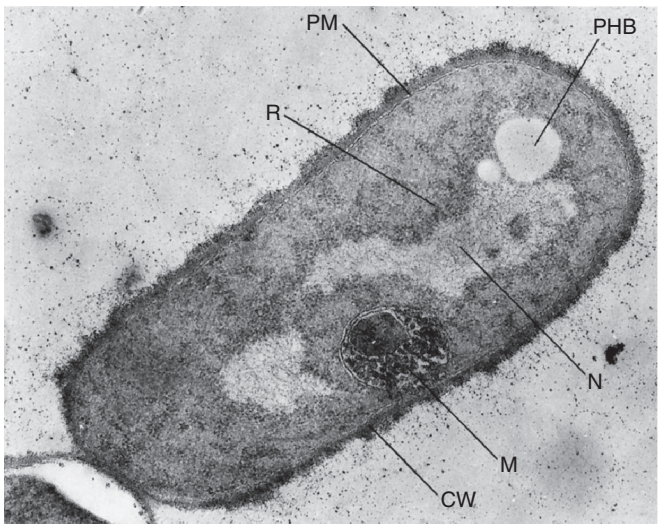


FIGURE 2-9 Thin section of *Synechocystis* during division. Many structures are visible. (Reproduced from Stanier RY: The position of cyanobacteria in the world of phototrophs. Carlsberg Res Commun 42:77-98, 1977. With kind permission of Springer + Business Media.)

β-hydroxybutyric acid units connected through ester linkages. PHB is produced when the source of nitrogen, sulfur, or phosphorous is limited and there is excess carbon in the medium (Figure 2-10A). Another storage product formed by prokaryotes when carbon is in excess is **glycogen**, which is a polymer of glucose. PHB and glycogen are used as carbon sources when protein and nucleic acid synthesis are resumed. A variety of prokaryotes are capable of oxidizing reduced sulfur compounds, such as hydrogen sulfide and thiosulfate, producing intracellular granules of elemental **sulfur** (Figure 2-10B). As the reduced sulfur source becomes limiting, the sulfur in the granules is oxidized, usually to sulfate, and the granules slowly disappear. Many bacteria accumulate large reserves of inorganic phosphate in the form of granules of **polyphosphate**. These granules can be degraded and used as sources of phosphate for nucleic acid and phospholipid synthesis to support growth. These granules are sometimes termed **volutin granules** or **metachromatic granules** because they stain red with a blue dye. They are characteristic features of *Corynebacterium* (see Chapter 13).

Certain groups of autotrophic bacteria that fix carbon dioxide to make their biochemical building blocks contain polyhedral bodies surrounded by a protein shell **(carboxysomes)** containing the key enzyme of CO_2 fixation, **ribulosebisphosphate carboxylase** (see Figure 2-9). **Magnetosomes** are intracellular crystal particles of the iron mineral magnetite (Fe_3O_4) that allow certain aquatic bacteria to exhibit **magnetotaxis** (ie, migration or orientation of the cell with respect to the earth's magnetic field). Magnetosomes are surrounded by a nonunit membrane containing phospholipids, proteins, and glycoproteins. **Gas vesicles** are found almost exclusively in microorganisms from aquatic habitats, where they provide buoyancy. The gas vesicle membrane is a 2-nm-thick layer of protein, impermeable to water and solutes but permeable to gases; thus, gas vesicles exist as gas-filled structures surrounded by the constituents of the cytoplasm (Figure 2-11).

The most numerous intracellular structure in most bacteria is the **ribosome**, the site of protein synthesis in all



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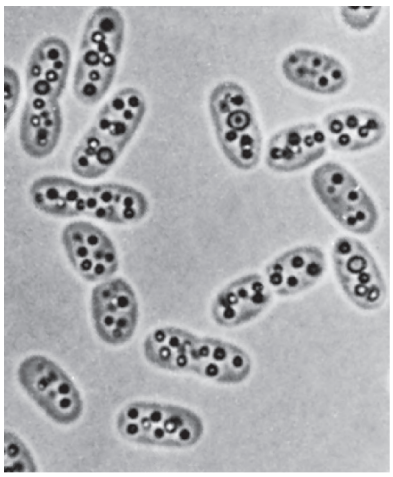


B

FIGURE 2-10 Inclusion bodies in bacteria. **A:** Electron micrograph of *B. megaterium* (30,500×) showing poly-β-hydroxybutyric acid inclusion body, PHB; cell wall, CW; nucleoid, N; plasma membrane, PM; "mesosome," M; and ribosomes, R. (Reproduced with permission. © Ralph A. Slepecky/Visuals Unlimited.) **B:** *Cromatium vinosum*, a purple sulfur bacterium, with intracellular sulfur granules, bright field microscopy (2000×). (Reproduced with permission from Holt J (editor): *The Shorter Bergey's Manual of Determinative Bacteriology*, 8th ed. Williams & Wilkins, 1977. Copyright Bergey's Manual Trust.)

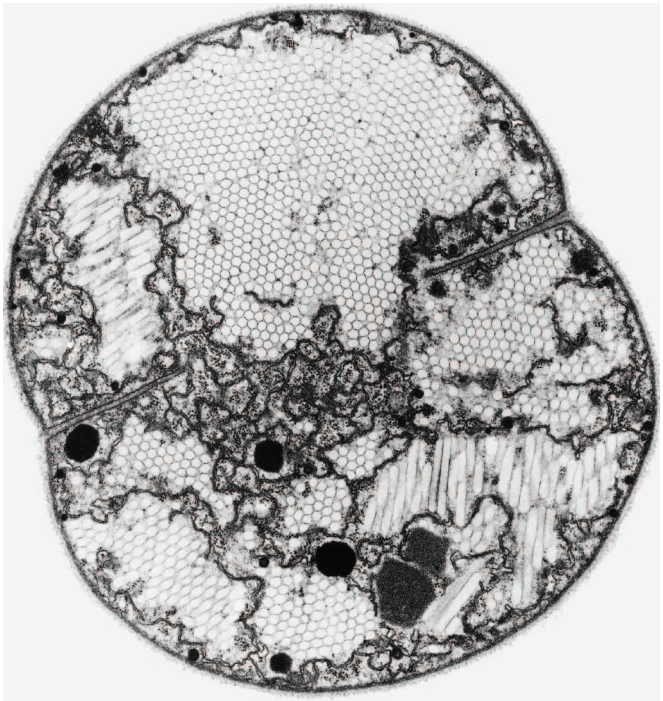


FIGURE 2-11 Transverse section of a dividing cell of the cyanobacterium *Microcystis* species showing hexagonal stacking of the cylindric gas vesicles (31,500×). (Micrograph by HS Pankratz. Reproduced with permission from Walsby AE: Gas vesicles. *Microbiol Rev* 1994;58:94.)

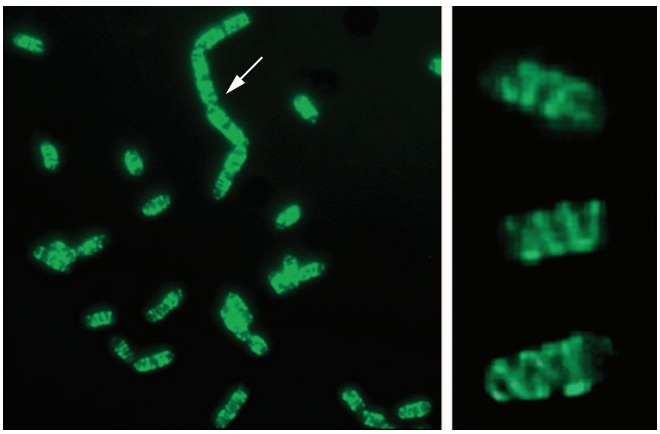


FIGURE 2-12 The prokaryotic cytoskeleton. Visualization of the MreB-like cytoskeletal protein (Mbl) of *B. subtilis*. The Mbl protein has been fused with green fluorescent protein, and live cells have been examined by fluorescence microscopy. **A:** *Arrows* point to the helical cytoskeleton cables that extend the length of the cells. **B:** Three of the cells from *A* are shown at a higher magnification. (Courtesy of Rut Carballido-Lopez and Jeff Errington.)

living organisms. All prokaryotes have 70S ribosomes, while eukaryotes contain larger 80S ribosomes in their cytoplasm. The 70S ribosome is made up of 50S and 30S subunits. The 50S subunit contains the 23S and 5S ribosomal RNA (rRNA), while the 30S subunit contains the 16S rRNA. These rRNA molecules are complexed with a large number of ribosomal proteins. The bacterial cytoplasm also contains homologs of all the major cytoskeletal proteins of eukaryotic cells as well as additional proteins that play cytoskeletal roles (Figure 2-12). Actin homologs (eg, MreB and Mbl) perform a variety of functions, helping to determine cell shape, segregate chromosomes, and localize proteins within the cell. Nonactin homologs (eg, FtsZ) and unique bacterial cytoskeletal proteins (eg, SecY and MinD) are involved in determining cell shape and in regulation of cell division and chromosome segregation.

The Cell Envelope

Prokaryotic cells are surrounded by complex envelope layers that differ in composition among the major groups. These structures protect the organisms from hostile environments, such as extreme osmolarity, harsh chemicals, and even antibiotics.

The Plasma Membrane

A. Structure

The plasma membrane, also called the **bacterial cytoplasmic membrane**, is visible in electron micrographs of thin sections (see Figure 2-9). It is a typical "unit membrane" composed of phospholipids and upward of 200 different proteins. Proteins account for approximately 70% of the mass of the membrane, which is a considerably higher proportion than that of mammalian cell membranes. Figure 2-13 illustrates a model of membrane organization. The membranes of prokaryotes are distinguished from those of eukaryotic cells by the absence of sterols (with some exceptions, eg, mycoplasmas, which also lack a cell wall, incorporate sterols, such as cholesterol, into their membranes when growing in sterol-containing media). However, many bacteria contain structurally related compounds called **hopanoids**, which

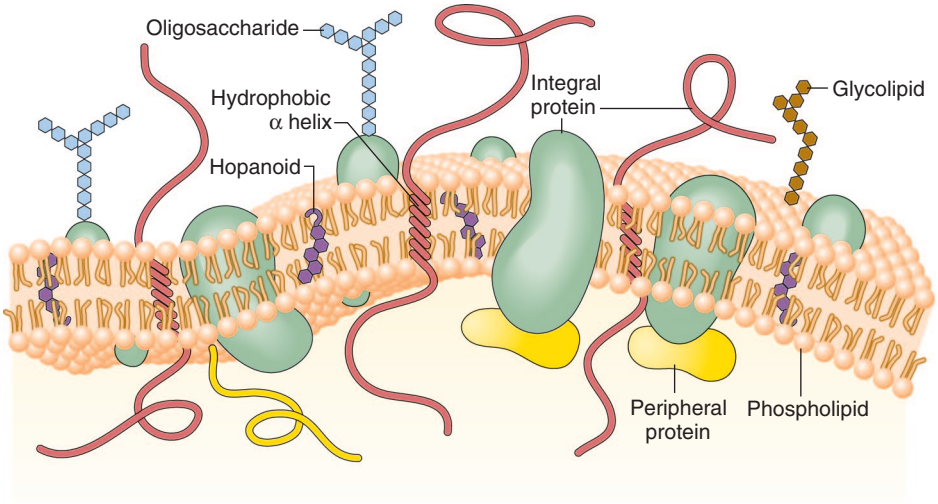


FIGURE 2-13 Bacterial plasma membrane structure. This diagram of the fluid mosaic model of bacterial membrane structure shown the integral proteins (*green* and *red*) floating in a lipid bilayer. Peripheral proteins (*yellow*) are associated loosely with the inner membrane surface. Small spheres represent the hydrophilic ends of membrane phospholipids and wiggly tails, the hydrophobic fatty acid chains. Other membrane lipids such as hopanoids (*purple*) may be present. For the sake of clarity, phospholipids are shown proportionately much larger size than in real membranes. (Reproduced with permission from Willey JM, Sherwood LM, Woolverton CJ: *Prescott, Harley, and Klein's Microbiology*, 7th ed. McGraw-Hill; 2008. © McGraw-Hill Education.)

likely fulfill the same function. Unlike eukaryotes, bacteria can have a wide variety of fatty acids within their membranes. Along with the typical saturated and unsaturated fatty acids, bacterial membranes can contain fatty acids with additional methyl, hydroxy, or cyclic groups. The relative proportions of these fatty acids can be modulated by the bacterium to maintain the optimum fluidity of the membrane. For example, at least 50% of the cytoplasmic membrane must be in the semifluid state for cell growth to occur. At low temperatures, this is achieved by greatly increased synthesis and incorporation of unsaturated fatty acids into the phospholipids of the cell membrane.

The cell membranes of the *Archaea* (see Chapter 1) differ from those of the *Bacteria*. Some Archaeal cell membranes contain unique lipids, **isoprenoids**, rather than fatty acids, linked to glycerol by ether rather than an ester linkage. Some of these lipids have no phosphate groups, and therefore, they are not phospholipids. In other species, the cell membrane is made up of a lipid monolayer consisting of long lipids (about twice as long as a phospholipid) with glycerol ethers at both ends (diglycerol tetraethers). The molecules orient themselves with the polar glycerol groups on the surfaces and the nonpolar hydrocarbon chain in the interior. These unusual lipids contribute to the ability of many *Archaea* to grow under environmental conditions such as high salt, low pH, or very high temperature.

B. Function

The major functions of the cytoplasmic membrane are (1) selective permeability and transport of solutes; (2) electron transport and oxidative phosphorylation in aerobic species; (3) excretion of hydrolytic exoenzymes; (4) contain the enzymes and carrier molecules that function in the biosynthesis of DNA, cell wall polymers, and membrane lipids; and (5) bear the receptors and other proteins of the chemotactic and other sensory transduction systems.

1. Permeability and transport—The cytoplasmic membrane forms a hydrophobic barrier impermeable to most hydrophilic molecules. However, several mechanisms **(transport systems)** exist that enable the cell to transport nutrients into and waste products out of the cell. These transport systems work against a concentration gradient to increase the nutrient concentrations inside the cell, a function that requires energy in some form. There are three general transport mechanisms involved in membrane transport: **passive transport**, **active transport**, and **group translocation**.

a. Passive transport—This mechanism relies on diffusion, uses no energy, and operates only when the solute is at higher concentration outside than inside the cell. **Simple diffusion** accounts for the entry of very few nutrients, including dissolved oxygen, carbon dioxide, and water itself. Simple diffusion provides neither speed nor selectivity. **Facilitated diffusion** also uses no energy, so the solute never achieves an internal concentration greater than what exists outside the cell. However, facilitated diffusion is selective. **Channel proteins** form selective channels that facilitate the passage of specific molecules. Facilitated diffusion is common in eukaryotic microorganisms (eg, yeast) but is rare in prokaryotes. Glycerol is one of the few compounds that enters prokaryotic cells by facilitated diffusion.

b. Active transport—Many nutrients are concentrated more than a thousandfold as a result of active transport. There are two types of active transport mechanisms depending on the source of energy used: **ion-coupled transport** and **ATP-binding cassette (ABC) transport**.

1) *Ion-coupled transport*—These systems move a molecule across the cell membrane at the expense of a previously established ion gradient such as **proton-** or **sodium-motive force**. There are three basic types: **uniport**, **symport**, and **antiport** (Figure 2-14). Ion-coupled transport is particularly common in aerobic organisms, which have an easier time generating an ion-motive force than do anaerobes. Uniporters catalyze the transport of a substrate independent of any coupled ion. Symporters catalyze the simultaneous transport of two substrates in the same direction by a single carrier; for example, an H^+ gradient can permit symport of an oppositely charged ion (eg, glycine) or a neutral molecule (eg, galactose). Antiporters catalyze the simultaneous transport of two like-charged compounds in opposite directions by a common carrier (eg, H^+:Na^+). Approximately, 40% of the substrates transported by *E. coli* use this mechanism.

2) *ABC transport*—This mechanism uses ATP directly to transport solutes into the cell. In Gram-negative bacteria, the transport of many nutrients is facilitated by specific **binding proteins** located in the periplasmic space; in Gram-positive cells, the binding proteins are attached to the outer surface of the cell membrane. These proteins function by transferring the bound substrate to a membrane-bound protein complex. Hydrolysis of ATP is then triggered, and the energy is used to open the membrane pore and allow the unidirectional movement of the substrate into the cell. Approximately 40% of the substrates transported by *E. coli* use this mechanism.

c. Group translocation—In addition to true transport, in which a solute is moved across the membrane without change in structure, bacteria use a process called group translocation **(vectorial metabolism)** to effect the net uptake of certain sugars (eg, glucose and mannose), the substrate becoming phosphorylated during the transport process. In a strict sense, group translocation is not active transport because no concentration gradient is involved. This process allows bacteria to use their energy resources efficiently by coupling transport with metabolism. In this process, a membrane carrier protein is first phosphorylated in the cytoplasm at the expense of **phosphoenolpyruvate**; the phosphorylated carrier protein then binds the free sugar at the exterior membrane face and transports it into the cytoplasm, releasing it as a sugar phosphate. Such systems of sugar transport are called

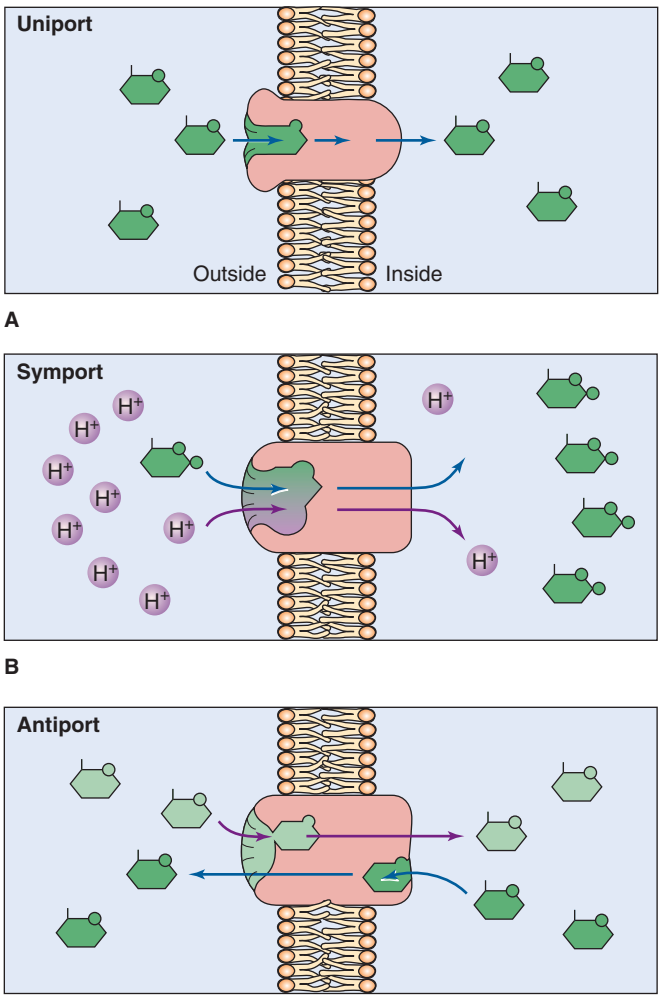


FIGURE 2-14 Three types of porters: **A:** uniporters, **B:** symporters, and **C:** antiporters. Uniporters catalyze the transport of a single species independently of any other, symporters catalyze the cotransport of two dissimilar species (usually a solute and a positively charged ion, H^+) in the same direction, and antiporters catalyze the exchange transport of two similar solutes in opposite directions. A single transport protein may catalyze just one of these processes, two of these processes, or even all three of these processes, depending on conditions. Uniporters, symporters, and antiporters have been found to be structurally similar and evolutionarily related, and they function by similar mechanisms. (Reproduced with permission from Saier MH Jr: Peter Mitchell and his chemiosmotic theories. *ASM News* 1997;63:13.)

phosphotransferase systems. Phosphotransferase systems are also involved in movement toward these carbon sources **(chemotaxis)** and in the regulation of several other metabolic pathways **(catabolite repression)**.

d. Special transport processes—Iron (Fe) is an essential nutrient for the growth of almost all bacteria. Under anaerobic conditions, Fe is generally in the +2 oxidation state and soluble. However, under aerobic conditions, Fe is generally in the +3 oxidation state and insoluble. The internal compartments of animals contain virtually no free Fe; it is sequestered in complexes with such proteins as **transferrin** and **lactoferrin**. Some bacteria solve this problem by secreting **siderophores**—compounds that chelate Fe and promote its transport as a soluble complex. One major group of siderophores consists of derivatives of hydroxamic acid ($-CONH_2OH$), which chelate Fe^{3+} very strongly. The iron–hydroxamate complex is actively transported into the cell by the cooperative action of a group of proteins that span the outer membrane, periplasm, and inner membrane. The iron is released, and the hydroxamate can exit the cell and be used again for iron transport.

Some pathogenic bacteria use a fundamentally different mechanism involving specific receptors that bind host transferrin and lactoferrin (as well as other iron-containing host proteins). The Fe is removed and transported into the cell using an ABC transporter.

2. Electron transport and oxidative phosphorylation—The cytochromes and other enzymes and components of the respiratory chain, including certain dehydrogenases, are located in the cytoplasmic membrane. The bacterial cytoplasmic membrane is thus a functional analog of the mitochondrial membrane—a relationship which has been taken by many biologists to support the theory that mitochondria have evolved from symbiotic bacteria. The mechanism by which ATP generation is coupled to electron transport is discussed in Chapter 6.

3. Excretion of hydrolytic exoenzymes and pathogenicity proteins—All organisms that rely on macromolecular organic polymers as a source of nutrients (eg, proteins, polysaccharides, and lipids) excrete hydrolytic enzymes that degrade these polymers to subunits small enough to penetrate the cell membrane. Higher animals secrete such enzymes into the lumen of the digestive tract; bacteria (both Gram-positive and Gram-negative) secrete them directly into the external medium or into the periplasmic space between the peptidoglycan layer and the outer membrane of the cell wall in the case of Gram-negative bacteria (see The Cell Wall).

In Gram-positive bacteria, proteins are secreted directly across the cytoplasmic membrane, but in Gram-negative bacteria, secreted proteins must traverse the outer membrane as well. At least six pathways of protein secretion have been described in bacteria: the type I, type II, type III, type IV, type V, and type VI secretion systems. A schematic overview of the type I to V systems is presented in Figure 2-15. The type I and IV secretion systems have been described in both Gram-negative and Gram-positive bacteria, but the type II, III, V, and VI secretion systems have been found only in Gram-negative bacteria. Proteins secreted by the type I and III pathways traverse the inner (cytoplasmic) membrane (IM) and outer membrane (OM) in one step, but proteins secreted by the type II and V pathways cross the IM and OM in separate steps. Proteins secreted by the type II and V pathways are

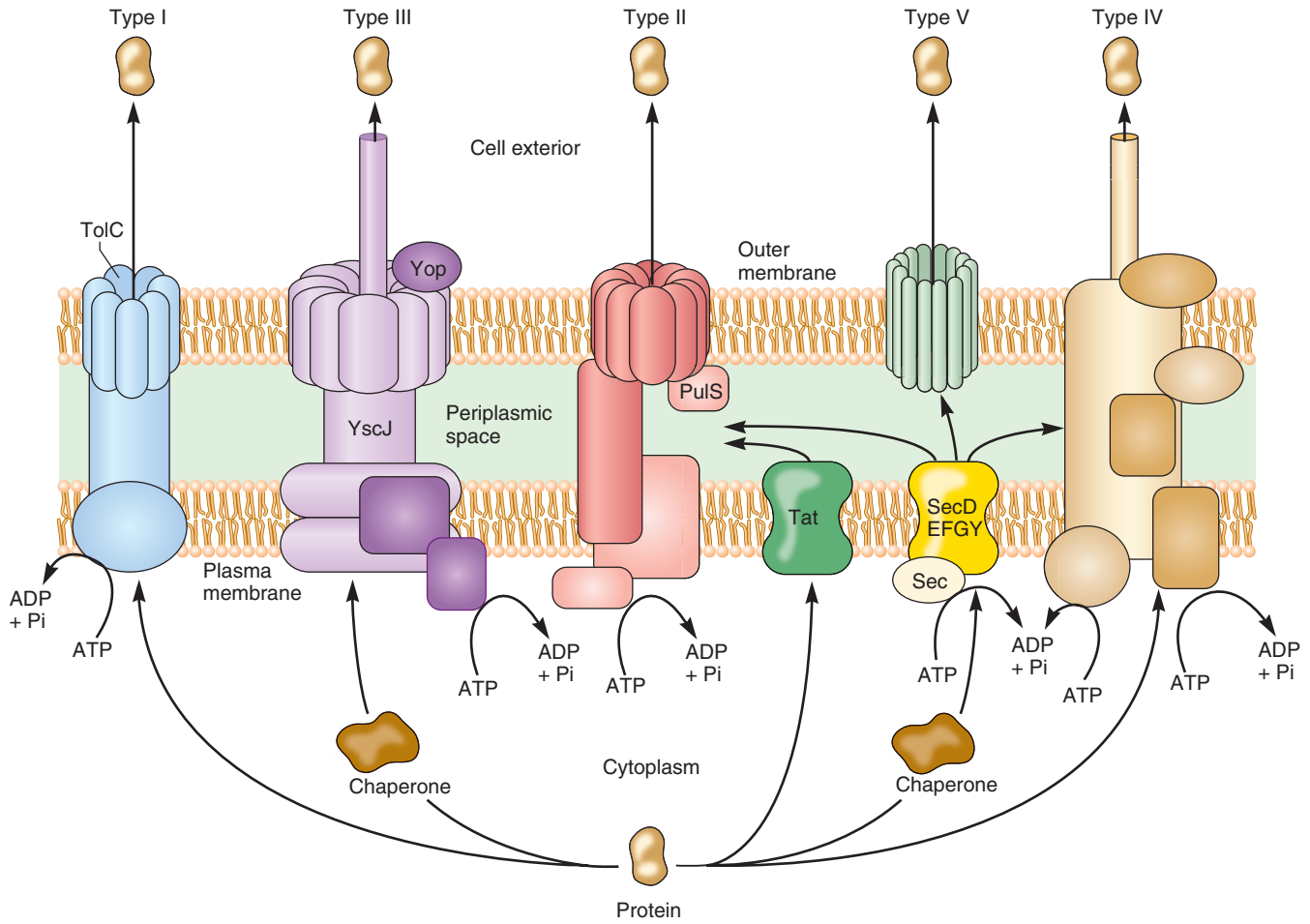


FIGURE 2-15 The protein secretion systems of Gram-negative bacteria. Five secretion systems of Gram-negative bacteria are shown. The Sec-dependent and Tat pathways deliver proteins from the cytoplasm to the periplasmic space. The type II, type V, and sometimes type IV systems complete the secretion process begun by the Sec-dependent pathway. The Tat system appears to deliver proteins only to the type II pathway. The type I and III systems bypass the Sec-dependent and Tat pathways, moving proteins directly from the cytoplasm, through the outer membrane, to the extracellular space. The type IV system can work either with the Sec-dependent pathway or can work alone to transport proteins to the extracellular space. Proteins translocated by the Sec-dependent pathway and the type III pathway are delivered to those systems by chaperone proteins. ADP, adenosine diphosphate; ATP, adenosine triphosphate; EFGY; PulS; SecD; TolC; Yop. (Reproduced with permission from Willey JM, Sherwood LM, Woolverton CJ: *Prescott, Harley, and Klein's Microbiology*, 7th ed. McGraw-Hill; 2008. © McGraw-Hill Education.)

synthesized on cytoplasmic ribosomes as preproteins containing an extra **leader** or **signal sequence** of 15–40 amino acids—most commonly about 30 amino acids—at the amino terminal and require the *sec* system for transport across the IM. In *E. coli*, the *sec* pathway comprises a number of IM proteins (SecD to SecF, SecY), a cell membrane-associated ATPase (SecA) that provides energy for export, a **chaperone** (SecB) that binds to the preprotein, and the periplasmic **signal peptidase**. After translocation, the leader sequence is cleaved off by the membrane-bound signal peptidase, and the mature protein is released into the periplasmic space. In contrast, proteins secreted by the type I and III systems do not have a leader sequence and are exported intact.

In Gram-negative and Gram-positive bacteria, another cytoplasmic membrane system that uses the twin-arginine targeting translocase (***tat* pathway**) can move proteins across the IM. In Gram-negative bacteria, these proteins are then delivered to the type II system (Figure 2-15). The *tat* pathway is distinct from the *sec* system in that it translocates already folded proteins.

Although proteins secreted by the type II and V systems are similar in the mechanism by which they cross the IM, differences exist in how they traverse the OM. Proteins secreted by the type II system are transported across the OM by a multiprotein complex (see Figure 2-15). This is the primary pathway for the secretion of extracellular degradative enzymes by Gram-negative bacteria. Elastase, phospholipase C, and exotoxin A are secreted by this system in *Pseudomonas aeruginosa*. However, proteins secreted by the type V system autotransport across the outer membrane by virtue of a

carboxyl terminal sequence, which is enzymatically removed upon release of the protein from the OM. Some extracellular proteins—eg, the IgA protease of *Neisseria gonorrhoeae* and the vacuolating cytotoxin of *Helicobacter pylori*—are secreted by this system.

The type I and III secretion pathways are *sec* independent and thus do not involve amino terminal processing of the secreted proteins. Protein secretion by these pathways occurs in a continuous process without the presence of a cytoplasmic intermediate. Type I secretion is exemplified by the α-hemolysin of *E. coli* and the adenylyl cyclase of *Bordetella pertussis*. Type I secretion requires three secretory proteins: an IM ATP-binding cassette (ABC transporter), which provides energy for protein secretion; an OM protein; and a membrane fusion protein, which is anchored in the inner membrane and spans the periplasmic space (see Figure 2-15). Instead of a signal peptide, the information is located within the carboxyl terminal 60 amino acids of the secreted protein.

The type III secretion pathway is a **contact-dependent** system. It is activated by contact with a host cell, and then injects a toxin protein into the host cell directly. The type III secretion apparatus is composed of approximately 20 proteins, most of which are located in the IM. Many of these IM components are homologous to the flagellar biosynthesis apparatus of both Gram-negative and Gram-positive bacteria. As in type I secretion, the proteins secreted via the type III pathway are not subject to amino terminal processing during secretion.

Type IV pathways secrete either polypeptide toxins (directed against eukaryotic cells) or protein–DNA complexes either between two bacterial cells or between a bacterial and a eukaryotic cell. Type IV secretion is exemplified by the protein–DNA complex delivered by *Agrobacterium tumefaciens* into a plant cell. Additionally, *B. pertussis* and *H. pylori* possess type IV secretion systems that mediate secretion of pertussis toxin and interleukin-8–inducing factor, respectively. The *sec*-independent type VI secretion was recently described in *P. aeruginosa*, where it contributes to pathogenicity in patients with cystic fibrosis. This secretion system is composed of 15–20 proteins whose biochemical functions are not well understood. However, recent studies suggest that some of these proteins share homology with bacteriophage tail proteins.

The characteristics of the protein secretion systems of bacteria are summarized in Table 9-5.

4. Biosynthetic functions—The cell membrane is the site of the carrier lipids on which the subunits of the cell wall are assembled (see the discussion of synthesis of cell wall substances in Chapter 6) as well as of the enzymes of cell wall biosynthesis. The enzymes of phospholipid synthesis are also localized in the cell membrane.

5. Chemotactic systems—Attractants and repellents bind to specific receptors in the bacterial membrane (see Flagella). There are at least 20 different chemoreceptors in the membrane of *E. coli*, some of which also function as a first step in the transport process.

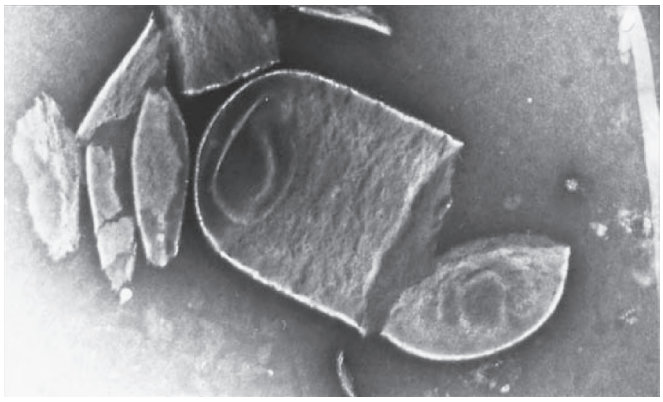


FIGURE 2-16 The rigid cell wall determines the shape of the bacterium. Even though the cell has split apart, the cell wall maintains its original shape. (Courtesy of Dale C. Birdsell.)

The Cell Wall

The internal osmotic pressure of most bacteria ranges from 5 to 20 atm as a result of solute concentration via active transport. In most environments, this pressure would be sufficient to burst the cell were it not for the presence of a high-tensile-strength cell wall (Figure 2-16). The bacterial cell wall owes its strength to a layer composed of a substance variously referred to as **murein**, **mucopeptide**, or **peptidoglycan** (all, including "cell wall," are synonyms). The structure of peptidoglycan is discussed as follows.

Most bacteria are classified as Gram-positive or Gram-negative according to their response to the Gram-staining procedure. This procedure was named for the histologist Hans Christian Gram, who developed this differential staining procedure in an attempt to identify bacteria in infected tissues. The Gram-stain depends on the ability of certain bacteria (the Gram-positive bacteria) to retain a complex of crystal violet (a purple dye) and iodine after a brief wash with alcohol or acetone. Gram-negative bacteria do not retain the dye–iodine complex and become translucent, but they can then be counterstained with safranin (a red dye). Thus, Gram-positive bacteria look purple under the microscope, and Gram-negative bacteria look red. The distinction between these two groups turns out to reflect fundamental differences in their cell envelopes (Table 2-1).

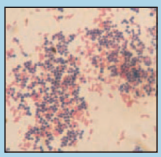
In addition to providing osmotic protection, the cell wall plays an essential role in cell division as well as serving as a primer for its own biosynthesis. The cell wall is, in general, nonselectively permeable; one layer of the Gram-negative wall, however—the outer membrane—hinders the passage of relatively large molecules (see next).

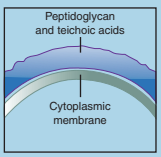
The biosynthesis of the cell wall and the antibiotics that interfere with this process are discussed in Chapter 6.

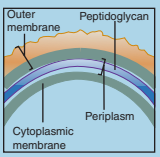
A. The Peptidoglycan Layer

Peptidoglycan is a complex polymer consisting, for the purposes of description, of three parts: a backbone, composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid

TABLE 2-1 Comparison of Features of Gram-Positive and Gram-Negative Bacteria







	Gram-Positive	Gram-Negative
Color of Gram-Stained Cell	Purple	Reddish-pink
Representative Genera	*Bacillus, Staphylococcus, Streptococcus*	*Escherichia, Neisseria, Pseudomonas*
Distinguishing Structures/Components		
Peptidoglycan	Thick layer	Thin layer
Teichoic acids	Present	Absent
Outer membrane	Absent	Present
Lipopolysaccharide (endotoxin)	Absent	Present
Porin proteins	Absent (unnecessary because there is no outer membrane)	Present; allow passage of molecules through outer membrane
Periplasm	Absent	Present
General Characteristics		
Sensitivity to penicillin	Generally more susceptible (with notable exceptions)	Generally less susceptible (with notable exceptions)
Sensitivity to lysozyme	Yes	No

connected by β1→4 linkages; a set of identical tetrapeptide side chains attached to *N*-acetylmuramic acid; and a set of identical peptide cross-bridges (Figure 2-17). The backbone is the same in all bacterial species; the tetrapeptide side chains and the peptide cross-bridges vary from species to species. In many Gram-negative cell walls, the cross-bridge consists of a direct peptide linkage between the diaminopimelic acid (DAP) amino group of one side chain and the carboxyl group of the terminal D-alanine of a second side chain.

The tetrapeptide side chains of all species, however, have certain notable features in common. Most have L-alanine at position 1 (attached to *N*-acetylmuramic acid), D-glutamate or substituted D-glutamate at position 2, and D-alanine at position 4. Position 3 is the most variable one: Most Gram-negative bacteria have diaminopimelic acid at this position, to which is linked the lipoprotein cell wall component discussed as follows. Gram-positive bacteria usually have L-lysine at position 3; however, some may have diaminopimelic acid or another amino acid at this position.

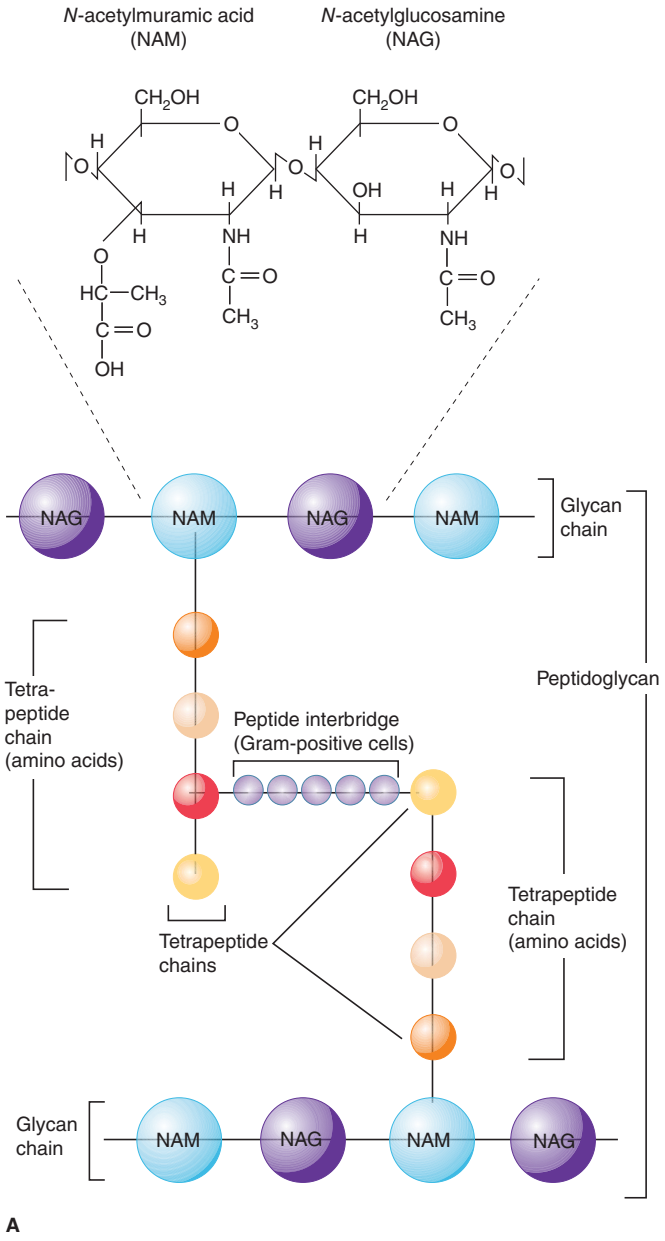
Diaminopimelic acid is a unique element of bacterial cell walls. It is never found in the cell walls of *Archaea* or eukaryotes. Diaminopimelic acid is the immediate precursor of lysine in the bacterial biosynthesis of that amino acid (see Figure 6-19). Bacterial mutants that are blocked before diaminopimelic acid in the biosynthetic pathway grow normally when provided with diaminopimelic acid in the medium; when given L-lysine alone, however, they lyse, because they continue to grow but are specifically unable to make new cell wall peptidoglycan.

The fact that all peptidoglycan chains are cross-linked means that each peptidoglycan layer is a single giant molecule. In Gram-positive bacteria, there are as many as 40 sheets of peptidoglycan, comprising up to 50% of the cell wall material; in Gram-negative bacteria, there appears to be only one or two sheets, comprising 5–10% of the wall material. Bacteria owe their shapes, which are characteristic of particular species, to their cell wall structure.

B. Special Components of Gram-Positive Cell Walls

Most Gram-positive cell walls contain considerable amounts of **teichoic** and **teichuronic acids**, which may account for up to 50% of the dry weight of the wall and 10% of the dry weight of the total cell. In addition, some Gram-positive walls may contain polysaccharide molecules.

1. Teichoic and teichuronic acids—The term *teichoic acids* encompass all wall, membrane, or capsular polymers containing glycerophosphate or ribitol phosphate residues. These polyalcohols are connected by phosphodiester linkages and usually have other sugars and D-alanine attached (Figure 2-18A). Because they are negatively charged, teichoic acids are partially responsible for the net negative



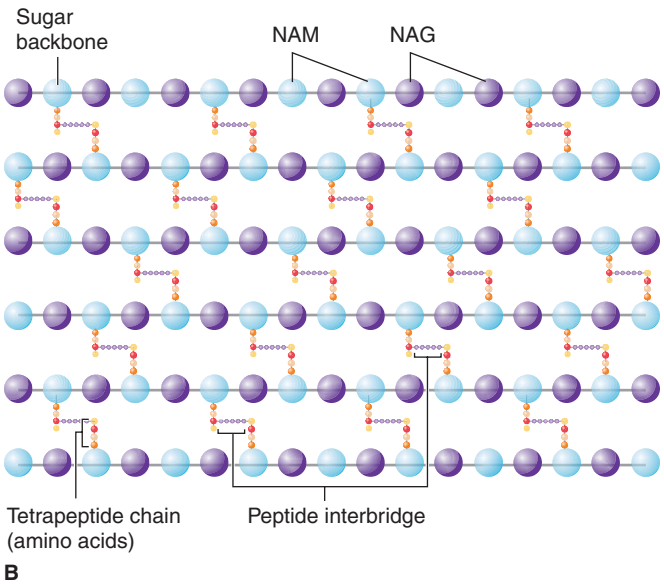


FIGURE 2-17 Components and structure of peptidoglycan. **A:** Chemical structure of *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM); the ring structures of the two molecules are glucose. Glycan chains are composed of alternating subunits of NAG and NAM joined by covalent bonds. Adjacent glycan chains are cross-linked via their tetrapeptide chains to create peptidoglycan. **B:** Interconnected glycan chains form a very large three-dimensional molecule of peptidoglycan. The β1→4 linkages in the backbone are cleaved by lysozyme. (Reproduced with permission from Nester EW, Anderson DG, Roberts CE, et al: *Microbiology: A Human Perspective,* 6th ed. McGraw-Hill, 2009. © McGraw-Hill Education.)

charge of the cell surface. There are two types of teichoic acids: **wall teichoic acid (WTA)**, covalently linked to peptidoglycan; and **membrane teichoic acid**, covalently linked to membrane glycolipid. Because the latter are intimately associated with lipids, they have been called **lipoteichoic acids (LTA)**. Together with peptidoglycan, WTA and LTA make up a polyanionic network or matrix that provides functions relating to the elasticity, porosity, tensile strength, and electrostatic properties of the envelope. Although not all Gram-positive bacteria have conventional LTA and WTA, those that lack these polymers generally have functionally similar ones.

Most teichoic acids contain substantial amounts of D-alanine, usually attached to position 2 or 3 of glycerol or position 3 or 4 of ribitol. In some of the more complex teichoic acids, however, D-alanine is attached to one of the sugar residues. In addition to D-alanine, other substituents may be attached to the free hydroxyl groups of glycerol and ribitol (eg, glucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine, or succinate). A given species may have more than one type of sugar substituent in addition to D-alanine; in such cases, it is not certain whether the different sugars occur on the same or on separate teichoic acid molecules. The composition of the teichoic acid formed by a given bacterial species can vary with the composition of the growth medium.

The teichoic acids constitute major surface antigens of those Gram-positive species that possess them, and their

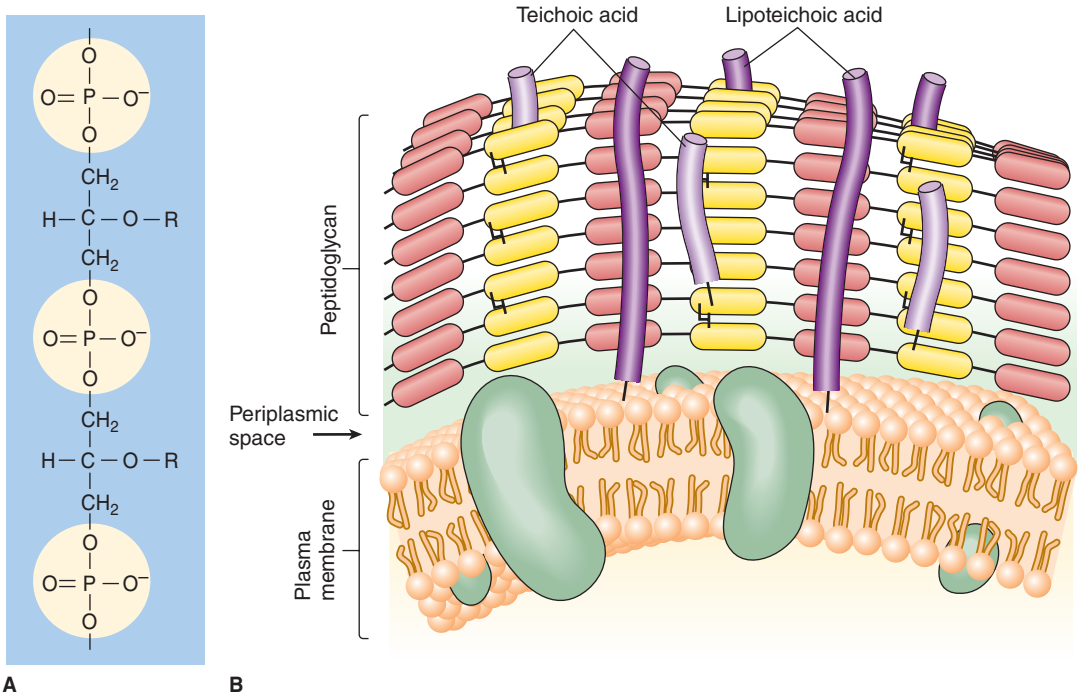


FIGURE 2-18 **A:** Teichoic acid structure. The segment of a teichoic acid made of phosphate, glycerol, and a side chain, R. R may represent D-alanine, glucose, or other molecules. **B:** Teichoic and lipoteichoic acids of the Gram-positive envelope. (Reproduced with permission from Willey JM, Sherwood LM, Woolverton CJ: *Prescott, Harley, and Klein's Microbiology*, 7th ed. McGraw-Hill; 2008. © McGraw-Hill Education.)

accessibility to antibodies has been taken as evidence that they lie on the outside surface of the peptidoglycan. Their activity is often increased, however, by partial digestion of the peptidoglycan; thus, much of the teichoic acid may lie between the cytoplasmic membrane and the peptidoglycan layer, possibly extending upward through pores in the latter (Figure 2-18B). In the pneumococcus (*Streptococcus pneumoniae*), the teichoic acids bear the antigenic determinants called **Forssman antigen**. In *Streptococcus pyogenes*, LTA is associated with the M protein that protrudes from the cell membrane through the peptidoglycan layer. The long M protein molecules together with the LTA form microfibrils that facilitate the attachment of *S. pyogenes* to animal cells (see Chapter 14).

The **teichuronic acids** are similar polymers, but the repeat units include sugar acids (eg, *N*-acetylmannosuronic or D-glucosuronic acid) instead of phosphoric acids. They are synthesized in place of teichoic acids when phosphate is limiting.

2. Polysaccharides—The hydrolysis of Gram-positive walls has yielded, from certain species, neutral sugars, such as mannose, arabinose, rhamnose, and glucosamine, and acidic sugars, such as glucuronic acid and mannuronic acid. It has been proposed that these sugars exist as subunits of polysaccharides in the cell wall; the discovery, however, that teichoic and teichuronic acids may contain a variety of sugars (see Figure 2-18A) leaves the true origin of these sugars uncertain.

C. Special Components of Gram-Negative Cell Walls

Gram-negative cell walls contain three components that lie outside of the peptidoglycan layer: outer membrane, lipopolysaccharide, and lipoprotein (Figure 2-19).

1. Outer membrane—The outer membrane is chemically distinct from all other biological membranes. It is a bilayered structure; its inner leaflet resembles in composition that of the cytoplasmic membrane, and its outer leaflet contains a distinctive component, a **lipopolysaccharide (LPS)** (see next). As a result, this is an asymmetrical membrane, and the properties of this bilayer differ considerably from those of a symmetrical biologic membrane such as the cell membrane.

The ability of the outer membrane to exclude hydrophobic molecules is an unusual feature among biologic membranes and serves to protect the cell (in the case of enteric bacteria) from deleterious substances such as bile salts. Because of its lipid nature, the outer membrane would be expected to exclude hydrophilic molecules as well. However, the outer membrane has special channels, consisting of protein molecules called **porins** that permit the passive diffusion of low-molecular-weight hydrophilic compounds, such as sugars, amino acids, and certain ions. Large antibiotic molecules penetrate the outer membrane relatively slowly, which accounts for the relatively high resistance of Gram-negative bacteria to some antibiotics. The permeability of the outer membrane varies widely from one Gram-negative species to another; in *P. aeruginosa*, for example, which is extremely

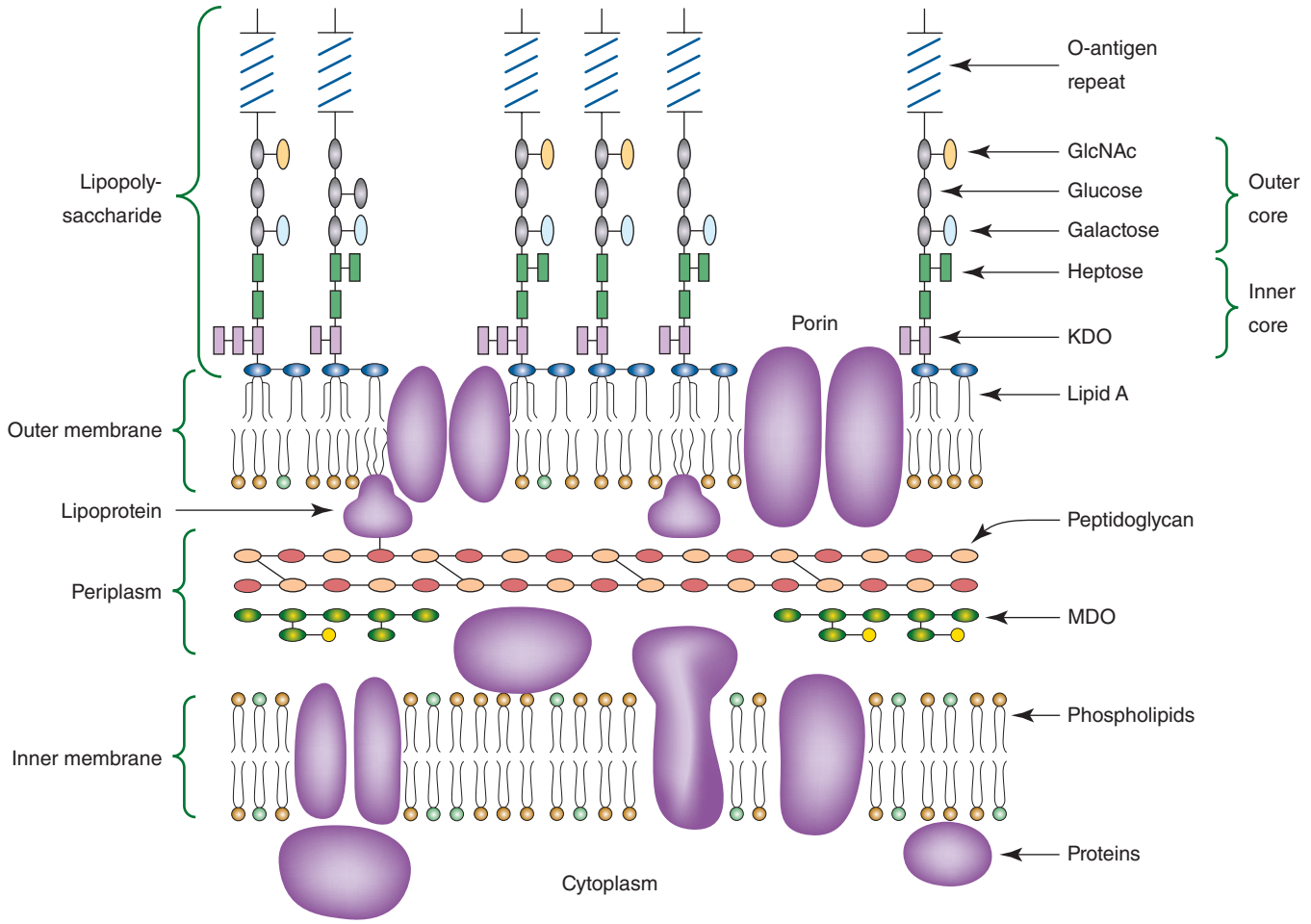


FIGURE 2-19 Molecular representation of the envelope of a Gram-negative bacterium. *Ovals* and *rectangles* represent sugar residues, and *circles* depict the polar head groups of the glycerophospholipids (phosphatidylethanolamine and phosphatidylglycerol). The core region shown is that of *E. coli* K-12, a strain that does not normally contain an O-antigen repeat unless transformed with an appropriate plasmid. MDO, membrane-derived oligosaccharides. (Reproduced with permission from Raetz CRH: Bacterial endotoxins: Extraordinary lipids that activate eucaryotic signal transduction. *J Bacteriol* 1993;175:5745.)

resistant to antibacterial agents, the outer membrane is 100 times less permeable than that of *E. coli*.

The major proteins of the outer membrane, named according to the genes that code for them, have been placed into several functional categories on the basis of mutants in which they are lacking and on the basis of experiments in which purified proteins have been reconstituted into artificial membranes. Porins, exemplified by OmpC, D, and F and PhoE of *E. coli* and *Salmonella typhimurium*, are trimeric proteins that penetrate both the inner and outer leaflets of the outer membrane (Figure 2-20). They form relatively nonspecific pores that permit the free diffusion of small hydrophilic solutes across the outer membrane. The porins of different species have different exclusion limits, ranging from molecular weights of about 600 in *E. coli* and *S. typhimurium* to more than 3000 in *P. aeruginosa*.

Members of a second group of outer membrane proteins, which resemble porins in many ways, are exemplified by LamB and Tsx. LamB, an inducible porin that is also the receptor for lambda bacteriophage, is responsible for most of the transmembrane diffusion of maltose and maltodextrins; Tsx, the receptor for T6 bacteriophage, is responsible for the transmembrane diffusion of nucleosides and some amino acids. LamB allows some passage of other solutes; however, its relative specificity may reflect weak interactions of solutes with configuration-specific sites within the channel.

The OmpA protein is an abundant protein in the outer membrane. The OmpA protein participates in the anchoring of the outer membrane to the peptidoglycan layer; it is also the sex pilus receptor in F-mediated bacterial conjugation (see Chapter 7).

The outer membrane also contains a set of less abundant proteins that are involved in the transport of specific molecules, such as vitamin B_{12} and iron–siderophore complexes. They show high affinity for their substrates and probably function like the classic carrier transport systems of the cytoplasmic membrane. The proper function of these proteins requires energy coupled through a protein called **TonB**.

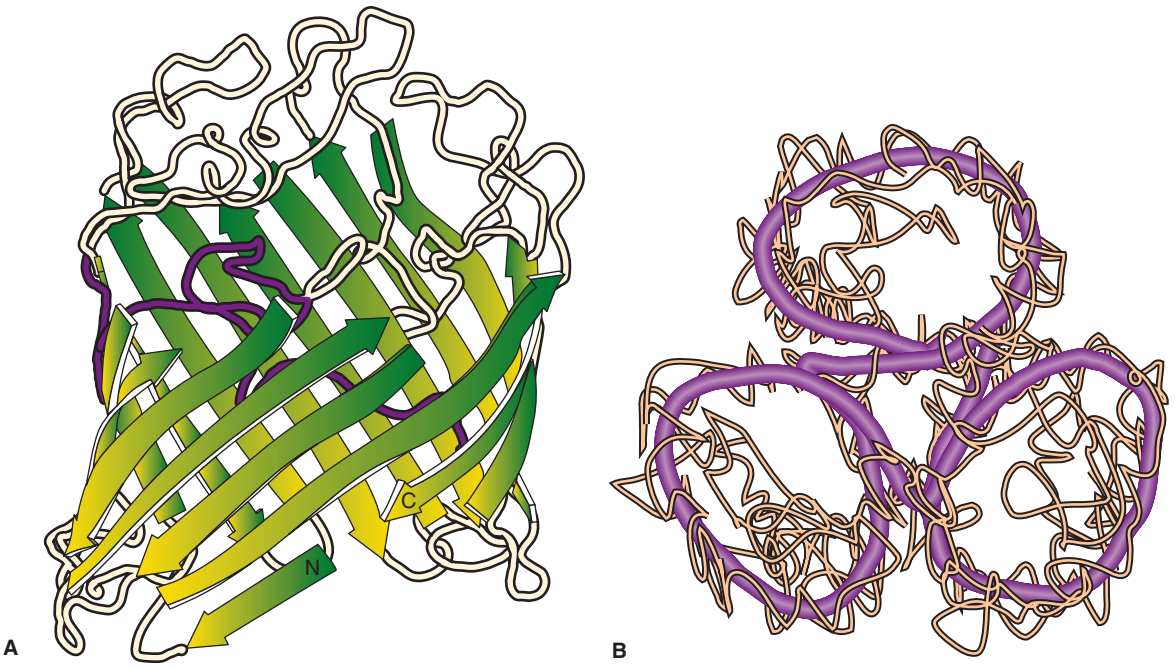


FIGURE 2-20 **A**: General fold of a porin monomer (OmpF porin from *Escherichia coli*). The large hollow β-barrel structure is formed by antiparallel arrangement of 16 β-strands. The strands are connected by short loops or regular turns on the periplasmic rim (*bottom*), and long irregular loops face the cell exterior (*top*). The internal loop, which connects β-strands 5 and 6 and extends inside the barrel, is highlighted in *dark*. The chain terminals are marked. The surface closest to the viewer is involved in subunit contacts. **B**: Schematic representation of the OmpF trimer. The view is from the extracellular space along the molecular threefold symmetry axis. (Reproduced with permission from Schirmer T: General and specific porins from bacterial outer membranes. *J Struct Biol* 1998;121:101.)

Additional minor proteins include a limited number of enzymes, among them phospholipases and proteases.

The topology of the major proteins of the outer membrane, based on cross-linking studies and analyses of functional relationships, is shown in Figure 2-19. The outer membrane is connected to both the peptidoglycan layer and the cytoplasmic membrane. The connection with the peptidoglycan layer is primarily mediated by the outer membrane lipoprotein (see next). About one-third of the lipoprotein molecules are covalently linked to peptidoglycan and help hold the two structures together. A noncovalent association of some of the porins with the peptidoglycan layer plays a lesser role in connecting the outer membrane with this structure. Outer membrane proteins are synthesized on ribosomes bound to the cytoplasmic surface of the cell membrane. They are translocated into the periplasm via the Sec translocase. They then fold in the periplasm before being inserted into the outer membrane. In *E. coli*, YaeT appears to function primarily in outer membrane protein insertion.

2. Lipopolysaccharide (LPS)—The LPS of Gram-negative cell walls consists of a complex glycolipid, called lipid A, to which is attached a polysaccharide made up of a core and a terminal series of repeat units (Figure 2-21A). The lipid A component is embedded in the outer leaflet of the membrane anchoring the LPS. LPS is synthesized on the cytoplasmic membrane and transported to its final exterior position. In *E. coli*, LPS insertion is mediated by OstA. The presence of LPS is required for the function of many outer membrane proteins.

Lipid A consists of phosphorylated glucosamine disaccharide units to which are attached several long-chain fatty acids (Figure 2-21). β-Hydroxymyristic acid, a C14 fatty acid, is always present and is unique to this lipid; the other fatty acids, along with substituent groups on the phosphates, vary according to the bacterial species.

The polysaccharide **core**, shown in Figure 2-21A and B, is similar in all Gram-negative species that have LPS and includes two characteristic sugars, **ketodeoxyoctanoic acid (KDO)** and a heptose. Each species, however, contains a unique repeat unit, that of *Salmonella* being shown in Figure 2-21A. The repeat units are usually linear trisaccharides or branched tetra- or pentasaccharides. The repeat unit is referred to as the **O antigen**. The hydrophilic carbohydrate chains of the O antigen cover the bacterial surface and exclude hydrophobic compounds.

The negatively charged LPS molecules are noncovalently cross-bridged by divalent cations (ie, Ca^{2+} and Mg^{2+}); this stabilizes the membrane and provides a barrier to hydrophobic molecules. Removal of the divalent cations with chelating agents or their displacement by polycationic antibiotics, such as polymyxins and aminoglycosides, renders the outer membrane permeable to large hydrophobic molecules.

LPS, which is extremely toxic to animals, has been called the **endotoxin** of Gram-negative bacteria because it is firmly bound to the cell surface and is released only when the cells

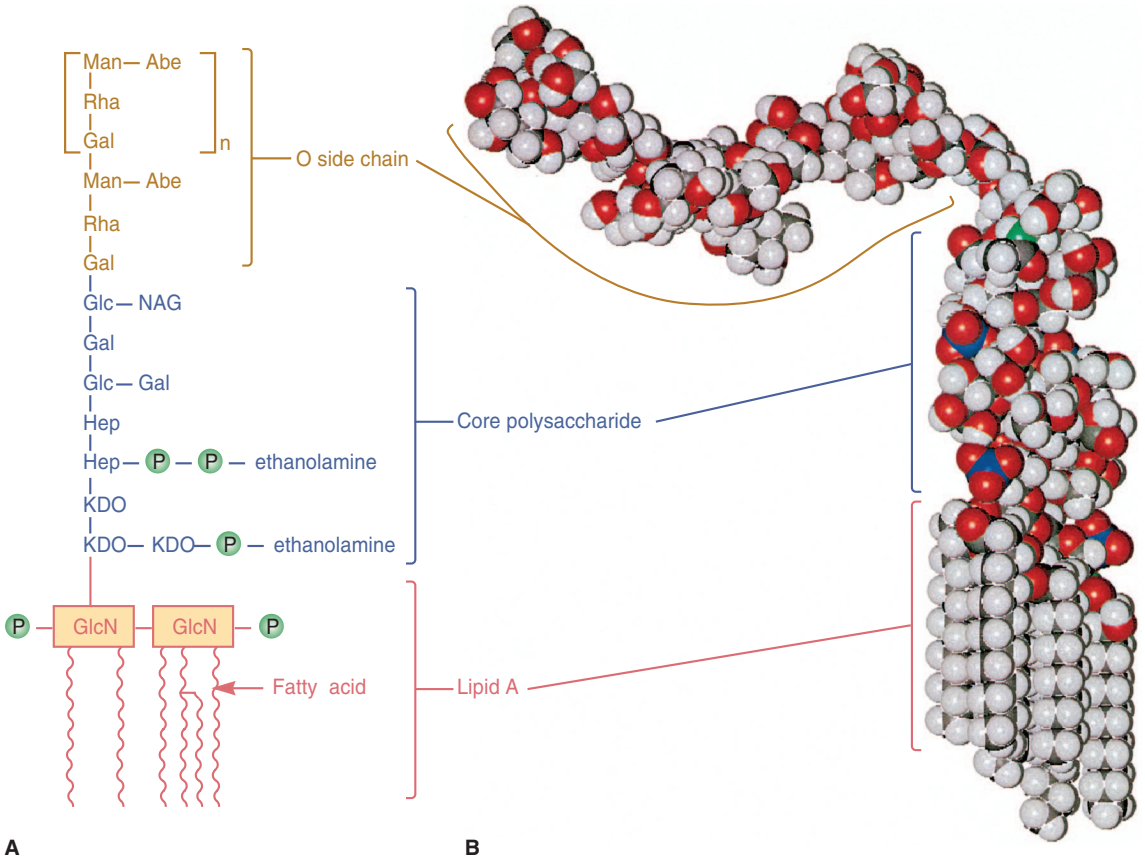


FIGURE 2-21 Lipopolysaccharide structure. **A:** The lipopolysaccharide from *Salmonella*. This slightly simplified diagram illustrates one form of the LPS. Abe, abequose; Gal, galactose; GlcN, glucosamine; Hep, heptulose; KDO, 2-keto-3-deoxyoctonate; Man, mannose; NAG, *N*-acetylglucosamine; P, phosphate; Rha, L-rhamnose. Lipid A is buried in the outer membrane. **B:** Molecular model of an *E. coli* lipopolysaccharide. The lipid A and core polysaccharide are straight; the O side chain is bent at an angle in this model. (Reproduced with permission from Willey JM, Sherwood LM, Woolverton CJ: *Prescott, Harley, and Klein's Microbiology*, 7th ed. McGraw-Hill; 2008. © McGraw-Hill Education.)

are lysed. When LPS is split into lipid A and polysaccharide, all the toxicity is associated with the former. The O antigen is highly immunogenic in a vertebrate animal. Antigenic specificity is conferred by the O antigen because this antigen is highly variable among species and even in strains within a species. The number of possible antigenic types is very great: Over 1000 have been recognized in *Salmonella* alone. Not all Gram-negative bacteria have outer membrane LPS composed of a variable number of repeated oligosaccharide units (see Figure 2-21); the outer membrane glycolipids of bacteria that colonize mucosal surfaces (eg, *Neisseria meningitidis, N. gonorrhoeae, Haemophilus influenzae*, and *Haemophilus ducreyi*) have relatively short, multiantennary (ie, branched) glycans. These smaller glycolipids have been compared with the "R-type" truncated LPS structures, which lack O antigens and are produced by rough mutants of enteric bacteria such as *E. coli*. However, the structures of these glycolipids more closely resemble those of the glycosphingolipids of mammalian cell membranes, and they are more properly termed **lipooligosaccharides (LOS)**. These molecules exhibit extensive antigenic and structural diversity even within a single strain. LOS is an important virulence factor. Epitopes have been identified on LOS that mimic host structures and may enable these organisms to evade the immune response of the host. Some LOS (eg, those from *N. gonorrhoeae, N. meningitidis*, and *H. ducreyi*) have a terminal *N*-acetyllactosamine (Galβ-1→4-GlcNAc) residue that is immunochemically similar to the precursor of the human erythrocyte I antigen. In the presence of a bacterial enzyme called **sialyltransferase** and a host or bacterial substrate (cytidine monophospho-*N*-acetylneuraminic acid, CMP-NANA), the *N*-acetyllactosamine residue is sialylated. This sialylation, which occurs in vivo, provides the organism with the environmental advantages of molecular mimicry of a host antigen and the biologic masking thought to be provided by sialic acids.

3. Lipoprotein—Molecules of an unusual **lipoprotein** cross-link the outer membrane and peptidoglycan layers (see Figure 2-19). The lipoprotein contains 57 amino acids, representing repeats of a 15-amino-acid sequence; it is peptide-linked to DAP residues of the peptidoglycan tetrapeptide side chains. The lipid component, consisting of a diglyceride

thioether linked to a terminal cysteine, is noncovalently inserted in the outer membrane. Lipoprotein is numerically the most abundant protein of Gram-negative cells (ca 700,000 molecules per cell). Its function (inferred from the behavior of mutants that lack it) is to stabilize the outer membrane and anchor it to the peptidoglycan layer.

4. The periplasmic space—The space between the inner and outer membranes, called the **periplasmic space**, contains the peptidoglycan layer and a gel-like solution of proteins. The periplasmic space is approximately 20–40% of the cell volume, which is far from insignificant. The periplasmic proteins include binding proteins for specific substrates (eg, amino acids, sugars, vitamins, and ions), hydrolytic enzymes (eg, alkaline phosphatase and 5′-nucleotidase) that break down nontransportable substrates into transportable ones, and detoxifying enzymes (eg, β-lactamase and aminoglycoside-phosphorylase) that inactivate certain antibiotics. The periplasm also contains high concentrations of highly branched polymers of D-glucose, 8 to 10 residues long, which are variously substituted with glycerol phosphate and phosphatidylethanolamine residues; some contain O-succinyl esters. These so-called **membrane-derived oligosaccharides** appear to play a role in osmoregulation because cells grown in media of low osmolarity increase their synthesis of these compounds 16-fold.

D. The Acid-Fast Cell Wall

Some bacteria, notably the tubercle bacillus (*M. tuberculosis*) and its relatives, have cell walls that contain substantial amounts of **waxes**, complex branched hydrocarbons (70–90 carbons long) known as **mycolic acids**. The cell wall is composed of peptidoglycan and an external asymmetric lipid bilayer; the inner leaflet contains mycolic acids linked to an arabinoglycan, and the outer leaflet contains other extractable lipids. This is a highly ordered lipid bilayer in which proteins are embedded, forming water-filled pores through which nutrients and certain drugs can pass slowly. Some compounds can also penetrate the lipid domains of the cell wall albeit slowly. This hydrophobic structure renders these bacteria resistant to many harsh chemicals, including detergents and strong acids. If a dye is introduced into these cells by brief heating or treatment with detergents, the dye cannot be removed by dilute hydrochloric acid, as in other bacteria. These organisms are therefore called **acid fast.** The permeability of the cell wall to hydrophilic molecules is 100- to 1000-fold lower than for *E. coli* and may be responsible for the slow growth rate of mycobacteria.

E. Cell Walls of the *Archaea*

The *Archaea* do not have cell walls like the *Bacteria*. Some have a simple S-layer (see next) often composed of glycoproteins. Some *Archaea* have a rigid cell wall composed of polysaccharides or a macromolecule called **pseudomurein**. The pseudomurein differs from the peptidoglycan of bacteria by having L-amino acids rather than D-amino acids and disaccharide units with an α-1→3 rather than a β1→4 linkage. *Archaea* that have a pseudomurein cell wall are Gram-positive.

F. Crystalline Surface Layers

Many bacteria, both Gram-positive and Gram-negative bacteria as well as Archaebacteria, possess a two-dimensional crystalline, subunit-type layer lattice of protein or glycoprotein molecules **(S-layer)** as the outermost component of the cell envelope. In both Gram-positive and Gram-negative bacteria, this structure is sometimes several molecules thick. In some *Archaea*, it is the only layer external to the cell membrane.

S-layers are generally composed of a single kind of protein molecule, sometimes with carbohydrates attached. The isolated molecules are capable of self-assembly (ie, they make sheets similar or identical to those present on the cells). S-layer proteins are resistant to proteolytic enzymes and protein-denaturing agents. The function of the S-layer is uncertain but is probably protective. In some cases, it has been shown to protect the cell from wall-degrading enzymes, from invasion by *Bdellovibrio bacteriovorus* (a predatory bacterium), and from bacteriophages. It also plays a role in the maintenance of cell shape in some species of Archaebacteria, and it may be involved in cell adhesion to host epidermal surfaces.

G. Enzymes That Attack Cell Walls

The β1→4 glycan linkage of the peptidoglycan backbone is hydrolyzed by the enzyme **lysozyme** (see Figure 2-17), which is found in animal secretions (tears, saliva, nasal secretions) as well as in egg white. Gram-positive bacteria treated with lysozyme in low-osmotic-strength media lyse; if the osmotic strength of the medium is raised to balance the internal osmotic pressure of the cell, free spherical bodies called **protoplasts** are liberated. The outer membrane of the Gram-negative cell wall prevents access of lysozyme unless disrupted by an agent such as ethylene-diaminetetraacetic acid (EDTA), a compound that chelates divalent cations; in osmotically protected media, cells treated with EDTA-lysozyme form **spheroplasts** that still possess remnants of the complex Gram-negative wall, including the outer membrane.

Bacteria themselves possess a number of **autolysins**, hydrolytic enzymes that attack peptidoglycan, including muramidases, glucosaminidases, endopeptidases, and carboxypeptidases. These enzymes catalyze the turnover or degradation of peptidoglycan in bacteria. These enzymes presumably participate in cell wall growth and turnover and in cell separation, but their activity is most apparent during the dissolution of dead cells (autolysis).

Enzymes that degrade bacterial cell walls are also found in cells that digest whole bacteria (eg, protozoa and the phagocytic cells of higher animals).

H. Cell Wall Growth

Cell wall synthesis is necessary for cell division; however, the incorporation of new cell wall material varies with the