
Jawetz, Melnick & Adelberg's
MEDICAL
MICROBIOLOGY
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Steve Miller
28th Edition
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Jawetz, Melnick, & Adelberg's Medical Microbiology

Twenty-Eighth Edition

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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**

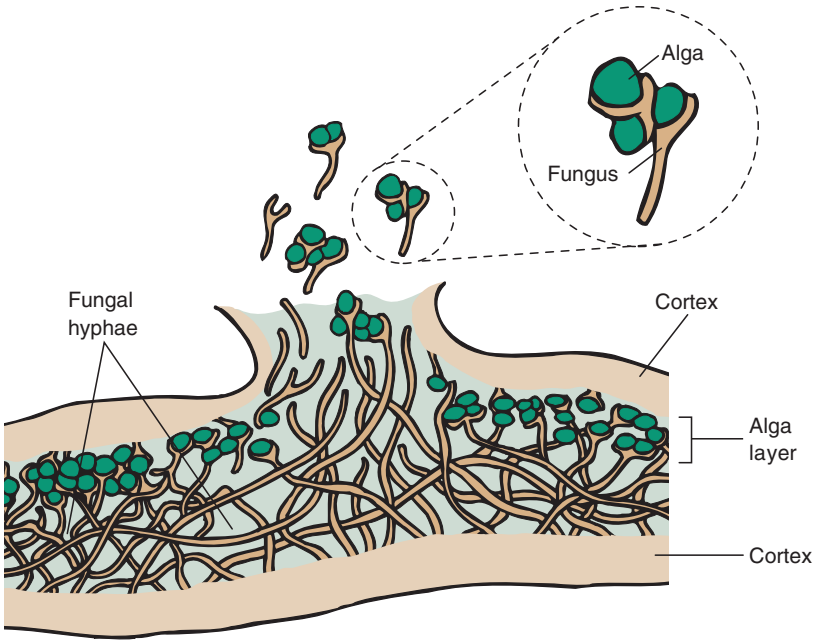

FIGURE 1-1 Diagram of a lichen, consisting of cells of a phototroph, either an alga or a cyanobacterium, entwined within the hyphae of the fungal partner. (Reproduced with permission from Nester EW, Anderson DG, Roberts CE, et al: *Microbiology: A Human Perspective*, 6th ed. McGraw-Hill, 2009, p. 293.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

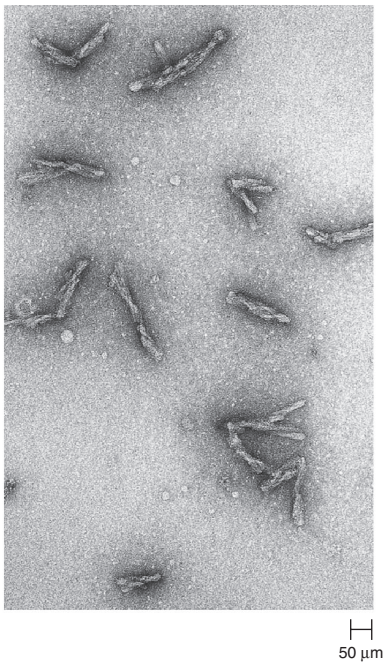

FIGURE 1-2 Prion. Prions isolated from the brain of a scrapie-infected hamster. This neurodegenerative disease is caused by a prion. (Reproduced with permission from Stanley B. Prusiner.)

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

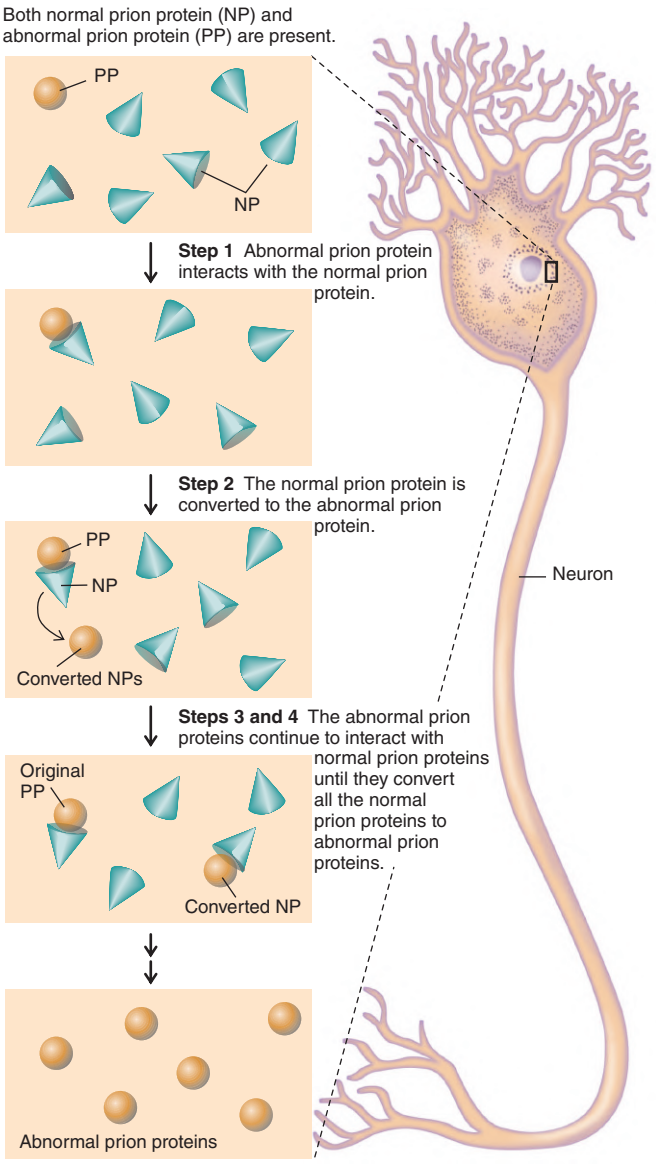

FIGURE 1-3 Proposed mechanism by which prions replicate. The normal and abnormal prion proteins differ in their tertiary structure. (Reproduced with permission from Nester EW, Anderson DG, Roberts CE, et al: *Microbiology: A Human Perspective*, 6th ed. McGraw-Hill, 2009, p. 342. © McGraw-Hill Education.)

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

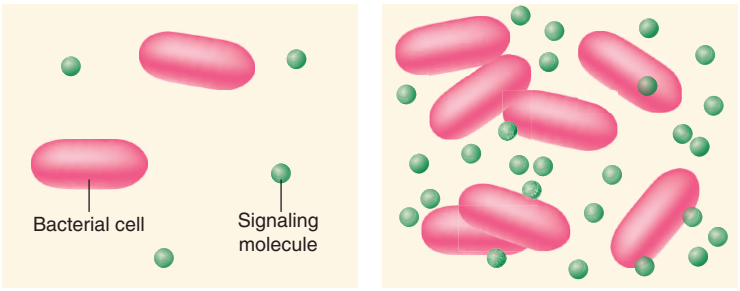

FIGURE 1-4 Quorum sensing. (Reproduced with permission from Nester EW, Anderson DG, Roberts CE, et al: *Microbiology: A Human Perspective*, 6th ed. McGraw-Hill, 2009, p. 181. © McGraw-Hill Education.)

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

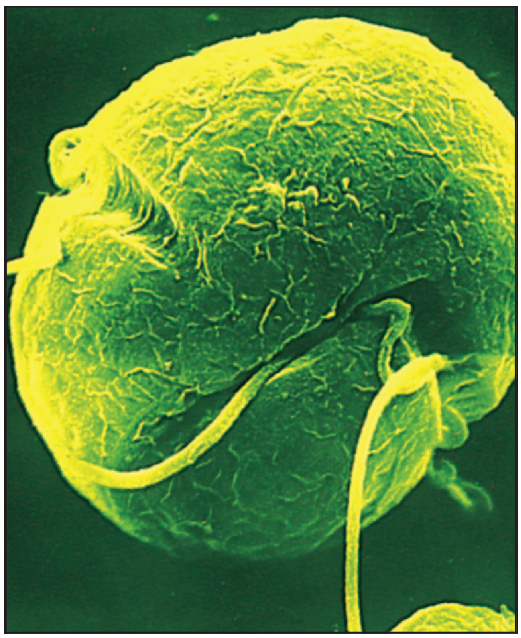

FIGURE 1-5 The dinoflagellate *Gymnodinium* scanning electron micrograph (4000×). (Reproduced with permission from Dr. David Phillips/Visuals Unlimited.)

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

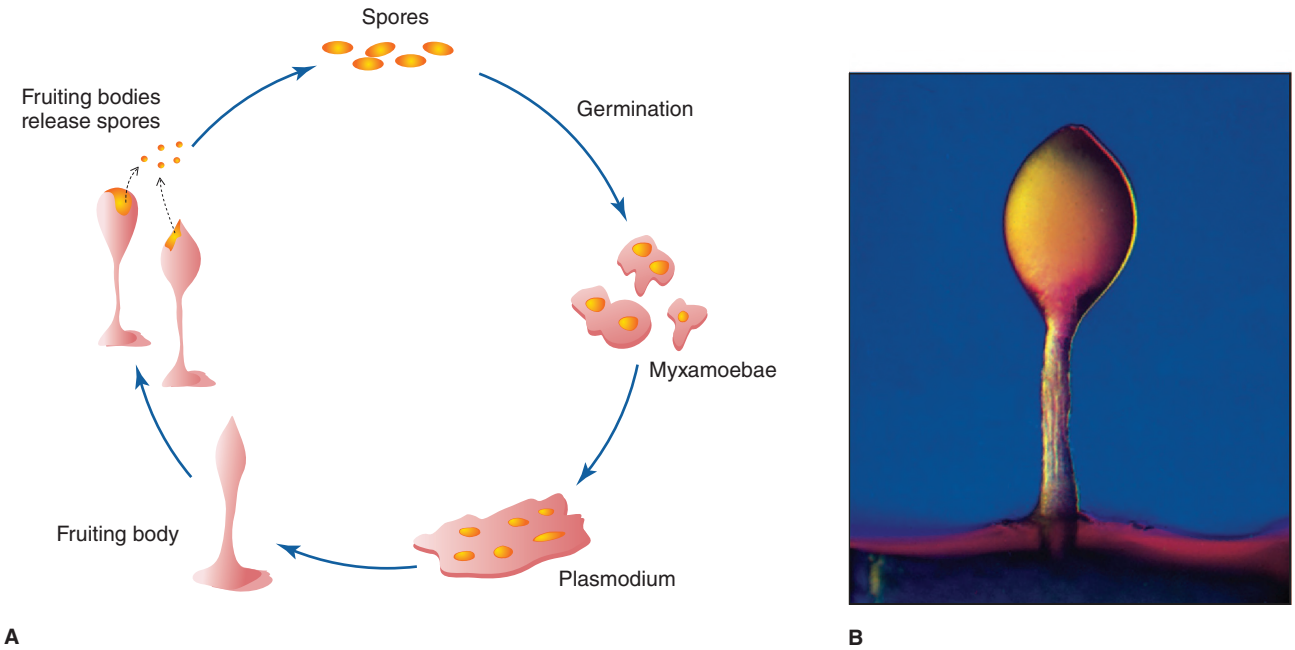

FIGURE 1-6 Slime molds. **A:** Life cycle of an acellular slime mold. **B:** Fruiting body of a cellular slime mold. (Reproduced with permission from Carolina Biological Supply/DIOMEDIA.)

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

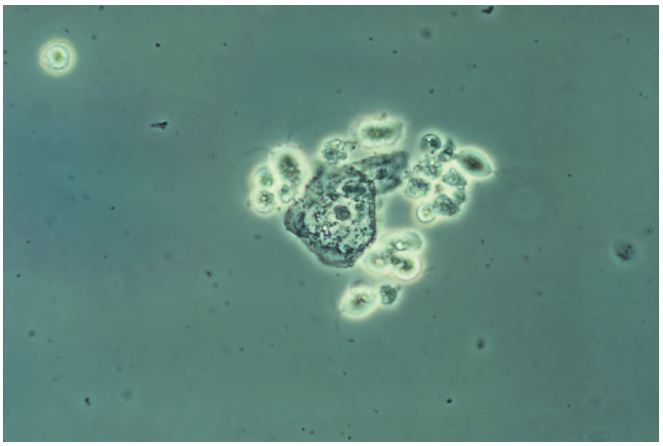


FIGURE 2-1 Using the phase contrast illumination technique, this photomicrograph of a wet mount of a vaginal discharge specimen revealed the presence of the flagellated protozoan, *Trichomonas vaginalis*. (Courtesy of Centers for Disease Control and Prevention, Public Health Image Library, ID# 5238.)

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

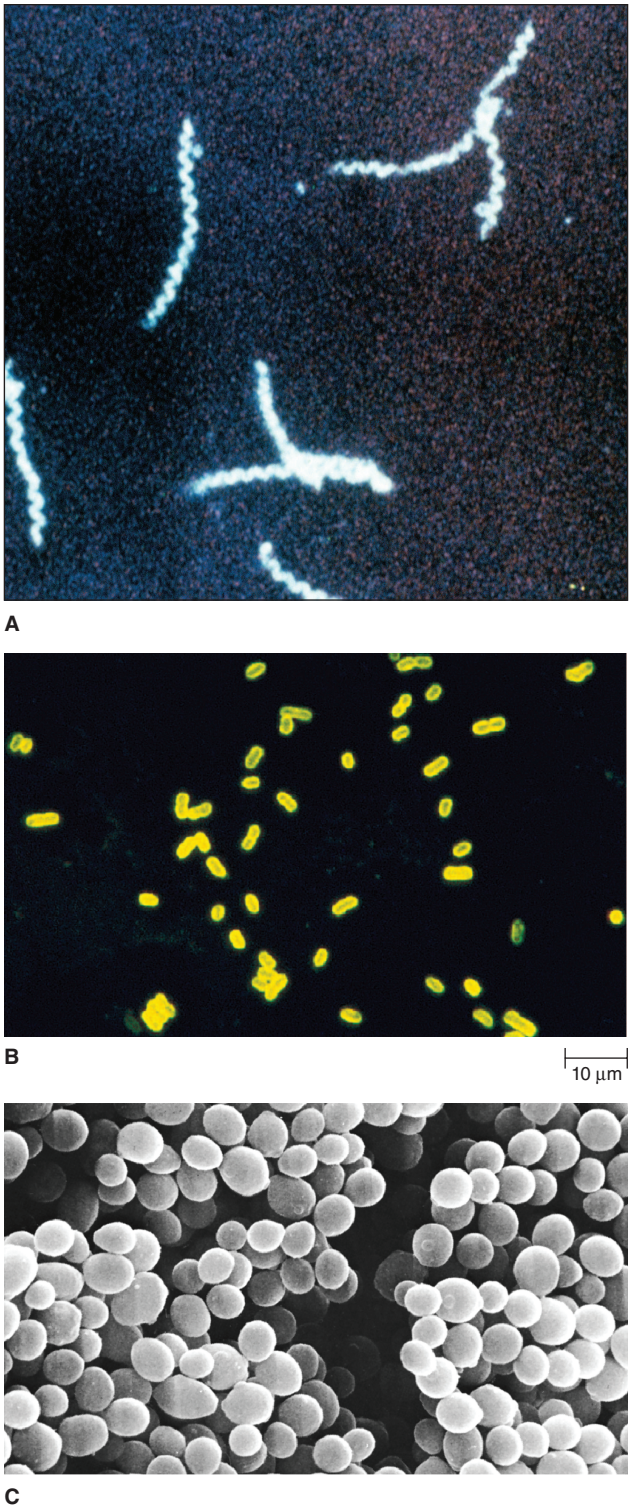


FIGURE 2-2 **A:** Positive dark-field examination. Treponemes are recognizable by their characteristic corkscrew shape and deliberate forward and backward movement with rotation about the longitudinal axis. (Reproduced with permission. © Charles Stratton/Visuals Unlimited.) **B:** Fluorescence photomicrograph. 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.

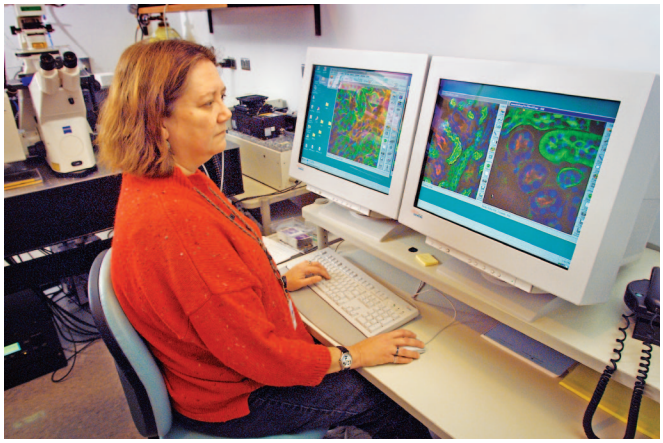


FIGURE 2-3 Using laser light, CDC laboratory scientists sometimes work with a confocal microscope when studying various pathogens. (Courtesy of James Gathany, Centers for Disease Control and Prevention, Public Health Image Library, ID# 1960.)

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

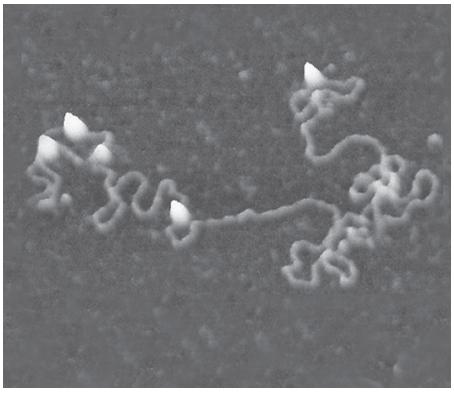


FIGURE 2-4 Atomic force microscopy. Micrograph of a fragment of DNA. The bright peaks are enzymes attached to the DNA. (Reproduced with permission from Torunn Berg, Photo Researchers, Inc.)

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

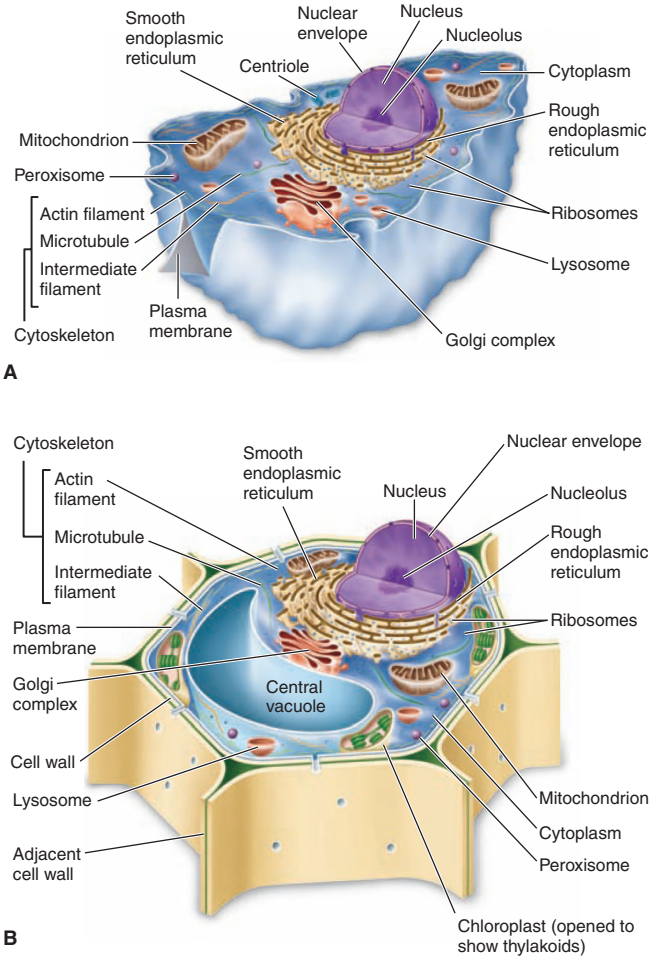


FIGURE 2-5 Eukaryotic cells. **A:** Diagrammatic representation of an animal cell. **B:** Diagrammatic representation of a plant cell. **C:** Micrograph of an animal cell shows several membrane-bound structures, including mitochondria and a nucleus. (Fig. 2-3(A) and (B) Reproduced with permission from 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

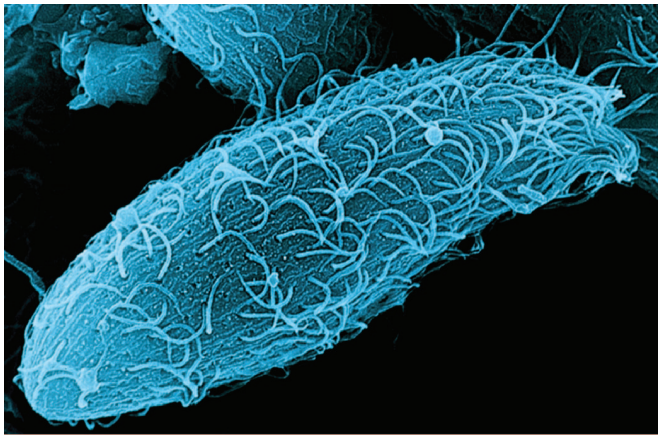


FIGURE 2-6 A paramecium moves with the aid of cilia on the cell surface. (© Manfred Kage.)

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.

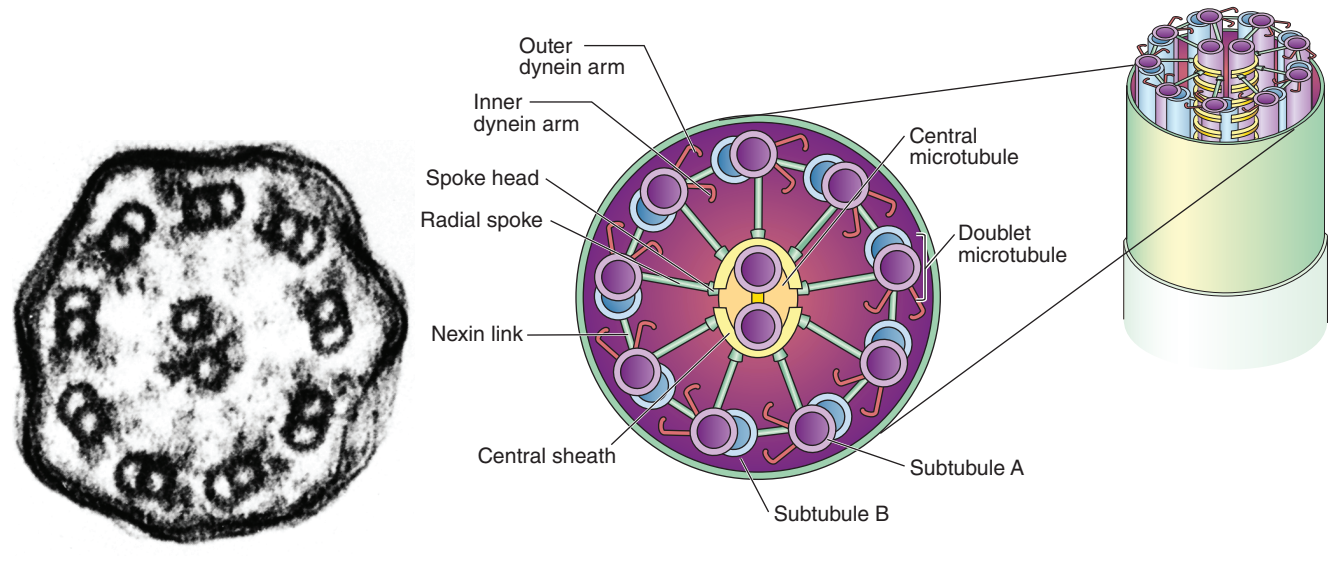
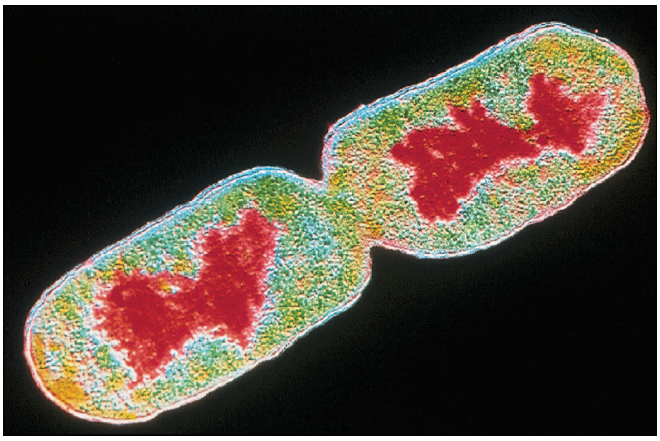


FIGURE 2-7 Cilia and flagella structure. **A:** An electron micrograph of a cilium cross section. Note the two central microtubles surrounded by nine microtubule doublets (160,000×). (Reproduced with permission. © Kallista Images/Visuals Unlimited, Inc.) **B:** A diagram of cilia and flagella structure.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



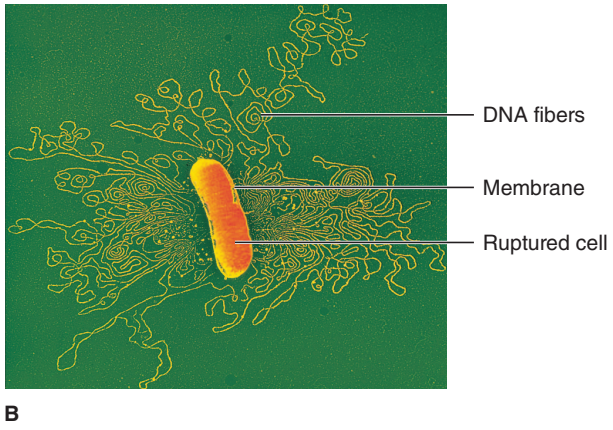


FIGURE 2-8 The nucleoid. **A:** Color-enhanced transmission electron micrograph of *E. coli* with the DNA shown in *red*. (Reproduced with permission. © CNRI/SPL/Photo Researchers, Inc.) **B:** Chromosome released from a gently lysed cell of *E. coli*. Note how tightly packaged the DNA must be inside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

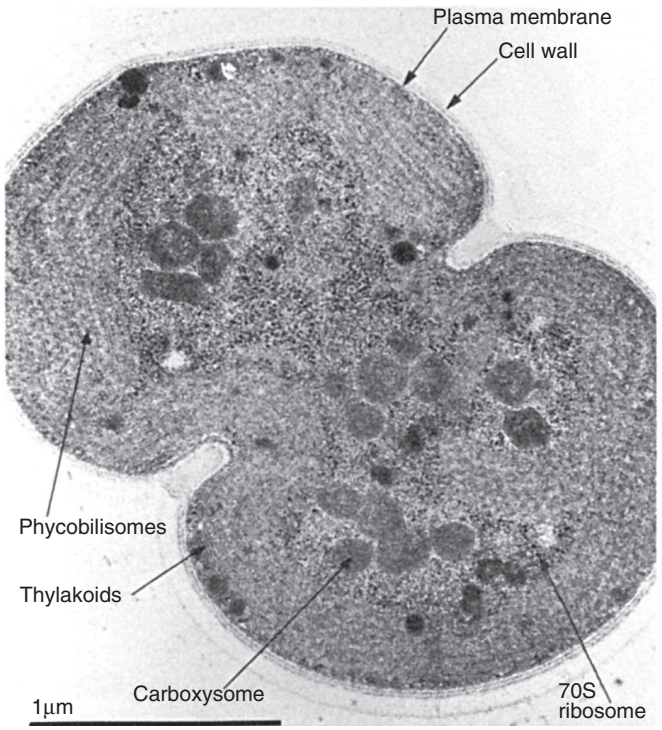
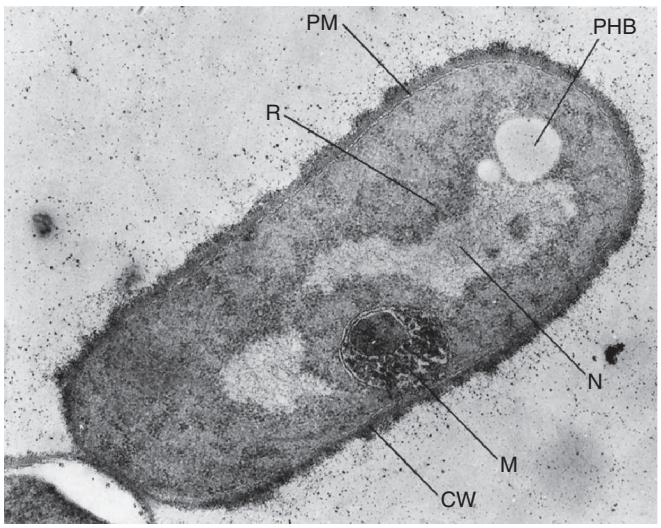


FIGURE 2-9 Thin section of *Synechocystis* during division. Many structures are visible. (Reproduced from Stanier RY: The position of cyanobacteria in the world of phototrophs. Carlsberg Res Commun 42:77-98, 1977. With kind permission of Springer + Business Media.)

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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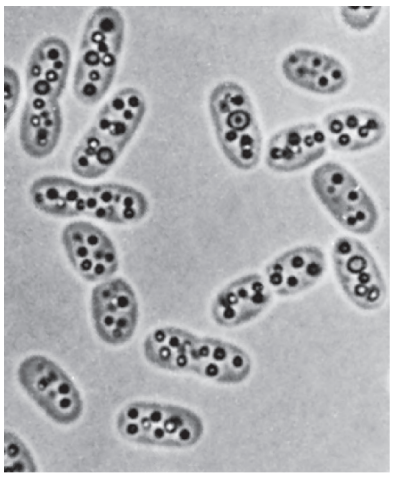

B

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.)

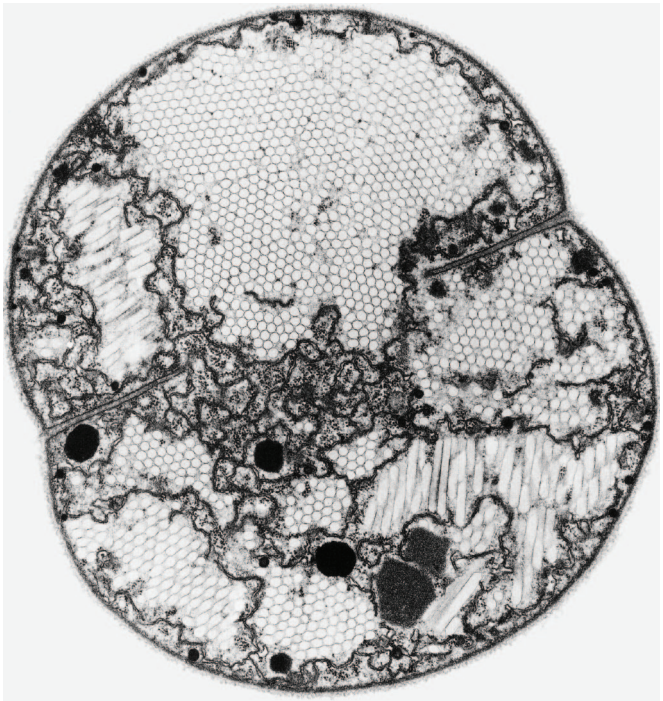

FIGURE 2-11 Transverse section of a dividing cell of the cyanobacterium *Microcystis* species showing hexagonal stacking of the cylindric gas vesicles (31,500×). (Micrograph by HS Pankratz. Reproduced with permission from Walsby AE: Gas vesicles. *Microbiol Rev* 1994;58:94.)

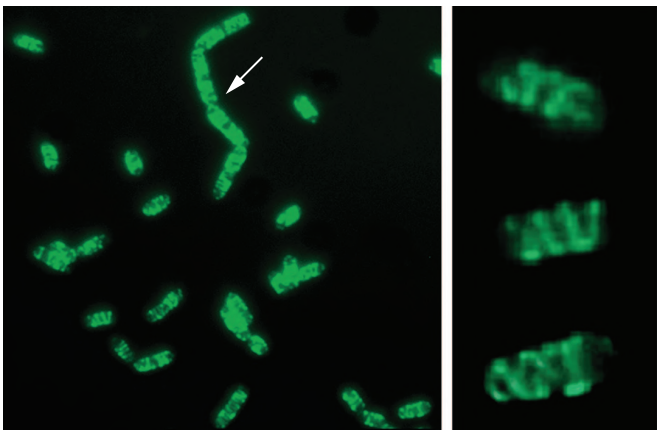

FIGURE 2-12 The prokaryotic cytoskeleton. Visualization of the MreB-like cytoskeletal protein (Mbl) of *B. subtilis*. The Mbl protein has been fused with green fluorescent protein, and live cells have been examined by fluorescence microscopy. **A:** *Arrows* point to the helical cytoskeleton cables that 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

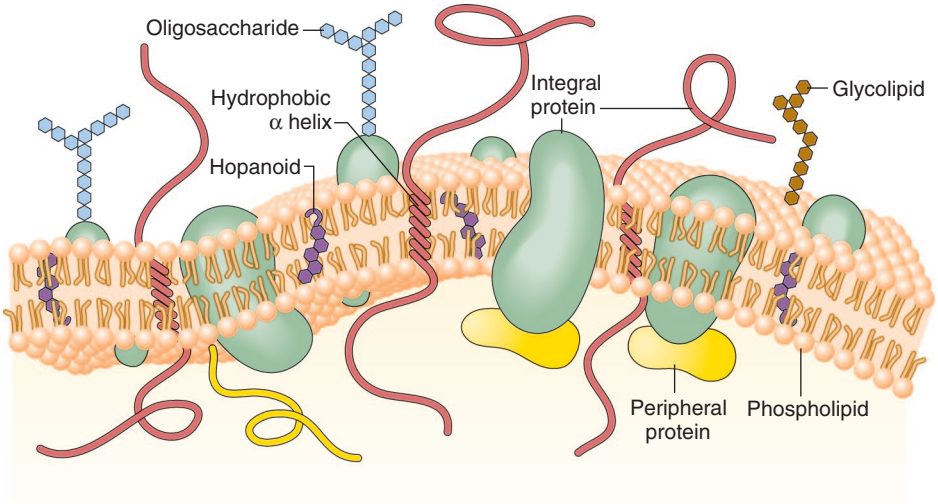

FIGURE 2-13 Bacterial plasma membrane structure. This diagram of the fluid mosaic model of bacterial membrane structure shown the integral proteins (*green* and *red*) floating in a lipid bilayer. Peripheral proteins (*yellow*) are associated loosely with the inner membrane surface. Small spheres represent 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