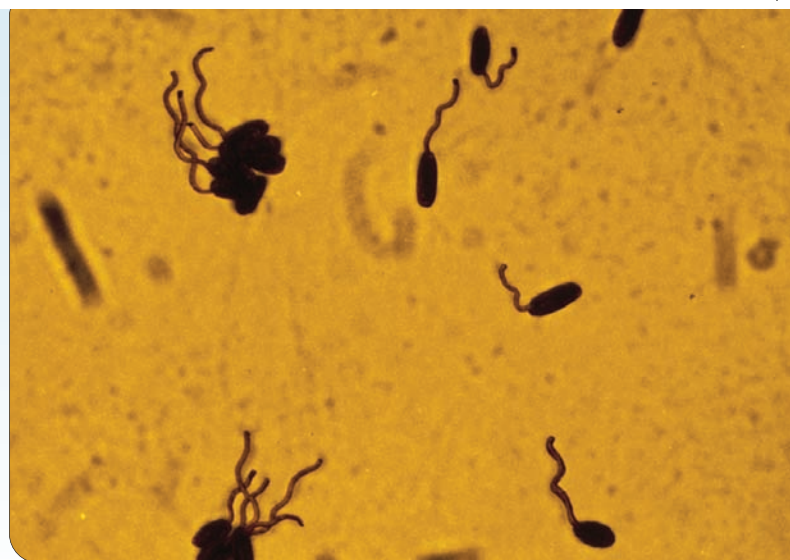


3

Prokaryotic Cell Structure and Function



Bacterial species may differ in their patterns of flagella distribution. These *Pseudomonas* cells have a single polar flagellum used for locomotion.

PREVIEW

- Prokaryotes can be distinguished from eukaryotes in terms of their size, cell structure, and molecular make-up. Most prokaryotes lack extensive, complex, internal membrane systems.
- Although some cell structures are observed in both eukaryotic and prokaryotic cells, some structures are unique to prokaryotes.
- Prokaryotes can be divided into two major groups: *Bacteria* and *Archaea*. Although similar in overall structure, *Bacteria* and *Archaea* exhibit important differences in their cell walls and membranes.
- Most bacteria can be divided into two broad groups based on cell wall structure; the differences in cell wall structure correlate with the reaction to the Gram staining procedure.
- Many prokaryotes are motile; several mechanisms for motility have been identified.
- Some bacteria form resistant endospores to survive harsh environmental conditions in a dormant state.

Even a superficial examination of the microbial world shows that prokaryotes are one of the most important groups by any criterion: numbers of organisms, general ecological importance, or practical importance for humans. Indeed, much of our understanding of phenomena in biochemistry and molecular biology comes from research on prokaryotes. Although considerable space in this text is devoted to eukaryotic microorganisms, the major focus is on prokaryotes. Therefore the unit on microbial morphology begins with the structure of prokaryotes. As mentioned in chapter 1, there are two quite different groups of prokaryotes: *Bacteria* and *Archaea*. Although considerably less is known about archaeal cell structure and biochemistry, certain features distinguish the two domains. Whenever possible, these distinctions will be noted. A more detailed discus-

sion of the *Archaea* is provided in chapter 20. A comment about nomenclature is necessary to avoid confusion. The word prokaryote will be used in a general sense to include both the *Bacteria* and *Archaea*; the term bacterium will refer specifically to a member of the *Bacteria* and archaeon to a member of the *Archaea*. [Members of the microbial world \(section 1.1\)](#)

3.1 AN OVERVIEW OF PROKARYOTIC CELL STRUCTURE

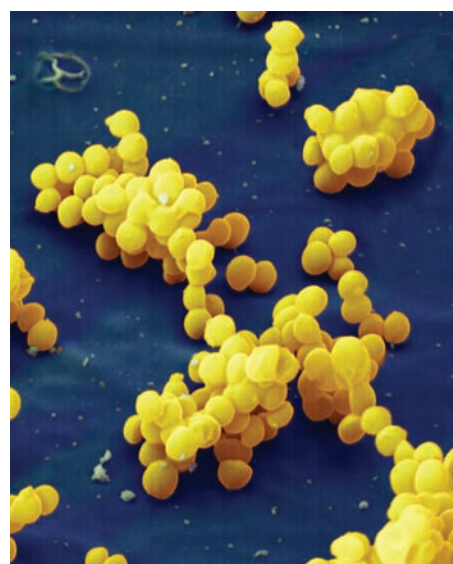
Because much of this chapter is devoted to a discussion of individual cell components, a preliminary overview of the prokaryotic cell as a whole is in order.

Shape, Arrangement, and Size

One might expect that small, relatively simple organisms like prokaryotes would be uniform in shape and size. This is not the case, as the microbial world offers almost endless variety in terms of morphology (**figures 3.1 and 3.2**). However, most commonly encountered prokaryotes have one of two shapes. **Cocci** (s., **coccus**) are roughly spherical cells. They can exist as individual cells, but also are associated in characteristic arrangements that are frequently useful in their identification. **Diplococci** (s., **diplococcus**) arise when cocci divide and remain together to form pairs. Long chains of cocci result when cells adhere after repeated divisions in one plane; this pattern is seen in the genera *Streptococcus*, *Enterococcus*, and *Lactococcus* (figure 3.1b). *Staphylococcus* divides in random planes to generate irregular grapelike clumps (figure 3.1a). Divisions in two or three planes

The era in which workers tended to look at bacteria as very small bags of enzymes has long passed.

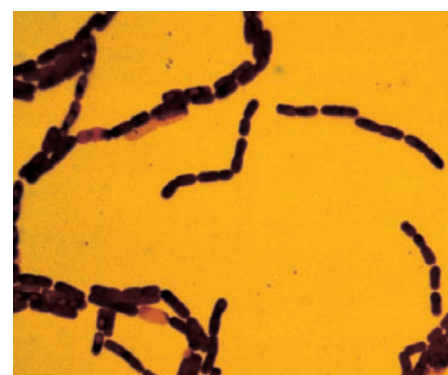
—Howard J. Rogers



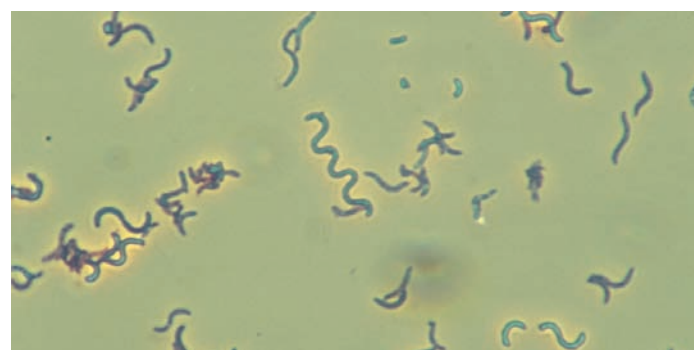
(a) *S. aureus*



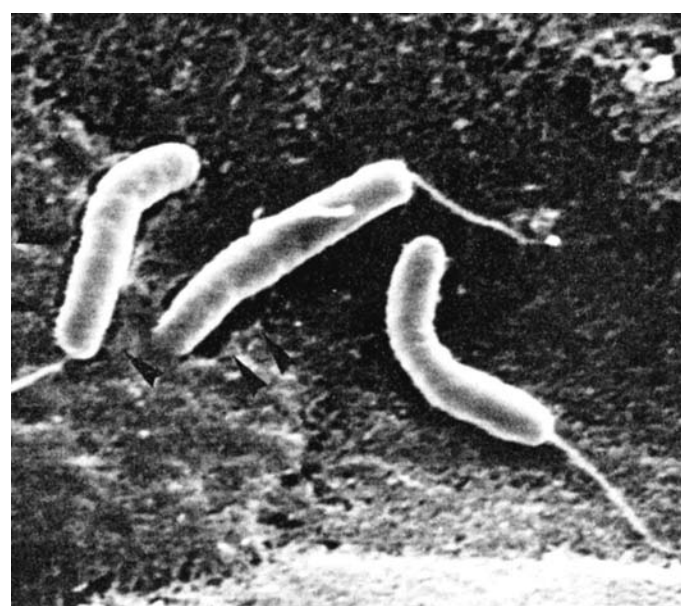
(b) *S. agalactiae*



(c) *B. megaterium*



(d) *R. rubrum*



(e) *V. cholerae*

Figure 3.1 Common Prokaryotic Cell Shapes. (a) *Staphylococcus aureus* cocci arranged in clusters; color-enhanced scanning electron micrograph; average cell diameter is about 1 μm . (b) *Streptococcus agalactiae*, the cause of Group B streptococcal infections; cocci arranged in chains; color-enhanced scanning electron micrograph ($\times 4,800$). (c) *Bacillus megaterium*, a rod-shaped bacterium arranged in chains, Gram stain ($\times 600$). (d) *Rhodospirillum rubrum*, phase contrast ($\times 500$). (e) *Vibrio cholerae*, curved rods with polar flagella; scanning electron micrograph.

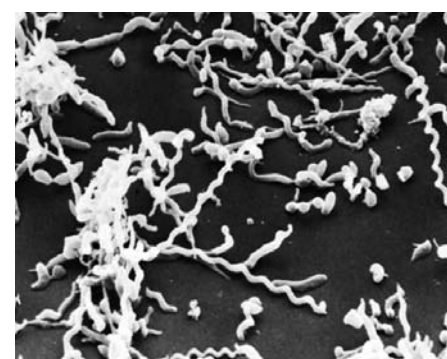
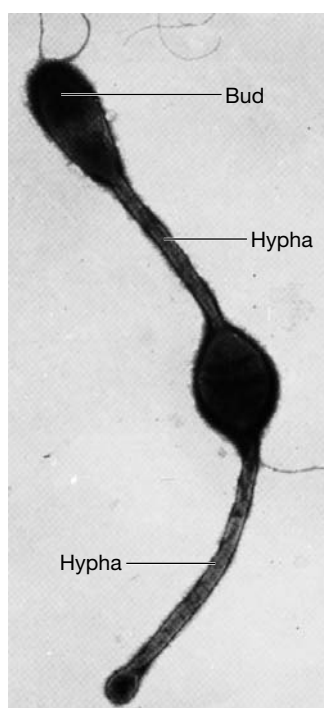
can produce symmetrical clusters of cocci. Members of the genus *Micrococcus* often divide in two planes to form square groups of four cells called tetrads. In the genus *Sarcina*, cocci divide in three planes producing cubical packets of eight cells.

The other common shape is that of a **rod**, sometimes called a **bacillus** (pl., **bacilli**). *Bacillus megaterium* is a typical example of a bacterium with a rod shape (figure 3.1c). Bacilli differ considerably in their length-to-width ratio, the coccobacilli being so short and wide that they resemble cocci. The shape of the rod's end often varies between species and may be flat, rounded, cigar-shaped, or bifurcated. Although many rods occur singly, some re-

main together after division to form pairs or chains (e.g., *Bacillus megaterium* is found in long chains).

Although procaryotes are often simple spheres or rods, other cell shapes and arrangements are not uncommon. **Vibrios** most closely resemble rods, as they are comma-shaped (figure 3.1e). Spiral-shaped procaryotes can be either classified as **spirilla**, which usually have tufts of flagella at one or both ends of the cell (figure 3.1d and 3.2c), or spirochetes. **Spirochetes** are more flexible and have a unique, internal flagellar arrangement. Actinomycetes typically form long filaments called hyphae that may branch to produce a network called a **mycelium** (figure 3.2a). In this sense, they are


(a) *Actinomyces*

(b) *M. pneumoniae*

(c) *Spiroplasma*

(d) *Hyphomicrobium*


(e) Walsby's square archaeon

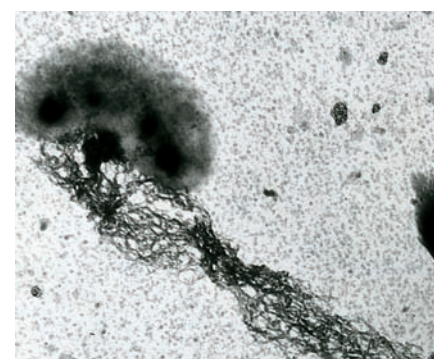

(f) *G. ferruginea*

Figure 3.2 Unusually Shaped Prokaryotes.

Examples of prokaryotes with shapes quite different from bacillus and coccus types. (a) *Actinomyces*, SEM ($\times 21,000$). (b) *Mycoplasma pneumoniae*, SEM ($\times 62,000$). (c) *Spiroplasma*, SEM ($\times 13,000$). (d) *Hyphomicrobium* with hyphae and bud, electron micrograph with negative staining. (e) Walsby's square archaeon. (f) *Gallionella ferruginea* with stalk.

similar to filamentous fungi, a group of eucaryotic microbes. The oval- to pear-shaped *Hyphomicrobium* (figure 3.2d) produces a bud at the end of a long hypha. Other bacteria such as *Gallionella* produce nonliving stalks (figure 3.2f). A few prokaryotes actually are flat. For example, Anthony Walsby has discovered square archaea living in salt ponds (figure 3.2e). They are shaped like flat, square-to-rectangular boxes about $2\ \mu\text{m}$ by 2 to $4\ \mu\text{m}$, and only $0.25\ \mu\text{m}$ thick. Finally, some prokaryotes are variable in shape and lack a single, characteristic form (figure 3.2b). These are called **pleomorphic** even though they may, like *Corynebacterium*, have a generally rod-like form. [Phylum Spirochaetes \(section 21.6\)](#)

Bacteria vary in size as much as in shape (figure 3.3). *Escherichia coli* is a rod of about average size, 1.1 to $1.5\ \mu\text{m}$ wide by 2.0 to $6.0\ \mu\text{m}$ long. Near the small end of the size continuum

are members of the genus *Mycoplasma*, an interesting group of bacteria that lack cell walls. For many years, it was thought that they were the smallest prokaryotes at about $0.3\ \mu\text{m}$ in diameter, approximately the size of the poxviruses. However, even smaller prokaryotes have been discovered. Nanobacteria range from around $0.2\ \mu\text{m}$ to less than $0.05\ \mu\text{m}$ in diameter. Only a few strains have been cultured, and these appear to be very small, bacteria-like organisms. The discovery of nanobacteria was quite surprising because theoretical calculations predicted that the smallest cells were about 0.14 to $0.2\ \mu\text{m}$ in diameter. At the other end of the continuum are bacteria such as the spirochaetes, which can reach $500\ \mu\text{m}$ in length, and the photosynthetic bacterium *Oscillatoria*, which is about $7\ \mu\text{m}$ in diameter (the same diameter as a red blood cell). A huge bacterium lives in the intestine of

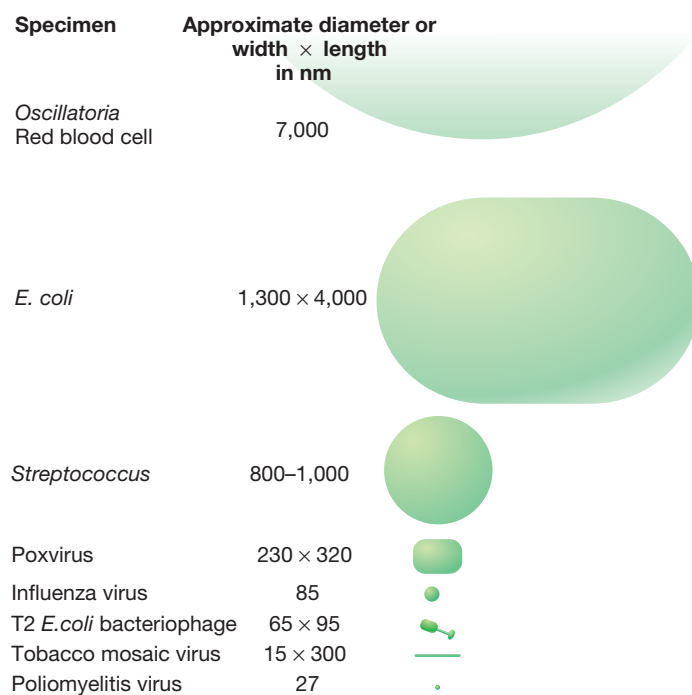


Figure 3.3 **Sizes of Prokaryotes and Viruses.** The sizes of selected bacteria relative to a red blood cell and viruses.

the brown surgeonfish, *Acanthurus nigrofasciatus*. *Epulopiscium fishelsoni* grows as large as 600 by 80 μm , a little smaller than a printed hyphen. More recently an even larger bacterium, *Thiomargarita namibiensis*, has been discovered in ocean sediment (**Microbial Diversity & Ecology 3.1**). Thus a few bacteria are much larger than the average eucaryotic cell (typical plant and animal cells are around 10 to 50 μm in diameter).

Prokaryotic Cell Organization

Prokaryotic cells are morphologically simpler than eucaryotic cells, but they are not just simpler versions of eucaryotes. Although many structures are common to both cell types, some are unique to prokaryotes. The major prokaryotic structures and their functions are summarized and illustrated in **table 3.1** and **figure 3.4**, respectively. Note that no single prokaryote possesses all of these structures at all times. Some are found only in certain cells in certain conditions or in certain phases of the life cycle. However, despite these variations prokaryotes are consistent in their fundamental structure and most important components.

Prokaryotic cells almost always are bounded by a chemically complex cell wall. Interior to this wall lies the plasma membrane. This membrane can be invaginated to form simple internal membranous structures such as the light-harvesting membrane of some photosynthetic bacteria. Since the prokaryotic cell does not contain internal membrane-bound organelles, its interior appears morphologically simple. The genetic material is localized in a discrete region, the nucleoid, and usually is not separated from the

surrounding cytoplasm by membranes. Ribosomes and larger masses called inclusion bodies are scattered about the cytoplasmic matrix. Many prokaryotes use flagella for locomotion. In addition, many are surrounded by a capsule or slime layer external to the cell wall.

In the remaining sections of this chapter we describe the major prokaryotic structures in more detail. We begin with the plasma membrane, a structure that defines all cells. We then proceed inward to consider structures located within the cytoplasm. Then the discussion moves outward, first to the cell wall and then to structures outside the cell wall. Finally, we consider a structure unique to bacteria, the bacterial endospore.

1. What characteristic shapes can bacteria assume? Describe the ways in which bacterial cells cluster together.
2. Draw a bacterial cell and label all important structures.

3.2 PROCARYOTIC CELL MEMBRANES

Membranes are an absolute requirement for all living organisms. Cells must interact in a selective fashion with their environment, whether it is the internal environment of a multicellular organism or a less protected and more variable external environment. Cells must not only be able to acquire nutrients and eliminate wastes, but they also have to maintain their interior in a constant, highly organized state in the face of external changes.

The **plasma membrane** encompasses the cytoplasm of both prokaryotic and eucaryotic cells. It is the chief point of contact with the cell's environment and thus is responsible for much of its relationship with the outside world. The plasma membranes of prokaryotic cells are particularly important because they must fill an incredible variety of roles. In addition to retaining the cytoplasm, the plasma membrane also serves as a selectively permeable barrier: it allows particular ions and molecules to pass, either into or out of the cell, while preventing the movement of others. Thus the membrane prevents the loss of essential components through leakage while allowing the movement of other molecules. Because many substances cannot cross the plasma membrane without assistance, it must aid such movement when necessary. Transport systems are used for such tasks as nutrient uptake, waste excretion, and protein secretion. The prokaryotic plasma membrane also is the location of a variety of crucial metabolic processes: respiration, photosynthesis, and the synthesis of lipids and cell wall constituents. Finally, the membrane contains special receptor molecules that help prokaryotes detect and respond to chemicals in their surroundings. Clearly the plasma membrane is essential to the survival of microorganisms. [Uptake of nutrients by cells \(section 5.6\)](#)

As will be evident in the following discussion, all membranes apparently have a common, basic design. However, prokaryotic membranes can differ dramatically in terms of the lipids they contain. Indeed, membrane chemistry can be used to identify particular bacterial species. To understand these chemical differences and to understand the many functions of the



Microbial Diversity & Ecology

3.1 Monstrous Microbes

Biologists often have distinguished between procaryotes and eucaryotes based in part on cell size. Generally, procaryotic cells are supposed to be smaller than eucaryotic cells. Procaryotes grow extremely rapidly compared to most eucaryotes and lack the complex vesicular transport systems of eucaryotic cells described in chapter 4. It has been assumed that they must be small because of the slowness of nutrient diffusion and the need for a large surface-to-volume ratio. Thus when Fishelson, Montgomery, and Myrberg discovered a large, cigar-shaped microorganism in the intestinal tract of the Red Sea brown surgeonfish, *Acanthurus nigrofusus*, they suggested in their 1985 publication that it was a protist. It seemed too large to be anything else. In 1993 Esther Angert, Kendall Clemens, and Norman Pace used rRNA sequence comparisons to identify the microorganism, now called *Epulopiscium fishelsoni*, as a procaryote related to the gram-positive bacterial genus *Clostridium*.

E. fishelsoni [Latin, *epulum*, a feast or banquet, and *piscium*, fish] can reach a size of 80 μm by 600 μm , and normally ranges from 200 to 500 μm in length (see **Box figure**). It is about a million times larger in volume than *Escherichia coli*. Despite its huge size the organism has a procaryotic cell structure. It is motile and swims at about two body lengths a second (approximately 2.4 cm/min) using the flagella that cover its surface. The cytoplasm contains large nucleoids and many ribosomes, as would be required for such a large cell. *Epulopiscium* appears to overcome the size limits set by diffusion by having a highly convoluted plasma membrane. This increases the cell's surface area and aids in nutrient transport.

It appears that *Epulopiscium* is transmitted between hosts through fecal contamination of the fish's food. The bacterium can be eliminated by starving the surgeonfish for a few days. If juvenile fish that lack the bacterium are placed with infected hosts, they are reinoculated. Interestingly this does not work with uninfected adult surgeonfish.

In 1997, Heidi Schulz discovered an even larger procaryote in the ocean sediment off the coast of Namibia. *Thiomargarita namibiensis* is a spherical bacterium, between 100 and 750 μm in diameter, that often forms chains of cells enclosed in slime sheaths. It is over 100 times larger in volume than *E. fishelsoni*. A vacuole occupies about 98% of the cell and contains fluid rich in nitrate; it is surrounded by a 0.5 to 2.0 μm layer of cytoplasm filled with sulfur granules. The cytoplasmic layer is the same thickness as most bacteria and sufficiently thin for adequate diffusion rates. It uses sulfur as an energy source and nitrate as the electron acceptor for the electrons released when sulfur is oxidized in energy-conserving processes.

The discovery of these procaryotes greatly weakens the distinction between procaryotes and eucaryotes based on cell size. They are certainly larger than a normal eucaryotic cell. The size distinction between procaryotes and eucaryotes has been further weakened by the discovery of eucaryotic cells that are smaller than

previously thought possible. The best example is *Nanochlorum eukaryotum*. *Nanochlorum* is only about 1 to 2 μm in diameter, yet is truly eucaryotic and has a nucleus, a chloroplast, and a mitochondrion. Our understanding of the factors limiting procaryotic cell size must be reevaluated. It is no longer safe to assume that large cells are eucaryotic and small cells are procaryotic.



(a)



(b)

Giant Bacteria. (a) This photograph, taken with pseudo dark-field illumination, shows *Epulopiscium fishelsoni* at the top of the figure dwarfing the paramecia at the bottom ($\times 200$). (b) A chain of *Thiomargarita namibiensis* cells as viewed with the light microscope. Note the external mucous sheath and the internal sulfur globules.

Sources: Angert, E. R., Clements, K. D., and Pace, N. R. 1993 The largest bacterium. *Nature* 362:239–41; and Schulz, H. N., Brinkhoff, T., Ferdelman, T. G., Mariné, M. H., Teske, A., and Jorgensen, B.B. 1999. Dense populations of a giant sulfur bacterium in Namibian shelf sediments. *Science* 284:493–95.

Table 3.1	Functions of Prokaryotic Structures
Plasma membrane	Selectively permeable barrier, mechanical boundary of cell, nutrient and waste transport, location of many metabolic processes (respiration, photosynthesis), detection of environmental cues for chemotaxis
Gas vacuole	Buoyancy for floating in aquatic environments
Ribosomes	Protein synthesis
Inclusion bodies	Storage of carbon, phosphate, and other substances
Nucleoid	Localization of genetic material (DNA)
Periplasmic space	Contains hydrolytic enzymes and binding proteins for nutrient processing and uptake
Cell wall	Gives prokaryotes shape and protection from osmotic stress
Capsules and slime layers	Resistance to phagocytosis, adherence to surfaces
Fimbriae and pili	Attachment to surfaces, bacterial mating
Flagella	Movement
Endospore	Survival under harsh environmental conditions

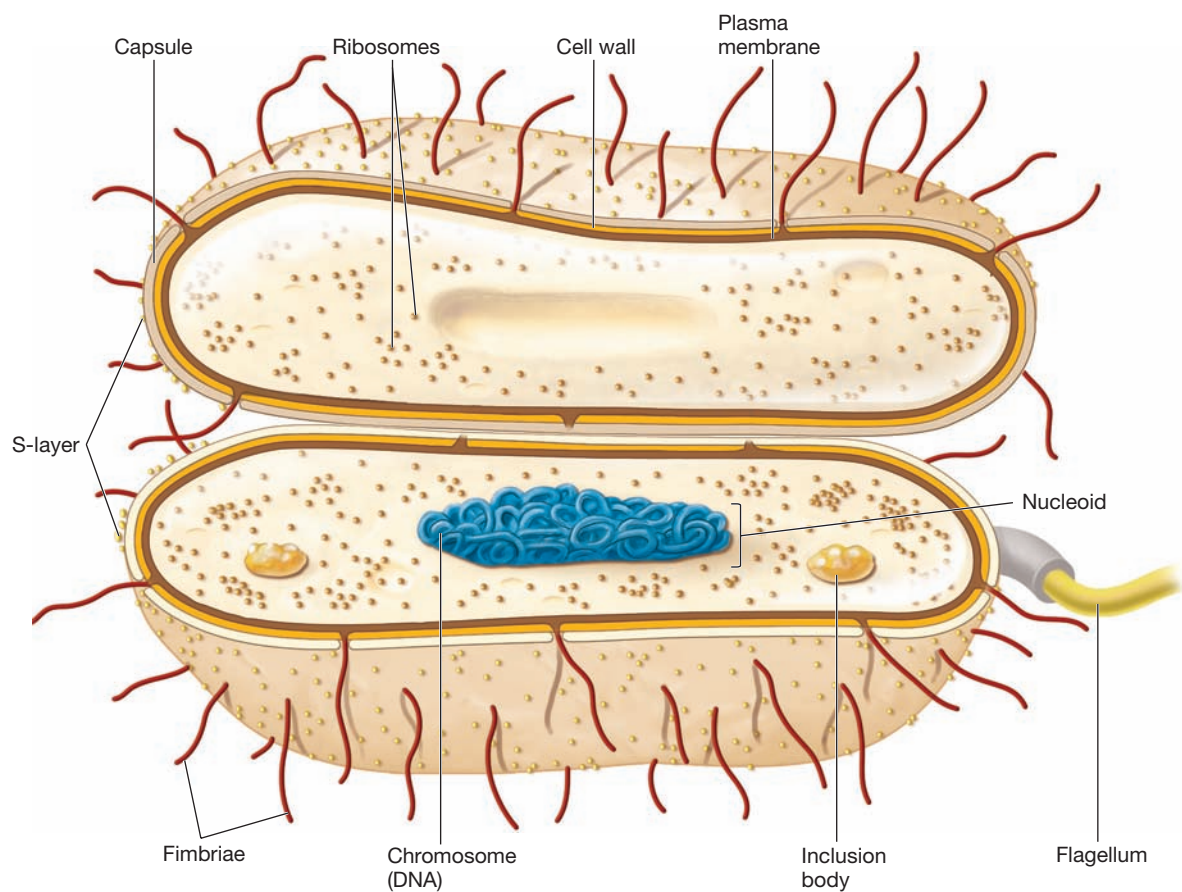


Figure 3.4 Morphology of a Prokaryotic Cell.

plasma membrane, it is necessary to become familiar with membrane structure. In this section, the common basic design of all membranes is discussed. This is followed by a consideration of the significant differences between bacterial and archaeal membranes.

The Fluid Mosaic Model of Membrane Structure

The most widely accepted model for membrane structure is the **fluid mosaic model** of Singer and Nicholson (**figure 3.5**), which proposes that membranes are lipid bilayers within which proteins float. The model is based on studies of eucaryotic and bac-

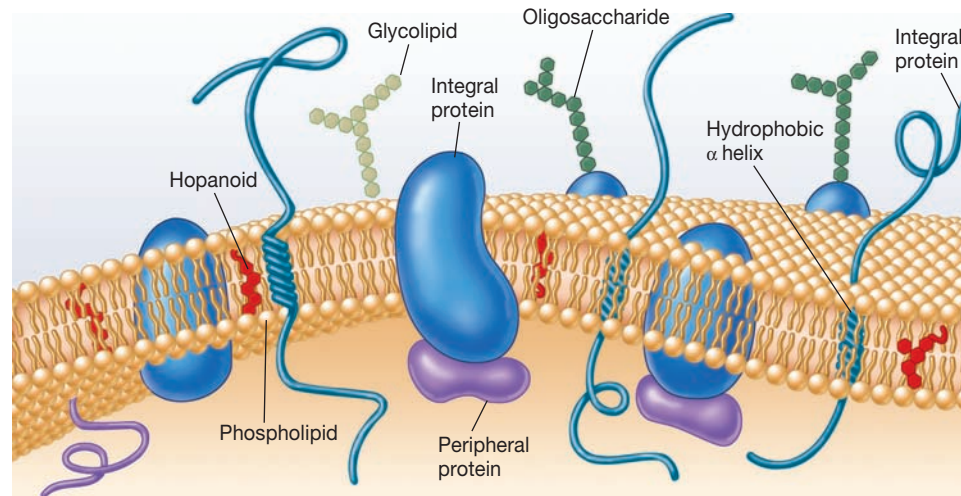


Figure 3.5 Bacterial Plasma Membrane Structure. This diagram of the fluid mosaic model of bacterial membrane structure shows the integral proteins (blue) floating in a lipid bilayer. Peripheral proteins (purple) are associated loosely with the inner membrane surface. Small spheres represent the hydrophilic ends of membrane phospholipids and wiggly tails, the hydrophobic fatty acid chains. Other membrane lipids such as hopanoids (red) may be present. For the sake of clarity, phospholipids are shown in proportionately much larger size than in real membranes.

terial membranes, and a variety of experimental approaches were used to establish it. Transmission electron microscopy (TEM) studies were particularly important. When membranes are stained and examined by TEM, it can be seen that cell membranes are very thin structures, about 5 to 10 nm thick, and that they appear as two dark lines on either side of a nonstained interior. This characteristic appearance has been interpreted to mean that the membrane lipid is organized in two sheets of molecules arranged end-to-end (figure 3.5). When membranes are cleaved by the freeze-etching technique, they can be split down the center of the lipid bilayer, exposing the complex internal structure. Within the lipid bilayer, small globular particles are visible; these have been suggested to be membrane proteins lying within the membrane lipid bilayer. The use of atomic force microscopy has provided powerful images to support this interpretation.

[Electron microscopy \(section 2.4\)](#); [Newer techniques in microscopy: Scanning probe microscopy \(section 2.5\)](#)

The chemical nature of membrane lipids is critical to their ability to form bilayers. Most membrane-associated lipids are structurally asymmetric, with polar and nonpolar ends (**figure 3.6**) and are called **amphipathic**. The polar ends interact with water and are **hydrophilic**; the nonpolar **hydrophobic** ends are insoluble in water and tend to associate with one another. In aqueous environments, amphipathic lipids can interact to form a bilayer. The outer surfaces of the bilayer membrane are hydrophilic, whereas hydrophobic ends are buried in the interior away from the surrounding water (figure 3.5). [Lipids \(appendix I\)](#)

Two types of membrane proteins have been identified based on their ability to be separated from the membrane. **Peripheral proteins** are loosely connected to the membrane and can be easily removed (figure 3.5). They are soluble in aqueous solutions

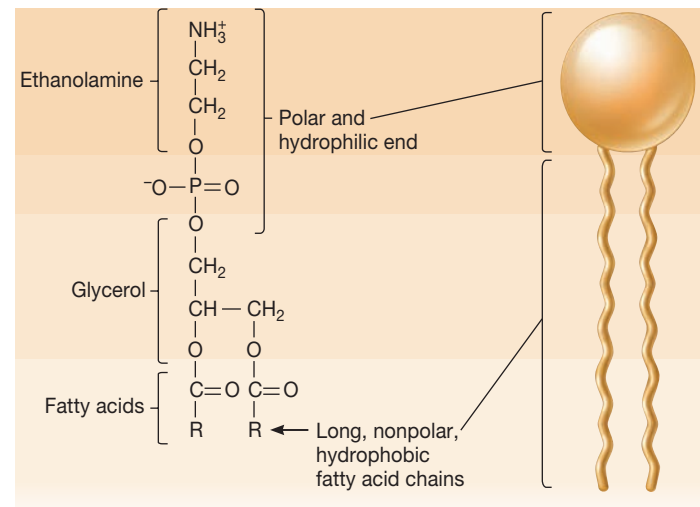


Figure 3.6 The Structure of a Polar Membrane Lipid.

Phosphatidylethanolamine, an amphipathic phospholipid often found in bacterial membranes. The R groups are long, nonpolar fatty acid chains.

and make up about 20 to 30% of total membrane protein. About 70 to 80% of membrane proteins are **integral proteins**. These are not easily extracted from membranes and are insoluble in aqueous solutions when freed of lipids. Integral proteins, like membrane lipids, are amphipathic; their hydrophobic regions are buried in the lipid while the hydrophilic portions project from the membrane surface (figure 3.5). Integral proteins can diffuse laterally in the membrane to new locations, but do not flip-flop or

rotate through the lipid layer. Carbohydrates often are attached to the outer surface of plasma membrane proteins, where they have important functions. [Proteins \(appendix I\)](#)

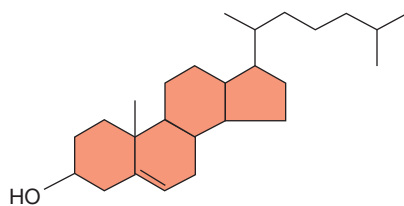
Bacterial Membranes

Bacterial membranes are similar to eucaryotic membranes in that many of their amphipathic lipids are phospholipids (figure 3.6), but they usually differ from eucaryotic membranes in lacking sterols (steroid-containing lipids) such as cholesterol (**figure 3.7a**). However, many bacterial membranes contain sterol-like molecules called **hopanoids** (figure 3.7b). Hopanoids are synthesized from the same precursors as sterols, and like the sterols in eucaryotic membranes, they probably stabilize the membrane. Hopanoids are also of interest to ecologists and geologists: it has been estimated that the total mass of hopanoids in sediments is around 10^{11-12} tons—about as much as the total mass of organic carbon in all living organisms (10^{12} tons)—and there is evidence that hopanoids have contributed significantly to the formation of petroleum.

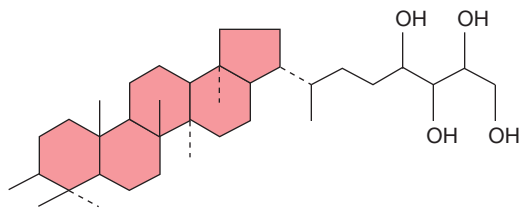
The emerging picture of bacterial plasma membranes is one of a highly organized and asymmetric system that also is flexible and dynamic. Numerous studies have demonstrated that lipids are not homogeneously distributed in the plasma membrane. Rather, there are domains in which particular lipids are concentrated. It has also been demonstrated that the lipid composition of bacterial membranes varies with environmental temperature in such a way that the membrane remains fluid during growth. For example, bacteria growing at lower temperatures will have fatty acids with lower melting points in their membrane phospholipids.

[The influence of environmental factors on growth: Temperature \(section 6.5\)](#)

Although procaryotes do not contain complex membranous organelles like mitochondria or chloroplasts, internal membranous structures can be observed in some bacteria (**figure 3.8**).



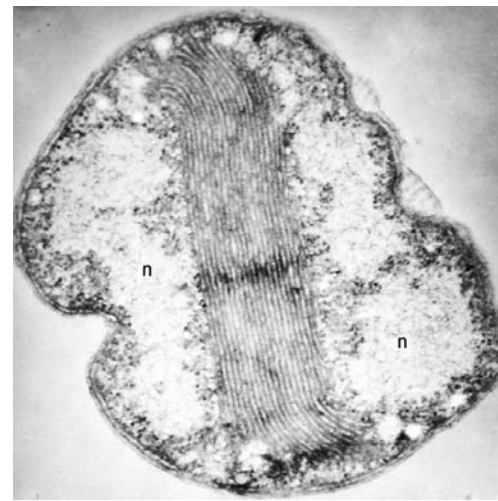
(a) Cholesterol (a steroid) is found in eucaryotes



(b) A bacteriohopanetetrol (a hopanoid), as found in bacteria

Figure 3.7 Membrane Steroids and Hopanoids. Common examples.

Plasma membrane infoldings are common in many bacteria and can become extensive and complex in photosynthetic bacteria such as the cyanobacteria and purple bacteria or in bacteria with very high respiratory activity, like the nitrifying bacteria. These internal membranous structures may be aggregates of spherical vesicles, flattened vesicles, or tubular membranes. Their function may be to provide a larger membrane surface for greater metabolic activity. One membranous structure sometimes reported in bacteria is the mesosome. Mesosomes appear to be invaginations of the plasma membrane in the shape of vesicles, tubules, or lamellae. Although a variety of functions have been ascribed to



(a)



(b)

Figure 3.8 Internal Bacterial Membranes. Membranes of nitrifying and photosynthetic bacteria. (a) *Nitrocystis oceanus* with parallel membranes traversing the whole cell. Note nucleoplasm (n) with fibrillar structure. (b) *Ectothiorhodospira mobilis* with an extensive intracytoplasmic membrane system ($\times 60,000$).

mesosomes, many bacteriologists believe that they are artifacts generated during the chemical fixation of bacteria for electron microscopy.

Archaeal Membranes

One of the most distinctive features of the *Archaea* is the nature of their membrane lipids. They differ from both *Bacteria* and *Eucarya* in having branched chain hydrocarbons attached to glycerol by ether links rather than fatty acids connected by ester links (**figure 3.9**). Sometimes two glycerol groups are linked to form an extremely long tetraether. Usually the diether hydrocarbon chains are 20 carbons in length, and the tetraether chains are 40 carbons. Cells can adjust the overall length of the tetraethers by cyclizing the chains to form pentacyclic rings (figure 3.9).

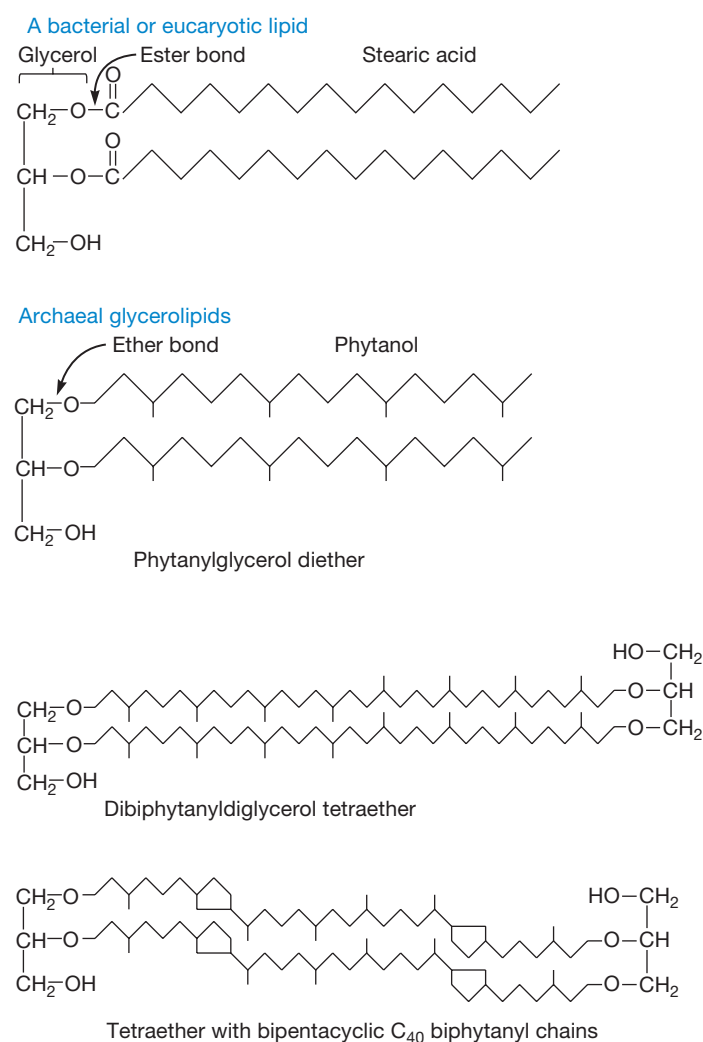


Figure 3.9 Archaeal Membrane Lipids. An illustration of the difference between archaeal lipids and those of *Bacteria*. Archaeal lipids are derivatives of isopranyl glycerol ethers rather than the glycerol fatty acid esters in *Bacteria*. Three examples of common archaeal glycerolipids are given.

Phosphate-, sulfur- and sugar-containing groups can be attached to the third carbons of the diethers and tetraethers, making them polar lipids. These predominate in the membrane, and 70 to 93% of the membrane lipids are polar. The remaining lipids are non-polar and are usually derivatives of squalene (**figure 3.10**).

Despite these significant differences in membrane lipids, the basic design of archaeal membranes is similar to that of *Bacteria* and eucaryotes—there are two hydrophilic surfaces and a hydrophobic core. When C_{20} diethers are used, a regular bilayer membrane is formed (**figure 3.11a**). When the membrane is constructed of C_{40} tetraethers, a monolayer membrane with much more rigidity is formed (**figure 3.11b**). As might be expected from

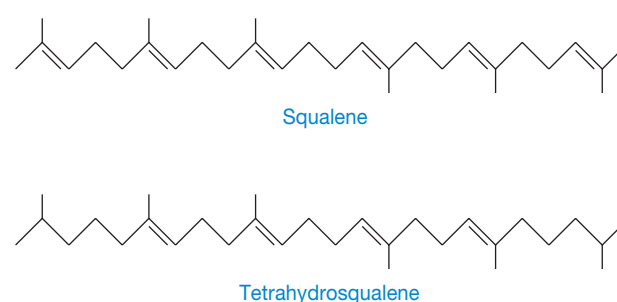


Figure 3.10 Nonpolar Lipids of *Archaea*. Two examples of the most predominant nonpolar lipids are the C₃₀ isoprenoid squalene and one of its hydroisoprenoid derivatives, tetrahydrosqualene.

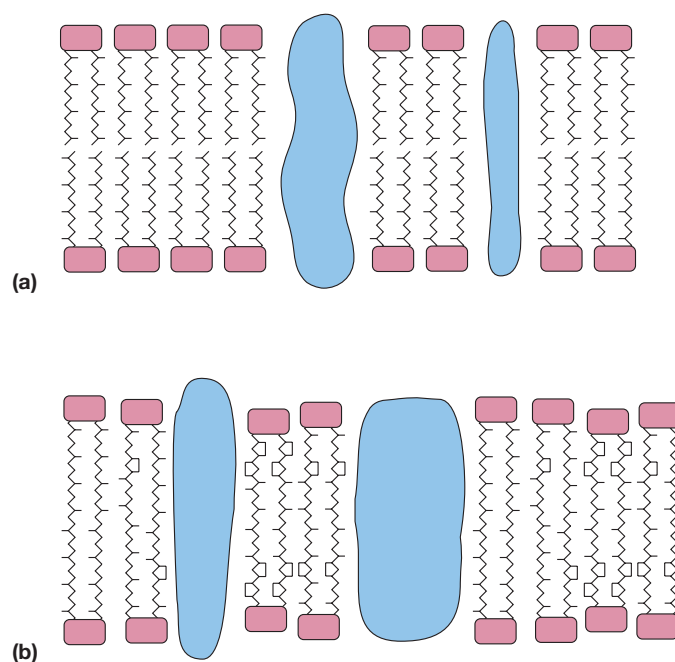


Figure 3.11 Examples of Archaeal Membranes. (a) A membrane composed of integral proteins and a bilayer of C_{20} diethers. (b) A rigid monolayer composed of integral proteins and C_{40} tetraethers.

their need for stability, the membranes of extreme thermophiles such as *Thermoplasma* and *Sulfolobus*, which grow best at temperatures over 85°C, are almost completely tetraether monolayers. Archaea that live in moderately hot environments have a mixed membrane containing some regions with monolayers and some with bilayers. [Phylum Euryarchaeota: Thermoplasms \(section 20.3\)](#); [Phylum Crenarchaeota: Sulfolobus \(section 20.2\)](#)

1. List the functions of the procaryotic plasma membrane.
2. Describe in words and with a labeled diagram the fluid mosaic model for cell membranes.
3. Compare and contrast bacterial and archaeal membranes.
4. Discuss the ways bacteria and archaea adjust the lipid content of their membranes in response to environmental conditions.

3.3 THE CYTOPLASMIC MATRIX

The **cytoplasmic matrix** is the substance in which the nucleoid, ribosomes, and inclusion bodies are suspended. It lacks organelles bound by lipid bilayers (often called unit membranes), and is largely water (about 70% of bacterial mass is water). Until recently, it was thought to lack a cytoskeleton. The plasma membrane and everything within is called the **protoplast**; thus the cytoplasmic matrix is a major part of the protoplast.

The Procaryotic Cytoskeleton

When examined with the electron microscope, the cytoplasmic matrix of procaryotes is packed with ribosomes. For many years it was thought that procaryotes lacked the high level of cytoplasmic organization present in eucaryotic cells because they lacked a cytoskeleton. Recently homologs of all three eucaryotic cytoskeletal elements (microfilaments, intermediate filaments, and microtubules) have been identified in bacteria, and one has been identified in archaea ([table 3.2](#)). The cytoskeletal filaments of procaryotes are structurally similar to their eucaryotic counterparts and carry out similar functions: they participate in cell division, localize proteins to certain sites in the cell, and determine cell shape ([table 3.2](#) and [figure 3.12](#)). [Procaryotic cell cycle: Septation \(section 6.1\)](#)

Inclusion Bodies

Inclusion bodies, granules of organic or inorganic material that often are clearly visible in a light microscope, are present in the cytoplasmic matrix. These bodies usually are used for storage (e.g., carbon compounds, inorganic substances, and energy), and also reduce osmotic pressure by tying up molecules in particulate form. Some inclusion bodies lie free in the cytoplasm—for example, polyphosphate granules, cyanophycin granules, and some glycogen granules. Other inclusion bodies are enclosed by a shell about 2.0 to 4.0 nm thick, which is single-layered and may consist of proteins or a membranous structure composed of proteins and phospholipids. Examples of enclosed inclusion bodies are poly-β-hydroxybutyrate granules, some glycogen and sulfur granules, carboxysomes, and gas vacuoles. Many inclusion bodies are used for storage; their quantity will vary with the nutritional status of the cell. For example, polyphosphate granules will

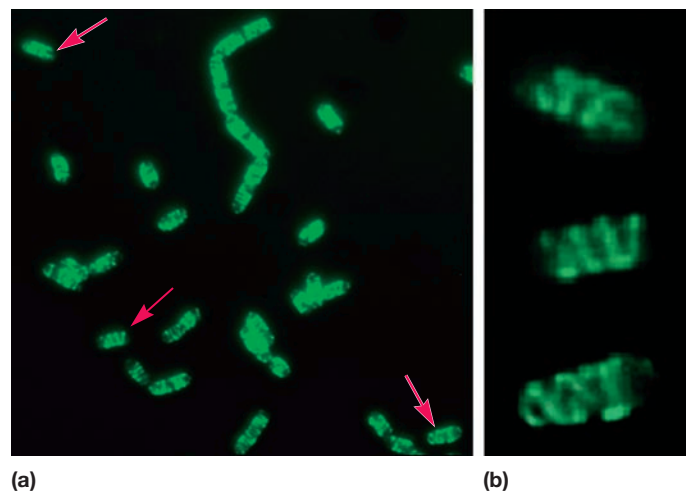


Figure 3.12 The Procaryotic Cytoskeleton. Visualization of the MreB-like cytoskeletal protein (Mbl) of *Bacillus subtilis*. The Mbl protein has been fused with green fluorescent protein and live cells have been examined by fluorescence microscopy. **(a)** Arrows point to the helical cytoskeletal cables that extend the length of the cells. **(b)** Three of the cells from (a) are shown at a higher magnification.

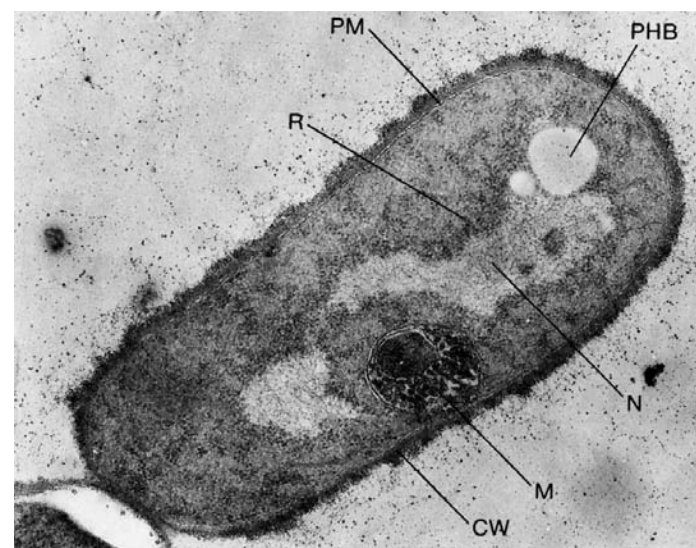
Table 3.2 Procaryotic Cytoskeletal Proteins		
Procaryotic Protein (Eucaryotic Counterpart)	Function	Comments
FtsZ (tubulin)	Cell division	Widely observed in <i>Bacteria</i> and <i>Archaea</i>
MreB (actin)	Cell shape	Observed in many rod-shaped bacteria; in <i>Bacillus subtilis</i> is called Mbl
Crescentin (intermediate filament proteins)	Cell shape	Discovered in <i>Caulobacter crescentus</i>

be depleted in freshwater habitats that are phosphate limited. A brief description of several important inclusion bodies follows.

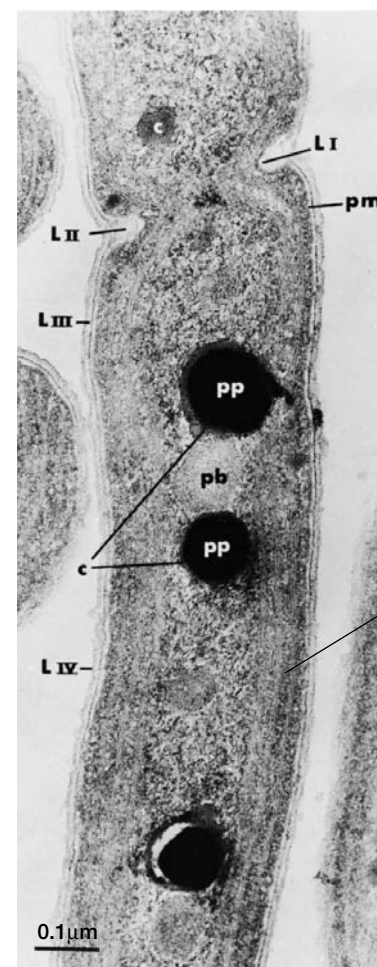
Organic inclusion bodies usually contain either glycogen or poly- β -hydroxyalkanoates (e.g., poly- β -hydroxybutyrate). **Glycogen** is a polymer of glucose units composed of long chains formed by $\alpha(1\rightarrow4)$ glycosidic bonds and branching chains connected to them by $\alpha(1\rightarrow6)$ glycosidic bonds. **Poly- β -hydroxybutyrate (PHB)** contains β -hydroxybutyrate molecules joined by ester bonds between the carboxyl and hydroxyl groups of adjacent molecules. Usually only one of these polymers is found in a species, but some photosynthetic bacteria have both glycogen and PHB. Poly- β -hydroxybutyrate accumulates in distinct bodies, around 0.2 to 0.7 μm in diameter, that are readily stained with Sudan black for light microscopy and are seen as empty “holes” in the electron microscope (**figure 3.13a**). This is because the solvents used to prepare specimens for electron microscopy dissolve these hydrophobic inclusion bodies. Glycogen is dispersed more evenly throughout the matrix as small granules (about 20 to 100 nm in diameter) and often can be seen only with the electron microscope. If cells contain a large amount of glycogen, staining with an iodine solution will turn them reddish-brown. Glycogen and PHB inclusion bodies are carbon storage reservoirs providing material for energy and biosynthesis. Many bacteria also store carbon as lipid droplets. [Carbohydrates \(appendix I\)](#)

Cyanobacteria, a group of photosynthetic bacteria, have two distinctive organic inclusion bodies. **Cyanophycin granules** (**figure 3.13b**) are composed of large polypeptides containing approximately equal amounts of the amino acids arginine and aspartic acid. The granules often are large enough to be visible in the light microscope and store extra nitrogen for the bacteria. **Carboxysomes** are present in many cyanobacteria and other CO_2 -fixing bacteria. They are polyhedral, about 100 nm in diameter, and contain the enzyme ribulose-1, 5-bisphosphate carboxylase, called Rubisco. **Rubisco** is the critical enzyme for CO_2 fixation, the process of converting CO_2 from the atmosphere into sugar. The enzyme assumes a paracrystalline arrangement in the carboxysome, which serves as a reserve of the enzyme. Carboxysomes also may be a site of CO_2 fixation. [The fixation of \$\text{CO}_2\$ by autotrophs \(section 10.3\)](#)

Figure 3.13 Inclusion Bodies in Bacteria. (a) Electron micrograph of *Bacillus megaterium* ($\times 30,500$). Poly- β -hydroxybutyrate inclusion body, PHB; cell wall, CW; nucleoid, N; plasma membrane, PM; “mesosome,” M; and ribosomes, R. (b) Ultrastructure of the cyanobacterium *Anacystis nidulans*. The bacterium is dividing and a septum is partially formed, LI and LII. Several structural features can be seen, including cell wall layers, LIII and LIV; polyphosphate granules, pp; a polyhedral body, pb; cyanophycin material, c; and plasma membrane, pm. Thylakoids run along the length of the cell. (c) *Chromatium vinosum*, a purple sulfur bacterium, with intracellular sulfur granules, bright-field microscopy ($\times 2,000$).



(a)



(b)



(c)

A most remarkable organic inclusion body is the **gas vacuole**, a structure that provides buoyancy to some aquatic procaryotes. Gas vacuoles are present in many photosynthetic bacteria and a few other aquatic procaryotes such as *Halobacterium* (a salt-loving archaeon) and *Thiothrix* (a filamentous bacterium). Gas vacuoles are aggregates of enormous numbers of small, hollow, cylindrical structures called **gas vesicles** (figure 3.14). Gas vesicle walls are composed entirely of a single small protein. These protein subunits assemble to form a rigid enclosed cylinder that is hollow and impermeable to water but freely permeable to atmospheric gases. Procaryotes with gas vacuoles can regulate their buoyancy to float at the depth necessary for proper light intensity, oxygen concentration, and nutrient levels. They descend by simply collapsing vesicles and float upward when new ones are constructed.

Two major types of **inorganic inclusion bodies** are seen in procaryotes: polyphosphate granules and sulfur granules. Many bacteria store phosphate as **polyphosphate granules** or **volutin granules** (figure 3.13b). Polyphosphate is a linear polymer of orthophosphates joined by ester bonds. Thus volutin granules function as storage reservoirs for phosphate, an important component

of cell constituents such as nucleic acids. In some cells they act as an energy reserve, and polyphosphate can serve as an energy source in reactions. These granules are sometimes called **metachromatic granules** because they show the metachromatic effect; that is, they appear red or a different shade of blue when stained with the blue dyes methylene blue or toluidine blue. Sulfur granules are used by some procaryotes to store sulfur temporarily (figure 3.13c). For example, photosynthetic bacteria can use hydrogen sulfide as a photosynthetic electron donor and accumulate the resulting sulfur in either the periplasmic space or in special cytoplasmic globules. **Phototrophy: The light reaction in anoxygenic photosynthesis** (section 9.12)

Inorganic inclusion bodies can be used for purposes other than storage. An excellent example is the **magnetosome**, which is used by some bacteria to orient in the Earth's magnetic field. Many of these inclusion bodies contain iron in the form of magnetite (**Microbial Diversity & Ecology 3.2**).

Ribosomes

As mentioned earlier, the cytoplasmic matrix often is packed with **ribosomes**; they also may be loosely attached to the plasma membrane. Ribosomes are very complex structures made of both protein and ribonucleic acid (RNA). They are the site of protein synthesis; cytoplasmic ribosomes synthesize proteins destined to remain within the cell, whereas plasma membrane ribosomes make proteins for transport to the outside. The newly formed polypeptide folds into its final shape either as it is synthesized by the ribosome or shortly after completion of protein synthesis. The shape of each protein is determined by its amino acid sequence. Special proteins called molecular chaperones, or simply chaperones, aid the polypeptide in folding to its proper shape. Protein synthesis, including a detailed treatment of ribosomes and chaperones, is discussed at considerable length in chapter 11.

Procaryotic ribosomes are smaller than the cytoplasmic or endoplasmic reticulum-associated ribosomes of eucaryotic cells. Procaryotic ribosomes are called 70S ribosomes (as opposed to 80S in eucaryotes), have dimensions of about 14 to 15 nm by 20 nm, a molecular weight of approximately 2.7 million, and are constructed of a 50S and a 30S subunit (figure 3.15). The S in 70S and similar values stands for **Svedberg unit**. This is the unit of the sedimentation coefficient, a measure of the sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged, the greater its Svedberg value or sedimentation coefficient. The sedimentation coefficient is a function of a particle's molecular weight, volume, and shape (see figure 16.19). Heavier and more compact particles normally have larger Svedberg numbers or sediment faster.

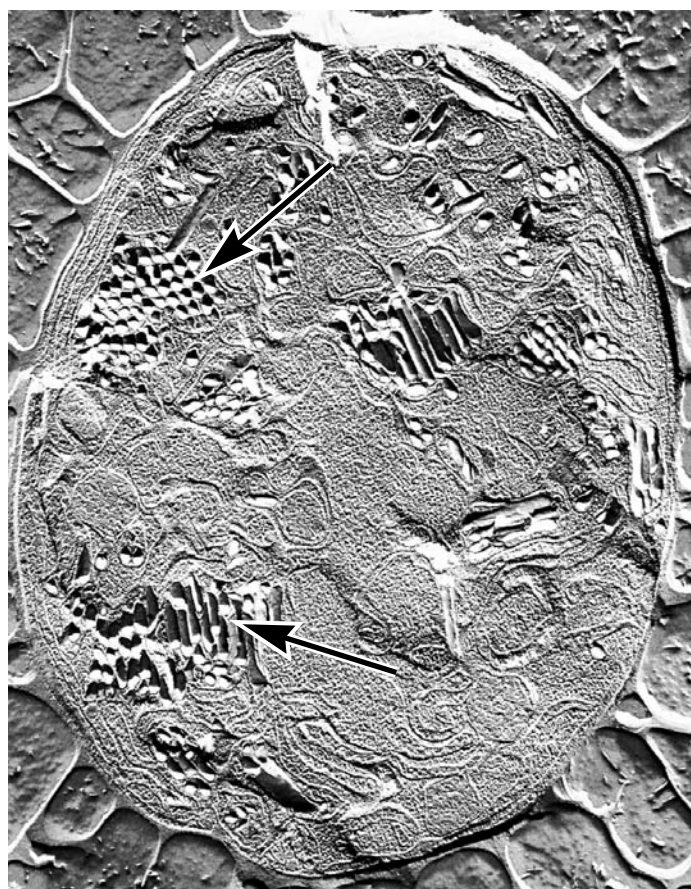


Figure 3.14 Gas Vesicles and Vacuoles. A freeze-fracture preparation of *Anabaena flosaquaе* ($\times 89,000$). Clusters of the cigar-shaped vesicles form gas vacuoles. Both longitudinal and cross-sectional views of gas vesicles can be seen (arrows).

1. Briefly describe the nature and function of the cytoplasmic matrix.
2. List and describe the functions of cytoskeletal proteins, inclusion bodies, and ribosomes.
3. List the most common kinds of inclusion bodies.
4. Relate the structure of a gas vacuole to its function.

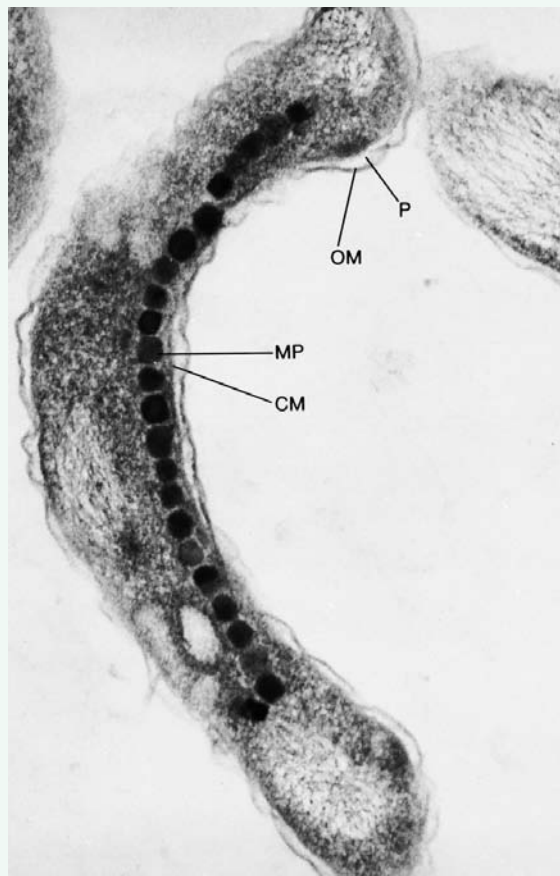


Microbial Diversity & Ecology

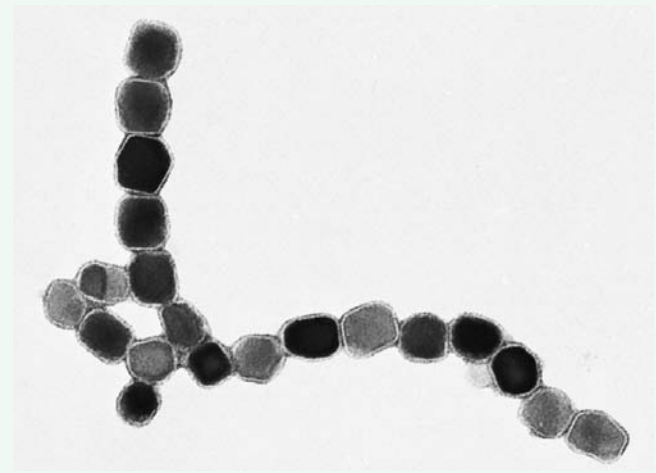
3.2 Living Magnets

Bacteria can respond to environmental factors other than chemicals. A fascinating example is that of the aquatic magnetotactic bacteria that orient themselves in the Earth's magnetic field. Most of these bacteria have intracellular chains of magnetite (Fe_3O_4) particles that are called magnetosomes. Magnetosomes are around 35 to 125 nm in diameter and are bounded by a lipid bilayer (see **Box figure**). Some species from sulfidic habitats have magnetosomes containing greigite (Fe_3S_4) and pyrite (FeS_2). Since each iron particle is a tiny magnet, the Northern Hemisphere bacteria use their magnetosome chain to determine northward and downward directions, and swim down to nutrient-rich sediments or locate the optimum depth in

freshwater and marine habitats. Magnetotactic bacteria in the Southern Hemisphere generally orient southward and downward, with the same result. Magnetosomes also are present in the heads of birds, tuna, dolphins, green turtles, and other animals, presumably to aid navigation. Animals and bacteria share more in common behaviorally than previously imagined.



(a)



(b)



(c)

Magnetotactic Bacteria. (a) Transmission electron micrograph of the magnetotactic bacterium *Aquaspirillum magnetotacticum* ($\times 123,000$). Note the long chain of electron-dense magnetite particles, MP. Other structures: OM, outer membrane; P, periplasmic space; CM, cytoplasmic membrane. (b) Isolated magnetosomes ($\times 140,000$). (c) Bacteria migrating in waves when exposed to a magnetic field.

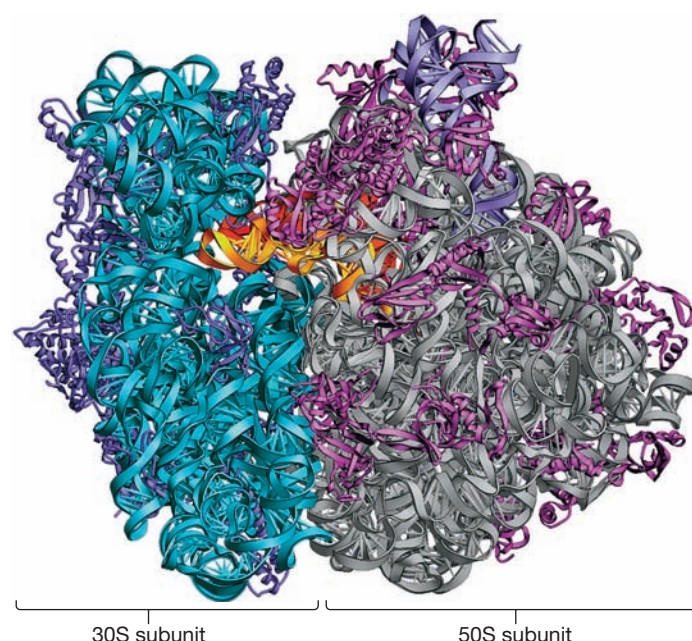


Figure 3.15 Prokaryotic Ribosome. The two subunits of a bacterial ribosome are shown. The 50S subunit includes 23S rRNA (gray) and 5S rRNA (light blue), while 16S rRNA (cyan) is found in the 30S subunit. A molecule of tRNA (gold) is shown in the A site. To generate this ribbon diagram, crystals of purified bacterial ribosomes were grown, exposed to X rays, and the resulting diffraction pattern analyzed.

3.4 THE NUCLEOID

Probably the most striking difference between prokaryotes and eukaryotes is the way in which their genetic material is packaged. Eukaryotic cells have two or more chromosomes contained within a membrane-delimited organelle, the nucleus. In contrast, prokaryotes lack a membrane-delimited nucleus. The prokaryotic chromosome is located in an irregularly shaped region called the **nucleoid** (other names are also used: the nuclear body, chromatin body, nuclear region) (**figure 3.16**). Usually prokaryotes contain a single circle of double-stranded **deoxyribonucleic acid (DNA)**, but some have a linear DNA chromosome and some, such as *Vibrio cholerae* and *Borrelia burgdorferi* (the causative agents of cholera and Lyme disease, respectively), have more than one chromosome.

Both electron and light microscopic studies have been important for understanding nucleoid structure and function, especially during active cell growth and division. The nucleoid has a fibrous appearance in electron micrographs; the fibers are probably DNA. In actively growing cells, the nucleoid has projections that extend into the cytoplasmic matrix. Presumably these projections contain DNA that is being actively transcribed to produce mRNA. Other studies have shown that more than one nucleoid can be observed within a single cell when genetic material has been duplicated but cell division has not yet occurred (**figure 3.16a**).

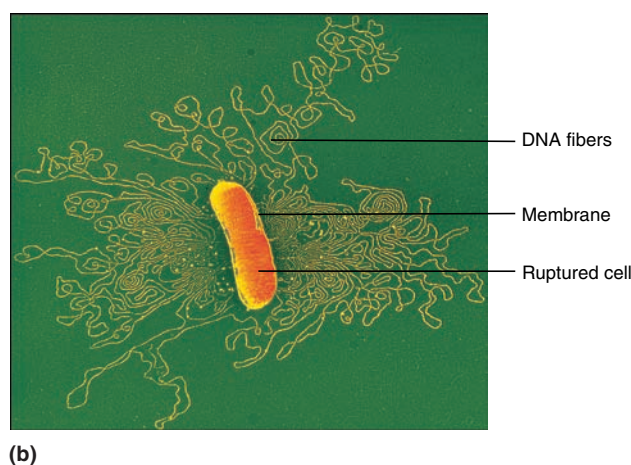
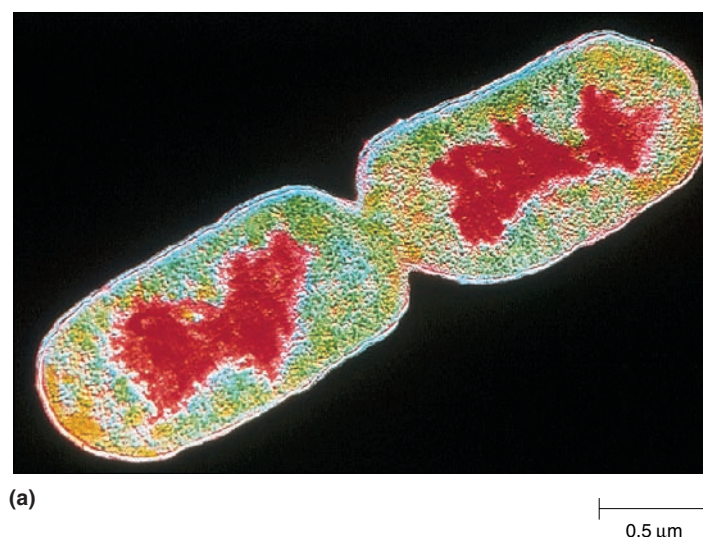


Figure 3.16 Prokaryotic Nucleoids and Chromosomes.

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

It is possible to isolate pure nucleoids. Chemical analysis of purified nucleoids reveals that they are composed of about 60% DNA, 30% RNA, and 10% protein by weight. In *Escherichia coli*, the closed DNA circle measures approximately 1,400 μm or about 230–700 times longer than the cell (**figure 3.16b**). Obviously it must be very efficiently packaged to fit within the nucleoid. The DNA is looped and coiled extensively (*see figure 11.8*), probably with the aid of RNA and a variety of nucleoid proteins. These include condensing proteins, which are conserved in both *Bacteria* and *Archaea*. Unlike the eukaryotes and some archaea, *Bacteria* do not use histone proteins to package their DNA.

There are a few exceptions to the preceding picture. Membrane-bound DNA-containing regions are present in two genera of the unusual bacterial phylum *Planctomycetes* (see figure 21.12). *Pirellula* has a single membrane that surrounds a region, the pirellulosome, which contains a fibrillar nucleoid and ribosome-like particles. The nuclear body of *Gemmata obscuriglobus* is bounded by two membranes. More work will be required to determine the functions of these membranes and how widespread this phenomenon is. [Phylum Planctomycetes \(section 21.4\)](#)

1. Describe the structure and function of the nucleoid and the DNA it contains.
2. List three genera that are exceptional in terms of their chromosome or nucleoid structure. Suggest how the differences observed in these genera might impact how they function.

3.5 PLASMIDS

In addition to the genetic material present in the nucleoid, many procaryotes (and some yeasts and other fungi) contain extrachromosomal DNA molecules called plasmids. Indeed, most of the bacterial and archaeal genomes sequenced thus far include plasmids. In some cases, numerous different plasmids within a single species have been identified. For instance, *B. burgdorferi*, carries 12 linear and 9 circular plasmids. Plasmids play many important roles in the lives of the organisms that have them. They also have proved invaluable to microbiologists and molecular geneticists in constructing and transferring new genetic combinations and in cloning genes, as described in chapter 14. This section discusses the different types of procaryotic plasmids.

Plasmids are small, double-stranded DNA molecules that can exist independently of the chromosome. Both circular and linear plasmids have been documented, but most known plasmids are circular. Linear plasmids possess special structures or sequences at their ends to prevent their degradation and to permit their replication. Plasmids have relatively few genes, generally less than 30. Their genetic information is not essential to the host, and cells that lack them usually function normally. However, many plasmids carry genes that confer a selective advantage to their hosts in certain environments.

Plasmids are able to replicate autonomously. Single-copy plasmids produce only one copy per host cell. Multicopy plasmids may be present at concentrations of 40 or more per cell. Some plasmids are able to integrate into the chromosome and are thus replicated with the chromosome. Such plasmids are called **episomes**. Plasmids are inherited stably during cell division, but they are not always equally apportioned into daughter cells and sometimes are lost. The loss of a plasmid is called **curing**. It can occur spontaneously or be induced by treatments that inhibit plasmid replication but not host cell reproduction. Some commonly used curing treatments are acridine mutagens, UV and ionizing radiation, thymine starvation, antibiotics, and growth above optimal temperatures.

Plasmids may be classified in terms of their mode of existence, spread, and function. A brief summary of the types of bacterial

plasmids and their properties is given in **table 3.3. Conjugative plasmids** are of particular note. They have genes for the construction of hairlike structures called pili and can transfer copies of themselves to other bacteria during conjugation. Perhaps the best-studied conjugative plasmid is the **F factor** (fertility factor or F plasmid) of *E. coli*, which was the first conjugative factor to be described. The F factor contains genes that direct the formation of sex pili that attach an F⁺ cell (a cell containing an F plasmid) to an F⁻ cell (a cell lacking an F plasmid). Other plasmid-encoded gene products aid DNA transfer from the F⁺ cell to the F⁻ cell. The F factor also has several segments called insertion sequences that enable it to integrate into the host cell chromosome. Thus the F factor is an episome. [Transposable elements \(section 13.5\); Bacterial conjugation \(section 13.7\)](#)

Resistance factors (R factors, R plasmids) are another group of important plasmids. They confer antibiotic resistance on the cells that contain them. R factors typically have genes that code for enzymes capable of destroying or modifying antibiotics. Some R plasmids have only a single resistance gene, whereas others have as many as eight. Often the resistance genes are within mobile genetic elements called transposons, and thus it is possible for multiple-resistance plasmids to evolve. R factors usually are not integrated into the host chromosome.

R factors are of major concern to public health officials because they can spread rapidly throughout a population of cells. This is possible for several reasons. One is that many R factors also are conjugative plasmids. However, a nonconjugative R factor can be spread to other cells if it is present in a cell that also contains a conjugative plasmid. In such a cell, the R factor can sometimes be transferred when the conjugative plasmid is transferred—that is, it is “mobilized.” Even more troubling is the fact that some R factors are readily transferred *between* species. When humans and other animals consume antibiotics, the growth of host bacteria with R factors is promoted. The R factors then can be transferred to more pathogenic genera such as *Salmonella* or *Shigella*, causing even greater public health problems. [Drug resistance \(section 34.6\)](#)

Several other important types of plasmids have been discovered. These include bacteriocin-encoding plasmids, virulence plasmids, and metabolic plasmids. Bacteriocin-encoding plasmids may give the bacteria that harbor them a competitive advantage in the microbial world. **Bacteriocins** are bacterial proteins that destroy other bacteria. They usually act only against closely related strains. Some bacteriocins kill cells by forming channels in the plasma membrane, thus breaching the critical selective permeability required for cell viability. They also may degrade DNA and RNA or attack peptidoglycan and weaken the cell wall. **Col plasmids** contain genes for the synthesis of bacteriocins known as colicins, which are directed against *E. coli*. Other plasmids carry genes for bacteriocins against other species. For example, cloacins kill *Enterobacter* species. Some Col plasmids are conjugative and carry resistance genes. It should be noted that not all bacteriocin genes are on plasmids. For example, the bacteriocin genes of *Pseudomonas aeruginosa*, which code for proteins called pyocins, are located on the chromosome. Bacteriocins produced by the normal flora of humans (and other animals) also are

Table 3.3 Major Types of Bacterial Plasmids					
Type	Representatives	Approximate Size (kbp)	Copy Number (Copies/Chromosome)	Hosts	Phenotypic Features ^a
Fertility Factor ^b	F factor	95–100	1–3	<i>E. coli</i> , <i>Salmonella</i> , <i>Citrobacter</i>	Sex pilus, conjugation
R Plasmids	RP4	54	1–3	<i>Pseudomonas</i> and many other gram-negative bacteria	Sex pilus, conjugation, resistance to Amp, Km, Nm, Tet
	R1	80	1–3	Gram-negative bacteria	Resistance to Amp, Km, Su, Cm, Sm
	R6	98	1–3	<i>E. coli</i> , <i>Proteus mirabilis</i>	Su, Sm, Cm, Tet, Km, Nm
	R100	90	1–3	<i>E. coli</i> , <i>Shigella</i> , <i>Salmonella</i> , <i>Proteus</i>	Cm, Sm, Su, Tet, Hg
	pSH6	21		<i>Staphylococcus aureus</i>	Gm, Tet, Km
	pSJ23a	36		<i>S. aureus</i>	Pn, Asa, Hg, Gm, Km, Nm, Em, etc.
	pAD2	25		<i>Enterococcus faecalis</i>	Em, Km, Sm
Col Plasmids	ColE1	9	10–30	<i>E. coli</i>	Colicin E1 production
	ColE2		10–15	<i>Shigella</i>	Colicin E2
	CloDF13			<i>Enterobacter cloacae</i>	Cloacin DF13
Virulence Plasmids	Ent (P307)	83		<i>E. coli</i>	Enterotoxin production
	K88 plasmid			<i>E. coli</i>	Adherence antigens
	ColV-K30	2		<i>E. coli</i>	Siderophore for iron uptake; resistance to immune mechanisms
	pZA10	56		<i>S. aureus</i>	Enterotoxin B
	Ti	200		<i>Agrobacterium tumefaciens</i>	Tumor induction
	CAM	230		<i>Pseudomonas</i>	Camphor degradation
	SAL	56		<i>Pseudomonas</i>	Salicylate degradation
Metabolic Plasmids	TOL	75		<i>Pseudomonas putida</i>	Toluene degradation
	pJP4			<i>Pseudomonas</i>	2,4-dichlorophenoxyacetic acid degradation
				<i>E. coli</i> , <i>Klebsiella</i> , <i>Salmonella</i>	Lactose degradation
				<i>Providencia</i>	Urease
	sym			<i>Rhizobium</i>	Nitrogen fixation and symbiosis
^a Abbreviations used for resistance to antibiotics and metals: Amp, ampicillin; Asa, arsenate; Cm, chloramphenicol; Em, erythromycin; Gm, gentamycin; Hg, mercury; Km, kanamycin; Nm, neomycin; Pn, pencillin; Sm, streptomycin; Su, sulfonamides; Tet, tetracycline.					
^b Many R plasmids, metabolic plasmids and others are also conjugative.					

components of our defenses against invading pathogens. **Virulence plasmids** encode factors that make their hosts more pathogenic. For example, enterotoxigenic strains of *E. coli* cause traveler’s diarrhea because they contain a plasmid that codes for an enterotoxin. **Metabolic plasmids** carry genes for enzymes that degrade substances such as aromatic compounds (toluene), pesticides (2,4-dichlorophenoxyacetic acid), and sugars (lactose). Metabolic plasmids even carry the genes required for some strains

of *Rhizobium* to induce legume nodulation and carry out nitrogen fixation.

1. Give the major features of plasmids. How do they differ from chromosomes?
 2. What is an episome? A conjugative plasmid?
 3. Describe each of the following plasmids and explain their importance: F factor, R factor, Col plasmid, virulence plasmid, and metabolic plasmid.

3.6 THE BACTERIAL CELL WALL

The cell wall is the layer, usually fairly rigid, that lies just outside the plasma membrane. It is one of the most important procaryotic structures for several reasons: it helps determine the shape of the cell; it helps protect the cell from osmotic lysis; it can protect the cell from toxic substances; and in pathogens, it can contribute to pathogenicity. The importance of the cell wall is reflected in the fact that relatively few procaryotes lack cell walls. Those that do have other features that fulfill cell wall function. The procaryotic cell wall also is the site of action of several antibiotics. Therefore, it is important to understand its structure.

The cell walls of *Bacteria* and *Archaea* are distinctive and are another example of the important features distinguishing these organisms. In this section, we focus on bacterial cell walls. An overview of bacterial cell wall structure is provided first. This is followed by more detailed discussions of particular aspects of cell wall structure and function. Archaeal cell walls are discussed in section 3.7.

Overview of Bacterial Cell Wall Structure

After Christian Gram developed the Gram stain in 1884, it soon became evident that most bacteria could be divided into two major groups based on their response to the Gram-stain procedure (see table 19.9). Gram-positive bacteria stained purple, whereas gram-negative bacteria were colored pink or red by the technique. The true structural difference between these two groups did not become clear until the advent of the transmission electron microscope. The gram-positive cell wall consists of a single 20 to 80 nm thick homogeneous layer of **peptidoglycan (murein)** lying outside the plasma membrane (figure 3.17). In contrast, the

gram-negative cell wall is quite complex. It has a 2 to 7 nm peptidoglycan layer covered by a 7 to 8 nm thick **outer membrane**. Because of the thicker peptidoglycan layer, the walls of gram-positive cells are more resistant to osmotic pressure than those of gram-negative bacteria. Microbiologists often call all the structures from the plasma membrane outward the **cell envelope**. Therefore this includes the plasma membrane, cell wall, and structures like capsules (p. 65) when present. [Preparation and staining of specimens: Differential staining \(section 2.3\)](#)

One important feature of the cell envelope is a space that is frequently seen between the plasma membrane and the outer membrane in electron micrographs of gram-negative bacteria, and is sometimes observed between the plasma membrane and the wall in gram-positive bacteria. This space is called the **periplasmic space**. The substance that occupies the periplasmic space is the **periplasm**. The nature of the periplasmic space and periplasm differs in gram-positive and gram-negative bacteria. These differences are pointed out in the more detailed discussions that follow.

Peptidoglycan Structure

Peptidoglycan, or murein, is an enormous meshlike polymer composed of many identical subunits. The polymer contains two sugar derivatives, *N*-acetylglucosamine and *N*-acetylmuramic acid (the lactyl ether of *N*-acetylglucosamine), and several different amino acids. Three of these amino acids are not found in proteins: D-glutamic acid, D-alanine, and *meso*-diaminopimelic acid. The presence of D-amino acids protects against degradation by most peptidases, which recognize only the L-isomers of amino acid residues. The peptidoglycan subunit present in most gram-negative bacteria and many gram-positive ones is shown in figure 3.18. The backbone of this

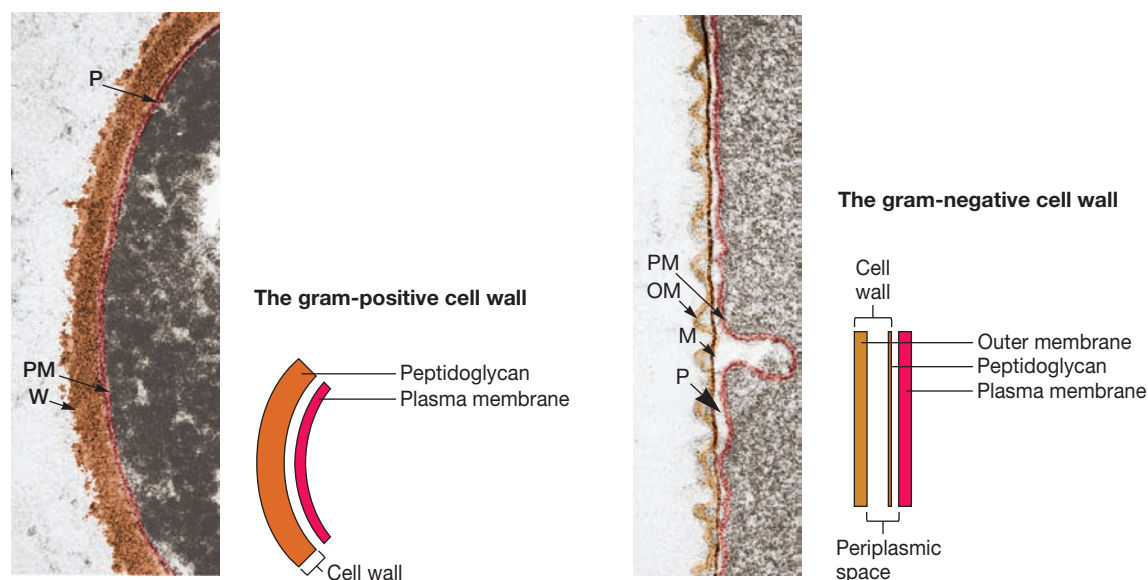


Figure 3.17 Gram-Positive and Gram-Negative Cell Walls. The gram-positive envelope is from *Bacillus licheniformis* (left), and the gram-negative micrograph is of *Aquaspirillum serpens* (right). M; peptidoglycan or murein layer; OM, outer membrane; PM, plasma membrane; P, periplasmic space; W, gram-positive peptidoglycan wall.

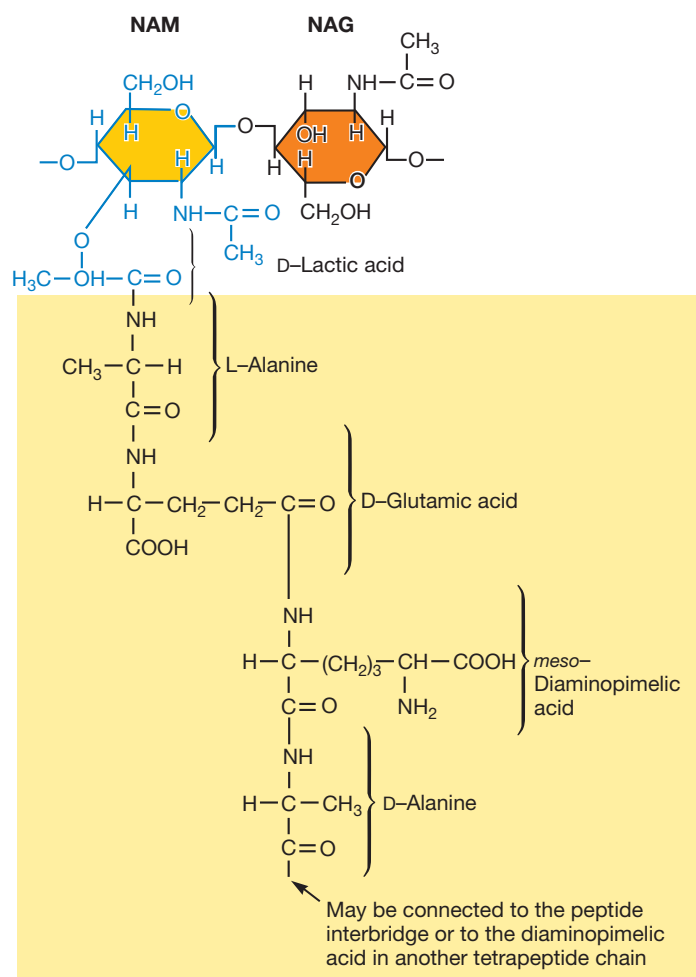


Figure 3.18 Peptidoglycan Subunit Composition. The peptidoglycan subunit of *E. coli*, most other gram-negative bacteria, and many gram-positive bacteria. NAG is *N*-acetylglucosamine. NAM is *N*-acetylmuramic acid (NAG with lactic acid attached by an ether linkage). The tetrapeptide side chain is composed of alternating D- and L-amino acids since *meso*-diaminopimelic acid is connected through its L-carbon. NAM and the tetrapeptide chain attached to it are shown in different shades of color for clarity.

polymer is composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid residues. A peptide chain of four alternating D- and L-amino acids is connected to the carboxyl group of *N*-acetylmuramic acid. Many bacteria replace *meso*-diaminopimelic acid with another diaminoacid, usually L-lysine (**figure 3.19**). [Carbohydrates \(appendix I\); Peptidoglycan and endospore structure \(section 22.3\); Proteins \(appendix I\)](#)

In order to make a strong, meshlike polymer, chains of linked peptidoglycan subunits must be joined by cross-links between the peptides. Often the carboxyl group of the terminal D-alanine is connected directly to the amino group of diaminopimelic acid,

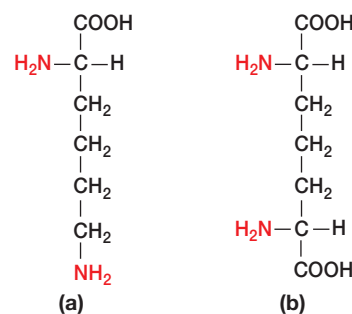


Figure 3.19 Diaminoacids Present in Peptidoglycan. (a) L-Lysine. (b) *meso*-Diaminopimelic acid.

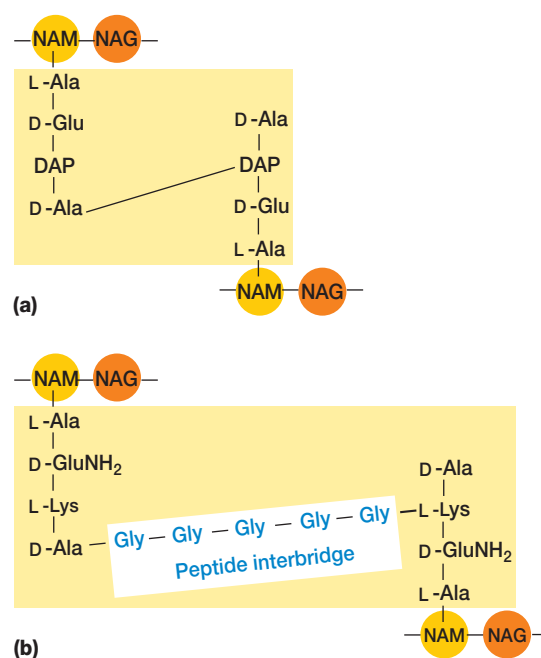


Figure 3.20 Peptidoglycan Cross-Links. (a) *E. coli* peptidoglycan with direct cross-linking, typical of many gram-negative bacteria. (b) *Staphylococcus aureus* peptidoglycan. *S. aureus* is a gram-positive bacterium. NAM is *N*-acetylmuramic acid. NAG is *N*-acetylglucosamine. Gly is glycine. Although the polysaccharide chains are drawn opposite each other for the sake of clarity, two chains lying side-by-side may be linked together (see figure 3.21).

but a **peptide interbridge** may be used instead (**figure 3.20**). Most gram-negative cell wall peptidoglycan lacks the peptide interbridge. This cross-linking results in an enormous peptidoglycan sac that is actually one dense, interconnected network (**figure 3.21**). These sacs have been isolated from gram-positive bacteria and are strong enough to retain their shape and integrity (**figure 3.22**), yet they are relatively porous, elastic, and somewhat stretchable.

Gram-Positive Cell Walls

Gram-positive bacteria normally have cell walls that are thick and composed primarily of peptidoglycan. Peptidoglycan in gram-positive bacteria often contains a peptide interbridge (figure 3.21 and figure 3.23). In addition, gram-positive cell walls usually contain large amounts of **teichoic acids**, polymers of glycerol or ribitol

joined by phosphate groups (figure 3.23 and figure 3.24). Amino acids such as D-alanine or sugars like glucose are attached to the glycerol and ribitol groups. The teichoic acids are covalently connected to either the peptidoglycan itself or to plasma membrane lipids; in the latter case they are called lipoteichoic acids. Teichoic acids appear to extend to the surface of the peptidoglycan, and, because they are negatively charged, help give the gram-positive cell

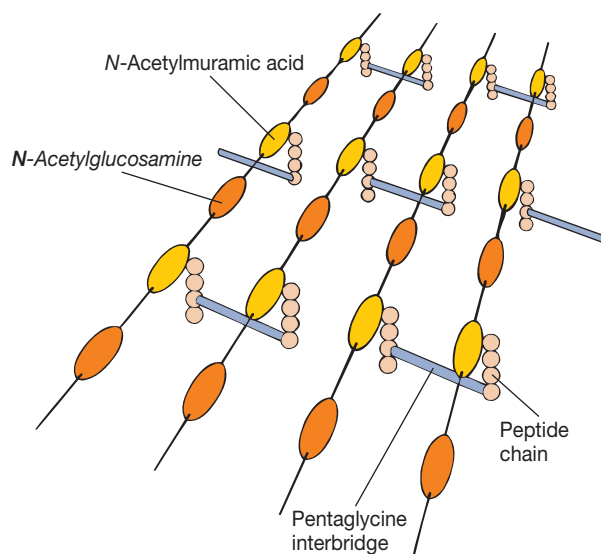


Figure 3.21 Peptidoglycan Structure. A schematic diagram of one model of peptidoglycan. Shown are the polysaccharide chains, tetrapeptide side chains, and peptide interbridges.

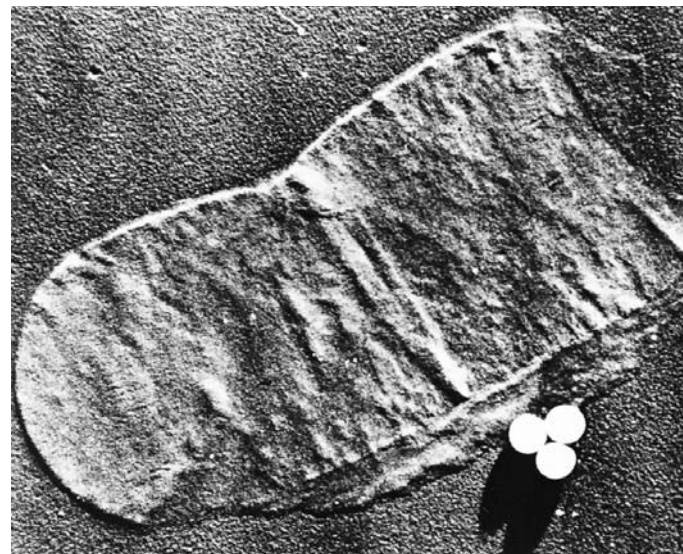


Figure 3.22 Isolated Gram-Positive Cell Wall. The peptidoglycan wall from *Bacillus megaterium*, a gram-positive bacterium. The latex spheres have a diameter of 0.25 μm .

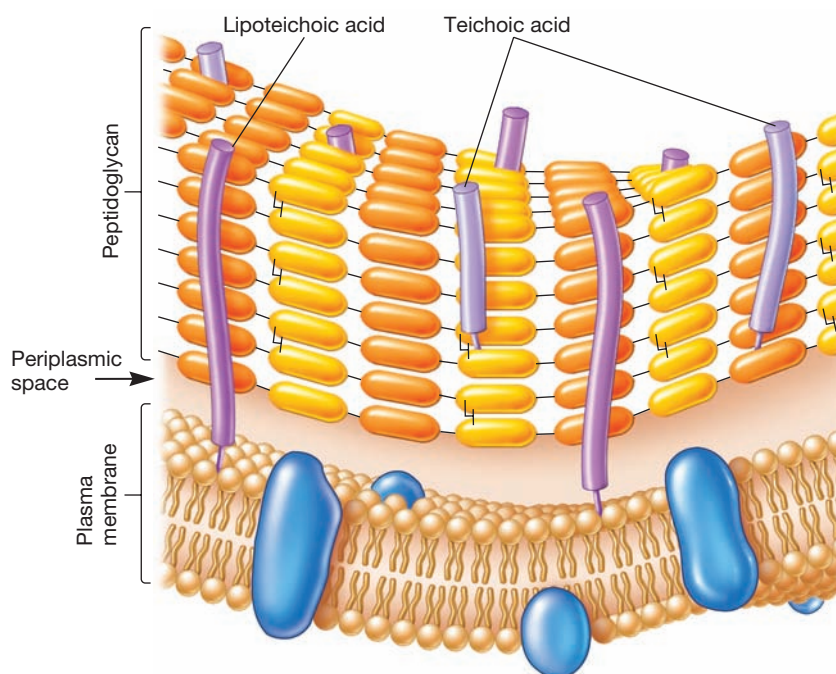


Figure 3.23 The Gram-Positive Envelope.

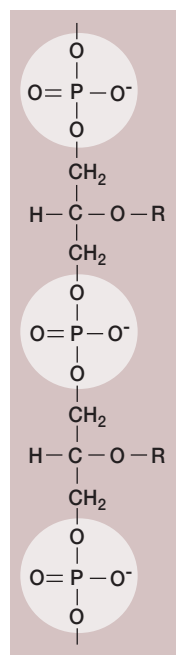


Figure 3.24 Teichoic Acid Structure. The segment of a teichoic acid made of phosphate, glycerol, and a side chain, R. R may represent D-alanine, glucose, or other molecules.

wall its negative charge. The functions of these molecules are still unclear, but they may be important in maintaining the structure of the wall. Teichoic acids are not present in gram-negative bacteria.

The periplasmic space of gram-positive bacteria, when observed, lies between the plasma membrane and the cell wall and is smaller than that of gram-negative bacteria. Even if gram-positive bacteria lack a discrete, obvious periplasmic space, they may have periplasm. The periplasm has relatively few proteins; this is probably because the peptidoglycan sac is porous and any proteins secreted by the cell usually pass through it. Enzymes secreted by gram-positive bacteria are called **exoenzymes**. They often serve to degrade polymeric nutrients that would otherwise be too large for transport across the plasma membrane. Those proteins that remain in the periplasmic space are usually attached to the plasma membrane.

Staphylococci and most other gram-positive bacteria have a layer of proteins on the surface of their cell wall peptidoglycan. These proteins are involved in the interactions of the cell with its environment. Some are noncovalently attached by binding to the peptidoglycan, teichoic acids, or other receptors. For example, the S-layer proteins (see p. 66) bind noncovalently to polymers scattered throughout the wall. Enzymes involved in peptidoglycan synthesis and turnover also seem to interact noncovalently with the cell wall. Other surface proteins are covalently attached to the peptidoglycan. Many covalently attached proteins, such as the M protein of pathogenic streptococci, have roles in virulence, such as aiding in adhesion to host tissues and interfering with host defenses. In staphylococci, these surface proteins are covalently joined to the pentaglycine bridge of the cell wall peptidoglycan.

An enzyme called sortase catalyzes the attachment of these surface proteins to the gram-positive peptidoglycan. Sortases are attached to the plasma membrane of the bacterial cell.

Gram-Negative Cell Walls

Even a brief inspection of figure 3.17 shows that gram-negative cell walls are much more complex than gram-positive walls. The thin peptidoglycan layer next to the plasma membrane and bounded on either side by the periplasmic space may constitute not more than 5 to 10% of the wall weight. In *E. coli* it is about 2 nm thick and contains only one or two sheets of peptidoglycan.

The periplasmic space of gram-negative bacteria is also strikingly different than that of gram-positive bacteria. It ranges in size from 1 nm to as great as 71 nm. Some recent studies indicate that it may constitute about 20 to 40% of the total cell volume, and it is usually 30 to 70 nm wide. When cell walls are disrupted carefully or removed without disturbing the underlying plasma membrane, periplasmic enzymes and other proteins are released and may be easily studied. Some periplasmic proteins participate in nutrient acquisition—for example, hydrolytic enzymes and transport proteins. Some periplasmic proteins are involved in energy conservation. For example, the denitrifying bacteria, which convert nitrate to nitrogen gas, and bacteria that use inorganic molecules as energy sources (chemolithotrophs) have electron transport proteins in their periplasm. Other periplasmic proteins are involved in peptidoglycan synthesis and the modification of toxic compounds that could harm the cell. [Chemolithotrophic metabolism \(section 9.10\)](#); [Biogeochemical cycling: The nitrogen cycle \(section 27.2\)](#)

The outer membrane lies outside the thin peptidoglycan layer (**figures 3.25 and 3.26**) and is linked to the cell in two ways. The first is by Braun's lipoprotein, the most abundant protein in the outer membrane. This small lipoprotein is covalently joined to the underlying peptidoglycan, and is embedded in the outer membrane by its hydrophobic end. The outer membrane and peptidoglycan are so firmly linked by this lipoprotein that they can be isolated as one unit. The second linking mechanism involves the many adhesion sites joining the outer membrane and the plasma membrane. The two membranes appear to be in direct contact at these sites. In *E. coli*, 20 to 100 nm areas of contact between the two membranes can be seen. Adhesion sites may be regions of direct contact or possibly true membrane fusions. It has been proposed that substances can move directly into the cell through these adhesion sites, rather than traveling through the periplasm.

Possibly the most unusual constituents of the outer membrane are its **lipopolysaccharides (LPSs)**. These large, complex molecules contain both lipid and carbohydrate, and consist of three parts: (1) lipid A, (2) the core polysaccharide, and (3) the O side chain. The LPS from *Salmonella* has been studied most, and its general structure is described here (**figure 3.27**). The **lipid A** region contains two glucosamine sugar derivatives, each with three fatty acids and phosphate or pyrophosphate attached. The fatty acids attach the lipid A to the outer membrane, while the remainder of the LPS molecule projects from the surface. The **core polysaccharide** is joined to lipid A. In *Salmonella* it is constructed of

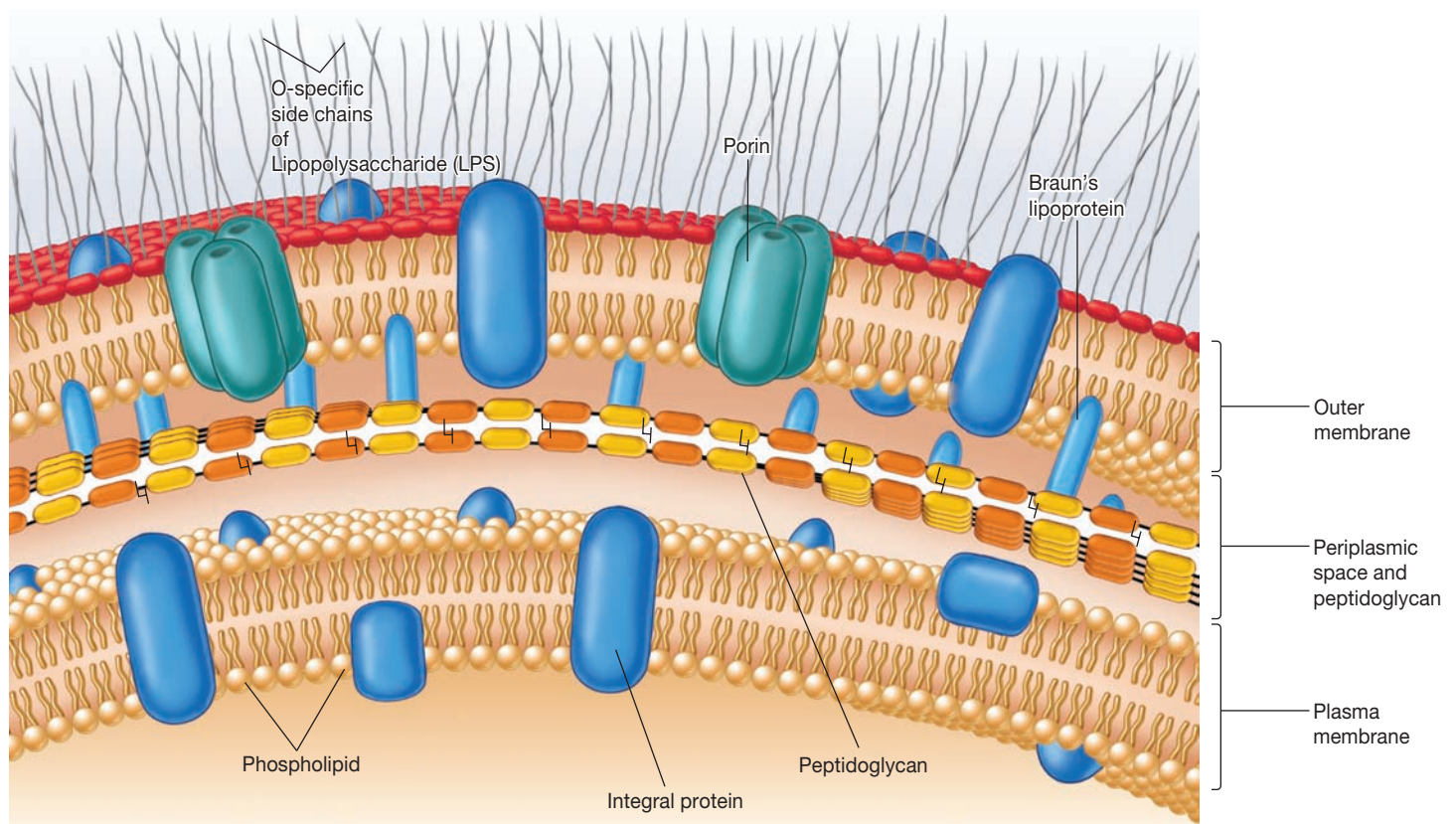


Figure 3.25 The Gram-Negative Envelope.

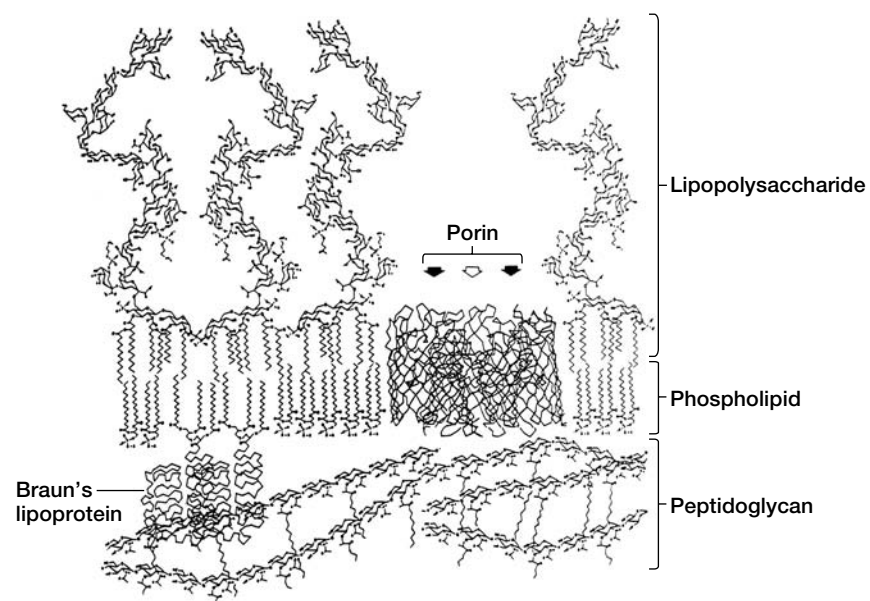


Figure 3.26 A Chemical Model of the *E. coli* Outer Membrane and Associated Structures. This cross-section is to scale. The porin OmpF has two channels in the front (solid arrows) and one channel in the back (open arrow) of the trimeric protein complex. LPS molecules can be longer than the ones shown here.

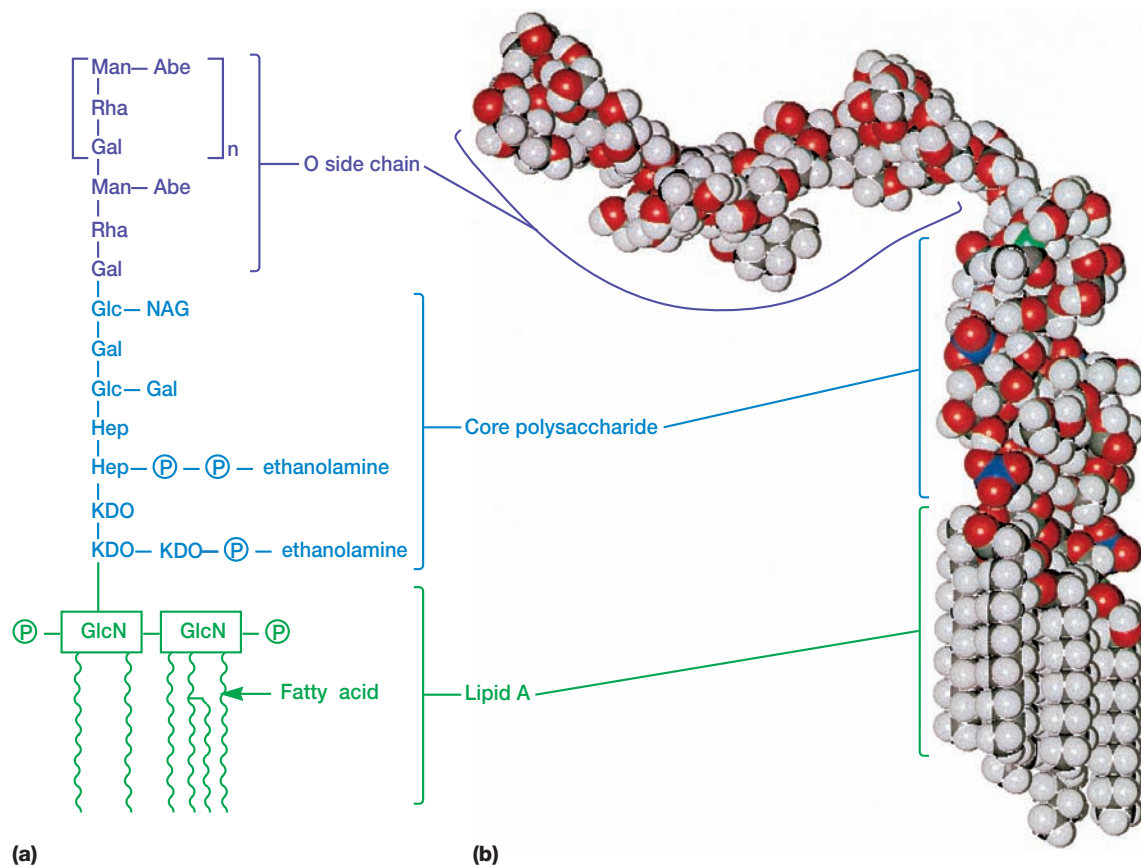


Figure 3.27 Lipopolysaccharide Structure. (a) The lipopolysaccharide from *Salmonella*. This slightly simplified diagram illustrates one form of the LPS. Abbreviations: Abe, abequose; Gal, galactose; Glc, glucose; GlcN, glucosamine; Hep, heptulose; KDO, 2-keto-3-deoxyoctonate; Man, mannose; NAG, *N*-acetylglucosamine; P, phosphate; Rha, L-rhamnose. Lipid A is buried in the outer membrane. (b) Molecular model of an *Escherichia coli* lipopolysaccharide. The lipid A and core polysaccharide are straight; the O side chain is bent at an angle in this model.

10 sugars, many of them unusual in structure. The **O side chain** or **O antigen** is a polysaccharide chain extending outward from the core. It has several peculiar sugars and varies in composition between bacterial strains.

LPS has many important functions. Because the core polysaccharide usually contains charged sugars and phosphate (figure 3.27), LPS contributes to the negative charge on the bacterial surface. As a major constituent of the exterior leaflet of the outer membrane, lipid A also helps stabilize outer membrane structure. LPS may contribute to bacterial attachment to surfaces and biofilm formation. A major function of LPS is that it aids in creating a permeability barrier. The geometry of LPS (figure 3.27b) and interactions between neighboring LPS molecules are thought to restrict the entry of bile salts, antibiotics, and other toxic substances that might kill or injure the bacterium. LPS also plays a role in protecting pathogenic gram-negative bacteria from host defenses. The O side chain of LPS is also called the O antigen because it elicits an immune response. This response involves the production of antibodies that bind the strain-specific form of LPS that elicited the response. However, many gram-

negative bacteria are able to rapidly change the antigenic nature of their O side chains, thus thwarting host defenses. Importantly, the lipid A portion of LPS often is toxic; as a result, the LPS can act as an **endotoxin** and cause some of the symptoms that arise in gram-negative bacterial infections. If the bacterium enters the bloodstream, LPS endotoxin can cause a form of septic shock for which there is no direct treatment. [Pathogenesis of bacterial disease \(section 33.3\)](#)

Despite the role of LPS in creating a permeability barrier, the outer membrane is more permeable than the plasma membrane and permits the passage of small molecules like glucose and other monosaccharides. This is due to the presence of **porin proteins** (figures 3.25 and 3.26). Most porin proteins cluster together to form a trimer in the outer membrane (figure 3.25 and **figure 3.28**). Each porin protein spans the outer membrane and is more or less tube-shaped; its narrow channel allows passage of molecules smaller than about 600 to 700 daltons. However, larger molecules such as vitamin B₁₂ also cross the outer membrane. Such large molecules do not pass through porins; instead, specific carriers transport them across the outer membrane.

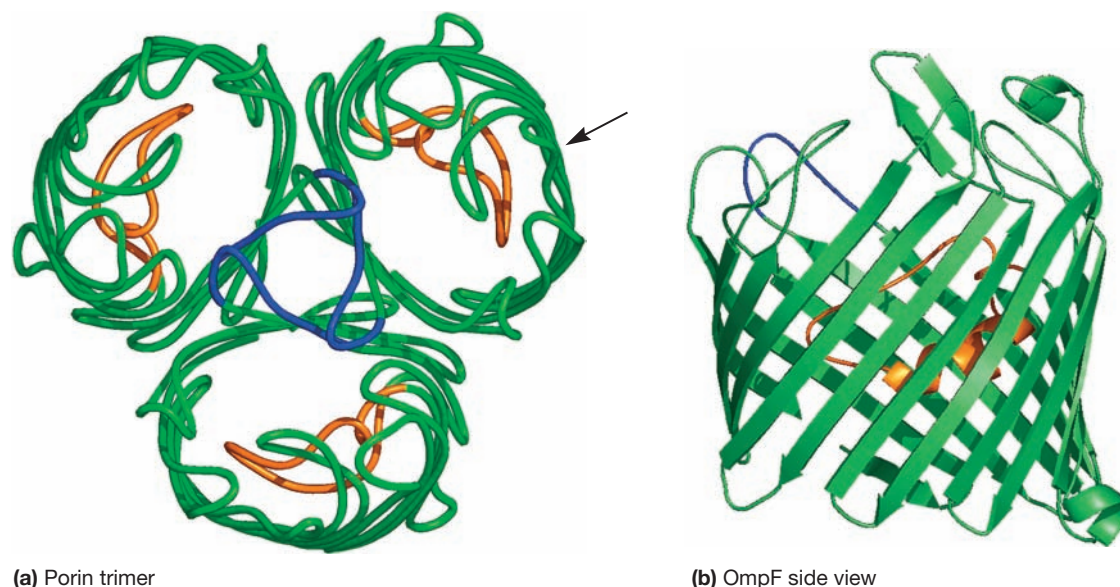


Figure 3.28 Porin Proteins. Two views of the OmpF porin of *E. coli*. **(a)** Porin structure observed when looking down at the outer surface of the outer membrane (i.e., top view). The three porin proteins forming the protein each form a channel. Each porin can be divided into three loops: the green loop forms the channel, the blue loop interacts with other porin proteins to help form the trimer, and the orange loop narrows the channel. The arrow indicates the area of a porin molecule viewed from the side in panel **(b)**. **(b)** Side view of a porin monomer showing the β -barrel structure characteristic of porin proteins.

The Mechanism of Gram Staining

Although several explanations have been given for the Gram-stain reaction results, it seems likely that the difference between gram-positive and gram-negative bacteria is due to the physical nature of their cell walls. If the cell wall is removed from gram-positive bacteria, they stain gram negative. Furthermore, genetically wall-less bacteria such as the mycoplasmas also stain gram negative. The peptidoglycan itself is not stained; instead it seems to act as a permeability barrier preventing loss of crystal violet. During the procedure the bacteria are first stained with crystal violet and next treated with iodine to promote dye retention. When gram-positive bacteria then are treated with ethanol, the alcohol is thought to shrink the pores of the thick peptidoglycan. Thus the dye-iodine complex is retained during this short decolorization step and the bacteria remain purple. In contrast, recall that gram-negative peptidoglycan is very thin, not as highly cross-linked, and has larger pores. Alcohol treatment also may extract enough lipid from the gram-negative outer membrane to increase its porosity further. For these reasons, alcohol more readily removes the purple crystal violet-iodine complex from gram-negative bacteria. Thus gram-negative bacteria are then easily stained red or pink by the counterstain safranin.

The Cell Wall and Osmotic Protection

Microbes have several mechanisms for responding to changes in osmotic pressure. This pressure arises when the concentration of solutes inside the cell differs from that outside, and the adaptive responses work to equalize the solute concentrations. However, in

certain situations, the osmotic pressure can exceed the cell's ability to adapt. In these cases, additional protection is provided by the cell wall. When cells are in hypotonic solutions—ones in which the solute concentration is less than that in the cytoplasm—water moves into the cell, causing it to swell. Without the cell wall, the pressure on the plasma membrane would become so great that it would be disrupted and the cell would burst—a process called **lysis**. Conversely, in hypertonic solutions, water flows out and the cytoplasm shrivels up—a process called **plasmolysis**.

The protective nature of the cell wall is most clearly demonstrated when bacterial cells are treated with lysozyme or penicillin. The enzyme **lysozyme** attacks peptidoglycan by hydrolyzing the bond that connects *N*-acetylmuramic acid with *N*-acetylglucosamine (figure 3.18). **Penicillin** works by a different mechanism. It inhibits peptidoglycan synthesis. If bacteria are treated with either of these substances while in a hypotonic solution, they will lyse. However, if they are in an isotonic solution, they can survive and grow normally. If they are gram positive, treatment with lysozyme or penicillin results in the complete loss of the cell wall, and the cell becomes a protoplast. When gram-negative bacteria are exposed to lysozyme or penicillin, the peptidoglycan layer is lost, but the outer membrane remains. These cells are called **spheroplasts**. Because they lack a complete cell wall, both protoplasts and spheroplasts are osmotically sensitive. If they are transferred to a dilute solution, they will lyse due to uncontrolled water influx (figure 3.29). [Antimicrobial drugs \(section 34.4\)](#)

Although most bacteria require an intact cell wall for survival, some have none at all. For example, the mycoplasmas lack a cell wall and are osmotically sensitive, yet often can grow in dilute

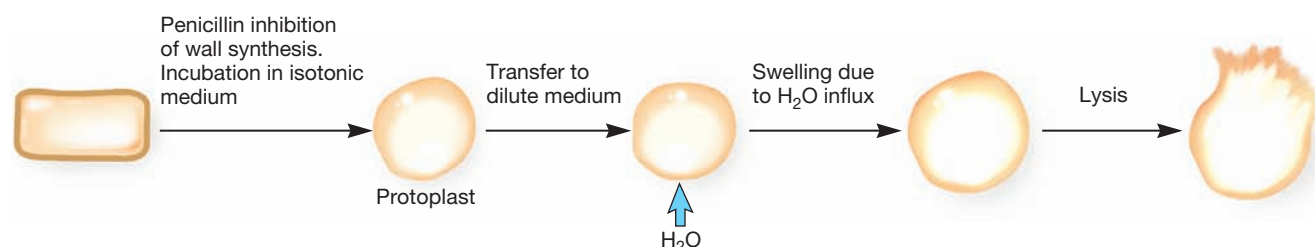


Figure 3.29 Protoplast Formation and Lysis. Protoplast formation induced by incubation with penicillin in an isotonic medium. Transfer to dilute medium will result in lysis.

media or terrestrial environments because their plasma membranes are more resistant to osmotic pressure than those of bacteria having walls. The precise reason for this is not clear, although the presence of sterols in the membranes of many species may provide added strength. Without a rigid cell wall, mycoplasmas tend to be pleomorphic or variable in shape.

1. List the functions of the cell wall.
2. Describe in detail the composition and structure of peptidoglycan. Why does peptidoglycan contain the unusual D isomers of alanine and glutamic acid rather than the L isomers observed in proteins?
3. Compare and contrast the cell walls of gram-positive bacteria and gram-negative bacteria. Include labeled drawings in your discussion.
4. Define or describe the following: outer membrane, periplasmic space, periplasm, envelope, teichoic acid, adhesion site, lipopolysaccharide, porin protein, protoplasts, and spheroplasts.
5. Design an experiment that illustrates the cell wall's role in protecting against lysis.
6. With a few exceptions, the cell walls of gram-positive bacteria lack porins. Why is this the case?

3.7 ARCHAEAL CELL WALLS

Before they were distinguished as a unique domain of life, the *Archaea* were characterized as being either gram positive or gram negative. However, their staining reaction does not correlate as reliably with a particular cell wall structure as does the Gram reaction of *Bacteria*. Archaeal wall structure and chemistry differ from those of the *Bacteria*. Archaeal cell walls lack peptidoglycan and also exhibit considerable variety in terms of their chemical make-up. Some of the major features of archaeal cell walls are described in this section.

Many archaea have a wall with a single, thick homogeneous layer resembling that in gram-positive bacteria (figure 3.30a). These archaea often stain gram positive. Their wall chemistry varies from species to species but usually consists of complex heteropolysaccharides. For example, *Methanobacterium* and some other methane-generating archaea (methanogens) have walls containing **pseudomurein** (figure 3.31), a peptidoglycan-like polymer that has L-amino acids instead of D-amino acids in its cross-links, N-acetylalosaminuronic acid instead of N-acetylmuramic acid, and β (1 $\rightarrow$ 3) glycosidic bonds instead of β (1 $\rightarrow$ 4) glycosidic

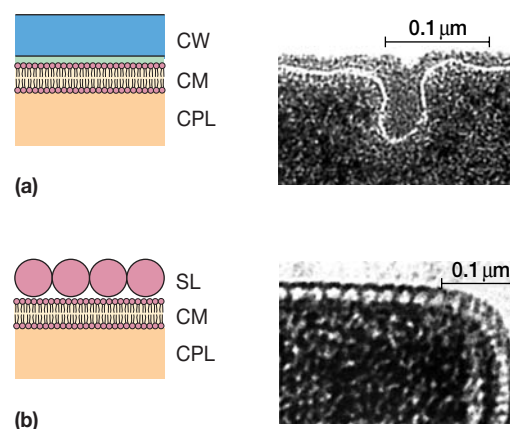


Figure 3.30 Cell Envelopes of Archaea. Schematic representations and electron micrographs of (a) *Methanobacterium formicum*, and (b) *Thermoproteus tenax*. CW, cell wall; SL, surface layer; CM, cell membrane or plasma membrane; CPL, cytoplasm.

bonds. Other archaea, such as *Methanosarcina* and the salt-loving *Halococcus*, contain complex polysaccharides similar to the chondroitin sulfate of animal connective tissue. **Phylum Euryarchaeota:** methanogens; halobacteria (section 20.3)

Many archaea that stain gram negative have a layer of glycoprotein or protein outside their plasma membrane (figure 3.30b). The layer may be as thick as 20 to 40 nm. Sometimes there are two layers—an electron-dense layer and a sheath surrounding it. Some methanogens (*Methanobolus*), salt-loving archaea (*Halobacterium*), and extreme thermophiles (*Sulfolobus*, *Thermoproteus*, and *Pyrodictium*) have glycoproteins in their walls. In contrast, other methanogens (*Methanococcus*, *Methanomicrobium*, and *Methanogenium*) and the extreme thermophile *Desulfurococcus* have protein walls. **Phylum Crenarchaeota** (section 20.2); **Phylum Euryarchaeota:** Extremely thermophilic S^0 -metabolizers (section 20.3)

1. How do the cells walls of *Archaea* differ from those of *Bacteria*?
2. What is pseudomurein? How is it similar to peptidoglycan? How is it different?
3. Archaea with cell walls consisting of a thick, homogeneous layer of complex polysaccharides often retain the crystal violet dye when stained using the Gram-staining procedure. Why do you think this is so?

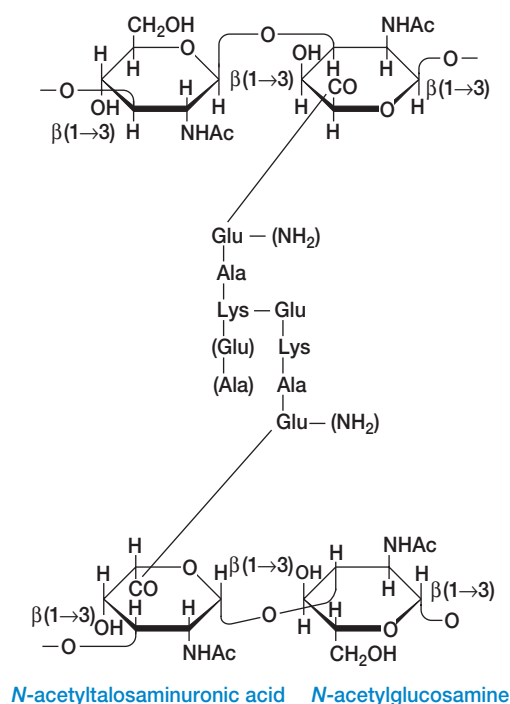


Figure 3.31 The Structure of Pseudomurein. The amino acids and amino groups in parentheses are not always present. Ac represents the acetyl group.

3.8 PROTEIN SECRETION IN PROCARYOTES

The membranes of the procaryotic cell envelope (i.e., the plasma membrane of *Archaea* and gram-positive bacteria and the plasma and outer membranes of gram-negative bacteria) present a considerable barrier to the movement of large molecules into or out of the cell. Yet, as will be discussed in section 3.9, many important structures are located outside the wall. How are the large molecules from which some of these structures are made transported out of the cell for assembly? Furthermore, exoenzymes and other proteins are released from procaryotes into their surroundings. How do these proteins get through the membrane(s) of the cell envelope? Clearly procaryotes must be able to secrete proteins. The research on protein secretion pathways has mushroomed in the last few decades in part because of the fundamental importance of protein secretion, but also because certain protein secretion mechanisms are common to pathogenic bacteria. Furthermore, an understanding of protein secretion can be exploited for vaccine development and a variety of industrial processes. Because relatively little is known about archaeal protein secretion systems, this section provides an overview of bacterial protein secretion pathways.

Overview of Bacterial Protein Secretion

Protein secretion poses different difficulties depending on whether the bacterium is gram-positive or gram-negative. In order for gram-positive bacteria to secrete proteins, the proteins must be

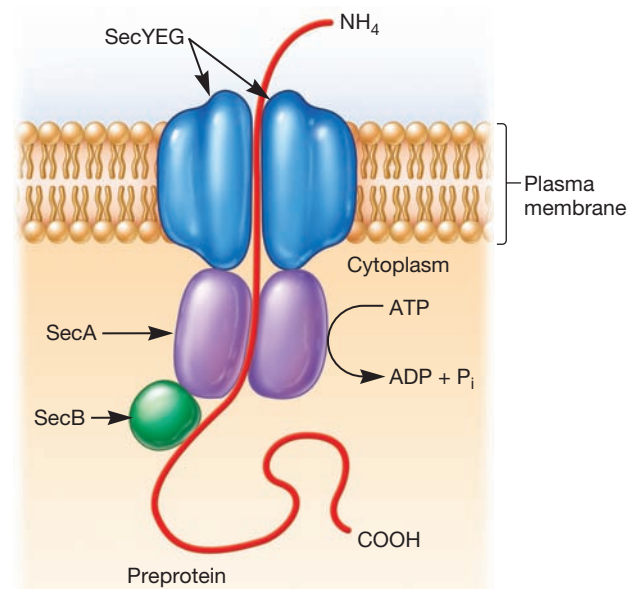


Figure 3.32 The Sec-Dependent Pathway. The Sec-dependent pathway of *E. coli*.

transported across the plasma membrane. Once across the plasma membrane, the protein either passes through the relatively porous peptidoglycan into the external environment or it becomes embedded in or attached to the peptidoglycan. Gram-negative bacteria have more hurdles to jump when they secrete proteins. They, too, must transport the proteins across the plasma membrane, but in order to complete the secretion process, the proteins must be able to escape attack from protein-degrading enzymes in the periplasmic space, and they must be transported across the outer membrane. In both gram-positive and gram-negative bacteria, the major pathway for transporting proteins across the plasma membrane is the Sec-dependent (secretion-dependent) pathway (**figure 3.32**). In gram-negative bacteria, proteins can be transported across the outer membrane by several different mechanisms, some of which bypass the Sec-dependent pathway altogether, moving proteins directly from the cytoplasm to the outside of the cell (**figure 3.33**). All protein secretion pathways described here require the expenditure of energy at some step in the process. The energy is usually supplied by the hydrolysis of high-energy molecules such as ATP and GTP, but another form of energy, the proton motive force, also sometimes plays a role. [The role of ATP in metabolism \(section 8.5\); Electron transport and oxidative phosphorylation \(section 9.5\)](#)

The Sec-Dependent Pathway

The **Sec-dependent pathway**, sometimes called the general secretion pathway, is highly conserved and has been identified in all three domains of life (**figure 3.32**). It translocates proteins across the plasma membrane or integrates them into the membrane itself. Proteins to be transported across the plasma membrane by this pathway are synthesized as presecretory proteins called preproteins. The preprotein has a **signal peptide** at its

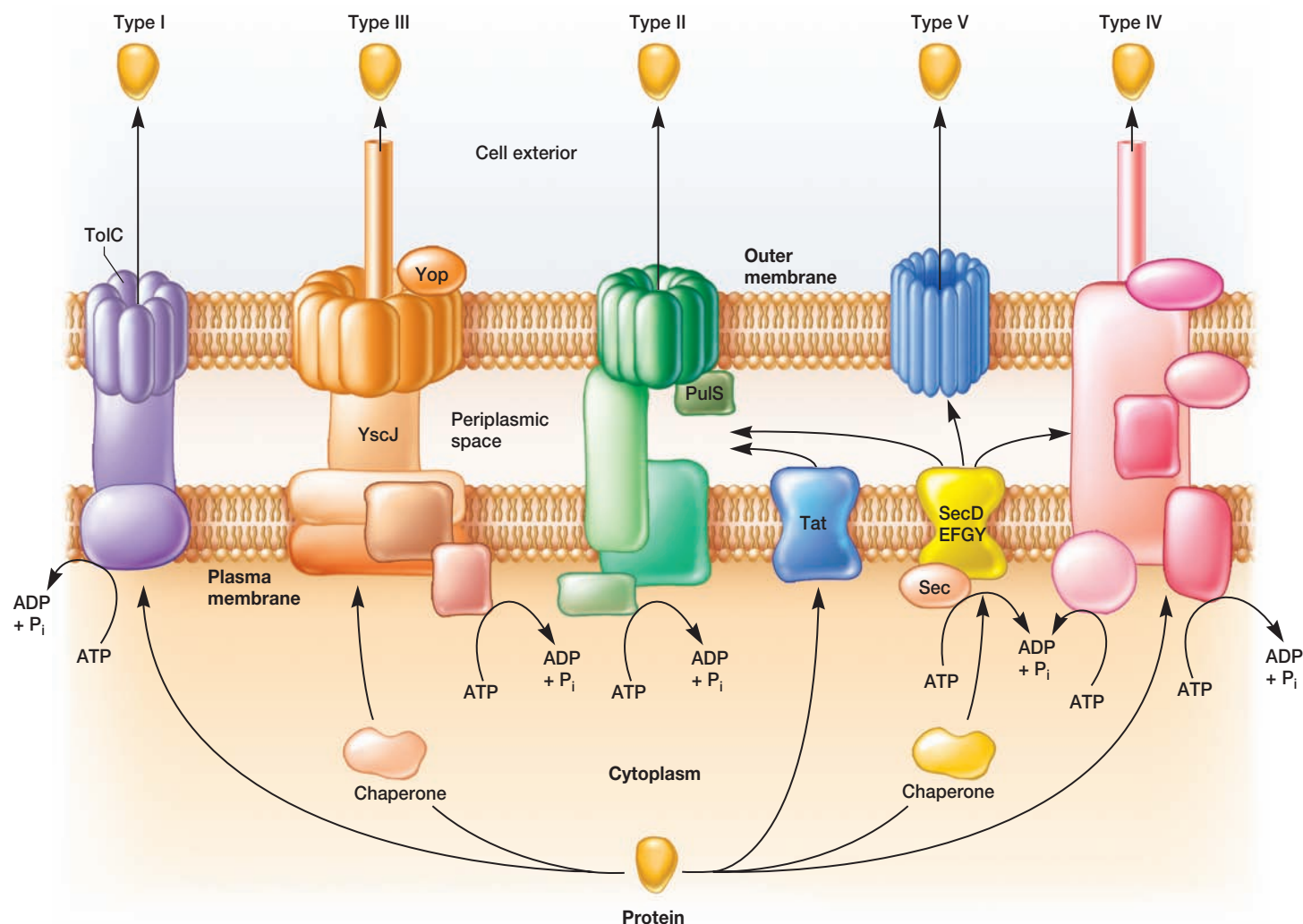


Figure 3.33 The Protein Secretion Systems of Gram-Negative Bacteria. The five secretion systems of gram-negative bacteria are shown. The Sec-dependent and Tat pathways deliver proteins from the cytoplasm to the periplasmic space. The type II, type V, and sometimes type IV systems complete the secretion process begun by the Sec-dependent pathway. The Tat system appears to deliver proteins only to the type II pathway. The type I and type 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.

amino-terminus, which is recognized by the Sec machinery. Soon after the signal peptide is synthesized, special proteins called chaperone proteins (e.g., SecB) bind it. This helps delay protein folding, thereby helping the preprotein reach the Sec transport machinery in the conformation needed for transport. There is evidence that translocation of proteins can begin before the completion of their synthesis by ribosomes. Certain Sec proteins (SecY, SecE and SecG) are thought to form a channel in the membrane through which the preprotein passes. Another protein (SecA) binds to the SecYEG proteins and the SecB-preprotein complex. SecA acts as a motor to translocate the preprotein (but

not the chaperone protein) through the plasma membrane using ATP hydrolysis. When the preprotein emerges from the plasma membrane, free from chaperones, an enzyme called signal peptidase removes the signal peptide. The protein then folds into the proper shape, and disulfide bonds are formed when necessary.

Protein Secretion in Gram-Negative Bacteria

Currently five protein secretion pathways have been identified in gram-negative bacteria (figure 3.33). Recall that gram-negative bacteria have a second, outer membrane that proteins must cross.

Gram-negative bacteria use the type II and type V pathways to transport proteins across the outer membrane after the protein has first been translocated across the plasma membrane by the Sec-dependant pathway. The type I and type III pathways do not interact with proteins that are first translocated by the Sec system, so they are said to be Sec-independent. The type IV pathway sometimes is linked to the Sec-dependent pathway but usually functions on its own.

The **type II protein secretion pathway** is present in a number of plant and animal pathogens, including *Erwinia carotovora*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Vibrio cholerae*. It is responsible for the secretion of proteins such as the degradative enzymes pullulanases, cellulases, pectinases, proteases, and lipases, as well as other proteins like cholera toxin and pili proteins. Type II systems are quite complex and can contain as many as 12 to 14 proteins, most of which appear to be integral membrane proteins (figure 3.33). Even though some components of type II systems span the plasma membrane, they apparently translocate proteins only across the outer membrane. In most cases, the Sec-dependent pathway first translocates the protein across the plasma membrane and then the type II system completes the secretion process. In gram-negative and gram-positive bacteria, another plasma membrane translocation system called the **Tat pathway** can move proteins across the plasma membrane. In gram-negatives, these proteins are then delivered to the type II system. The Tat pathway is distinct from the Sec system in that it translocates already folded proteins.

The **type V protein secretion pathways** are the most recently discovered protein secretion systems. They, too, rely on the Sec-dependent pathway to move proteins across the plasma membrane. However, once in the periplasmic space, many of these proteins are able to form a channel in the outer membrane through which they transport themselves; these proteins are referred to as autotransporters. Other proteins are secreted by the type V pathway with the aid of a separate helper protein.

The **ABC protein secretion pathway**, which derives its name from ATP binding cassette, is ubiquitous in procaryotes—that is, it is present in gram-positive and gram-negative bacteria as well as *Archaea*. It is sometimes called the **type I protein secretion pathway** (figure 3.33). In pathogenic gram-negative bacteria, it is involved in the secretion of toxins (α -hemolysin), as well as proteases, lipases, and specific peptides. Secreted proteins usually contain C-terminal secretion signals that help direct the newly synthesized protein to the type I machinery, which spans the plasma membrane, the periplasmic space, and the outer membrane. These systems translocate proteins in one step across both membranes, bypassing the Sec-dependent pathway. Gram-positive bacteria use a modified version of the type I system to translocate proteins across the plasma membrane. Analysis of the *Bacillus subtilis* genome has identified 77 ABC transporters. This may reflect the fact that ABC transporters transport a wide variety of solutes in addition to proteins, including sugars and amino acids, as well as exporting drugs from the cell interior.

Several gram-negative pathogens have the **type III protein secretion pathway**, another secretion system that bypasses the Sec-dependent pathway. Most type III systems inject virulence

factors directly into the plant and animal host cells these pathogens attack. These virulence factors include toxins, phagocytosis inhibitors, stimulators of cytoskeleton reorganization in the host cell, and promoters of host cell suicide (apoptosis). However, in some cases the virulence factor is simply secreted into the extracellular milieu. Type III systems also transport other proteins, including (1) some of the proteins from which the system is built, (2) proteins that regulate the secretion process, and (3) proteins that aid in the insertion of secreted proteins into target cells. Type III systems are structurally complex and often are shaped like a syringe (figure 3.33). The slender, needlelike portion extends from the cell surface; a cylindrical base is connected to both the outer membrane and the plasma membrane and looks somewhat like the flagellar basal body (see p. 67). It is thought that proteins may move through a translocation channel. Important examples of bacteria with type III systems are *Salmonella*, *Yersinia*, *Shigella*, *E. coli*, *Bordetella*, *Pseudomonas aeruginosa*, and *Erwinia*. The participation of type III systems in bacterial virulence is further discussed in chapter 33.

Type IV protein secretion pathways are unique in that they are used to secrete proteins as well as to transfer DNA from a donor bacterium to a recipient during bacterial conjugation. Type IV systems are composed of many different proteins, and like the type III systems, these proteins form a syringelike structure. Type IV systems and conjugation are described in more detail in chapter 13.

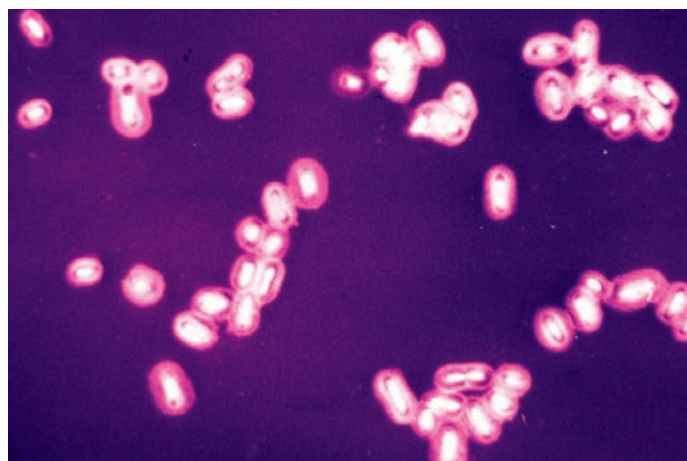
1. Give the major characteristics and functions of the protein secretion pathways described in this section.
2. Which secretion pathway is most widespread?
3. What is a signal peptide? Why do you think a protein's signal peptide is not removed until after the protein is translocated across the plasma membrane?

3.9 COMPONENTS EXTERNAL TO THE CELL WALL

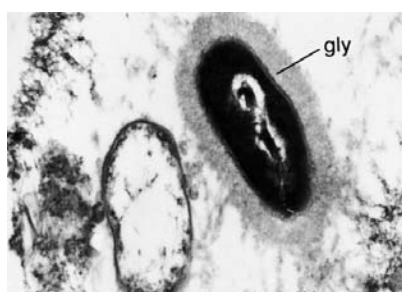
Procaryotes have a variety of structures outside the cell wall that can function in protection, attachment to objects, and cell movement. Several of these are discussed.

Capsules, Slime Layers, and S-Layers

Some procaryotes have a layer of material lying outside the cell wall. This layer has different names depending on its characteristics. When the layer is well organized and not easily washed off, it is called a **capsule** (figure 3.34a). It is called a **slime layer** when it is a zone of diffuse, unorganized material that is removed easily. When the layer consists of a network of polysaccharides extending from the surface of the cell, it is referred to as the **glycocalyx** (figure 3.34b), a term that can encompass both capsules and slime layers because they usually are composed of polysaccharides. However, some slime layers and capsules are constructed of other materials. For example, *Bacillus anthracis* has a



(a) *K. pneumoniae*



(b) *Bacteroides*

Figure 3.34 Bacterial Capsules. (a) *Klebsiella pneumoniae* with its capsule stained for observation in the light microscope ($\times 1,500$). (b) *Bacteroides* glycolyx (gly), TEM ($\times 71,250$).

proteinaceous capsule composed of poly-D-glutamic acid. Capsules are clearly visible in the light microscope when negative stains or special capsule stains are employed (figure 3.34a); they also can be studied with the electron microscope (figure 3.34b).

Although capsules are not required for growth and reproduction in laboratory cultures, they do confer several advantages when prokaryotes grow in their normal habitats. They help pathogenic bacteria resist phagocytosis by host phagocytes. *Streptococcus pneumoniae* provides a dramatic example. When it lacks a capsule, it is destroyed easily and does not cause disease, whereas the capsulated variant quickly kills mice. Capsules contain a great deal of water and can protect against desiccation. They exclude viruses and most hydrophobic toxic materials such as detergents. The glycocalyx also aids in attachment to solid surfaces, including tissue surfaces in plant and animal hosts (figure 3.35). Gliding bacteria often produce slime, which in some cases, has been shown to facilitate motility. [Microbial Diversity & Ecology 21.1: The mechanism of gliding motility](#); [Phagocytosis \(section 31.3\)](#); [Pathogenesis of bacterial disease \(section 33.3\)](#)

Many prokaryotes have a regularly structured layer called an **S-layer** on their surface. In bacteria, the S-layer is external to the cell wall. In archaea, the S-layer may be the only wall structure outside the plasma membrane. The S-layer has a pattern something like

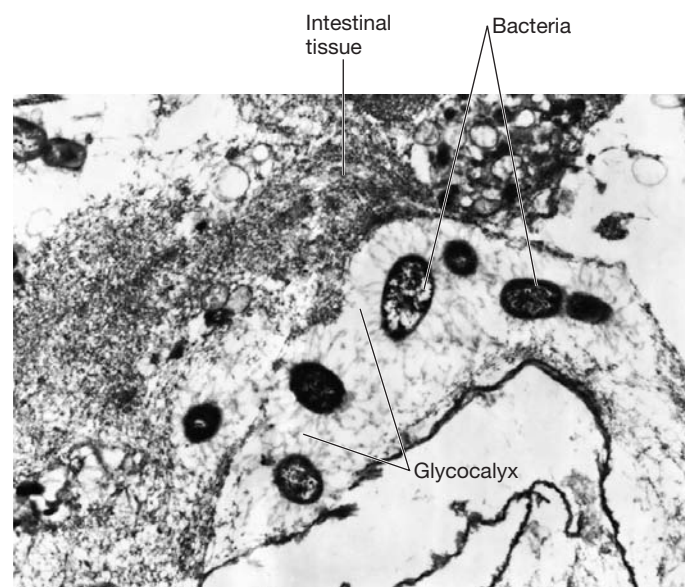


Figure 3.35 Bacterial Glycocalyx. Bacteria connected to each other and to the intestinal wall, by their glycocalyxes, the extensive networks of fibers extending from the cells ($\times 17,500$).

floor tiles and is composed of protein or glycoprotein (figure 3.36). In gram-negative bacteria the S-layer adheres directly to the outer membrane; it is associated with the peptidoglycan surface in gram-positive bacteria. It may protect the cell against ion and pH fluctuations, osmotic stress, enzymes, or the predacious bacterium *Bdellovibrio*. The S-layer also helps maintain the shape and envelope rigidity of some cells. It can promote cell adhesion to surfaces. Finally, the S-layer seems to protect some bacterial pathogens against host defenses, thus contributing to their virulence. [Class Deltaproteobacteria: Order Bdellovibrionales \(section 22.4\)](#)

Pili and Fimbriae

Many prokaryotes have short, fine, hairlike appendages that are thinner than flagella. These are usually called **fimbriae** (s., **fimbria**). Although many people use the terms fimbriae and pili interchangeably, we shall distinguish between fimbriae and sex pili. A cell may be covered with up to 1,000 fimbriae, but they are only visible in an electron microscope due to their small size (figure 3.37). They are slender tubes composed of helically arranged protein subunits and are about 3 to 10 nm in diameter and up to several micrometers long. At least some types of fimbriae attach bacteria to solid surfaces such as rocks in streams and host tissues.

Fimbriae are responsible for more than attachment. Type IV fimbriae are present at one or both poles of bacterial cells. They can aid in attachment to objects, and also are required for the twitching motility that occurs in some bacteria such as *P. aeruginosa*, *Neisseria gonorrhoeae*, and some strains of *E. coli*. Movement is by short, intermittent jerky motions of up to several micrometers in length and normally is seen on very moist surfaces. There is evidence that the fimbriae actively retract to move these bacteria. Type

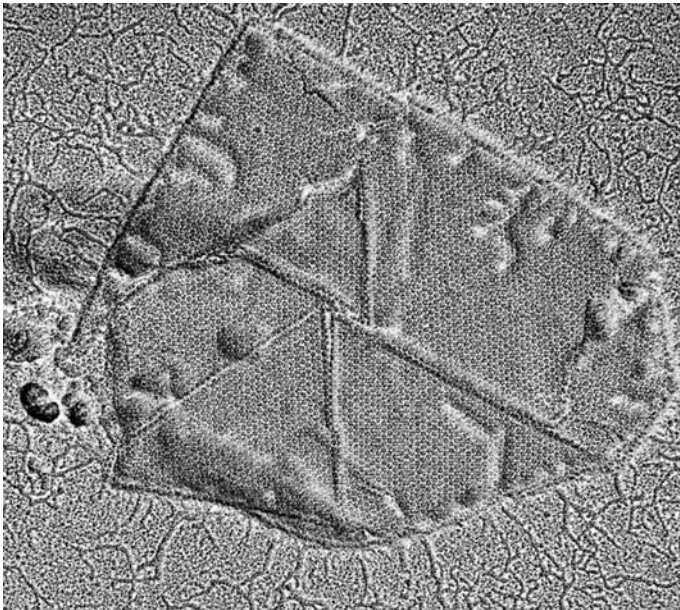


Figure 3.36 The S-Layer. An electron micrograph of the S-layer of the bacterium *Deinococcus radiodurans* after shadowing.

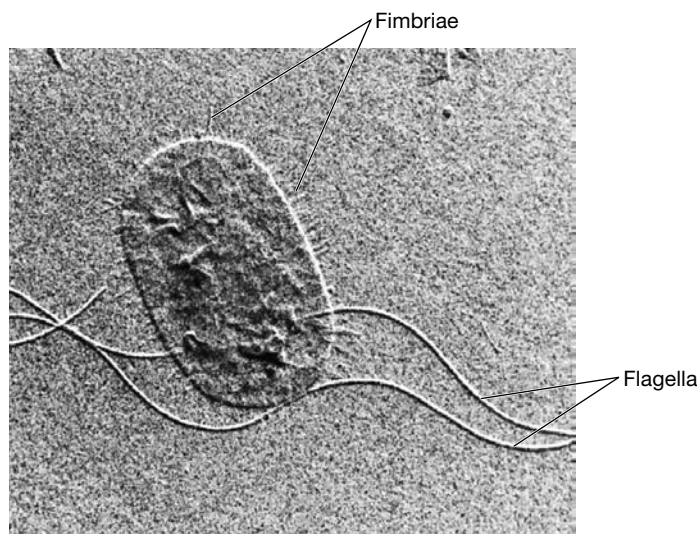


Figure 3.37 Flagella and Fimbriae. The long flagella and the numerous shorter fimbriae are very evident in this electron micrograph of the bacterium *Proteus vulgaris* ($\times 39,000$).

IV fimbriae are also involved in gliding motility by myxobacteria. These bacteria are also of interest because they have complex life cycles that include the formation of a fruiting body. [Class Deltaproteobacteria: Order Myxococcales \(section 22.4\)](#).

Many bacteria have about 1-10 **sex pili** (s., **pilus**) per cell. These are hairlike structures that differ from fimbriae in the following ways. Pili often are larger than fimbriae (around 9 to 10 nm in diameter). They are genetically determined by conjugative

plasmids and are required for conjugation. Some bacterial viruses attach specifically to receptors on sex pili at the start of their reproductive cycle. [Bacterial conjugation \(section 13.7\)](#)

Flagella and Motility

Most motile procaryotes move by use of **flagella** (s., **flagellum**), threadlike locomotor appendages extending outward from the plasma membrane and cell wall. Bacterial flagella are the best studied and they are the focus of this discussion.

Bacterial flagella are slender, rigid structures, about 20 nm across and up to 15 or 20 μm long. Flagella are so thin they cannot be observed directly with a bright-field microscope, but must be stained with special techniques designed to increase their thickness. The detailed structure of a flagellum can only be seen in the electron microscope (figure 3.37).

Bacterial species often differ distinctively in their patterns of flagella distribution and these patterns are useful in identifying bacteria. **Monotrichous** bacteria (*trichous* means hair) have one flagellum; if it is located at an end, it is said to be a **polar flagellum** (figure 3.38a). **Amphitrichous** bacteria (*amphi* means on both sides) have a single flagellum at each pole. In contrast, **lophotrichous** bacteria (*lopho* means tuft) have a cluster of flagella at one or both ends (figure 3.38b). Flagella are spread fairly evenly over the whole surface of **peritrichous** (*peri* means around) bacteria (figure 3.38c).

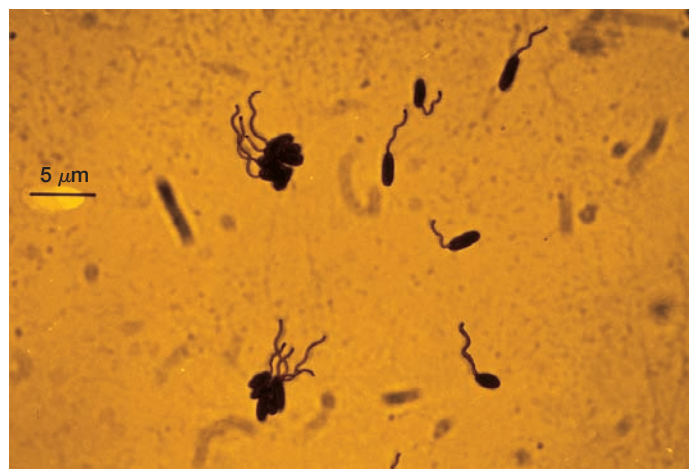
Flagellar Ultrastructure

Transmission electron microscope studies have shown that the bacterial flagellum is composed of three parts. (1) The longest and most obvious portion is the **flagellar filament**, which extends from the cell surface to the tip. (2) A **basal body** is embedded in the cell; and (3) a short, curved segment, the **flagellar hook**, links the filament to its basal body and acts as a flexible coupling. The filament is a hollow, rigid cylinder constructed of subunits of the protein **flagellin**, which ranges in molecular weight from 30,000 to 60,000 daltons, depending on the bacterial species. The filament ends with a capping protein. Some bacteria have sheaths surrounding their flagella. For example, *Bdellovibrio* has a membranous structure surrounding the filament. *Vibrio cholerae* has a lipopolysaccharide sheath.

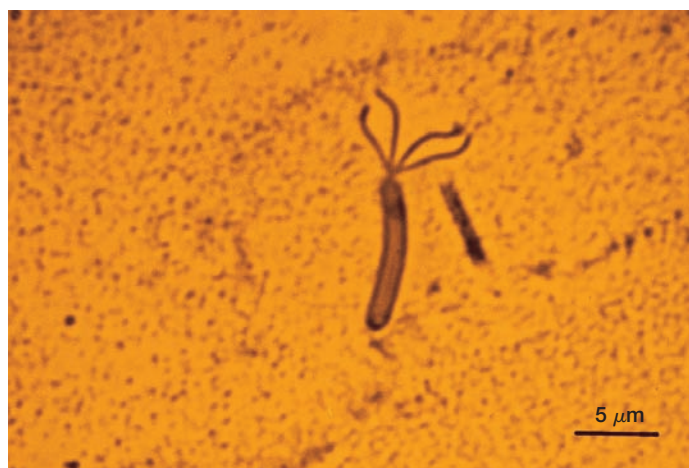
The hook and basal body are quite different from the filament (figure 3.39). Slightly wider than the filament, the hook is made of different protein subunits. The basal body is the most complex part of a flagellum. In *E. coli* and most gram-negative bacteria, the basal body has four rings connected to a central rod (figure 3.39a,d). The outer L and P rings associate with the lipopolysaccharide and peptidoglycan layers, respectively. The inner M ring contacts the plasma membrane. Gram-positive bacteria have only two basal body rings—an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan (figure 3.39b).

Flagellar Synthesis

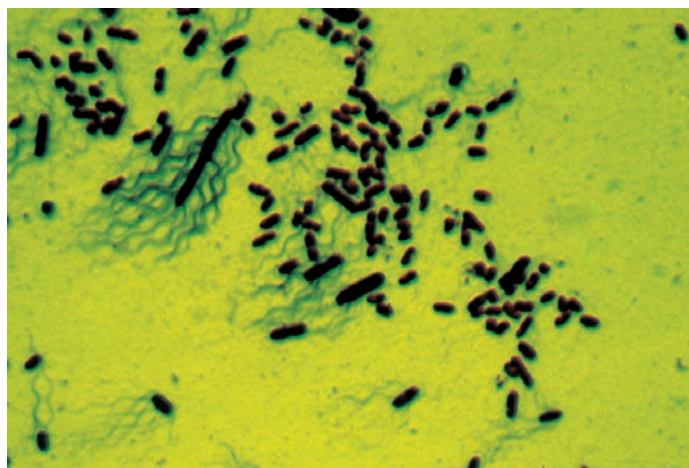
The synthesis of bacterial flagella is a complex process involving at least 20 to 30 genes. Besides the gene for flagellin, 10 or more genes code for hook and basal body proteins; other genes



(a) *Pseudomonas*—monotrichous polar flagellation



(b) *Spirillum*—lophotrichous flagellation



(c) *P. vulgaris*—peritrichous flagellation

Figure 3.38 Flagellar Distribution. Examples of various patterns of flagellation as seen in the light microscope.

(a) Monotrichous polar (*Pseudomonas*). (b) Lophotrichous (*Spirillum*). (c) Peritrichous (*Proteus vulgaris*, $\times 600$).

are concerned with the control of flagellar construction or function. How the cell regulates or determines the exact location of flagella is not known.

When flagella are removed, the regeneration of the flagellar filament can then be studied. Transport of many flagellar components is carried out by an apparatus in the basal body that is a specialized type III protein secretion system. It is thought that flagellin subunits are transported through the filament's hollow internal core. When they reach the tip, the subunits spontaneously aggregate under the direction of a special filament cap so that the filament grows at its tip rather than at the base (**figure 3.40**). Filament synthesis is an excellent example of **self-assembly**. Many structures form spontaneously through the association of their component parts without the aid of any special enzymes or other factors. The information required for filament construction is present in the structure of the flagellin subunit itself.

The Mechanism of Flagellar Movement

Prokaryotic flagella operate differently from eucaryotic flagella. The filament is in the shape of a rigid helix, and the cell moves when this helix rotates. Considerable evidence shows that flagella act just like propellers on a boat. Bacterial mutants with straight flagella or abnormally long hook regions cannot swim. When bacteria are tethered to a glass slide using antibodies to filament or hook proteins, the cell body rotates rapidly about the stationary flagellum. If polystyrene-latex beads are attached to flagella, the beads spin about the flagellar axis due to flagellar rotation. The flagellar motor can rotate very rapidly. The *E. coli* motor rotates 270 revolutions per second; *Vibrio alginolyticus* averages 1,100 rps. [Cilia and flagella \(section 4.10\)](#)

The direction of flagellar rotation determines the nature of bacterial movement. Monotrichous, polar flagella rotate counterclockwise (when viewed from outside the cell) during normal forward movement, whereas the cell itself rotates slowly clockwise. The rotating helical flagellar filament thrusts the cell forward in a run with the flagellum trailing behind (**figure 3.41**). Monotrichous bacteria stop and tumble randomly by reversing the direction of flagellar rotation. Peritrichously flagellated bacteria operate in a somewhat similar way. To move forward, the flagella rotate counterclockwise. As they do so, they bend at their hooks to form a rotating bundle that propels the cell forward. Clockwise rotation of the flagella disrupts the bundle and the cell tumbles.

Because bacteria swim through rotation of their rigid flagella, there must be some sort of motor at the base. A rod extends from the hook and ends in the M ring, which can rotate freely in the plasma membrane (**figure 3.42**). It is thought that the S ring is attached to the cell wall in gram-positive cells and does not rotate. The P and L rings of gram-negative bacteria would act as bearings for the rotating rod. There is some evidence that the basal body is a passive structure and rotates within a membrane-embedded protein complex much like the rotor of an electrical motor turns in the center of a ring of electromagnets (the stator).

The exact mechanism that drives basal body rotation is not entirely clear. Figure 3.42 provides a more detailed depiction of

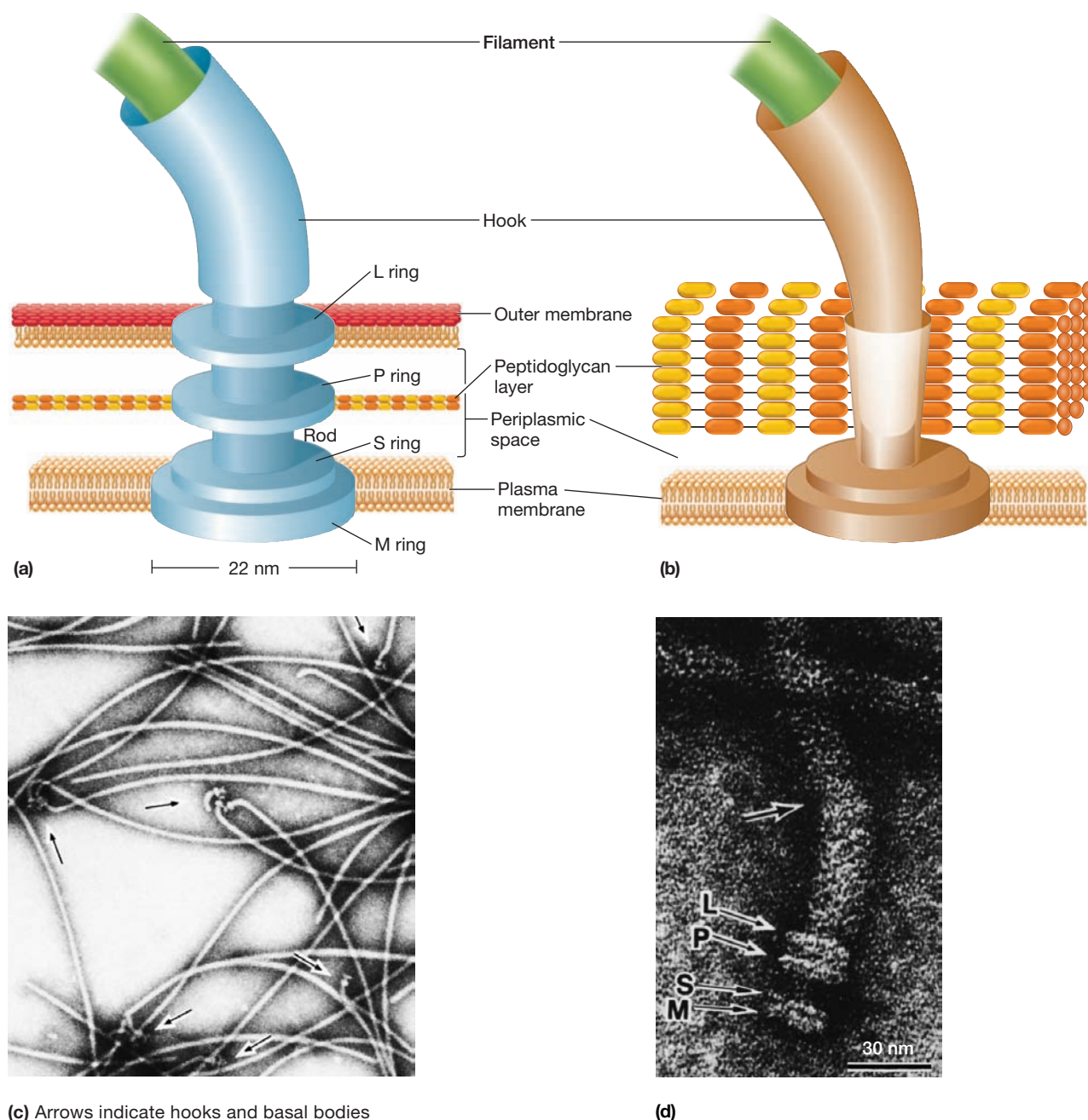


Figure 3.39 The Ultrastructure of Bacterial Flagella. Flagellar basal bodies and hooks in (a) gram-negative and (b) gram-positive bacteria. (c) Negatively stained flagella from *Escherichia coli* ($\times 66,000$). (d) An enlarged view of the basal body of an *E. coli* flagellum ($\times 485,000$). All four rings (L, P, S, and M) can be clearly seen. The uppermost arrow is at the junction of the hook and filament.

the basal body in gram-negative bacteria. The rotor portion of the motor seems to be made primarily of a rod, the M ring, and a C ring joined to it on the cytoplasmic side of the basal body. These two rings are made of several proteins; FliG is particularly important in generating flagellar rotation. The two most important proteins in the stator part of the motor are MotA and MotB. These form a proton channel through the plasma membrane, and MotB also anchors the Mot complex to cell wall peptidoglycan.

There is some evidence that MotA and FliG directly interact during flagellar rotation. This rotation is driven by proton or sodium gradients in procaryotes, not directly by ATP as is the case with eucaryotic flagella. [The electron transport chain and oxidative phosphorylation \(section 9.5\)](#)

The flagellum is a very effective swimming device. From the bacterium's point of view, swimming is quite a task because the surrounding water seems as thick and viscous as molasses. The cell

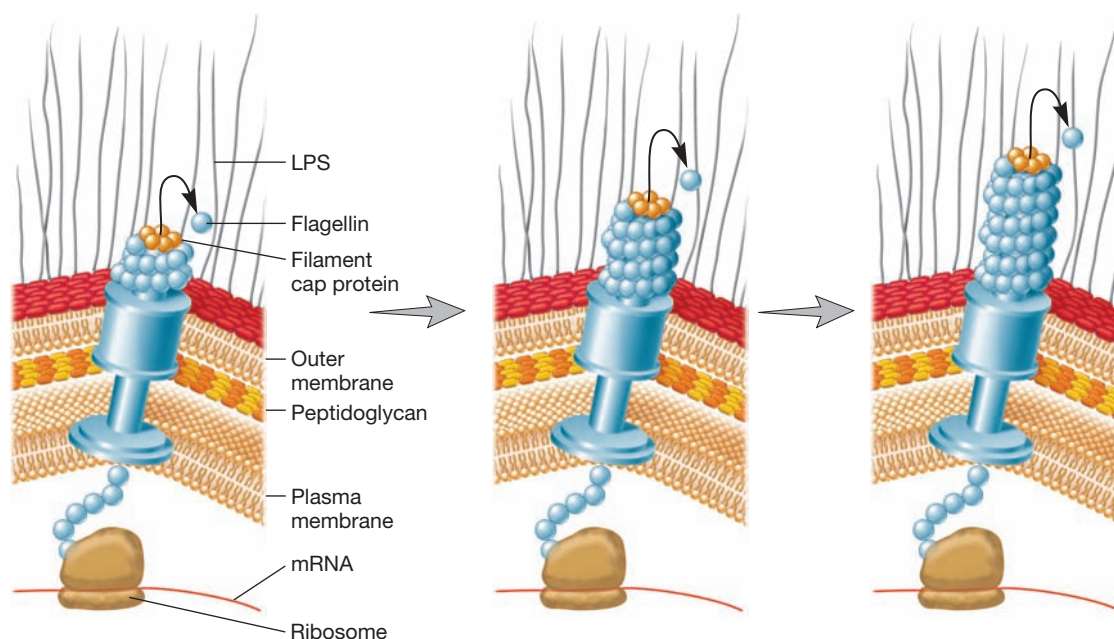


Figure 3.40 Growth of Flagellar Filaments. Flagellin subunits travel through the flagellar core and attach to the growing tip. Their attachment is directed by the filament cap protein.

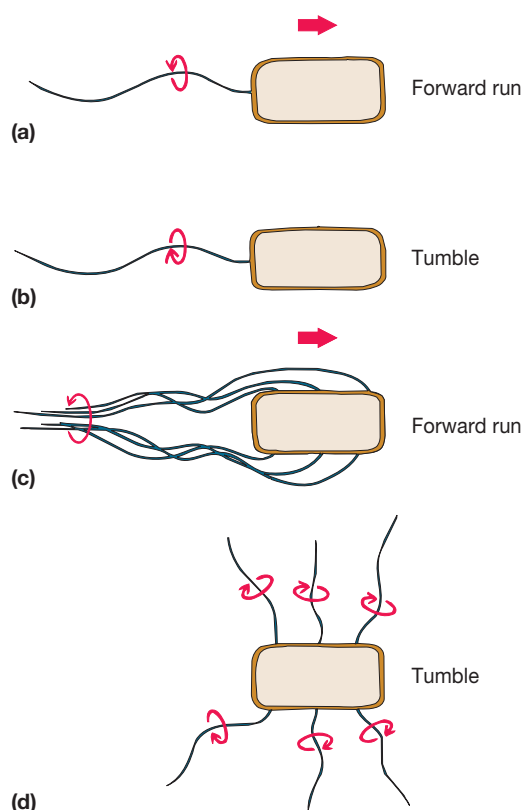


Figure 3.41 Flagellar Motility. The relationship of flagellar rotation to bacterial movement. Parts (a) and (b) describe the motion of monotrichous, polar bacteria. Parts (c) and (d) illustrate the movements of peritrichous organisms.

must bore through the water with its corkscrew-shaped flagella, and if flagellar activity ceases, it stops almost instantly. Despite such environmental resistance to movement, bacteria can swim from 20 to almost 90 $\mu\text{m}/\text{second}$. This is equivalent to traveling from 2 to over 100 cell lengths per second. In contrast, an exceptionally fast 6-ft human might be able to run around 5 body lengths per second.

Bacteria can move by mechanisms other than flagellar rotation. Spirochetes are helical bacteria that travel through viscous substances such as mucus or mud by flexing and spinning movements caused by a special **axial filament** composed of periplasmic flagella. The swimming motility of the helical bacterium *Spiroplasma* is accomplished by the formation of kinks in the cell body that travel the length of the bacterium. A very different type of motility, **gliding motility**, is employed by many bacteria: cyanobacteria, myxobacteria and cytophagas, and some mycoplasmas. Although there are no visible external structures associated with gliding motility, it enables movement along solid surfaces at rates up to 3 $\mu\text{m}/\text{second}$. [Microbial Diversity & Ecology 21.1: The mechanism of gliding motility; Phylum Spirochaetes \(section 21.6\); Photosynthetic bacteria \(section 21.3\); Class Deltaproteobacteria: Order Myxococcales \(section 22.4\); Class Mollicutes \(the Mycoplasmas\) \(section 23.2\)](#)

1. Briefly describe capsules, slime layers, glycocalyxes, and S-layers. What are their functions?
2. Distinguish between fimbriae and sex pili, and give the function of each.
3. Be able to discuss the following: flagella distribution patterns, flagella structure and synthesis, and the way in which flagella operate to move a bacterium.
4. What is self-assembly? Why does it make sense that the flagellar filament is assembled in this way?

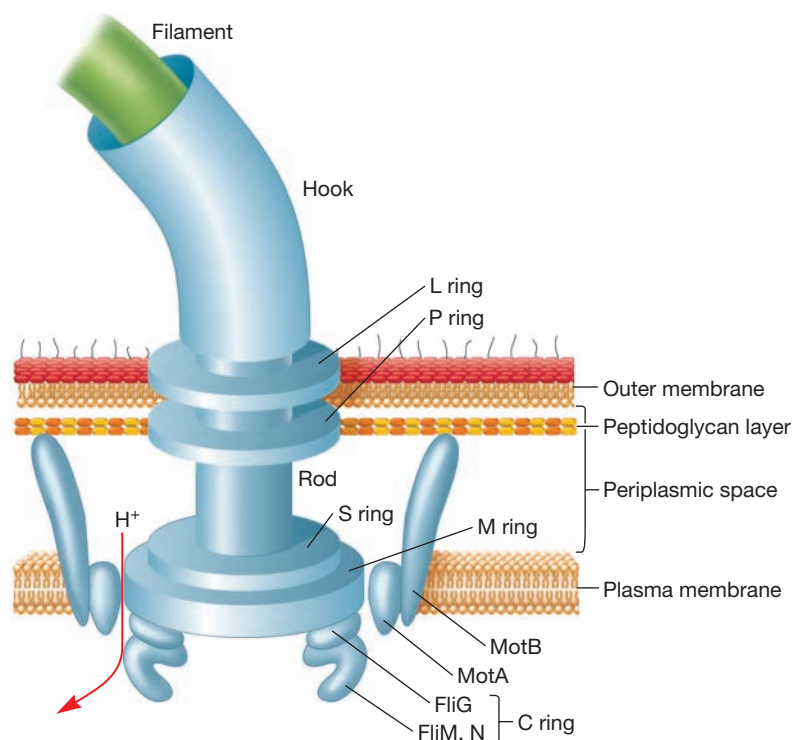


Figure 3.42 Mechanism of Flagellar Movement.

This diagram of a gram-negative flagellum shows some of the more important components and the flow of protons that drives rotation. Five of the many flagellar proteins are labeled (MotA, MotB, FliG, FliM, FliN).

3.10 CHEMOTAXIS

Bacteria do not always move aimlessly but are attracted by such nutrients as sugars and amino acids, and are repelled by many harmful substances and bacterial waste products. Bacteria also can respond to other environmental cues such as temperature (thermotaxis), light (phototaxis), oxygen (aerotaxis), osmotic pressure (osmotaxis), and gravity; (Microbial Diversity & Ecology 3.2.) Movement toward chemical attractants and away from repellents is known as **chemotaxis**. Such behavior is of obvious advantage to bacteria.

Chemotaxis may be demonstrated by observing bacteria in the chemical gradient produced when a thin capillary tube is filled with an attractant and lowered into a bacterial suspension. As the attractant diffuses from the end of the capillary, bacteria collect and swim up the tube. The number of bacteria within the capillary after a short length of time reflects the strength of attraction and rate of chemotaxis. Positive and negative chemotaxis also can be studied with petri dish cultures (**figure 3.43**). If bacteria are placed in the center of a dish of semisolid agar containing an attractant, the bacteria will exhaust the local supply and then swim outward following the attractant gradient they have created. The result is an expanding ring of bacteria. When a disk of repellent is placed in a petri dish of semisolid agar and bacteria, the bacteria will swim away from the repellent, creating a clear zone around the disk (**figure 3.44**).

Bacteria can respond to very low levels of attractants (about 10^{-8} M for some sugars), the magnitude of their response increasing with attractant concentration. Usually they sense repellents only at higher concentrations. If an attractant and a repellent

are present together, the bacterium will compare both signals and respond to the chemical with the most effective concentration.

Attractants and repellents are detected by **chemoreceptors**, special proteins that bind chemicals and transmit signals to the other components of the chemosensing system. About 20 attractant chemoreceptors and 10 chemoreceptors for repellents have been discovered thus far. These chemoreceptor proteins may be located in the periplasmic space or the plasma membrane. Some receptors participate in the initial stages of sugar transport into the cell.

The chemotactic behavior of bacteria has been studied using the tracking microscope, a microscope with a moving stage that automatically keeps an individual bacterium in view. In the absence of a chemical gradient, *E. coli* and other bacteria move randomly. For a few seconds, the bacterium will travel in a straight or slightly curved line called a **run**. When a bacterium is running, its flagella are organized into a coordinated, corkscrew-shaped bundle (**figure 3.41c**). Then the flagella “fly apart” and the bacterium will stop and **tumble**. The tumble results in the random reorientation of the bacterium so that it often is facing in a different direction. Therefore when it begins the next run, it usually goes in a different direction (**figure 3.45a**). In contrast, when the bacterium is exposed to an attractant, it tumbles less frequently (or has longer runs) when traveling towards the attractant. Although the tumbles can still orient the bacterium away from the attractant, over time, the bacterium gets closer and closer to the attractant (**figure 3.45b**). The opposite response occurs with a repellent. Tumbling frequency decreases (the run time lengthens) when the bacterium moves away from the repellent.

Figure 3.43 Positive Bacterial Chemotaxis.

Chemotaxis can be demonstrated on an agar plate that contains various nutrients. Positive chemotaxis by *E. coli* on the left. The outer ring is composed of bacteria consuming serine. The second ring was formed by *E. coli* consuming aspartate, a less powerful attractant. The upper right colony is composed of motile, but nonchemotactic mutants. The bottom right colony is formed by nonmotile bacteria.

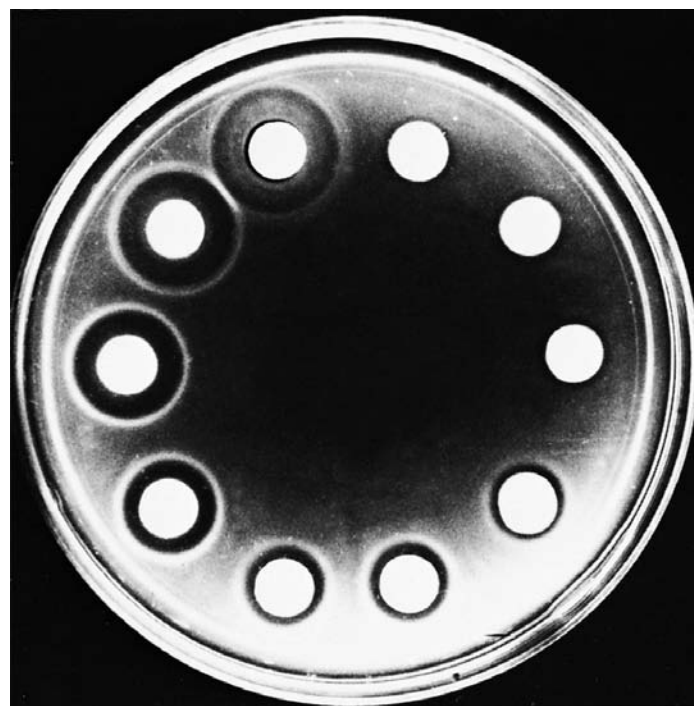
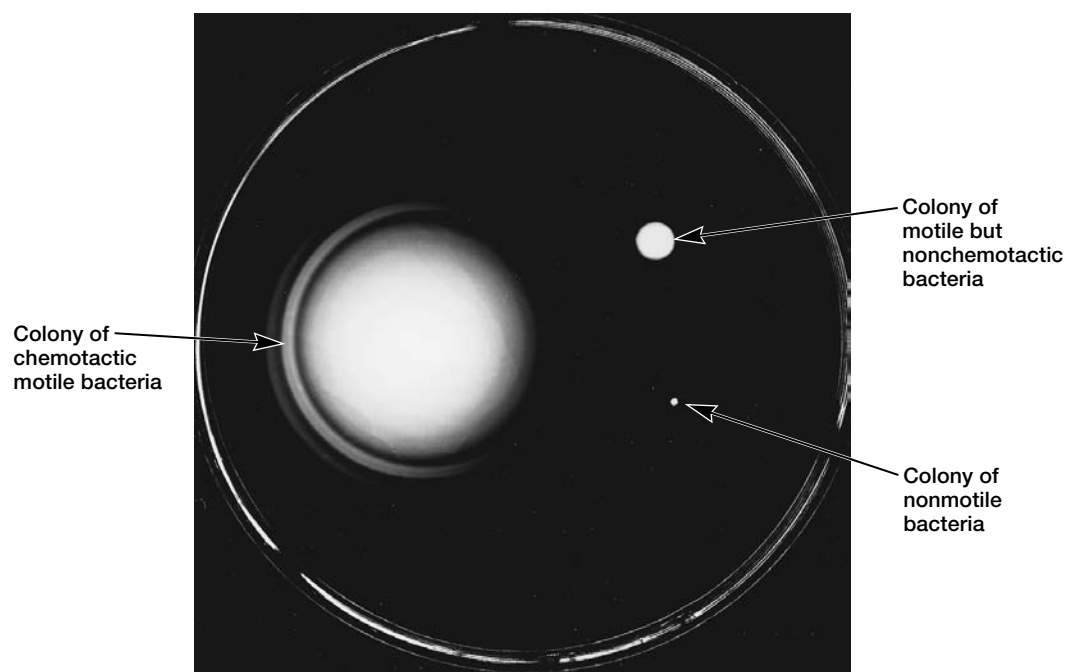
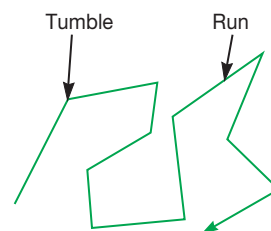
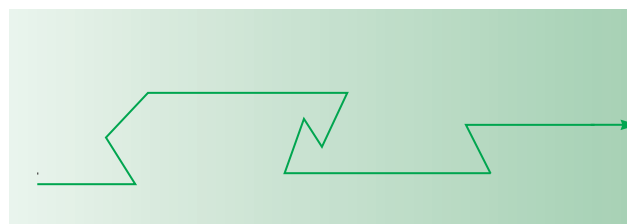


Figure 3.44 Negative Bacterial Chemotaxis. Negative chemotaxis by *E. coli* in response to the repellent acetate. The bright disks are plugs of concentrated agar containing acetate that have been placed in dilute agar inoculated with *E. coli*. Acetate concentration increases from zero at the top right to 3 M at top left. Note the increasing size of bacteria-free zones with increasing acetate. The bacteria have migrated for 30 minutes.



(a)



(b)

Figure 3.45 Directed Movement in Bacteria. (a) Random movement of a bacterium in the absence of a concentration gradient. Tumbling frequency is fairly constant. (b) Movement in an attractant gradient. Tumbling frequency is reduced when the bacterium is moving up the gradient. Therefore, runs in the direction of increasing attractant are longer.

Clearly, the bacterium must have some mechanism for sensing that it is getting closer to the attractant (or is moving away from the repellent). The behavior of the bacterium is shaped by temporal changes in chemical concentration. The bacterium moves toward the attractant because it senses that the concentration of the attractant is increasing. Likewise, it moves away from a repellent because it senses that the concentration of the repellent is decreasing. The bacterium's chemoreceptors play a critical role in this process. The molecular events that enable bacterial cells to sense a chemical gradient and respond appropriately are presented in chapter 8.

1. Define chemotaxis, run, and tumble.
2. Explain in a general way how bacteria move toward substances like nutrients and away from toxic materials.

3.11 THE BACTERIAL ENDOSPORE

A number of gram-positive bacteria can form a special resistant, dormant structure called an **endospore**. Endospores develop within vegetative bacterial cells of several genera: *Bacillus* and *Clostridium* (rods), *Sporosarcina* (cocci), and others. These structures are extraordinarily resistant to environmental stresses such as heat, ultraviolet radiation, gamma radiation, chemical disinfectants, and desiccation. In fact, some endospores have remained viable for around 100,000 years. Because of their resistance and the fact that several species of endospore-forming bacteria are dangerous pathogens, endospores are of great practical importance in food, industrial, and medical microbiology. This is because it is essential to be able to sterilize solutions and solid objects. Endospores often survive boiling for an hour or more; therefore autoclaves must be used to sterilize many materials. Endospores are also of considerable theoretical interest. Because bacteria manufacture these intricate structures in a very organized fashion over a period of a few hours, spore formation is well suited for research on the construction of complex biological structures. In the environment, endospores aid in survival when moisture or nutrients are scarce. [The use of physical methods in control: Heat \(section 7.4\)](#)

Endospores can be examined with both light and electron microscopes. Because endospores are impermeable to most stains, they often are seen as colorless areas in bacteria treated with methylene blue and other simple stains; special endospore stains are used to make them clearly visible. Endospore position in the mother cell (**sporangium**) frequently differs among species, making it of considerable value in identification. Endospores may be centrally located, close to one end (subterminal), or definitely terminal (**figure 3.46**). Sometimes an endospore is so large that it swells the sporangium. [Preparation and staining of specimens \(section 2.3\)](#)

Electron micrographs show that endospore structure is complex (**figure 3.47**). The spore often is surrounded by a thin, delicate covering called the **exosporium**. A **spore coat** lies beneath the exosporium, is composed of several protein layers, and may be fairly thick. It is impermeable to many toxic molecules and is responsible for the spore's resistance to chemicals. The coat also is thought to contain enzymes that are involved in germination.

The **cortex**, which may occupy as much as half the spore volume, rests beneath the spore coat. It is made of a peptidoglycan that is less cross-linked than that in vegetative cells. The **spore cell wall** (or core wall) is inside the cortex and surrounds the protoplast or **spore core**. The core has normal cell structures such as ribosomes and a nucleoid, but is metabolically inactive.

It is still not known precisely why the endospore is so resistant to heat and other lethal agents. As much as 15% of the spore's dry weight consists of dipicolinic acid complexed with calcium ions (**figure 3.48**), which is located in the core. It has long been thought that dipicolinic acid was directly involved in

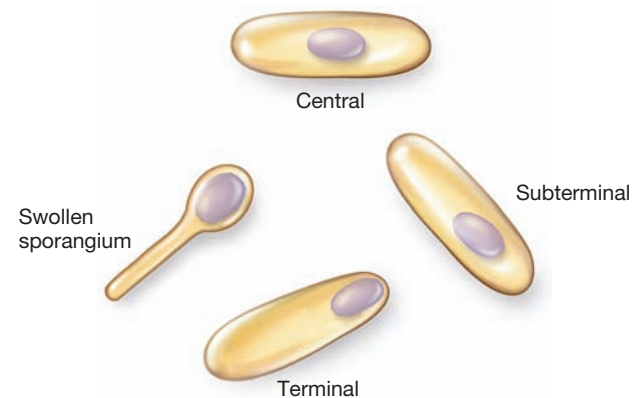


Figure 3.46 Examples of Endospore Location and Size.

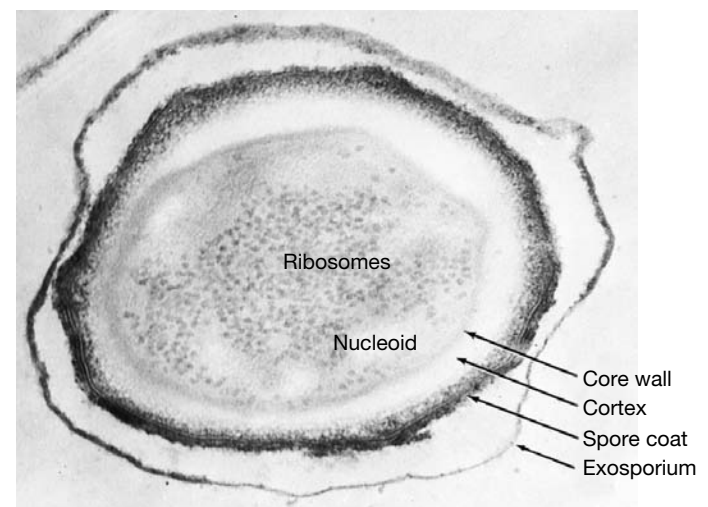


Figure 3.47 Endospore Structure. *Bacillus anthracis* endospore ($\times 151,000$).

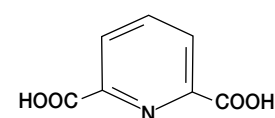


Figure 3.48 Dipicolinic Acid.

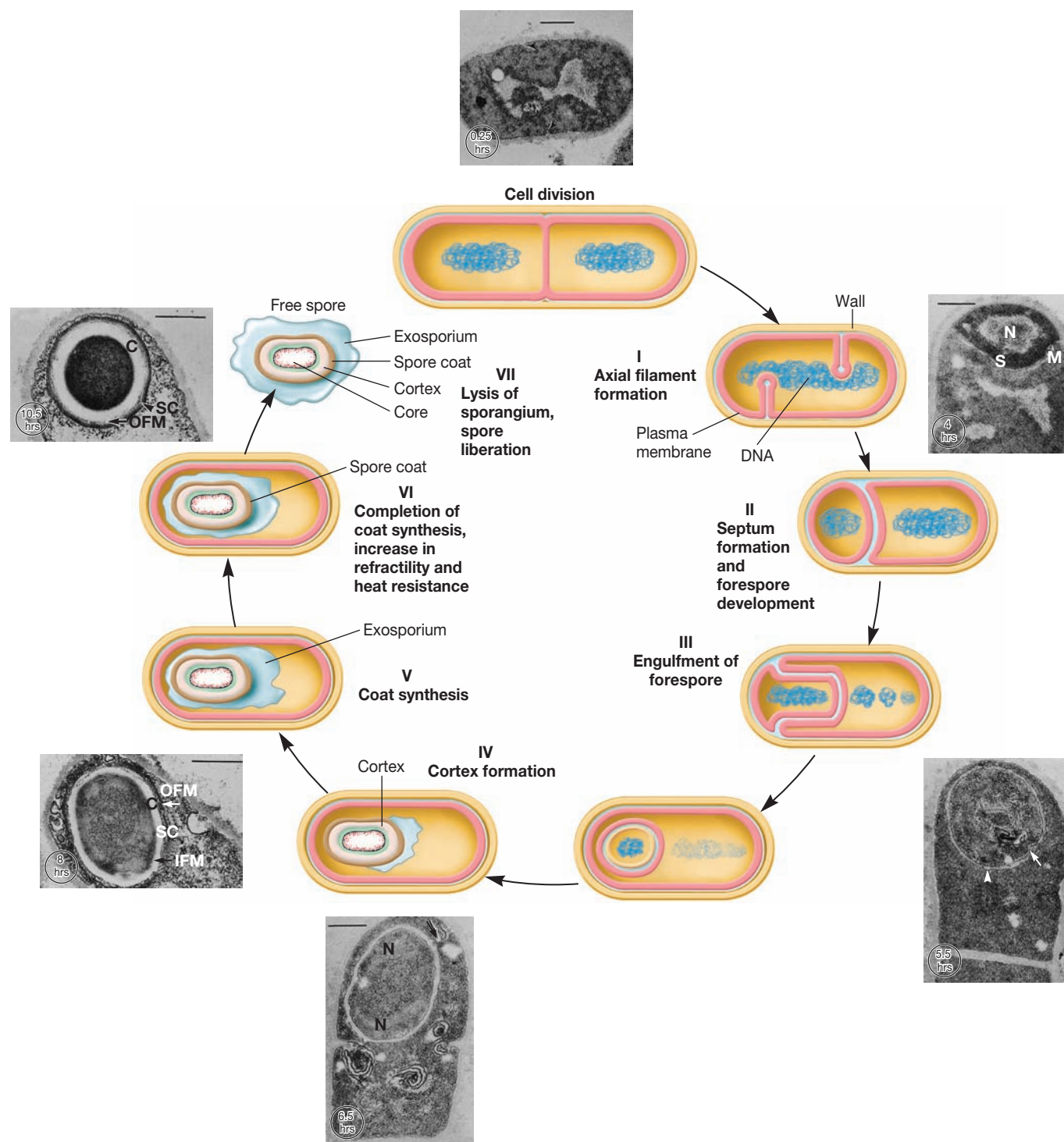


Figure 3.49 Endospore Formation: Life Cycle of *Bacillus megaterium*. The stages are indicated by Roman numerals. The circled numbers in the photographs refer to the hours from the end of the logarithmic phase of growth: 0.25 h—a typical vegetative cell; 4 h—stage II cell, septation; 5.5 h—stage III cell, engulfment; 6.5 h—stage IV cell, cortex formation; 8 h—stage V cell, coat formation; 10.5 h—stage VI cell, mature spore in sporangium. Abbreviations used: C, cortex; IFM and OFM, inner and outer forespore membranes; M, mesosome; N, nucleoid; S, septum; SC, spore coats. Bars = 0.5 μ m.

heat resistance, but heat-resistant mutants lacking dipicolinic acid have been isolated. Calcium does aid in resistance to wet heat, oxidizing agents, and sometimes dry heat. It may be that calcium-dipicolinate stabilizes the spore's nucleic acids. In addition, specialized small, acid-soluble DNA-binding proteins (SASPs), are found in the endospore. They saturate spore DNA and protect it from heat, radiation, dessication, and chemicals. Dehydration of the protoplast appears to be very important in heat resistance. The cortex may osmotically remove water from the protoplast, thereby protecting it from both heat and radiation damage. The spore coat also seems to protect against enzymes and chemicals such as hydrogen peroxide. Finally, spores contain some DNA repair enzymes. DNA is repaired once the spore germinates and the cell becomes active again. In summary, endospore heat resistance probably is due to several factors: calcium-dipicolinate and acid-soluble protein stabilization of DNA, protoplast dehydration, the spore coat, DNA repair, the greater stability of cell proteins in bacteria adapted to growth at high temperatures, and others.

Endospore formation, also called **sporogenesis** or **sporulation**, normally commences when growth ceases due to lack of nutrients. It is a complex process and may be divided into seven stages (**figure 3.49**). An axial filament of nuclear material forms (stage I), followed by an inward folding of the cell membrane to enclose part of the DNA and produce the forespore septum (stage II). The membrane continues to grow and engulfs the immature endospore in a second membrane (stage III). Next, cortex is laid down in the space between the two membranes, and both calcium and dipicolinic acid are accumulated (stage IV). Protein coats then are formed around the cortex (stage V), and maturation of the endospore occurs (stage VI). Finally, lytic enzymes destroy the sporangium releasing the spore (stage VII). Sporulation requires about 10 hours in *Bacillus megaterium*. [Global regulatory systems: Sporulation in *Bacillus subtilis* \(section 12.5\)](#)

The transformation of dormant spores into active vegetative cells seems almost as complex a process as sporogenesis. It occurs in three stages: (1) activation, (2) germination, and (3) outgrowth (**figure 3.50**). Often a spore will not germinate successfully, even in a nutrient-rich medium, unless it has been activated. **Activation**

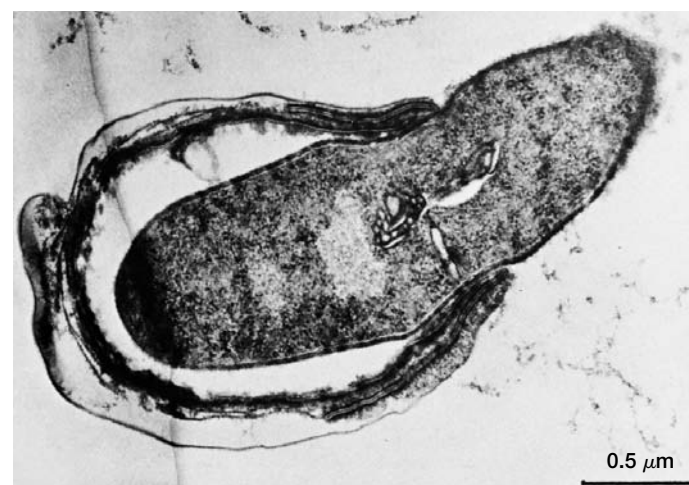


Figure 3.50 Endospore Germination. *Clostridium pectinovorum* emerging from the spore during germination.

is a process that prepares spores for germination and usually results from treatments like heating. It is followed by **germination**, the breaking of the spore's dormant state. This process is characterized by spore swelling, rupture or absorption of the spore coat, loss of resistance to heat and other stresses, loss of refractility, release of spore components, and increase in metabolic activity. Many normal metabolites or nutrients (e.g., amino acids and sugars) can trigger germination after activation. Germination is followed by the third stage, **outgrowth**. The spore protoplast makes new components, emerges from the remains of the spore coat, and develops again into an active bacterium.

1. Describe the structure of the bacterial endospore using a labeled diagram.
2. Briefly describe endospore formation and germination. What is the importance of the endospore? What might account for its heat resistance?
3. How might one go about showing that a bacterium forms true endospores?
4. Why do you think dehydration of the protoplast is an important factor in the ability of endospores to resist environmental stress?

Summary

3.1 An Overview of Prokaryotic Cell Structure

- a. Prokaryotes may be spherical (cocci), rod-shaped (bacilli), spiral, or filamentous; they may form buds and stalks; or they may even have no characteristic shape at all (pleomorphic) (**figure 3.1** and **3.2**).
- b. Prokaryotic cells can remain together after division to form pairs, chains, and clusters of various sizes and shapes.
- c. Prokaryotes are much simpler structurally than eukaryotes, but they do have unique structures. **Table 3.1** summarizes the major functions of prokaryotic cell structures.

3.2 Prokaryotic Cell Membranes

- a. The plasma membrane fulfills many roles, including acting as a semipermeable barrier, carrying out respiration and photosynthesis, and detecting and responding to chemicals in the environment.
- b. The fluid mosaic model proposes that cell membranes are lipid bilayers in which integral proteins are buried (**figure 3.5**). Peripheral proteins are loosely associated with the membrane.
- c. Bacterial membranes are composed of phospholipids constructed of fatty acids connected to glycerol by ester linkages (**figure 3.6**). Bacterial membranes usually lack sterols, but often contain hopanoids (**figure 3.7**).

76 Chapter 3 Prokaryotic Cell Structure and Function

- d. The plasma membrane of some bacteria invaginates to form simple membrane systems containing photosynthetic and respiratory assemblies. Other bacteria, like the cyanobacteria, have internal membranes (**figure 3.8**).
- e. Archaeal membranes are composed of glycerol diether and diglycerol tetraether lipids (**figure 3.9**). Membranes composed of glycerol diether are lipid bilayers. Membranes composed of diglycerol tetraethers are lipid monolayers (**figure 3.11**). The overall structure of a monolayer membrane is similar to that of the bilayer membrane in that the membrane has a hydrophobic core and its surfaces are hydrophilic.

3.3 The Cytoplasmic Matrix

- a. The cytoplasm of prokaryotes contains proteins that are similar in structure and function to the cytoskeletal proteins observed in eukaryotes.
- b. The cytoplasmic matrix of prokaryotes contains inclusion bodies. Most are used for storage (glycogen inclusions, PHB inclusions, cyanophycin granules, carboxysomes, and polyphosphate granules), but others are used for other purposes (magnetosomes and gas vacuoles).
- c. The cytoplasm of prokaryotes is packed with 70S ribosomes (**figure 3.15**).

3.4 The Nucleoid

- a. Prokaryotic genetic material is located in an area called the nucleoid and is not usually enclosed by a membrane (**figure 3.16**).
- b. In most prokaryotes, the nucleoid contains a single chromosome. The chromosome consists of a double-stranded, covalently closed, circular DNA molecule.

3.5 Plasmids

- a. Plasmids are extrachromosomal DNA molecules. They are found in many prokaryotes.
- b. Although plasmids are not required for survival in most conditions, they can encode traits that confer selective advantage in some environments.
- c. Episomes are plasmids that are able to exist freely in the cytoplasm or can be integrated into the chromosome.
- d. Conjugative plasmids encode genes that promote their transfer from one cell to another.
- e. Resistance factors are plasmids that have genes conferring resistance to antibiotics.
- f. Col plasmids contain genes for the synthesis of colicins, proteins that kill *E. coli*. Other plasmids encode virulence factors or metabolic capabilities.

3.6 The Bacterial Cell Wall

- a. The vast majority of prokaryotes have a cell wall outside the plasma membrane to give them shape and protect them from osmotic stress.
- b. Bacterial walls are chemically complex and usually contain peptidoglycan (**figures 3.17–3.21**).
- c. Bacteria often are classified as either gram-positive or gram-negative based on differences in cell wall structure and their response to Gram staining.
- d. Gram-positive walls have thick, homogeneous layers of peptidoglycan and teichoic acids (**figure 3.23**). Gram-negative bacteria have a thin peptidoglycan layer surrounded by a complex outer membrane containing lipopolysaccharides (LPSs) and other components (**figure 3.25**).
- e. The mechanism of the Gram stain is thought to depend on the peptidoglycan, which binds crystal violet tightly, preventing the loss of crystal violet during the ethanol wash.

3.7 Archaeal Cell Walls

- a. Archaeal cell walls do not contain peptidoglycan (**figure 3.30**).
- b. Archaea exhibit great diversity in their cell wall make-up. Some archaeal cell walls are composed of heteropolysaccharides, some are composed of glycoprotein, and some are composed of protein.

3.8 Protein Secretion in Prokaryotes

- a. The Sec-dependent protein secretion pathway (**figure 3.32**) has been observed in all domains of life. It transports proteins across or into the cytoplasmic membrane.
- b. Gram-negative bacteria have additional protein secretion systems that allow them to move proteins from the cytoplasm, across both the cytoplasmic and outer membranes, to the outside of the cell (**figure 3.33**). Some of these systems work with the Sec-dependent pathway to accomplish this (Type II, Type V, and usually Type IV). Some pathways function alone to move proteins across both membranes (Types I and III).
- c. ABC transporters (Type I protein secretion system) are used by all prokaryotes for protein translocation.

3.9 Components External to the Cell Wall

- a. Capsules, slime layers, and glycocalyxes are layers of material lying outside the cell wall. They can protect prokaryotes from certain environmental conditions, allow prokaryotes to attach to surfaces, and protect pathogenic bacteria from host defenses (**figures 3.34 and 3.35**).
- b. S-layers are observed in some bacteria and many archaea. They are composed of proteins or glycoprotein and have a characteristic geometric shape. In many archaea the S-layer serves as the cell wall (**figure 3.36**).
- c. Pili and fimbriae are hairlike appendages. Fimbriae function primarily in attachment to surfaces, but some types of bacterial fimbriae are involved in a twitching motility. Sex pili participate in the transfer of DNA from one bacterium to another (**figure 3.37**).
- d. Many prokaryotes are motile, usually by means of threadlike, locomotory organelles called flagella (**figure 3.38**).
- e. Bacterial species differ in the number and distribution of their flagella.
- f. In bacteria, the flagellar filament is a rigid helix that rotates like a propeller to push the bacterium through water (**figure 3.41**).

3.10 Chemotaxis

- a. Motile prokaryotes can respond to gradients of attractants and repellents, a phenomenon known as chemotaxis.
- b. A bacterium accomplishes movement toward an attractant by increasing the length of time it spends moving toward the attractant, shortening the time it spends tumbling. Conversely, a bacterium increases its run time when it moves away from a repellent.

3.11 The Bacterial Endospore

- a. Some bacteria survive adverse environmental conditions by forming endospores, dormant structures resistant to heat, desiccation, and many chemicals (**figure 3.47**).
- b. Both endospore formation and germination are complex processes that begin in response to certain environmental signals and involve numerous stages (**figures 3.49 and 3.50**).

Key Terms

ABC protein secretion pathway 65	fimbriae 66	mycelium 40	run 71
activation 75	flagellar filament 67	nucleoid 52	Sec-dependent pathway 63
amphipathic 45	flagellar hook 67	O antigen 60	self-assembly 68
amphitrichous 67	flagellin 67	O side chain 60	sex pili 67
axial filament 70	flagellum 67	outer membrane 55	signal peptide 63
bacillus 40	fluid mosaic model 44	outgrowth 75	S-layer 66
bacteriocin 53	gas vacuole 50	penicillin 61	slime layer 65
basal body 67	gas vesicles 50	peptide interbridge 56	spheroplast 61
capsule 65	germination 75	peptidoglycan 55	spirilla 40
carboxysomes 49	gliding motility 70	peripheral proteins 45	spirochete 40
cell envelope 55	glycocalyx 65	periplasm 55	sporangium 73
chemoreceptors 71	glycogen 49	periplasmic space 55	spore cell wall 73
chemotaxis 71	hopanoids 46	peritrichous 67	spore coat 73
coccus 39	hydrophilic 45	plasma membrane 42	spore core 73
Col plasmid 53	hydrophobic 45	plasmid 53	sporogenesis 75
conjugative plasmid 53	inclusion body 48	plasmolysis 61	sporulation 75
core polysaccharide 58	integral proteins 45	pleomorphic 41	Svedberg unit 50
cortex 73	lipid A 58	polar flagellum 67	teichoic acid 57
curing 53	lipopolysaccharides (LPSs) 58	poly- β -hydroxybutyrate (PHB) 49	tumble 71
cyanophycin granules 49	lophotrichous 67	polyphosphate granules 50	type I protein secretion pathway 65
cytoplasmic matrix 48	lysis 61	porin proteins 60	type II protein secretion pathway 65
deoxyribonucleic acid (DNA) 52	lysozyme 61	protoplast 48	type III protein secretion pathway 65
diplococcus 39	magnetosomes 50	pseudomurein 62	type IV protein secretion pathway 65
endospore 73	metabolic plasmid 54	resistance factor (R factor, R plasmid) 53	type V protein secretion pathway 65
episome 53	metachromatic granules 50	ribosome 50	vibrio 40
exoenzyme 58	monotrichous 67	rod 40	virulence plasmid 54
exosporium 73	murein 55		volutin granules 50
F factor 53			

Critical Thinking Questions

1. Propose a model for the assembly of a flagellum in a gram-positive cell envelope. How would that model need to be modified for the assembly of a flagellum in a gram-negative cell envelope?
2. If you could not use a microscope, how would you determine whether a cell is procaryotic or eucaryotic? Assume the organism can be cultured easily in the laboratory.
3. The peptidoglycan of bacteria has been compared with the chain mail worn beneath a medieval knight's suit of armor. It provides both protection and flexibility. Can you describe other structures in biology that have an analogous function? How are they replaced or modified to accommodate the growth of the inhabitant?

Learn More

- Cannon, G. C.; Bradburne, C. E.; Aldrich, H. C.; Baker, S. H.; Heinhorst, S.; and Shively, J. M. 2001. Microcompartments in prokaryotes: Carboxysomes and related polyhedra. *Appl. Env. Microbiol.* 67(12):5351–61.
- Drews, G. 1992. Intracytoplasmic membranes in bacterial cells: Organization, function and biosynthesis. In *Prokaryotic structure and function*, S. Mohan, C. Dow, and J. A. Coles, editors, 249–74. New York: Cambridge University Press.
- Frankel, R. B., and Bazylynski, D. A. 2004. Magnetosome mysteries. *ASM News* 70(4):176–83.
- Ghuysen, J.-M., and Hekenbeck, R., editors. 1994. *Bacterial cell wall*. New York: Elsevier.
- Gital, Z. 2005. The new bacterial cell biology: Moving parts and subcellular architecture. *Cell* 120:577–86.

- Harshey, R. M. 2003. Bacterial motility on a surface: Many ways to a common goal. *Annu. Rev. Microbiol.* 57:249–73.
- Henderson, I. R.; Navarro-Garcia, F.; Desvaux, M.; Fernandez, R. C.; and Ala'Aldeen, D. 2004. Type V protein secretion pathway: The autotransporter story. *Microbiol. Mol. Biol. Rev.* 68(4):692–744.
- Hoppert, M., and Mayer, F. 1999. Prokaryotes. *American Scientist* 87:518–25.
- Kerfeld, C. A.; Sawaya, M. R.; Tanaka, A.; Nguyen, C. V.; Phillips, M.; Beeby, M.; and Yeates, T. O. 2005. Protein structures forming the shell of primitive bacterial organelles. *Science* 309:936–38.
- Kostakioti, M.; Newman, C. L.; Thanassi, D. G.; and Stathopoulos, C. 2005. Mechanisms of protein export across the bacterial outer membrane. *J. Bacteriol.* 187(13):4306–14.
- Macnab, R. M. 2003. How bacteria assemble flagella. *Annu. Rev. Microbiol.* 57:77–100.
- Mattick, J. S. 2002. Type IV pili and twitching motility. *Annu. Rev. Microbiol.* 56:289–314.
- Nicholson, W. L.; Munakata, N.; Horneck, G.; Melosh, H. J.; and Setlow, P. 2000. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol. Mol. Biol. Rev.* 64(3):548–72.
- Nikaido, H. 2003. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.* 67(4):593–656.
- Parkinson, J. S. 2004. Signal amplification in bacterial chemotaxis through receptor teamwork. *ASM News* 70(12):575–82.
- Robinow, C., and Kellenberger, E. 1994. The bacterial nucleoid revisited. *Microbiol. Rev.* 58(2):211–32.
- Sára, M., and Sleytr, U. B. 2000. S-layer proteins. *J. Bacteriol.* 182(4):859–68.
- Scherrer, R. 1984. Gram's staining reaction, Gram types and cell walls of bacteria. *Trends Biochem. Sci.* 9:242–45.
- Schulz, H. N., and Jorgensen, B. B. 2001. Big bacteria. *Annu. Rev. Microbiol.* 55:105–37.
- Trun, N. J., and Marko, J. F. 1998. Architecture of a bacterial chromosome. *ASM News* 64(5):276–83.
- Walsby, A. E. 1994. Gas vesicles. *Microbiol. Rev.* 58(1):94–144.
- Walsby, A. E. 2005. Archaea with square cells. *Trends Microbiol.* 13(5):193–95.
- Wätermann, M., and Steinbüchel, A. 2005. Neutral lipid bodies in prokaryotes: Recent insights into structure, formation, and relationship to eukaryotic lipid depots. *J. Bacteriol.* 187(11):3607–19.

**Please visit the Prescott website at www.mhhe.com/prescott7
for additional references.**
