3

Cell Structure and Function in *Bacteria* and *Archaea*

Bacteria are keenly attuned to their environment and respond by directing their movements toward or away from chemical and physical stimuli.

I Cell Shape and Size

In this chapter we examine key structures of the prokaryotic cell: the cytoplasmic membrane, the cell wall, cell surface structures and inclusions, and mechanisms of motility. Our overarching theme will be structure and function. We begin this chapter by considering two key features of prokaryotic cells—their shape and small size. Prokaryotes typically have defined shapes and are extremely small cells. Shape is useful for differentiating cells of the *Bacteria* and the *Archaea* and size has profound effects on their biology.

3.1 Cell Morphology

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

Major Cell Morphologies

Examples of bacterial morphologies are shown in **Figure 3.1**. A bacterium that is spherical or ovoid in morphology is called a *coccus* (plural, cocci). A bacterium with a cylindrical shape is called a *rod* or a *bacillus*. Some rods twist into spiral shapes and are called *spirilla*. The cells of many prokaryotic species remain together in groups or clusters after cell division, and the arrangements are often characteristic of certain genera. For instance, some cocci form long chains (for example, the bacterium *Streptococcus*), others occur in three-dimensional cubes (*Sarcina*), and still others in grapelike clusters (*Staphylococcus*).

Several groups of bacteria are immediately recognizable by the unusual shapes of their individual cells. Examples include spirochetes, which are tightly coiled bacteria; appendaged bacteria, which possess extensions of their cells as long tubes or stalks; and filamentous bacteria, which form long, thin cells or chains of cells (Figure 3.1).

The cell morphologies shown here should be viewed with the understanding that they are *representative* shapes; many variations of these key morphologies are known. For example, there are fat rods, thin rods, short rods, and long rods, a rod simply being a cell that is longer in one dimension than in the other. As we will see, there are even square bacteria and star-shaped bacteria! Cell morphologies thus form a continuum, with some shapes, such as rods, being very common and others more unusual.

Morphology and Biology

Although cell morphology is easily recognized, it is in general a poor predictor of other properties of a cell. For example, under the microscope many rod-shaped *Archaea* look identical to rod-shaped *Bacteria*, yet we know they are of different phylogenetic

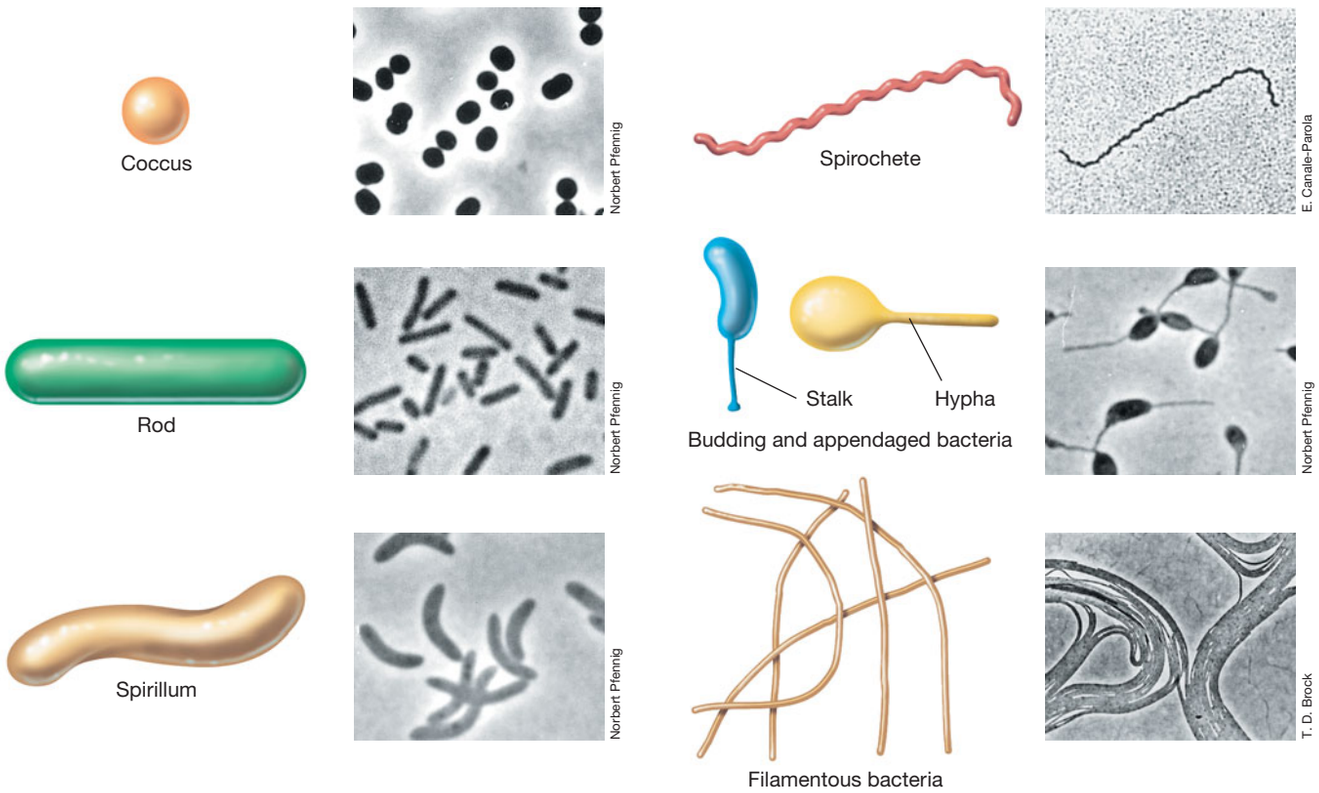

Figure 3.1 Representative cell morphologies of prokaryotes. Next to each drawing is a phase-contrast photomicrograph showing an example of that morphology. Organisms are coccus, *Thiocapsa roseopersicina* (diameter of a single cell = 1.5 μm); rod, *Desulfuromonas acetoxidans* (diameter = 1 μm); spirillum, *Rhodospirillum rubrum* (diameter = 1 μm); spirochete, *Spirochaeta stenostrepta* (diameter = 0.25 μm); budding and appendaged, *Rhodomicrobium vannielii* (diameter = 1.2 μm); filamentous, *Chloroflexus aurantiacus* (diameter = 0.8 μm).

domains (↻ Section 2.7). Thus, with very rare exceptions, it is impossible to predict the physiology, ecology, phylogeny, or virtually any other property of a prokaryotic cell, by simply knowing its morphology.

What sets the morphology of a particular species? Although we know something about *how* cell shape is controlled, we know little about *why* a particular cell evolved the morphology it has. Several selective forces are likely to be in play in setting the morphology of a given species. These include optimization for nutrient uptake (small cells and those with high surface-to-volume ratios), swimming motility in viscous environments or near surfaces (helical or spiral-shaped cells), gliding motility (filamentous bacteria), and so on. Thus morphology is not a trivial feature of a microbial cell. A cell's morphology is a genetically directed characteristic and has evolved to maximize fitness for the species in a particular habitat.

MiniQuiz

- How do cocci and rods differ in morphology?
- Is cell morphology a good predictor of other properties of the cell?

3.2 Cell Size and the Significance of Smallness

Prokaryotes vary in size from cells as small as about 0.2 μm in diameter to those more than 700 μm in diameter (**Table 3.1**). The vast majority of rod-shaped prokaryotes that have been cultured in the laboratory are between 0.5 and 4 μm wide and less than 15 μm long, but a few very large prokaryotes, such as *Epulopiscium fishelsoni*, are huge, with cells longer than 600 μm (0.6 millimeter) (**Figure 3.2**). This bacterium, phylogenetically related to the endospore-forming bacterium *Clostridium* and found in the gut of the surgeonfish, is interesting not only because it is so large, but also because it has an unusual form of cell division and contains multiple copies of its genome. Multiple offspring are formed and are then released from the *Epulopiscium* "mother cell." A mother cell of *Epulopiscium* contains several thousand genome copies, each of which is about the same size as the genome of *Escherichia coli* (4.6 million base pairs). The many copies are apparently necessary because the cell volume of *Epulopiscium* is so large (Table 3.1) that a single copy of its genome would not be sufficient to support the transcriptional and translational needs of the cell.

Cells of the largest known prokaryote, the sulfur chemolithotroph *Thiomargarita* (Figure 3.2*b*), can be 750 μm in diameter, nearly visible to the naked eye. Why these cells are so large is not well understood, although for sulfur bacteria a large cell size may be a mechanism for storing sulfur (an energy source). It is hypothesized that problems with nutrient uptake ultimately dictate the upper limits for the size of prokaryotic cells. Since the metabolic rate of a cell varies inversely with the square of its size, for very large cells nutrient uptake eventually limits metabolism to the point that the cell is no longer competitive with smaller cells.

Very large cells are not common in the prokaryotic world. In contrast to *Thiomargarita* or *Epulopiscium* (Figure 3.2), the dimensions of an average rod-shaped prokaryote, the bacterium *E. coli*, for example, are about 1 × 2 μm; these dimensions are typical of most prokaryotes. For comparison, average eukaryotic cells can be 10 to more than 200 μm in diameter. In general, then, it can be said that prokaryotes are very small cells compared with eukaryotes.

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(a)

Heidi Schulz

(b)

Figure 3.2 Some very large prokaryotes. *(a)* Dark-field photomicrograph of a giant prokaryote, *Epulopiscium fishelsoni*. The rod-shaped cell in this field is about 600 μm (0.6 mm) long and 75 μm wide and is shown with four cells of the protist (eukaryote) *Paramecium,* each of which is about 150 μm long. *E. fishelsoni* is a species of *Bacteria*, phylogenetically related to *Clostridium*. *(b) Thiomargarita namibiensis,* a large sulfur chemolithotroph (phylum *Proteobacteria* of the *Bacteria*) and currently the largest known prokaryote. Cell widths vary from 400 to 750 μm.

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

There are significant advantages to being small. Small cells have more surface area relative to cell volume than do large cells; that is, they have a higher *surface-to-volume ratio*. Consider a spherical coccus. The volume of such a cell is a function of the cube of

Table 3.1 *Cell size and volume of some prokaryotic cells, from the largest to the smallest*

Organism	*Characteristics*	*Morphology*	*Size[a] (μm)*	*Cell volume (μm³)*	E. coli *volumes*
Thiomargarita namibiensis	Sulfur chemolithotroph	Cocci in chains	750	200,000,000	100,000,000
Epulopiscium fishelsoni[a]	Chemoorganotroph	Rods with tapered ends	80 × 600	3,000,000	1,500,000
Beggiatoa species[a]	Sulfur chemolithotroph	Filaments	50 × 160	1,000,000	500,000
Achromatium oxaliferum	Sulfur chemolithotroph	Cocci	35 × 95	80,000	40,000
Lyngbya majuscula	Cyanobacterium	Filaments	8 × 80	40,000	20,000
Thiovulum majus	Sulfur chemolithotroph	Cocci	18	3,000	1500
Staphylothermus marinus[a]	Hyperthermophile	Cocci in irregular clusters	15	1,800	900
Magnetobacterium bavaricum	Magnetotactic bacterium	Rods	2 × 10	30	15
Escherichia coli	Chemoorganotroph	Rods	1 × 2	2	1
Pelagibacter ubique[a]	Marine chemoorganotroph	Rods	0.2 × 0.5	0.014	0.007
Mycoplasma pneumoniae	Pathogenic bacterium	Pleomorphic[b]	0.2	0.005	0.0025

[a]Where only one number is given, this is the diameter of spherical cells. The values given are for the largest cell size observed in each species. For example, for *T. namibiensis*, an average cell is only about 200 μm in diameter. But on occasion, giant cells of 750 μm are observed. Likewise, an average cell of *S. marinus* is about 1 μm in diameter. The species of *Beggiatoa* here is unclear and *E. fishelsoni* and *P. ubique* are not formally recognized names in taxonomy.
[b]*Mycoplasma* is a cell wall–less bacterium and can take on many shapes (*pleomorphic* means "many shapes").
Source: Data obtained from Schulz, H.N., and B.B. Jørgensen. 2001. *Ann. Rev. Microbiol. 55*: 105–137.

1. nutrient exchange 2. Growth rate
3. Genetic diversity

its radius ($V = \frac{4}{3}\pi r^3$), while its surface area is a function of the square of the radius ($S = 4\pi r^2$). Therefore, the S/V ratio of a spherical coccus is $3/r$ (**Figure 3.3**). As a cell increases in size, its S/V ratio decreases. To illustrate this, consider the S/V ratio for some of the cells of different sizes listed in Table 3.1: *Pelagibacter ubique*, 22; *E. coli*, 4.5; and *E. fishelsoni*, 0.05.

The S/V ratio of a cell affects several aspects of its biology, including its evolution. For instance, because a cell's growth rate depends, among other things, on the rate of nutrient exchange, the higher S/V ratio of smaller cells supports a faster rate of nutrient exchange per unit of cell volume compared with that of larger cells. Because of this, smaller cells, in general, grow faster than larger cells, and a given amount of resources (the nutrients available to support growth) will support a larger population of small cells than of large cells. How can this affect evolution?

Each time a cell divides, its chromosome replicates. As DNA is replicated, occasional errors, called *mutations*, occur. Because mutation rates appear to be roughly the same in all cells, large or small, the more chromosome replications that occur, the greater the total number of mutations in the population. Mutations are the "raw material" of evolution; the larger the pool of mutations, the greater the evolutionary possibilities. Thus, because prokaryotic cells are quite small and are also genetically haploid (allowing mutations to be expressed immediately), they have, in general, the capacity for more rapid growth and evolution than larger, genetically diploid cells. In the latter, not only is the S/V ratio smaller but the effects of a mutation in one gene can be masked by a second, unmutated gene copy. These fundamental differences in size and genetics between prokaryotic and eukaryotic cells underlie the fact that prokaryotes can adapt quite rapidly to changing environmental conditions and can more easily exploit new habitats than can eukaryotic cells. We will see this concept in action in later chapters when we consider, for example, the enormous metabolic diversity of prokaryotes, or the spread of antibiotic resistance.

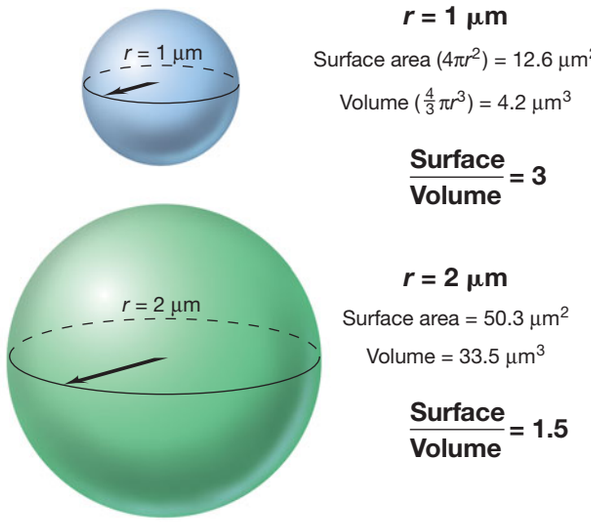


Figure 3.3 **Surface area and volume relationships in cells.** As a cell increases in size, its S/V ratio decreases.

Lower Limits of Cell Size

From the foregoing discussion one might predict that smaller and smaller bacteria would have greater and greater selective advantages in nature. However, this is not true, as there are lower limits to cell size. If one considers the volume needed to house the essential components of a free-living cell—proteins, nucleic acids, ribosomes, and so on—a structure of 0.1 μm in diameter or less is simply insufficient to do the job, and structures 0.15 μm

in diameter are marginal. Thus, structures occasionally observed in nature of 0.1 μm or smaller that "look" like bacterial cells are almost certainly not so. Despite this, many very small prokaryotic cells are known and many have been grown in the laboratory. The open oceans, for example, contain 10^4–10^5 prokaryotic cells per milliliter, and these tend to be very small cells, 0.2–0.4 μm in diameter. We will see later that many pathogenic bacteria are also very small. When the genomes of these pathogens are examined, they are found to be highly streamlined and missing many genes whose functions are supplied to them by their hosts.

MiniQuiz

- What physical property of cells increases as cells become smaller?
- How can the small size and haploid genetics of prokaryotes accelerate their evolution?

II The Cytoplasmic Membrane and Transport

We now consider the structure and function of a critical cell component, the cytoplasmic membrane. The cytoplasmic membrane plays many roles, chief among them as the "gatekeeper" for substances that enter and exit the cell.

3.3 The Cytoplasmic Membrane

The **cytoplasmic membrane** is a thin barrier that surrounds the cell and separates the cytoplasm from the cell's environment. If the membrane is broken, the integrity of the cell is destroyed, the cytoplasm leaks into the environment, and the cell dies. We will see that the cytoplasmic membrane confers little protection from osmotic lysis but is ideal as a selective permeability barrier.

Composition of Membranes

The general structure of the cytoplasmic membrane is a phospholipid bilayer. Phospholipids contain both hydrophobic (fatty acid) and hydrophilic (glycerol–phosphate) components and can be of many different chemical forms as a result of variation in the groups attached to the glycerol backbone (**Figure 3.4**) As phospholipids aggregate in an aqueous solution, they naturally form bilayer structures. In a phospholipid membrane, the fatty acids point inward toward each other to form a hydrophobic environment, and the hydrophilic portions remain exposed to the external environment or the cytoplasm (Figure 3.4*b*).

The cell's cytoplasmic membrane, which is 6–8 nanometers wide, can be seen with the electron microscope, where it appears as two dark-colored lines separated by a lighter area (Figure 3.4*c*). This *unit membrane*, as it is called (because each phospholipid leaf forms half of the "unit"), consists of a phospholipid bilayer with proteins embedded in it (**Figure 3.5**). Although in a diagram the cytoplasmic membrane may appear rather rigid, in reality it is somewhat fluid, having a consistency approximating that of a low-viscosity oil. Some freedom of movement of proteins within the membrane is possible, although it remains unclear exactly

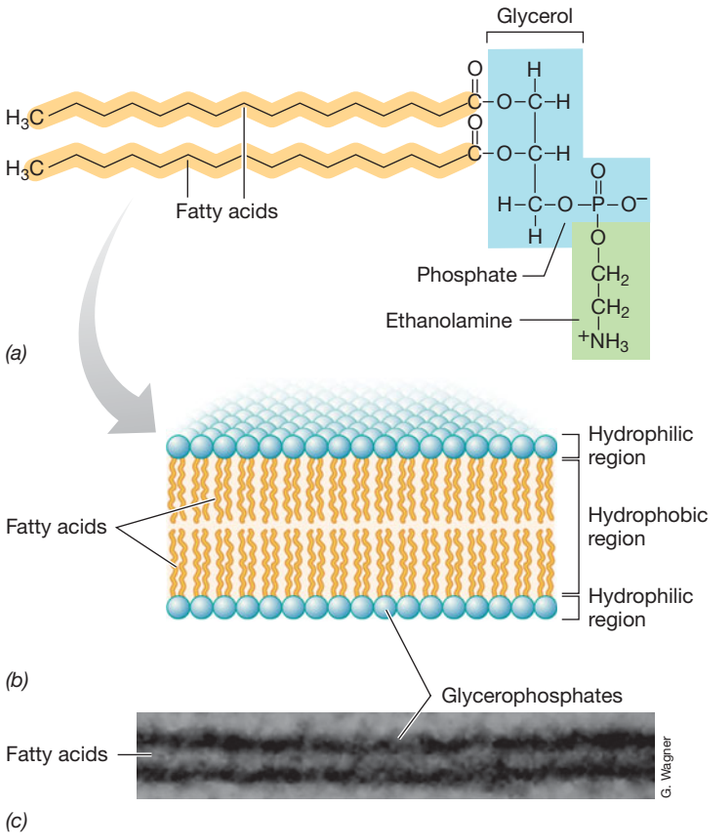


Figure 3.4 Phospholipid bilayer membrane. *(a)* Structure of the phospholipid phosphatidylethanolamine. *(b)* General architecture of a bilayer membrane; the blue balls depict glycerol with phosphate and (or) other hydrophilic groups. *(c)* Transmission electron micrograph of a membrane. The light inner area is the hydrophobic region of the model membrane shown in part b.

how extensive this is. The cytoplasmic membranes of some *Bacteria* are strengthened by molecules called *hopanoids*. These somewhat rigid planar molecules are structural analogs of sterols, compounds that strengthen the membranes of eukaryotic cells, many of which lack a cell wall.

Membrane Proteins

The major proteins of the cytoplasmic membrane have hydrophobic surfaces in their regions that span the membrane and hydrophilic surfaces in their regions that contact the environment and the cytoplasm (Figures 3.4 and 3.5). The *outer* surface of the cytoplasmic membrane faces the environment and in gram-negative bacteria interacts with a variety of proteins that bind substrates or process large molecules for transport into the cell (periplasmic proteins, see Section 3.7). The *inner* side of the cytoplasmic membrane faces the cytoplasm and interacts with proteins involved in energy-yielding reactions and other important cellular functions.

Many membrane proteins are firmly embedded in the membrane and are called *integral* membrane proteins. Other proteins have one portion anchored in the membrane and extramembrane regions that point into or out of the cell (Figure 3.5). Still

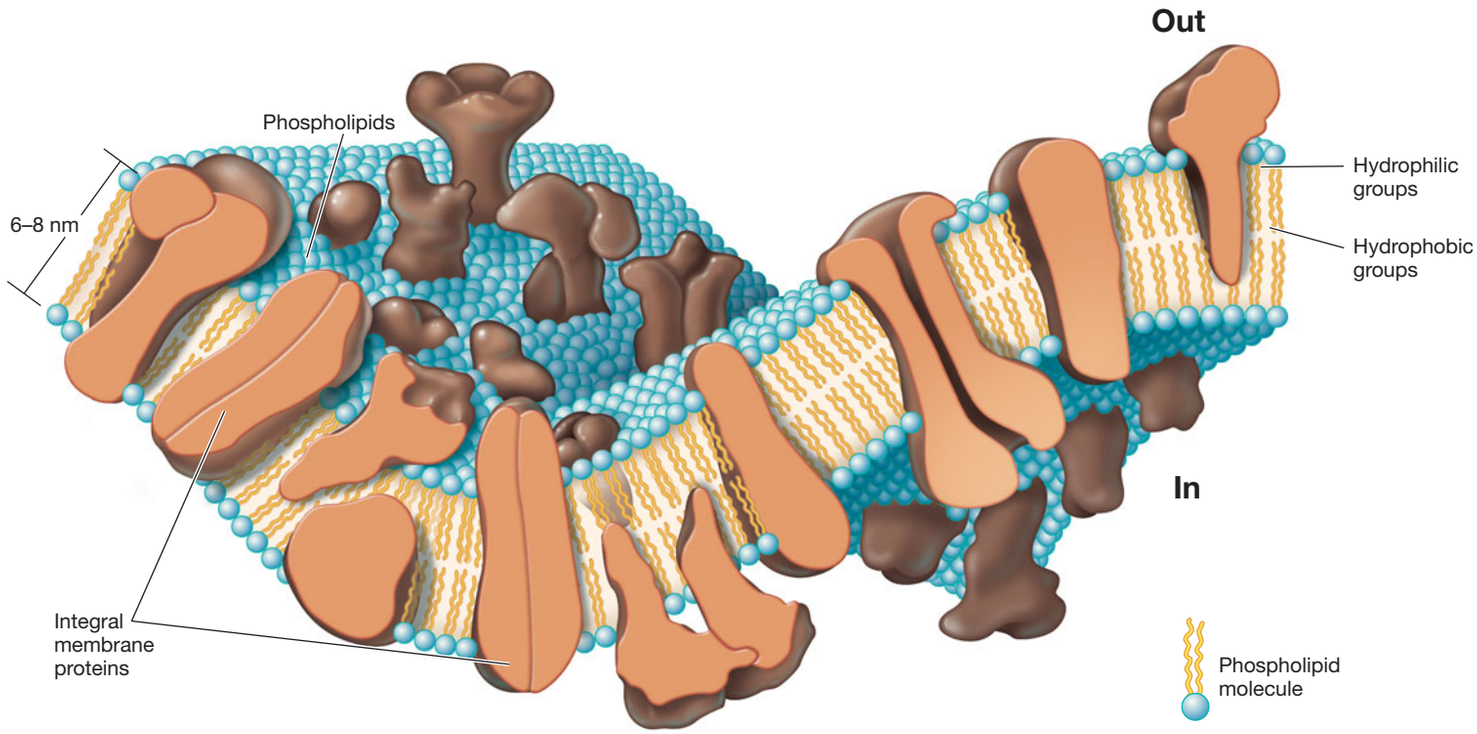


Figure 3.5 **Structure of the cytoplasmic membrane.** The inner surface (**In**) faces the cytoplasm and the outer surface (**Out**) faces the environment. Phospholipids compose the matrix of the cytoplasmic membrane with proteins embedded or surface associated. Although there are some chemical differences, the overall structure of the cytoplasmic membrane shown is similar in both prokaryotes and eukaryotes (but an exception to the bilayer design is shown in Figure 3.7*e*).

other proteins, called *peripheral* membrane proteins, are not membrane-embedded but nevertheless remain firmly associated with membrane surfaces. Some of these peripheral membrane proteins are lipoproteins, molecules that contain a lipid tail that anchors the protein into the membrane. Peripheral membrane proteins typically interact with integral membrane proteins in important cellular processes such as energy metabolism and transport.

Proteins in the cytoplasmic membrane are arranged in clusters (Figure 3.5), a strategy that allows proteins that need to interact to be adjacent to one another. The overall protein content of the membrane is quite high, and it is thought that the variation in lipid bilayer thickness (6–8 nm) is necessary to accommodate thicker and thinner patches of membrane proteins.

Archaeal Membranes

In contrast to the lipids of *Bacteria* and *Eukarya* in which *ester* linkages bond the fatty acids to glycerol, the lipids of *Archaea* contain *ether* bonds between glycerol and their hydrophobic side chains (**Figure 3.6**). Archaeal lipids lack true fatty acid side chains and instead, the side chains are composed of repeating units of the hydrophobic five-carbon hydrocarbon isoprene (Figure 3.6*c*).

The cytoplasmic membrane of *Archaea* can be constructed of either glycerol diethers (**Figure 3.7*a***), which have 20-carbon side chains (the 20-C unit is called a *phytanyl* group), or diglycerol tetraethers (Figure 3.7*b*), which have 40-carbon side chains. In the tetraether lipid, the ends of the phytanyl side chains that point inward from each glycerol molecule are covalently linked. This forms a lipid *monolayer* instead of a lipid *bilayer* membrane (Figure 3.7*d*, *e*). In contrast to lipid bilayers, lipid monolayer membranes are extremely resistant to heat denaturation and are therefore widely distributed in hyperthermophiles, prokaryotes that grow best at temperatures above 80°C. Membranes with a mixture of bilayer and monolayer character are also possible, with some of the inwardly opposing hydrophobic groups covalently bonded while others are not.

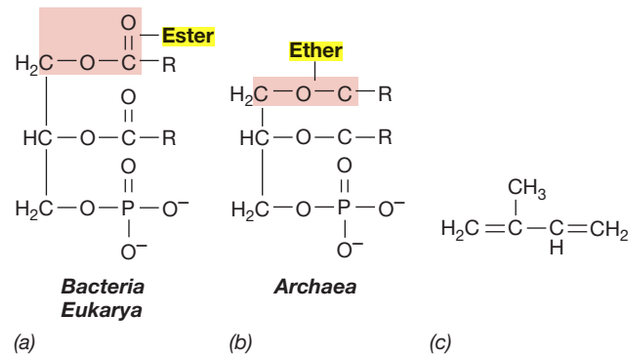


Figure 3.6 **General structure of lipids.** *(a)* The ester linkage and *(b)* the ether linkage. *(c)* Isoprene, the parent structure of the hydrophobic side chains of archaeal lipids. By contrast, in lipids of *Bacteria* and *Eukarya,* the side chains are composed of fatty acids (see Figure 3.4*a*).

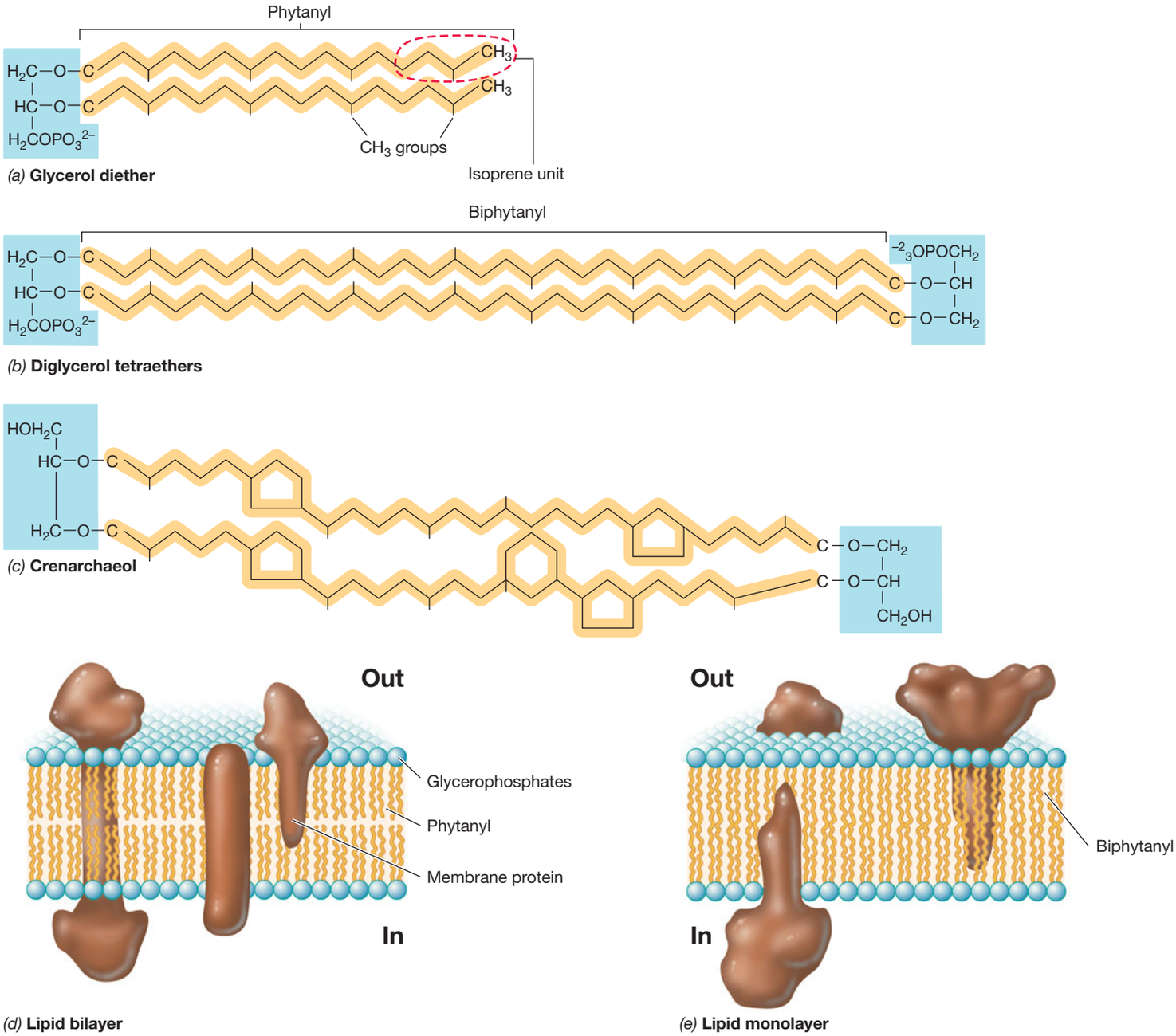


Figure 3.7 **Major lipids of *Archaea* and the architecture of archaeal membranes.** *(a, b)* Note that the hydrocarbon of the lipid is attached to the glycerol by an ether linkage in both cases. The hydrocarbon is phytanyl (C_{20}) in part a and biphytanyl (C_{40}) in part b. *(c)* A major lipid of *Crenarchaeota* is crenarchaeol, a lipid containing 5- and 6-carbon rings. *(d, e)* Membrane structure in *Archaea* may be bilayer or monolayer (or a mix of both).

Many archaeal lipids also contain rings within the hydrocarbon chains. For example, *crenarchaeol*, a lipid widespread among species of *Crenarchaeota* (↻ Section 2.10), contains four cyclopentyl rings and one cyclohexyl ring (Figure 3.7*c*). The predominant membrane lipids of many *Euryarchaeota*, such as the methanogens and extreme halophiles, are glycolipids, lipids with a carbohydrate bonded to glycerol. Rings formed in the hydrocarbon side chains affect the properties of the lipids (and thus overall membrane function), and considerable variation in the number and position of the rings has been discovered in the lipids of different species.

Despite the differences in chemistry between the cytoplasmic membranes of *Archaea* and organisms in the other domains, the fundamental construction of the archaeal cytoplasmic membrane—inner and outer hydrophilic surfaces and a hydrophobic interior—is the same as that of membranes in *Bacteria* and

Eukarya. Evolution has selected this design as the best solution to the main function of the cytoplasmic membrane—permeability—and we consider this problem now.

MiniQuiz

- Draw the basic structure of a lipid bilayer and label the hydrophilic and hydrophobic regions.
- How are the membrane lipids of *Bacteria* and *Archaea* similar, and how do they differ?

3.4 Functions of the Cytoplasmic Membrane

The cytoplasmic membrane is more than just a barrier separating the inside from the outside of the cell. The membrane plays critical roles in cell function. First and foremost, the membrane functions as a *permeability barrier*, preventing the passive leakage of solutes into or out of the cell (**Figure 3.8**). Secondly, the membrane is an *anchor* for many proteins. Some of these are enzymes that catalyze bioenergetic reactions and others transport solutes into and out of the cell. We will learn in the next chapter that the cytoplasmic membrane is also a major *site of energy conservation* in the cell. The membrane has an energetically charged form in which protons (H^+) are separated from hydroxyl ions (OH^-) across its surface (Figure 3.8). This charge separation is a form of energy, analogous to the potential energy present in a charged battery. This energy source, called the *proton motive force*, is responsible for driving many energy-requiring functions in the cell, including some forms of transport, motility, and biosynthesis of ATP.

The Cytoplasmic Membrane as a Permeability Barrier

The cytoplasm is a solution of salts, sugars, amino acids, nucleotides, and many other substances. The hydrophobic portion of the cytoplasmic membrane (Figure 3.5) is a tight barrier to diffusion of these substances. Although some small hydrophobic molecules pass the cytoplasmic membrane by diffusion, polar and charged molecules do not diffuse but instead must be transported. Even a substance as small as a proton (H^+) cannot diffuse across the membrane.

Table 3.2 *Comparative permeability of membranes to various molecules*

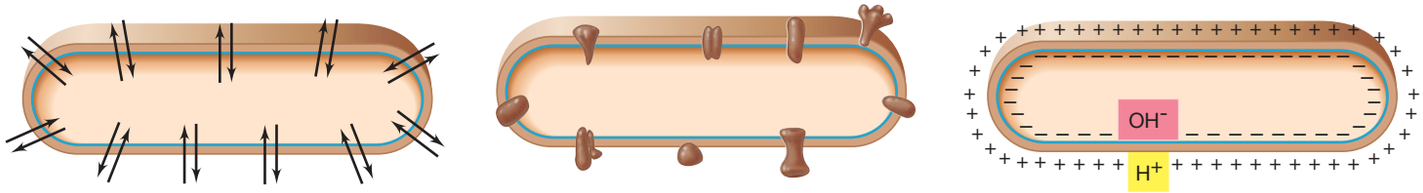
Substance	*Rate of permeability*[a]	*Potential for diffusion into a cell*
Water	100	Excellent
Glycerol	0.1	Good
Tryptophan	0.001	Fair/Poor
Glucose	0.001	Fair/Poor
Chloride ion (Cl^-)	0.000001	Very poor
Potassium ion (K^+)	0.0000001	Extremely poor
Sodium ion (Na^+)	0.00000001	Extremely poor

[a]Relative scale—permeability with respect to permeability to water given as 100. Permeability of the membrane to water may be affected by aquaporins (see text).

One substance that does freely pass the membrane in both directions is water, a molecule that is weakly polar but sufficiently small to pass between phospholipid molecules in the lipid bilayer (**Table 3.2**). But in addition, the movement of water across the membrane is accelerated by dedicated transport proteins called *aquaporins*. For example, aquaporin AqpZ of *Escherichia coli* imports or exports water depending on whether osmotic conditions in the cytoplasm are high or low, respectively. The relative permeability of the membrane to a few biologically relevant substances is shown in Table 3.2. As can be seen, most substances cannot diffuse into the cell and thus must be transported.

Transport Proteins

Transport proteins do more than just ferry substances across the membrane—they *accumulate* solutes against the concentration gradient. The necessity for carrier-mediated transport is easy to understand. If diffusion were the only mechanism by which solutes entered a cell, cells would never achieve the intracellular concentrations necessary to carry out biochemical reactions; that is, their rate of uptake and intracellular concentration would never exceed the external concentration, which in nature is often quite low (**Figure 3.9**). Hence, cells must have mechanisms for accumulating solutes—most of which are vital nutrients—to levels higher than those in their habitats, and this is the job of transport proteins.



(a) **Permeability barrier:** Prevents leakage and functions as a gateway for transport of nutrients into, and wastes out of, the cell

(b) **Protein anchor:** Site of many proteins that participate in transport, bioenergetics, and chemotaxis

(c) **Energy conservation:** Site of generation and use of the proton motive force

Figure 3.8 The major functions of the cytoplasmic membrane. Although structurally weak, the cytoplasmic membrane has many important cellular functions.

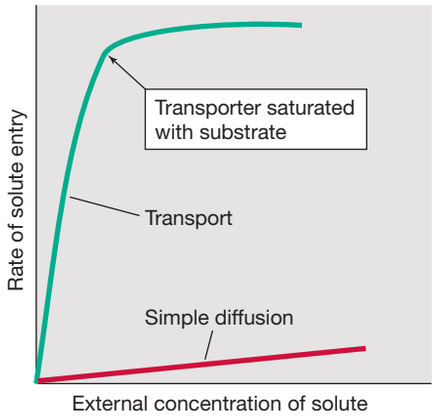


Figure 3.9 Transport versus diffusion. In transport, the uptake rate shows saturation at relatively low external concentrations.

Transport systems show several characteristic properties. First, in contrast with diffusion, transport systems show a *saturation effect*. If the concentration of substrate is high enough to saturate the transporter, which can occur at even the very low substrate concentrations found in nature, the rate of uptake becomes maximal and the addition of more substrate does not increase the rate (Figure 3.9). This characteristic feature of transport proteins is essential for a system that must concentrate nutrients from an often very dilute environment. A second characteristic of carrier-mediated transport is the *high specificity* of the transport event. Many carrier proteins react only with a single molecule, whereas a few show affinities for a closely related class of molecules, such as sugars or amino acids. This economy in uptake reduces the need for separate transport proteins for each different amino acid or sugar.

And finally, a third major characteristic of transport systems is that their biosynthesis is typically *highly regulated* by the cell. That is, the specific complement of transporters present in the cytoplasmic membrane of a cell at any one time is a function of both the resources available and their concentrations. Biosynthetic control of this type is important because a particular nutrient may need to be transported by one type of transporter when the nutrient is present at high concentration and by a different, higher-affinity transporter, when present at low concentration.

MiniQuiz

- List two reasons why a cell cannot depend on diffusion as a means of acquiring nutrients.
- Why is physical damage to the cytoplasmic membrane such a critical issue for the cell?

3.5 Transport and Transport Systems

Nutrient transport is a vital process. To fuel metabolism and support growth, cells need to import nutrients and export wastes on a continuous basis. To fulfill these requirements, several different mechanisms for transport exist in prokaryotes, each with its own unique features, and we explore this subject here.

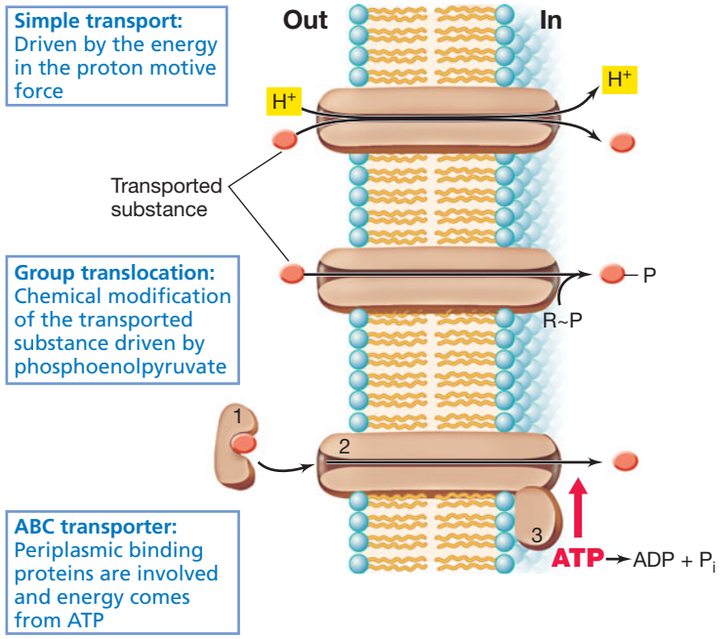


Figure 3.10 The three classes of transport systems. Note how simple transporters and the ABC system transport substances without chemical modification, whereas group translocation results in chemical modification (in this case phosphorylation) of the transported substance. The three proteins of the ABC system are labeled 1, 2, and 3.

Structure and Function of Membrane Transport Proteins

At least three transport systems exist in prokaryotes: simple transport, group translocation, and ABC transport. **Simple transport** consists only of a membrane-spanning transport protein, group translocation involves a series of proteins in the transport event, and the ABC system consists of three components: a substrate-binding protein, a membrane-integrated transporter, and an ATP-hydrolyzing protein (**Figure 3.10**). All transport systems require energy in some form, either from the proton motive force, or ATP, or some other energy-rich organic compound.

Figure 3.10 contrasts these transport systems. Regardless of the system, the membrane-spanning proteins typically show significant similarities in amino acid sequence, an indication of the common evolutionary roots of these structures. Membrane transporters are composed of 12 alpha helices that weave back and forth through the membrane to form a channel. It is through this channel that a solute is actually carried into the cell (**Figure 3.11**). The transport event requires that a conformational change occur in the membrane protein following binding of its solute. Like a gate swinging open, the conformational change then brings the solute into the cell.

Actual transport events can be of three types: uniport, symport, and antiport (Figure 3.11). *Uniporters* are proteins that transport a molecule unidirectionally across the membrane, either in or out. *Symporters* are cotransporters; they transport one molecule along with another substance, typically a proton. *Antiporters* are proteins that transport one molecule into the cell while simultaneously transporting a second molecule out of the cell.

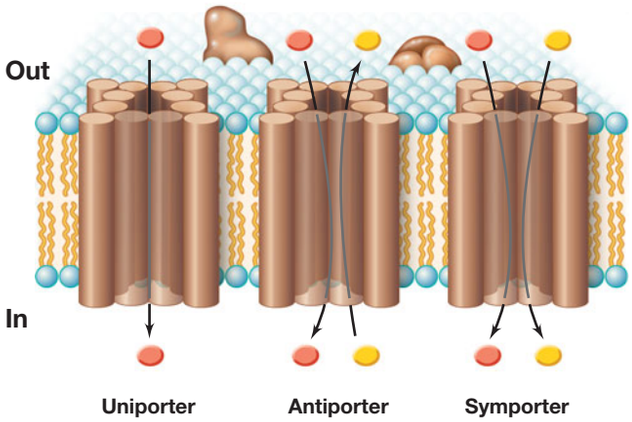


Figure 3.11 Structure of membrane-spanning transporters and types of transport events. Membrane-spanning transporters are made of 12 α-helices (each shown here as a cylinder) that aggregate to form a channel through the membrane. Shown here are three different transport events; for antiporters and symporters, the cotransported substance is shown in yellow.

Simple Transport: Lac Permease of *Escherichia coli*

The bacterium *Escherichia coli* metabolizes the disaccharide sugar lactose. Lactose is transported into cells of *E. coli* by the activity of a simple transporter, *lac permease*, a type of symporter. This is shown in **Figure 3.12**, where the activity of lac permease is compared with that of some other simple transporters, including uniporters and antiporters. We will see later that lac permease is one of three proteins required to metabolize lactose in *E. coli* and that the synthesis of these proteins is highly regulated by the cell (↻ Section 8.5).

As is true of all transport systems, the activity of lac permease is energy-driven. As each lactose molecule is transported into the cell, the energy in the proton motive force (Figure 3.8*c*) is diminished by the cotransport of protons into the cytoplasm. The membrane is reenergized through energy-yielding reactions that we will describe in Chapter 4. Thus the net result of lac permease activity is the energy-driven accumulation of lactose in the cytoplasm against the concentration gradient.

Group Translocation: The Phosphotransferase System

Group translocation is a form of transport in which the substance transported is chemically modified during its uptake across the membrane. One of the best-studied group translocation systems transports the sugars glucose, mannose, and fructose in *E. coli*. These compounds are modified by phosphorylation during transport by the *phosphotransferase system*.

The phosphotransferase system consists of a family of proteins that work in concert; five proteins are necessary to transport any given sugar. Before the sugar is transported, the proteins in the phosphotransferase system are themselves alternately phosphorylated and dephosphorylated in a cascading fashion until the actual transporter, Enzyme II_c, phosphorylates the sugar during the transport event (**Figure 3.13**). A small protein called *HPr*, the enzyme that phosphorylates HPr (Enzyme I), and Enzyme II_a are all cytoplasmic proteins. By contrast, Enzyme II_b lies on the inner surface of the membrane and Enzyme II_c is an integral membrane protein. HPr and Enzyme I are nonspecific components of the phosphotransferase system and participate in the uptake of several different sugars. Several different versions of Enzyme II exist, one for each different sugar transported (Figure 3.13). Energy for the phosphotransferase system comes from the energy-rich compound phosphoenolpyruvate, which is a key intermediate in glycolysis, a major pathway for glucose metabolism present in most cells (↻ Section 4.8).

Periplasmic Binding Proteins and the ABC System

We will learn a bit later in this chapter that gram-negative bacteria contain a region called the *periplasm* that lies between the cytoplasmic membrane and a second membrane layer called the *outer membrane*, part of the gram-negative cell wall (Section 3.7). The periplasm contains many different proteins, several of which function in transport and are called *periplasmic binding proteins*.

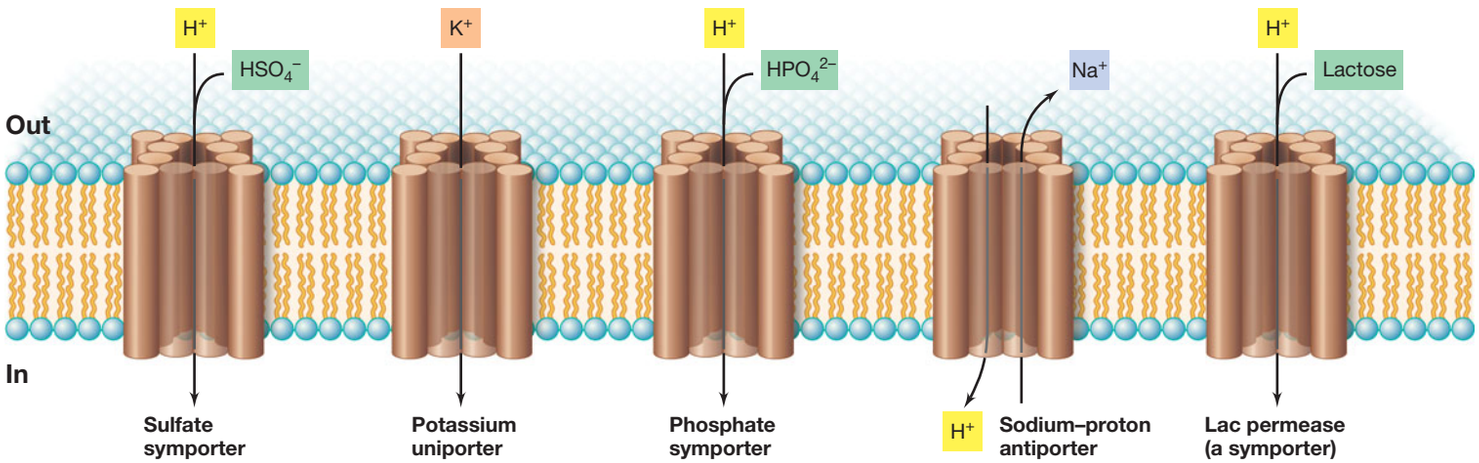


Figure 3.12 The lac permease of *Escherichia coli* and several other well-characterized simple transporters. Note the different classes of transport events depicted.

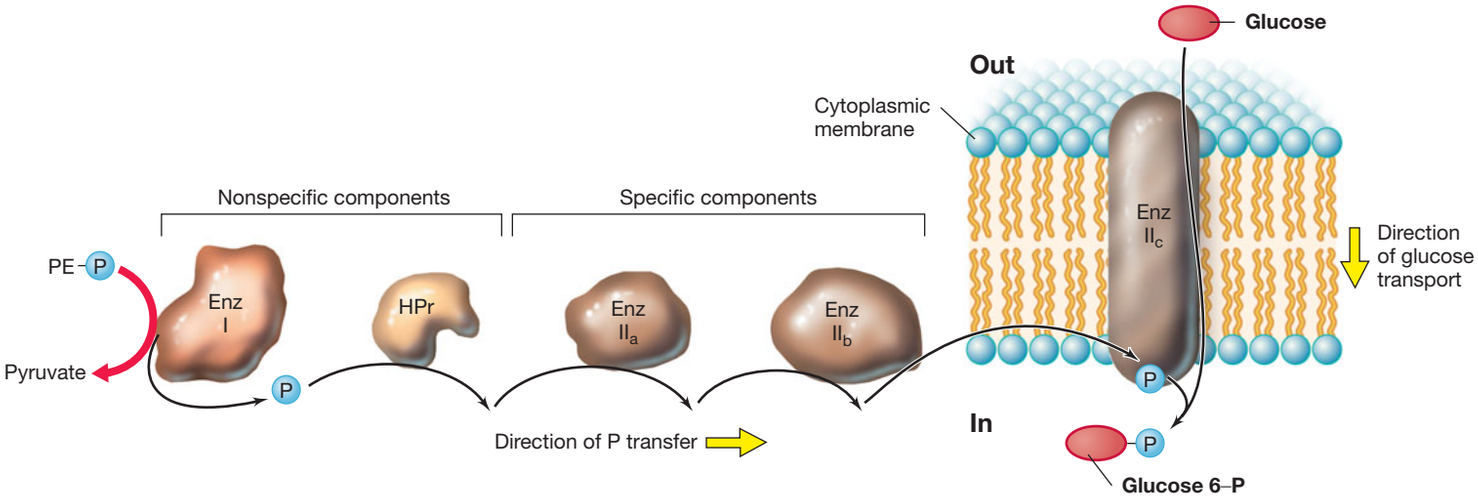


Figure 3.13 **Mechanism of the phosphotransferase system of *Escherichia coli*.** For glucose uptake, the system consists of five proteins: Enzyme (Enz) I, Enzymes II$_a$, II$_b$, and II$_c$, and HPr. A phosphate cascade occurs from phosphoenolpyruvate (PE-P) to Enzyme II$_c$ and the latter actually transports and phosphorylates the sugar. Proteins HPr and Enz I are nonspecific and transport any sugar. The Enz II components are specific for each particular sugar.

Transport systems that employ periplasmic binding proteins along with a membrane transporter and ATP-hydrolyzing proteins are called **ABC transport systems**, the "ABC" standing for *A*TP-*b*inding *c*assette, a structural feature of proteins that bind ATP (**Figure 3.14**). More than 200 different ABC transport systems have been identified in prokaryotes. ABC transporters exist for the uptake of organic compounds such as sugars and amino acids, inorganic nutrients such as sulfate and phosphate, and trace metals.

A characteristic property of periplasmic binding proteins is their high substrate affinity. These proteins can bind their substrate(s) even when they are at extremely low concentration; for example, less than 1 micromolar (10^{-6} M). Once its substrate is bound, the periplasmic binding protein interacts with its respective membrane transporter to transport the substrate into the cell driven by ATP hydrolysis (Figure 3.14).

Even though gram-positive bacteria lack a periplasm, they have ABC transport systems. In gram-positive bacteria, however, substrate-binding proteins are anchored to the external surface of the cytoplasmic membrane. Nevertheless, once these proteins bind substrate, they interact with a membrane transporter to catalyze uptake of the substrate at the expense of ATP hydrolysis, just as they do in gram-negative bacteria (Figure 3.14).

Protein Export

Thus far our discussion of transport has focused on small molecules. How do large molecules, such as proteins, get out of cells? Many proteins need to be either transported outside the cytoplasmic membrane or inserted in a specific way into the membrane in order to function properly. Proteins are exported through and inserted into prokaryotic membranes by the activities of other proteins called *translocases*, a key one being the Sec (*sec* for *sec*retory) system. The Sec system both exports proteins and inserts integral membrane proteins into the membrane. Proteins destined for transport are recognized by the Sec system because they are tagged in a specific way. We discuss this process later (↻ Section 6.21).

Protein export is important to bacteria because many bacterial enzymes are designed to function outside the cell (exoenzymes). For example, hydrolytic exoenzymes such as amylase or cellulase are excreted directly into the environment where they cleave starch or cellulose, respectively, into glucose; the glucose is then used by the cell as a carbon and energy source. In gram-negative

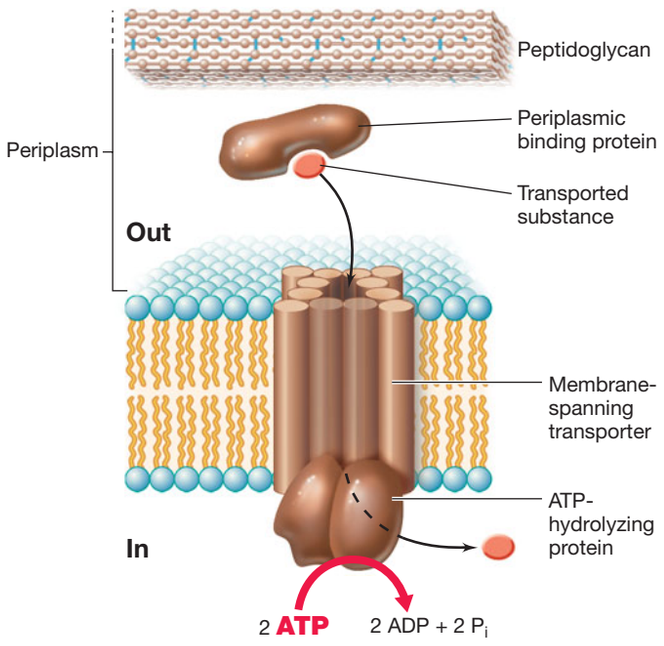


Figure 3.14 **Mechanism of an ABC transporter.** The periplasmic binding protein has high affinity for substrate, the membrane-spanning proteins form the transport channel, and the cytoplasmic ATP-hydrolyzing proteins supply the energy for the transport event.

bacteria, many enzymes are periplasmic enzymes, and these must traverse the cytoplasmic membrane in order to function. Moreover, many pathogenic bacteria excrete protein toxins or other harmful proteins into the host during infection. Many toxins are excreted by a second translocase system called the *type III secretion system*. This system differs from the Sec system in that the secreted protein is translocated from the bacterial cell directly into the host, for example, a human cell. However, all of these large molecules need to move through the cytoplasmic membrane, and translocases such as SecYEG and the type III secretion system assist in these transport events.

MiniQuiz

- Contrast simple transporters, the phosphotransferase system, and ABC transporters in terms of (1) energy source, (2) chemical alterations of the solute transported, and (3) number of proteins involved.
- Which transport system is best suited for the transport of nutrients present at extremely low levels, and why?
- Why is protein excretion important to cells?

III Cell Walls of Prokaryotes

3.6 The Cell Wall of *Bacteria*: Peptidoglycan

Because of the activities of transport systems, the cytoplasm of bacterial cells maintains a high concentration of dissolved solutes. This causes a significant osmotic pressure—about 2 atmospheres in a typical bacterial cell. This is roughly the same as the pressure in an automobile tire. To withstand these pressures and prevent bursting (cell lysis), bacteria employ cell walls. Besides protecting against osmotic lysis, cell walls also confer shape and rigidity on the cell.

Species of *Bacteria* can be divided into two major groups, called **gram-positive** and **gram-negative**. The distinction between gram-positive and gram-negative bacteria is based on the **Gram stain** reaction (Section 2.2). But differences in cell wall structure are at the heart of the Gram stain reaction. The surface of gram-positive and gram-negative cells as viewed in the electron microscope differs markedly, as shown in **Figure 3.15**. The gram-negative cell wall, or cell envelope as it is sometimes called, is chemically complex and consists of at least two layers, whereas the gram-positive cell wall is typically much thicker and consists primarily of a single type of molecule.

The focus of this section is on the polysaccharide component of the cell walls of *Bacteria*, both gram-positive and gram-negative. In the next section we describe the special wall components present in gram-negative *Bacteria*. And finally, in Section 3.8 we briefly describe the cell walls of *Archaea*.

Peptidoglycan

The walls of *Bacteria* have a rigid layer that is primarily responsible for the strength of the wall. In gram-negative bacteria, additional layers are present outside this rigid layer. The rigid layer, called **peptidoglycan**, is a polysaccharide composed of two sugar derivatives—*N-acetylglucosamine* and *N-acetylmuramic acid*—and a few amino acids, including L-alanine, D-alanine, D-glutamic acid, and either lysine or the structurally similar amino acid analog, diaminopimelic acid (DAP). These constituents are connected to form a repeating structure, the *glycan tetrapeptide* (**Figure 3.16**).

Long chains of peptidoglycan are biosynthesized adjacent to one another to form a sheet surrounding the cell (see Figure 3.18). The chains are connected through cross-links of amino acids. The glycosidic bonds connecting the sugars in the glycan strands are covalent bonds, but these provide rigidity to the structure in only one direction. Only after cross-linking is peptidoglycan strong in both the X and Y directions (**Figure 3.17**). Cross-linking occurs to different extents in different species of *Bacteria*; more extensive cross-linking results in greater rigidity.

In gram-negative bacteria, peptidoglycan cross-linkage occurs by peptide bond formation from the amino group of DAP of one glycan chain to the carboxyl group of the terminal D-alanine on the adjacent glycan chain (Figure 3.17). In gram-positive bacteria, cross-linkage may occur through a short peptide interbridge, the kinds and numbers of amino acids in the interbridge varying from species to species. For example, in the gram-positive *Staphylococcus aureus*, the interbridge peptide is composed of five glycine residues, a common interbridge amino acid (Figure 3.17*b*). The overall structure of peptidoglycan is shown in Figure 3.17*c*.

Peptidoglycan can be destroyed by certain agents. One such agent is the enzyme *lysozyme*, a protein that cleaves the β-1,4-glycosidic bonds between *N*-acetylglucosamine and *N*-acetylmuramic acid in peptidoglycan (Figure 3.16), thereby weakening the wall; water can then enter the cell and cause lysis. Lysozyme is found in animal secretions including tears, saliva, and other body fluids, and functions as a major line of defense against bacterial infection. When we consider peptidoglycan biosynthesis in Chapter 5 we will see that the important antibiotic penicillin also targets peptidoglycan, but in a different way from that of lysozyme. Whereas lysozyme destroys preexisting peptidoglycan, penicillin instead prevents its biosynthesis, leading eventually to osmotic lysis.

Diversity of Peptidoglycan

Peptidoglycan is present only in species of *Bacteria*—the sugar *N*-acetylmuramic acid and the amino acid analog DAP have never been found in the cell walls of *Archaea* or *Eukarya*. However, not all *Bacteria* examined have DAP in their peptidoglycan; some have lysine instead. An unusual feature of peptidoglycan is the presence of two amino acids of the D stereoisomer, D-alanine and D-glutamic acid. Proteins, by contrast, are always constructed of L-amino acids.

More than 100 different peptidoglycans are known, with diversity typically governed by the peptide cross-links and interbridge. In every form of peptidoglycan the glycan portion is constant; only the sugars *N*-acetylglucosamine and *N*-acetylmuramic acid are present and are connected in β-1,4 linkage (Figure 3.16). Moreover, the tetrapeptide shows major variation in only one amino acid, the lysine–DAP alternation. Thus, although the

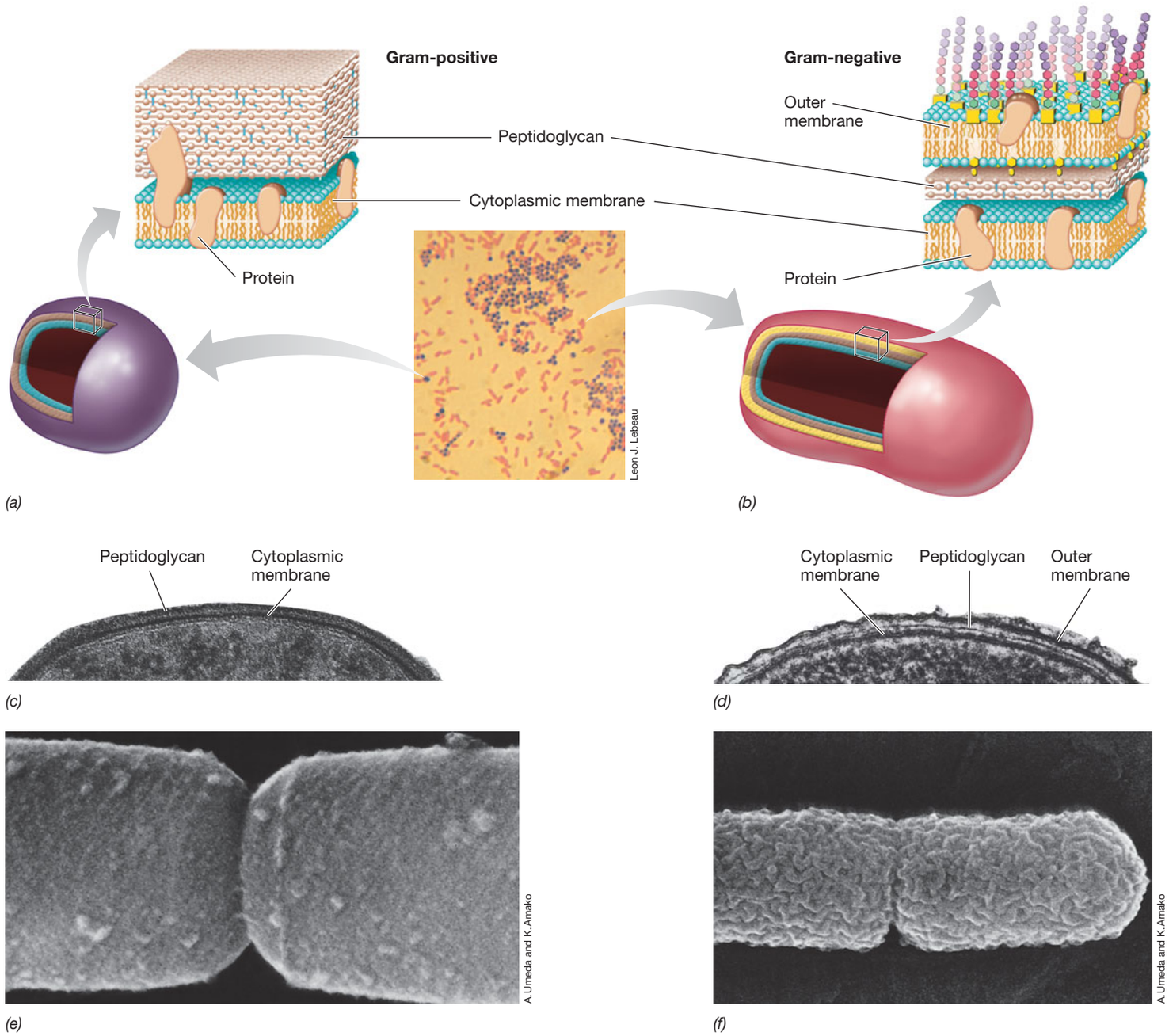


Figure 3.15 **Cell walls of *Bacteria*.** *(a, b)* Schematic diagrams of gram-positive and gram-negative cell walls. The Gram stain photo in the center shows cells of *Staphylococcus aureus* (purple, gram-positive) and *Escherichia coli* (pink, gram-negative). *(c, d)* Transmission electron micrographs (TEMs) showing the cell wall of a gram-positive bacterium and a gram-negative bacterium. *(e, f)* Scanning electron micrographs of gram-positive and gram-negative bacteria, respectively. Note differences in surface texture. Each cell in the TEMs is about 1 μm wide.

peptide composition of peptidoglycan can vary, the peptidoglycan backbone—alternating repeats of *N*-acetylglucosamine and *N*-acetylmuramic acid—is invariant.

The Gram-Positive Cell Wall

In gram-positive bacteria, as much as 90% of the wall is peptidoglycan. And, although some bacteria have only a single layer of peptidoglycan surrounding the cell, many gram-positive bacteria have several sheets of peptidoglycan stacked one upon another (Figure 3.15*a*). It is thought that the peptidoglycan is laid down by the cell in "cables" about 50 nm wide, with each cable consisting of several cross-linked glycan strands (**Figure 3.18*a***). As the peptidoglycan "matures," the cables themselves become cross-linked to form an even stronger cell wall structure.

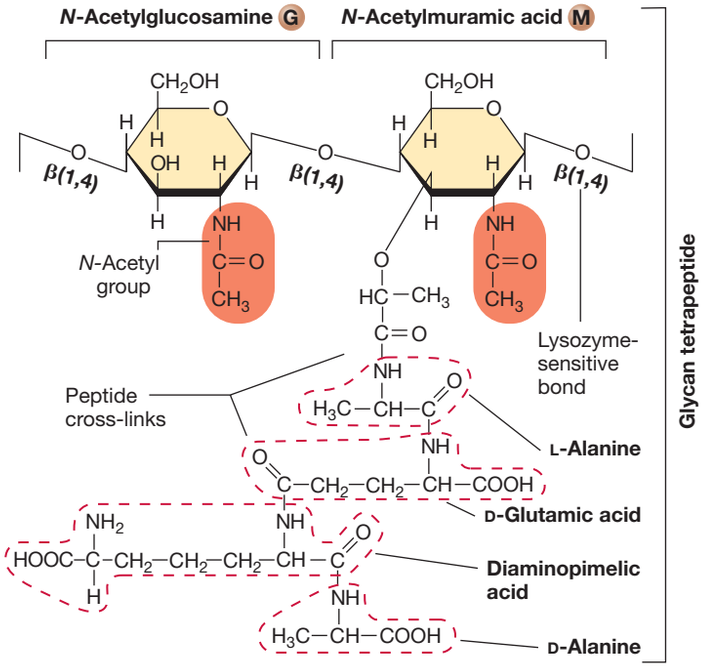


Figure 3.16 **Structure of the repeating unit in peptidoglycan, the glycan tetrapeptide.** The structure given is that found in *Escherichia coli* and most other gram-negative *Bacteria*. In some *Bacteria*, other amino acids are present as discussed in the text.

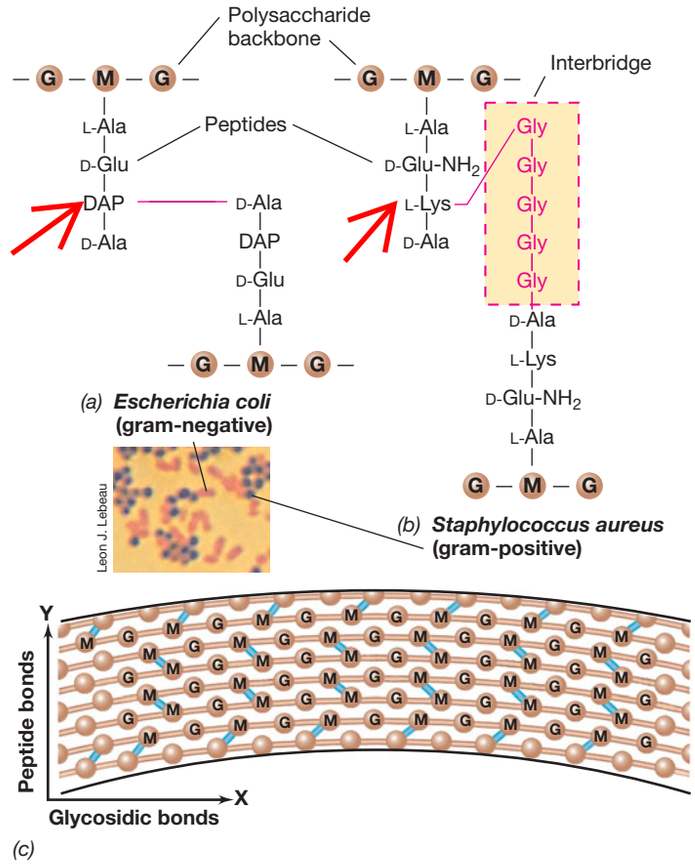


Figure 3.17 **Peptidoglycan in *Escherichia coli* and *Staphylococcus aureus*.** *(a)* No interbridge is present in *E. coli* peptidoglycan nor that of other gram-negative *Bacteria*. *(b)* The glycine interbridge in *S. aureus* (gram-positive). *(c)* Overall structure of peptidoglycan. G, *N*-acetylglucosamine; M, *N*-acetylmuramic acid. Note how glycosidic bonds confer strength on peptidoglycan in the X direction whereas peptide bonds confer strength in the Y direction.

Wall teicchic and lipoteichoic

Many gram-positive bacteria have acidic components called **teichoic acids** embedded in their cell wall. The term "teichoic acids" includes all cell wall, cytoplasmic membrane, and capsular polymers composed of glycerol phosphate or ribitol phosphate. These polyalcohols are connected by phosphate esters and typically contain sugars or D-alanine (Figure 3.18*b*). Teichoic acids are covalently bonded to muramic acid in the wall peptidoglycan. Because the phosphates are negatively charged, teichoic acids are at least in part responsible for the overall negative electrical charge of the cell surface. Teichoic acids also function to bind Ca^{2+} and Mg^{2+} for eventual transport into the cell. Certain teichoic acids are covalently bound to membrane lipids, and these are called *lipoteichoic* acids (Figure 3.18*c*).

Figure 3.18 summarizes the structure of the cell wall of gram-positive *Bacteria* and shows how teichoic acids and lipoteichoic acids are arranged in the overall wall structure. It also shows how the peptidoglycan cables run perpendicular to the long axis of a rod-shaped bacterium.

Cells That Lack Cell Walls

Although most prokaryotes cannot survive in nature without their cell walls, some do so naturally. These include the mycoplasmas, a group of pathogenic bacteria that causes several infectious diseases of humans and other animals, and the *Thermoplasma* group, species of *Archaea* that naturally lack cell walls. These bacteria are able to survive without cell walls because they either contain unusually tough cytoplasmic membranes or because they live in osmotically protected habitats such as the animal body. Most mycoplasmas have sterols in their cytoplasmic membranes, and these probably function to add strength and rigidity to the membrane as they do in the cytoplasmic membranes of eukaryotic cells.

MiniQuiz

- Why do bacterial cells need cell walls? Do all bacteria have cell walls?
- Why is peptidoglycan such a strong molecule?
- What does the enzyme lysozyme do?

3.7 The Outer Membrane

In gram-negative bacteria only about 10% of the total cell wall consists of peptidoglycan (Figure 3.15*b*). Instead, most of the wall is composed of the **outer membrane**. This layer is effectively a second lipid bilayer, but it is not constructed solely of phospholipid and protein, as is the cytoplasmic membrane (Figure 3.5). The gram-negative cell outer membrane also contains polysaccharide. The lipid and polysaccharide are linked in the outer

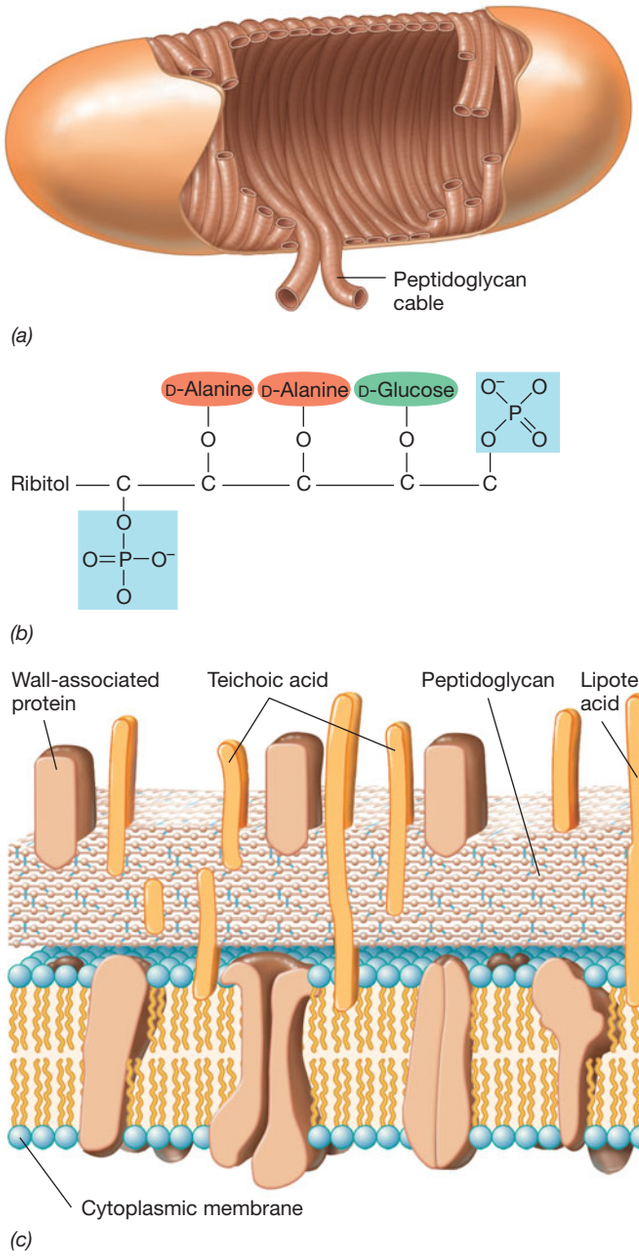


Figure 3.18 **Structure of the gram-positive bacterial cell wall.** *(a)* Schematic of a gram-positive rod showing the internal architecture of the peptidoglycan "cables." *(b)* Structure of a ribitol teichoic acid. The teichoic acid is a polymer of the repeating ribitol unit shown here. *(c)* Summary diagram of the gram-positive bacterial cell wall.

membrane to form a complex. Because of this, the outer membrane is also called the **lipopolysaccharide** layer, or simply **LPS**.

Chemistry and Activity of LPS

The chemistry of LPS from several bacteria is known. As seen in **Figure 3.19**, the polysaccharide portion of LPS consists of two components, the *core polysaccharide* and the *O-polysaccharide*. In *Salmonella* species, where LPS has been best studied, the core polysaccharide consists of ketodeoxyoctonate (KDO), various seven-carbon sugars (heptoses), glucose, galactose, and *N*-acetylglucosamine. Connected to the core is the O-polysaccharide, which typically contains galactose, glucose, rhamnose, and mannose, as well as one or more dideoxyhexoses, such as abequose, colitose, paratose, or tyvelose. These sugars are connected in four- or five-membered sequences, which often are branched. When the sequences repeat, the long O-polysaccharide is formed.

The relationship of the LPS layer to the overall gram-negative cell wall is shown in **Figure 3.20**. The lipid portion of the LPS, called *lipid A*, is not a typical glycerol lipid (see Figure 3.4*a*), but instead the fatty acids are connected through the amine groups from a disaccharide composed of glucosamine phosphate (Figure 3.19). The disaccharide is attached to the core polysaccharide through KDO (Figure 3.19). Fatty acids commonly found in lipid A include caproic (C_6), lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), and stearic (C_{18}) acids.

LPS replaces much of the phospholipid in the outer half of the outer membrane bilayer. By contrast, lipoprotein is present on the inner half of the outer membrane, along with the usual phospholipids (Figure 3.20*a*). Lipoprotein functions as an anchor tying the outer membrane to peptidoglycan. Thus, although the overall structure of the outer membrane is considered a lipid bilayer, its structure is distinct from that of the cytoplasmic membrane (compare Figures 3.5 and 3.20*a*).

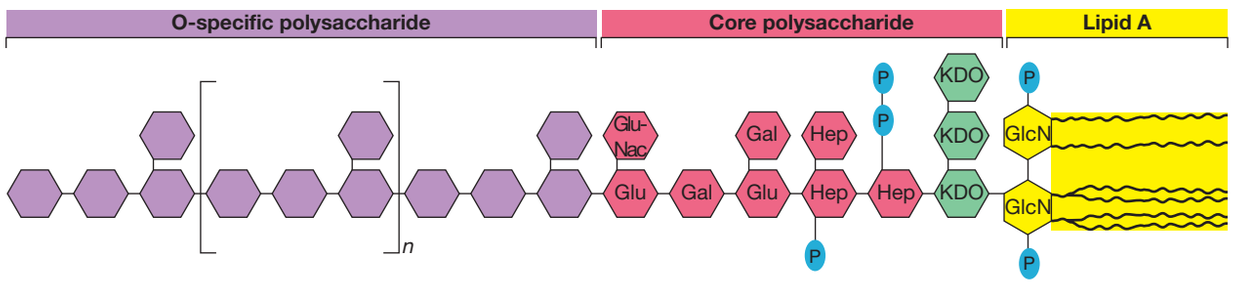


Figure 3.19 **Structure of the lipopolysaccharide of gram-negative *Bacteria*.** The chemistry of lipid A and the polysaccharide components varies among species of gram-negative *Bacteria*, but the major components (lipid A–KDO–core–O-specific) are typically the same. The O-specific polysaccharide varies greatly among species. KDO, ketodeoxyoctonate; Hep, heptose; Glu, glucose; Gal, galactose; GluNac, *N*-acetylglucosamine; GlcN, glucosamine; P, phosphate. Glucosamine and the lipid A fatty acids are linked through the amine groups. The lipid A portion of LPS can be toxic to animals and comprises the endotoxin complex. Compare this figure with Figure 3.20 and follow the LPS components by the color-coding.

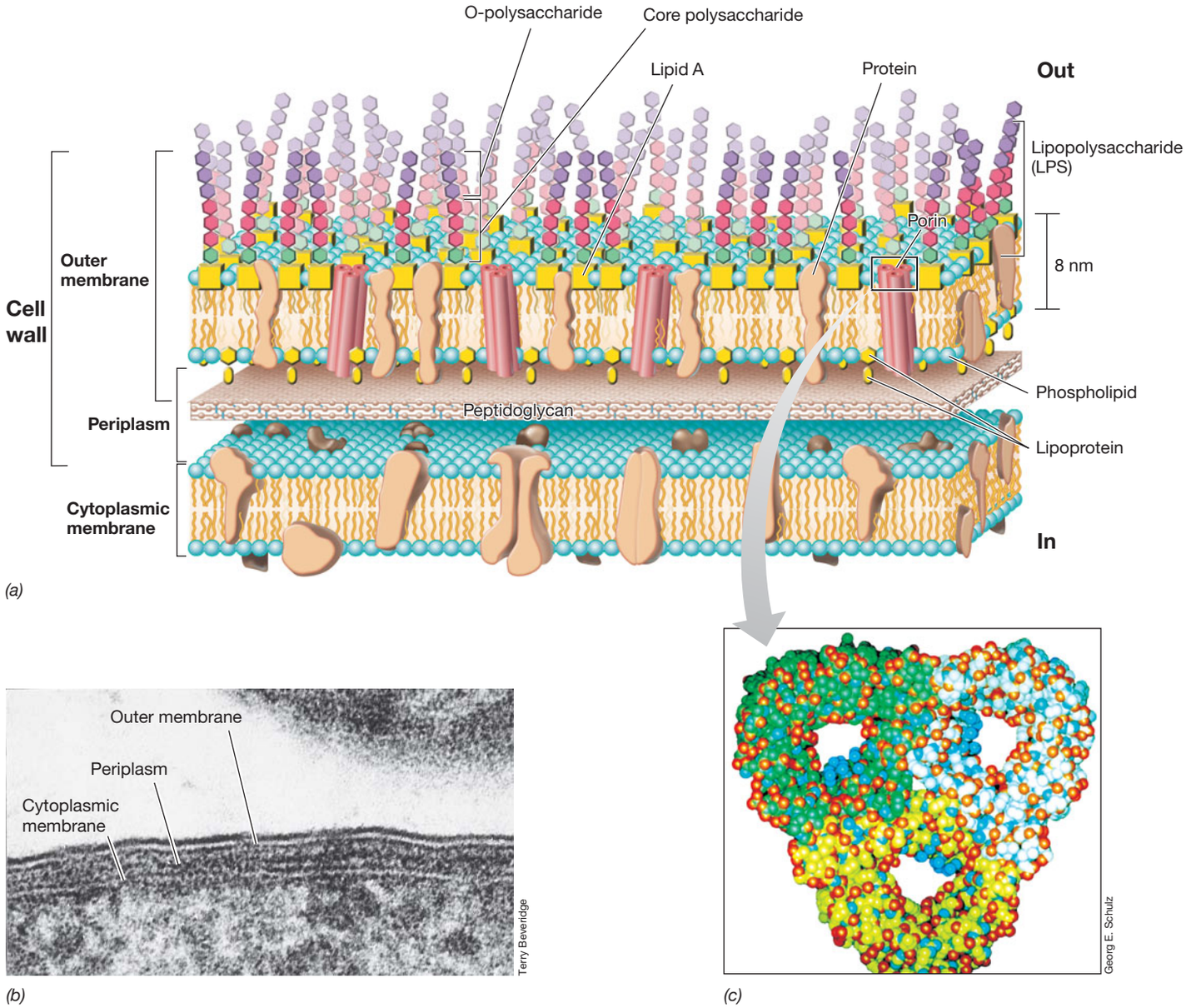


Figure 3.20 **The gram-negative cell wall.** *(a)* Arrangement of lipopolysaccharide, lipid A, phospholipid, porins, and lipoprotein in the outer membrane. See Figure 3.19 for details of the structure of LPS. *(b)* Transmission electron micrograph of a cell of *Escherichia coli* showing the cytoplasmic membrane and wall. *(c)* Molecular model of porin proteins. Note the four pores present, one within each of the proteins forming a porin molecule and a smaller central pore between the porin proteins. The view is perpendicular to the plane of the membrane.

Although the major function of the outer membrane is undoubtedly structural, one of its important biological activities is its toxicity to animals. Gram-negative bacteria that are pathogenic for humans and other mammals include species of *Salmonella*, *Shigella*, and *Escherichia*, among many others, and some of the intestinal symptoms these pathogens elicit are due to toxic outer membrane components. Toxicity is associated with the LPS layer, in particular, lipid A. The term *endotoxin* refers to this toxic component of LPS. Some endotoxins cause violent symptoms in humans, including gas, diarrhea, and vomiting, and the endotoxins produced by *Salmonella* and enteropathogenic strains of *E. coli* transmitted in contaminated foods are classic examples of this.

The Periplasm and Porins

Although permeable to small molecules, the outer membrane is not permeable to proteins or other large molecules. In fact, one of the major functions of the outer membrane is to keep proteins whose activities occur outside the cytoplasmic membrane from diffusing away from the cell. These proteins are present in a

region called the **periplasm** (see Figure 3.20). This space, located between the outer surface of the cytoplasmic membrane and the inner surface of the outer membrane, is about 15 nm wide. The periplasm is gel-like in consistency because of the high concentration of proteins present there.

Depending on the organism, the periplasm can contain several different classes of proteins. These include hydrolytic enzymes, which function in the initial degradation of food molecules; binding proteins, which begin the process of transporting substrates (Section 3.5); and chemoreceptors, which are proteins involved in the chemotaxis response (Section 3.15). Most of these proteins reach the periplasm by way of the Sec protein-exporting system in the cytoplasmic membrane (Section 3.5).

The outer membrane of gram-negative bacteria is relatively permeable to small molecules even though it is a lipid bilayer. This is due to *porins* embedded in the outer membrane that function as channels for the entrance and exit of solutes (Figure 3.20). Several porins are known, including both specific and nonspecific classes.

Nonspecific porins form water-filled channels through which any small substance can pass. By contrast, specific porins contain a binding site for only one or a small group of structurally related substances. Porins are transmembrane proteins that consist of three identical subunits. Besides the channel present in each barrel of the porin, the barrels of the porin proteins associate in such a way that a hole about 1 nm in diameter is formed in the outer membrane through which very small solutes can travel (Figure 3.20*c*).

Relationship of Cell Wall Structure to the Gram Stain

The structural differences between the cell walls of gram-positive and gram-negative *Bacteria* are thought to be responsible for differences in the Gram stain reaction. In the Gram stain, an insoluble crystal violet–iodine complex forms inside the cell. This complex is extracted by alcohol from gram-negative but not from gram-positive bacteria (↻ Section 2.2). As we have seen, gram-positive bacteria have very thick cell walls consisting primarily of peptidoglycan (Figure 3.18); these become dehydrated by the alcohol, causing the pores in the walls to close and preventing the insoluble crystal violet–iodine complex from escaping. By contrast, in gram-negative bacteria, alcohol readily penetrates the lipid-rich outer membrane and extracts the crystal violet–iodine complex from the cell. After alcohol treatment, gram-negative cells are nearly invisible unless they are counterstained with a second dye, a standard procedure in the Gram stain (↻ Figure 2.4).

MiniQuiz

- What components constitute the outer membrane of gram-negative bacteria?
- What is the function of porins and where are they located in a gram-negative cell wall?
- What component of the cell has endotoxin properties?
- Why does alcohol readily decolorize gram-negative but not gram-positive bacteria?

3.8 Cell Walls of *Archaea*

Peptidoglycan, a key biomarker for *Bacteria*, is absent from the cell walls of *Archaea*. An outer membrane is typically lacking in *Archaea* as well. Instead, a variety of chemistries are found in the cell walls of *Archaea*, including polysaccharides, proteins, and glycoproteins.

Pseudomurein and Other Polysaccharide Walls

The cell walls of certain methanogenic *Archaea* contain a molecule that is remarkably similar to peptidoglycan, a polysaccharide called *pseudomurein* (the term "murein" is from the Latin word for "wall" and was an old term for peptidoglycan; **Figure 3.21**). The backbone of pseudomurein is composed of alternating repeats of *N*-acetylglucosamine (also found in peptidoglycan) and *N*-acetyltalosaminuronic acid; the latter replaces the *N*-acetylmuramic acid of peptidoglycan. Pseudomurein also differs from peptidoglycan in that the glycosidic bonds between the sugar derivatives are β-1,3 instead of β-1,4, and the amino acids are all of the L stereoisomer. It is thought that peptidoglycan and pseudomurein either arose by convergent evolution after *Bacteria* and *Archaea* had diverged or, more likely, by evolution from a common polysaccharide present in the cell walls of the common ancestor of the domains *Bacteria* and *Archaea*.

Cell walls of some other *Archaea* lack pseudomurein and instead contain other polysaccharides. For example, *Methanosarcina* species have thick polysaccharide walls composed of polymers of glucose, glucuronic acid, galactosamine uronic acid, and acetate. Extremely halophilic (salt-loving) *Archaea* such as *Halococcus*, which are related to *Methanosarcina*, have similar cell walls that

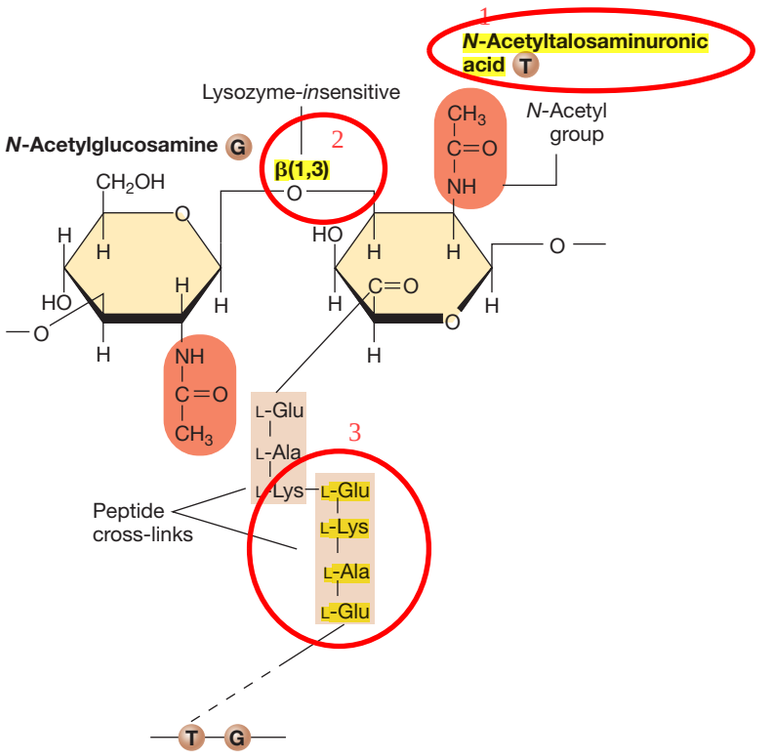


Figure 3.21 Pseudomurein. Structure of pseudomurein, the cell wall polymer of *Methanobacterium* species. Note the similarities and differences between pseudomurein and peptidoglycan (Figure 3.16).

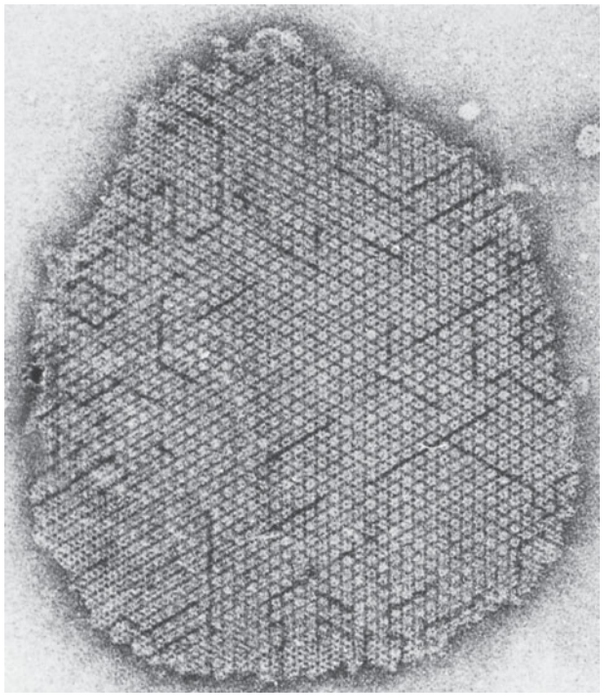


Figure 3.22 **The S-layer.** Transmission electron micrograph of an S-layer showing the paracrystalline structure. Shown is the S-layer from *Aquaspirillum serpens* (a species of *Bacteria*); this S-layer shows hexagonal symmetry as is common in S-layers of *Archaea* as well.

also contain sulfate (SO_4^{2-}). The negative charge on the sulfates bind the high concentration of Na^+ present in the habitats of *Halococcus*, salt evaporation ponds and saline seas and lakes; this helps stabilize the cell wall in such strongly polar environments.

S-Layers

The most common cell wall in species of *Archaea* is the paracrystalline surface layer, or **S-layer**. S-layers consist of interlocking protein or glycoprotein molecules that show an ordered appearance when viewed with the electron microscope (**Figure 3.22**). The paracrystalline structure of S-layers is arranged to yield various symmetries, such as hexagonal, tetragonal, or trimeric, depending upon the number and structure of the protein or glycoprotein subunits of which they are composed. S-layers have been found in representatives of all major lineages of *Archaea* and also in several species of *Bacteria* (Figure 3.22).

The cell walls of some *Archaea*, for example the methanogen *Methanocaldococcus jannaschii*, consist only of an S-layer. Thus, S-layers are themselves sufficiently strong to withstand osmotic bursting. However, in many organisms S-layers are present in addition to other cell wall components, usually polysaccharides. For example, in *Bacillus brevis*, a species of *Bacteria*, an S-layer is present along with peptidoglycan. However, when an S-layer is present along with other wall components, the S-layer is always the *outermost* wall layer, the layer that is in direct contact with the environment.

Besides serving as protection from osmotic lysis, S-layers may have other functions. For example, as the interface between the cell and its environment, it is likely that the S-layer functions as a selective sieve, allowing the passage of low-molecular-weight solutes while excluding large molecules and structures (such as viruses). The S-layer may also function to retain proteins near the cell surface, much as the outer membrane (Section 3.7) does in gram-negative bacteria.

We thus see several cell wall chemistries in species of *Archaea*, varying from molecules that closely resemble peptidoglycan to those that totally lack a polysaccharide component. But with rare exception, all *Archaea* contain a cell wall of some sort, and as in *Bacteria*, the archaeal cell wall functions to prevent osmotic lysis and gives the cell its shape. In addition, because they lack peptidoglycan in their cell walls, *Archaea* are naturally resistant to the activity of lysozyme (Section 3.6) and the antibiotic penicillin, agents that either destroy peptidoglycan or prevent its proper synthesis.

MiniQuiz

- How does pseudomurein resemble peptidoglycan? How do the two molecules differ?
- What is the composition of an S-layer?
- Why are *Archaea* insensitive to penicillin?

IV Other Cell Surface Structures and Inclusions

In addition to cell walls, prokaryotic cells can have other layers or structures in contact with the environment. Moreover, cells often contain one or more types of cellular inclusions. We examine some of these here.

Differencess between capsules and slime layers

3.9 Cell Surface Structures

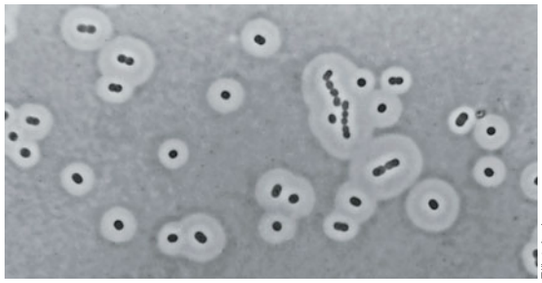
Many prokaryotes secrete slimy or sticky materials on their cell surface. These materials consist of either polysaccharide or protein. These are not considered part of the cell wall because they do not confer significant structural strength on the cell. The terms "capsule" and "slime layer" are used to describe these layers.

Function

Capsules and Slime Layers

Capsules and slime layers may be thick or thin and rigid or flexible, depending on their chemistry and degree of hydration. Traditionally, if the layer is organized in a tight matrix that excludes small particles, such as India ink, it is called a **capsule** (**Figure 3.23**). By contrast, if the layer is more easily deformed, it will not exclude particles and is more difficult to see; this form is called a *slime layer*. In addition, capsules typically adhere firmly to the cell wall, and some are even covalently linked to peptidoglycan. Slime layers, by contrast, are loosely attached and can be lost from the cell surface. 1.

Polysaccharide layers have several functions in bacteria. Surface polysaccharides assist in the attachment of microorganisms to solid surfaces. As we will see later, pathogenic microorganisms that enter the animal body by specific routes usually do so by first binding specifically to surface components of host tissues, and this binding is often mediated by bacterial cell surface polysaccharides. Many nonpathogenic bacteria also bind to solid surfaces in nature, sometimes forming a thick layer of cells called a *biofilm*. Extracellular polysaccharides play a key role in 2.



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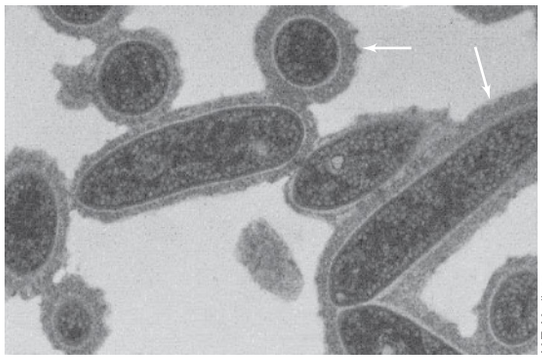

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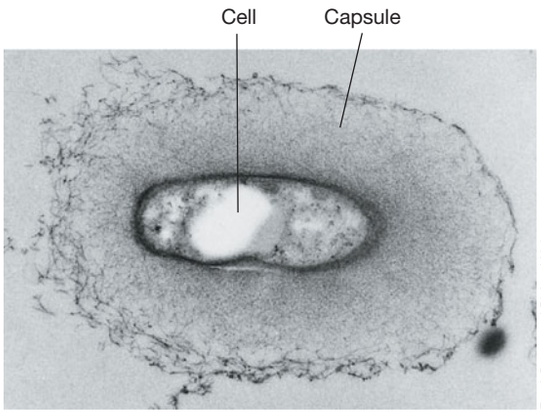

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Figure 3.23 **Bacterial capsules.** *(a)* Capsules of *Acinetobacter* species observed by phase-contrast microscopy after negative staining of cells with India ink. India ink does not penetrate the capsule and so the capsule appears as a light area surrounding the cell, which appears black. *(b)* Transmission electron micrograph of a thin section of cells of *Rhodobacter capsulatus* with capsules (arrows) clearly evident; cells are about 0.9 μm wide. *(c)* Transmission electron micrograph of *Rhizobium trifolii* stained with ruthenium red to reveal the capsule. The cell is about 0.7 μm wide.

the development of biofilms (↔ Microbial Sidebar in Chapter 5, "Microbial Growth in the Real World: Biofilms").

Capsules can play other roles as well. For example, encapsulated pathogenic bacteria are typically more difficult for phagocytic cells of the immune system to recognize and subsequently destroy. In addition, because outer polysaccharide layers bind a significant amount of water, it is likely that these layers play some role in resistance of the cell to desiccation.

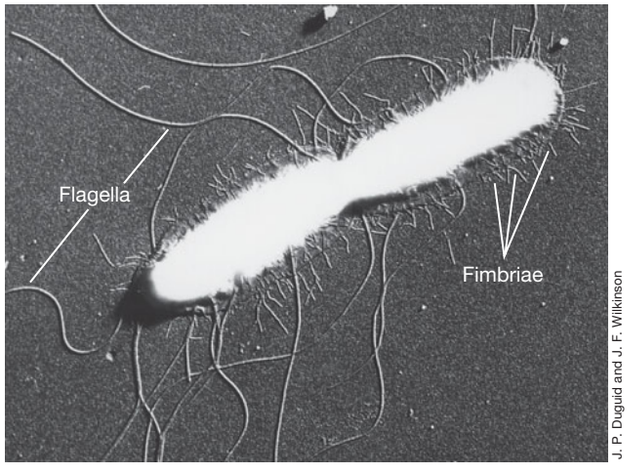


Figure 3.24 **Fimbriae.** Electron micrograph of a dividing cell of *Salmonella typhi*, showing flagella and fimbriae. A single cell is about 0.9 μm wide.

Fimbriae and Pili

Fimbriae and pili are filamentous structures composed of protein that extend from the surface of a cell and can have many functions. *Fimbriae* (**Figure 3.24**) enable cells to stick to surfaces, including animal tissues in the case of pathogenic bacteria, or to form pellicles (thin sheets of cells on a liquid surface) or biofilms on surfaces. Notorious human pathogens in which fimbriae assist in the disease process include *Salmonella* species (salmonellosis), *Neisseria gonorrhoeae* (gonorrhea), and *Bordetella pertussis* (whooping cough).

Pili are similar to fimbriae, but are typically longer and only one or a few pili are present on the surface of a cell. Because pili can be receptors for certain types of viruses, they can best be seen under the electron microscope when they become coated with virus particles (**Figure 3.25**). Many classes of pili are known, distinguished by their structure and function. Two very important functions of pili include facilitating genetic exchange between cells in a process called conjugation (Figure 3.25) and in the adhesion of pathogens to specific host tissues and subsequent invasion. The latter function has been best studied in gram-negative pathogens such as *Neisseria*, species of which cause gonorrhea and meningitis, but pili are also present on certain gram-positive pathogens such as *Streptococcus pyogenes*, the cause of strep throat and scarlet fever.

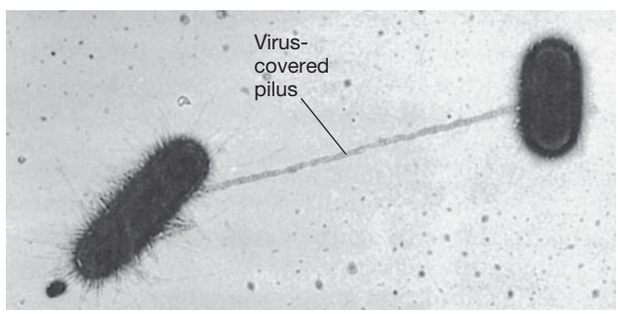


Figure 3.25 **Pili.** The pilus on an *Escherichia coli* cell that is undergoing conjugation (a form of genetic transfer) with a second cell is better resolved because viruses have adhered to it. The cells are about 0.8 μm wide.

One important class of pili, called *type IV pili*, assist cells in adhesion but also allow for an unusual form of cell motility called *twitching motility*. Type IV pili are 6 nm in diameter and present only at the poles of those rod-shaped cells that contain them. Twitching motility is a type of gliding motility, movement along a solid surface (Section 3.14). In twitching motility, extension of pili followed by their retraction drags the cell along a solid surface, with energy supplied by ATP. Certain species of *Pseudomonas* and *Moraxella* are well known for their twitching motility. Colonization

Type IV pili have also been implicated as key colonization factors for certain human pathogens, including *Vibrio cholerae* (cholera) and *Neisseria gonorrhoeae* (gonorrhea). The twitching motility of these pathogens presumably assists the organism to locate specific sites for attachment to initiate the disease process. Type IV pili are also thought to mediate genetic transfer by the process of transformation in some bacteria, which, along with conjugation and transduction, are the three known means of horizontal gene transfer in prokaryotes (Chapter 10).

MiniQuiz

- Could a bacterial cell dispense with a cell wall if it had a capsule? Why or why not?
- How do fimbriae differ from pili, both structurally and functionally?

3.10 Cell Inclusions

Granules or other inclusions are often present in prokaryotic cells. Inclusions function as energy reserves and as reservoirs of structural building blocks. Inclusions can often be seen directly with the light microscope and are usually enclosed by single layer (nonunit) membranes that partition them off in the cell. Storing carbon or other substances in an insoluble inclusion confers an advantage on the cell because it reduces the osmotic stress that would be encountered if the same amount of the substance was dissolved in the cytoplasm.

Carbon Storage Polymers

One of the most common inclusion bodies in prokaryotic organisms is **poly-β-hydroxybutyric acid (PHB)**, a lipid that is formed from β-hydroxbutyric acid units. The monomers of PHB bond by ester linkage to form the PHB polymer, and then the polymer aggregates into granules; the latter can be observed by either light or electron microscope (**Figure 3.26**).

The monomer in the polymer is not only hydroxybutyrate (C_4) but can vary in length from as short as C_3 to as long as C_{18}. Thus, the more generic term *poly-β-hydroxyalkanoate* (PHA) is often used to describe this class of carbon- and energy-storage polymers. PHAs are synthesized by cells when there is an excess of carbon and are broken down for biosynthetic or energy purposes when conditions warrant. Many prokaryotes, including species of both *Bacteria* and *Archaea*, produce PHAs.

Another storage product is *glycogen*, which is a polymer of glucose. Like PHA, glycogen is a storehouse of both carbon and energy. Glycogen is produced when carbon is in excess in the environment and is consumed when carbon is limited. Glycogen

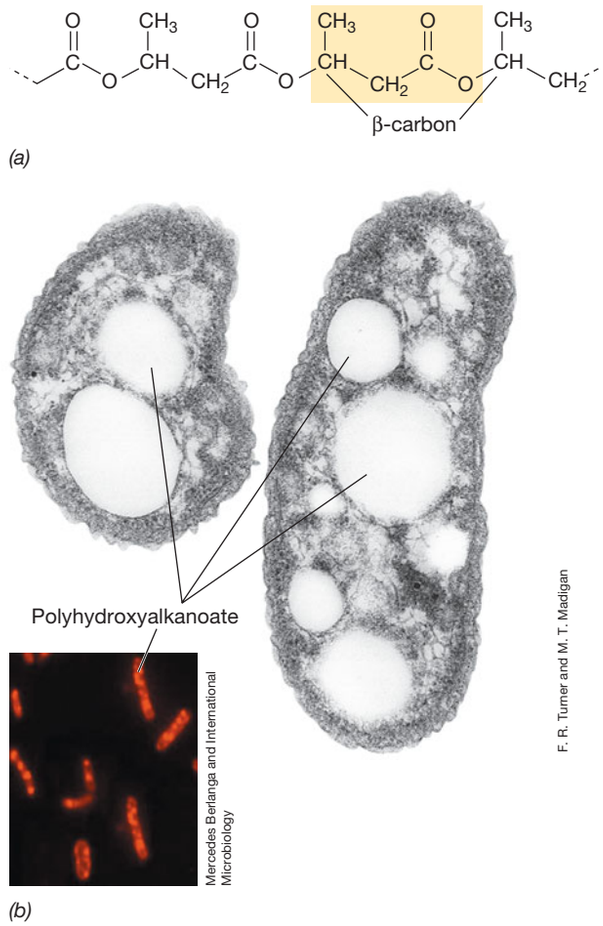


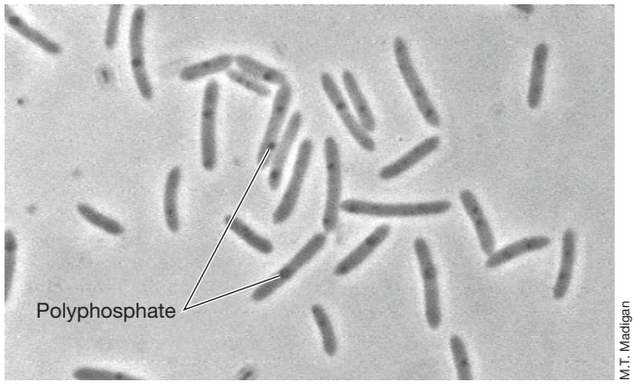
Figure 3.26 **Poly-β-hydroxyalkanoates.** *(a)* Chemical structure of poly-β-hydroxybutyrate, a common PHA. A monomeric unit is shown in color. Other PHAs are made by substituting longer-chain hydrocarbons for the $-CH_3$ group on the β carbon. *(b)* Electron micrograph of a thin section of cells of a bacterium containing granules of PHA. Color photo: Nile red–stained cells of a PHA-containing bacterium.

resembles starch, the major storage reserve of plants, but differs slightly from starch in the manner in which the glucose units are linked together.

Polyphosphate and Sulfur

Many microorganisms accumulate inorganic phosphate (PO_4^{3-}) in the form of granules of *polyphosphate* (**Figure 3.27*a***). These granules can be degraded and used as sources of phosphate for nucleic acid and phospholipid biosyntheses and in some organisms can be used to make the energy-rich compound ATP. Phosphate is often a limiting nutrient in natural environments. Thus if a cell happens upon an excess of phosphate, it is advantageous to be able to store it as polyphosphate for future use.

Many gram-negative prokaryotes can oxidize reduced sulfur compounds, such as hydrogen sulfide (H_2S). The oxidation of sulfide is linked to either reactions of energy metabolism (chemolithotrophy) or CO_2 fixation (autotrophy). In either case, *elemental sulfur* (S^0) may accumulate in the cell in microscopically visible globules (Figure 3.27*b*). This sulfur remains as long as the source of reduced sulfur from which it was derived is still



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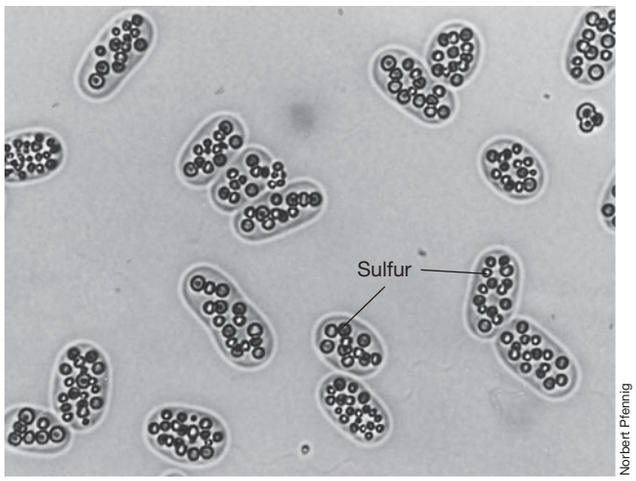
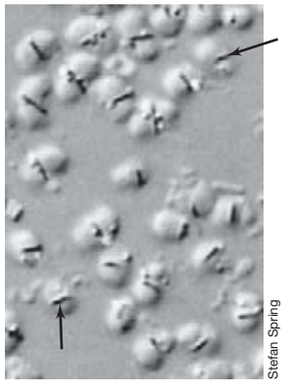


(b)

Figure 3.27 **Polyphosphate and sulfur storage products.** *(a)* Phase-contrast photomicrograph of cells of *Heliobacterium modesticaldum* showing polyphosphate as dark granules; a cell is about 1 μm wide. *(b)* Bright-field photomicrograph of cells of the purple sulfur bacterium *Isochromatium buderi*. The intracellular inclusions are sulfur globules formed from the oxidation of hydrogen sulfide (H_2S). A single cell is about 4 μm wide.



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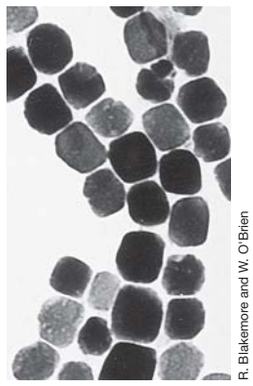

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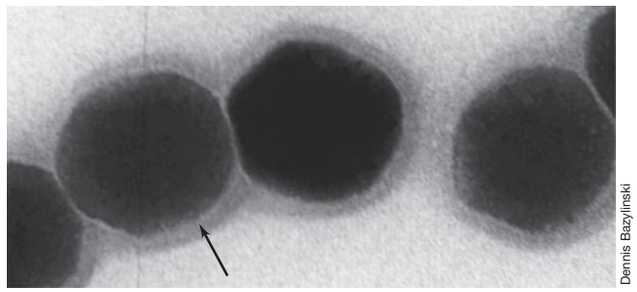


(c)

Figure 3.28 **Magnetotactic bacteria and magnetosomes.** *(a)* Differential interference contrast micrograph of coccoid magnetotactic bacteria; note chains of magnetosomes (arrows). A single cell is 2.2 μm wide. *(b)* Magnetosomes isolated from the magnetotactic bacterium *Magnetospirillum magnetotacticum*; each particle is about 50 nm wide. *(c)* Transmission electron micrograph of magnetosomes from a magnetic coccus. The arrow points to the membrane that surrounds each magnetosome. A single magnetosome is about 90 nm wide.

present. However, as the reduced sulfur source becomes limiting, the sulfur in the granules is oxidized to sulfate (SO_4^{2-}), and the granules slowly disappear as this reaction proceeds. Interestingly, although the sulfur globules appear to be in the cytoplasm they actually reside in the periplasm. The periplasm expands outward to accommodate the globules as H_2S is oxidized to S^0 and then contracts inward as S^0 is oxidized to SO_4^{2-}.

Magnetic Storage Inclusions: Magnetosomes

Some bacteria can orient themselves specifically within a magnetic field because they contain **magnetosomes**. These structures are intracellular particles of the iron mineral magnetite—Fe_3O_4 (**Figure 3.28**). Magnetosomes impart a magnetic dipole on a cell, allowing it to respond to a magnetic field. Bacteria that produce magnetosomes exhibit *magnetotaxis*, the process of orienting and migrating along Earth's magnetic field lines. Although the suffix "-taxis" is used in the word magnetotaxis, there is no evidence that magnetotactic bacteria employ the sensory systems of chemotactic or phototactic bacteria (Section 3.15). Instead, the alignment of magnetosomes in the cell simply imparts a magnetic moment that orients the cell in a particular direction in its environment.

The major function of magnetosomes is unknown. However, magnetosomes have been found in several aquatic organisms that grow best in laboratory culture at low O_2 concentrations. It has thus been hypothesized that one function of magnetosomes may be to guide these primarily aquatic cells downward (the direction of Earth's magnetic field) toward the sediments where O_2 levels are lower.

Magnetosomes are surrounded by a thin membrane containing phospholipids, proteins, and glycoproteins (Figure 3.28*b*, *c*). This membrane is not a true unit (bilayer) membrane, as is the cytoplasmic membrane (Figure 3.5), and the proteins present play a role in precipitating Fe^{3+} (brought into the cell in soluble form by chelating agents) as Fe_3O_4 in the developing magnetosome. A similar nonunit membrane surrounds granules of PHA. The morphology of magnetosomes appears to be species-specific, varying in shape from square to rectangular to spike-shaped in different species, forming into chains inside the cell (Figure 3.28).

MiniQuiz

- Under what growth conditions would you expect PHAs or glycogen to be produced?
- Why would it be impossible for gram-positive bacteria to store sulfur as gram-negative sulfur-oxidizing chemolithotrophs can?
- What form of iron is present in magnetosomes?

3.11 Gas Vesicles

Some prokaryotes are *planktonic*, meaning that they live a floating existence within the water column of lakes and the oceans. These organisms can float because they contain **gas vesicles**. These structures confer buoyancy on cells, allowing them to position themselves in a water column in response to environmental cues.

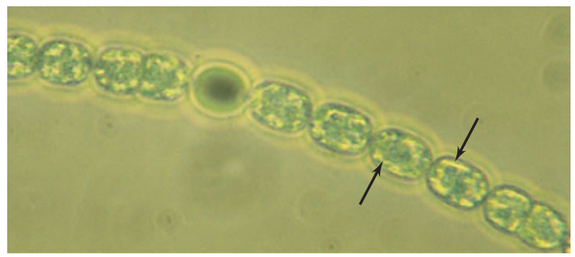
The most dramatic examples of gas-vesiculate bacteria are cyanobacteria that form massive accumulations called *blooms* in lakes or other bodies of water (**Figure 3.29**). Gas-vesiculate cells rise to the surface of the lake and are blown by winds into dense masses. Many primarily aquatic bacteria have gas vesicles and the property is found in both *Bacteria* and *Archaea*. By contrast, gas vesicles have never been found in eukaryotic microorganisms.

General Structure of Gas Vesicles

Gas vesicles are spindle-shaped structures made of protein; they are hollow yet rigid and of variable length and diameter (**Figure 3.30**). Gas vesicles in different organisms vary in length from about 300 to more than 1000 nm and in width from 45 to 120 nm, but the vesicles of a given organism are more or less of constant size. Gas vesicles may number from a few to hundreds per cell and are impermeable to water and solutes but permeable to gases. The presence of gas vesicles in cells can be determined either by light microscopy, where clusters of vesicles, called *gas vacuoles*, appear as irregular bright inclusions, or by transmission electron microscopy (Figure 3.30).

T. D. Brock

Figure 3.29 Buoyant cyanobacteria. Flotation of gas-vesiculate cyanobacteria that formed a bloom in a freshwater lake, Lake Mendota, Madison, Wisconsin (USA).



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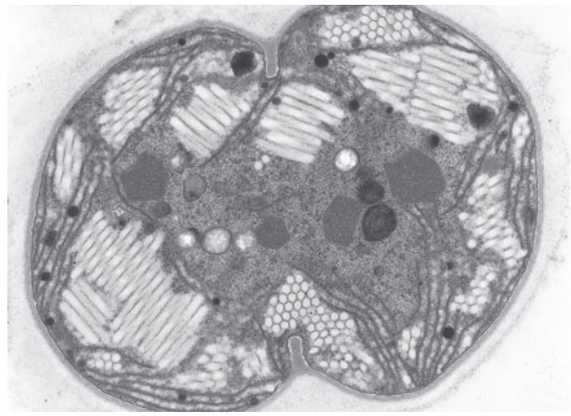


S. Pellegrini and M. Grilli Caiola

(b)

Figure 3.30 Gas vesicles of the cyanobacteria *Anabaena* and *Microcystis*. *(a)* Phase-contrast photomicrograph of *Anabaena*. Clusters of gas vesicles form phase-bright gas vacuoles (arrows). *(b)* Transmission electron micrograph of *Microcystis*. Gas vesicles are arranged in bundles, here seen in both longitudinal and cross section.

Molecular Structure of Gas Vesicles

The conical-shaped gas vesicle is composed of two different proteins. The major protein, called *GvpA*, forms the vesicle shell itself and is a small, hydrophobic, and very rigid protein. The rigidity is essential for the structure to resist the pressures exerted on it from outside. The minor protein, called *GvpC*, functions to strengthen the shell of the gas vesicle by cross-linking copies of GvpA (**Figure 3.31**).

Gas vesicles consist of copies of GvpA that align to yield parallel "ribs" that form the watertight shell. The ribs are then clamped by the GvpC protein, which binds the ribs at an angle to group several GvpA molecules together (Figure 3.31). Gas vesicles vary in shape in different organisms from long and thin to short and fat (compare Figures 3.30 and 3.31*a*), and shape is governed by how the GvpA and GvpC proteins interact to form the intact vesicle.

How do gas vesicles confer buoyancy, and what ecological benefit does buoyancy confer? The composition and pressure of the gas inside a gas vesicle is that of the gas in which the organism is suspended. However, because an inflated gas vesicle has a density of only about 10% of that of the cell proper, gas vesicles decrease cell density, thereby increasing its buoyancy. Phototrophic organisms in particular benefit from gas vesicles because they allow cells to adjust their vertical position in a water column to reach regions where the light intensity for photosynthesis is optimal.

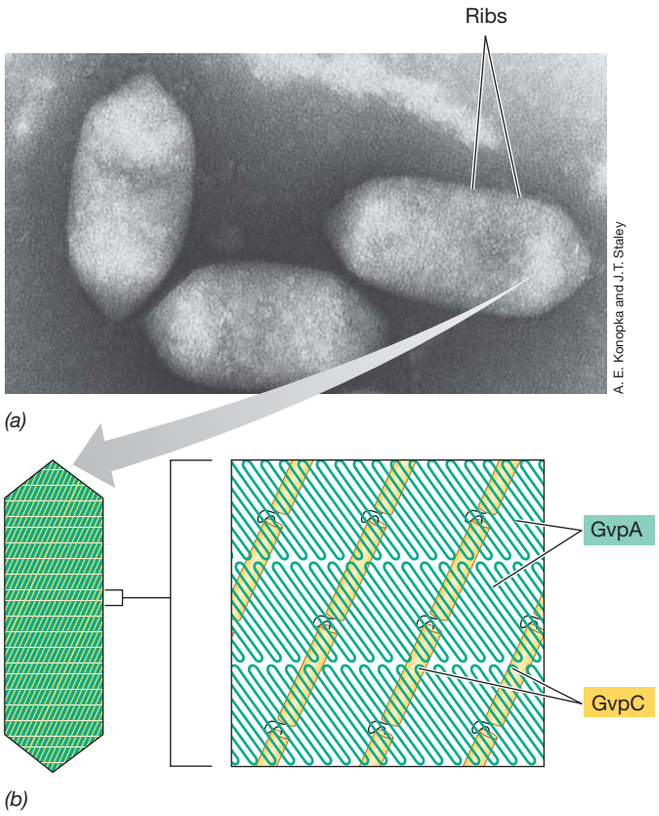


Figure 3.31 **Gas vesicle architecture.** Transmission electron micrographs of gas vesicles purified from the bacterium *Ancylobacter aquaticus* and examined in negatively stained preparations. A single vesicle is about 100 nm in diameter. *(b)* Model of how gas vesicle proteins GvpA and GvpC interact to form a watertight but gas-permeable structure. GvpA, a rigid β-sheet, makes up the rib, and GvpC, an α-helix structure, is the cross-linker.

MiniQuiz

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

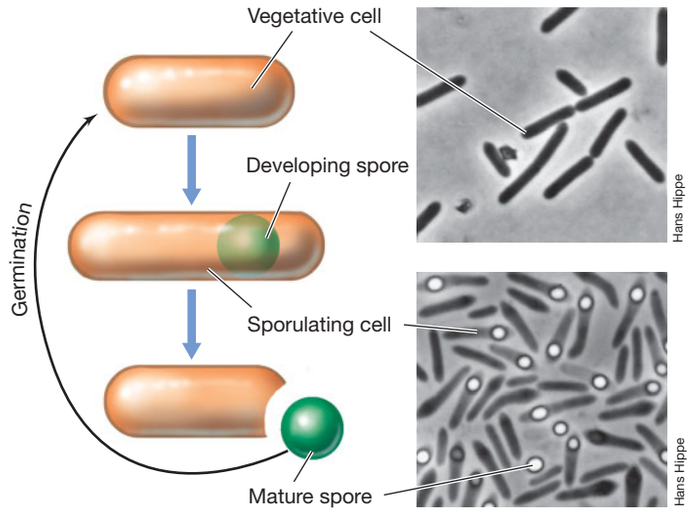


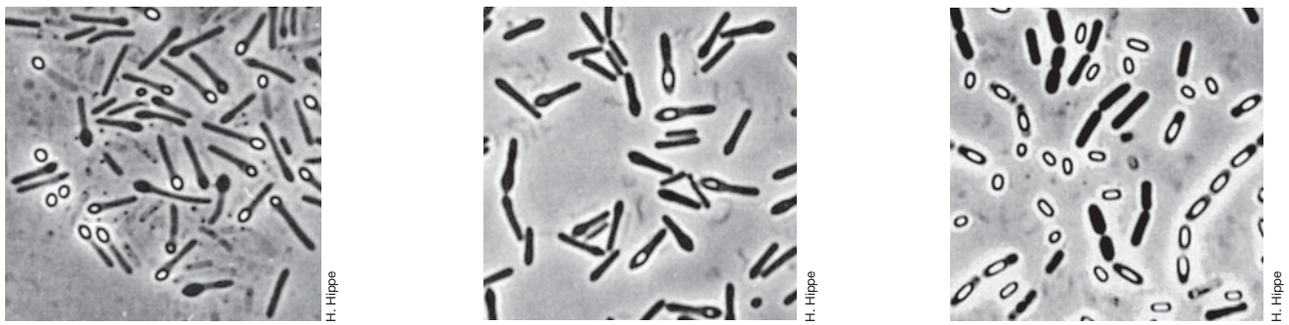
Figure 3.33 **The life cycle of an endospore-forming bacterium.** The phase-contrast photomicrographs are of cells of *Clostridium pascui*. A cell is about 0.8 μm wide.

3.12 Endospores

Certain species of *Bacteria* produce structures called **endospores** (**Figure 3.32**) during a process called *sporulation*. Endospores (the prefix *endo* means "within") are highly differentiated cells that are extremely resistant to heat, harsh chemicals, and radiation. Endospores function as survival structures and enable the organism to endure unfavorable growth conditions, including but not limited to extremes of temperature, drying, or nutrient depletion. Endospores can thus be thought of as the dormant stage of a bacterial life cycle: vegetative cell → endospore → vegetative cell. Endospores are also easily dispersed by wind, water, or through the animal gut. Endospore-forming bacteria are commonly found in soil, and species of *Bacillus* are the best-studied representatives.

Endospore Formation and Germination

During endospore formation, a vegetative cell is converted into a nongrowing, heat-resistant structure (**Figure 3.33**). Cells do not sporulate when they are actively growing but only when growth ceases owing to the exhaustion of an essential nutrient. Thus,



(a) **Terminal spores** *(b)* **Subterminal spores** *(c)* **Central spores**

Figure 3.32 **The bacterial endospore.** Phase-contrast photomicrographs illustrating endospore morphologies and intracellular locations in different species of endospore-forming bacteria. Endospores appear bright by phase-contrast microscopy.

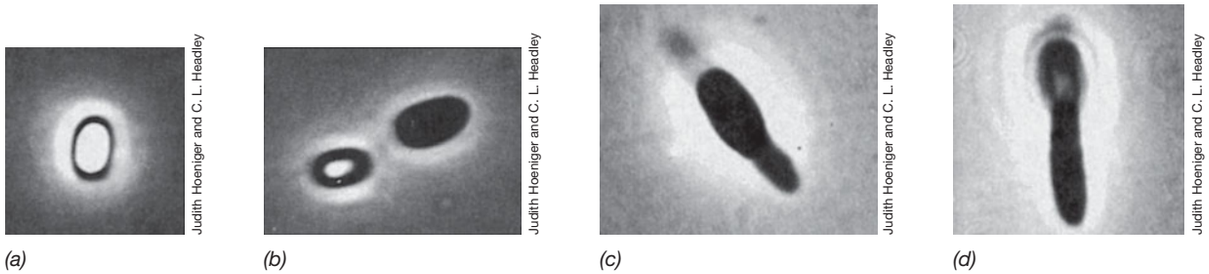


Figure 3.34 Endospore germination in *Bacillus*. Conversion of an endospore into a vegetative cell. The series of phase-contrast photomicrographs shows the sequence of events starting from *(a)* a highly refractile free endospore. *(b)* Activation: Refractility is being lost. *(c, d)* Outgrowth: The new vegetative cell is emerging.

cells of *Bacillus*, a typical endospore-forming bacterium, cease vegetative growth and begin sporulation when, for example, a key nutrient such as carbon or nitrogen becomes limiting.

An endospore can remain dormant for years (see the Microbial Sidebar, "Can an Endospore Live Forever?"), but it can convert back to a vegetative cell relatively rapidly. This process involves three steps: *activation*, *germination*, and *outgrowth* (**Figure 3.34**). Activation occurs when endospores are heated for several minutes at an elevated but sublethal temperature. Activated endospores are then conditioned to germinate when placed in the presence of specific nutrients, such as certain amino acids. Germination, typically a rapid process (on the order of several minutes), involves loss of microscopic refractility of the endospore, increased ability to be stained by dyes, and loss of resistance to heat and chemicals. The final stage, outgrowth, involves visible swelling due to water uptake and synthesis of RNA, proteins, and DNA. The cell emerges from the broken endospore and begins to grow, remaining in vegetative growth until environmental signals once again trigger sporulation.

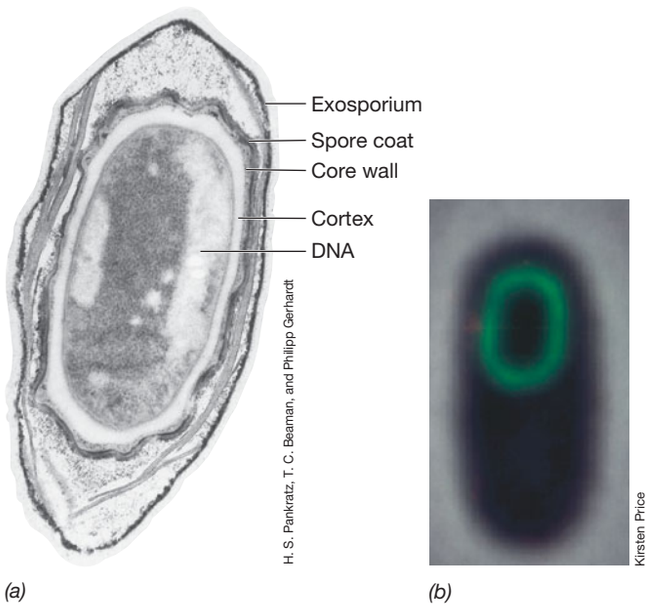


Figure 3.35 Structure of the bacterial endospore. *(a)* Transmission electron micrograph of a thin section through an endospore of *Bacillus megaterium*. *(b)* Fluorescent photomicrograph of a cell of *Bacillus subtilis* undergoing sporulation. The green color is a dye that specifically stains a sporulation protein in the spore coat.

Endospore Structure

Endospores stand out under the light microscope as strongly refractile structures (see Figures 3.32–3.34). Endospores are impermeable to most dyes, so occasionally they are seen as unstained regions within cells that have been stained with basic dyes such as methylene blue. To stain endospores, special stains and procedures must be used. In the classical endospore-staining protocol, malachite green is used as a stain and is infused into the spore with steam.

The structure of the endospore as seen with the electron microscope differs distinctly from that of the vegetative cell (**Figure 3.35**). In particular, the endospore is structurally more complex in that it has many layers that are absent from the vegetative cell. The outermost layer is the *exosporium*, a thin protein covering. Within this are the *spore coats*, composed of layers of spore-specific proteins (Figure 3.35*b*). Below the spore coat is the *cortex*, which consists of loosely cross-linked peptidoglycan, and inside the cortex is the *core*, which contains the core wall, cytoplasmic membrane, cytoplasm, nucleoid, ribosomes, and other cellular essentials. Thus, the endospore differs structurally from the vegetative cell primarily in the kinds of structures found outside the core wall.

One substance that is characteristic of endospores but absent from vegetative cells is **dipicolinic acid** (**Figure 3.36**), which accumulates in the core. Endospores are also enriched in calcium (Ca^{2+}), most of which is complexed with dipicolinic acid (Figure 3.36*b*). The calcium–dipicolinic acid complex represents about

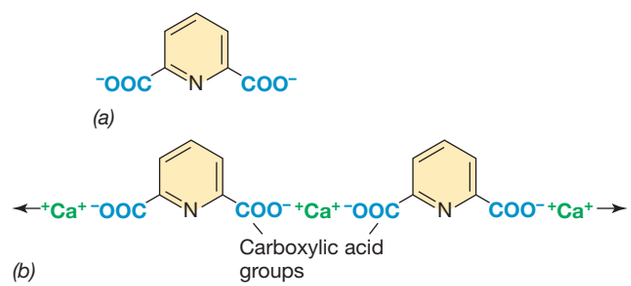
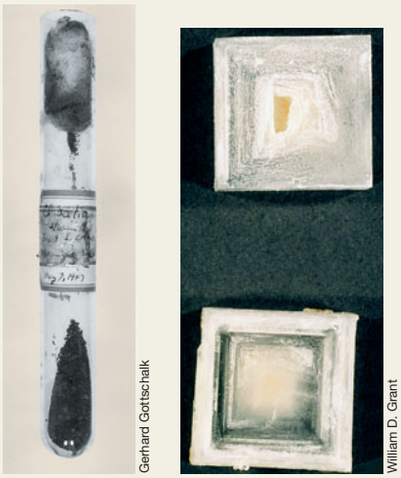


Figure 3.36 Dipicolinic acid (DPA). *(a)* Structure of DPA. *(b)* How Ca^{2+} cross-links DPA molecules to form a complex.

Can an Endospore Live Forever?

In this chapter we have emphasized the dormancy and resistance of bacterial endospores and have pointed out that endospores can survive for long periods in a dormant state. But how long is *long*?

It is clear from experiments that endospores can remain alive for at least several decades. For example, a suspension of endospores of the bacterium *Clostridium aceticum* (Figure 1) prepared in 1947 was placed in sterile growth medium in 1981, 34 years later, and in less than 12 h growth commenced, leading to a robust pure culture. *C. aceticum* was originally isolated by the Dutch scientist K.T. Wieringa in 1940 but was thought to have been lost until the 1947 vial of *C. aceticum* endospores was found in a storage room at the University of California at Berkeley and revived.[1]



(a) *(b)*

Figure 1 **Longevity of endospores.** *(a)* A tube containing endospores from the bacterium *Clostridium aceticum* prepared on May 7, 1947. After remaining dormant for over 30 years, the endospores were suspended in a culture medium after which growth occurred within 12 h. *(b)* Halophilic bacteria trapped within salt crystals. These two crystals (about 1 cm in diameter) were grown in the laboratory in the presence of *Halobacterium* cells (orange) that remain viable in the crystals. Crystals similar to these but of Permian age (~250 million years old) were reported to contain viable halophilic endosporulating bacteria.

Other, more dramatic examples of endospore longevity have been well documented. Bacteria of the genus *Thermoactinomyces* are widespread in soil, plant litter, and fermenting plant material. Microbiological examination of a 2000-year-old Roman archaeological site in the United Kingdom yielded significant numbers of viable *Thermoactinomyces* endospores in various pieces of debris. Additionally, *Thermoactinomyces* endospores were recovered from lake sediments known to be over 9000 years old. Although contamination is always a possibility in such studies, samples in both of these cases were processed in such a way as to virtually rule out contamination with "recent" endospores. Thus, endospores can last for several thousands of years, but is this the limit? As we will see, apparently not.

What factors could limit the age of an endospore? Cosmic radiation has been considered a major factor because it can introduce mutations in DNA. It has been hypothesized that over thousands of years, the cumulative effects of cosmic radiation could introduce so many mutations into the genome of an organism that even highly radiation-resistant structures such as endospores would succumb to the genetic damage. However, if the endospores were partially shielded from cosmic radiation, for example, by being embedded in layers of organic matter (such as in the Roman archaeological dig or the lake sediments described above), they might well be able to survive several hundred thousand years. Amazing, but is *this* the upper limit?

In 1995 a group of scientists reported the revival of bacterial endospores they claimed were 25–40 million years old.[2] The endospores were allegedly preserved in the gut of an extinct bee trapped in amber of known geological age. The presence of endospore-forming bacteria in these bees was previously suspected because electron microscopic studies of the insect gut showed endospore-like structures (see Figure 3.35*a*) and because *Bacillus* DNA was recovered from the insect. Incredibly, samples of bee tissue incubated in a sterile culture medium quickly yielded endospore-forming bacteria. Rigorous precautions were taken to demonstrate that the endospore-forming bacterium revived from the amber-encased bee was not a modern-day contaminant. Subsequently, an even more spectacular claim was made that halophilic (salt-loving) endospore-forming bacteria had been isolated from fluid inclusions in salt crystals of Permian age, over 250 million years old.[3] These cells were presumably trapped in brines within the crystal (Figure 1*b*) as it formed and then remained dormant for more than a quarter billion years! Molecular experiments on even older material, 425-million-year-old halite, showed evidence for prokaryotic inhabitants as well.[4]

If these astonishing claims are supported by repetition of the results in independent laboratories, then it appears that endospores stored under the proper conditions can remain viable indefinitely. This is remarkable testimony to a structure that undoubtedly evolved as a means of surviving relatively brief dormant periods or as a mechanism to withstand drying, but that turned out to be so well designed that survival for millions or even billions of years may be possible.

[1]Braun, M., F. Mayer, and G. Gottschalk. 1981. *Clostridium aceticum* (Wieringa), a microorganism producing acetic acid from molecular hydrogen and carbon dioxide. *Arch. Microbiol. 128*: 288–293.

[2]Cano, R.J., and M.K. Borucki. 1995. Revival and identification of bacterial spores in 25- to 40-million-year-old Dominican amber. *Science 268*: 1060–1064.

[3]Vreeland, R.H., W.D. Rosenzweig, and D.W. Powers. 2000. Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. *Nature 407*: 897–900.

[4]Fish, S.A., T.J. Shepherd, T.J. McGenity, and W.D. Grant. 2002. Recovery of 16S ribosomal RNA gene fragments from ancient halite. *Nature 417*: 432–436.

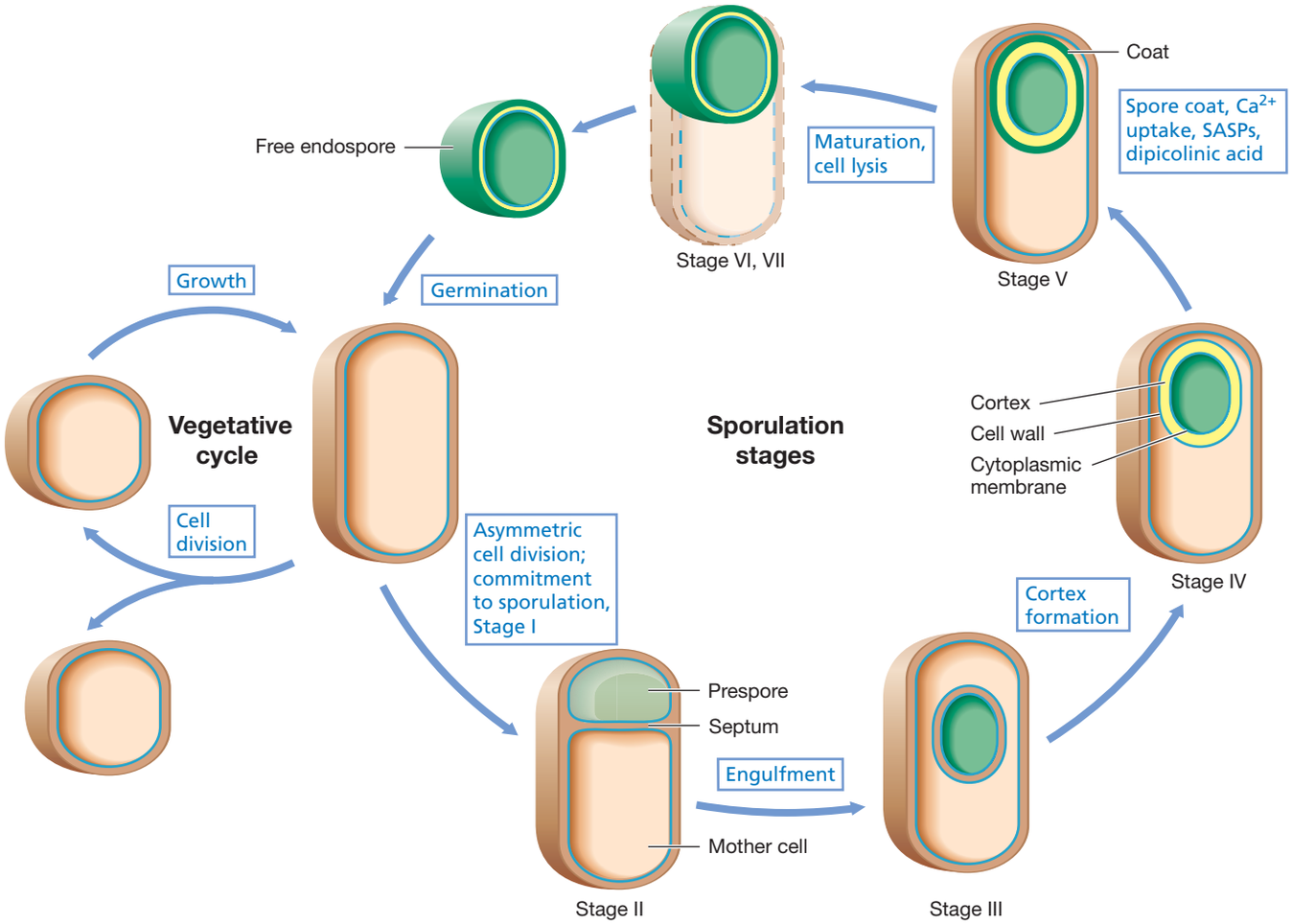


Figure 3.37 Stages in endospore formation. The stages are defined from genetic and microscopic analyses of sporulation in *Bacillus subtilis*, the model organism for studies of sporulation.

10% of the dry weight of the endospore, and functions to bind free water within the endospore, thus helping to dehydrate it. In addition, the complex intercalates (inserts between bases) in DNA, which stabilizes DNA against heat denaturation.

The Endospore Core and SASPs

Although both contain a copy of the chromosome and other essential cellular components, the core of a mature endospore differs greatly from the vegetative cell from which it was formed. Besides the high levels of calcium dipicolinate (Figure 3.36), which help reduce the water content of the core, the core becomes greatly dehydrated during the sporulation process. The core of a mature endospore has only 10–25% of the water content of the vegetative cell, and thus the consistency of the core cytoplasm is that of a gel. Dehydration of the core greatly increases the heat resistance of macromolecules within the spore. Some bacterial endospores survive heating to temperatures as high as 150°C, although 121°C, the standard for microbiological sterilization (121°C is autoclave temperature, ↻ Section 26.1), kills the endospores of most species. Boiling has essentially no effect on endospore viability. Dehydration has also been shown to confer resistance in the endospore to chemicals, such as hydrogen peroxide (H_2O_2), and causes enzymes remaining in the core to become inactive. In addition to the low water content of the endospore, the pH of the core is about one unit lower than that of the vegetative cell cytoplasm.

The endospore core contains high levels of *small acid-soluble proteins* (SASPs). These proteins are made during the sporulation process and have at least two functions. SASPs bind tightly to DNA in the core and protect it from potential damage from ultraviolet radiation, desiccation, and dry heat. Ultraviolet resistance is conferred when SASPs change the molecular structure of DNA from the normal "B" form to the more compact "A" form. A-form DNA better resists pyrimidine dimer formation by UV radiation, a means of mutation (↻ Section 10.4), and resists the denaturing effects of dry heat. In addition, SASPs function as a carbon and energy source for the outgrowth of a new vegetative cell from the endospore during germination.

The Sporulation Process

Sporulation is a complex series of events in cellular differentiation; many genetically directed changes in the cell underlie the conversion from vegetative growth to sporulation. The structural changes occurring in sporulating cells of *Bacillus* are shown in **Figure 3.37**. Sporulation can be divided into several stages. In

Bacillus subtilis, where detailed studies have been done, the entire sporulation process takes about 8 hours and begins with asymmetric cell division (Figure 3.37). Genetic studies of mutants of *Bacillus*, each blocked at one of the stages of sporulation, indicate that more than 200 spore-specific genes exist. Sporulation requires a significant regulatory response in that the synthesis of many vegetative proteins must cease while endospore proteins are made. This is accomplished by the activation of several families of endospore-specific genes in response to an environmental trigger to sporulate. The proteins encoded by these genes catalyze the series of events leading from a moist, metabolizing, vegetative cell to a relatively dry, metabolically inert, but extremely resistant endospore (**Table 3.3**). In Section 8.12 we examine some of the molecular events that control the sporulation process.

Diversity and Phylogenetic Aspects of Endospore Formation

Nearly 20 genera of *Bacteria* form endospores, although the process has only been studied in detail in a few species of *Bacillus* and *Clostridium*. Nevertheless, many of the secrets to endospore survival, such as the formation of calcium–dipicolinate complexes (Figure 3.36) and the production of endospore-specific proteins, seem universal. Although some of the details of sporulation may vary from one organism to the next, the general principles seem to be the same in all endosporulating bacteria.

From a phylogenetic perspective, the capacity to produce endospores is found only in a particular sublineage of the gram-positive bacteria. Despite this, the physiologies of endospore-forming bacteria are highly diverse and include anaerobes, aerobes, phototrophs, and chemolithotrophs. In light of this physiological diversity, the actual triggers for endospore formation may vary with different species and could include signals other than simple nutrient starvation, the major trigger for endospore formation in *Bacillus*. No *Archaea* have been shown to form endospores, suggesting that the capacity to produce endospores evolved sometime after the major prokaryotic lineages diverged billions of years ago (↻ Figure 1.6).

Table 3.3 *Differences between endospores and vegetative cells*

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

MiniQuiz

- What is dipicolinic acid and where is it found?
- What are SASPs and what is their function?
- What happens when an endospore germinates?

V Microbial Locomotion

We finish our survey of microbial structure and function by considering cell locomotion. Most microbial cells can move under their own power, and motility allows cells to reach different parts of their environment. In nature, movement may present new opportunities and resources for a cell and be the difference between life and death.

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

3.13 Flagella and Motility

Many prokaryotes are motile by swimming, and this function is due to a structure called the **flagellum** (plural, flagella) (**Figure 3.38**). The flagellum functions by rotation to push or pull the cell through a liquid medium.

Flagella of *Bacteria*

Bacterial flagella are long, thin appendages free at one end and attached to the cell at the other end. Bacterial flagella are so thin (15–20 nm, depending on the species) that a single flagellum can be seen with the light microscope only after being stained with special stains that increase their diameter (Figure 3.38). However, flagella are easily seen with the electron microscope (**Figure 3.39**).

Flagella can be attached to cells in different places. In **polar flagellation**, the flagella are attached at one or both ends of a cell. Occasionally a group of flagella (called a *tuft*) may arise at one end of the cell, a type of polar flagellation called *lophotrichous* (Figure 3.38*c*). Tufts of flagella can often be seen in unstained

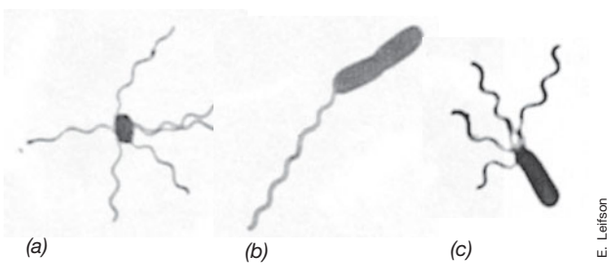
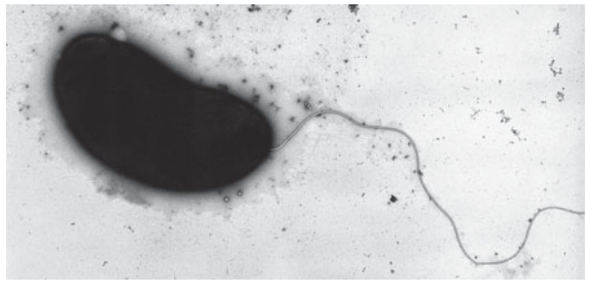
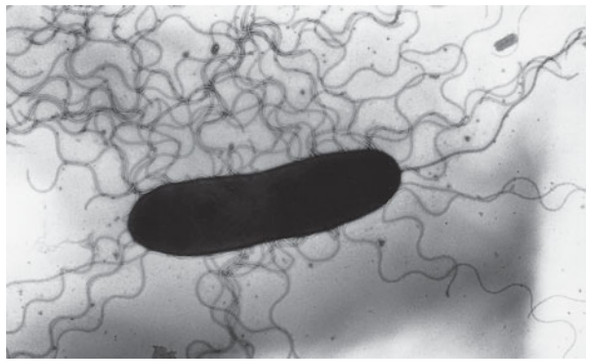


Figure 3.38 **Bacterial flagella.** Light photomicrographs of prokaryotes containing different arrangements of flagella. Cells are stained with Leifson flagella stain. *(a)* Peritrichous. *(b)* Polar. *(c)* Lophotrichous.



(a)



(b)

Figure 3.39 **Bacterial flagella as observed by negative staining in the transmission electron microscope.** *(a)* A single polar flagellum. *(b)* Peritrichous flagella. Both micrographs are of cells of the phototrophic bacterium *Rhodospirillum centenum*, which are about 1.5 μm wide. Cells of *R. centenum* are normally polarly flagellated but under certain growth conditions form peritrichous flagella. See Figure 3.49*b* for a photo of colonies of *R. centenum* cells that move toward an increasing gradient of light (phototaxis).

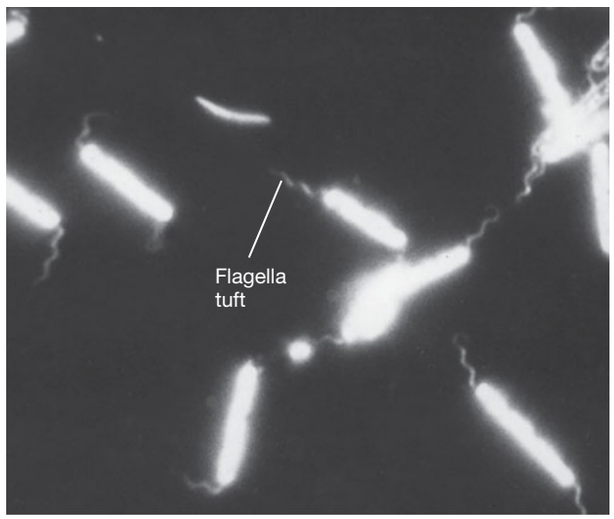
cells by dark-field or phase-contrast microscopy (**Figure 3.40**). When a tuft of flagella emerges from both poles of the cell, flagellation is called *amphitrichous*. In **peritrichous flagellation** (Figures 3.38*a* and 3.39*b*), flagella are inserted at many locations around the cell surface. The type of flagellation, polar or peritrichous, is a characteristic used in the classification of bacteria.

Flagellar Structure

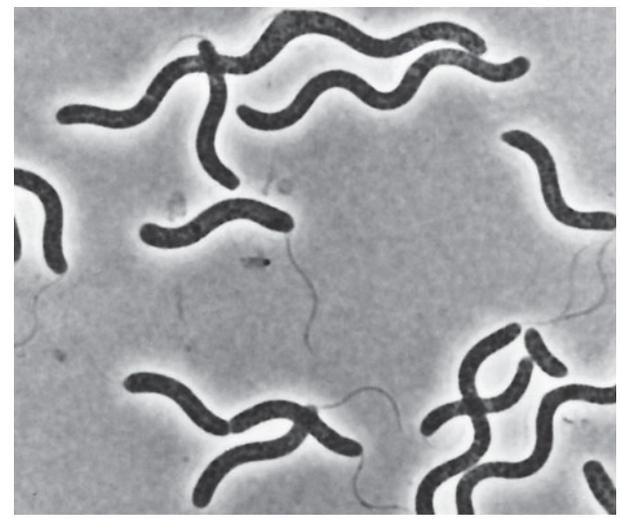
Flagella are not straight but are actually helical. When flattened, flagella show a constant distance between adjacent curves, called the *wavelength*, and this wavelength is characteristic for the flagella of any given species (Figures 3.38–3.40). The filament of a bacterial flagellum is composed of many copies of a protein called *flagellin*. The shape and wavelength of the flagellum are in part determined by the structure of the flagellin protein and also to some extent by the direction of rotation of the filament. Flagellin is highly conserved in amino acid sequences in species of *Bacteria*, suggesting that flagellar motility evolved early and has deep roots within this domain.

A flagellum consists of several components and moves by rotation, much like a propeller of a boat motor. The base of the flagellum is structurally different from the filament. There is a wider region at the base of the filament called the *hook*. The hook consists of a single type of protein and connects the filament to the motor portion in the base (**Figure 3.41**).

The motor is anchored in the cytoplasmic membrane and cell wall. The motor consists of a central rod that passes through a series of rings. In gram-negative bacteria, an outer ring, called the *L ring*, is anchored in the lipopolysaccharide layer. A second ring, called the *P ring*, is anchored in the peptidoglycan layer of the cell wall. A third set of rings, called the *MS* and *C rings*, are located within the cytoplasmic membrane and the cytoplasm,



(a)



(b)

Figure 3.40 **Bacterial flagella observed in living cells.** *(a)* Dark-field photomicrograph of a group of large rod-shaped bacteria with flagellar tufts at each pole (amphitrichous flagellation). A single cell is about 2 μm wide. *(b)* Phase-contrast photomicrograph of cells of the large phototrophic purple bacterium *Rhodospirillum photometricum* with a tuft of lophotrichous flagella that emanate from one of the poles. A single cell measures about 3 × 30 μm.

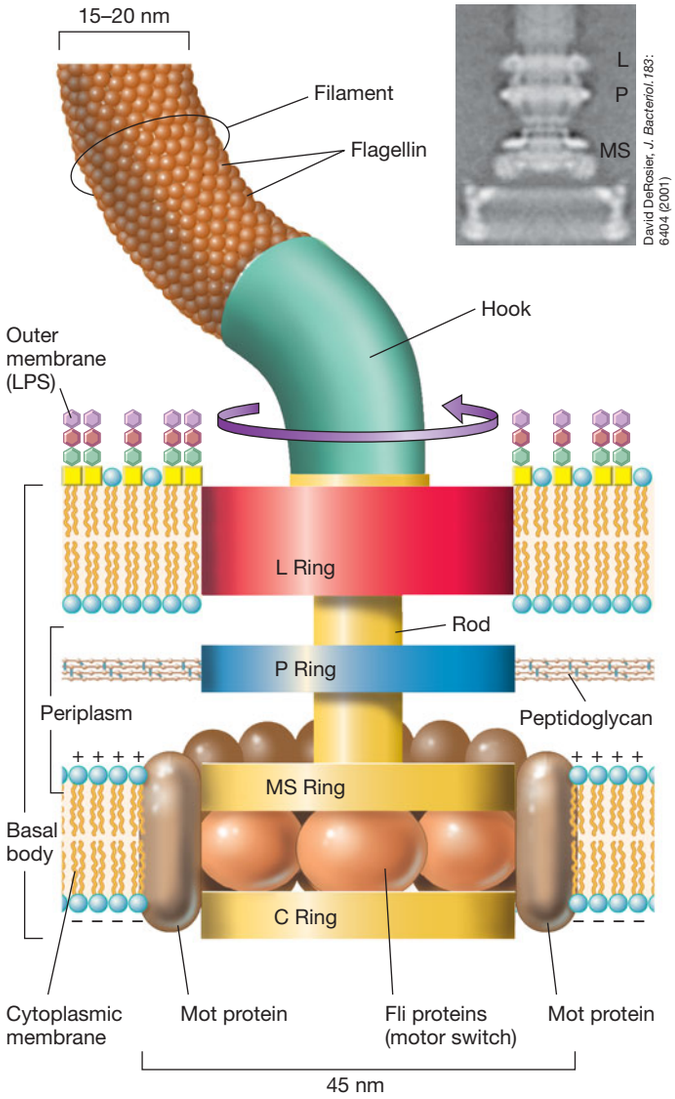


Figure 3.41 **Structure and function of the flagellum in gram-negative *Bacteria*.** *(a)* Structure. The L ring is embedded in the LPS and the P ring in peptidoglycan. The MS ring is embedded in the cytoplasmic membrane and the C ring in the cytoplasm. A narrow channel exists in the rod and filament through which flagellin molecules diffuse to reach the site of flagellar synthesis. The Mot proteins function as the flagellar motor, whereas the Fli proteins function as the motor switch. The flagellar motor rotates the filament to propel the cell through the medium. Inset: transmission electron micrograph of a flagellar basal body from *Salmonella enterica* with the various rings labeled. *(b)* Function. A "proton turbine" model has been proposed to explain rotation of the flagellum. Protons, flowing through the Mot proteins, may exert forces on charges present on the C and MS rings, thereby spinning the rotor.

respectively (Figure 3.41*a*). In gram-positive bacteria, which lack an outer membrane, only the inner pair of rings is present. Surrounding the inner ring and anchored in the cytoplasmic membrane are a series of proteins called *Mot proteins*. A final set of proteins, called the *Fli proteins* (Figure 3.41*a*), function as the motor switch, reversing the direction of rotation of the flagella in response to intracellular signals.

Flagellar Movement

The flagellum is a tiny rotary motor. How does this motor work? Rotary motors contain two main components: the *rotor* and the *stator*. In the flagellar motor, the rotor consists of the central rod and the L, P, C, and MS rings. Collectively, these structures make up the **basal body**. The stator consists of the Mot proteins that surround the basal body and function to generate torque.

Rotation of the flagellum is imparted by the basal body. The energy required for rotation of the flagellum comes from the proton motive force (↻ Section 4.10). Proton movement across the cytoplasmic membrane through the Mot complex drives rotation of the flagellum (Figure 3.41). About 1000 protons are translocated per rotation of the flagellum, and a model for how this could work is shown in Figure 3.41*b*. In this model called the proton turbine model, protons flowing through channels in the Mot proteins exert electrostatic forces on helically arranged charges on the rotor proteins. Attractions between positive and negative charges would then cause the basal body to rotate as protons flow though the Mot proteins. www.microbiologyplace.com **Online Tutorial 3.1: The Prokaryotic Flagellum**

Archaeal Flagella

Besides *Bacteria*, flagellar motility is also widespread among species of *Archaea*; major genera of methanogens, extreme halophiles, thermoacidophiles, and hyperthermophiles are all capable of swimming motility. Archaeal flagella are roughly half the diameter of bacterial flagella, measuring only 10–13 nm in width (**Figure 3.42**), but impart movement to the cell by rotating, as do flagella in *Bacteria*. However, unlike *Bacteria*, in which a single type of protein makes up the flagellar filament, several different flagellin proteins are known from *Archaea*, and their amino acid sequences and genes that encode them bear no relationship to those of bacterial flagellin.

Studies of swimming cells of the extreme halophile *Halobacterium* show that they swim at speeds only about one-tenth that of cells of *Escherichia coli*. Whether this holds for all *Archaea* is

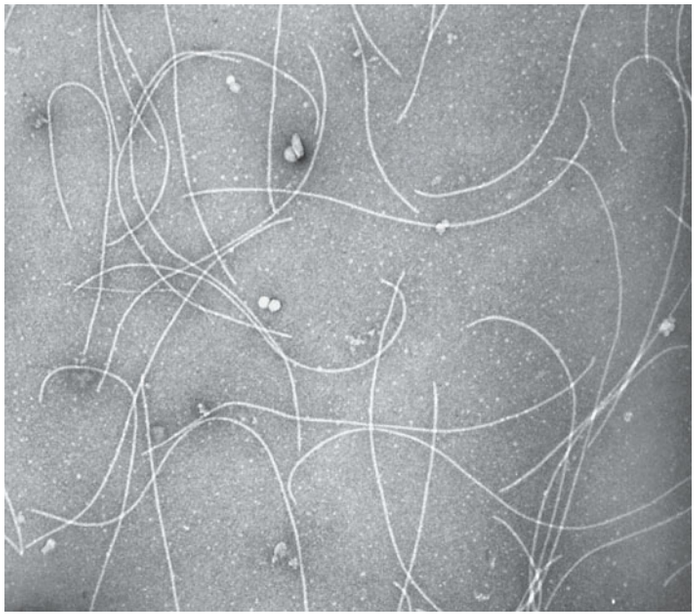


Figure 3.42 Archaeal flagella. Transmission electron micrograph of flagella isolated from cells of the methanogen *Methanococcus maripaludis*. A single flagellum is about 12 nm wide.

unknown, but the significantly smaller diameter of the archaeal flagellum compared with the bacterial flagellum would naturally reduce the torque and power of the flagellar motor such that slower swimming speeds would be expected. Moreover, from biochemical experiments with *Halobacterium* it appears that archaeal flagella are powered directly by ATP rather than by the proton motive force, the source of energy for the flagella of *Bacteria* (Figure 3.41). If this holds for the flagella of all motile *Archaea*, it would mean that the flagellar motors of *Archaea* and *Bacteria* employ fundamentally different mechanisms. Coupled with the clear differences in flagellar protein structure, this suggests that flagellar motility in *Bacteria* and *Archaea* evolved after the two prokaryotic domains had diverged over 3 billion years ago (↻ Figure 1.6*b*).

Flagellar Synthesis

Several gene products are required to support motility in *Bacteria*. In *Escherichia coli* and *Salmonella enterica* (*typhimurium*), where studies have been most extensive, over 50 genes are linked to motility. These genes have several functions, including encoding structural proteins of the flagellum and motor apparatus, export of flagellar proteins through the cytoplasmic membrane to the outside of the cell, and regulation of the many biochemical events surrounding the synthesis of new flagella.

A flagellar filament grows not from its base, as does an animal hair, but from its tip. The MS ring is synthesized first and inserted into the cytoplasmic membrane. Then other anchoring proteins are synthesized along with the hook before the filament forms (**Figure 3.43**). Flagellin molecules synthesized in the cytoplasm pass up through a 3-nm channel inside the filament and add on at the terminus to form the mature flagellum. At the end of the growing flagellum a protein "cap" exists. Cap proteins assist flagellin molecules that have diffused through the channel to organize at the flagellum termini to form new filament (Figure 3.43). Approximately 20,000 flagellin protein molecules are needed to make one filament. The flagellum grows more or less continuously until it reaches its final length. Broken flagella still rotate and can be repaired with new flagellin units passed through the filament channel to replace the lost ones.

Cell Speed and Motion

In *Bacteria*, flagella do not rotate at a constant speed but instead increase or decrease their rotational speed in relation to the strength of the proton motive force. Flagella can rotate at up to 300 revolutions per second and propel cells through a liquid at up to 60 cell lengths/sec. By contrast, the fastest known animal, the cheetah, moves at a maximum rate of about 25 body lengths/sec. Thus, when size is taken into account, a bacterial cell swimming at 60 lengths/sec is actually moving twice as fast as the fastest animal!

The swimming motions of polarly and lophotrichously flagellated organisms differ from those of peritrichously flagellated organisms, and these can be distinguished microscopically (**Figure 3.44**). Peritrichously flagellated organisms typically move in a

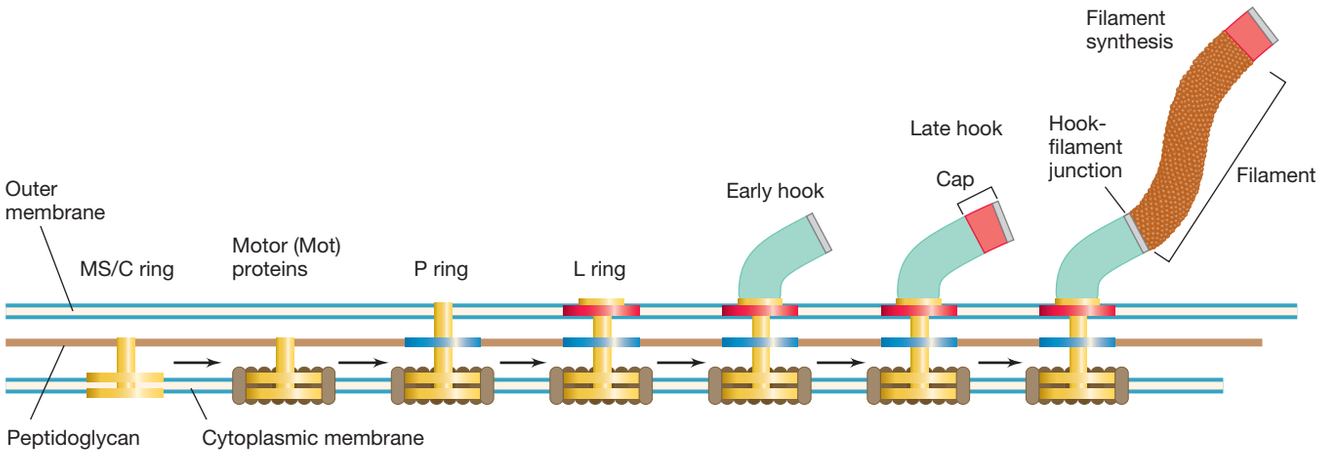
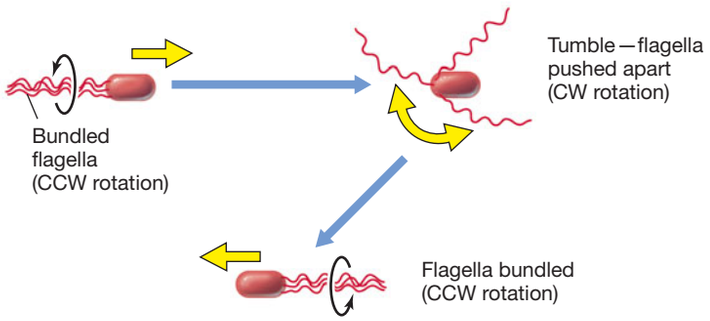
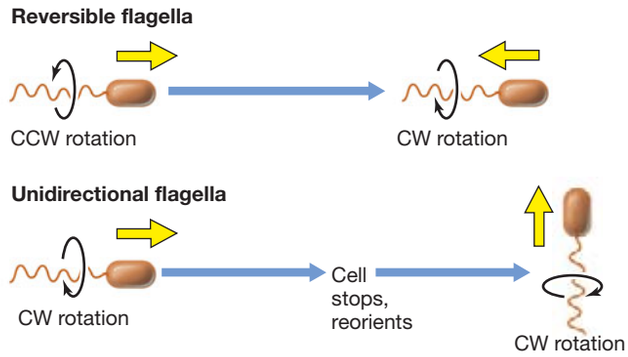


Figure 3.43 Flagella biosynthesis. Synthesis begins with assembly of MS and C rings in the cytoplasmic membrane, followed by the other rings, the hook, and the cap. Flagellin protein flows through the hook to form the filament and is guided into position by cap proteins.



(a) **Peritrichous**



(b) **Polar**

Figure 3.44 **Movement in peritrichously and polarly flagellated prokaryotes.** *(a)* Peritrichous: Forward motion is imparted by all flagella rotating counterclockwise (CCW) in a bundle. Clockwise (CW) rotation causes the cell to tumble, and then a return to counterclockwise rotation leads the cell off in a new direction. *(b)* Polar: Cells change direction by reversing flagellar rotation (thus pulling instead of pushing the cell) or, with unidirectional flagella, by stopping periodically to reorient, and then moving forward by clockwise rotation of its flagella. The yellow arrows show the direction the cell is traveling.

straight line in a slow, deliberate fashion. Polarly flagellated organisms, on the other hand, move more rapidly, spinning around and seemingly dashing from place to place. The different behavior of flagella on polar and peritrichous organisms, including differences in reversibility of the flagellum, is illustrated in Figure 3.44.

Swimming speed is a genetically governed property because different motile species, even different species that are the same cell size, can swim at different maximum speeds. When assessing the capacity of a laboratory culture of a bacterium for swimming motility and swimming speed, observations should only be made on young cultures. In old cultures, otherwise motile cells often stop swimming and the culture may appear to be nonmotile.

MiniQuiz

- Cells of the rod-shaped *Salmonella* are peritrichously flagellated, those of the rod-shaped *Pseudomonas* polarly flagellated, and those of *Spirillum* lophotrichously flagellated. Sketch the three different cells here, showing how their flagella are arranged.
- Compare the flagella of *Bacteria* and *Archaea* in terms of their structure and function.

3.14 Gliding Motility

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

Diversity of Gliding Motility

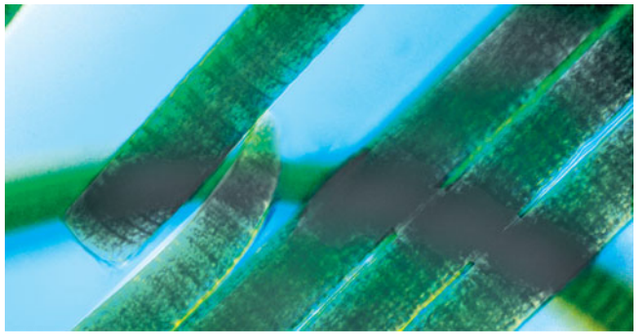
Gliding motility is widely distributed among *Bacteria* but has been well studied in only a few groups. The gliding movement itself—up to 10 μm/sec in some gliding bacteria—is considerably slower than propulsion by flagella but still offers the cell a means of moving about its habitat.

Gliding prokaryotes are filamentous or rod-shaped cells (**Figure 3.45**), and the gliding process requires that the cells be in contact with a solid surface. The morphology of colonies of a typical gliding bacterium are distinctive, because cells glide out and move away from the center of the colony (Figure 3.45*c*). Perhaps the best-known gliding bacteria are the filamentous cyanobacteria (Figure 3.45*a*, *b*), certain gram-negative *Bacteria* such as *Myxococcus* and other myxobacteria, and species of *Cytophaga* and *Flavobacterium* (Figure 3.45*c*, *d*). No gliding *Archaea* are known, but once some of the *Archaea* that have been detected in soil using molecular techniques (↻ Section 2.11) are isolated, gliding species would not be surprising.

Mechanisms of Gliding Motility

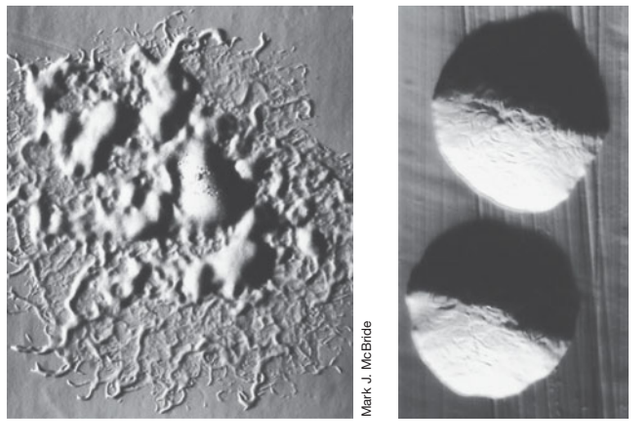
Although no gliding mechanism is thoroughly understood, it is clear that more than one mechanism is responsible for gliding motility. Cyanobacteria (phototrophic bacteria, Figure 3.45*a*, *b*) glide by secreting a polysaccharide slime on the outer surface of the cell. The slime contacts both the cell surface and the solid surface against which the cell moves. As the excreted slime adheres to the surface, the cell is pulled along. This mechanism is supported by the identification of slime-excreting pores on the cell surface of gliding filamentous cyanobacteria. The nonphototrophic gliding bacterium *Cytophaga* also moves at the expense of slime excretion, rotating along its long axis as it does.

Cells capable of "twitching motility" also display a form of gliding motility using a mechanism by which repeated extension and retraction of type IV pili propel the cell along a surface (Section 3.9). The gliding myxobacterium *Myxococcus xanthus* has two forms of gliding motility. One form is driven by type IV pili whereas the other is distinct from either the type IV pili or the slime extrusion methods. In this form of *M. xanthus* motility a protein adhesion complex is formed at one pole of the rod-shaped cell and remains at a fixed position on the surface as the cell glides forward. This means that the adhesion complex moves in the direction opposite that of the cell, presumably fueled by some sort of cytoplasmic motility engine perhaps linked to the cell cytoskeleton (↻ Section 5.3). These different forms of motility can be expressed at the same time and are somehow coordinated by the cell, presumably in response to various signals from the environment (Section 3.15).



(a)

(b)



(c) (d)

Figure 3.45 **Gliding bacteria.** *(a, b)* The filamentous cyanobacterium *Oscillatoria* has cells about 35 μm wide. *(b) Oscillatoria* filaments gliding on an agar surface. *(c)* Masses of the bacterium *Flavobacterium johnsoniae* gliding away from the center of the colony (the colony is about 2.7 mm wide). *(d)* Nongliding mutant strain of *F. johnsoniae* showing typical colony morphology of nongliding bacteria (the colonies are 0.7–1 mm in diameter). See also Figure 3.46.

Neither slime extrusion nor twitching is the mechanism of gliding in other gliding bacteria. In *Flavobacterium johnsoniae* (Figure 3.45*c*), for example, no slime is excreted and the cells lack type IV pili. Instead, the movement of proteins on the cell surface may be the mechanism of gliding in this organism. Specific motility proteins anchored in the cytoplasmic and outer membranes are thought to propel cells of *F. johnsoniae* forward by a ratcheting mechanism (**Figure 3.46**). Movement of gliding-specific proteins in the cytoplasmic membrane is driven by energy from

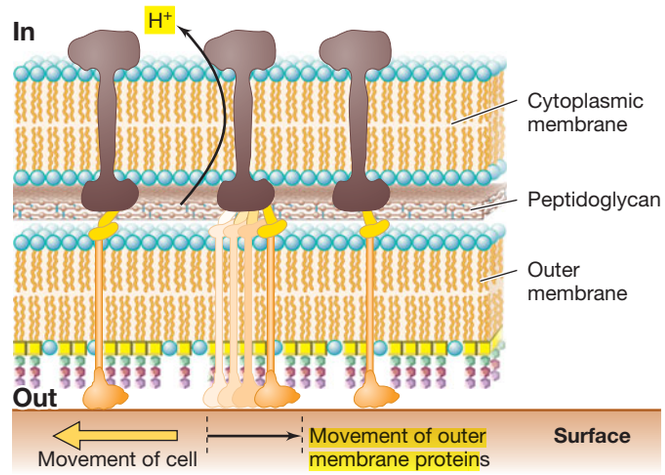


Figure 3.46 **Gliding motility in *Flavobacterium johnsoniae*.** Tracks (yellow) exist in the peptidoglycan that connect cytoplasmic proteins (brown) to outer membrane proteins (orange) and propel the outer membrane proteins along the solid surface. Note that the outer membrane proteins and the cell proper move in opposite directions.

the proton motive force that is somehow transmitted to gliding-specific proteins in the outer membrane. It is thought that movement of these proteins against the solid surface literally pulls the cell forward (Figure 3.46).

Like other forms of motility, gliding motility has significant ecological relevance. Gliding allows a cell to exploit new resources and to interact with other cells. In the latter regard, it is of interest that myxobacteria, such as *Myxococcus xanthus*, have a very social and cooperative lifestyle. In these bacteria gliding motility may play an important role in the cell-to-cell interactions that are necessary to complete their life cycle (↻ Section 17.17).

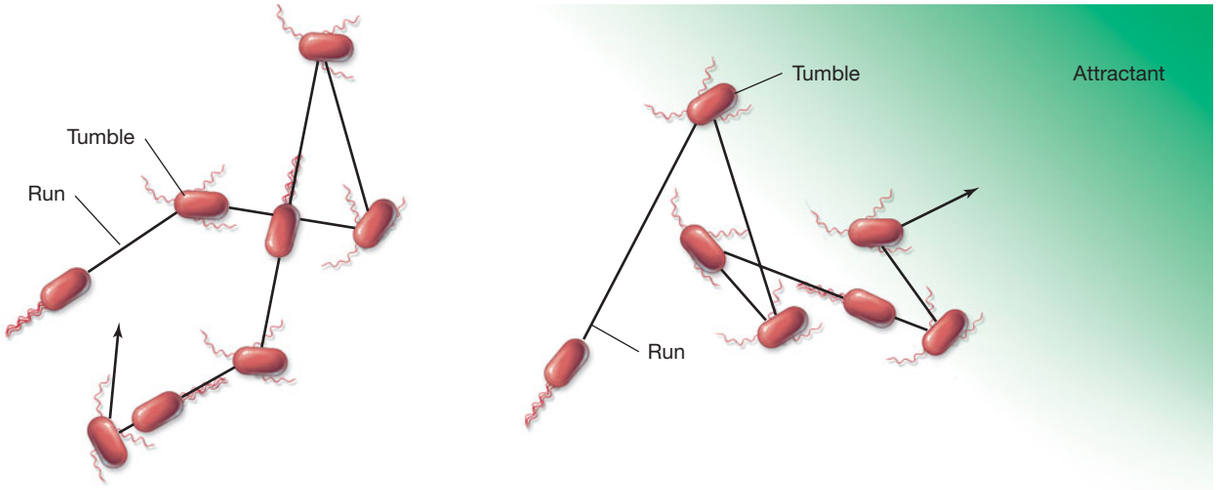
MiniQuiz

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

3.15 Microbial Taxes

Prokaryotes often encounter gradients of physical or chemical agents in nature and have evolved means to respond to these gradients by moving either toward or away from the agent. Such a directed movement is called a *taxis* (plural, taxes). **Chemotaxis**, a response to chemicals, and **phototaxis**, a response to light, are two well-studied taxes. Here we discuss these taxes in a general way. In Section 8.8 we examine the mechanism of chemotaxis and its regulation in *Escherichia coli* as a model for all prokaryotic taxes.

Chemotaxis has been well studied in swimming bacteria, and much is known at the genetic level concerning how the chemical state of the environment is communicated to the flagellar assembly. Our discussion here will thus deal solely with swimming bacteria. However, some gliding bacteria (Section 3.14) are also



(a) **No attractant present: Random movement** *(b)* **Attractant present: Directed movement**

Figure 3.47 **Chemotaxis in a peritrichously flagellated bacterium such as *Escherichia coli.*** *(a)* In the absence of a chemical attractant the cell swims randomly in runs, changing direction during tumbles. *(b)* In the presence of an attractant runs become biased, and the cell moves up the gradient of the attractant. The attractant gradient is depicted in green, with the highest concentration where the color is most intense.

chemotactic, and there are phototactic movements in filamentous cyanobacteria (Figure 3.45*b*). In addition, although they reside in a different evolutionary domain, many species of *Archaea* are also chemotactic and many of the same types of proteins that control chemotaxis in *Bacteria* are present in motile *Archaea* as well.

Chemotaxis

Much research on chemotaxis has been done with the peritrichously flagellated bacterium *E. coli*. To understand how chemotaxis affects the behavior of *E. coli*, consider the situation in which a cell experiences a gradient of some chemical in its environment (**Figure 3.47**). In the absence of a gradient, cells move in a random fashion that includes *runs*, in which the cell is swimming forward in a smooth fashion, and *tumbles*, when the cell stops and jiggles about. During forward movement in a run, the flagellar motor rotates counterclockwise. When flagella rotate clockwise, the bundle of flagella pushes apart, forward motion ceases, and the cells tumble (Figure 3.47).

Following a tumble, the direction of the next run is random. Thus, by means of runs and tumbles, the cell moves about its environment in a random fashion but does not really go anywhere. However, if a gradient of a chemical attractant is present, these random movements become biased. As the organism senses that it is moving toward higher concentrations of the attractant, runs become longer and tumbles are less frequent. The result of this behavioral response is that the organism moves up the concentration gradient of the attractant (Figure 3.47*b*). If the organism senses a repellent, the same general mechanism applies, although in this case it is the decrease in concentration of the repellent (rather than the increase in concentration of an attractant) that promotes runs.

How are chemical gradients sensed? Prokaryotic cells are too small to sense a gradient of a chemical along the length of a single cell. Instead, while moving, the cell monitors its environment, comparing its chemical or physical state with that sensed a few moments before. Bacterial cells are thus responding to *temporal* rather than *spatial* differences in the concentration of a chemical as they swim. Sensory information is fed through an elaborate cascade of proteins that eventually affect the direction of rotation of the flagellar motor. The attractants and repellents are sensed by a series of membrane proteins called *chemoreceptors*. These proteins bind the chemicals and begin the process of sensory transduction to the flagellum (Section 8.8). In a way, chemotaxis can be considered a type of sensory response system, analogous to sensory responses in the nervous system of animals.

Chemotaxis in Polarly Flagellated Bacteria

Chemotaxis in polarly flagellated cells shows similarities to and differences from that in peritrichously flagellated cells such as *E. coli*. Many polarly flagellated bacteria, such as *Pseudomonas* species, can reverse the direction of rotation of their flagella and in so doing reverse their direction of movement (Figure 3.44*b*). However, some polarly flagellated bacteria, such as the phototrophic bacterium *Rhodobacter sphaeroides*, have flagella that rotate only in a clockwise direction. How do such cells change direction, and are they chemotactic?

In cells of *R. sphaeroides*, which have only a single flagellum inserted subpolarly, rotation of the flagellum stops periodically. When it stops, the cell becomes reoriented in a random way by Brownian motion. As the flagellum begins to rotate again, the cell moves in a new direction. Nevertheless, cells of *R. sphaeroides* are strongly chemotactic to certain organic compounds and also show tactic responses to oxygen and light. *R. sphaeroides* cannot reverse its flagellar motor and tumble as *E. coli* can, but there is a

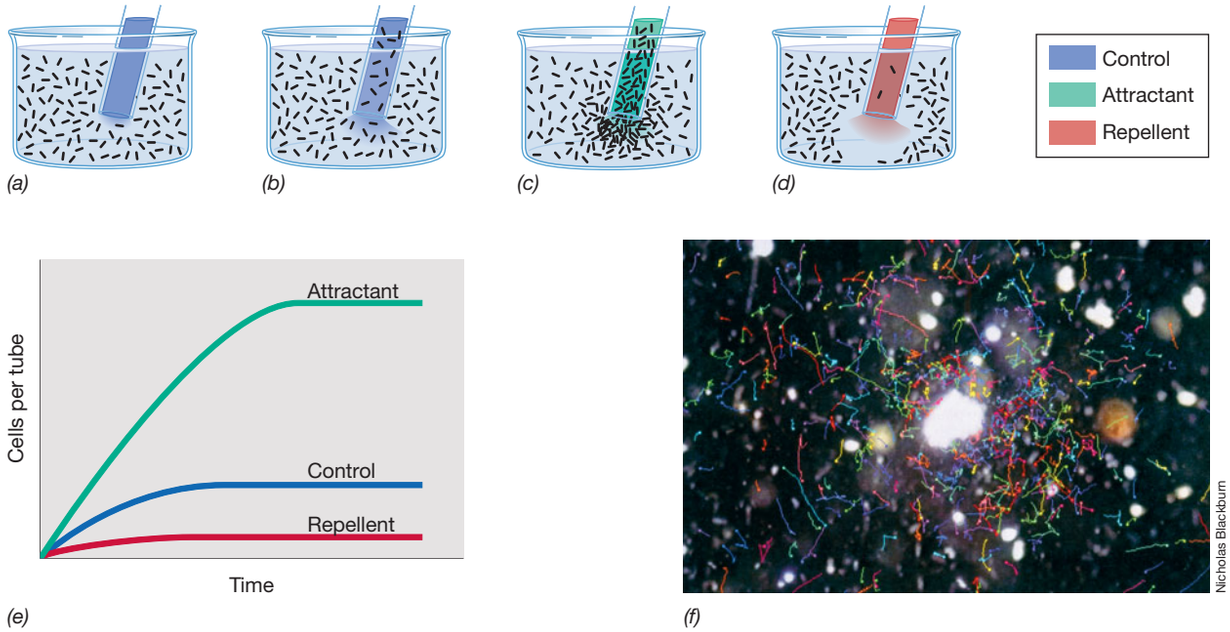


Figure 3.48 Measuring chemotaxis using a capillary tube assay. *(a)* Insertion of the capillary into a bacterial suspension. As the capillary is inserted, a gradient of the chemical begins to form. *(b)* Control capillary contains a salt solution that is neither an attractant nor a repellent. Cell concentration inside the capillary becomes the same as that outside. *(c)* Accumulation of bacteria in a capillary containing an attractant. *(d)* Repulsion of bacteria by a repellent. *(e)* Time course showing cell numbers in capillaries containing various chemicals. *(f)* Tracks of motile bacteria in seawater swarming around an algal cell (large white spot, center) photographed with a tracking video camera system attached to a microscope. The bacterial cells are showing positive aerotaxis by moving toward the oxygen-producing algal cell. The alga is about 60 μm in diameter.

similarity in that the cells maintain runs as long as they sense an increasing concentration of attractant; movement ceases if the cells sense a decreasing concentration of attractant. By random reorientation, a cell eventually finds a path of increasing attractant and maintains a run until either its chemoreceptors are saturated or it begins to sense a decrease in the level of attractant.

Measuring Chemotaxis

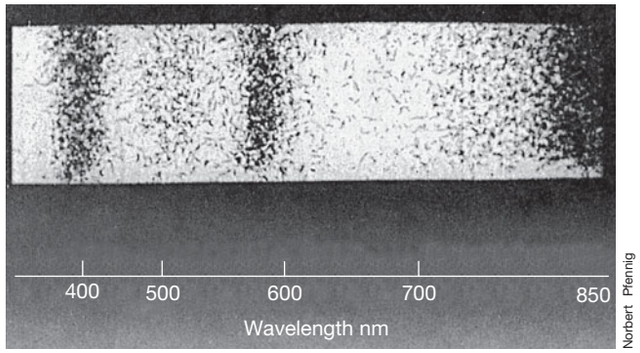
Bacterial chemotaxis can be demonstrated by immersing a small glass capillary tube containing an attractant in a suspension of motile bacteria that does not contain the attractant. From the tip of the capillary, a gradient forms into the surrounding medium, with the concentration of chemical gradually decreasing with distance from the tip (**Figure 3.48**). When an attractant is present, the bacteria will move toward it, forming a swarm around the open tip (Figure 3.48*c*) with many of the bacteria swimming into the capillary itself. Of course, because of random movements some bacteria will move into the capillary even if it contains a solution of the same composition as the medium (control solution, Figure 3.48*b*). However, when an attractant is present, movements become biased, and the number of bacteria within the capillary can be many times higher than external cell numbers. If the capillary is removed after a time period and the cells within the capillary are counted and compared with that of the control, attractants can easily be identified (Figure 3.48*e*).

If the inserted capillary contains a repellent, just the opposite occurs; the cells sense an increasing gradient of repellent and the appropriate chemoreceptors affect flagellar rotation to move the cells away from the repellent. In this case, the number of bacteria within the capillary will be fewer than in the control (Figure 3.48*d*). Using the capillary method, it is possible to screen chemicals to see if they are attractants or repellents for a given bacterium.

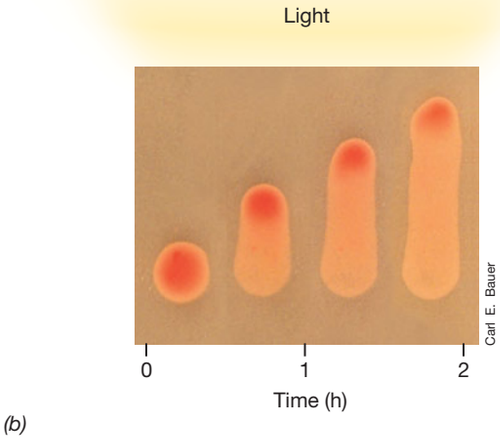
Chemotaxis can also be observed under a microscope. Using a video camera that captures the position of bacterial cells with time and shows the motility tracks of each cell, it is possible to see the chemotactic movements of cells (Figure 3.48*f*). This method has been adapted to studies of chemotaxis of bacteria in natural environments. In nature it is thought that the major chemotactic agents for bacteria are nutrients excreted from larger microbial cells or from live or dead macroorganisms. Algae, for example, produce both organic compounds and oxygen (O_2, from photosynthesis) that can trigger chemotactic movements of bacteria toward the algal cell (Figure 3.48*f*).

Phototaxis

Many phototrophic microorganisms can move toward light, a process called *phototaxis*. The advantage of phototaxis for a phototrophic organism is that it allows it to orient itself most efficiently to receive light for photosynthesis. This can be seen if a light spectrum is spread across a microscope slide on which there are motile phototrophic purple bacteria. On such a slide the bacteria accumulate at wavelengths at which their photosynthetic pigments absorb (**Figure 3.49**; Sections 13.1–13.5 cover photosynthesis). These pigments include, in particular, bacteriochlorophylls and carotenoids.



(a)



(b)

Figure 3.49 Phototaxis of phototrophic bacteria. *(a)* Scotophobic accumulation of the phototrophic purple bacterium *Thiospirillum jenense* at wavelengths of light at which its pigments absorb. A light spectrum was displayed on a microscope slide containing a dense suspension of the bacteria; after a period of time, the bacteria had accumulated selectively and the photomicrograph was taken. The wavelengths at which the bacteria accumulated are those at which the photosynthetic pigment bacteriochlorophyll *a* absorbs (compare with Figure 13.3*b*). *(b)* Phototaxis of an entire colony of the purple phototrophic bacterium *Rhodospirillum centenum*. These strongly phototactic cells move in unison toward the light source at the top. See Figure 3.39 for electron micrographs of flagellated *R. centenum* cells.

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

True phototaxis differs from scotophobotaxis; in phototaxis, cells move up a gradient of light from lower to higher intensities. Phototaxis is analogous to chemotaxis except the attractant in this case is light instead of a chemical. In some species, such as the highly motile phototrophic organism *Rhodospirillum centenum* (Figure 3.39), entire colonies of cells show phototaxis and move in unison toward the light (Figure 3.49*b*).

Several components of the regulatory system that govern chemotaxis also control phototaxis. This conclusion has emerged from the study of mutants of phototrophic bacteria defective in phototaxis; such mutants show defective chemotaxis systems as well. A *photoreceptor*, a protein that functions similar to a chemoreceptor but senses a gradient of light instead of chemicals, is the initial sensor in the phototaxis response. The photoreceptor then interacts with the same cytoplasmic proteins that control flagellar rotation in chemotaxis, maintaining the cell in a run if it is swimming toward an increasing intensity of light. Thus, although the stimulus in chemotaxis and phototaxis is different—chemicals versus light—the same molecular machinery processes both signals. We discuss this cytoplasmic machinery in detail in Section 8.8.

Other Taxes

Other bacterial taxes, such as movement toward or away from oxygen (*aerotaxis*, see Figure 3.48*f*) or toward or away from conditions of high ionic strength (*osmotaxis*), are known among various swimming prokaryotes. In some gliding cyanobacteria an unusual taxis, *hydrotaxis* (movement toward water), has also been observed. Hydrotaxis allows gliding cyanobacteria that inhabit dry environments, such as soils, to glide toward a gradient of increasing hydration.

It should be clear from our consideration of microbial taxes that motile prokaryotes do not just swim around at random, but instead remain keenly attuned to the chemical and physical state of their habitat. When gradients of virtually any nutrient form in nature, motile cells are "constantly on the move" exploiting them, and by so doing, improve their chances for survival. And from a mechanistic standpoint, prokaryotic cells monitor these gradients by periodically sampling their environment for chemicals, light, oxygen, salt, or other substances, and then processing the results through a common network of proteins that ultimately control the direction of flagellar rotation. By being able to move toward or away from various stimuli, prokaryotic cells have a better chance of competing successfully for resources and avoiding the harmful effects of substances that could damage or kill them.

MiniQuiz

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

Big Ideas

3.1
Prokaryotic cells can have many different shapes; rods, cocci, and spirilla are common cell morphologies. Morphology is a poor predictor of other cell properties and is a genetically directed characteristic that has evolved to best serve the ecology of the cell.

3.2
Prokaryotes are typically smaller in size than eukaryotes, although some very large and some very small prokaryotes are known. The typical small size of prokaryotic cells affects their physiology, growth rate, ecology, and evolution. The lower limit for the diameter of a coccus-shaped cell is about 0.15 μm.

3.3
The cytoplasmic membrane is a highly selective permeability barrier constructed of lipids and proteins that form a bilayer, hydrophobic inside and hydrophilic outside. In contrast to *Bacteria* and *Eukarya*, *Archaea* contain ether-linked lipids, and hyperthermophilic species have membranes of monolayer construction.

3.4
The major functions of the cytoplasmic membrane are permeability, transport, and energy conservation. To accumulate nutrients against the concentration gradient, transport mechanisms are employed that are characterized by their specificity, saturation effect, and biosynthetic regulation.

3.5
At least three types of transporters are known: simple transporters, phosphotransferase systems, and ABC systems. Transport requires energy from either ATP directly or from the proton motive force to accumulate solutes in the cell against the concentration gradient.

3.6
The cell walls of *Bacteria* contain peptidoglycan. Peptidoglycan is a polysaccharide consisting of an alternating repeat of *N*-acetylglucosamine and *N*-acetylmuramic acid, the latter in adjacent strands cross-linked by tetrapeptides. One to several sheets of peptidoglycan can be present, depending on the organism. The enzyme lysozyme and the antibiotic penicillin target peptidoglycan, leading to cell lysis.

3.7
In addition to peptidoglycan, gram-negative bacteria have an outer membrane consisting of LPS, protein, and lipoprotein. Proteins called porins allow for permeability across the outer membrane. The gap between the outer and cytoplasmic membranes is called the periplasm and contains proteins involved in transport, sensing chemicals, and other important cell functions.

3.8
Cell walls of *Archaea* can be of several types, including pseudomurein, various polysaccharides, and S-layers, which are composed of protein or glycoprotein. As for *Bacteria*, the walls of *Archaea* protect the cell from osmotic lysis.

3.9
Many prokaryotic cells contain capsules, slime layers, pili, or fimbriae. These structures have several functions, including attachment, genetic exchange, and twitching motility.

3.10
Prokaryotic cells can contain inclusions of sulfur, polyphosphate, carbon polymers, or magnetosomes. These substances function as storage materials or in magnetotaxis.

3.11
Gas vesicles are cytoplasmic gas-filled structures that confer buoyancy on cells. Gas vesicles are composed of two different proteins arranged to form a gas-permeable but watertight structure.

3.12
The endospore is a highly resistant and differentiated bacterial cell produced by certain gram-positive *Bacteria*. Endospores are dehydrated and contain various protective agents such as calcium dipicolinate and small acid-soluble proteins, absent from vegetative cells. Endospores can remain dormant indefinitely but can germinate quickly when conditions warrant.

3.13
Swimming motility is due to flagella. The flagellum is a complex structure made of several proteins anchored in the cell wall and cytoplasmic membrane. The flagellum filament is made of a single kind of protein in *Bacteria* and rotates at the expense of the proton motive force. The flagella of *Archaea* and *Bacteria* differ in structure and probably also in their rotational mechanism.

3.14
Bacteria that move by gliding motility do not employ rotating flagella but instead creep along a solid surface by employing any of several different mechanisms.

3.15
Motile bacteria respond to chemical and physical gradients in their environment. In swimming bacteria, movement of a cell is biased either toward or away from a stimulus by controlling the lengths of runs and frequency of tumbles. Tumbles are controlled by the direction of rotation of the flagellum, which in turn is controlled by a network of sensory and response proteins.

Q1. Describe the function of the following bacterial components
a. Type IV pili
b.

Q2. Compare and contrast between the slime layers and capsule in bacteria