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

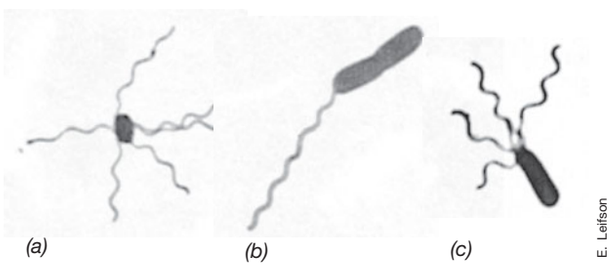
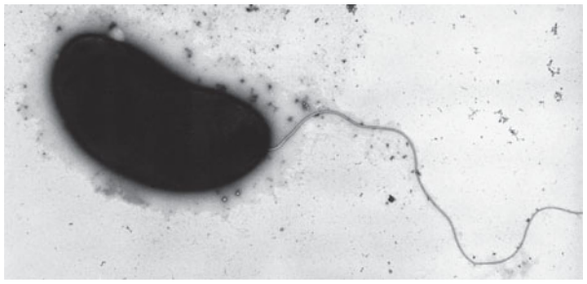
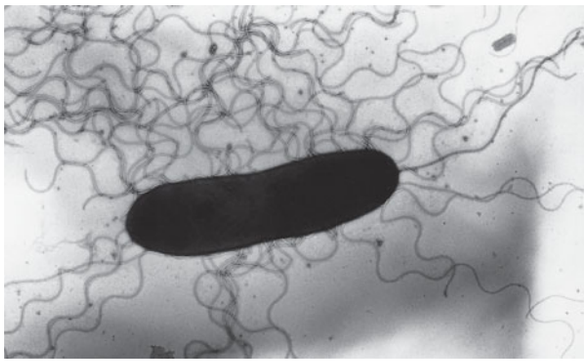


Figure 3.38 Bacterial flagella. Light photomicrographs of prokaryotes containing different arrangements of flagella. Cells are stained with Leifson flagella stain. *(a)* Peritrichous. *(b)* Polar. *(c)* Lophotrichous.



(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).

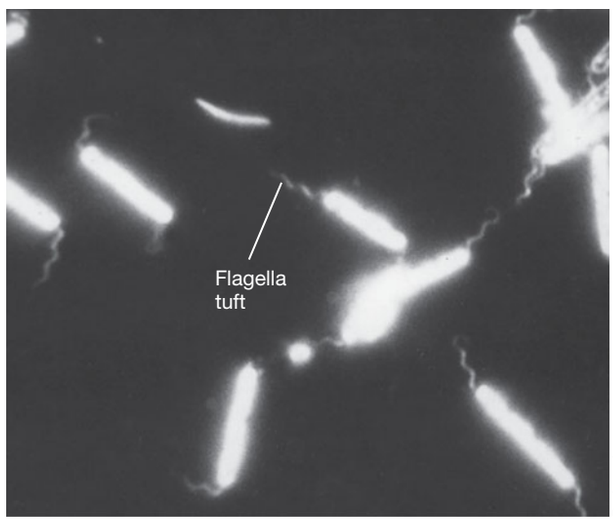
cells by dark-field or phase-contrast microscopy (**Figure 3.40**). When a tuft of flagella emerges from both poles of the cell, flagellation is called *amphitrichous*. In **peritrichous flagellation** (Figures 3.38*a* and 3.39*b*), flagella are inserted at many locations around the cell surface. The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

Flagellar Structure

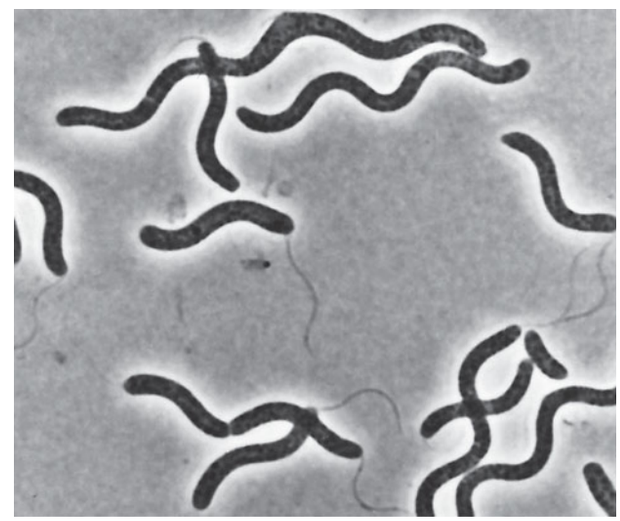
Flagella are not straight but are actually helical. When flattened, flagella show a constant distance between adjacent curves, called the *wavelength*, and this wavelength is characteristic for the flagella of any given species (Figures 3.38–3.40). The filament of a bacterial flagellum is composed of 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.

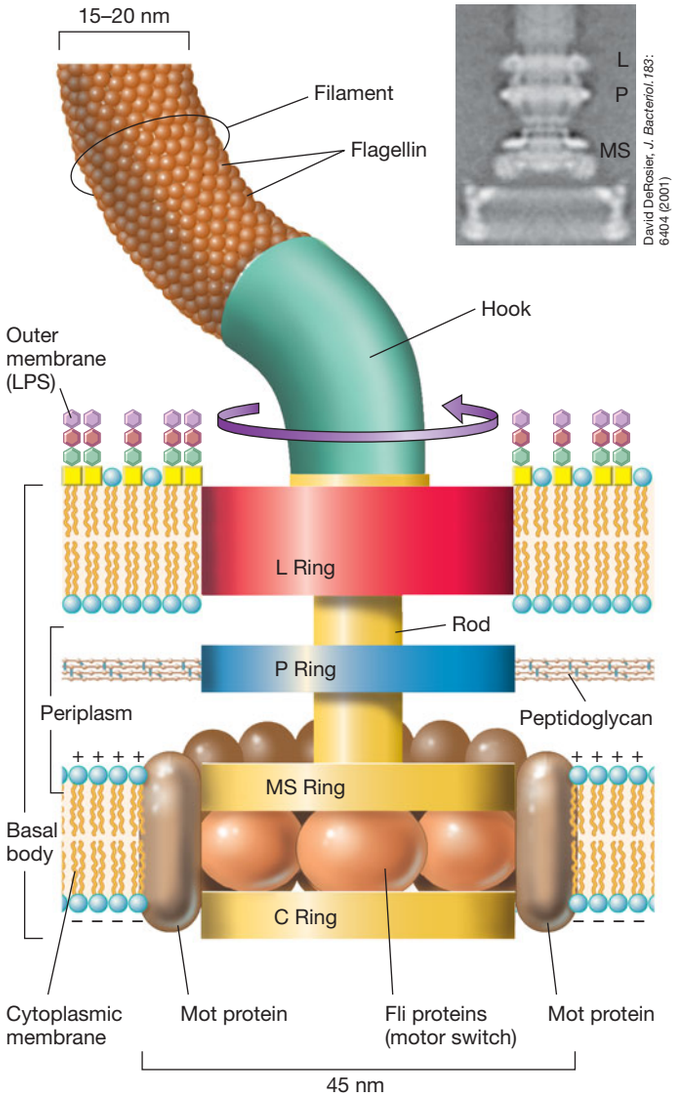


Figure 3.41 **Structure and function of the flagellum in gram-negative *Bacteria*.** *(a)* Structure. The L ring is embedded in the LPS and the P ring in peptidoglycan. The MS ring is embedded in the cytoplasmic membrane and the C ring in the cytoplasm. A narrow channel exists in the rod and filament 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

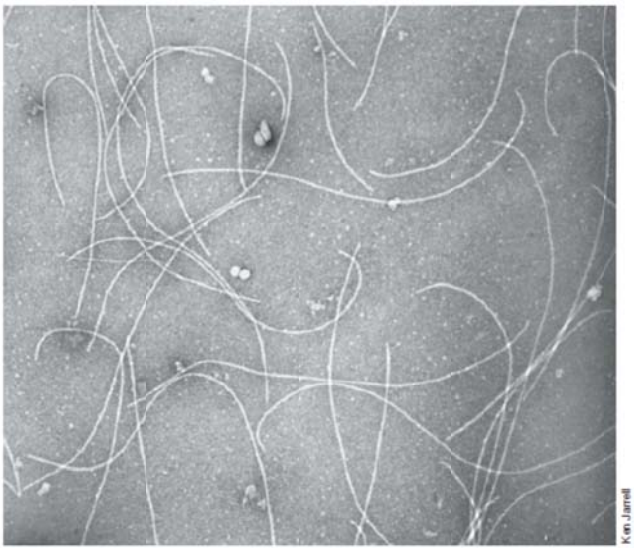


Figure 3.42 Archaeal flagella. Transmission electron micrograph of flagella isolated from cells of the methanogen *Methanococcus maripaludis*. A single flagellum is about 12 nm wide.

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

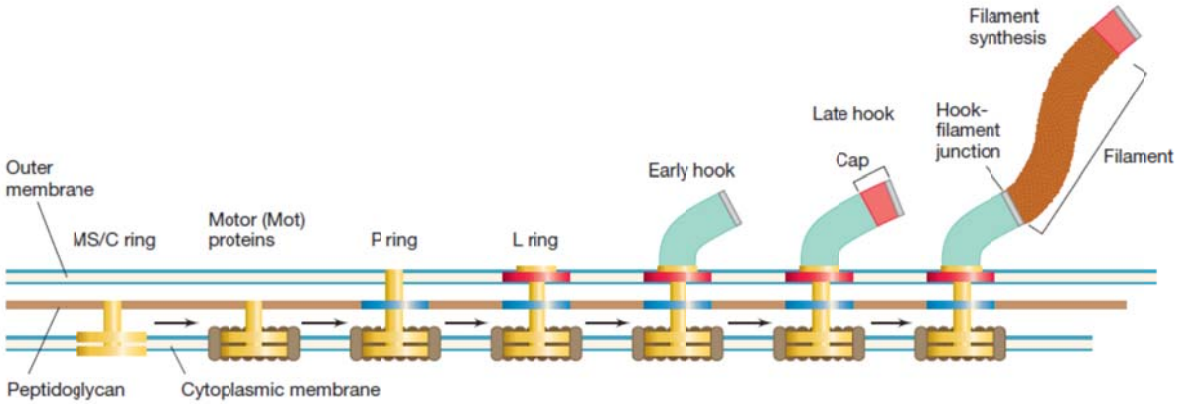
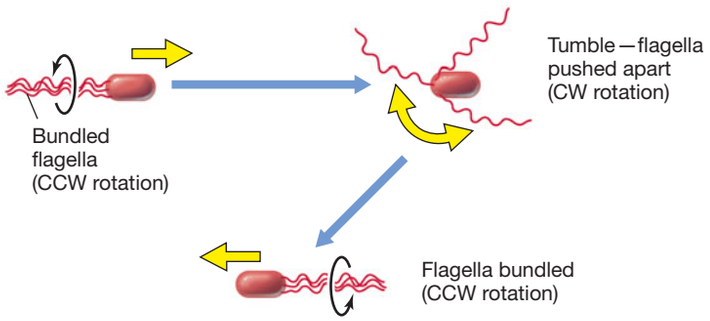
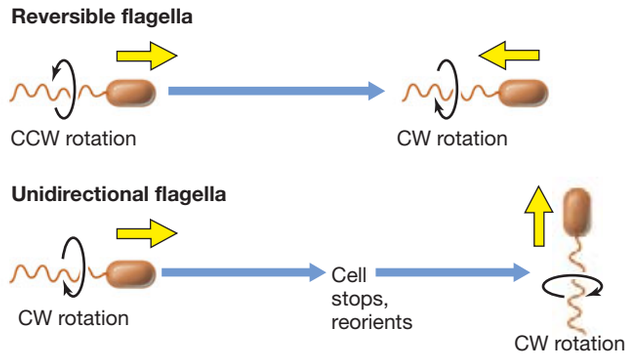


Figure 3.43 Flagella biosynthesis. Synthesis begins with assembly of MS and C rings in the cytoplasmic membrane, followed by the other rings, the hook, and the cap. Flagellin protein flows through the hook to form the filament and is guided into position by cap proteins.



(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

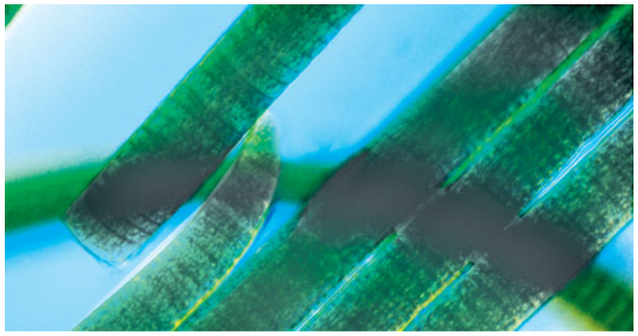
Gliding motility is widely distributed among *Bacteria* but has been well studied in only a few groups. The gliding movement itself—up to 10 μm/sec in some gliding bacteria—is considerably slower than propulsion by flagella but still offers the cell a means of moving about its habitat.

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

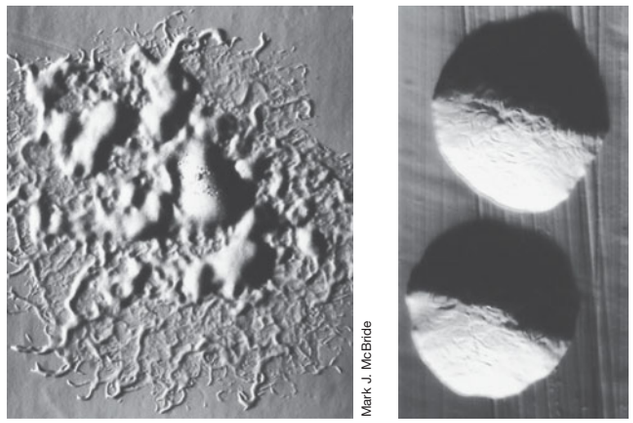
Although no gliding mechanism is thoroughly understood, it is clear that more than one mechanism is responsible for gliding motility. Cyanobacteria (phototrophic bacteria, Figure 3.45*a*, *b*) glide by secreting a polysaccharide slime on the outer surface of the cell. The slime contacts both the cell 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

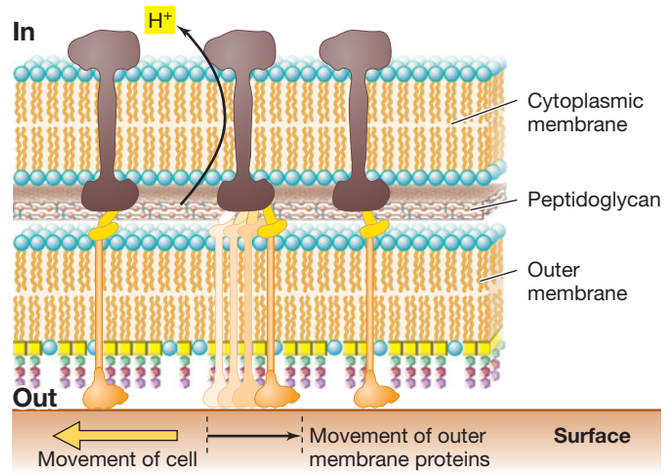


Figure 3.46 **Gliding motility in *Flavobacterium johnsoniae*.** Tracks (yellow) exist in the peptidoglycan that connect cytoplasmic proteins (brown) to outer membrane proteins (orange) and propel the outer membrane proteins along the solid surface. Note that the outer membrane proteins and the cell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

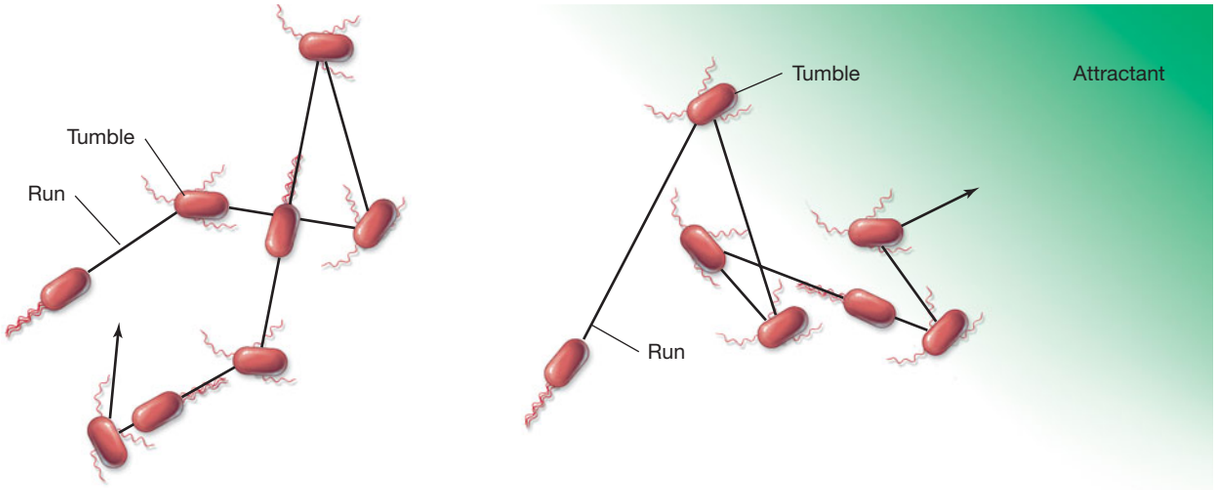
MiniQuiz

- 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

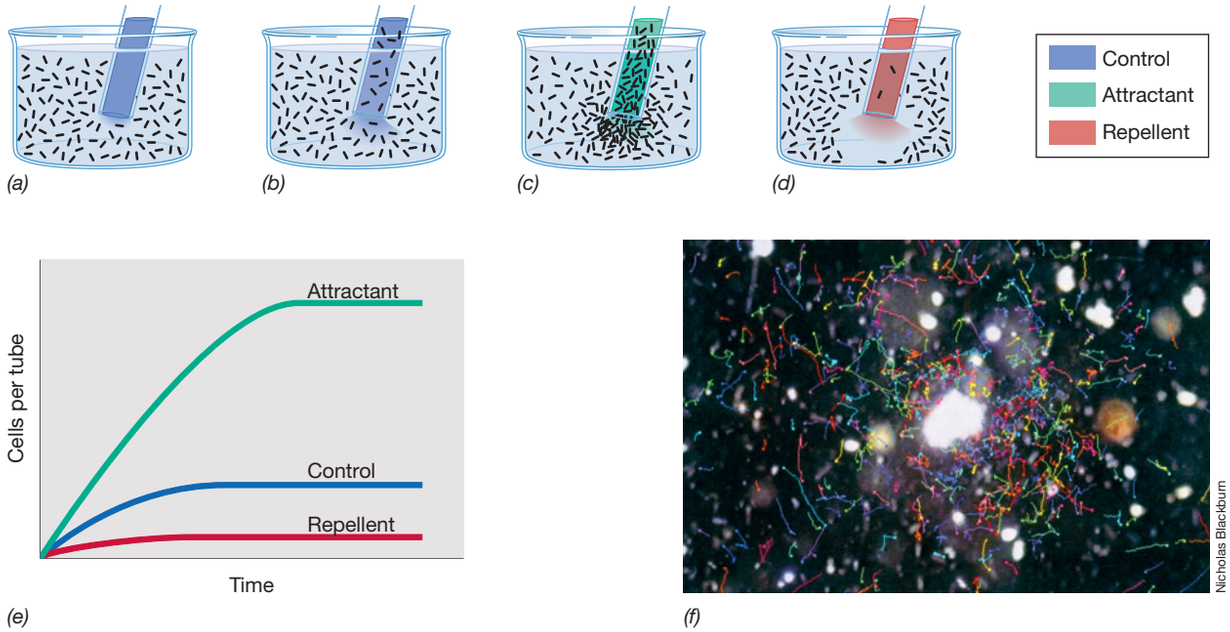


Figure 3.48 Measuring chemotaxis using a capillary tube assay. *(a)* Insertion of the capillary into a bacterial suspension. As the capillary is inserted, a gradient of the chemical begins to form. *(b)* Control capillary contains a salt solution that is neither an attractant nor a repellent. Cell concentration 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

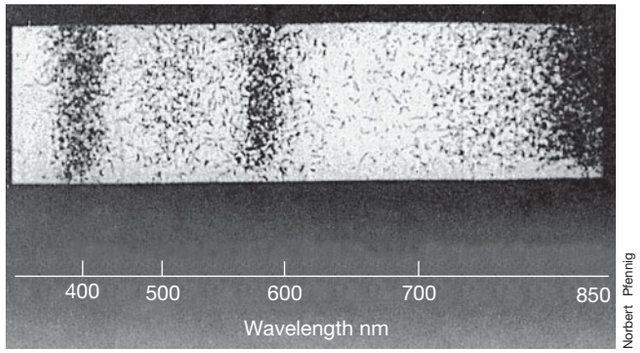
Bacterial chemotaxis can be demonstrated by immersing a small glass capillary tube containing an attractant in a suspension of motile bacteria that does not contain the attractant. From the tip of the capillary, a gradient forms into the surrounding medium, with the concentration of chemical gradually 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.

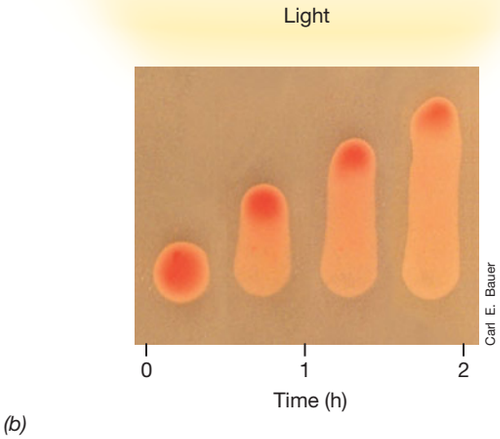
Chemotaxis can also be observed under a microscope. Using a video camera that captures the position of bacterial cells with time and shows the motility tracks of each cell, it is possible to see the chemotactic movements of cells (Figure 3.48*f*). This method has been adapted to studies of chemotaxis 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.

Review of Key Terms

ABC (ATP-binding cassette) transport system a membrane transport system consisting of three proteins, one of which hydrolyzes ATP; the system transports specific nutrients into the cell

Basal body the "motor" portion of the bacterial flagellum, embedded in the cytoplasmic membrane and wall

Capsule a polysaccharide or protein outermost layer, usually rather slimy, present on some bacteria

Chemotaxis directed movement of an organism toward (positive chemotaxis) or away from (negative chemotaxis) a chemical gradient

Cytoplasmic membrane the permeability barrier of the cell, separating the cytoplasm from the environment

Dipicolinic acid a substance unique to endospores that confers heat resistance on these structures

Endospore a highly heat-resistant, thick-walled, differentiated structure produced by certain gram-positive *Bacteria*

Flagellum a long, thin cellular appendage capable of rotation and responsible for swimming motility in prokaryotic cells

Gas vesicles gas-filled cytoplasmic structures bounded by protein and conferring buoyancy on cells

Gram-negative a bacterial cell with a cell wall containing small amounts of peptidoglycan, and an outer membrane containing lipopolysaccharide, lipoprotein, and other complex macromolecules

Gram-positive a bacterial cell whose cell wall consists chiefly of peptidoglycan; it lacks the outer membrane of gram-negative cells

Gram stain a differential staining procedure that stains cells either purple (gram-positive cells) or pink (gram-negative cells)

Group translocation an energy-dependent transport system in which the substance transported is chemically modified during the process of being transported by a series of proteins

Lipopolysaccharide (LPS) a combination of lipid with polysaccharide and protein that forms the major portion of the outer membrane in gram-negative *Bacteria*

Magnetosome a particle of magnetite (Fe_3O_4) enclosed by a nonunit membrane in the cytoplasm of magnetotactic *Bacteria*

Morphology the *shape* of a cell—rod, coccus, spirillum, and so on

Outer membrane a phospholipid- and polysaccharide-containing unit membrane that lies external to the peptidoglycan layer in cells of gram-negative *Bacteria*

Peptidoglycan a polysaccharide composed of alternating repeats of *N*-acetylglucosamine and *N*-acetylmuramic acid arranged in adjacent layers and cross-linked by short peptides

Periplasm a gel-like region between the outer surface of the cytoplasmic membrane and the inner surface of the lipopolysaccharide layer of gram-negative *Bacteria*

Peritrichous flagellation having flagella located in many places around the surface of the cell

Phototaxis movement of an organism toward light

Pili thin, filamentous structures that extend from the surface of a cell and, depending on type, facilitate cell attachment, genetic exchange, or twitching motility

Polar flagellation having flagella emanating from one or both poles of the cell

Poly-β-hydroxybutyrate (PHB) a common storage material of prokaryotic cells consisting of a polymer of β-hydroxybutyrate or another β-alkanoic acid or mixtures of β-alkanoic acids

S-layer an outermost cell surface layer composed of protein or glycoprotein present on some *Bacteria* and *Archaea*

Simple transport system a transporter that consists of only a membrane-spanning protein and is typically driven by energy from the proton motive force

Teichoic acid a phosphorylated polyalcohol found in the cell wall of some gram-positive *Bacteria*

Review Questions

1. What are the major morphologies of prokaryotes? Draw cells for each morphology you list (Section 3.1).
2. How large can a prokaryote be? How small? Why is it that we likely know the lower limit more accurately than the upper limit? What are the dimensions of the rod-shaped bacterium *Escherichia coli* (Section 3.2)?
3. Describe in a single sentence the structure of a unit membrane (Section 3.3).
4. Describe a major chemical difference between membranes of *Bacteria* and *Archaea* (Section 3.3).
5. Explain in a single sentence why ionized molecules do not readily pass through the cytoplasmic membrane of a cell. How do such molecules get through the cytoplasmic membrane (Sections 3.4 and 3.5)?
6. Cells of *Escherichia coli* take up lactose via lac permease, glucose via the phosphotransferase system, and maltose via an ABC-type transporter. For each of these sugars describe: (1) the components of the transport system and (2) the source of energy that drives the transport event (Section 3.5).
7. Why is the rigid layer of the bacterial cell wall called peptidoglycan? What are the chemical reasons for the rigidity that is conferred on the cell wall by the peptidoglycan structure (Section 3.6)?
8. List several functions of the outer membrane in gram-negative *Bacteria*. What is the chemical composition of the outer membrane (Section 3.7)?
9. What cell wall polysaccharide common in *Bacteria* is absent from *Archaea*? What is unusual about S-layers compared to other cell walls of prokaryotes ? What types of cell walls are found in *Archaea* (Section 3.8)?
10. What function(s) do polysaccharide layers outside the cell wall have in prokaryotes (Section 3.9)?
11. What types of cytoplasmic inclusions are formed by prokaryotes? How does an inclusion of poly-β-hydroxybutyric acid differ from a magnetosome in composition and metabolic role (Section 3.10)?
12. What is the function of gas vesicles? How are these structures made such that they can remain gas tight (Section 3.11)?

13. In a few sentences, indicate how the bacterial endospore differs from the vegetative cell in structure, chemical composition, and ability to resist extreme environmental conditions (Section 3.12).
14. Define the following terms: mature endospore, vegetative cell, and germination (Section 3.12).
15. Describe the structure and function of a bacterial flagellum. What is the energy source for the flagellum? How do the flagella of *Bacteria* differ from those of *Archaea* in both size and composition (Section 3.13)?
16. How do the mechanism and energy requirements for motility in *Flavobacterium* differ from that in *Escherichia coli* (Sections 3.13 and 3.14)?
17. In a few sentences, explain how a motile bacterium is able to sense the direction of an attractant and move toward it (Section 3.15).
18. In the experiment described in Figure 3.48, why is it essential to have a control (Section 3.15)?

Application Questions

1. Calculate the surface-to-volume ratio of a spherical cell 15 μm in diameter and of a cell 2 μm in diameter. What are the consequences of these differences in surface-to-volume ratio for cell function?
2. Assume you are given two cultures, one of a species of gram-negative *Bacteria* and one of a species of *Archaea*. Other than by phylogenetic analyses, discuss at least four different ways you could tell which culture was which.
3. Calculate the amount of time it would take a cell of *Escherichia coli* (1×2 μm) swimming at maximum speed (60 cell lengths per second) to travel all the way up a 3-cm-long capillary tube containing a chemical attractant.
4. Assume you are given two cultures of rod-shaped bacteria, one gram-positive and the other gram-negative. How could you differentiate them using (a) light microscopy; (b) electron microscopy; (c) chemical analyses of cell walls; and (d) phylogenetic analyses?

Need more practice? Test your understanding with Quantitative Questions; access additional study tools including tutorials, animations, and videos; and then test your knowledge with chapter quizzes and practice tests at **www.microbiologyplace.com**.