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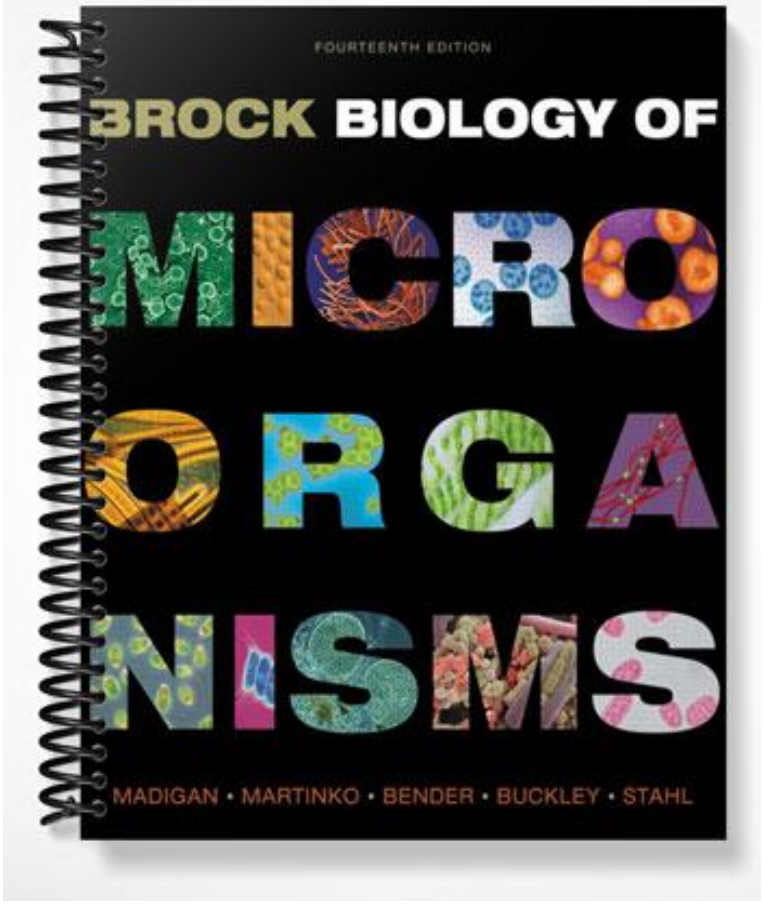
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CHAPTER

2 Microbial Cell Structure and Function

Summary

Chapter 2 is an excellent introductory overview of microscopic techniques and the structure and function of both prokaryotic and eukaryotic cells. For courses designed for nonscience majors, this chapter provides general details on each topic that, if supplemented with material from related chapters later in the text, may be sufficient background for most students. However, it is recommended that Chapter 2 be used to set the stage for more detailed coverage later in the course.

2.1–2.4 | Microscopy

The variety of microscopic methods available for observing microorganisms must be introduced early, as much of the presentation of structure–function relationships depends upon the excellent micrographs that appear throughout the book. Although details of microscopy are more easily introduced in the laboratory portion of the course, the material included here is pertinent to effective lecture presentation.

- Discuss the basic principles and components of the compound light microscope, including the relationships between resolution and magnification, and numerical aperture (Figure 2.1). Note that although *bright-field microscopy* is fine for visualizing pigmented cells (Figure 2.2), it is not an efficient tool for viewing unstained cells with no natural pigmentation, such as nonphototrophic bacteria.
- This deficiency will lead to a discussion of various methods employed to increase contrast. Discuss the various simple dyes used to stain cells, most of which are positively charged, basic dyes capable of binding to negatively charged cell surfaces (e.g., methylene blue and crystal violet; Figure 2.3). Continue the discussion of *differential stains*, the most widely used of which is the Gram stain (Figure 2.4).
- Students should understand that while staining procedures increase the contrast of cells against the background to make them more visible, they also kill cells and often distort their appearance. Discuss *phase-contrast microscopy* and *dark-field microscopy* (Figure 2.5), two tools that allow one to look at living cells without the need for staining.
- *Fluorescence microscopy* is widely used in clinical diagnostic microbiology and environmental microbiology (Figure 2.6). Most students who enter the biotechnology industry or medical profession will work with fluorescent molecules (such as those used for fluorescence antibody staining methods). The variety and sensitivity of these molecules has

increased dramatically over the past decade. This has allowed the development of a wide variety of nonradioactive alternatives to biological assays that are now routinely used in research.

- Students should be interested in the micrographs from three-dimensional imaging of cells. Depending upon the level of the course, you may choose to discuss the principles of *differential interference contrast microscopy* (Figure 2.7) and *confocal scanning laser microscopy* (Figure 2.8). Lastly, show and discuss the micrographs obtained from *electron microscopy* (Figures 2.9 and 2.10). Note the differences between scanning electron microscopy (SEM), which provides an image of the external features of a specimen, and transmission electron microscopy (TEM), in which thin sections of the specimen show its detailed internal structure.

2.5 | Cell Morphology

Using Figure 2.11, point out the three major morphologies of prokaryotic cells (*coccus, rod,* and *spirillum*). Inform your students that, in some species, the cells remain attached following cell division, giving rise to different arrangements that are often genus-specific. For example, coccus cells may exist as short chains (*Streptococcus*) or grapelike clusters (*Staphylococcus*). Less common cell morphologies also exist, such as *spirochetes*, *appendaged* (budding) bacteria, and *filamentous* bacteria (Figure 2.11). Stress to students that these morphologies are only *representative* of those found in nature. Other unusual shapes have also been described in rare cases (for example, square and star-shaped cells!).

Before the molecular era, morphological and physiological properties were used to classify bacterial species. However, we now know that these criteria are poor predictors of evolutionary relationships. For example, certain species of *Archaea* may appear identical in size and shape to species of *Bacteria* under the microscope, but these organisms are of different phylogenetic domains and thus are not closely related to one another on an evolutionary basis. The cell morphology of a particular species is primarily a result of selective pressures in a given habitat that favored a particular cell shape for enhanced reproductive success.

2.6 | Cell Size and the Significance of Being Small

The presentation in the text on *the significance of being small* is an important concept for students to internalize as they progress in their study of microbiology. Table 2.1 shows the wide size range variability of prokaryotic cells, which range from a diameter of about 0.2 μm to over 700 μm. Use the two examples of unusually large prokaryotes discussed in this section to illustrate the current upper limit of prokaryotic cell size: (1) the surgeonfish gut symbiont *Epulopiscium fishelsonsi* (>600 μm in length; Figure 2.12*a*), and (2) the sulfur chemolithotroph *Thiomargarita namibiensis* (750 μm; Figure 2.12*b*). The evolutionary "rationale" for the existence of unusually large-celled prokaryotes is a mystery when one considers that the metabolic rate of a cell varies inversely with the square of its size. Ask your students for ideas and/or hypotheses that might explain the selective advantage of large cell size in these two prokaryotes.

The fact that bacteria can live independently as single cells (unlike an individual cell of a multicellular organism) suggests that they must possess some capabilities that provide a

selective advantage over their multicellular counterparts that ensure their survival on the planet. Small cells have more *surface area* to *volume* (i.e., a higher *surface-to-volume ratio*), and this alone confers many of the evolutionary advantages of being small, including the following:

- Rapid nutrient and waste transport into and out of the cell allows for faster metabolic rates and growth rates.
- Rapid growth rates result in the rapid production of large populations of cells. These populations, in turn, can greatly affect the physiochemical conditions of an ecosystem within a short time period.
- Transport rates are a function of the surface area of the cell membrane relative to cell volume. Use Figure 2.13 to mathematically demonstrate to students that the *surface area* of a sphere is a function of the square of the radius, whereas the *volume* of a sphere is a function of the cube of the radius. This means that the surface-to-volume ratio of a spherical cell can be expressed as $3/r$, where r equals the radius of the cell. Therefore, a coccus cell having a smaller radius has more surface area per volume, and thus more efficient transport capabilities, than a coccus cell having a larger radius.
- Rates of evolutionary change are higher in smaller, faster growing haploid cells than in larger, slower growing diploid cells. This allows for greater adaptive potential through rapid selection for advantageous mutations and counterselection against deleterious mutations.

The theoretical lower limit of size for a living cell is likely near 0.2 μm in diameter. This limit is dictated by the amount of volume required to contain cellular components that are crucial for maintaining life, such as (1) the presence of essential genes on the chromosome; (2) having a sufficient number of ribosomes; and (3) containing a minimal number of metabolic, structural, and transport proteins within the cell. Challenge students to list these and other molecular components a cell would have to contain to maintain life. Remind students that some cells are parasitic in nature. Inform them that, much like viruses, such microorganisms often have streamlined genomes that lack important genes and may make them dependent upon their hosts for growth. Can such organisms truly be considered living? *This might make a good outside project for group debate, requiring students to view the cell as a three-dimensional physical structure constrained in space and to research a problem that is currently being debated.*

2.7 | Membrane Structure

The structure of the cytoplasmic membrane, a phospholipid bilayer, should be discussed in considerable detail because it plays a critical role in establishing and maintaining the cell's internal environment. Students must understand that the cytoplasmic membrane is the *selectively permeable* boundary between the cytoplasm of the cell and the cell's immediate environment. If the integrity of the membrane becomes compromised, then essential cellular components can leak out of the cytoplasm and into the environment, thereby destroying the cell. Convey to students that the cytoplasmic membrane generally does *not* confer a specific shape and provide rigid support to the cell (these are roles of the cell wall, to be discussed later), but rather the membrane has a fluid nature that allows for a degree of lateral movement of phospholipids and proteins (Figures 2.14 and 2.15). Proteins embedded in the membrane

consist of both hydrophobic regions that are situated within the lipid portion of the phospholipid bilayer and hydrophilic regions that are oriented toward either the external environment or the aqueous cytoplasm of the cell.

In contrast to eukaryotic cells, which contain rigid sterol molecules to strengthen and stabilize membranes (especially those of animal cells, which lack cell walls), most prokaryotic membranes instead contain planar molecules called *hopanoids* that serve a similar function. Exceptions to this generalization include methanotrophic bacteria, which contain large amounts of sterols in internal membranes, and the mycoplasmas, a group of parasitic bacteria that lack cell walls.

While members of the *Bacteria* and *Eukarya* contain *ester* linkages that bond the fatty acids to glycerol in their membranes (Figure 2.16*a* and *b*), *Archaea* contain *ether* linkages between the glycerol and lipid portions of their membranes. In addition, archaeal membrane lipids are not composed of fatty acids but instead consist of repeating five-carbon isoprene units that combine to form 20-carbon *phytanyl* side chains (Figures 2.16*c* and 2.17*a* and *b*). Together, the glycerol and phytanyl form a *glycerol diether*. In some *Archaea*, glycerol diethers are joined at their hydrophobic ends to create a lipid monolayer of diglycerol tetraethers (Figure 2.17*b* and *e*). This structural conformation provides superior thermostability of the membrane, and indeed lipid monolayers are most commonly found in hyperthermophilic archaeal species. Finally, members of the *Crenarchaeota* often contain *crenarchaeol*, a unique monolayer membrane lipid having four cyclopentyl rings and one cyclohexyl ring (Figure 2.17*c*). Despite the molecular differences between archaeal membranes and bacterial/eukaryotic membranes, their basic structural properties are the same in that each possesses hydrophobic interior hydrocarbon chains attached to polar (hydrophilic) glycerophosphate molecules.

Although molecular adaptations of membranes to high and low temperatures are discussed in some detail in Chapter 5, this may be a good opportunity to introduce the topic of saturated versus unsaturated hydrocarbon chains and discuss how they relate to membrane fluidity under high and low temperature extremes (e.g., why vegetable shortening is a solid at room temperature, and vegetable oil is a liquid under the same conditions).

2.8 | Membrane Function

The major functions of the cytoplasmic membrane are summarized in Figure 2.18 and include its role as (1) a *permeability barrier*, (2) a *protein anchor*, and (3) a means of *energy conservation*. With respect to acting as a permeability barrier, impress upon students that even extremely small ions do not freely pass through the hydrophobic interior of the membrane due to their charges (Table 2.2). While water molecules do diffuse through membranes (due to their small size and only weak polarity) in a process called *osmosis*, the movement of water across membranes is greatly accelerated by water transport proteins called *aquaporins*. These transport proteins have been identified in the membranes of organisms from all domains of life but are perhaps best studied in the bacterium *Escherichia coli*.

Introduce students to the concept that a membrane can function much like a battery in that it can store potential energy. By separating protons to the outside of the membrane from hydroxyl ions on the inside, the membrane becomes "energized" (i.e., polarized), and this energized state is referred to as the *proton motive force (PMF)*. The dissipation of this force results in the conversion of potential energy to kinetic energy. When protons stored outside of the membrane return to the inside of the cell through an ATPase enzyme complex, ADP

and P_i are converted to ATP, the cell's energy currency. This concept will be discussed in detail in Chapter 3.

Discuss with your students the necessity for membrane-bound transport proteins by comparing the rate of simple diffusion of a solute across a membrane to the greatly accelerated rate of carrier-mediated transport of a solute across a membrane (Figure 2.19). Transport proteins allow for the accumulation inside a cell of a solute that may be in very low concentration in the environment. Point out that each carrier-mediated transport protein shows *high specificity* for a given solute.

2.9 | Nutrient Transport

Some students may find the variety of nutrient transport mechanisms difficult to comprehend initially, so discuss these mechanisms in detail using Figures 2.20–2.23 to illustrate the concepts and provide examples of each type of transport event. When describing the three classes of membrane transport systems—*simple transport*, *group translocation*, and the *ABC (ATP-binding cassette) system*—highlight the following points to your students:

- Some transport mechanisms require only a membrane-spanning component (e.g., the simple transporters shown in Figure 2.21).
- Some require a series of proteins that cooperate in a phosphorylation/dephosphorylation cascade to carry out the transport event (e.g., the group translocation phosphotransferase system; Figure 2.22).
- Some require a membrane-spanning transporter, a substrate-binding protein, and an ATP-hydrolyzing protein (e.g., the monosaccharide ABC transporters; Figure 2.23).

In addition to small molecule transport, larger molecules, such as proteins, need to be inserted into membranes or transported outside the cell (e.g., toxins, amylases, and cellulases). This movement of materials is accomplished by translocases, the most well-characterized being the *SecYEG system* that is found in many prokaryotes and the *Type III Secretion System* employed for the export of toxins by several pathogenic bacteria.

2.10 | Peptidoglycan

The bacterial cell wall warrants extensive coverage in the classroom because research on its structure and function can be traced back to the early history of microbiology. It began with Ferdinand Cohn's early observation of the differential reaction of various bacterial cells to the Gram stain. This stain distinguished two types of bacteria based on the composition of the cell wall: gram-positive and gram-negative. Research proceeded with the discovery that both lysozyme and penicillin induced cell lysis and with the realization that some of the bacterial cell wall constituents (diaminopimelic acid and N-acetylmuramic acid) were unique. These discoveries were exciting. They increased our understanding of prokaryotic cells and helped to obtain better chemotherapy with which to combat bacterial diseases. The mechanisms of peptidoglycan biosynthesis, cell division (covered in Chapter 5), osmotic lysis, and the activity of penicillin are important topics of discussion because they provide striking examples of the interrelationship of basic knowledge and practical applications of great significance.

Figure 2.24 provides an excellent summary of the differences in structure and appearance of gram-positive versus gram-negative cell walls. Point out the fundamental repeating

structure of peptidoglycan (the *glycan tetrapeptide*; Figure 2.25), which consists of alternating *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) residues. Indicate that the latter of these two sugars is connected to a short peptide chain consisting of four amino acids, including in many bacteria the unique lysine analog diaminopimelic acid (DAP). The peptide chains provide structural rigidity to peptidoglycan by cross-linking the polysaccharide layers such that tensile strength is conferred on the cell wall in both the X and Y directions (Figure 2.26). Although there is some variation in the amino acid composition of these peptide cross-linkages, there is great unity within the bacteria regarding the presence of *N*-acetylmuramic acid, DAP (which may be replaced by lysine), and D-amino acids (D-alanine and D-glutamic acid) rather than the usual L stereoisomers found in proteins. However, in contrast to gram-negative bacteria, some NAM residues in the peptidoglycan of gram-positive bacteria contain covalently bound teichoic acids, polyalcohols joined by phosphate esters (Figure 2.27). Teichoic acids contribute to the overall negative charge of the gram-positive cell surface and help to sequester cationic micronutrients (e.g., Ca^{2+} and Mg^{2+}) from the environment.

At this time you may want to foreshadow the structure of the gram-positive bacterial cell wall in the context of antibiotic therapy and design. Emphasize that examples of unique cell chemistry often provide targets for successful chemotherapy without the problems of host toxicity (Figures 2.25–2.29). The mechanisms of action of both lysozyme and penicillin are good examples of how a chemical agent can destroy peptidoglycan, resulting in bacterial cell lysis.

Finally, note that there are also prokaryotic cells that lack a cell wall, including the mycoplasmas, a group of pathogenic bacteria (see Chapter 15), and species of the archaeal genera *Thermoplasma* and *Ferroplasma* (see Chapter 16). As previously noted, the mycoplasmas are unusual among bacteria in that they contain sterols in their membranes. The structural rigidity provided by these molecules presumably helps to maintain cell integrity during mild osmotic stress.

2.11 | LPS: The Outer Membrane

The outer membrane of gram-negative bacteria is obvious in TEM sections, where it is seen as a wavy lipid bilayer outside of a thin layer of peptidoglycan. In the outer membrane, *lipopolysaccharide* (*LPS*) (Figure 2.28) replaces most of the phospholipids in the outer leaflet, whereas lipoproteins in the inner leaflet function to anchor the outer membrane to peptidoglycan (Figure 2.29). Depending upon the chemistry background of your students, discuss the chemical components of the LPS: (1) *Lipid A*, the phosphoglycolipid portion of the LPS; (2) the *core polysaccharide,* consisting of ketodeoxyoctonate (KDO), heptoses (7-carbon sugars), hexoses, and *N*-acetylglucosamine; and (3) the *O-polysaccharide*, consisting of repeating sequences of hexoses that form long chains and may be branched (Figure 2.28).

Although the purpose of the outer membrane is structural, it is toxic to animals due to the lipid A component of the LPS. Toxins that are part of the cell wall of gram-negative bacteria are called *endotoxins*. Provide examples for your students of endotoxic human pathogens (e.g., *Shigella*, *Salmonella*, and *Escherichia*) that elicit ill effects in the host, most of which include gastrointestinal distress.

In contrast to the cytoplasmic membrane, the outer membrane is permeable to small molecules due to membrane channels called *porins* (Figure 2.29), which vary in specificity from nonspecific to highly specific. Students should be made aware that the *periplasm* of

gram-negative bacteria contains binding proteins that are not present in gram-positive bacteria. The periplasm contains a number of different classes of enzymes, some of which facilitate transport (Section 2.9) or chemotaxis (Section 2.19).

2.12 | Archaeal Cell Walls

Cell walls of *Archaea* do not contain peptidoglycan, but they do possess diverse chemistries that include proteins, polysaccharides, and glycoproteins. Some methanogens (Chapter 16) produce a polysaccharide similar to peptidoglycan called *pseudomurein* (Figure 2.30). Point out that the β-1,3 glycosidic linkage in pseudomurein is different from the β-1,4 linkage in peptidoglycan, thus making the former insensitive to the action of lysozyme. There are no known human pathogens from the *Archaea*, and thus the evolution of lysozyme most probably arose from the interactions of *Bacteria* with animal hosts over time.

Although not all *Archaea* contain pseudomurein, nearly all contain a cell wall of some type (exceptions were noted in Section 2.10). Extremely halophilic *Archaea* have sulfate (SO_4^{2-}) incorporated into their cell walls to bind excessive Na^+ and help shield the cell from its extremely salty environment. Other *Archaea* (and some *Bacteria*) have a paracrystalline surface layer, the *S-layer*, composed of protein or glycoprotein (Figure 2.31). Discuss the potential functions of S-layers, which are varied and may include protecting the cell from osmotic lysis; preventing the access of larger particles, such as viruses, to the cell membrane; and retaining secreted proteins near the cell surface.

2.13 | Cell Surface Structures

Cell surface structures produced by bacteria that are not an integral part of the cell wall are generally not considered essential to cell survival. However, the presence of *capsules* and *slime layers* (Figure 2.32), *fimbriae* (Figure 2.33), and *pili* (Figure 2.34) on many prokaryotes suggests that such structures play important ecological roles for these organisms, including the establishment of pathogenic associations with host cells. The attachment of one cell to another is a specific molecular interaction between host and pathogen, and this contact often initiates changes in the host cell resulting in internalization of the pathogen and continuation of its life cycle. Although details of host–pathogen interaction are not part of the material presented here, you could pique student interest by showing a specific example of the role played by these cell surface structures in a specific pathogenesis (e.g., *Streptococcus pneumoniae*, *Yersinia pestis*, and *Listeria monocytogenes*). Clearly define the structural and functional differences of fimbriae, pili, and flagella to students, who may equate these structures based on their similar microscopic appearance.

2.14 | Cell Inclusions

Many bacterial cells contain inclusions, such as the storage granules *polyhydroxybutyrate* (*PHB*; Figure 2.35) and *glycogen*, both of which serve as carbon and energy reserves. Additional nutrient inclusions include polyphosphate and elemental sulfur granules (Figure 2.36). The latter of these inclusions serves as an important secondary energy source for a variety of phototrophic and chemolithotrophic bacteria that oxidize sulfide (H_2S) as an electron donor (see Chapter 13).

Other storage inclusions are not necessarily for nutritional purposes. Many prokaryotes catalyze *biomineralization*, the process of mineral formation by microorganisms. Figure 2.37 shows a beautiful example of benstonite granule accumulation inside a cell of the cyanobacterium *Gleomargarita*; the function of these structures is unknown but it may be to provide ballast for the cell in its aqueous environment. Your discussion of *magnetosomes* and magnetotactic bacteria should be of interest to students, who will likely find the idea of "magnetic bacteria" intriguing. Although the function of magnetosomes is also unknown, they are most certainly important to species that form them (Figure 2.38). One hypothesis is that the magnetite in these inclusions acts like a compass, pulling the microaerophilic aquatic bacteria that contain them downward toward the Earth's magnetic poles and into the sediments where dissolved O_2 concentrations are lower. Unlike polyphosphate and elemental sulfur granules, both magnetosomes and PHA inclusions have a "nonunit" (single layer) phospholipid membrane.

2.15 | Gas Vesicles

Gas vesicles are rigid, hollow structures in the cytoplasm of some cells that allow vertical migration in a water column. They are therefore considered a mechanism of motility (Figures 2.39–2.41). The proteinaceous shell is permeable to gases, but not to water and solutes. At the molecular level, the shell contains two proteins: GvpA, a rigid β-sheet that makes up 97% of the shell; and GvpC, a cross-linking protein made up of α-helices (Figure 2.41). These structures are found mostly in aquatic phototrophs, allowing them to regulate their position in the water column where the light intensity required for photosynthesis is optimal. Some nonphototrophs, including some species of *Archaea*, also contain gas vesicles.

2.16 | Endospores

Introduce your discussion of endospores by reminding students of Ferdinand Cohn's discovery of the endospore-forming genus *Bacillus* and his research demonstrating the incredible heat resistance of these structures (Chapter 1). Because of Cohn's efforts, important new methods of sterilization were developed that are still used by the food and medical industries. To spark student interest, note that many endospore-forming bacteria are also pathogenic and cause some of the most serious diseases known. For example, pathogenic members of the genera *Bacillus* and *Clostridium* often produce potent toxins that cause fatal diseases if not treated within a short time. Examples include botulism (*C. botulinum*), tetanus (*C. tetani*), gas gangrene (*C. perfringens*), and anthrax (*B. anthracis*).

Depending on the level of your course, discuss the structure of endospores and the endosporulation and germination processes in some detail (Figures 2.42–2.47). Some key points to stress include the following:

- Describe the unique nature of the core, stressing the functions of the dipicolinic acid (DPA) and Ca^{2+} complexes, the low water content and low pH, and the role of small acid-soluble spore proteins (SASPs) in protecting the DNA and in serving as a carbon and energy source for the cell during germination.
- Discuss endospore formation as an example of cellular differentiation in prokaryotes, using *Bacillus subtilis* as a model (Figure 2.47). To impress upon students the remarkable complexity of the differentiation process, mention that more than 200 genes are involved in

sporulation, and many details of the process are still being investigated in laboratories around the world.

Finally, students should show interest in a discussion concerning how long endospores can remain viable. The debate on the longevity of these structures has now pushed their life span to millions of years. If experimental evidence from independent research laboratories repeatedly supports these claims, this would indeed be an extraordinary testament to the life-preserving design of these structures.

2.17 | Flagella and Swimming Motility

The ability of prokaryotes to move via flagella is intimately connected to their ability to sense and respond to environmental signals through complex signal transduction pathways. Bacteria arrange flagella on their surfaces in a variety of ways (Figures 2.48–2.50), and even many archaea are flagellated (Figure 2.52). The flagellar structure is complex, and its synthesis and assembly involve more than 40 genes in *E. coli* (Figures 2.51 and 2.53). Rotation of bacterial flagella requires significant energy directly from a *proton motive force* (*PMF*; Section 2.8 and Chapter 3). In fact, a single rotation requires the translocation of about 1000 protons across the membrane through the Mot complex (Figure 2.51*b*). Although bacterial flagella do not rotate at a constant speed, up to 300 revolutions per second are possible, resulting in extremely fast movement of about 60 cell lengths per second. *When measured as the number of body lengths moved per second*, a bacterium swimming at full speed would be moving nearly 2.5 times faster than a cheetah can run!

Flagella from the different domains of life exhibit significant structural and operational differences. Prokaryotic flagella rotate instead of moving in a whip-like motion (Figure 2.54), as is the case in eukaryotes. Several differences also exist between the flagella of different prokaryotes. Bacterial flagella are about twice as thick as archaeal flagella. In addition, the filament portion of all bacterial flagella is composed of a single type of flagellin protein, whereas the protein composition of archaeal flagellar filaments varies depending on the species. Perhaps the most significant difference between bacterial and archaeal flagella comes from recent evidence indicating that, like eukaryotes, archaeal flagella are powered directly by the hydrolysis of ATP rather than by a PMF, as in bacteria. As is the case for many of their cellular properties, it is interesting to note that *Archaea* exhibit flagellar characteristics similar to those of both *Bacteria* and *Eukarya* without being identical to either one. The fundamental differences between the flagella of the three domains of life suggest that these mechanisms have arisen independently as a result of convergent evolution rather than from a common origin.

2.18 | Gliding Motility

Gliding motility (motility without flagella) in bacteria is a relatively underrepresented phenomenon in microbiology texts, and this is probably because of the lack of knowledge of the molecular mechanisms involved in the process. Consequently, it is a good puzzle to present to students following your discussion of flagellar locomotion, about which much is known. Gliding motility has never been observed in *Archaea*, but several species of gliding *Bacteria* are known, including species of cyanobacteria and the genera *Myxococcus*, *Cytophaga*, and *Flavobacterium* (Figure 2.55). Movement by gliding requires a solid surface and is

considerably slower than flagellar motility. Several mechanisms of gliding motility have been described. Some bacteria secrete a polysaccharide slime that adheres to a surface and pulls the cell forward. Others exhibit a "twitching motility" in which cell movements are carried out by the repeated extension and retraction of type IV pili. Cells of *Flavobacterium johnsoniae* appear to glide via the coordinated ratcheting of cytoplasmic membrane proteins with outer membrane proteins, where the latter move along a surface in the opposite direction of the cell itself, much like the movement of a tank on its tracks (Figure 2.56).

2.19 | Chemotaxis and Other Taxes

The ability of bacteria to exhibit taxes (i.e., directed movement) confers a selective advantage depending upon environmental conditions. Students should understand that prokaryotes (unlike larger organisms) sense gradients in a *temporal* (an effect lasting for only a short time) rather than a *spatial* (a lingering effect) manner. In other words, they must continually compare their current external conditions with those of a few moments before. The studies of chemotaxis in *E. coli* provided the first genetic model of the process in swimming bacteria. Chemotaxis will be discussed in Chapter 7 in the context of two-component signal transduction systems, but use Figures 2.57 and 2.58 to show the run and tumble "directed" response and capillary assay system used to evaluate and identify signal molecules that act as attractants or repellants.

Many of the protein components that function in chemotactic pathways are also activated during phototaxis, and flagellar rotation is controlled accordingly. The response of *Rhodospirillum centenum* to light is a fascinating example of phototaxis. Mention to students that *scotophobotaxis* (movement away from dark) is not the same as true phototaxis, which involves movement up a light gradient (Figure 2.59). *R. centenum* is also unusual in that an entire colony of cells on solid media will move toward an infrared light source (the wavelengths absorbed by their photosynthetic pigments) and away from fluorescent light. If one observes the cells within the colony as the colony moves in one direction, the individual cells appear to be moving more or less randomly, suggesting there must be some intercellular communication occurring to generate a net directional movement.

Other taxes have been observed in microorganisms, including directed movement toward or away from oxygen (*aerotaxis*), toward a specific osmotic condition (*osmotaxis*), or toward water (*hydrotaxis*).

2.20–2.22 | Eukaryotic Microbial Cells

Students should be familiar with the organelles and general structure of the eukaryotic cell from general biology courses, but you should still present a brief overview of the topic, focusing first on the nucleus and chromosome organization (Figures 2.60 and 2.61). Remind students that eukaryotic DNA is packaged in the nucleus by being wound around positively charged *histone* proteins. Note that transport of proteins and nucleic acids through *nuclear pores* requires the energy of GTP. The *nucleolus* is the site of ribosomal RNA synthesis and assembly of the large and small subunits of the ribosome. Also review *mitosis* (Figure 2.62) and *meiosis* with your students, reminding them that these processes, which occur only in eukaryotes, are mechanisms by which a cell divides to create two diploid daughter cells or four haploid gametes (or spores), respectively.

Review mitochondrial structure (Figure 2.63) and function with your students, and allow this to lead into a discussion of the *hydrogenosome* (Figure 2.64). The latter organelle is found in certain eukaryotes that lack mitochondria, the present-day representatives of which arose early after the divergence of *Eukarya* from *Archaea* (see Chapter 12). Unlike mitochondria, hydrogenosomes usually lack cristae and do not contain citric acid cycle enzymes. Therefore, organisms possessing hydrogenosomes are strictly fermentative. Several are parasites, such as the sexually transmitted pathogen *Trichomonas vaginalis*, and all are either aerotolerant or obligate anaerobes. Figure 2.64 shows the pyruvate oxidation scheme carried out by the hydrogenosome for ATP synthesis.

Chloroplasts are the structures found in plant cells and many microbial eukaryotes (e.g., dinoflagellates, euglenids, diatoms, and various algae) that carry out photosynthesis and allow for photoautotrophic growth (Figure 2.65). The relationship of chloroplasts, mitochondria, and hydrogenosomes to *Bacteria* is well-documented and is discussed briefly here as the *endosymbiotic hypothesis*. Although this topic will be addressed in more detail in future chapters (see Sections 12.3 and 17.1), you may wish to point out key features supporting endosymbiosis to your students at this time:

- All three structures contain their own DNA in covalently closed circles. This DNA encodes rRNAs, tRNAs, and some respiratory enzymes.
- These organelles contain their own ribosomes that are bacterial in structure and are sensitive to antibiotics that affect bacterial ribosomes.
- The nuclear DNA of eukaryotic cells contains bacterially derived genes, lending additional evidence to support the endosymbiotic hypothesis (Section 17.1).
- 16S rRNA gene sequence analyses show the evolutionary relatedness of these structures to *Bacteria*.

Finally, review the function of other eukaryotic cell structures (e.g., the endoplasmic reticulum, Golgi complex, ribosomes, and cytoskeletal elements) mentioned in Section 2.22, which should already be familiar to most students (Figures 2.66–2.68). Point out that eukaryotic flagella are powered by ATP hydrolysis and propel the cell via a whiplike motion rather than by rotation, as seen in prokaryotic cells.

Answers to Review Questions

1. Magnification is the optical enlargement of an object, whereas resolution is the ability to distinguish two objects as distinct and separate, especially in reference to microscopic observation. Although magnification can be increased virtually without limit, increases in resolution are limited by the physical properties of light.
2. Cells are stained to increase their contrast so that they can be more easily seen against the background. In bright-field microscopy, cationic (positively charged) dyes are used because they combine with negatively charged cellular constituents, such as nucleic acids, teichoic acids in the gram-positive cell wall, and acidic polysaccharides. Phase-contrast microscopy exploits the principle that different substances refract light differently. By amplifying these differences using a phase ring, living, unstained specimens can be quickly and easily viewed. Therefore, unlike bright-field microscopy, phase-contrast microscopes can be used to observe living specimens in wet mount preparations with good contrast against the lit background. A differential interference contrast (DIC) microscope polarizes light, generating two distinct beams. When these beams recombine, they are not totally in phase, and therefore subtle differences within the cell are

intensified. Because of this, specific cellular structures have a three-dimensional appearance, and harsh staining techniques become unnecessary. Imaging of this type is not possible with a bright-field microscope.

3. The major advantage the electron microscope has over the light microscope is greatly increased resolution due to the use of an electron beam for imaging rather than a beam of light. The increased resolution allows for clear imaging at very high magnifications. A scanning electron microscope would be used to view the three-dimensional features of a cell.

4. The major prokaryotic morphologies are coccus, rod, spirillum, spirochete, appendaged, and filamentous. Several variations and subcategories exist.

5. Prokaryotic cell sizes typically range from about 0.4 μm in length to several hundred micrometers, with most cells averaging 1–3 μm. The maximum size of a prokaryotic cell appears to be in excess of 750 μm, and the smallest is likely to be about 0.2 μm. We are better able to know the lower limit of size of a bacterial cell than the upper limit because we know the minimum amount of volume required for genetic material, ribosomes, proteins, and so on necessary to carry out metabolism. An *E. coli* cell is 1–2 μm in length and 0.5–0.6 μm wide.

6. Because phospholipids, the major structural component of unit membranes, contain both hydrophobic and hydrophilic moieties, their aggregation in aqueous solution leads to the formation of a bilayer in which hydrophobic lipids face each other in the internal region and polar glycerophosphates communicate with the external environment.

7. In contrast to *Bacteria* and *Eukarya,* which use ester linkages to bond fatty acids to the glycerol molecule, lipids from *Archaea* have ether linkages between the glycerol and their hydrophobic side chains. Moreover, archaeal lipids lack fatty acids and instead have side chains composed of repeating units of the C_5 hydrocarbon isoprene that combine to form the C_{20} molecule phytanyl. Glycerol diethers, diglycerol tetraethers, and crenarchaeol are the major classes of lipids present in species of *Archaea.* The phytanyl side chains of diglycerol tetraether are covalently bonded together at their ends, thus forming a phospholipid monolayer instead of a bilayer.

8. Ionized molecules, which have a net charge, are hydrophilic and are therefore repelled by the hydrophobic interior of the membrane. Such molecules are brought into the cell through membrane-spanning transport proteins.

9. The LacY permease system is a symporter system that takes up one molecule of lactose along with one proton, thereby using the energy of the proton motive force to drive uptake of lactose. The phosphotransferase system is a group translocation system in which a series of proteins are alternately phosphorylated and dephosphorylated in a cascading fashion. In the end, the sugar itself (in this case, glucose) is phosphorylated upon being transported into the cell through a membrane-spanning transport protein. The energy to drive the uptake of glucose originates from the high-energy anhydride bond in phosphoenolpyruvate. The ABC transport system uses three components to transport a substance (in this case, maltose) across a membrane: a periplasmic binding protein, a membrane-spanning transporter, and an ATP-hydrolyzing protein, the latter of which uses the energy released from the hydrolysis of ATP to drive the transport event.

10. Because the rigid layer is a polymer composed of sugars linked by β-1,4 glycosidic bonds and polypeptides that cross-link *N*-acetylmuramic acid residues, the term peptidoglycan (“protein” + “sugar”) is appropriate. Together, the covalent glycosidic and

peptide bonds of peptidoglycan provide tensile strength in both the X and Y directions, respectively.

11. Functions of the outer membrane of gram-negative bacteria include antigenicity, adhesion to surfaces, toxicity for animals, provision of membrane channels and porins for the influx and efflux of low molecular weight substances, and a permeability barrier to the passage of high molecular weight substances. The chemical composition of the outer membrane is a phospholipid bilayer containing lipopolysaccharide.

12. Peptidoglycan is a polysaccharide present in *Bacteria,* but absent in *Archaea*. S-layers consist of protein or glycoprotein in a paracrystalline array. They are found in both *Bacteria* and *Archaea* and may serve as an outermost selective sieve around the cell to prevent access of large particles, such as viruses, to the cytoplasmic membrane. Methanogenic *Archaea* have cell walls composed of pseudomurein, which is similar in structure to peptidoglycan but contains lysozyme-insensitive β-1,3 glycosidic bonds. Extremely halophilic *Archaea* have polysaccharide cell walls containing large amounts of sulfate, which buffers the cell from its very salty environment by binding Na^+.

13. Polysaccharide layers produced by bacteria are referred to as *capsules* and/or *slime layers*, and they have several potential functions. They play a major role in the attachment of cells to a surface (a property that allows for biofilm formation), they prevent phagocytosis by immune system macrophages, and they may function to prevent desiccation.

14. Cytoplasmic inclusions include carbon polymers (e.g., poly-β-hydroxybutyrate and glycogen), inorganic phosphate (polyphosphate), elemental sulfur, and magnetite. Poly-β-hydroxybutyrate granules serve as a cell energy reserve and consist of a polymeric fatty acid, whereas magnetosomes consist of magnetite (Fe_3O_4) and serve as small magnetic compasses. Aquatic cells may use magnetosomes as a means to align themselves within zones of favorable oxygen concentration.

15. Because of their buoyancy, gas vesicles provide a means of motility for aquatic cells. These structures are water impermeable because their membranes consist of highly hydrophobic, rigid, cross-linked proteins. Gas-filled vesicles decrease the density of the cell. Therefore, the number of inflated vesicles in a cell determines its buoyancy and position in the water column.

16. The endospore is a different type of cell than the vegetative cell for several reasons. The cytoplasm of endospores is considerably dehydrated compared to vegetative cells, and endospores have a unique cell surface consisting of multiple layers of proteins. In addition, endospores are "shut down" in the sense that they are essentially metabolically dormant. All of these factors permit significant resistance to heat, radiation, desiccation, and extremes of pH, and they allow for remarkable longevity of these structures.

17. *Mature endospores* are a form of metabolically inactive, differentiated cells produced by some species of *Bacteria* in which a marked degree of dehydration has occurred, and the surface has changed to a tough, multilayered protein composition. While endospores represent a dormant stage of an endospore-forming bacterium, *vegetative cells* are metabolically active cells capable of growth, reproduction, and other activities characteristic of living systems. *Germination* is the process by which a free, mature endospore is converted back into a vegetative cell upon encountering favorable environmental conditions.

18. The bacterial flagellum is a long, rigid, protein filament of polymerized flagellin that extends from the cytoplasmic membrane and cell wall. It generates cellular motility by rapidly rotating, much like a miniature propeller. A wider hook at the base of the

flagellum serves to connect the flagellum to the motor portion (basal body), which is anchored to the cell wall and cytoplasmic membrane. In *Bacteria*, the proton motive force provides the energy for rotation via the flow of H^+ across the membrane. Archaeal flagella are thinner by comparison and may be composed of a diversity of flagellin-type proteins, depending on the species.

19. The gliding motility exhibited by species of *Flavobacterium* differs from the flagellar motility of *E. coli* in several respects. Gliding motility, which requires a surface for the cells to move across, is considerably slower than the swimming motility of flagellated cells. The mechanism of gliding in *Flavobacterium* appears to be by a ratcheting movement between proteins anchored in the cytoplasmic membrane and the outer membrane. However, gliding motility in species of *Flavobacterium* and swimming motility in *E. coli* are both powered by energy from the proton motive force.

20. Motile bacteria move toward attractants by comparing the strength of stimuli (using chemoreceptors) obtained over different moments in time, during which they have changed their position (i.e., the response is temporal). If an environmental stimulus is desirable, flagellar activity results in fewer tumbles and longer runs. If a stimulus is not desirable, then the opposite will occur. Although the new direction following a tumble is random, adjusting the duration of runs and the frequency of tumbles allows the cell to ultimately move toward the attractant, albeit in a somewhat erratic manner. Chemotaxis, therefore, resembles smell rather than sight.

21. In the experiment shown in Figure 2.58, the control is a capillary that contains neither an attractant nor a repellant. The control is essential because it provides an unmanipulated point of comparison and, therefore, serves to validate the results obtained when a variable (i.e., the attractant or repellant) is introduced to the capillary.

22. Three features that clearly differentiate eukaryotic from prokaryotic cells include the following: (1) Almost all eukaryotes possess a membrane-bound nucleus containing their genomic DNA (although, interestingly, there are a few nucleated prokaryotes among the *Planctomycetes*, described in Chapter 15); (2) unlike nearly all prokaryotes, eukaryotic cells contain various membrane-bound organelles (e.g., mitochondria and the Golgi complex); and (3) because prokaryotes have no nucleus, they carry out coupled transcription and translation; these processes are separate in eukaryotes because of the presence of a nuclear membrane. Histones are positively charged protein complexes that bind and compact DNA in the nucleus of eukaryotic cells.

23. The hydrogenosome and mitochondrion are about the same size, and they both function to catabolize pyruvate. In mitochondria, pyruvate is oxidized completely using enzymes of the citric acid cycle. CO_2 and H_2O are the products of this aerobic respiration, and ATP is synthesized by oxidative phosphorylation using a proton motive force established by the mitochondrial electron transport chain. The hydrogenosome is found only in certain anaerobic eukaryotes. Because it lacks citric acid cycle enzymes and cristae containing electron transport chain components, pyruvate is fermented to acetate, CO_2, and H_2, and ATP is synthesized via substrate-level phosphorylation.

24. The chloroplast is the site of photosynthesis. The light-induced reaction center and electron transport chain generate energy (ATP) and reducing power (NAD[P]H) required to reduce CO_2 to organic carbon (e.g., phosphoglyceric acid) via the Calvin cycle for biosynthetic and metabolic requirements. The important Calvin cycle enzyme RubisCO comprises over 50% of the total chloroplast protein.

25. Certain eukaryotic organelles, specifically mitochondria, chloroplasts, and hydrogenosomes, contain circular DNA that is more similar to bacterial DNA than eukaryotic DNA in sequence composition. In addition, these organelles contain their own ribosomes that resemble those of bacteria and, therefore, are sensitive to many of the same antibiotics that target bacterial ribosomes.

26. The endoplasmic reticulum (ER) is a network of membranes continuous with the outer leaflet of the nuclear membrane that is involved in the synthesis of lipids and carbohydrates (smooth ER) and glycoproteins and new membrane material (rough ER). The Golgi complex is a stack of membranes that works in concert with the ER in the production, sorting, and modification of membrane and secretory proteins. Such modifications help to direct these products to their final destinations. Lysosomes contain digestive enzymes to break down various macromolecules and recycle them for biosynthesis.

Answers to Application Questions

1. The diameter of the smallest object resolvable by any lens in a light microscope is equal to 0.5λ/numerical aperture (NA). Using a 600-nm wavelength and an NA of 1.32, this would be 227 nm, or ~0.23 μm. The resolution could be improved using this same lens by using a shorter wavelength in the blue light range.

2. The surface/volume ratio for a cell having a diameter of 15 μm is 0.4. The surface/volume ratio for a cell with a diameter of 2 μm is 1.5. As the diameter of the cell *decreases*, there is more surface area per unit volume. Thus, with a higher surface-to-volume ratio, there is an increased capacity for the cell to interact with its environment per unit of cytoplasm. This generally leads to higher levels of metabolic activity in the cell and, therefore, a faster growth rate.

3. Five ways to tell which culture is a species of *Bacteria* or *Archaea*:

Bacteria	***Archaea***
Ester links from side chains of glycerol	Ether links from side chains of glycerol
Fatty acid side chains from glycerol	Polyisoprene side chains from glycerol
N-acetylmuramic acid in cell wall	*N*-acetyltalosaminuronic acid in cell wall
Peptidoglycan in cell wall	Other polysaccharides in cell wall
Lysozyme-sensitive	Lysozyme-resistant

4. Sixty cell lengths per second for an *E. coli* cell that is 2 μm long equals a speed of 120 μm/sec. A 3-cm capillary tube = 30,000 μm, so at this speed it would take 250 seconds, or nearly 4.2 minutes, for the cell to travel the length of the tube.

5. Unknown cultures can be differentiated in terms of Gram reaction using the following methods:

(a) *Light microscope*: Observe the Gram reaction of the cells following Gram staining of the cultures.

(b) *Electron microscope*: Observe the appearance of the cell wall in thin-section micrographs. Gram-positive cells will have a thick layer of peptidoglycan with no outer membrane, whereas gram-negative cells will have a thin layer of peptidoglycan within a periplasm and a wavy outer membrane containing the LPS layer.

(c) *Chemical analyses of cell walls*: Unlike gram-negative cells, gram-positive cells contain teichoic acids in their cell walls but lack an outer membrane containing the LPS. The presence of the endotoxin lipid A (a component of the LPS) would confirm a gram-negative culture.

(d) *Phylogenetic analyses*: The Gram reaction of an unknown culture can be inferred based on the relationship of the organism to its closest phylogenetic relatives (based on 16S rRNA gene sequence analysis). For example, an organism that is closely related to species of *Clostridium* would almost certainly be gram-positive.