two or more fused cyclobutane rings. They pack together very tightly, and this characteristic is thought to be important to anammoxosome function.

Inclusions

Inclusions are common in all cells. They are formed by the aggregation of substances that may be either organic or inorganic. The first bacterial inclusions were discovered in the late 1800s. Since then much has been learned about their structure and function. Inclusions can take the form of granules, crystals, or globules; some are amorphous. Some inclusions lie free in the cytoplasm. Other inclusions are enclosed by a shell or membrane that is single-layered and may consist of proteins or of both proteins and phospholipids. Some inclusions are surrounded by invaginations of the plasma membrane. Many inclusions are used for storage (e.g., of carbon compounds, inorganic substances, and energy) or to reduce osmotic pressure by tying up molecules in particulate form. The quantity of inclusions used for storage varies with the nutritional status of the cell. Some inclusions are so distinctive that they are increasingly being referred to as microcompartments. A brief description of several important inclusions follows.

Storage Inclusions

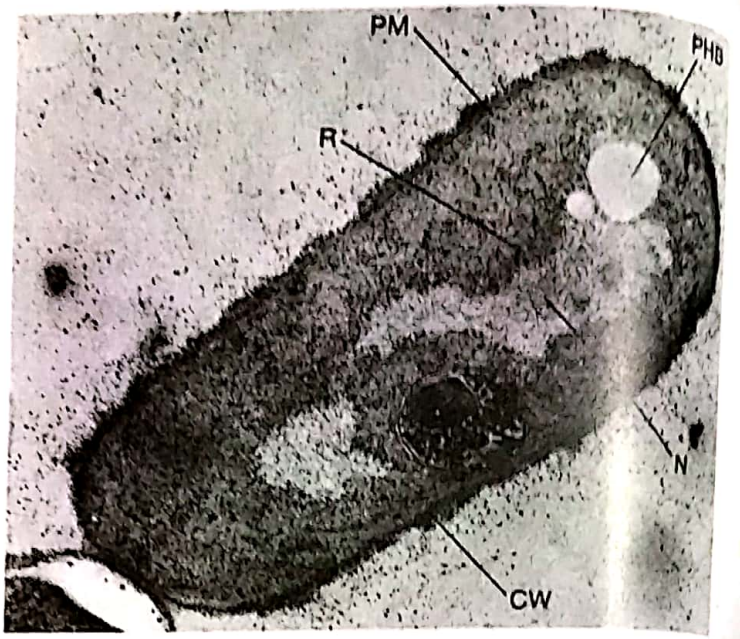
Cells have a wide variety of storage inclusions. Many are formed when one nutrient is in ready supply but another nutrient is not. Some store end products of metabolic processes. In some cases these end products are used by the microbe when it is in different environmental conditions. The most common storage inclusions are glycogen inclusions, polyhydroxyalkonate granules, sulfur globules, and polyphosphate granules. Some storage inclusions, such as the cyanophycin granules in cyanobacteria, are observed only in certain organisms.

Glycogen is a long branched chain of glucose units. Glycogen inclusions are found in both bacterial and archaeal cells but have been best studied in bacteria. Glycogen inclusions usually form when bacteria are growing in an environment that is limited for an important nutrient (e.g., phosphate) but contains excess carbon. Thus glycogen inclusions serve to store carbon until the missing nutrient becomes available. Most glycogen inclusions are not bound by a membrane, but some are surrounded by a single-layered membrane. Other glycogen inclusions that are not membrane bound are dispersed evenly throughout the cytoplasm as small granules (about 20 to 100 nm in diameter) and often can be seen only with the electron microscope. If cells contain a large amount of glycogen, staining with an iodine solution will turn the cells reddish-brown. ▶▶| *Carbohydrates (appendix I)*

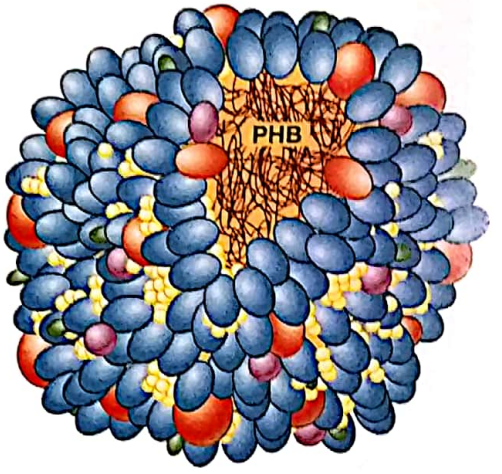
Carbon is also stored as polyhydroxyalkonate (PHA) granules. Several types of PHA granules have been identified, but the most common granules contain **poly-β-hydroxybutyrate (PHB).** PHB contains β-hydroxybutyrate molecules joined by ester bonds between the carboxyl and hydroxyl groups of adjacent molecules. PHB accumulates in distinct bodies, around 0.2 to 0.7 μm in diameter, that are readily stained with Sudan black for light microscopy and are seen as empty "holes" in the electron microscope (**figure 3.33*a***). This is because PHB is hydrophobic, so it is dissolved by the solvents used to prepare specimens for electron microscopy. The structure of PHB inclusions has been well studied, and PHB granules are now known to be surrounded by a single-layered membrane composed of proteins and a small amount of phospholipids (figure 3.33*b*). Much of the interest in PHB and other PHA granules is due to their industrial use in making biodegradeable plastics. ▶▶| *Biopolymers (section 41.3)*

Polyphosphate granules and sulfur globules are inorganic inclusions observed in many organisms. Many bacteria store phosphate as **polyphosphate granules,** also called volutin granules or metachromatic granules. Polyphosphate is a linear polymer of orthophosphates joined by ester bonds. Thus polyphosphate granules store the phosphate needed for synthesis of important cell constituents such as nucleic acids. In some cells they act as an energy reserve, and polyphosphate also can serve as an energy source in some reactions. Polyphosphate granules are called metachromatic granules because they show the metachromatic effect; that is, they appear red or blue when stained with the blue dyes methylene blue or toluidine blue. Sulfur globules are formed by bacteria that use reduced sulfur-containing compounds as a source of electrons during their energy-conserving metabolic processes (**figure 3.34**). For example, some photosynthetic bacteria can use hydrogen sulfide (rather than water) as an electron donor and accumulate the resulting sulfur either externally or internally. ▶▶| *Light reactions in anoxygenic photosynthesis (section 10.12);* Gammaproteobacteria *(section 20.3)*

Cyanophycin granules are observed in cyanobacteria, a group of photosynthetic bacteria. These inclusions are composed of large polypeptides containing approximately equal amounts of the amino acids arginine and aspartic acid. The formation of these granules is of particular interest because the cyanophycin granule polypeptide is not encoded by mRNA and is not synthesized by ribosomes. The granules often are large



(a)



(b)

FIGURE 3.33 PHB Inclusions in Bacteria. (a) Electron micrograph of *Bacillus megaterium* (×30,500). PHB, poly-β-hydroxybutyrate inclusion; CW, cell wall; N, nucleoid; PM, plasma membrane and R, ribosomes. (b) Structure of a PHB granule. PHB is enclosed by a membrane composed of several different proteins, including the PHB-synthesizing enzyme (red sphere) and the PHB-degrading enzyme (green sphere). Yellow spheres represent the phospholipids that are also found in the membrane. Note that the membrane is not a phospholipid bilayer.

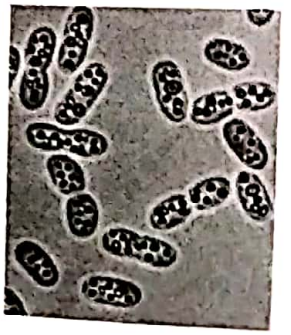


FIGURE 3.34 Sulfur Globules. *Chromatium vinosum,* a purple sulfur bacterium, with intracellular sulfur globules, bright-field microscopy (×2,000

enough to be visible in the light microscope and store extra nitrogen for the bacteria.

Microcompartments

Some bacterial inclusions are very unique and serve functions other than simply storing substances for later use by the cell. Many researchers now refer to these inclusions as microcompartments. Although microcompartments are not bound by a lipid bilayer, some scientists feel they are analogous to membrane-bound organelles such as mitochondria. The best studied microcompartment is the carboxysome.

Carboxysomes are present in many cyanobacteria and other CO_2-fixing bacteria **(figure 3.35)**. They, like other microcompartments, consist of a protein coat that is polyhedral, a shape similar to that of certain viruses. The polyhedral coat is composed of 6 to 10 different proteins and is about 100 nm in diameter. One of the proteins in the shell is the enzyme carbonic anhydrase, which converts carbonic acid into CO_2 and releases it into the lumen of the carboxysome. The nature of the carboxysome shell prevents the CO_2 from escaping; thus the carboxysome concentrates CO_2. Also enclosed within the polyhedron is the enzyme ribulose-1, 5-bisphosphate carboxylase (Rubisco). Rubisco is the critical enzyme for CO_2 fixation, the process of converting CO_2 into sugar. Thus the carboxysome also serves as a site for CO_2 fixation.

▶▶| *CO_2 fixation (section 11.3)*

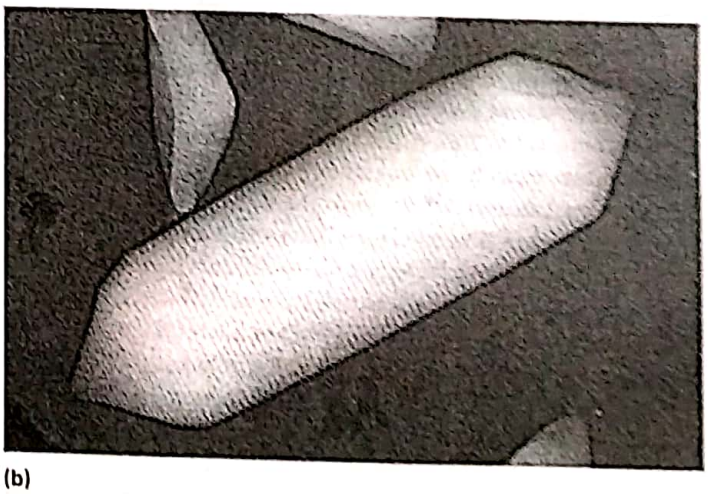
Other Inclusions

Inclusions can be used for functions other than storage or as microcompartments. Two of the most remarkable inclusions are gas vacuoles and magnetosomes. Both are involved in the movement of microbes.

FIGURE 3.35 Carboxysomes. Carboxysomes in the bacterium *Halothiobacillus neapolitanus*. This is one image of a tilt series taken for electron cryotomography. Each carboxysome is approximately 100 nm in diameter.

The gas vacuole provides buoyancy to some aquatic bacteria and archaea. Gas vacuoles are present in many photosynthetic bacteria, aquatic archaea such as *Halobacterium* (a salt-loving archaeon), and some aquatic bacteria that are not photosynthetic (e.g., *Thiothrix*, a filamentous bacterium). Gas vacuoles are aggregates of enormous numbers of small, hollow, cylindrical structures called **gas vesicles (figure 3.36)**. Gas vesicle walls are composed entirely of a single small protein. These protein subunits assemble to form a rigid cylinder that is impermeable to water but freely permeable to atmospheric gases. Cells with gas vacuoles can regulate their buoyancy to float at the depth necessary for proper light intensity, oxygen concentration, and nutrient levels. They descend by simply collapsing vesicles and float upward when new ones are constructed.

(a)



(b)

FIGURE 3.36 Gas Vacuoles and Gas Vesicles. (a) A freeze-fracture preparation of *Anabaena flosaquae* (×89,000) showing gas vesicles and gas vacuoles. Clusters of the cylindrical vesicles form gas vacuoles. Both longitudinal and cross-sectional views of gas vesicles are indicated by arrows. (b) Gas vesicles of *Halobacterium salinarum* (~×150,000).

Aquatic magnetotactic bacteria use **magnetosomes** to orient themselves in Earth's magnetic field. Magnetosomes are intracellular chains of magnetite (Fe_3O_4) particles (**figure 3.37**). They are around 35 to 125 nm in diameter and enclosed within invaginations of the plasma membrane. Since each iron particle is a tiny magnet, the Northern Hemisphere bacteria use their magnetosome chain to determine northward and downward directions, and swim down to nutrient-rich sediments or locate the optimum depth in freshwater and marine habitats. Magnetotactic bacteria in the Southern Hemisphere generally orient southward and downward, with the same result. For the cell to move properly within a magnetic field, the magnetosomes must be arranged in a chain. A cytoskeletal protein called MamK is currently thought to be responsible for establishing a framework upon which the chain can form (figure 3.37*b*).

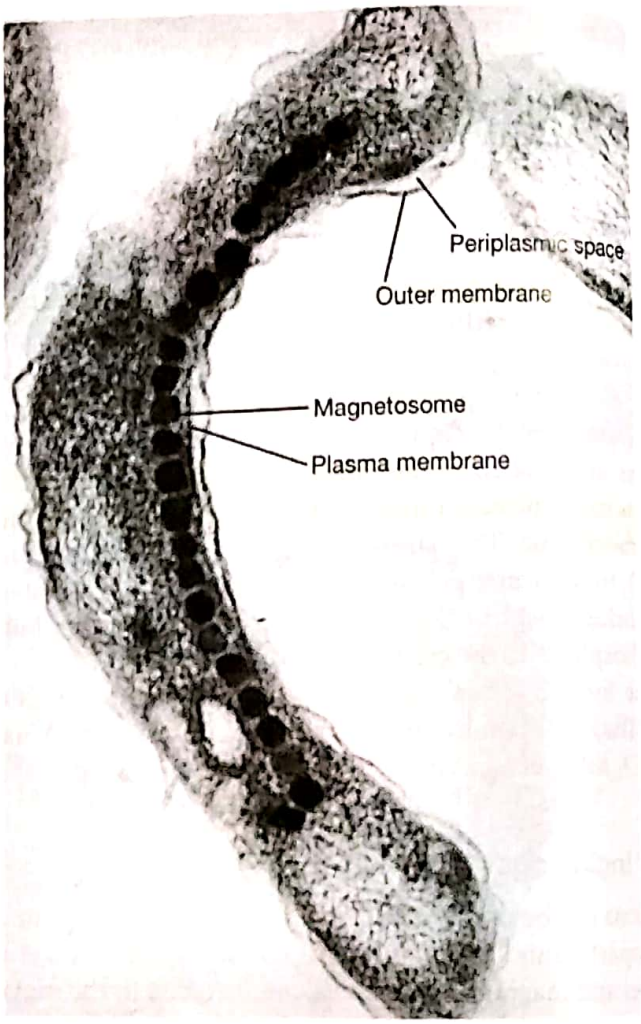
Ribosomes

Ribosomes are the site of protein synthesis, and large numbers of them are found in nearly all cells. The cytoplasm of bacterial and archaeal cells is often packed with ribosomes, and other ribosomes may be loosely attached to the plasma membrane. The cytoplasmic ribosomes synthesize proteins destined to remain within the cell, whereas plasma membrane-associated ribosomes make proteins that will reside in the cell envelope or are transported to the outside.

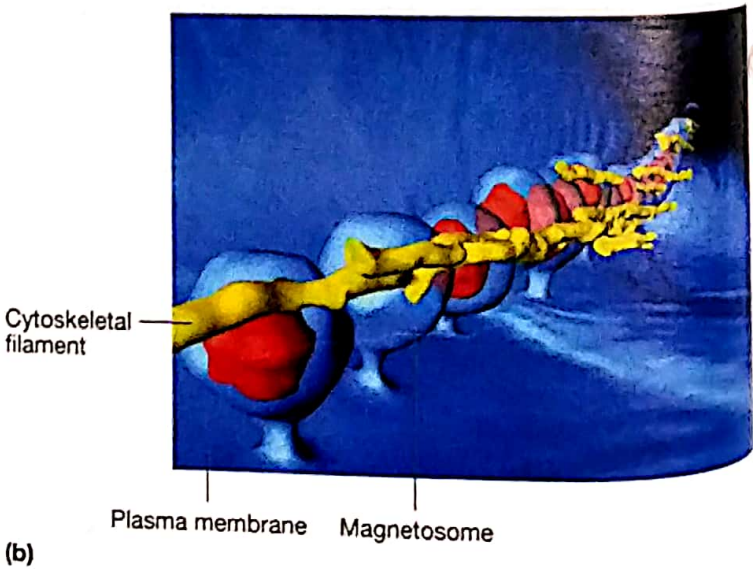
Translation, the process of protein synthesis, is amazingly complex and is discussed in detail in chapter 12. This complexity is evidenced in part by the structure of ribosomes, which are made of numerous proteins and several ribonucleic acid (RNA) molecules. Although the overall morphology and makeup of ribosomes is similar across all domains, there are some important differences. The similarities and some of the differences between bacterial and archaeal ribosomes are discussed here.

Bacterial and archaeal ribosomes are called 70S ribosomes (as opposed to 80S in eukaryotes) and are constructed of a 50S and a 30S subunit (**figure 3.38**). The S in 70S and similar values stands for **Svedberg unit.** This is the unit of the sedimentation coefficient, a measure of the sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged, the greater its Svedberg value or sedimentation coefficient. The sedimentation coefficient is a function of a particle's molecular weight, volume, and shape. Heavier and more compact particles normally have larger Svedberg numbers and sediment faster. Thus bacterial and archaeal ribosomes are smaller than the ribosomes of eukaryotic cells.

Despite their similar size, the makeup of bacterial ribosomes and their shape are somewhat different than archaeal ribosomes. Both have ribosomal RNA (rRNA) molecules of similar size: 16S in the small subunit, and 23S and 5S in the large subunit. However, at least one archaeon has an additional rRNA, a 5.8S rRNA, in the large subunit. This is of interest because the large subunit of eukaryotic ribosomes contains both 5S and 5.8S rRNA molecules. The protein composition of bacterial and archaeal ribosomes also differs. Bacterial ribosomes have about 55 proteins, archaeal ribosomes about 68, and eukaryotic ribosomes about 78. Some of the ribosomal proteins are similar across the three domains, but others are observed only in archaeal and eukaryotic ribosomes. All the ribosomal proteins present in both *Archaea* and *Bacteria* are also seen in the *Eukarya*.



(a)



(b)

FIGURE 3.37 Magnetosomes. (a) Transmission electron micrograph of the magnetotactic bacterium *Aquaspirillum magnetotacticum* (×123,000). (b) An electron cryotomography three-dimensional reconstruction of the magnetosomes of *Magnetospirillum magneticum*.

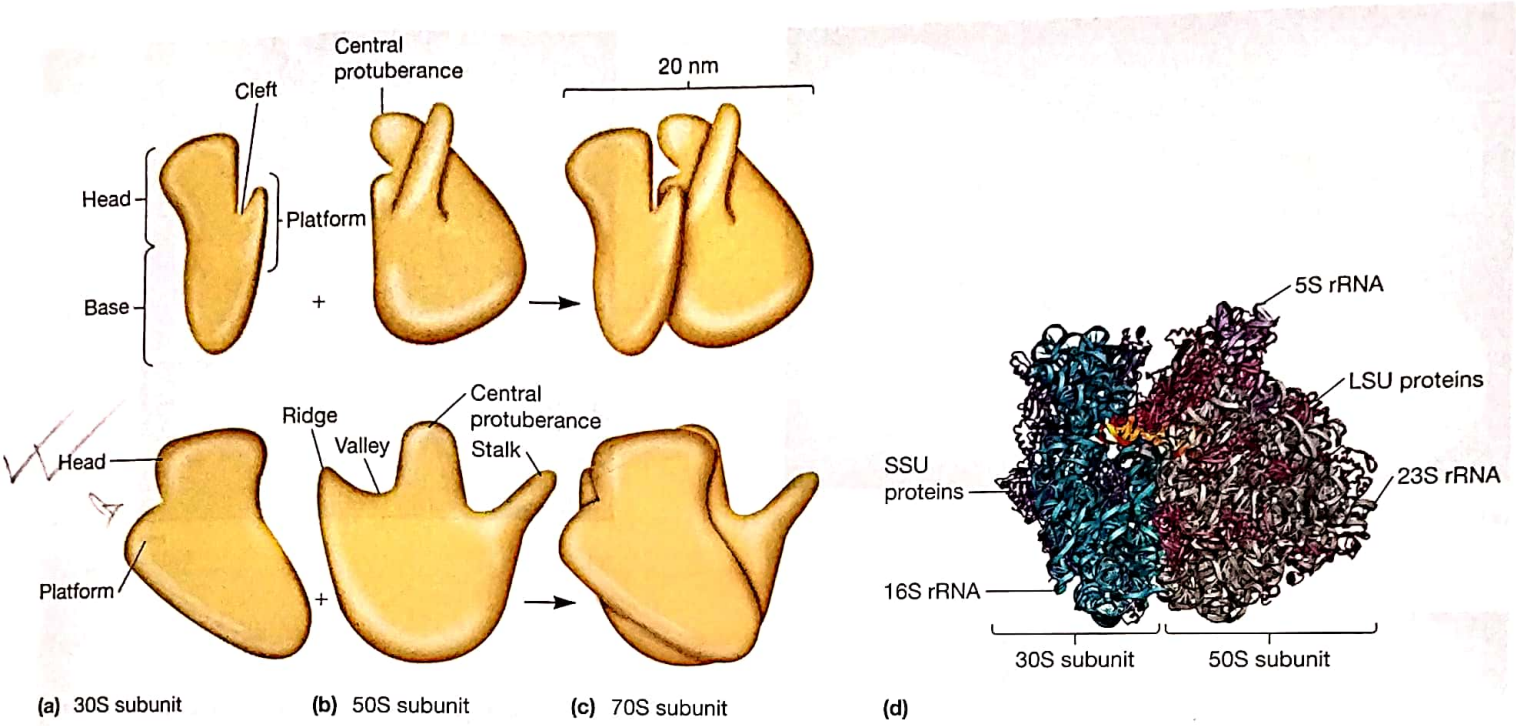


FIGURE 3.38 Bacterial Ribosomes. (a–c) Schematic representation of the two subunits and the complete 70S ribosome of *Escherichia coli.* (d) The molecular structure of the 70S ribosome of *Thermus thermophilus.* The 50S subunit (LSU) includes 23S rRNA (gray) and 5S rRNA (lavender), while 16S rRNA (turquoise) is found in the 30S subunit (SSU). A molecule of tRNA (gold) is shown in the A site. To generate this ribbon diagram, crystals of purified bacterial ribosomes were grown, exposed to X rays, and the resulting diffraction pattern analyzed.

there are no ribosomal proteins that might be referred to as prokaryotic (i.e., present only in the ribosomes of *Archaea* and *Bacteria*). The similarities in protein makeup between archaeal and eukaryotic ribosomes are consistent with the observation that the overall shape of archaeal ribosomes is more similar to that of eukaryotic ribosomes.

Nucleoid

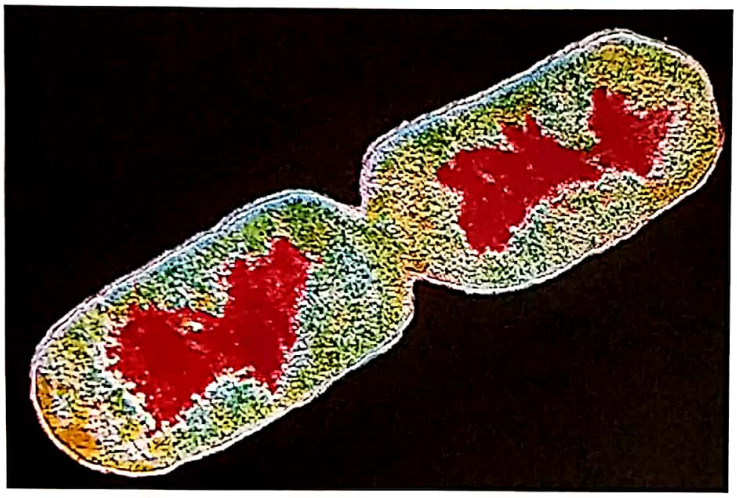
The **nucleoid** (other names are also used: the nuclear body, chromatin body, and nuclear region) is an irregularly shaped region that contains the cell's chromosome and numerous proteins (**figure 3.39**). The chromosomes of most bacteria and all known archaea are a single circle of double-stranded **deoxyribonucleic acid (DNA)**, but some bacteria have a linear chromosome, and some bacteria, such as *Vibrio cholerae* and *Borrelia burgdorferi* (the causative agents of cholera and Lyme disease, respectively), have more than one chromosome.

Bacterial and archaeal chromosomes are longer than the length of the cell. Thus an important and still unanswered question is how these microbes manage to fit their chromosomes into the relatively small space occupied by the nucleoid. For instance, *Escherichia coli*'s circular chromosome measures approximately 1,400 μm, or about 230–700 times longer than the cell (figure 3.39*b*). Thus the chromosome must be compacted in some way. It is thought that much of the compaction is the result of supercoiling, which produces a dense, central core of DNA with loops extending out from the core. There is evidence that some of the proteins found in the nucleoid also contribute to packing the DNA into a smaller space. In bacteria, the protein HU is thought to be important. An HU homologue is also found in some archaea. Most other archaea have histones associated with their chromosomes. These histones form nucleosomes that are similar to the nucleosomes observed in eukaryotes (*see figure 4.11*). During cell division, bacterial and archaeal chromosomes are further condensed by proteins called condensins. This extra level of packing is important for proper segregation of daughter chromosomes during cell division. ▶▶| *Nucleus (section 4.5)*

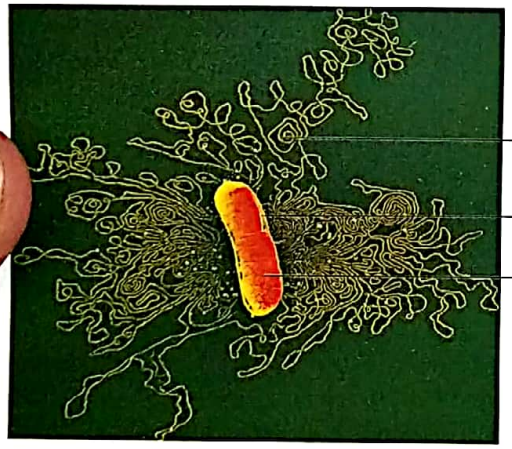
For most bacteria and all known archaea, the nucleoid is simply a region in the cytoplasm; it is not separated from other components of the cytoplasm by a membrane. However, there are a few exceptions. Membrane-bound DNA-containing regions are present in at least two genera of the unusual bacterial phylum *Planctomycetes (see figure 19.10). Pirellula* has a single membrane that surrounds a region called the pirellulosome, which contains a fibrillar nucleoid and ribosome-like particles. The nuclear body of *Gemmata obscuriglobus* is bounded by two membranes. More work is required to determine the functions of these membranes and how widespread this phenomenon is. ▶▶| *Phylum* Planctomycetes *(section 19.4)*

Plasmids

In addition to the genetic material present in the nucleoid, many bacteria, archaea, and some yeasts and other fungi contain extrachromosomal DNA molecules called plasmids. Indeed, most of



(a)



(b)

(c)

FIGURE 3.39 ***E. coli* Nucleoids and Chromosomes.** Bacteria and archaeal chromosomes are located in the nucleoid, an area in the cytoplasm. (a) A color-enhanced transmission electron micrograph of a thin section of a dividing *E. coli* cell. The red areas are the nucleoids present in the two daughter cells. (b) Chromosome released from a gently lysed *E. coli* cell. Note how tightly packaged the DNA must be inside the cell. (c) Atomic force micrograph of an *E. coli* nucleoid. The image was prepared from a culture in the stationary phase of the growth cycle. In that phase, the rate of cell division is the same as the rate of cell death; thus the culture does not increase in size.

the bacterial and archaeal genomes sequenced thus far include plasmids. In some cases, numerous different plasmids within a single species have been identified. For instance, *Borrelia burgdorferi,* which causes Lyme disease, carries 12 linear and nine circular plasmids. Plasmids play many important roles in the lives of the organisms that have them. They also have proved invaluable to microbiologists and molecular geneticists in constructing and transferring new genetic combinations and in cloning genes, as described in chapter 15.

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

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

Plasmids may be classified in terms of their mode of existence, spread, and function. A brief summary of the types of bacterial plasmids and their properties is given in **table 3.5**. **Conjugative plasmids** are of particular note because they can transfer copies of themselves to other bacteria during conjugation. Perhaps the best-studied conjugative plasmid is the **F factor** (fertility factor or F plasmid) of *E. coli,* which is discussed in detail in chapter 14. Some conjugative plasmids are also **R plasmids (resistance factors, R factors).** R plasmids confer antibiotic resistance to the cells that contain them. Conjugative R factors are therefore important in the spread of antibiotic resistance among bacteria. ▶▶| *Bacterial conjugation (section 14.7)*

Table 3.3 Major Types of Bacterial Plasmids

Type	Representatives	Approximate Size (kbp)	Copy Number (Copies/ Chromosome)	Hosts	Phenotypic Features[a]
Conjugative Plasmids[b]	F factor	95–100	1–3	*E. coli, Salmonella, Citrobacter*	Sex pilus, conjugation
R Plasmids	RP4	54	1–3	*Pseudomonas* and many other gram-negative bacteria	Sex pilus, conjugation, resistance to Amp, Km, Nm, Tet
	pSH6	21		*Staphylococcus aureus*	Resistance to Gm, Tet, Km
Col Plasmids	ColE1	9	10–30	*E. coli*	Colicin E1 production
	CloDF13	10	50–70	*E. coli*	Cloacin DF13
Virulence Plasmids	Ent (P307)	83		*E. coli*	Enterotoxin production
	Ti	200		*Agrobacterium tumefaciens*	Tumor induction in plants
Metabolic Plasmids	CAM	230		*Pseudomonas*	Camphor degradation
	TOL	75		*Pseudomonas putida*	Toluene degradation

[a]Abbreviations used for resistance to antibiotics: Amp, ampicillin; Gm, gentamycin; Km, kanamycin; Nm, neomycin; Tet, tetracycline.
[b]Many R plasmids, metabolic plasmids, and others are also conjugative.

Several other important types of plasmids have been discovered. These include bacteriocin-encoding plasmids, virulence plasmids, and metabolic plasmids. Bacteriocin-encoding plasmids may give the bacteria that harbor them a competitive advantage in the microbial world. Bacteriocins are proteins that destroy other, closely related bacteria. Col plasmids contain genes for the synthesis of bacteriocins known as colicins, which are produced by and directed against strains of *E. coli*. Plasmids in other bacteria carry genes for bacteriocins against other species. **Virulence plasmids** encode factors that make their hosts more pathogenic. For example, enterotoxigenic strains of *E. coli* cause traveler's diarrhea because they contain a plasmid that codes for an enterotoxin. **Metabolic plasmids** carry genes for enzymes that degrade substances such as aromatic compounds (toluene), pesticides (2,4-dichlorophenoxyacetic acid), and sugars (lactose). Metabolic plasmids even carry the genes required for some strains of *Rhizobium* to induce legume nodulation and carry out nitrogen fixation. ▶▶| *Order Rhizobiales (section 20.1)*

1. Briefly describe the nature and function of the cytoplasm, and the regions and structures within it.
2. What is the importance of bacterial cytoskeletal proteins? What do you think would be the outcome if you were able to "transplant" CreS into a rod-shaped bacterium such as *Bacillus subtilis*?
3. List the most common kinds of inclusions. How are they similar to eukaryotic organelles such as mitochondria and chloroplasts? How do they differ?
4. Relate the structure of a gas vacuole to its function. Why do you think gas vacuoles are bounded by proteins rather than a lipid bilayer membrane?
5. List three genera that are exceptional in terms of their chromosome or nucleoid structure. Suggest how the differences observed in these genera might impact how they function.
6. Give the major features of plasmids. How do they differ from chromosomes? What is an episome?
7. Describe each of the following plasmids and explain their importance: conjugative plasmid, F factor, R factor, Col plasmid, virulence plasmid, and metabolic plasmid.

3.6 External Structures

Many bacteria and archaea have structures that extend beyond the cell envelope. These external structures can function in protection, attachment to surfaces, horizontal gene transfer, and cell movement. Several are discussed in this section.

Pili and Fimbriae

Many bacteria and archaea have short, fine, hairlike appendages that are thinner than flagella. These are usually called **fimbriae** (s., **fimbria**) or **pili** (s., **pilus**). The terms are synonymous, although certain structures are always called pilus (e.g., sex pilus),

while others are always called fimbriae. We will use the terms interchangeably, except in those instances. A cell may be covered with up to 1,000 fimbriae, but they are only visible in an electron microscope due to their small size (**figure 3.40**). They are slender tubes composed of helically arranged protein subunits and are about 3 to 10 nm in diameter and up to several micrometers long. Several different types of fimbriae have been identified in gram-negative bacteria. Most function to attach bacteria to solid surfaces such as rocks in streams and host tissues. One type, called type IV pili, are involved in motility (section 3.7) and the uptake of DNA during the process of bacterial transformation. Gram-positive bacteria have at least two types of pili; both are involved in attaching the bacteria to surfaces. ▶▶| *Transformation (section 14.8)*

Many bacteria have up to 10 **sex pili** (s., **sex pilus**) per cell. These hairlike structures differ from other pili in the following ways. Sex pili often are larger than other pili (around 9 to 10 nm in diameter). They are genetically determined by conjugative plasmids and are required for conjugation. Some bacterial viruses attach specifically to receptors on sex pili at the start of their reproductive cycle. ▶▶| *Bacterial conjugation (section 14.7)*

Flagella

Many motile bacteria and archaea move by use of **flagella** (s., **flagellum**), threadlike locomotor appendages extending outward from the plasma membrane and cell wall. Although the main function of flagella is motility, they can have other roles. Flagella are important for certain types of swarming behavior. They can be involved in attachment to surfaces, and in some bacteria they are virulence factors. Bacterial flagella are the best studied and are considered first. ▶▶| *Microbial growth on solid surfaces (section 6.8)*

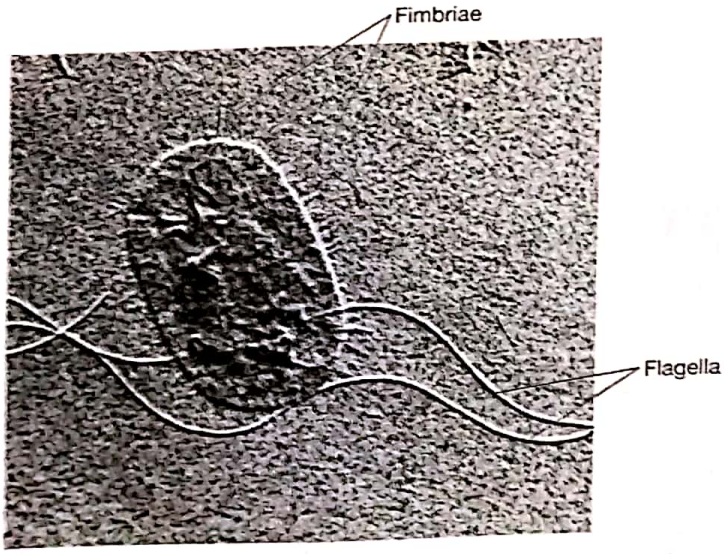


FIGURE 3.40 Flagella and Fimbriae. The long flagella and numerous shorter fimbriae are evident in this electron micrograph of the bacterium *Proteus vulgaris* (×39,000).

Bacterial Flagella

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

Bacterial species often differ distinctively in their patterns of flagella distribution, and these patterns are useful in identifying bacteria. **Monotrichous** bacteria (*trichous* means hair) have one flagellum; if it is located at an end, it is said to be a **polar flagellum** (**figure 3.41*a***). **Amphitrichous** bacteria (*amphi* means on both sides) have a single flagellum at each pole. In contrast, **lophotrichous** bacteria (*lopho* means tuft) have a cluster of flagella

(a) *Pseudomonas*—monotrichous polar flagellation

(b) *Spirillum*—lophotrichous flagellation

(c) *P. vulgaris*—peritrichous flagellation

FIGURE 3.41 Flagellar Distribution. Examples of various patterns of flagellation as seen in the light microscope. (a) Monotrichous polar (*Pseudomonas*). (b) Lophotrichous (*Spirillum*). (c) Peritrichous (*Proteus vulgaris*, ×600).

at one or both ends (figure 3.41*b*). Flagella are spread evenly over the whole surface of **peritrichous** (*peri* means around) bacteria (figure 3.41*c*).

Transmission electron microscope studies have shown that the bacterial flagellum is composed of three parts (**figure 3.42**). (1) The longest and most obvious portion is the **filament,** which extends from the cell surface to the tip. (2) The **basal body** is embedded in the cell; and (3) a short, curved segment, the **hook,** links the filament to its basal body and acts as a flexible coupling. The filament is a hollow, rigid cylinder constructed of subunits of the protein **flagellin,** which ranges in molecular weight from 30,000 to 60,000 daltons, depending on the bacterial species. The filament ends with a capping protein. Some bacteria have sheaths surrounding their flagella. For example, *Vibrio cholerae* has a lipopolysaccharide sheath.

The hook and basal body are quite different from the filament (figure 3.42). Slightly wider than the filament, the hook is made of different protein subunits. The basal body is the most complex part of a flagellum. In the earliest-made transmission electron micrographs of the basal bodies of *E. coli* and most other gram-negative bacteria, the basal body appeared to have four rings—L ring, P ring, S ring, and M ring—connected to a central rod (figure 3.42*a*). It is now known that the S ring and M ring are different portions of the same protein, and they are now referred to as the MS ring. A later discovery was the C ring, which is on the cytoplasmic side of the MS ring. Gram-positive bacteria have only two rings—an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan (figure 3.42*b*).

The synthesis of bacterial flagella is a complex process involving at least 20 to 30 genes. Besides the gene for flagellin, 10 or more genes code for hook and basal body proteins; other genes are concerned with the control of flagellar construction or function. How the cell regulates or determines the exact location of flagella is not known.

Because many components of the flagellum lie outside the cell wall, they must be transported across the plasma membrane and cell wall. Interestingly, evidence suggests that components of the basal body are evolutionarily related to a type of protein secretion system observed in gram-negative bacteria. This system, called a type III secretion system, has a needlelike structure through which proteins are secreted. The needle is thought to be analogous to the filament of the flagellum. Thus the flagellin subunits are transported by way of a type III-like secretion process through the filament's hollow internal core. When the subunits reach the tip, they spontaneously aggregate under the direction of a protein called the filament cap; thus the filament grows at its tip rather than at the base (**figure 3.43**). Filament synthesis, like S-layer formation, is an example of **self-assembly.** ▶▶| *Protein maturation and secretion (section 12.9)*

Archaeal Flagella

Archaeal flagella have not been as thoroughly studied as bacterial flagella. They are superficially similar to their bacterial counterparts, but important differences have been identified. Archaeal flagella are thinner than bacterial flagella (10 to 13 nm rather than 20 nm) and are composed of more than one type of flagellin subunit (**figure 3.44**). The flagellum is not hollow. Archaeal

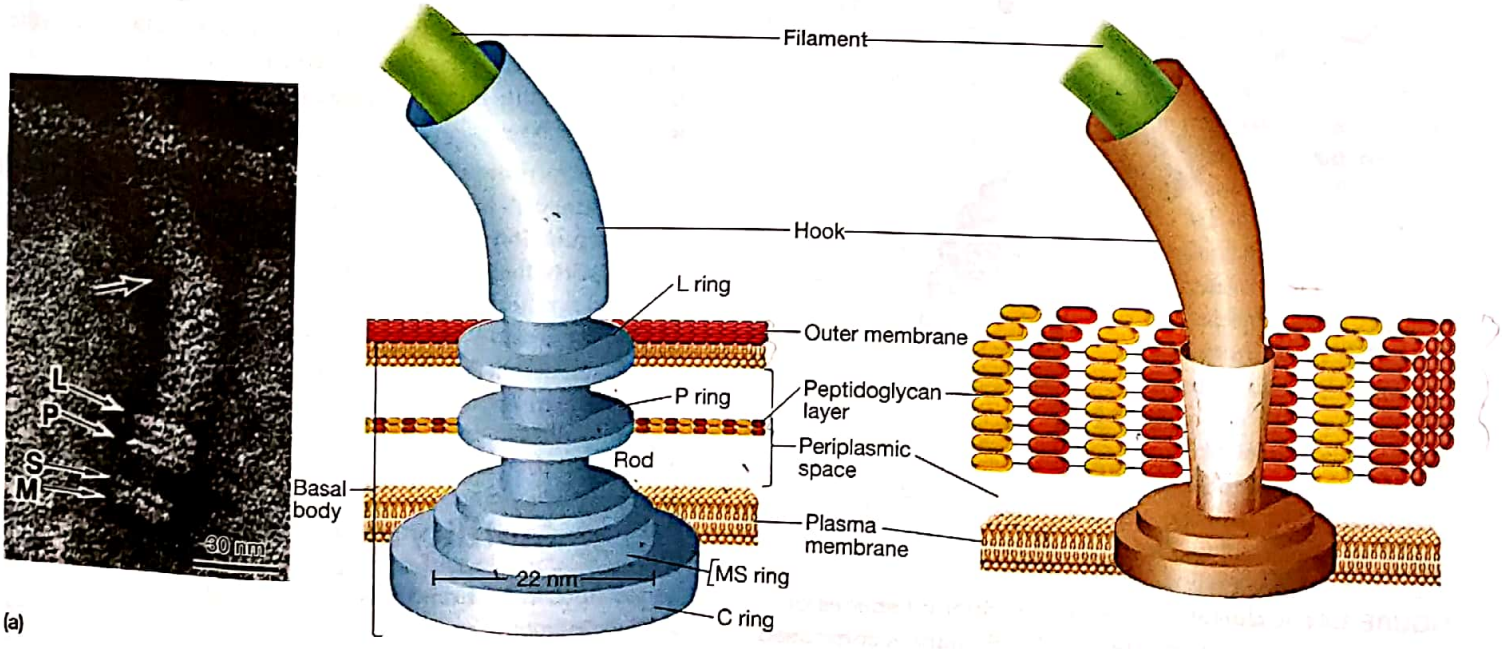


FIGURE 3.42 The Ultrastructure of Bacterial Flagella. Flagellar basal bodies and hooks in (a) gram-negative and (b) gram-positive bacteria. The photo shows an enlarged view of the basal body of an *E. coli* flagellum. All three rings (L, P, and MS) can be clearly seen. The uppermost arrow is at the junction of the hook and filament.

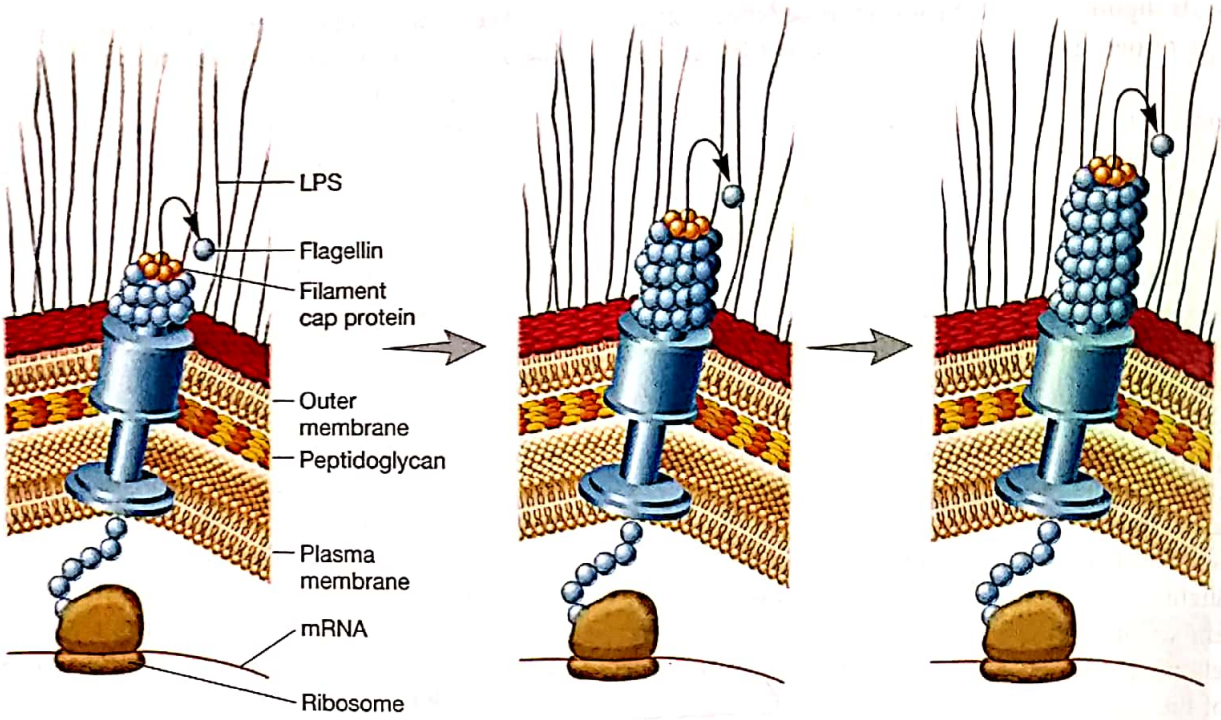


FIGURE 3.43 Growth of Flagellar Filaments. Flagellin subunits travel through the flagellar core and attach to the growing tip. Their attachment is directed by the filament cap protein.

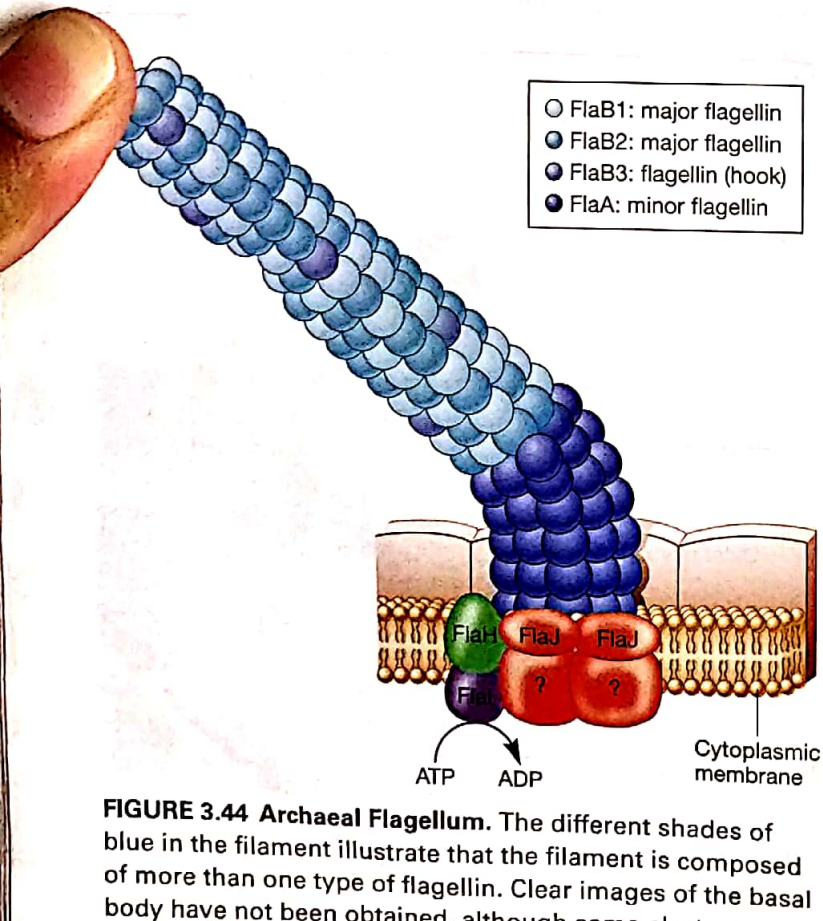


FIGURE 3.44 Archaeal Flagellum. The different shades of blue in the filament illustrate that the filament is composed of more than one type of flagellin. Clear images of the basal body have not been obtained, although some electron micrographs show a knob at the cell end of the flagellum, as illustrated here.

hooks are difficult to distinguish from the filament; they al tend to be longer than bacterial hooks. A basal body has n been identified, but some preparations of archaeal flagella have knoblike structure at the end embedded in the cell. One interes ing feature of the flagellar proteins thus far characterized is th they are more related to type IV bacterial pili than they are to t proteins in bacterial flagella. Like type IV pili, the filament of t archaeal flagellin increases in length as flagellin subunits a added at the base.

1. Distinguish between fimbriae (pili) and sex pili, and give the function of each.
2. Discuss flagella distribution patterns and the structure and synthesis of flagella.
3. What is self-assembly? Why does it make sense that the filament of a flagellum is assembled in this way?
4. Compare and contrast bacterial and archaeal flagella.

3.7 Motility and Chemotaxis

As we note in section 3.6, several structures outside the cell wall contribute to motility. Four major methods of movement have been observed in *Bacteria:* the swimming movement conferred by flagella; the corkscrew movement of spirochetes; the twitching motility associated with type IV pili; and gliding motility.

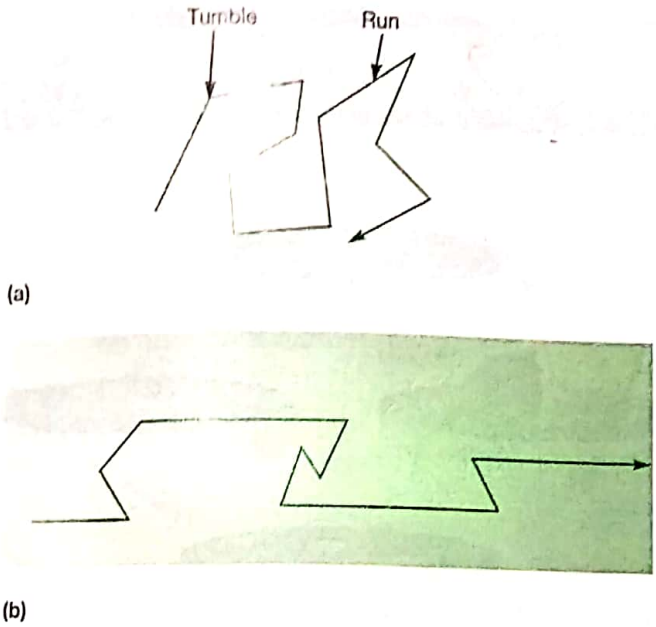


FIGURE 3.50 Directed Movement in Bacteria. (a) Random movement of a bacterium in the absence of a concentration gradient. Tumbling frequency is fairly constant. (b) Movement in an attractant gradient. Tumbling frequency is reduced when the bacterium is moving up the gradient. Therefore runs in the direction of increasing attractant are longer.

3.8 Bacterial Endospores

Several genera of gram-positive bacteria, including *Bacillus* and *Clostridium* (rods), and *Sporosarcina* (cocci), can form a resistant, dormant structure called an **endospore**. Endospore-forming bacteria are common in soil, where they must be able to withstand fluctuating levels of nutrients. Endospore formation (sporulation) normally commences when growth ceases due to lack of nutrients. Thus it is a survival mechanism that allows the bacterium to produce a dormant cell that can survive until nutrients are again available and vegetative growth can resume. Interestingly, some bacteria have modified the sporulation process and use it to produce live offspring within themselves (**Microbial Diversity & Ecology 3.1**).

Endospores are extraordinarily resistant to environmental stresses such as heat, ultraviolet radiation, gamma radiation, chemical disinfectants, and desiccation. In fact, some endospores have remained viable for around 100,000 years. They are of both practical and theoretical interest. Because of their resistance and the fact that several species of endospore-forming bacteria are dangerous pathogens, endospores are of great practical importance in food, industrial, and medical microbiology. In these areas, it is essential to be able to sterilize solutions and solid objects. Endospores often survive boiling for an hour or more; therefore autoclaves must be used to sterilize many materials. Endospores are of considerable theoretical interest to scientists studying the construction of complex biological structures. Bacteria

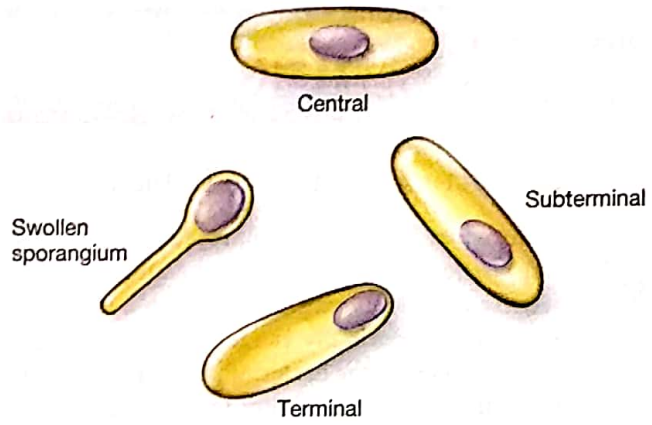


FIGURE 3.51 Examples of Endospore Location and Size.

manufacture these intricate structures in a very organized fashion over a period of a few hours. Thus spore formation is well suited for this type of research, and the endospore-forming *Bacillus subtilis* has become an important model organism. ▶▶| *Heat (section 8.4); Sporulation in* Bacillus subtilis *(section 13.6)*

Endospores can be examined with both light and electron microscopes. Because endospores are impermeable to most stains, they often are seen as colorless areas in bacteria treated with methylene blue and other simple stains; staining procedures specific for endospores are used to make them clearly visible. Endospore position in the mother cell (**sporangium**) frequently differs among species, making it of value in identification. Endospores may be centrally located, close to one end (subterminal), or terminal (**figure 3.51**). Sometimes an endospore is so large that it swells the sporangium. |◀◀ *Preparation and staining of specimens (section 2.3)*

Electron micrographs show that endospore structure is complex (**figure 3.52**). The spore often is surrounded by a thin, delicate covering called the exosporium. A coat lies beneath the exosporium. It is composed of several protein layers and may be fairly thick. The cortex, which may occupy as much as half the spore volume, rests beneath the coat. It is made of a peptidoglycan that is less cross-linked than that in vegetative cells. The core wall is inside the cortex and surrounds the core. The core has normal cell structures such as ribosomes and a nucleoid but has a very low water content and is metabolically inactive.

The various layers of the spore are thought to contribute to its resistance to heat and other lethal agents. The exosporium and spore coat are both thought to protect the spore from chemicals, although the mechanisms by which they do so are not completely understood. It is known that the spore coat is impermeable to many toxic molecules. The inner membrane, which separates the cortex from the core, is also impermeable to various chemicals, including those that cause DNA damage. The core plays a major role in resistance. Several factors may play a part in resistance (e.g., very low water content, high amounts of dipicolinic acid complexed with calcium ions, and a slightly lower pH). However, the major core-related factor is the protection of the spore's DNA by small, acid-soluble DNA-binding proteins (SASPs), which

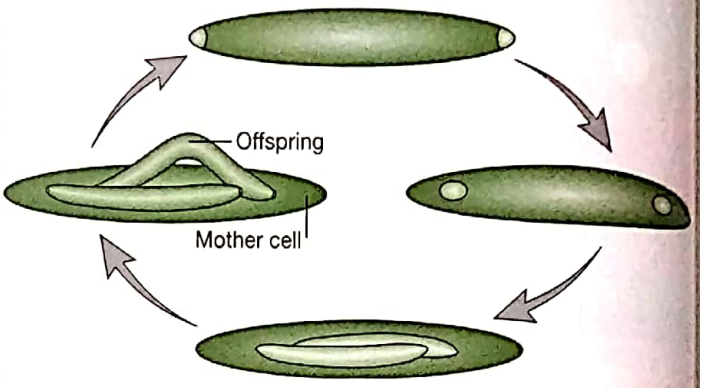
MICROBIAL DIVERSITY & ECOLOGY

3.1 Bacteria That Have Babies

Epulopscium fishelsoni (Latin, *epulum*, a feast or banquet, and *piscium*, fish) first gained the attention of biologists because of its large size. It can reach a size of 80 µm by 600 µm and normally ranges from 200 to 500 µm in length. However, the bacterium has other interesting characteristics. One is its ability to produce living offspring within itself (viviparity). Usually two offspring are formed.

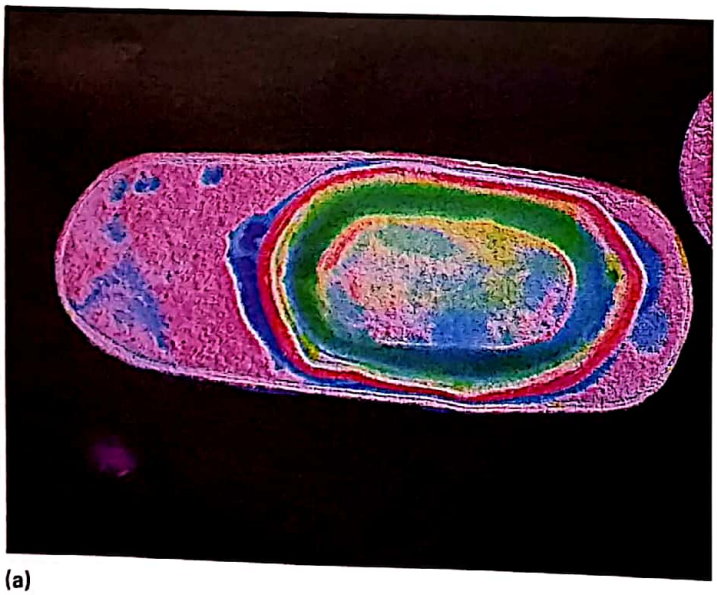
E. fishelsoni is related to the gram-positive, endospore-forming bacterial genus *Clostridium*. It appears that its mechanism of reproduction is a modified version of sporulation. The process begins with division of the cytoplasm into three compartments: two small compartments at each end and a larger, centrally located compartment. The central compartment is the mother cell, and the two smaller compartments will become the offspring (**box figure**). The mother cell engulfs the offspring, just as the mother cell of an endospore former engulfs a developing endospore. Initially both the offspring and mother cell grow, but later the mother cell's growth begins to slow. Eventually the mother cell dies and the offspring are released.

A central question being addressed by Esther Angert and others studying this bacterium is which came first: viviparity or endospore formation. Currently it is thought that endospore formation evolved first. One line of evidence is that *E. fishelsoni* is closely related to another interesting bacterium, *Metabacterium polyspora*. As its name implies, this bacterium produces multiple endospores rather than just one, as is the case for members of the genera *Bacillus* and *Clostridium*.

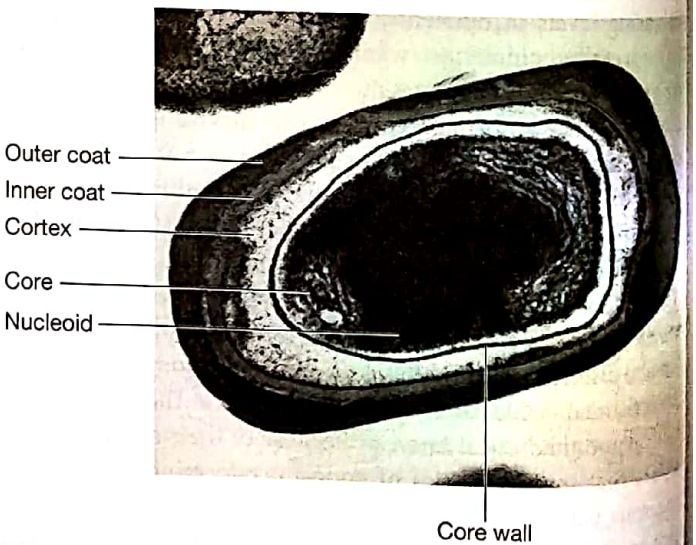


Production of Intracellular Living Offspring by *E. fishelsoni*. The life cycle begins with the formation of two small compartments within the mother cell and ends with the death of the mother cell and release of the offspring.

Source: Angert, E. R. 2005. Alternatives to binary fission in bacteria. *Nature Rev. Microbiol.* 3:214–24.



(a)



(b)

FIGURE 3.52 Bacterial Endospores. (a) A colorized cross section of a *Bacillus subtilis* cell undergoing sporulation. The oval in the center is an endospore that is almost mature; when it reaches maturity, the mother cell will lyse to release it. (b) A cross section of a mature *B. subtilis* spore showing the cortex and spore coat layers that surround the core. The endospore in (a) is 1.3 µm; the spore in (b) is 1.2 µm.

saturate spore DNA. There are several types of SASPs. The α/β type plays a major role in resistance. Cells that have been mutated and do not make α/β SASPs are considerably more sensitive to heat, UV radiation, dessication, and a variety of chemicals but are still resistant to other types of DNA damage. Thus other mechanisms for protecting the DNA must exist.

Sporulation is a complex process and may be divided into seven stages (**figure 3.53**). An axial filament of nuclear material

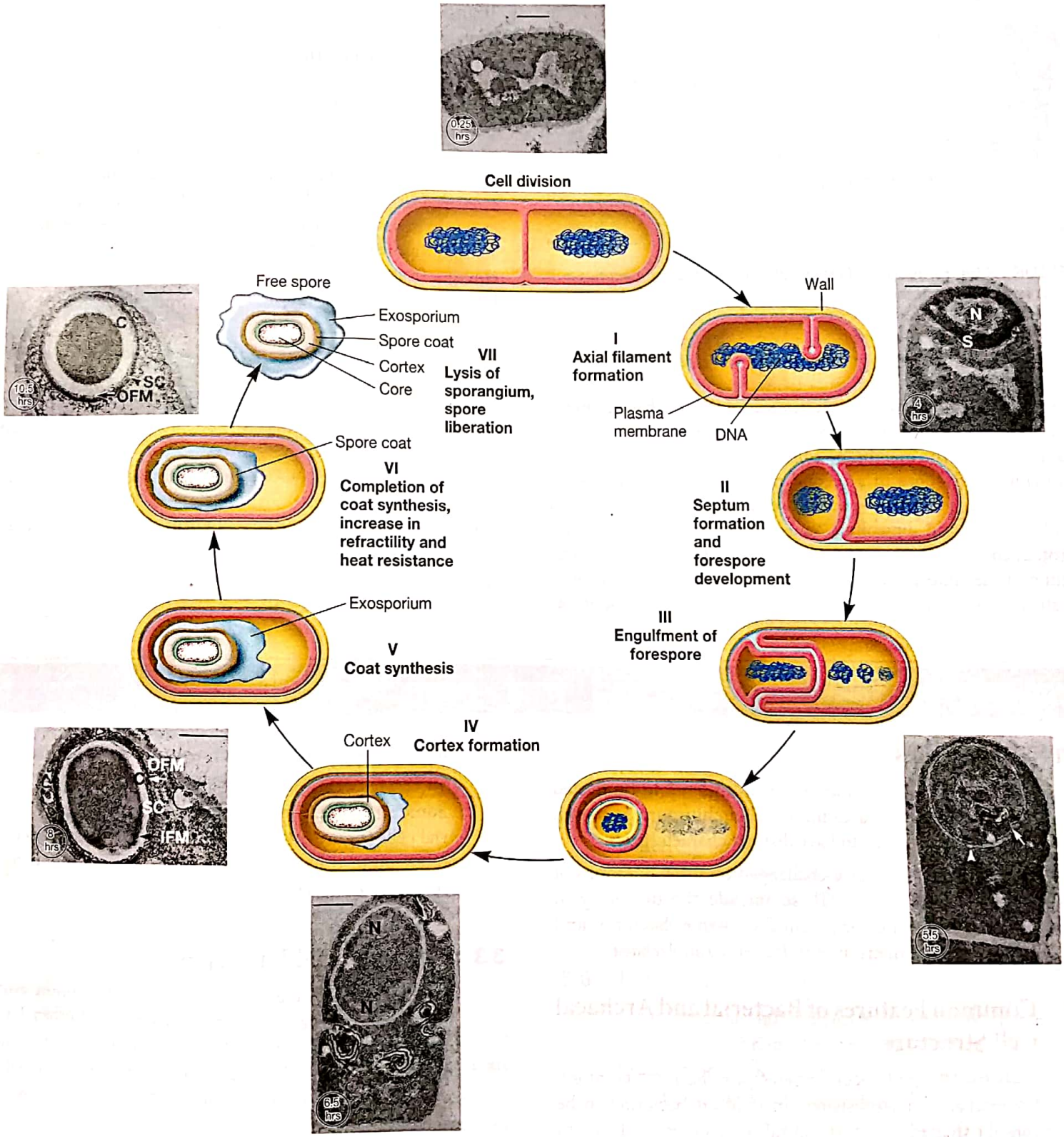


FIGURE 3.53 Endospore Formation: Life Cycle of *Bacillus megaterium*. The stages are indicated by Roman numerals. The circled numbers in the photographs refer to the hours from the end of the logarithmic phase of growth: 0.25 h—a typical vegetative cell; 4 h–stage II cell, septation; 5.5 h–stage III cell, engulfment; 6.5 h–stage IV cell, cortex formation; 8 h–stage V cell, coat formation; 10.5 h–stage VI cell, mature spore in sporangium. Abbreviations used: C, cortex; IFM and OFM, inner and outer forespore membranes; N, nucleoid; S, septum; SC, spore coats. Bars = 0.5 μm.

FIGURE 3.54 Endospore Germination. *Clostridium pectinovorum* emerging from the spore during germination.

forms (stage I), followed by an inward folding of the cell membrane to enclose part of the DNA and produce the forespore septum (stage II). The membrane continues to grow and engulfs the immature endospore in a second membrane (stage III). Next, cortex is laid down in the space between the two membranes, and both calcium and dipicolinic acid are accumulated (stage IV). Protein coats are formed around the cortex (stage V), and maturation of the endospore occurs (stage VI). Finally, lytic enzymes destroy the sporangium, releasing the spore (stage VII). Sporulation requires about 10 hours in *Bacillus megaterium*. Bacterial Spore Formation

The transformation of dormant spores into active vegetative cells seems almost as complex a process as sporulation. It occurs in three stages: (1) activation, (2) germination, and (3) outgrowth (**figure 3.54**). Activation is a process that prepares spores for germination and can result from treatments such as heating. This is followed by **germination,** the breaking of the spore's dormant state. Proteins in the exosporium, spore coat, and inner membrane are all thought to play a role. Some are involved in detecting the presence of certain compounds such as sugars and amino acids. Germination is characterized by spore swelling, rupture, or absorption of the spore coat, loss of resistance to heat and other stresses, loss of refractility, release of spore components, and increase in metabolic activity. Germination is followed by the third stage, outgrowth. The spore protoplast makes new components, emerges from the remains of the spore coat, and develops again into an active bacterium.

1. Describe the structure of the bacterial endospore using a labeled diagram.
2. Briefly describe endospore formation and germination. What is the importance of the endospore? What might account for its heat resistance?
3. How might one go about showing that a bacterium forms true endospores?
4. Why do you think the low water content of the spore contributes to its dormancy and resistance? Why are SASPs important?

of the cell becomes spherical regardless of the original cell shape. Why does this occur?

5. Design an experiment that illustrates the cell wall's role in protecting against lysis.
6. With a few exceptions, the cell walls of gram-positive bacteria lack porins. Why is this the case?

Layers Outside the Cell Wall

Many bacteria have another layer in their cell envelopes that lies outside the cell wall. This layer is given different names depending on its makeup and how it is organized.

Capsules and Slime Layers

Capsules are layers that are well organized and not easily washed off (**figure 3.23*a***). Capsules are most often composed of polysaccharides, but some are constructed of other materials. For example, *Bacillus anthracis* has a proteinaceous capsule composed of poly-D-glutamic acid. Capsules are clearly visible in the light microscope when negative stains or specific capsule stains are employed (figure 3.23*a*); they also can be studied with the electron microscope.

Although capsules are not required for growth and reproduction in laboratory cultures, they confer several advantages when bacteria grow in their normal habitats. They help pathogenic

They provide an extra layer of protection to bacterial cell.

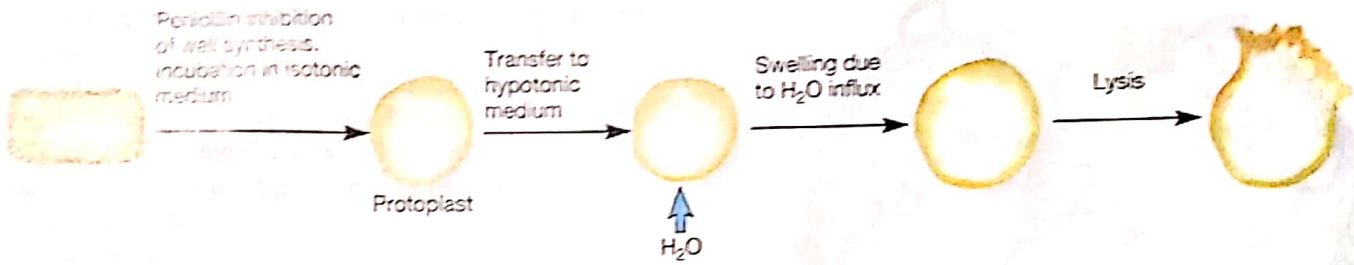
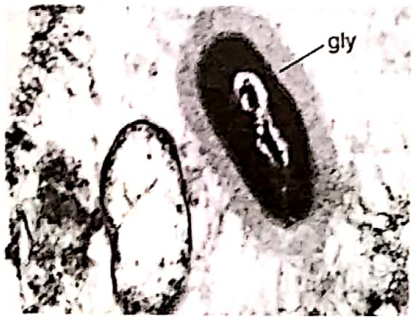


FIGURE 3.22 Protoplast Formation and Lysis. Protoplast formation induced by incubation with penicillin in an isotonic medium. Transfer to hypotonic medium will result in lysis.

(a) *K. pneumoniae*



(b) *Bacteroides*

FIGURE 3.23 Bacterial Capsules. (a) *Klebsiella pneumoniae* with its capsule stained for observation in the light microscope (×1,500). (b) *Bacteroides* glycocalyx (gly), TEM (×71,250).

bacteria resist phagocytosis by host phagocytes. *Streptococcus pneumoniae* provides a dramatic example. When it lacks a capsule, it is destroyed easily and does not cause disease. On the other hand, the capsulated variant quickly kills mice. Capsules contain a great deal of water and can protect against desiccation. They exclude viruses and most hydrophobic toxic materials such as detergents.

A **slime layer** is a zone of diffuse, unorganized material that is removed easily. It is usually composed of polysaccharides, but is not as easily observed by light microscopy. Gliding bacteria often

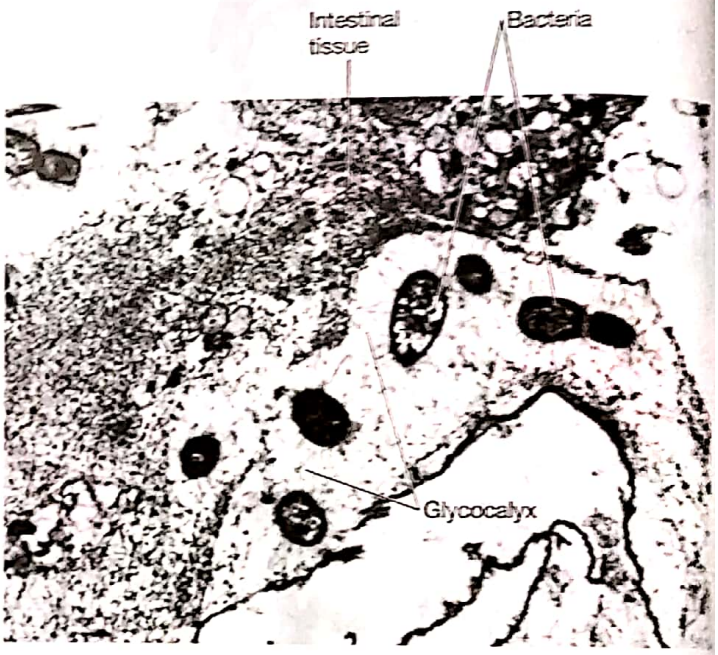


FIGURE 3.24 Bacterial Glycocalyx. Bacteria connected to each other and to the intestinal wall by their glycocalyxes, the extensive networks of fibers extending from the cells (×17,500).

produce slime, which in some cases has been shown to facilitate motility (section 3.7).

The term **glycocalyx** refers to a layer consisting of a network of polysaccharides extending from the surface of the cell (figure 3.23*b*). The term can encompass both capsules and slime layers because they usually are composed of polysaccharides. The glycocalyx aids in attachment to solid surfaces, including tissue surfaces in plant and animal hosts (**figure 3.24**). ▶▶| *Virulence factors (section 31.3)*

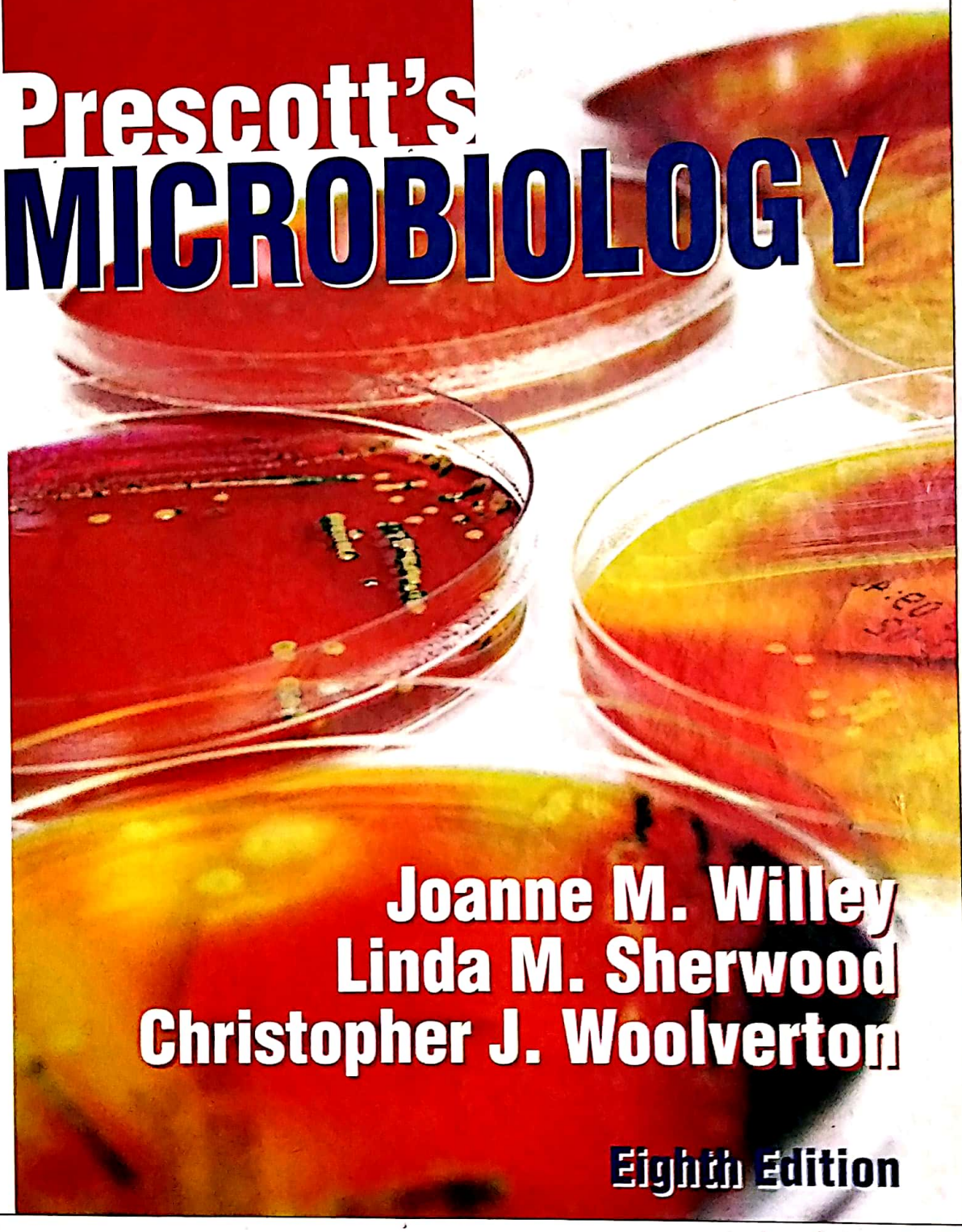
S-Layers

Many bacteria have a regularly structured layer called an **S-layer** on their surface. The S-layer has a pattern something like floor tiles and is composed of protein or glycoprotein (**figure 3.25**). In gram-negative bacteria, the S-layer adheres directly to the outer membrane; it is associated with the peptidoglycan surface in gram-positive bacteria.

FIGURE 3.25 The S-Layer. An electron micrograph of the S-layer of the bacterium *Deinococcus radiodurans* after shadowing.

Currently S-layers are of considerable interest not only for their biological roles but also in the growing field of nanotechnology. Their biological roles include protecting the cell against ion and pH fluctuations, osmotic stress, enzymes, or predacious bacteria. The S-layer also helps maintain the shape and envelope rigidity of some cells, and it can promote cell adhesion to surfaces. Finally, the S-layer seems to protect some bacterial pathogens against host defenses, thus contributing to their virulence. The potential use of S-layers in nanotechnology is due to the ability of S-layer proteins to self-assemble. That is, the S-layer proteins contain the information required to spontaneously associate and form the S-layer without the aid of any additional enzymes or other factors. Thus S-layer proteins could be used as building blocks for the creation of technologies such as drug-delivery systems and novel detection systems for toxic chemicals or bioterrorism agents.

Prescott's MICROBIOLOGY



Joanne M. Willey
Linda M. Sherwood
Christopher J. Woolverton

Eighth Edition

McGRAW-HILL INTERNATIONAL EDITION