

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

—Howard J. Rogers

Even a superficial examination of the microbial world shows that bacteria are one of the most important groups by any criterion: numbers of organisms, general ecological importance, or practical importance for humans. Indeed, much of our understanding of phenomena in biochemistry and molecular biology comes from research on bacteria. Although considerable space is devoted to eucaryotic microorganisms, the major focus is on procaryotes. Therefore the unit on microbial morphology begins with the structure of procaryotes. As mentioned in chapter 1 (*see p. 11*), there are two quite different groups of procaryotes: Bacteria and Archaea. This chapter focuses primarily on bacterial morphology; chapter 20 will discuss archaeal cell structure and composition. A comment about nomenclature is necessary to avoid confusion. The word procaryote will be used in a general sense to include both the bacteria and archaea; the term bacterium will refer specifically to bacteria. Eucaryotes, procaryotes, and the composition of the microbial world (pp. 11; 91–92). The Archaea (pp. 450–65)

3.1 An Overview of Prokaryotic Cell Structure

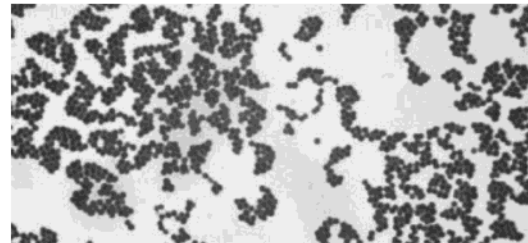
Because much of this chapter is devoted to a discussion of individual cell components, a preliminary overview of the procaryotic cell as a whole is in order.

Size, Shape, and Arrangement

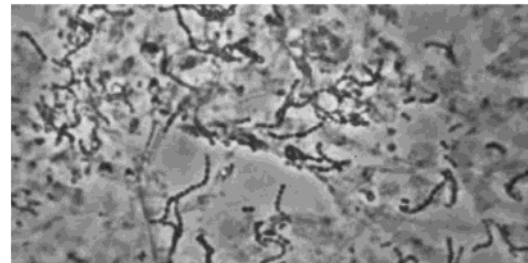
One might expect that small, relatively simple organisms like procaryotes would be uniform in shape and size. Although it is true that many procaryotes are similar in morphology, there is a remarkable amount of variation due to differences in genetics and ecology. (figures 3.1 and 3.2; *see also figures 2.8 and 2.15*). Major morphological patterns are described here, and interesting variants are mentioned in the procaryotic survey (*see chapters 20–24*).

Most commonly encountered bacteria have one of two shapes. **Cocci** (s., **coccus**) are roughly spherical cells. They can

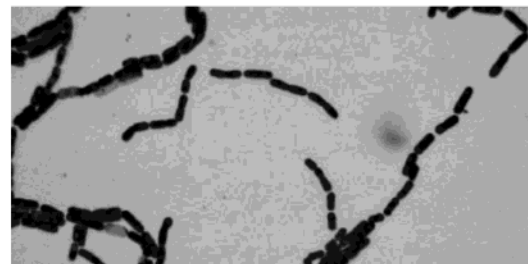
Figure 3.1 Representative Bacteria. Stained bacterial cultures as seen in the light microscope. (a) *Staphylococcus aureus*. Note the gram-positive spheres in irregular clusters. Gram stain ($\times 1,000$). (b) *Enterococcus faecalis*. Note the chains of cocci; phase contrast ($\times 200$). (c) *Bacillus megaterium*, a rod-shaped bacterium in chains. Gram stain ($\times 600$). (d) *Rhodospirillum rubrum*. Phase contrast ($\times 500$). (e) *Vibrio cholerae*. Curved rods with polar flagella ($\times 1,000$).



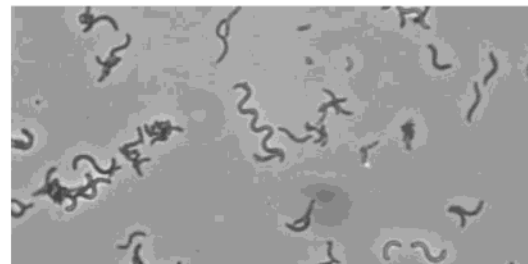
(a)



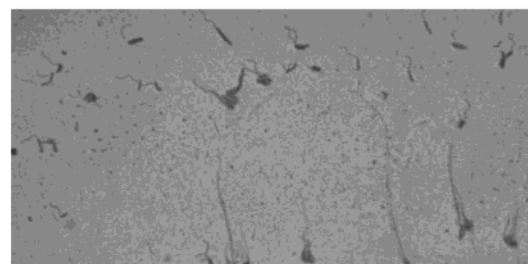
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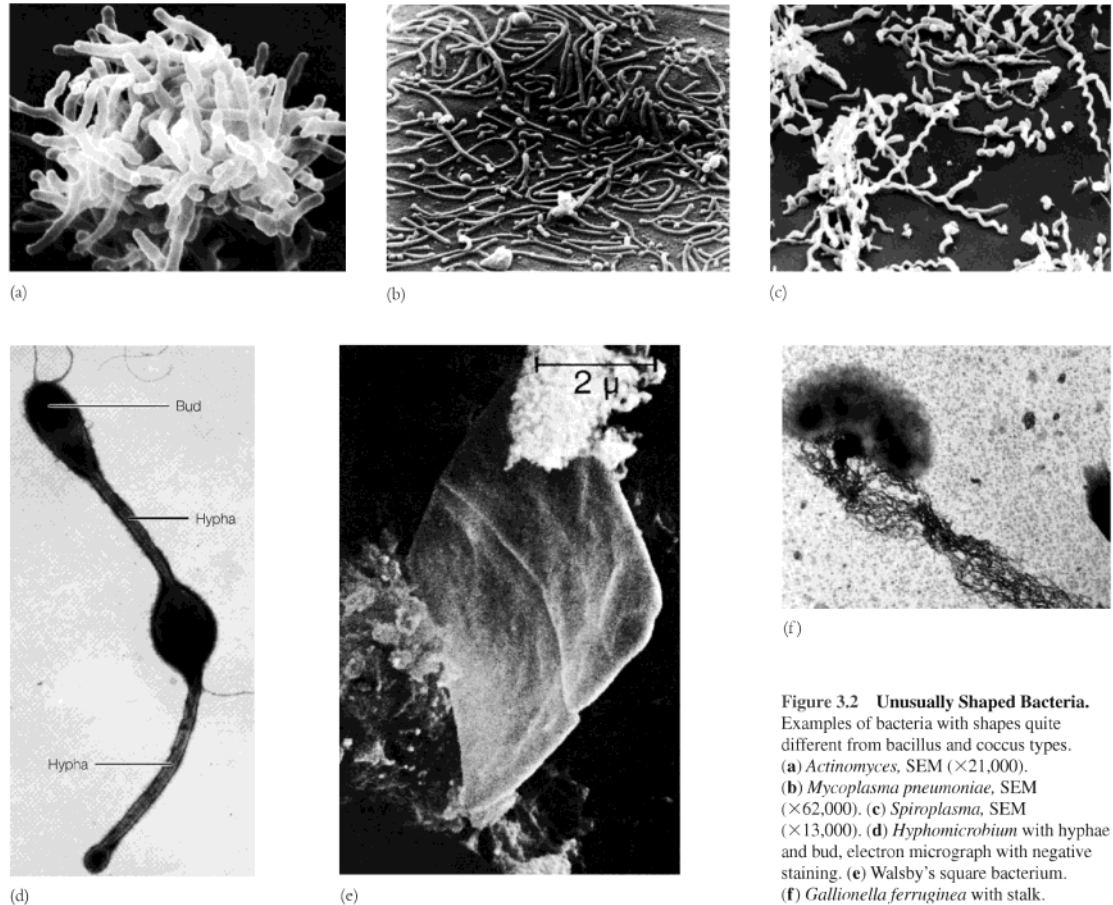


Figure 3.2 Unusually Shaped Bacteria. Examples of bacteria with shapes quite different from bacillus and coccus types. (a) *Actinomyces*, SEM ($\times 21,000$). (b) *Mycoplasma pneumoniae*, SEM ($\times 62,000$). (c) *Spiroplasma*, SEM ($\times 13,000$). (d) *Hyphomicrobium* with hyphae and bud, electron micrograph with negative staining. (e) Walsby's square bacterium. (f) *Gallionella ferruginea* with stalk.

exist as individual cells, but also are associated in characteristic arrangements that are frequently useful in bacterial identification. **Diplococci** (s., **diplococcus**) arise when cocci divide and remain together to form pairs (*Neisseria*; see figure 2.15d). Long chains of cocci result when cells adhere after repeated divisions in one plane; this pattern is seen in the genera *Streptococcus*, *Enterococcus*, and *Lactococcus* (figure 3.1b). *Staphylococcus* divides in random planes to generate irregular grapelike clumps (figure 3.1a). Divisions in two or three planes can produce symmetrical clusters of cocci. Members of the genus *Micrococcus* often divide in two planes to form square groups of four cells called tetrads. In the genus *Sarcina*, cocci divide in three planes producing cubical packets of eight cells.

The other common bacterial shape is that of a **rod**, often called a **bacillus** (pl., **bacilli**). *Bacillus megaterium* is a typical

example of a bacterium with a rod shape (figure 3.1c; see also figure 2.15a,c). Bacilli differ considerably in their length-to-width ratio, the coccobacilli being so short and wide that they resemble cocci. The shape of the rod's end often varies between species and may be flat, rounded, cigar-shaped, or bifurcated. Although many rods do occur singly, they may remain together after division to form pairs or chains (e.g., *Bacillus megaterium* is found in long chains). A few rod-shaped bacteria, the **vibrios**, are curved to form distinctive commas or incomplete spirals (figure 3.1e).

Bacteria can assume a great variety of shapes, although they often are simple spheres or rods. Actinomycetes characteristically form long multinucleate filaments or hyphae that may branch to produce a network called a **mycelium** (figure 3.2a). Many bacteria are shaped like long rods twisted into

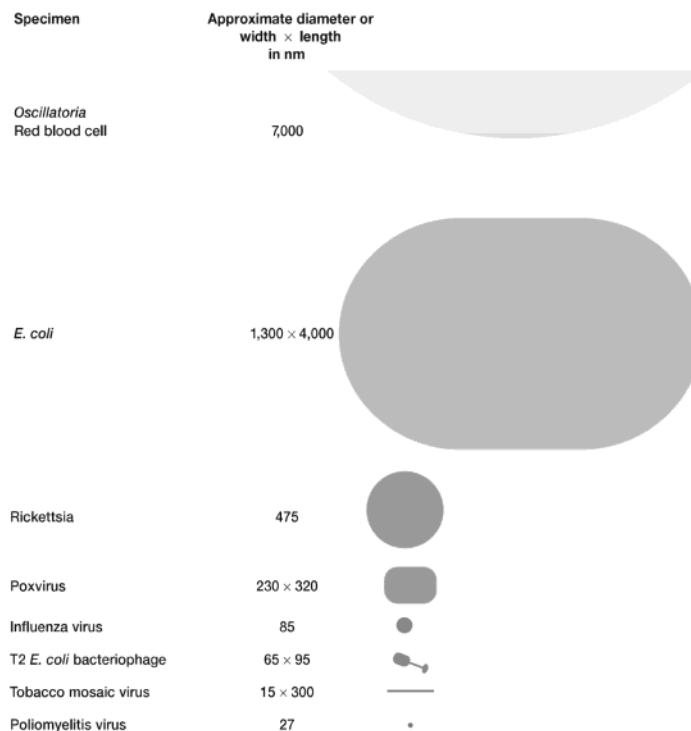


Figure 3.3 Sizes of Bacteria and Viruses. The sizes of selected bacteria relative to the red blood cell and viruses.

spirals or helices; they are called **spirilla** if rigid and **spirochetes** when flexible (figures 3.1d, 3.2c; see also figure 2.8a,c). The oval- to pear-shaped *Hyphomicrobium* (figure 3.2d) produces a bud at the end of a long hypha. Other bacteria such as *Gallionella* produce nonliving stalks (figure 3.2f). A few bacteria actually are flat. For example, Anthony E. Walsby has discovered square bacteria living in salt ponds (figure 3.2e). These bacteria are shaped like flat, square-to-rectangular boxes about 2 μm by 2 to 4 μm, and only 0.25 μm thick. Finally, some bacteria are variable in shape and lack a single, characteristic form (figure 3.2b). These are called **pleomorphic** even though they may, like *Corynebacterium*, have a generally rodlike form.

Bacteria vary in size as much as in shape (figure 3.3). The smallest (e.g., some members of the genus *Mycoplasma*) are about 0.3 μm in diameter, approximately the size of the largest viruses (the poxviruses). Recently there have been reports of even smaller cells. Nanobacteria or ultramicrobacteria appear to range from around 0.2 μm to less than 0.05 μm in diameter.

A few strains have been cultured, but most are simply very small bacteria-like objects only observed microscopically. It has been thought that the smallest possible cell is about 0.14 to 0.2 μm in diameter, but many nanobacteria are reported to be smaller. Some microbiologists think nanobacteria are artifacts, and more research will be required before the significance of these forms becomes clear. *Escherichia coli*, a bacillus of about average size, is 1.1 to 1.5 μm wide by 2.0 to 6.0 μm long. A few bacteria become fairly large; some spirochetes occasionally reach 500 μm in length, and the cyanobacterium *Oscillatoria* is about 7 μm in diameter (the same diameter as a red blood cell). A huge bacterium lives in the intestine of the brown surgeonfish, *Acanthurus nigrofuscus*. *Epulopiscium fishelsoni* grows as large as 600 by 80 μm, a little smaller than a printed hyphen. More recently an even larger bacterium, *Thiomargarita namibiensis*, has been discovered in ocean sediment (Box 3.1). Thus a few bacteria are much larger than the average eucaryotic cell (typical plant and animal cells are around 10–50 μm in diameter).

Box 3.1

Monstrous Microbes

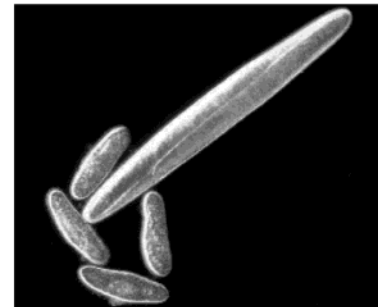
Biologists often have distinguished between prokaryotes and eukaryotes based in part on cell size. Generally, prokaryotic cells are supposed to be smaller than eukaryotic cells. Prokaryotes grow extremely rapidly compared to most eukaryotes and lack the complex vesicular transport systems of eukaryotic cells (see chapter 4). It has been assumed that they must be small because of the slowness of nutrient diffusion and the need for a large surface-to-volume ratio. Thus when Fishelson, Montgomery, and Myrberg discovered a large, cigar-shaped microorganism in the intestinal tract of the Red Sea brown surgeonfish, *Acanthurus nigrofuscus*, they suggested in their 1985 publication that it was a protist. It seemed too large to be anything else. In 1993 Esther Angert, Kendall Clemens, and Norman Pace used rRNA sequence comparisons (see p. 432) to identify the microorganism, now called *Epulopiscium fishelsoni*, as a prokaryote related to the gram-positive genus *Clostridium*.

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

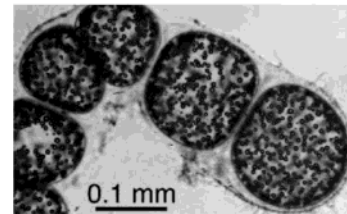
It appears that *Epulopiscium* is transmitted between hosts through fecal contamination of the fish's food. The bacterium can be eliminated by starving the surgeonfish for a few days. If juvenile fish that lack the bacterium are placed with infected hosts, they are reinoculated. Interestingly this does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

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

The discovery of these prokaryotes greatly weakens the distinction between prokaryotes and eukaryotes based on cell size. They are certainly larger than a normal eukaryotic cell. In addition, some eukaryotic



(a)



(b)

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

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

Prokaryotic Cell Organization

A variety of structures is found in prokaryotic cells. Their major functions are summarized in table 3.1, and figure 3.4 illustrates many of them. Not all structures are found in every genus. Furthermore, gram-negative and gram-positive cells differ, particularly with respect to their cell walls. Despite these variations prokaryotes are consistent in their fundamental structure and most important components.

Prokaryotic cells almost always are bounded by a chemically complex cell wall. Inside this wall, and separated from it by a

periplasmic space, lies the plasma membrane. This membrane can be invaginated to form simple internal membranous structures. Since the prokaryotic cell does not contain internal membrane-bound organelles, its interior appears morphologically simple. The genetic material is localized in a discrete region, the nucleoid, and is not separated from the surrounding cytoplasm by membranes. Ribosomes and larger masses called inclusion bodies are scattered about in the cytoplasmic matrix. Both gram-positive and gram-negative cells can use flagella for locomotion. In addition, many cells are surrounded by a capsule or slime layer external to the cell wall.

Table 3.1 Functions of Prokaryotic Structures

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

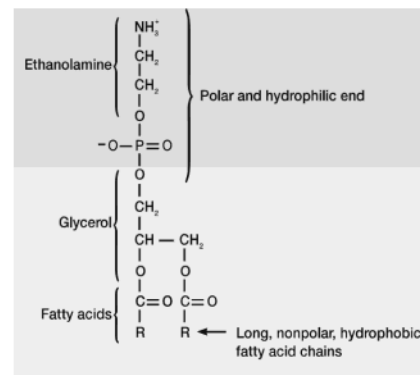


Figure 3.5 The Structure of a Polar Membrane Lipid.

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

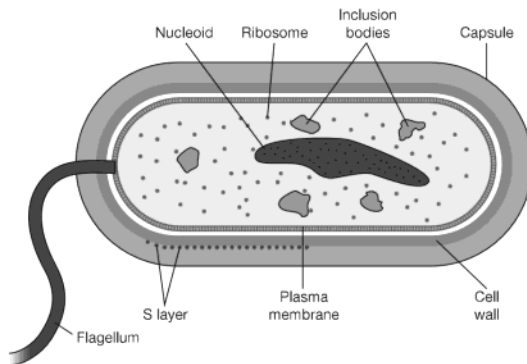


Figure 3.4 Morphology of a Gram-Positive Bacterium. The majority of the structures shown here are found in all gram-positive cells. Only a small stretch of surface proteins in the S-layer has been included to simplify the drawing; when present, these proteins cover the surface.

Prokaryotic cells are morphologically much simpler than eucaryotic cells. These two cell types are compared following the review of eucaryotic cell structure (*see pp. 91–92*).

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

3.2 Prokaryotic Cell Membranes

Membranes are an absolute requirement for all living organisms. Cells must interact in a selective fashion with their environment, whether it is the internal environment of a multicellular organism or a less protected and more variable external environment. Cells must not only be able to acquire nutrients and eliminate wastes, but they also have to maintain their interior in a constant, highly organized state in the face of external changes. The **plasma membrane** encompasses the cytoplasm of both prokaryotic and eucaryotic cells. This membrane is the chief point of contact with the cell's environment and thus is responsible for much of its relationship with the outside world. To understand membrane function, it is necessary to become familiar with membrane structure, and particularly with plasma membrane structure.

The Plasma Membrane

Membranes contain both proteins and lipids, although the exact proportions of protein and lipid vary widely. Bacterial plasma membranes usually have a higher proportion of protein than do eucaryotic membranes, presumably because they fulfill so many different functions that are carried out by other organelle membranes in eucaryotes. Most membrane-associated lipids are structurally asymmetric with polar and nonpolar ends (**figure 3.5**) and are called amphipathic. The polar ends interact with water and are **hydrophilic**; the nonpolar **hydrophobic** ends are insoluble in water and tend to associate with one another. This property of lipids enables them to form a bilayer in membranes. The outer surfaces are hydrophilic, whereas hydrophobic ends are buried in the interior away from the surrounding water. Many of these am-

Box 3.2

Bacteria and Fossil Fuels

For many years there has been great interest in the origin of fossil fuels such as coal and petroleum. In the oceans, there is a constant "snow" of prokaryotic membranes and other organic matter that settles on the bottom sediments. Fossil fuel formation begins when organic matter is buried before it can be oxidized to carbon dioxide by microorganisms. When organic matter is buried deeply and subjected to increasing temperature under anaerobic conditions, petroleum and coal are often formed. The quantities involved in these processes are enormous. It has been estimated that the earth contains about 10^{16} tons of carbon in its sediments.

There is increasing evidence that much of the organic material in sediments is bacterial in origin. About 90% of this material is in the

form of insoluble kerogen, an organic precursor of petroleum. Recently the hopanoid bacteriohopanetetrol (figure 3.6b) was isolated from kerogen, and evidence is accumulating that kerogen arises from bacterial activity. We may owe our supply of fossil fuels largely to bacteria that serve as the final degraders of the organic material in dead organisms.

It has been estimated that the total mass of hopanoids in sediments is around 10^{11-12} tons, about as much as the total mass of organic carbon in all living organisms (10^{12} tons). Hopanoids may be the most abundant biomolecules on our planet.

phospholipids are phospholipids (figure 3.5). Bacterial membranes usually differ from eucaryotic membranes in lacking sterols such as cholesterol (figure 3.6a). However, many bacterial membranes do contain pentacyclic sterol-like molecules called hopanoids (figure 3.6b), and huge quantities of hopanoids are present in our ecosystem (Box 3.2). Hopanoids are synthesized from the same precursors as steroids. Like steroids in eucaryotes, they probably stabilize the bacterial membrane. The membrane lipid is organized in two layers, or sheets, of molecules arranged end-to-end (figure 3.7).

Many archaeal membranes differ from other bacterial membranes in having a monolayer with lipid molecules spanning the whole membrane. Archaea (chapter 20)

Cell membranes are very thin structures, about 5 to 10 nm thick, and can only be seen with the electron microscope. The freeze-etching technique has been used to cleave membranes down the center of the lipid bilayer, splitting them in half and exposing the interior. In this way it has been discovered that many membranes, including the plasma membrane, have a complex internal structure. The small globular particles seen in these membranes are thought to be membrane proteins that lie within the membrane lipid bilayer (see figure 2.26). Freeze-etching technique (p. 33)

The most widely accepted current model for membrane structure is the **fluid mosaic model** of S. Jonathan Singer and Garth Nicholson (figure 3.7). They distinguish between two types of membrane proteins. **Peripheral proteins** are loosely connected to the membrane and can be easily removed. They are soluble in aqueous solutions and make up about 20 to 30% of total membrane protein. About 70 to 80% of membrane proteins are **integral proteins**. These are not easily extracted from membranes and are insoluble in aqueous solutions when freed of lipids. Protein and lipid chemistry (appendix I)

Integral proteins, like membrane lipids, are amphipathic; their hydrophobic regions are buried in the lipid while the hydrophilic portions project from the membrane surface (figure 3.7). Some of these proteins even extend all the way through the lipid layer. Integral proteins can diffuse laterally around the surface to new loca-

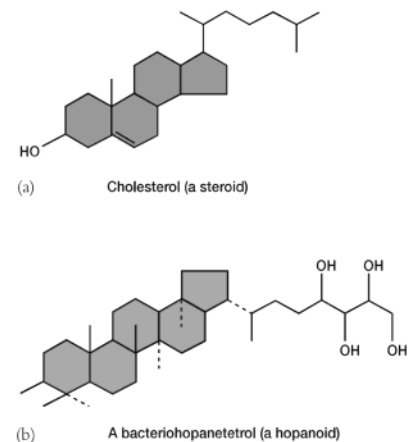


Figure 3.6 Membrane Steroids and Hopanoids. Common examples.

tions, but do not flip-flop or rotate through the lipid layer. Often carbohydrates are attached to the outer surface of plasma membrane proteins and seem to have important functions.

The emerging picture of the cell membrane is one of a highly organized and asymmetric system, which also is flexible and dynamic. Although membranes apparently have a common basic design, there are wide variations in both their structure and functional capacities. The differences are so large and characteristic that membrane chemistry can be used in bacterial identification.

The plasma membranes of prokaryotic cells must fill an incredible variety of roles successfully. Many major plasma membrane functions are noted here even though they are discussed individually at later points in the text. The plasma membrane retains the

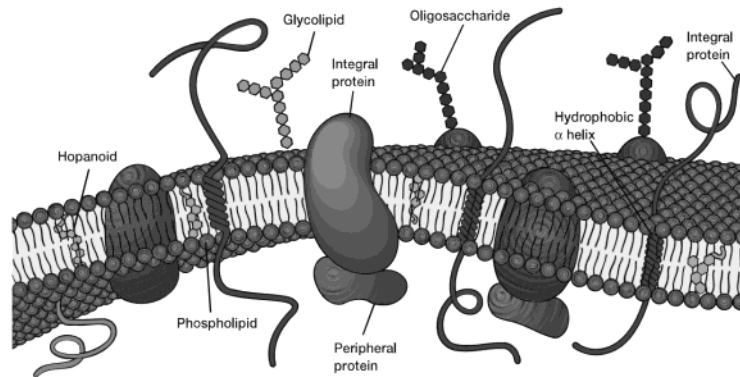


Figure 3.7 Plasma Membrane Structure. This diagram of the fluid mosaic model of bacterial membrane structure shows the integral proteins (blue) floating in a lipid bilayer. Peripheral proteins (purple) are associated loosely with the inner membrane surface. Small spheres represent the hydrophilic ends of membrane phospholipids and wiggly tails, the hydrophobic fatty acid chains. Other membrane lipids such as hopanoids (pink) may be present. For the sake of clarity, phospholipids are shown in proportionately much larger size than in real membranes.

cytoplasm, particularly in cells without cell walls, and separates it from the surroundings. The plasma membrane also serves as a selectively permeable barrier: it allows particular ions and molecules to pass, either into or out of the cell, while preventing the movement of others. Thus the membrane prevents the loss of essential components through leakage while allowing the movement of other molecules. Because many substances cannot cross the plasma membrane without assistance, it must aid such movement when necessary. Transport systems can be used for such tasks as nutrient uptake, waste excretion, and protein secretion. The prokaryotic plasma membrane also is the location of a variety of crucial metabolic processes: respiration, photosynthesis, the synthesis of lipids and cell wall constituents, and probably chromosome segregation. Finally, the membrane contains special receptor molecules that help prokaryotes detect and respond to chemicals in their surroundings. Clearly the plasma membrane is essential to the survival of microorganisms. Osmosis (p. 61); Transport of substances across membranes (pp. 100–4)

Internal Membrane Systems

Although prokaryotic cytoplasm does not contain complex membranous organelles like mitochondria or chloroplasts, membranous structures of several kinds can be observed. A commonly observed structure is the mesosome. Mesosomes are invaginations of the plasma membrane in the shape of vesicles, tubules, or lamellae (**figure 3.8** and **figure 3.11**). They are seen in both gram-positive and gram-negative bacteria, although they are generally more prominent in the former.

Mesosomes often are found next to septa or cross-walls in dividing bacteria and sometimes seem attached to the bacterial chromosome. Thus they may be involved in cell wall formation

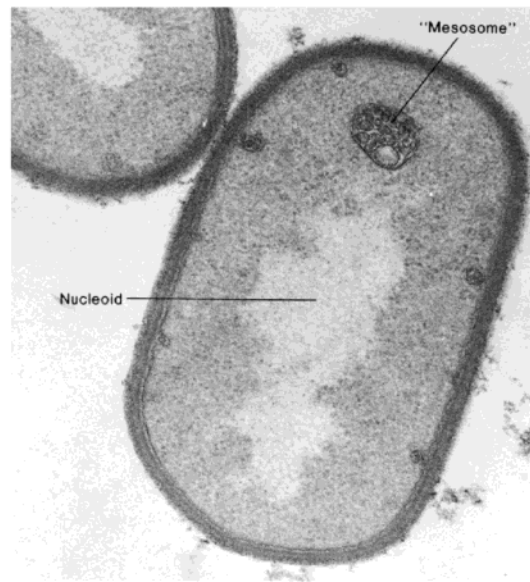
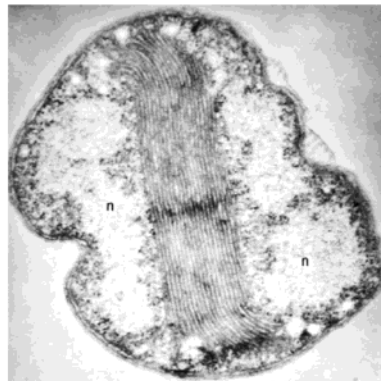


Figure 3.8 Mesosome Structure. *Bacillus fastidiosus* (×91,000). A large mesosome lies adjacent to the nucleoid.

during division or play a role in chromosome replication and distribution to daughter cells.

Currently many bacteriologists believe that mesosomes are artifacts generated during the chemical fixation of bacteria for elec-



(a)



(b)

Figure 3.9 Internal Bacterial Membranes. Membranes of nitrifying and photosynthetic bacteria. (a) *Nitrocystis oceanus* with parallel membranes traversing the whole cell. Note nucleoplasm (n) with fibrillar structure. (b) *Ectothiorhodospira mobilis* with an extensive intracytoplasmic membrane system ($\times 60,000$).

tron microscopy. Possibly they represent parts of the plasma membrane that are chemically different and more disrupted by fixatives.

Many bacteria have internal membrane systems quite different from the mesosome (figure 3.9). Plasma membrane infoldings can become extensive and complex in photosynthetic bacteria such as the cyanobacteria and purple bacteria or in bacteria with very high respiratory activity like the nitrifying bacteria (see chapter 22). They may be aggregates of spherical vesicles, flattened vesicles, or tubular membranes. Their function may be to provide a larger membrane surface for greater metabolic activity.

1. Describe with a labeled diagram and in words the fluid mosaic model for cell membranes.
2. List the functions of the plasma membrane.
3. Discuss the nature, structure, and possible functions of the mesosome.

3.3 The Cytoplasmic Matrix

Prokaryotic cytoplasm, unlike that of eucaryotes, lacks unit membrane-bound organelles. The **cytoplasmic matrix** is the substance lying between the plasma membrane and the nucleoid (p. 54). The matrix is largely water (about 70% of bacterial mass is water). It is featureless in electron micrographs but often is packed with ribosomes and highly organized (figure 3.10). Specific proteins are positioned at particular sites such as the cell pole and the place where the bacterial cell will divide. Thus although bacteria may lack a true cytoskeleton, they do have a cytoskeleton-like system of proteins in their cytoplasmic matrix. The plasma membrane and everything within is called the **protoplast**; thus the cytoplasmic matrix is a major part of the protoplast.

Inclusion Bodies

A variety of **inclusion bodies**, granules of organic or inorganic material that often are clearly visible in a light microscope, is present in the cytoplasmic matrix. These bodies usually are used for storage (e.g., carbon compounds, inorganic substances, and energy), and also reduce osmotic pressure by tying up molecules in particulate form. Some inclusion bodies are not bounded by a membrane and lie free in the cytoplasm—for example, polyphosphate granules, cyanophycin granules, and some glycogen granules. Other inclusion bodies are enclosed by a membrane about 2.0 to 4.0 nm thick, which is single-layered and not a typical bilayer membrane. Examples of membrane-enclosed inclusion bodies are poly- β -hydroxybutyrate granules, some glycogen and sulfur granules, carboxysomes, and gas vacuoles. Inclusion body membranes vary in composition. Some are protein in nature, whereas others contain lipid. Because inclusion bodies are used for storage, their quantity will vary with the nutritional status of the cell. For example, polyphosphate granules will be depleted in freshwater habitats that are phosphate limited. A brief description of several important inclusion bodies follows.

Organic inclusion bodies usually contain either glycogen or poly- β -hydroxybutyrate. **Glycogen** is a polymer of glucose units composed of long chains formed by $\alpha(1\rightarrow4)$ glycosidic bonds and branching chains connected to them by $\alpha(1\rightarrow6)$ glycosidic bonds (see appendix I). **Poly- β -hydroxybutyrate (PHB)** contains β -hydroxybutyrate molecules joined by ester bonds between the carboxyl and hydroxyl groups of adjacent molecules. Usually only one of these polymers is found in a species, but purple photosynthetic bacteria have both. Poly- β -hydroxybutyrate accumulates in distinct bodies, around 0.2 to 0.7 μm in diameter, that are readily stained with Sudan black for light microscopy and

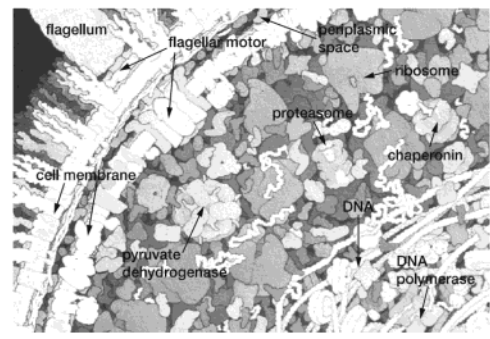
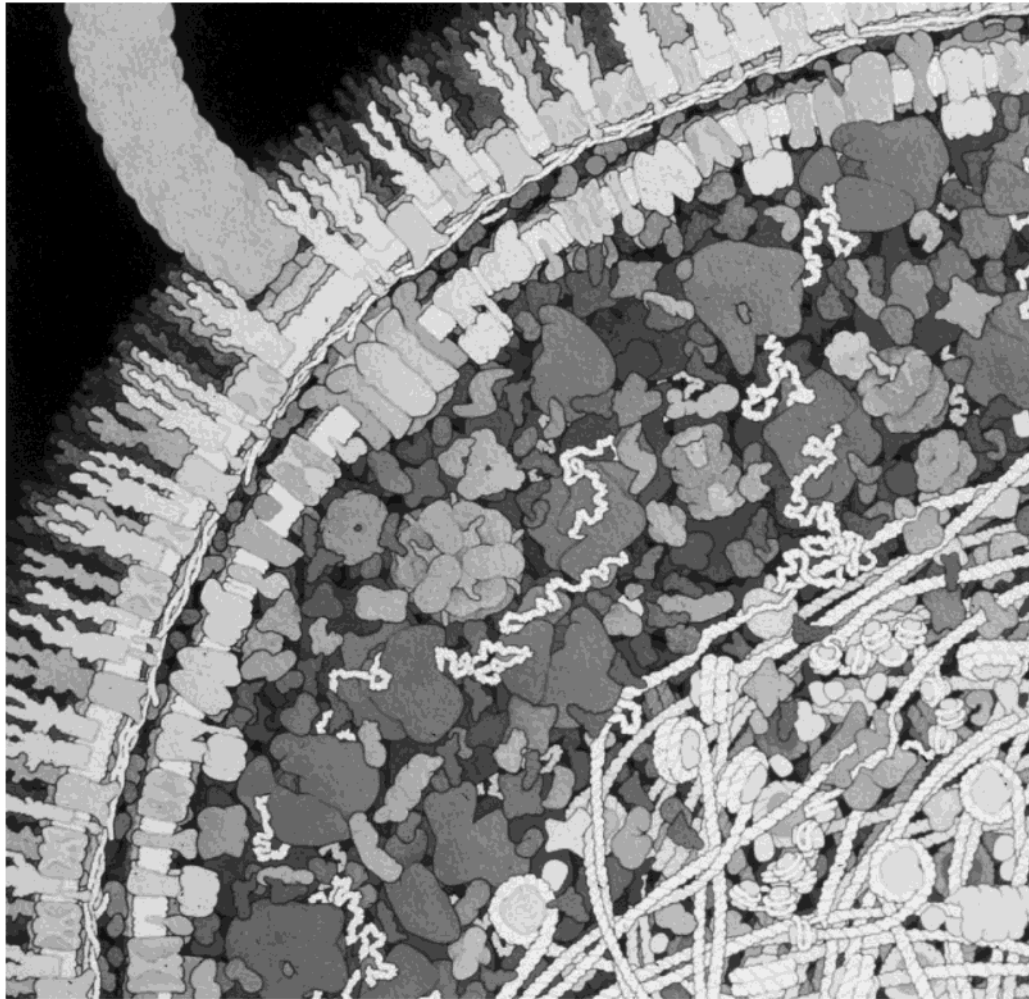


Figure 3.10 A Cross Section of the Bacterium *Escherichia coli* Drawn at a Magnification of a Million Times. The glycocalyx, flagellum, gram-negative cell wall, and plasma membrane are at the top. Ribosomes synthesizing proteins fill the underlying cytoplasmic matrix. At the bottom is the nucleoid with its dense tangle of DNA and associated proteins.

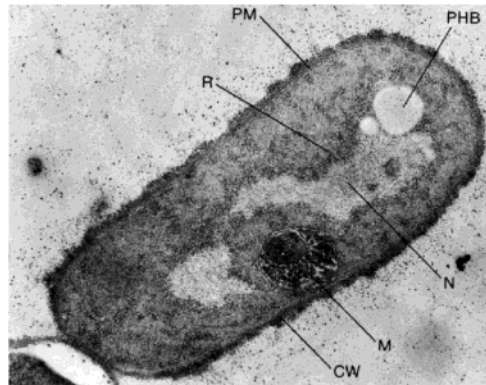


Figure 3.11 The Structure of a Typical Gram-Positive Cell. Electron micrograph of *Bacillus megaterium* ($\times 30,500$). Note the thick cell wall, CW; "mesosome," M; nucleoid, N; poly- β -hydroxybutyrate inclusion body, PHB; plasma membrane, PM; and ribosomes, R.

are clearly visible in the electron microscope (**figure 3.11**). Glycogen is dispersed more evenly throughout the matrix as small granules (about 20 to 100 nm in diameter) and often can be seen only with the electron microscope. If cells contain a large amount of glycogen, staining with an iodine solution will turn them reddish-brown. Glycogen and PHB inclusion bodies are carbon storage reservoirs providing material for energy and biosynthesis. Many bacteria also store carbon as lipid droplets.

Cyanobacteria have two distinctive organic inclusion bodies. **Cyanophycin granules** (**figure 3.13a**) are composed of large polypeptides containing approximately equal amounts of the amino acids arginine and aspartic acid. The granules often are large enough to be visible in the light microscope and store extra nitrogen for the bacteria. **Carboxysomes** are present in many cyanobacteria, nitrifying bacteria, and thiobacilli. They are polyhedral, about 100 nm in diameter, and contain the enzyme ribulose-1,5-bisphosphate carboxylase (*see p. 208*) in a paracrystalline arrangement. They serve as a reserve of this enzyme and may be a site of CO_2 fixation.

A most remarkable organic inclusion body, the **gas vacuole**, is present in many cyanobacteria (*see section 21.3*), purple and green photosynthetic bacteria, and a few other aquatic forms such as *Halobacterium* and *Thiothrix*. These bacteria float at or near the surface, because gas vacuoles give them buoyancy. This is vividly demonstrated by a simple but dramatic experiment. Cyanobacteria held in a full, tightly stoppered bottle will float, but if the stopper is struck with a hammer, the bacteria sink to the bottom. Examination of the bacteria at the beginning and end of the experiment shows that the sudden pressure increase has collapsed the gas vacuoles and destroyed the microorganisms' buoyancy.

Gas vacuoles are aggregates of enormous numbers of small, hollow, cylindrical structures called **gas vesicles** (**figure 3.12**). Gas vesicle walls do not contain lipid and are composed entirely

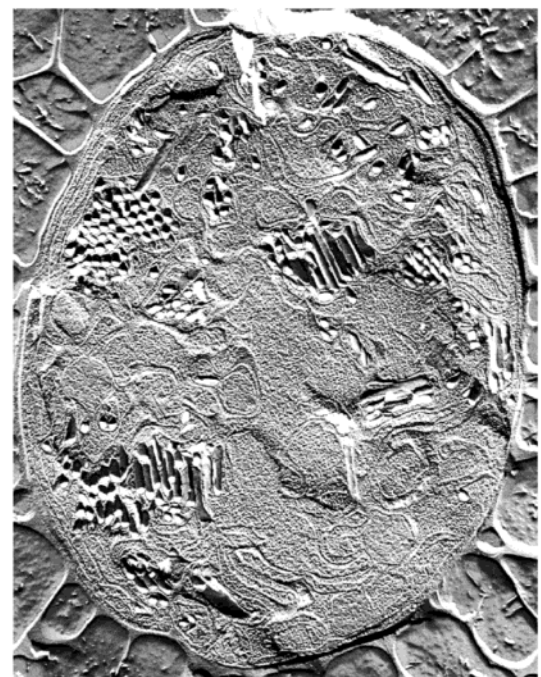
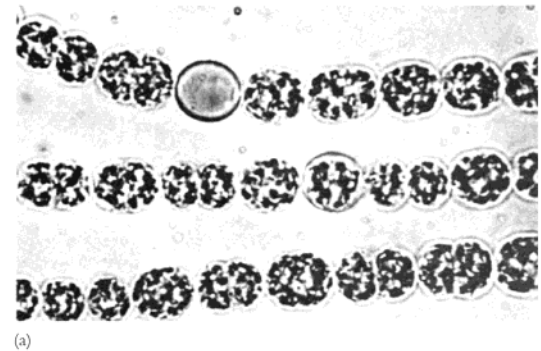


Figure 3.12 Gas Vesicles and Vacuoles. (a) Filaments of the cyanobacterium *Anabaena flos-aquae* as seen in the light microscope. (b) A freeze-fracture preparation of *Anabaena flos-aquae* ($\times 89,000$). Clusters of the cigar shaped vesicles form gas vacuoles. Both longitudinal and cross-sectional views of gas vesicles can be seen.

of a single small protein. These protein subunits assemble to form a rigid enclosed cylinder that is hollow and impermeable to water but freely permeable to atmospheric gases. Bacteria with gas vacuoles can regulate their buoyancy to float at the depth necessary

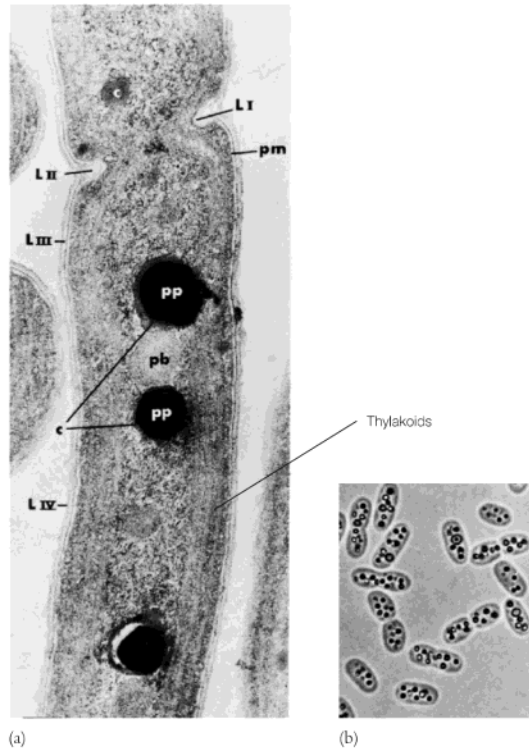


Figure 3.13 Inclusion Bodies in Bacteria. (a) Ultrastructure of the cyanobacterium *Anacystis nidulans*. The bacterium is dividing, and a septum is partially formed, LI and LII. Several structural features can be seen, including cell wall layers, LIII and LIV; the plasma membrane, pm; polyphosphate granules, pp; a polyhedral body, pb; and cyanophycin material, c. Thylakoids run along the length of the cell. Bar = 0.1 μm . (b) *Chromatium vinosum*, a purple sulfur bacterium, with intracellular sulfur granules, light field ($\times 2,000$).

for proper light intensity, oxygen concentration, and nutrient levels. They descend by simply collapsing vesicles and float upward when new ones are constructed.

Two major types of inorganic inclusion bodies are seen. Many bacteria store phosphate as **polyphosphate granules** or **volutin granules** (figure 3.13a). Polyphosphate is a linear polymer of orthophosphates joined by ester bonds. Thus volutin granules function as storage reservoirs for phosphate, an important component of cell constituents such as nucleic acids. In some cells they act as an energy reserve, and polyphosphate can serve as an energy source in reactions. These granules are sometimes called **metachromatic granules** because they show the metachromatic effect; that is, they appear red or a different shade

of blue when stained with the blue dyes methylene blue or toluidine blue. Some bacteria also store sulfur temporarily as sulfur granules, a second type of inorganic inclusion body (figure 3.13b). For example, purple photosynthetic bacteria can use hydrogen sulfide as a photosynthetic electron donor (see section 9.11) and accumulate the resulting sulfur in either the periplasmic space or in special cytoplasmic globules.

Inorganic inclusion bodies can be used for purposes other than storage. An excellent example is the **magnetosome**, which is used by some bacteria to orient in the earth's magnetic field. These inclusion bodies contain iron in the form of magnetite (Box 3.3).

Ribosomes

As mentioned earlier, the cytoplasmic matrix often is packed with **ribosomes**; they also may be loosely attached to the plasma membrane. Ribosomes look like small, featureless particles at low magnification in electron micrographs (figure 3.11) but are actually very complex objects made of both protein and ribonucleic acid (RNA). They are the site of protein synthesis; matrix ribosomes synthesize proteins destined to remain within the cell, whereas the plasma membrane ribosomes make proteins for transport to the outside. The newly formed polypeptide folds into its final shape either as it is synthesized by the ribosome or shortly after completion of protein synthesis. The shape of each protein is determined by its amino acid sequence. Special proteins called molecular chaperones, or chaperones, aid the polypeptide in folding to its proper shape. Protein synthesis, including a detailed treatment of ribosomes and chaperones, is discussed at considerable length in chapter 12.

Note that prokaryotic ribosomes are smaller than eucaryotic ribosomes. They commonly are called 70S ribosomes, have dimensions of about 14 to 15 nm by 20 nm, a molecular weight of approximately 2.7 million, and are constructed of a 50S and a 30S subunit. The S in 70S and similar values stands for **Svedberg unit**. This is the unit of the sedimentation coefficient, a measure of the sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged, the greater its Svedberg value or sedimentation coefficient. The sedimentation coefficient is a function of a particle's molecular weight, volume, and shape (see figure 16.7). Heavier and more compact particles normally have larger Svedberg numbers or sediment faster. Ribosomes in the cytoplasmic matrix of eucaryotic cells are 80S ribosomes and about 22 nm in diameter. Despite their overall difference in size, both types of ribosomes are similarly composed of a large and a small subunit.

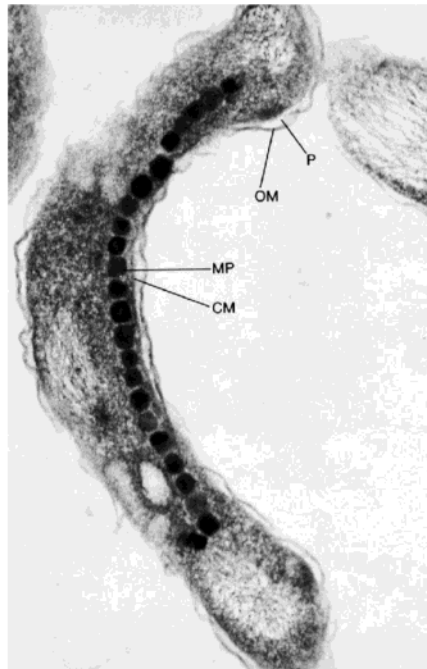
1. Briefly describe the nature and function of the cytoplasmic matrix and the ribosome. What is a protoplast?
2. What kinds of inclusion bodies do prokaryotes have? What are their functions?
3. What is a gas vacuole? Relate its structure to its function.

Box 3.3

Living Magnets

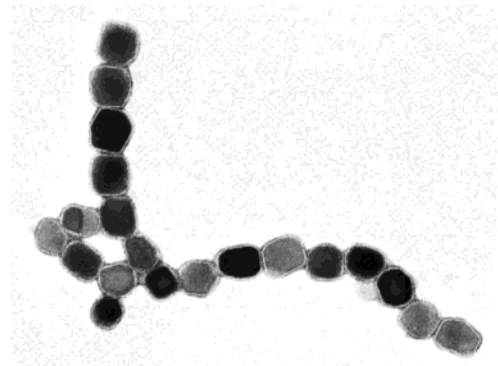
Bacteria can respond to environmental factors other than chemicals. A fascinating example is that of the aquatic magnetotactic bacteria that orient themselves in the earth's magnetic field. Most of these bacteria have intracellular chains of magnetite (Fe_3O_4) particles or magnetosomes, around 40 to 100 nm in diameter and bounded by a membrane (see **Box figure**). Some species from sulfidic habitats have magnetosomes containing greigite (Fe_3S_4) and pyrite (FeS_2). Since each iron particle is a tiny magnet, the Northern Hemisphere bacteria use their

magnetosome chain to determine northward and downward directions, and swim down to nutrient-rich sediments or locate the optimum depth in freshwater and marine habitats. Magnetotactic bacteria in the Southern Hemisphere generally orient southward and downward, with the same result. Magnetosomes also are present in the heads of birds, tuna, dolphins, green turtles, and other animals, presumably to aid navigation. Animals and bacteria share more in common behaviorally than previously imagined.



(a)

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



(b)



(c)

3.4 The Nucleoid

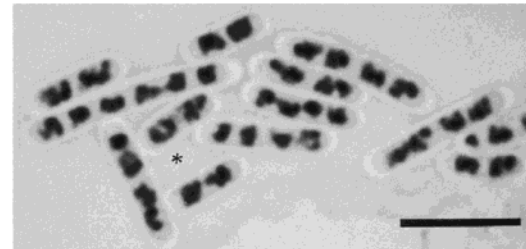
Probably the most striking difference between prokaryotes and eucaryotes is the way in which their genetic material is packaged. Eucaryotic cells have two or more chromosomes contained within a membrane-delimited organelle, the nucleus. In contrast, prokaryotes lack a membrane-delimited nucleus. The prokaryotic chromosome is located in an irregularly shaped region called the **nucleoid** (other names are also used: the nuclear body, chromatin body, nuclear region). Usually prokaryotes contain a single circle of double-stranded **deoxyribonucleic acid (DNA)**, but some have a linear DNA chromosome. Recently it has been discovered that some bacteria such as *Vibrio cholerae* have more than one chromosome. Although nucleoid appearance varies with the method of fixation and staining, fibers often are seen in electron micrographs (figure 3.11 and **figure 3.14**) and are probably DNA. The nucleoid also is visible in the light microscope after staining with the Feulgen stain, which specifically reacts with DNA. A cell can have more than one nucleoid when cell division occurs after the genetic material has been duplicated (figure 3.14*a*). In actively growing bacteria, the nucleoid has projections that extend into the cytoplasmic matrix (figure 3.14*b,c*). Presumably these projections contain DNA that is being actively transcribed to produce mRNA.

Careful electron microscopic studies often have shown the nucleoid in contact with either the mesosome or the plasma membrane. Membranes also are found attached to isolated nucleoids. Thus there is evidence that bacterial DNA is attached to cell membranes, and membranes may be involved in the separation of DNA into daughter cells during division.

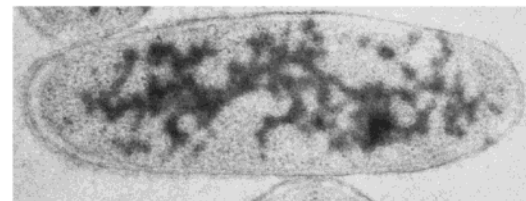
Nucleoids have been isolated intact and free from membranes. Chemical analysis reveals that they are composed of about 60% DNA, 30% RNA, and 10% protein by weight. In *Escherichia coli*, a rod-shaped cell about 2 to 6 μm long, the closed DNA circle measures approximately 1,400 μm . Obviously it must be very efficiently packaged to fit within the nucleoid. The DNA is looped and coiled extensively (see figure 11.8), probably with the aid of RNA and nucleoid proteins (these proteins differ from the histone proteins present in eucaryotic nuclei).

There are a few exceptions to the above picture. Membrane-bound DNA-containing regions are present in two genera of planctomycetes. *Pirellula* has a single membrane that surrounds a region, the pirellosome, which contains a fibrillar nucleoid and ribosome-like particles. The nuclear body of *Gemmata obscuriglobus* is bounded by two membranes (see figure 21.12). More work will be required to determine the functions of these membranes and how widespread this phenomenon is. The cell cycle and cell division (pp. 285–86). Prokaryotic DNA and its function (chapters 11 and 12)

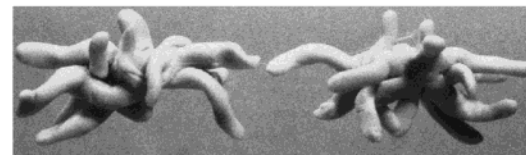
Many bacteria possess **plasmids** in addition to their chromosome. These are double-stranded DNA molecules, usually circular, that can exist and replicate independently of the chromosome or may be integrated with it; in either case they normally are inherited or passed on to the progeny. However, plasmids are not usually attached to the plasma membrane and sometimes are lost



(a)



(b)



(c)

Figure 3.14 The Bacterial Nucleoid. (a) Nucleoids in growing *Bacillus* cells stained using HCl-Giemsa stain and viewed with a light microscope (bar = 5 μm). (b) A section of actively growing *E. coli* immunostained specifically for DNA and examined in the transmission electron microscope. Coupled transcription and translation occur in parts of the nucleoid that extend out into the cytoplasm. (c) A model of two nucleoids in an actively growing *E. coli* cell. Note that a metabolically active nucleoid is not compact and spherical but has projections that extend into the cytoplasmic matrix.

to one of the progeny cells during division. Plasmids are not required for host growth and reproduction, although they may carry genes that give their bacterial host a selective advantage. Plasmid genes can render bacteria drug-resistant, give them new metabolic abilities, make them pathogenic, or endow them with a number of other properties. Because plasmids often move between bacteria, properties such as drug resistance can spread throughout a population. Plasmids (pp. 294–97)

1. Characterize the nucleoid with respect to its structure and function.
2. What is a plasmid?