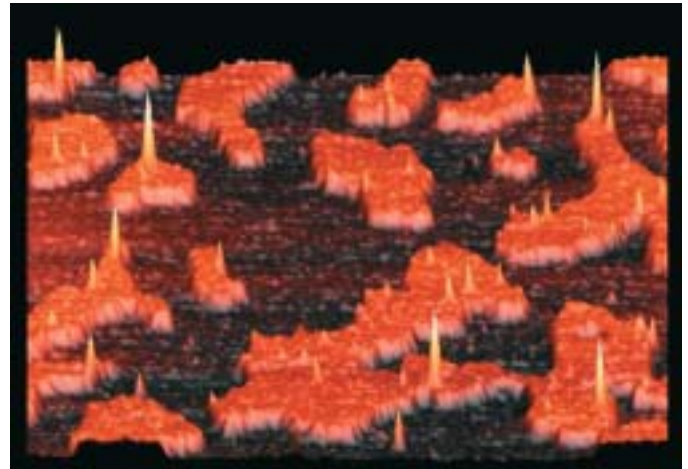


5

BIOMEMBRANES AND CELL ARCHITECTURE



Atomic force microscopy reveals sphyngomyelin rafts (orange) protruding from a dioleoylphosphatidylcholine background (black) in a mica-supported lipid bilayer. Placental alkaline phosphatase (yellow peaks), a glycosylphosphatidylinositol-anchored protein, is shown to be almost exclusively raft associated. [From D. E. Saslowsky et al., 2002, *J. Biol. Chem.* 277:26966–26970.]

Prokaryotes, which represent the simplest and smallest cells, about 1–2 μm in length, are surrounded by a **plasma membrane** but contain no internal membrane-limited subcompartments (see Figure 1-2a). Although DNA is concentrated in the center of these unicellular organisms, most enzymes and metabolites are thought to diffuse freely within the single internal aqueous compartment. Certain metabolic reactions, including protein synthesis and anaerobic glycolysis, take place there; others, such as the replication of DNA and the production of ATP, take place at the plasma membrane.

In the larger cells of eukaryotes, however, the rates of chemical reactions would be limited by the diffusion of small molecules if a cell were not partitioned into smaller subcompartments termed **organelles**. Each organelle is surrounded by one or more **biomembranes**, and each type of organelle contains a unique complement of proteins—some embedded in its membrane(s), others in its aqueous interior space, or **lumen**. These proteins enable each organelle to carry out its characteristic cellular functions. The **cytoplasm** is the part of the cell outside the largest organelle, the nucleus. The **cytosol**, the aqueous part of the cytoplasm outside all of the organelles, also contains its own distinctive proteins.

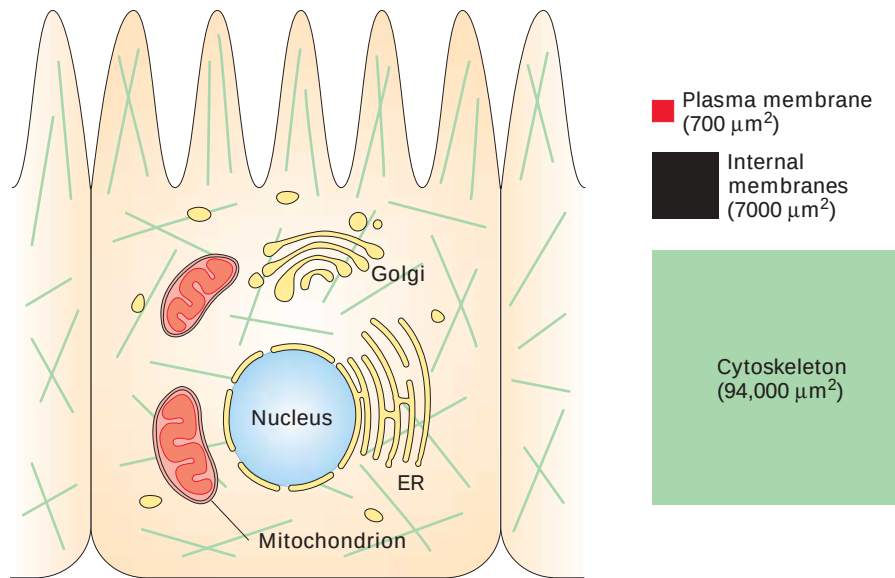
All biomembranes form closed structures, separating the lumen on the inside from the outside, and are based on a similar bilayer structure. They control the movement of molecules between the inside and the outside of a cell and into and out of the organelles of eukaryotic cells. In accord with the importance of internal membranes to cell function, the total surface area of these membranes is roughly tenfold as great as that of the plasma membrane (Figure 5-1).

Although the basic architecture of all eukaryotic cells is constructed from membranes, organelles, and the cytosol, each type of cell exhibits a distinctive design defined by the shape of the cell and the location of its organelles. The structural basis of the unique design of each cell type lies in the **cytoskeleton**, a dense network of three classes of protein filaments that permeate the cytosol and mechanically support cellular membranes. Cytoskeletal proteins are among the most abundant proteins in a cell, and the enormous surface area of the cytoskeleton (see Figure 5-1) constitutes a scaffold to which particular sets of proteins and membranes are bound.

We begin our examination of cell architecture by considering the basic structure of biomembranes. The lipid components of membranes not only affect their shape and

OUTLINE

- 5.1 Biomembranes: Lipid Composition and Structural Organization
- 5.2 Biomembranes: Protein Components and Basic Functions
- 5.3 Organelles of the Eukaryotic Cell
- 5.4 The Cytoskeleton: Components and Structural Functions
- 5.5 Purification of Cells and Their Parts
- 5.6 Visualizing Cell Architecture



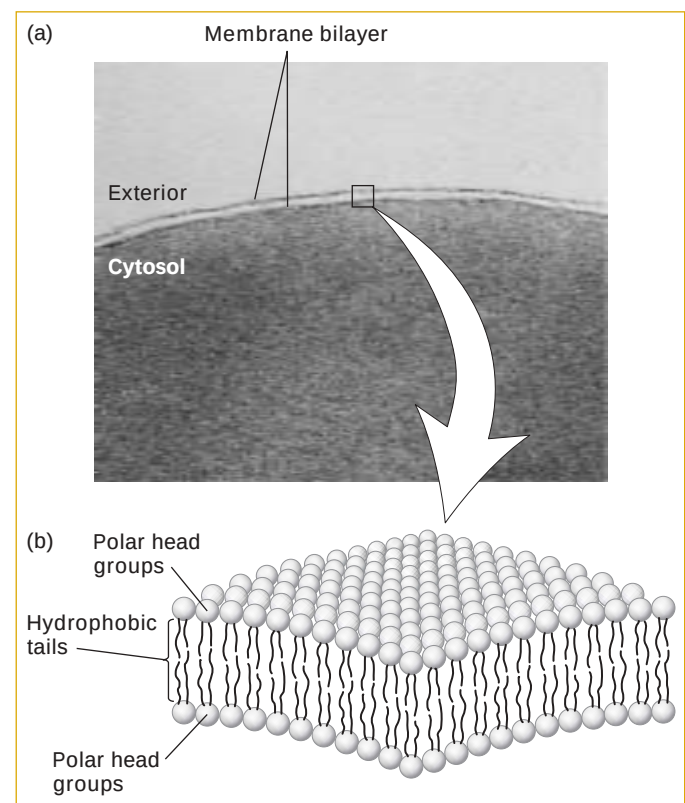
▲ **FIGURE 5-1 Schematic overview of the major components of eukaryotic cell architecture.** The plasma membrane (red) defines the exterior of the cell and controls the movement of molecules between the cytosol and the extracellular medium. Different types of organelles and smaller vesicles enclosed within their own distinctive membranes (black) carry out special functions such as gene expression, energy production, membrane synthesis, and intracellular transport.

Fibers of the cytoskeleton (green) provide structural support for the cell and its internal compartments. The internal membranes of organelles and vesicles possess more surface area than that of the plasma membrane, as schematically represented by the red, black, and green boxes. The enormous surface area of the cytoskeleton allows it to function as a scaffold on which cellular reactions can take place.

function but also play important roles in anchoring proteins to the membrane, modifying membrane protein activities, and transducing signals to the cytoplasm. We then consider the general structure of membrane proteins and how they can relate to different membranes. The unique function of each membrane is determined largely by the complement of proteins within and adjacent to it. The theme of membrane-limited compartments is continued with a review of the functions of various organelles. We then introduce the structure and function of the cytoskeleton, which is intimately associated with all biomembranes; changes in the organization of this filamentous network affect the structure and function of the attached membranes. In the remainder of the chapter, we describe common methods for isolating particular types of cells and subcellular structures and various microscopic techniques for studying cell structure and function.

► **FIGURE 5-2 The bilayer structure of biomembranes.**

(a) Electron micrograph of a thin section through an erythrocyte membrane stained with osmium tetroxide. The characteristic “railroad track” appearance of the membrane indicates the presence of two polar layers, consistent with the bilayer structure for phospholipid membranes. (b) Schematic interpretation of the phospholipid bilayer in which polar groups face outward to shield the hydrophobic fatty acyl tails from water. The hydrophobic effect and van der Waals interactions between the fatty acyl tails drive the assembly of the bilayer (Chapter 2). [Part (a) courtesy of J. D. Robertson.]



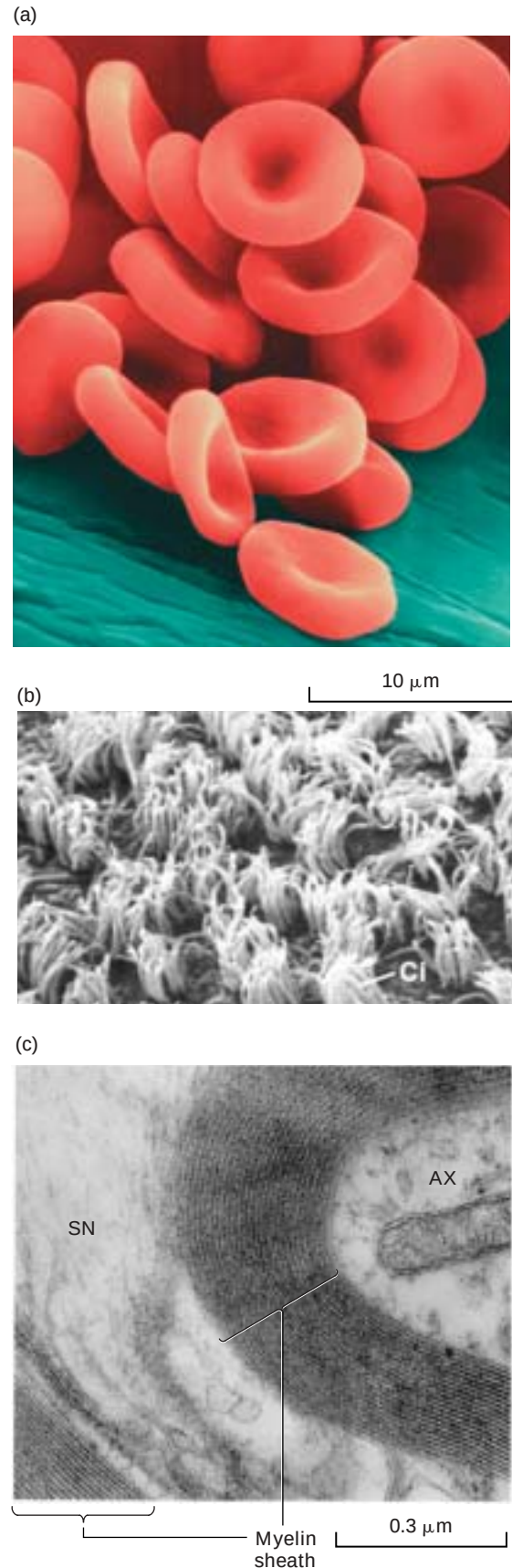
5.1 Biomembranes: Lipid Composition and Structural Organization

Phospholipids of the composition present in cells spontaneously form sheetlike **phospholipid bilayers**, which are two molecules thick. The hydrocarbon chains of the phospholipids in each layer, or *leaflet*, form a **hydrophobic** core that is 3–4 nm thick in most biomembranes. Electron microscopy of thin membrane sections stained with osmium tetroxide, which binds strongly to the polar head groups of phospholipids, reveals the bilayer structure (Figure 5-2). A cross section of all single membranes stained with osmium tetroxide looks like a railroad track: two thin dark lines (the stain-head group complexes) with a uniform light space of about 2 nm (the hydrophobic tails) between them.

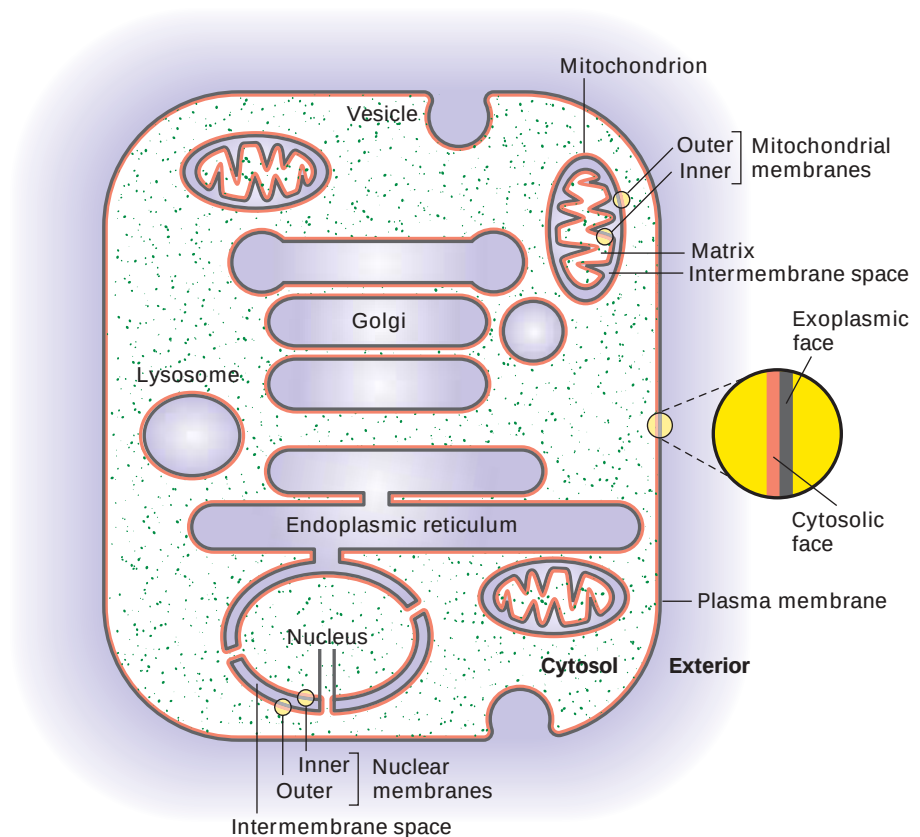
The lipid bilayer has two important properties. First, the hydrophobic core is an impermeable barrier that prevents the diffusion of water-soluble (**hydrophilic**) solutes across the membrane. Importantly, this simple barrier function is modulated by the presence of membrane proteins that mediate the transport of specific molecules across this otherwise impermeable bilayer. The second property of the bilayer is its stability. The bilayer structure is maintained by hydrophobic and van der Waals interactions between the lipid chains. Even though the exterior aqueous environment can vary widely in ionic strength and pH, the bilayer has the strength to retain its characteristic architecture.

Natural membranes from different cell types exhibit a variety of shapes, which complement a cell's function (Figure 5-3). The smooth flexible surface of the erythrocyte plasma membrane allows the cell to squeeze through narrow blood capillaries. Some cells have a long, slender extension of the plasma membrane, called a cilium or flagellum, which beats in a whiplike manner. This motion causes fluid to flow across the surface of an epithelium or a sperm cell to swim through the medium. The axons of many neurons are encased by multiple layers of modified plasma membrane called the **myelin sheath**. This membranous structure is elaborated by

► **FIGURE 5-3 Variation in biomembranes in different cell types.** (a) A smooth, flexible membrane covers the surface of the discoid erythrocyte cell. (b) Tufts of cilia (Ci) project from the ependymal cells that line the brain ventricles. (c) Many nerve axons are enveloped in a myelin sheath composed of multiple layers of modified plasma membrane. The individual myelin layers can be seen in this electron micrograph of a cross section of an axon (AX). The myelin sheath is formed by an adjacent supportive (glial) cell (SC). [Parts (a) and (b) from R. G. Kessel and R. H. Kardon, 1979, *Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy*, W. H. Freeman and Company. Part (c) from P. C. Cross and K. L. Mercer, 1993, *Cell and Tissue Ultrastructure: A Functional Perspective*, W. H. Freeman and Company, p. 137.]



► **FIGURE 5-4 The faces of cellular membranes.** The plasma membrane, a single bilayer membrane, encloses the cell. In this highly schematic representation, internal cytosol (green stipple) and external environment (purple) define the cytosolic (red) and exoplasmic (black) faces of the bilayer. Vesicles and some organelles have a single membrane and their internal aqueous space (purple) is topologically equivalent to the outside of the cell. Three organelles—the nucleus, mitochondrion, and chloroplast (which is not shown)—are enclosed by two membranes separated by a small intermembrane space. The exoplasmic faces of the inner and outer membranes around these organelles border the intermembrane space between them. For simplicity, the hydrophobic membrane interior is not indicated in this diagram.



an adjacent supportive cell and facilitates the conduction of nerve impulses over long distances (Chapter 7). Despite their diverse shapes and functions, these biomembranes and all other biomembranes have a common bilayer structure.

Because all cellular membranes enclose an entire cell or an internal compartment, they have an *internal face* (the surface oriented toward the interior of the compartment) and an *external face* (the surface presented to the environment). More commonly, the surfaces of a cellular membrane are designated as the **cytosolic face** and the **exoplasmic face**. This nomenclature is useful in highlighting the topological equivalence of the faces in different membranes, as diagrammed in Figure 5-4. For example, the exoplasmic face of the plasma membrane is directed away from the cytosol, toward the extracellular space or external environment, and defines the outer limit of the cell. For organelles and vesicles surrounded by a single membrane, however, the face directed away from the cytosol—the exoplasmic face—is on the inside in contact with an internal aqueous space equivalent to the extracellular space. This equivalence is most easily understood for vesicles that arise by invagination of the plasma membrane; this process results in the external face of the plasma membrane becoming the internal face of the vesicle membrane. Three organelles—the nucleus, mitochondrion, and chloroplast—are surrounded by two membranes; the exoplasmic

surface of each membrane faces the space between the two membranes.

Three Classes of Lipids Are Found in Biomembranes

A typical biomembrane is assembled from phosphoglycerides, sphingolipids, and steroids. All three classes of lipids are **amphipathic** molecules having a polar (hydrophilic) head group and hydrophobic tail. The hydrophobic effect and van der Waals interactions, discussed in Chapter 2, cause the tail groups to self-associate into a bilayer with the polar head groups oriented toward water (see Figure 5-2). Although the common membrane lipids have this amphipathic character in common, they differ in their chemical structures, abundance, and functions in the membrane.

Phosphoglycerides, the most abundant class of lipids in most membranes, are derivatives of glycerol 3-phosphate (Figure 5-5a). A typical phosphoglyceride molecule consists of a hydrophobic tail composed of two fatty acyl chains esterified to the two hydroxyl groups in glycerol phosphate and a polar head group attached to the phosphate group. The two fatty acyl chains may differ in the number of carbons that they contain (commonly 16 or 18) and their degree of saturation (0, 1, or 2 double bonds). A phosphoglyceride is

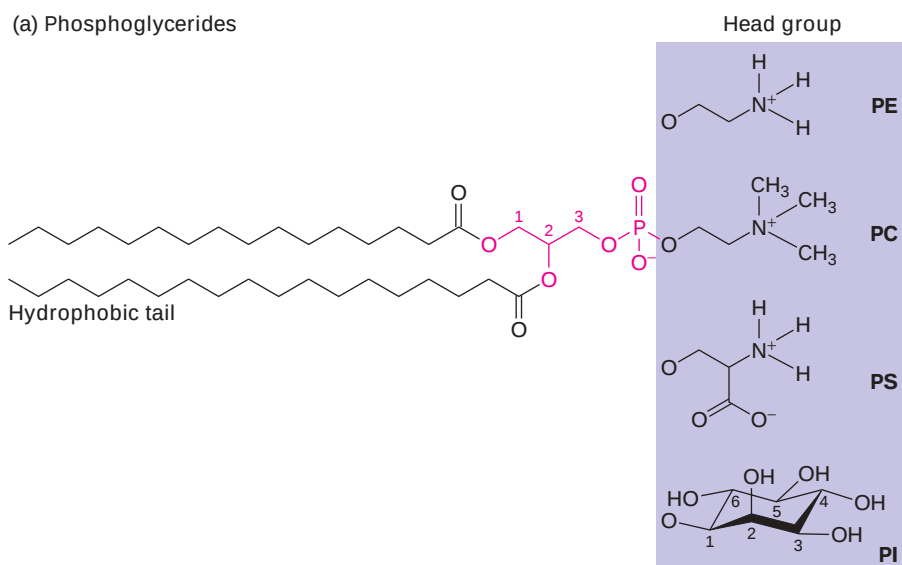
classified according to the nature of its head group. In phosphatidylcholines, the most abundant phospholipids in the plasma membrane, the head group consists of choline, a positively charged alcohol, esterified to the negatively charged phosphate. In other phosphoglycerides, an OH-containing molecule such as ethanolamine, serine, and the sugar derivative inositol is linked to the phosphate group. The negatively charged phosphate group and the positively charged groups or the hydroxyl groups on the head group interact strongly with water.

The *plasmalogens* are a group of phosphoglycerides that contain one fatty acyl chain, attached to glycerol by an ester linkage, and one long hydrocarbon chain, attached to glycerol by an ether linkage (C—O—C). These molecules constitute about 20 percent of the total phosphoglyceride content in humans. Their abundance varies among tissues and species but is especially high in human brain and heart tissue. The additional chemical stability of the ether linkage

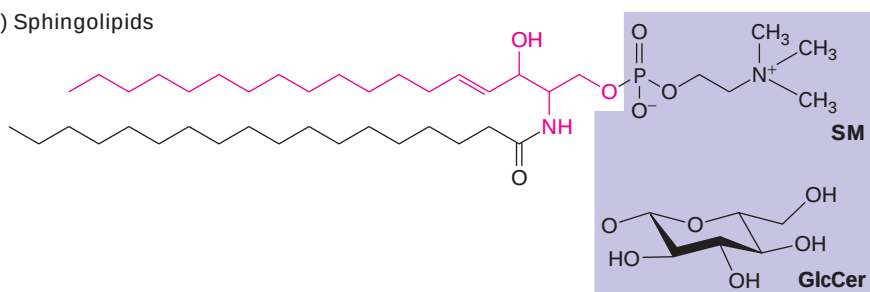
in plasmalogens or the subtle differences in their three-dimensional structure compared with that of other phosphoglycerides may have as-yet unrecognized physiologic significance.

A second class of membrane lipid is the **sphingolipids**. All of these compounds are derived from sphingosine, an amino alcohol with a long hydrocarbon chain, and contain a long-chain fatty acid attached to the sphingosine amino group. In sphingomyelin, the most abundant sphingolipid, phosphocholine is attached to the terminal hydroxyl group of sphingosine (Figure 5-5b). Thus sphingomyelin is a phospholipid, and its overall structure is quite similar to that of phosphatidylcholine. Other sphingolipids are amphipathic **glycolipids** whose polar head groups are sugars. Glucosylcerebroside, the simplest glycosphingolipid, contains a single glucose unit attached to sphingosine. In the complex glycosphingolipids called *gangliosides*, one or two branched sugar chains containing sialic acid groups are attached to

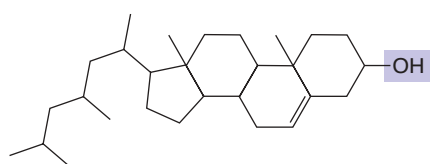
(a) Phosphoglycerides



(b) Sphingolipids



(c) Cholesterol



◀ **FIGURE 5-5 Three classes of membrane lipids.** (a) Most

phosphoglycerides are derivatives of glycerol 3-phosphate (red) containing two esterified fatty acyl chains, constituting the hydrophobic "tail" and a polar "head group" esterified to the phosphate. The fatty acids can vary in length and be saturated (no double bonds) or unsaturated (one, two, or three double bonds). In phosphatidylcholine (PC), the head group is choline. Also shown are the molecules attached to the phosphate group in three other common phosphoglycerides: phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI). (b) Sphingolipids are derivatives of sphingosine (red), an amino alcohol with a long hydrocarbon chain. Various fatty acyl chains are connected to sphingosine by an amide bond. The sphingomyelins (SM), which contain a phosphocholine head group, are phospholipids. Other sphingolipids are glycolipids in which a single sugar residue or branched oligosaccharide is attached to the sphingosine backbone. For instance, the simple glycolipid glucosylcerebroside (GlcCer) has a glucose head group. (c) Like other membrane lipids, the steroid cholesterol is amphipathic. Its single hydroxyl group is equivalent to the polar head group in other lipids; the conjugated ring and short hydrocarbon chain form the hydrophobic tail. [See H. Sprong et al., 2001, *Nature Rev. Mol. Cell Biol.* 2:504.]

sphingosine. Glycolipids constitute 2–10 percent of the total lipid in plasma membranes; they are most abundant in nervous tissue.

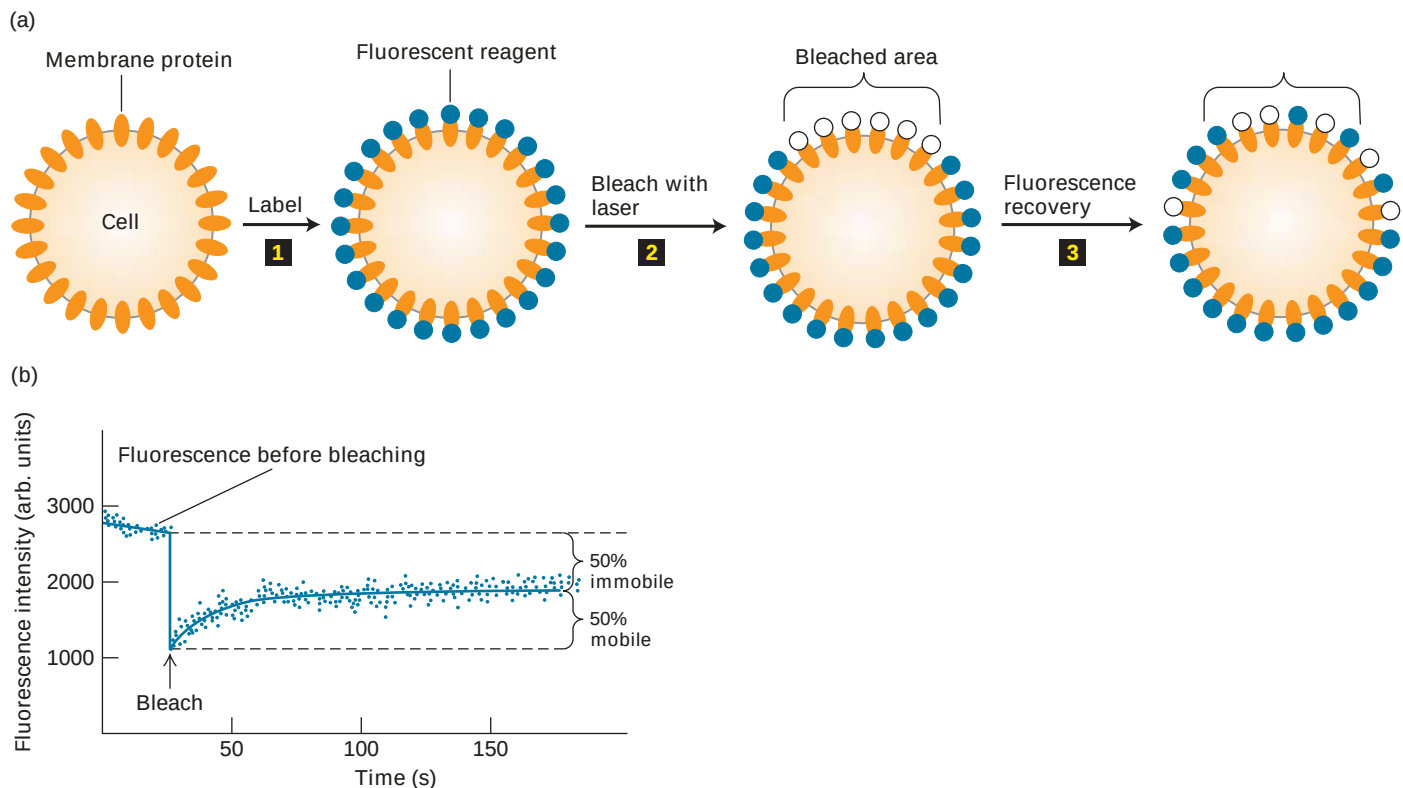
Cholesterol and its derivatives constitute the third important class of membrane lipids, the **steroids**. The basic structure of steroids is a four-ring hydrocarbon. Cholesterol, the major steroidal constituent of animal tissues, has a hydroxyl substituent on one ring (Figure 5-5c). Although cholesterol is almost entirely hydrocarbon in composition, it is amphipathic because its hydroxyl group can interact with water. Cholesterol is especially abundant in the plasma membranes of mammalian cells but is absent from most prokaryotic cells. As much as 30–50 percent of the lipids in plant plasma membranes consist of certain steroids unique to plants.

At neutral pH, some phosphoglycerides (e.g., phosphatidylcholine and phosphatidylethanolamine) carry no net

electric charge, whereas others (e.g., phosphatidylinositol and phosphatidylserine) carry a single net negative charge. Nonetheless, the polar head groups in all phospholipids can pack together into the characteristic bilayer structure. Sphingomyelins are similar in shape to phosphoglycerides and can form mixed bilayers with them. Cholesterol and other steroids are too hydrophobic to form a bilayer structure unless they are mixed with phospholipids.

Most Lipids and Many Proteins Are Laterally Mobile in Biomembranes

In the two-dimensional plane of a bilayer, thermal motion permits lipid molecules to rotate freely around their long axes and to diffuse laterally within each leaflet. Because such movements are lateral or rotational, the fatty acyl chains remain in the hydrophobic interior of the bilayer. In both natural and ar-



▲ **EXPERIMENTAL FIGURE 5-6 Fluorescence recovery after photobleaching (FRAP) experiments can quantify the lateral movement of proteins and lipids within the plasma membrane.** (a) Experimental protocol. Step 1: Cells are first labeled with a fluorescent reagent that binds uniformly to a specific membrane lipid or protein. Step 2: A laser light is then focused on a small area of the surface, irreversibly bleaching the bound reagent and thus reducing the fluorescence in the illuminated area. Step 3: In time, the fluorescence of the bleached patch increases as unbleached fluorescent surface molecules diffuse into it and bleached ones diffuse outward. The extent of recovery of fluorescence in the bleached patch is

proportional to the fraction of labeled molecules that are mobile in the membrane. (b) Results of FRAP experiment with human hepatoma cells treated with a fluorescent antibody specific for the asialoglycoprotein receptor protein. The finding that 50 percent of the fluorescence returned to the bleached area indicates that 50 percent of the receptor molecules in the illuminated membrane patch were mobile and 50 percent were immobile. Because the rate of fluorescence recovery is proportional to the rate at which labeled molecules move into the bleached region, the diffusion coefficient of a protein or lipid in the membrane can be calculated from such data. [See Y. I. Henis et al., 1990, *J. Cell Biol.* **111**:1409.]

tificial membranes, a typical lipid molecule exchanges places with its neighbors in a leaflet about 10^7 times per second and diffuses several micrometers per second at 37 °C. These diffusion rates indicate that the viscosity of the bilayer is 100 times as great as that of water—about the same as the viscosity of olive oil. Even though lipids diffuse more slowly in the bilayer than in an aqueous solvent, a membrane lipid could diffuse the length of a typical bacterial cell (1 μm) in only 1 second and the length of an animal cell in about 20 seconds.

The lateral movements of specific plasma-membrane proteins and lipids can be quantified by a technique called *fluorescence recovery after photobleaching* (FRAP). With this method, described in Figure 5-6, the rate at which membrane lipid or protein molecules move—the diffusion coefficient—can be determined, as well as the proportion of the molecules that are laterally mobile.

The results of FRAP studies with fluorescence-labeled phospholipids have shown that, in fibroblast plasma membranes, all the phospholipids are freely mobile over distances of about 0.5 μm , but most cannot diffuse over much longer distances. These findings suggest that protein-rich regions of the plasma membrane, about 1 μm in diameter, separate lipid-rich regions containing the bulk of the membrane phospholipid. Phospholipids are free to diffuse within such a region but not from one lipid-rich region to an adjacent one. Furthermore, the rate of lateral diffusion of lipids in the plasma membrane is nearly an order of magnitude slower than in pure phospholipid bilayers: diffusion constants of 10^{-8} cm^2/s and 10^{-7} cm^2/s are characteristic of the plasma membrane and a lipid bilayer, respectively. This difference suggests that lipids may be tightly but not irreversibly bound to certain integral proteins in some membranes.

Lipid Composition Influences the Physical Properties of Membranes

A typical cell contains myriad types of membranes, each with unique properties bestowed by its particular mix of lipids and proteins. The data in Table 5-1 illustrate the variation in lipid composition among different biomembranes. Several phenomena contribute to these differences. For instance, differences between membranes in the endoplasmic reticulum (ER) and the Golgi are largely explained by the fact that phospholipids are synthesized in the ER, whereas sphingolipids are synthesized in the Golgi. As a result, the proportion of sphingomyelin as a percentage of total membrane lipid phosphorus is about six times as high in Golgi membranes as it is in ER membranes. In other cases, the translocation of membranes from one cellular compartment to another can selectively enrich membranes in certain lipids.

Differences in lipid composition may also correspond to specialization of membrane function. For example, the plasma membrane of absorptive epithelial cells lining the intestine exhibits two distinct regions: the **apical** surface faces the lumen of the gut and is exposed to widely varying external conditions; the **basolateral** surface interacts with other epithelial cells and with underlying extracellular structures (see Figure 6-5). In these **polarized cells**, the ratio of sphingolipid to phosphoglyceride to cholesterol in the basolateral membrane is 0.5:1.5:1, roughly equivalent to that in the plasma membrane of a typical unpolarized cell subjected to mild stress. In contrast, the apical membrane of intestinal cells, which is subjected to considerable stress, exhibits a 1:1:1 ratio of these lipids. The relatively high concentration of sphingolipid in this membrane may increase its stability

TABLE 5-1 Major Lipid Components of Selected Biomembranes

Source/Location	Composition (mol %)			
	PC	PE + PS	SM	Cholesterol
Plasma membrane (human erythrocytes)	21	29	21	26
Myelin membrane (human neurons)	16	37	13	34
Plasma membrane (<i>E. coli</i>)	0	85	0	0
Endoplasmic reticulum membrane (rat)	54	26	5	7
Golgi membrane (rat)	45	20	13	13
Inner mitochondrial membrane (rat)	45	45	2	7
Outer mitochondrial membrane (rat)	34	46	2	11
Primary leaflet location	Exoplasmic	Cytosolic	Exoplasmic	Both

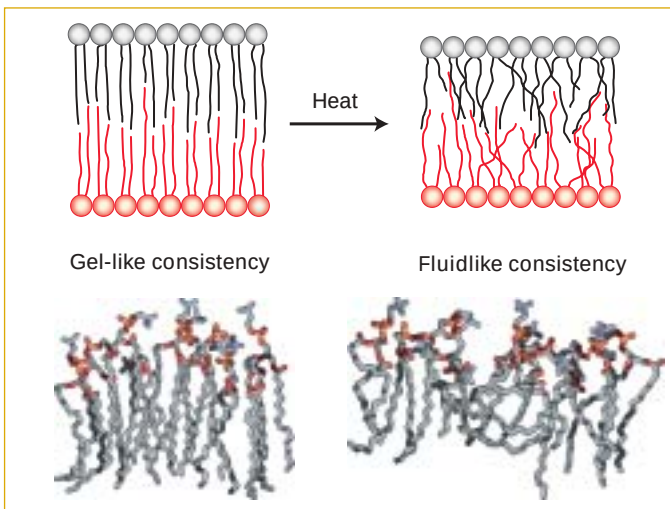
PC = phosphatidylcholine; PE = phosphatidylethanolamine; PS = phosphatidylserine; SM = sphingomyelin.

SOURCE: W. Dowhan and M. Bogdanov, 2002, in D. E. Vance and J. E. Vance, eds., *Biochemistry of Lipids, Lipoproteins, and Membranes*, Elsevier.

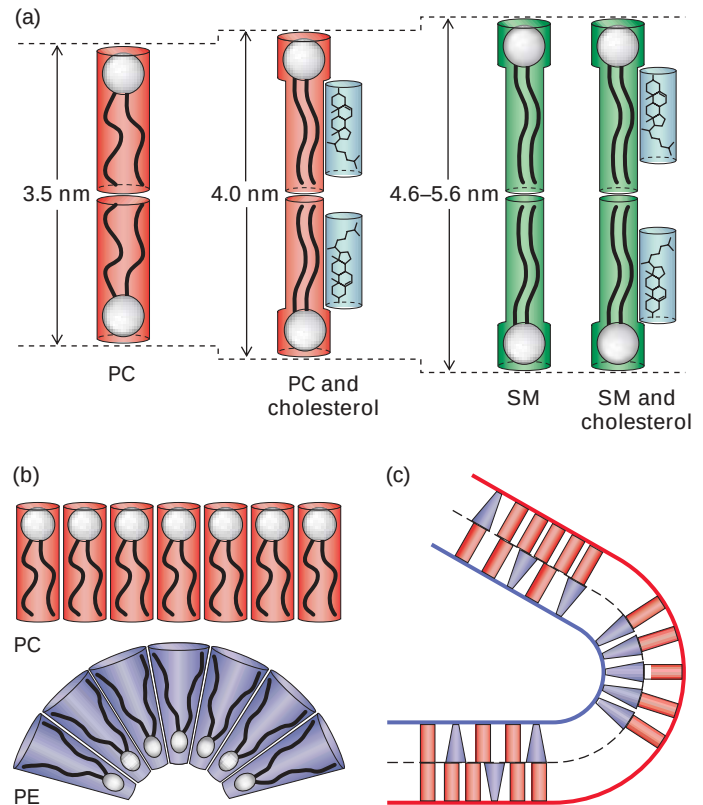
because of extensive hydrogen bonding by the free —OH group in the sphingosine moiety (see Figure 5-5).

The ability of lipids to diffuse laterally in a bilayer indicates that it can act as a fluid. The degree of bilayer fluidity depends on the lipid composition, structure of the phospholipid hydrophobic tails, and temperature. As already noted, van der Waals interactions and the hydrophobic effect cause the nonpolar tails of phospholipids to aggregate. Long, saturated fatty acyl chains have the greatest tendency to aggregate, packing tightly together into a gel-like state. Phospholipids with short fatty acyl chains, which have less surface area for interaction, form more fluid bilayers. Likewise, the kinks in unsaturated fatty acyl chains result in their forming less stable van der Waals interactions with other lipids than do saturated chains and hence more fluid bilayers. When a highly ordered, gel-like bilayer is heated, the increased molecular motions of the fatty acyl tails cause it to undergo a transition to a more fluid, disordered state (Figure 5-7).

At usual physiologic temperatures, the hydrophobic interior of natural membranes generally has a low viscosity and a fluidlike, rather than gel-like, consistency. Cholesterol is important in maintaining the fluidity of natural membranes, which appears to be essential for normal cell growth and reproduction. As noted previously, cholesterol cannot form a sheetlike bilayer on its own. At concentrations found in natural membranes, cholesterol is interca-



▲ **FIGURE 5-7 Gel and fluid forms of the phospholipid bilayer.** (Top) Depiction of gel-to-fluid transition. Phospholipids with long saturated fatty acyl chains tend to assemble into a highly ordered, gel-like bilayer in which there is little overlap of the nonpolar tails in the two leaflets. Heat disorders the nonpolar tails and induces a transition from a gel to a fluid within a temperature range of only a few degrees. As the chains become disordered, the bilayer also decreases in thickness. (Bottom) Molecular models of phospholipid monolayers in gel and fluid states, as determined by molecular dynamics calculations. [Bottom based on H. Heller et al., 1993, *J. Phys. Chem.* **97**:8343.]



▲ **FIGURE 5-8 Effect of lipid composition on bilayer thickness and curvature.** (a) A pure sphingomyelin (SM) bilayer is thicker than one formed from a phosphoglyceride such as phosphatidylcholine (PC). Cholesterol has a lipid-ordering effect on phosphoglyceride bilayers that increases their thickness but does not affect the thickness of the more ordered SM bilayer. (b) Phospholipids such as PC have a cylindrical shape and form more or less flat monolayers, whereas those with smaller head groups such as phosphatidylethanolamine (PE) have a conical shape. (c) A bilayer enriched with PC in the exoplasmic leaflet and with PE in the cytosolic face, as in many plasma membranes, would have a natural curvature. [Adapted from H. Sprong et al., 2001, *Nature Rev. Mol. Cell Biol.* **2**:504.]

lated (inserted) among phospholipids. Cholesterol restricts the random movement of phospholipid head groups at the outer surfaces of the leaflets, but its effect on the movement of long phospholipid tails depends on concentration. At the usual cholesterol concentrations, the interaction of the steroid ring with the long hydrophobic tails of phospholipids tends to immobilize these lipids and thus decrease biomembrane fluidity. At lower cholesterol concentrations, however, the steroid ring separates and disperses phospholipid tails, causing the inner regions of the membrane to become slightly more fluid.

The lipid composition of a bilayer also influences its thickness, which in turn may play a role in localizing proteins to a particular membrane. The results of studies on artificial membranes demonstrate that sphingomyelin associates into a

more gel-like and thicker bilayer than phospholipids do (Figure 5-8a). Similarly, cholesterol and other molecules that decrease membrane fluidity increase membrane thickness. Because sphingomyelin tails are already optimally stabilized, the addition of cholesterol has no effect on the thickness of a sphingomyelin bilayer.

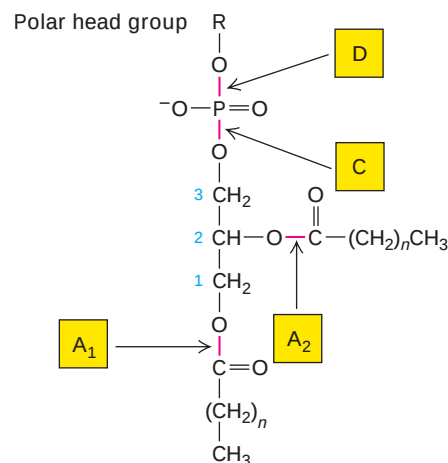
Another property dependent on the lipid composition of a bilayer is its local curvature, which depends on the relative sizes of the polar head groups and nonpolar tails of its constituent phospholipids. Lipids with long tails and large head groups are cylindrical in shape; those with small head groups are cone shaped (Figure 5-8b). As a result, bilayers composed of cylindrical lipids are relatively flat, whereas those containing large amounts of cone-shaped lipids form curved bilayers (Figure 5-8c). This effect of lipid composition on bilayer curvature may play a role in the formation of highly curved membrane pits and blebs, internal membrane vesicles, and specialized membrane structures such as microvilli.

Membrane Lipids Are Usually Distributed Unequally in the Exoplasmic and Cytosolic Leaflets

A characteristic of all membranes is an asymmetry in lipid composition across the bilayer. Although most phospholipids are present in both membrane leaflets, they are commonly more abundant in one or the other leaflet. For instance, in plasma membranes from human erythrocytes and certain canine kidney cells grown in culture, almost all the sphingomyelin and phosphatidylcholine, both of which form less fluid bilayers, are found in the exoplasmic leaflet. In contrast, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, which form more fluid bilayers, are preferentially located in the cytosolic leaflet. This segregation of lipids across the bilayer may influence membrane curvature (see Figure 5-8c). Unlike phospholipids, cholesterol is relatively evenly distributed in both leaflets of cellular membranes.

The relative abundance of a particular phospholipid in the two leaflets of a plasma membrane can be determined on the basis of its susceptibility to hydrolysis by **phospholipases**, enzymes that cleave various bonds in the hydrophilic ends of phospholipids (Figure 5-9). Phospholipids in the cytosolic leaflet are resistant to hydrolysis by phospholipases added to the external medium because the enzymes cannot penetrate to the cytosolic face of the plasma membrane.

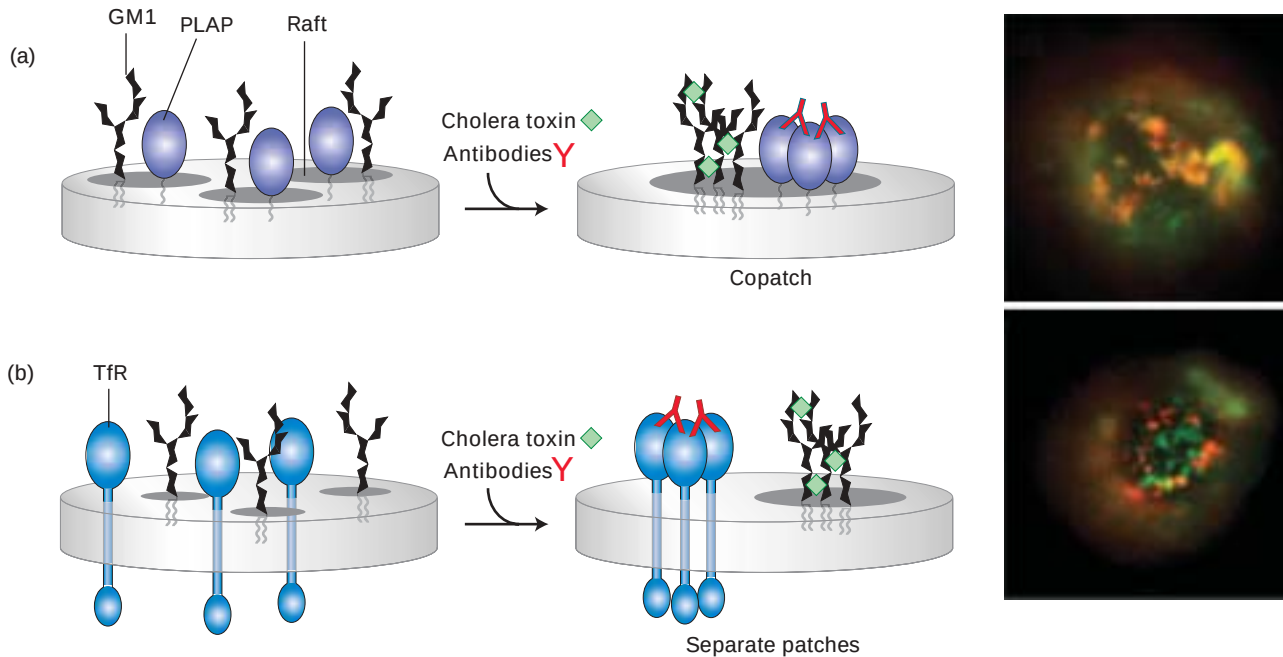
How the asymmetric distribution of phospholipids in membrane leaflets arises is still unclear. In pure bilayers, phospholipids do not spontaneously migrate, or flip-flop, from one leaflet to the other. Energetically, such flip-flopping is extremely unfavorable because it entails movement of the polar phospholipid head group through the hydrophobic interior of the membrane. To a first approximation, the asym-



▲ **FIGURE 5-9 Specificity of phospholipases.** Each type of phospholipase cleaves one of the susceptible bonds shown in red. The glycerol carbon atoms are indicated by small numbers. In intact cells, only phospholipids in the exoplasmic leaflet of the plasma membrane are cleaved by phospholipases in the surrounding medium. Phospholipase C, a cytosolic enzyme, cleaves certain phospholipids in the cytosolic leaflet of the plasma membrane.

metry in phospholipid distribution results from the vectorial synthesis of lipids in the endoplasmic reticulum and Golgi. Sphingomyelin is synthesized on the luminal (exoplasmic) face of the Golgi, which becomes the exoplasmic face of the plasma membrane. In contrast, phosphoglycerides are synthesized on the cytosolic face of the ER membrane, which is topologically identical with the cytosolic face of the plasma membrane (see Figure 5-4). Clearly, this explanation does not account for the preferential location of phosphatidylcholine in the exoplasmic leaflet. Movement of this phosphoglyceride and perhaps others from one leaflet to the other in some natural membranes is catalyzed by certain ATP-powered transport proteins called **flippases** discussed in Chapters 7 and 18.

The preferential location of lipids to one face of the bilayer is necessary for a variety of membrane-based functions. For example, the head groups of all phosphorylated forms of phosphatidylinositol face the cytosol. Certain of them are cleaved by phospholipase C located in the cytosol; this enzyme in turn is activated as a result of cell stimulation by many hormones. These cleavages generate cytosol-soluble phosphoinositols and membrane-soluble diacylglycerol. As we see in later chapters, these molecules participate in intracellular signaling pathways that affect many aspects of cellular metabolism. Phosphatidylserine also is normally most abundant in the cytosolic leaflet of the plasma membrane. In the initial stages of platelet stimulation by serum, phosphatidylserine is briefly translocated to the exoplasmic face, presumably by a flippase enzyme, where it activates enzymes participating in blood clotting.



▲ **EXPERIMENTAL FIGURE 5-10 Some membrane lipids and proteins colocalize in lipid rafts.** The results of biochemical studies suggested that GM1, a glycosphingolipid, and placental alkaline phosphatase (PLAP), a lipid-anchored membrane protein, aggregate together into lipid rafts, whereas the transferrin receptor (Tfr), which traverses the entire membrane, does not. To locate these components in the intact plasma membrane, cells were treated with fluorescence-labeled cholera toxin (green), which cross-links closely spaced GM1 molecules, and with fluorescence-labeled antibodies (red) specific for either PLAP or Tfr. Each antibody can cross-link closely spaced

molecules of the protein that it recognizes. Cross-linking causes the proteins or lipids to form larger patches that can be detected by fluorescence microscopy (see Figure 5-42). (a) Micrograph of a cell treated with toxin and with anti-PLAP antibody shows GM1 and PLAP colocalized in the same patches (yellow). This copatching suggests that both GM1 and PLAP are present in lipid rafts that coalesce in the presence of the cross-linking reagents. (b) Micrograph of a cell treated with toxin and with anti-Tfr antibody shows that GM1 and Tfr reside in separate patches (i.e., red and green), indicating that Tfr is not a raft-resident protein. [Micrographs from T. Harder et al., 1998, *J. Cell Biol.* **141**:929.]

Cholesterol and Sphingolipids Cluster with Specific Proteins in Membrane Microdomains

The results of recent studies have challenged the long-held belief that lipids are randomly mixed in each leaflet of a bilayer. The first hint that lipids may be organized within the leaflets was the discovery that the residues remaining after the extraction of plasma membranes with detergents contain two lipids: cholesterol and sphingomyelin. Because these two lipids are found in more ordered, less fluid bilayers, researchers hypothesized that they form microdomains, termed **lipid rafts**, surrounded by other more fluid phospholipids that are easily extracted by detergents.

Biochemical and microscopic evidence supports the existence of lipid rafts in natural membranes. For instance, fluorescence microscopy reveals aggregates of lipids and raft-specific proteins in the membrane (Figure 5-10). The rafts are heterogeneous in size but are typically 50 nm in diameter. Rafts can be disrupted by methyl- β -cyclodextrin, which depletes the membrane of cholesterol, or by antibiotics, such as filipin, that sequester cholesterol; such findings indicate the importance of cholesterol in maintaining the

integrity of these rafts. Besides their enrichment by cholesterol and sphingolipids, lipid rafts are enriched for many types of cell-surface receptor proteins, as well as many signaling proteins that bind to the receptors and are activated by them. These lipid-protein complexes can form only in the two-dimensional environment of a hydrophobic bilayer and, as discussed in later chapters, they are thought to facilitate the detection of chemical signals from the external environment and the subsequent activation of cytosolic events.

KEY CONCEPTS OF SECTION 5.1

Biomembranes: Lipid Composition and Structural Organization

- The eukaryotic cell is demarcated from the external environment by the plasma membrane and organized into membrane-limited internal compartments (organelles and vesicles).
- The total surface area of internal membranes far exceeds that of the plasma membrane.

- The phospholipid bilayer, the basic structural unit of all biomembranes, is a two-dimensional lipid sheet with hydrophilic faces and a hydrophobic core, which is impermeable to water-soluble molecules and ions (see Figure 5-2).
- Certain proteins present in biomembranes make them selectively permeable to water-soluble molecules and ions.
- The primary lipid components of biomembranes are phosphoglycerides, sphingolipids, and steroids (see Figure 5-5).
- Most lipids and many proteins are laterally mobile in biomembranes.
- Different cellular membranes vary in lipid composition (see Table 5-1). Phospholipids and sphingolipids are asymmetrically distributed in the two leaflets of the bilayer, whereas cholesterol is fairly evenly distributed in both leaflets.
- Natural biomembranes generally have a fluidlike consistency. In general, membrane fluidity is decreased by sphingolipids and cholesterol and increased by phosphoglycerides. The lipid composition of a membrane also influences its thickness and curvature (see Figure 5-8).
- Lipid rafts are microdomains containing cholesterol, sphingolipids, and certain membrane proteins that form in the plane of the bilayer. These aggregates are sites for signaling across the plasma membrane.

5.2 Biomembranes: Protein Components and Basic Functions

Membrane proteins are defined by their location within or at the surface of a phospholipid bilayer. Although every biological membrane has the same basic bilayer structure, the proteins associated with a particular membrane are responsible for its distinctive activities. The density and complement of proteins associated with biomembranes vary, depending on cell type and subcellular location. For example, the inner mitochondrial membrane is 76 percent protein; the myelin membrane, only 18 percent. The high phospholipid content of myelin allows it to electrically insulate a nerve cell from its environment. The importance of membrane proteins is suggested from the finding that approximately a third of all yeast genes encode a membrane protein. The relative abundance of genes for membrane proteins is even greater in multicellular organisms in which membrane proteins have additional functions in cell adhesion.

The lipid bilayer presents a unique two-dimensional hydrophobic environment for membrane proteins. Some proteins are buried within the lipid-rich bilayer; other proteins are associated with the exoplasmic or cytosolic leaflet of the bilayer. Protein domains on the extracellular surface of the plasma membrane generally bind to other molecules, including external signaling proteins, ions, and small metabolites (e.g., glucose, fatty acids), and to adhesion molecules on

other cells or in the external environment. Domains within the plasma membrane, particularly those that form channels and pores, move molecules in and out of cells. Domains lying along the cytosolic face of the plasma membrane have a wide range of functions, from anchoring cytoskeletal proteins to the membrane to triggering intracellular signaling pathways.

In many cases, the function of a membrane protein and the topology of its polypeptide chain in the membrane can be predicted on the basis of its homology with another, well-characterized protein. In this section, we examine the characteristic structural features of membrane proteins and some of their basic functions. More complete characterization of the structure and function of various types of membrane proteins is presented in several later chapters; the synthesis and processing of this large, diverse group of proteins are discussed in Chapters 16 and 17.

Proteins Interact with Membranes in Three Different Ways

Membrane proteins can be classified into three categories—integral, lipid-anchored, and peripheral—on the basis of the nature of the membrane–protein interactions (Figure 5-11).

Integral membrane proteins, also called *transmembrane proteins*, span a phospholipid bilayer and are built of three segments. The cytosolic and exoplasmic domains have hydrophilic exterior surfaces that interact with the aqueous solutions on the cytosolic and exoplasmic faces of the membrane. These domains resemble other water-soluble proteins in their amino acid composition and structure. In contrast, the 3-nm-thick membrane-spanning domain contains many hydrophobic amino acids whose side chains protrude outward and interact with the hydrocarbon core of the phospholipid bilayer. In all transmembrane proteins examined to date, the membrane-spanning domains consist of one or more α helices or of multiple β strands. In addition, most transmembrane proteins are glycosylated with a complex branched sugar group attached to one or several amino acid side chains. Invariably these sugar chains are localized to the exoplasmic domains.

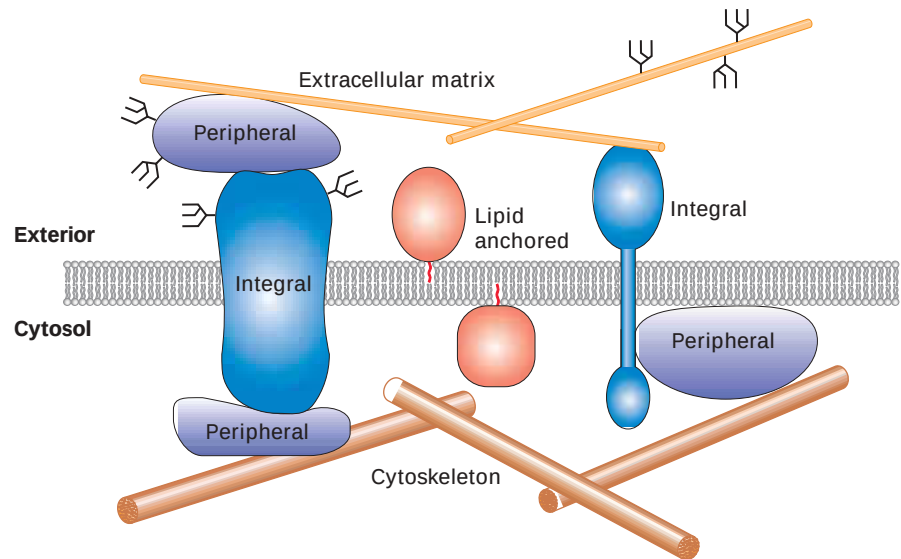
Lipid-anchored membrane proteins are bound covalently to one or more lipid molecules. The hydrophobic carbon chain of the attached lipid is embedded in one leaflet of the membrane and anchors the protein to the membrane. The polypeptide chain itself does not enter the phospholipid bilayer.

Peripheral membrane proteins do not interact with the hydrophobic core of the phospholipid bilayer. Instead they are usually bound to the membrane indirectly by interactions with integral membrane proteins or directly by interactions with lipid head groups. Peripheral proteins are localized to either the cytosolic or the exoplasmic face of the plasma membrane.

In addition to these proteins, which are closely associated with the bilayer, cytoskeletal filaments are more loosely associated with the cytosolic face, usually through one or more

► **FIGURE 5-11 Diagram of how various classes of proteins associate with the lipid bilayer.**

Integral (transmembrane) proteins span the bilayer. Lipid-anchored proteins are tethered to one leaflet by a long covalently attached hydrocarbon chain. Peripheral proteins associate with the membrane primarily by specific noncovalent interactions with integral proteins or membrane lipids. Farther from the membrane are membrane-associated proteins including the cytoskeleton, extracellular matrix in animal cells, and cell wall in plant and bacterial cells (not depicted). Carbohydrate chains are attached to many extracellular proteins and to the exoplasmic domains of many transmembrane proteins.



peripheral (adapter) proteins (see Figure 5-11). Such associations with the cytoskeleton provide support for various cellular membranes (see Section 5.4); they also play a role in the two-way communication between the cell interior and the cell exterior, as we learn in Chapter 6. Finally, peripheral proteins on the outer surface of the plasma membrane and the exoplasmic domains of integral membrane proteins are often attached to components of the **extracellular matrix** or to the **cell wall** surrounding bacterial and plant cells.

Membrane-Embedded α Helices Are the Primary Secondary Structures in Most Transmembrane Proteins

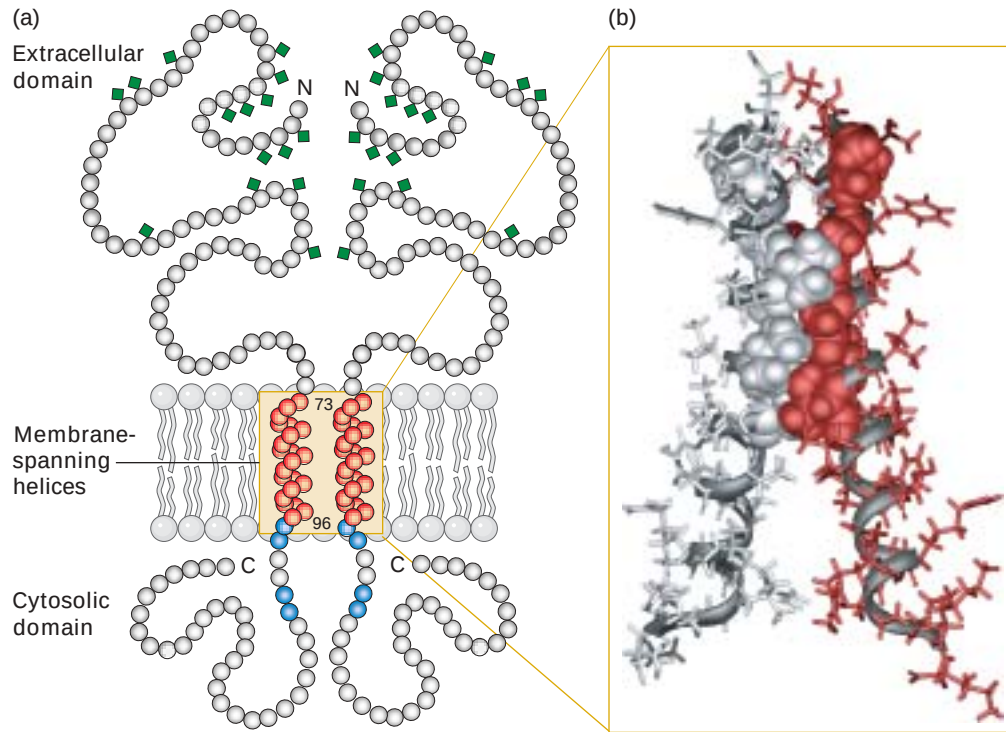
Soluble proteins exhibit hundreds of distinct localized folded structures, or motifs (see Figure 3-6). In comparison, the repertoire of folded structures in integral membrane proteins is quite limited, with the hydrophobic α helix predominating. Integral proteins containing membrane-spanning α -helical domains are embedded in membranes by hydrophobic interactions with specific lipids and probably also by ionic interactions with the polar head groups of the phospholipids.

Glycophorin A, the major protein in the erythrocyte plasma membrane, is a representative *single-pass* transmembrane protein, which contains only one membrane-spanning α helix (Figure 5-12). Typically, a membrane-embedded α helix is composed of 20–25 hydrophobic (uncharged) amino acids (see Figure 2-13). The predicted length of such a helix (3.75 nm) is just sufficient to span the hydrocarbon core of a phospholipid bilayer. The hydrophobic side chains protrude outward from the helix and form van der Waals interactions with the fatty acyl chains in the bilayer. In contrast, the carbonyl (C=O) and imino (NH) groups taking part in the formation of backbone peptide bonds through

hydrogen bonding are in the interior of the α helix (see Figure 3-3); thus these polar groups are shielded from the hydrophobic interior of the membrane. The transmembrane helix of one glycophorin A molecule associates with the helix in another to form a coiled-coil dimer (see Figure 5-12b). Such interaction of membrane-spanning helices is a common mechanism for creating dimeric membrane proteins. Many cell-surface receptors, for instance, are activated by dimerization.

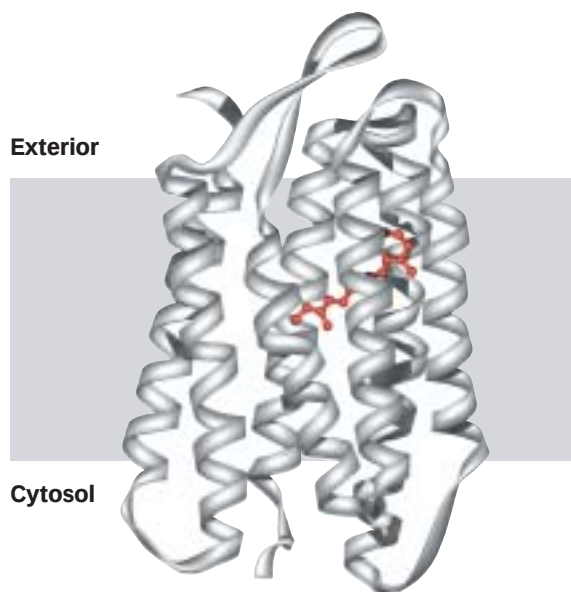
A large and important family of integral proteins is defined by the presence of seven membrane-spanning α helices. Among the more than 150 such “seven spanning” *multipass* proteins that have been identified are the G protein–coupled receptors described in Chapter 13. The structure of bacteriorhodopsin, a protein found in the membrane of certain photosynthetic bacteria, illustrates the general structure of all these proteins (Figure 5-13). Absorption of light by the retinal group covalently attached to bacteriorhodopsin causes a conformational change in the protein that results in the pumping of protons from the cytosol across the bacterial membrane to the extracellular space. The proton concentration gradient thus generated across the membrane is used to synthesize ATP (Chapter 8). In the high-resolution structure of bacteriorhodopsin now available, the positions of all the individual amino acids, retinal, and the surrounding lipids are determined. As might be expected, virtually all of the amino acids on the exterior of the membrane-spanning segments of bacteriorhodopsin are hydrophobic and interact with the hydrocarbon core of the surrounding lipid bilayer.

Ion channels compose a second large and important family of multipass transmembrane proteins. As revealed by the crystal structure of a resting K^+ channel, ion channels are typically tetrameric proteins. Each of the four subunits has a pair of membrane-spanning helices that bundle with helices



▲ **FIGURE 5-12 Structure of glycophorin A, a typical single-pass transmembrane protein.** (a) Diagram of dimeric glycophorin showing major sequence features and its relation to the membrane. The single 23-residue membrane-spanning α helix in each monomer is composed of amino acids with hydrophobic (uncharged) side chains (red spheres). By binding negatively charged phospholipid head groups, the positively charged arginine and lysine residues (blue spheres) near the cytosolic side of the helix help anchor glycophorin in the membrane. Both the extracellular and the cytosolic domains are rich in charged

residues and polar uncharged residues; the extracellular domain is heavily glycosylated, with the carbohydrate side chains (green diamonds) attached to specific serine, threonine, and asparagine residues. (b) Molecular model of the transmembrane domain of dimeric glycophorin corresponding to residues 73–96. The side chains of the α helix in one monomer are shown in red; those in the other monomer, in gray. Residues depicted as space-filling structures participate in intermonomer van der Waals interactions that stabilize the coiled-coil dimer. [Part (b) adapted from K. R. MacKenzie et al., 1997, *Science* **276**:131]



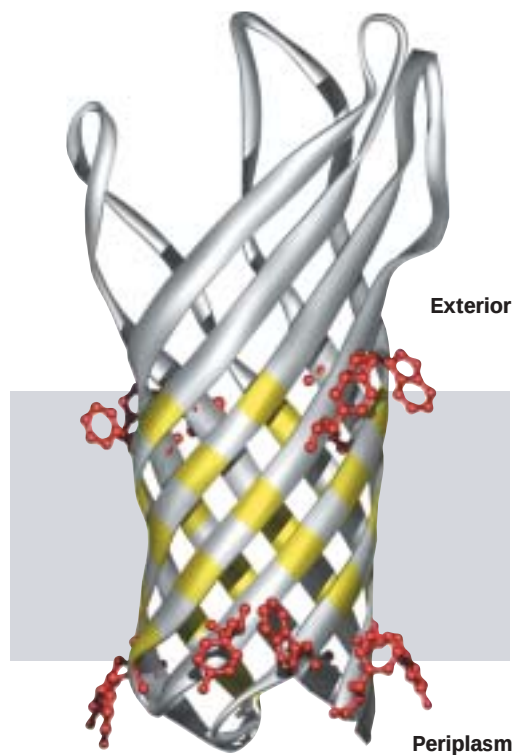
of other subunits, forming a central channel (see Figure 7-15). Polar and hydrophobic residues lining the center of the bundle form a channel in the membrane, but as with bacteriorhodopsin virtually all of the amino acids on the exterior of the membrane-spanning domain are hydrophobic. In many ion channels, external factors (e.g., a ligand, voltage, or mechanical strain) regulate ion flow across the bilayer by reorienting the helices. Details of ion channels and their structures are discussed in Chapter 7.

◀ **FIGURE 5-13 Structural model of bacteriorhodopsin, a multipass transmembrane protein that functions as a photoreceptor in certain bacteria.** The seven hydrophobic α helices in bacteriorhodopsin traverse the lipid bilayer. A retinal molecule (red) covalently attached to one helix absorbs light. The large class of G protein-coupled receptors in eukaryotic cells also has seven membrane-spanning α helices; their three-dimensional structure is similar to that of bacteriorhodopsin. [After H. Luecke et al., 1999, *J. Mol. Biol.* **291**:899.]

Multiple β Strands in Porins Form Membrane-Spanning “Barrels”

The **porins** are a class of transmembrane proteins whose structure differs radically from that of other integral proteins. Several types of porin are found in the outer membrane of gram-negative bacteria such as *E. coli* and in the outer membranes of mitochondria and chloroplasts. The outer membrane protects an intestinal bacterium from harmful agents (e.g., antibiotics, bile salts, and proteases) but permits the uptake and disposal of small hydrophilic molecules including nutrients and waste products. The porins in the outer membrane of an *E. coli* cell provide channels for the passage of disaccharides and other small molecules as well as phosphate.

The amino acid sequences of porins are predominantly polar and contain no long hydrophobic segments typical of integral proteins with α -helical membrane-spanning domains. X-ray crystallography has revealed that porins are trimers of identical subunits. In each subunit, 16 β strands form a barrel-shaped structure with a pore in the center (Fig-



▲ **FIGURE 5-14 Structural model of one subunit of OmpX, a porin found in the *E. coli* outer membrane.** All porins are trimeric transmembrane proteins. Each subunit is barrel shaped, with β strands forming the wall and a transmembrane pore in the center. A band of aliphatic (noncyclic) side chains (yellow) and a border of aromatic (ring-containing) side chains (red) position the protein in the bilayer. [After G. E. Schulz, 2000, *Curr. Opin. Struc. Biol.* 10:443.]

ure 5-14). Unlike a typical water-soluble globular protein, a porin has a hydrophilic inside and a hydrophobic exterior; in this sense, porins are inside-out. In a porin monomer, the outward-facing side groups on each of the β strands are hydrophobic and form a nonpolar ribbonlike band that encircles the outside of the barrel. This hydrophobic band interacts with the fatty acyl groups of the membrane lipids or with other porin monomers. The side groups facing the inside of a porin monomer are predominantly hydrophilic; they line the pore through which small water-soluble molecules cross the membrane.

As discussed in Chapter 7, the plasma membranes of animal cells contain a water channel called *aquaporin*. Like most other integral proteins, aquaporin contains multiple transmembrane α helices. Thus, despite its name, aquaporin differs structurally from the porins as well as functionally in that it mediates transport of a single molecule—namely, water.

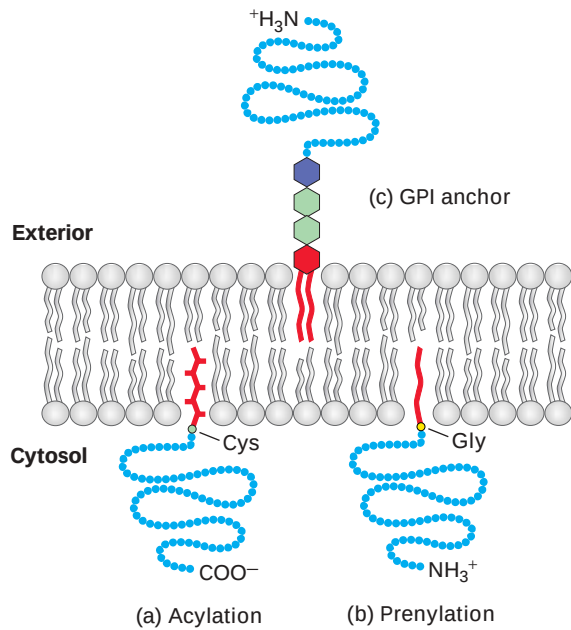
Covalently Attached Hydrocarbon Chains Anchor Some Proteins to Membranes

In eukaryotic cells, several types of covalently attached lipids anchor some proteins to one or the other leaflet of the plasma membrane and certain other cellular membranes. In these lipid-anchored proteins, the lipid hydrocarbon chains are embedded in the bilayer, but the protein itself does not enter the bilayer.

A group of cytosolic proteins are anchored to the cytosolic face of a membrane by a fatty acyl group (e.g., myristate or palmitate) attached to the N-terminal glycine residue (Figure 5-15a). Retention of such proteins at the membrane by the N-terminal acyl anchor may play an important role in a membrane-associated function. For example, v-Src, a mutant form of a cellular tyrosine kinase, is oncogenic and can transform cells only when it has a myristylated N-terminus.

A second group of cytosolic proteins are anchored to membranes by an unsaturated fatty acyl group attached to a cysteine residue at or near the C-terminus (Figure 5-15b). In these proteins, a farnesyl or geranylgeranyl group is bound through a thioether bond to the —SH group of a C-terminal cysteine residue. These *prenyl anchors* are built from isoprene units (C_5), which are also used in the synthesis of cholesterol (Chapter 18). In some cases, a second geranylgeranyl group or a palmitate group is linked to a nearby cysteine residue. The additional anchor is thought to reinforce the attachment of the protein to the membrane. Ras, a GTPase superfamily protein that functions in intracellular signaling, is localized to the cytosolic face of the plasma membrane by such a double anchor. Rab proteins, which also belong to the GTPase superfamily, are similarly bound to the cytosolic surface of intracellular vesicles by prenyl-type anchors; these proteins are required for the fusion of vesicles with their target membranes (Chapter 17).

Some cell-surface proteins and heavily glycosylated proteoglycans of the extracellular matrix are bound to the exo-



▲ **FIGURE 5-15 Anchoring of plasma-membrane proteins to the bilayer by covalently linked hydrocarbon groups.**

(a) Cytosolic proteins such as v-Src are associated with the plasma membrane through a single fatty acyl chain attached to the N-terminal glycine (Gly) residue of the polypeptide. Myristate (C_{14}) and palmitate (C_{16}) are common acyl anchors. (b) Other cytosolic proteins (e.g., Ras and Rab proteins) are anchored to the membrane by prenylation of one or two cysteine (Cys) residues, at or near the C-terminus. The anchors are farnesyl (C_{15}) and geranylgeranyl (C_{20}) groups, both of which are unsaturated. (c) The lipid anchor on the exoplasmic surface of the plasma membrane is glycosylphosphatidylinositol (GPI). The phosphatidylinositol part (red) of this anchor contains two fatty acyl chains that extend into the bilayer. The phosphoethanolamine unit (purple) in the anchor links it to the protein. The two green hexagons represent sugar units, which vary in number and arrangement in different GPI anchors. The complete structure of a yeast GPI anchor is shown in Figure 16-14. [Adapted from H. Sprong et al., 2001, *Nature Rev. Mol. Cell Biol.* 2:504.]

plasmic face of the plasma membrane by a third type of anchor group, glycosylphosphatidylinositol (GPI). The exact structures of *GPI anchors* vary greatly in different cell types, but they always contain phosphatidylinositol (PI), whose two fatty acyl chains extend into the lipid bilayer; phosphoethanolamine, which covalently links the anchor to the C-terminus of a protein; and several sugar residues (Figure 5-15c). Various experiments have shown that the GPI anchor is both necessary and sufficient for binding proteins to the membrane. For instance, the enzyme phospholipase C cleaves the phosphate–glycerol bond in phospholipids and in GPI anchors (see Figure 5-9). Treatment of cells with phospholipase C releases GPI-anchored proteins such as Thy-1 and placental alkaline phosphatase (PLAP) from the cell surface.

As already discussed, PLAP is concentrated in lipid rafts, the more ordered bilayer microdomains that are enriched in sphingolipids and cholesterol (see Figure 5-10). Although PLAP and other GPI-anchored proteins lie in the opposite membrane leaflet from acyl-anchored proteins, both types of membrane proteins are concentrated in lipid rafts. In contrast, prenylated proteins are not found in lipid rafts.

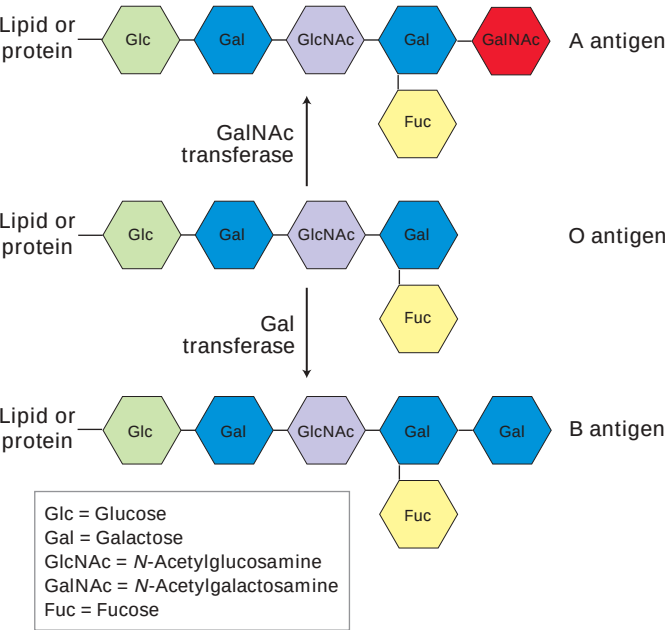
All Transmembrane Proteins and Glycolipids Are Asymmetrically Oriented in the Bilayer

Lipid-anchored proteins are just one example of membrane proteins that are asymmetrically located with respect to the faces of cellular membranes. Each type of transmembrane protein also has a specific orientation with respect to the membrane faces. In particular, the same part(s) of a particular protein always faces the cytosol, whereas other parts face the exoplasmic space. This asymmetry in protein orientation confers different properties on the two membrane faces. (We describe how the orientation of different types of transmembrane proteins is established during their synthesis in Chapter 16.) Membrane proteins have never been observed to flip-flop across a membrane; such movement, requiring a transient movement of hydrophilic amino acid residues through the hydrophobic interior of the membrane, would be energetically unfavorable. Accordingly, the asymmetry of a transmembrane protein, which is established during its biosynthesis and insertion into a membrane, is maintained throughout the protein's lifetime.

Many transmembrane proteins contain carbohydrate chains covalently linked to serine, threonine, or asparagine side chains of the polypeptide. Such transmembrane glycoproteins are always oriented so that the carbohydrate chains are in the exoplasmic domain (see Figures 5-11 and 5-12). Likewise, glycolipids, in which a carbohydrate chain is attached to the glycerol or sphingosine backbone, are always located in the exoplasmic leaflet with the carbohydrate chain protruding from the membrane surface. Both glycoproteins and glycolipids are especially abundant in the plasma membranes of eukaryotic cells; they are absent from the inner mitochondrial membrane, chloroplast lamellae, and several other intracellular membranes. Because the carbohydrate chains of glycoproteins and glycolipids in the plasma membrane extend into the extracellular space, they are available to interact with components of the extracellular matrix as well as lectins, growth factors, and antibodies.



One important consequence of such interactions is illustrated by the A, B, and O blood-group antigens. These three structurally related oligosaccharide components of certain glycoproteins and glycolipids are expressed on the surfaces of human erythrocytes and many other cell types (Figure 5-16). All humans have the enzymes for synthesizing O antigen. Persons with type A blood also have a glycosyltransferase that adds an extra



▲ FIGURE 5-16 Human ABO blood-group antigens. These antigens are oligosaccharide chains covalently attached to glycolipids or glycoproteins in the plasma membrane. The terminal oligosaccharide sugars distinguish the three antigens. The presence or absence of the glycosyltransferases that add galactose (Gal) or N-acetylgalactosamine (GalNAc) to O antigen determine a person's blood type.

N-acetylgalactosamine to O antigen to form A antigen. Those with type B blood have a different transferase that adds an extra galactose to O antigen to form B antigen. People with both transferases produce both A and B antigen (AB blood type); those who lack these transferases produce O antigen only (O blood type).

Persons whose erythrocytes lack the A antigen, B antigen, or both on their surface normally have antibodies against the missing antigen(s) in their serum. Thus if a type

A or O person receives a transfusion of type B blood, antibodies against the B epitope will bind to the introduced red cells and trigger their destruction. To prevent such harmful reactions, blood-group typing and appropriate matching of blood donors and recipients are required in all transfusions (Table 5-2). ■

Interactions with the Cytoskeleton Impede the Mobility of Integral Membrane Proteins

The results of experiments like the one depicted in Figure 5-6 and other types of studies have shown that many transmembrane proteins and lipid-anchored proteins, like phospholipids, float quite freely within the plane of a natural membrane. From 30 to 90 percent of all integral proteins in the plasma membrane are freely mobile, depending on the cell type. The lateral diffusion rate of a mobile protein in a pure phospholipid bilayer or isolated plasma membrane is similar to that of lipids. However, the diffusion rate of a protein in the plasma membrane of intact cells is generally 10–30 times lower than that of the same protein embedded in synthetic spherical bilayer structures (liposomes). These findings suggest that the mobility of integral proteins in the plasma membrane of living cells is restricted by interactions with the rigid submembrane cytoskeleton. Some integral proteins are permanently linked to the underlying cytoskeleton; these proteins are completely immobile in the membrane. In regard to mobile proteins, such interactions are broken and remade as the proteins diffuse laterally in the plasma membrane, slowing down their rate of diffusion. We consider the nature and functional consequences of linkages between integral membrane proteins and the cytoskeleton in Chapter 6.

Lipid-Binding Motifs Help Target Peripheral Proteins to the Membrane

Until the past decade or so, the interaction of peripheral proteins with integral proteins was thought to be the major

TABLE 5-2 ABO Blood Groups			
Blood-Group Type	Antigens on RBCs*	Serum Antibodies	Can Receive Blood Types
A	A	Anti-A	A and O
B	B	Anti-B	B and O
AB	A and B	None	All
O	O	Anti-A and anti-B	O

*See Figure 5-16 for antigen structures.

TABLE 5-3 Selected Lipid-Binding Motifs

Motif	Ligand*	Selected Proteins with Motif
PH	PIP ₂ , PIP ₃	Phospholipase C _γ 1, protein kinase B, pleckstrin
C2	Acidic phospholipids	Protein kinase C, PI-3 kinase, phospholipase, PTEN phosphatase
Ankyrin repeat	PS	Ankyrin [†]
FERM	PIP ₂	Band 4.1 protein; ezrin, radixin, moesin (ERM) [†]

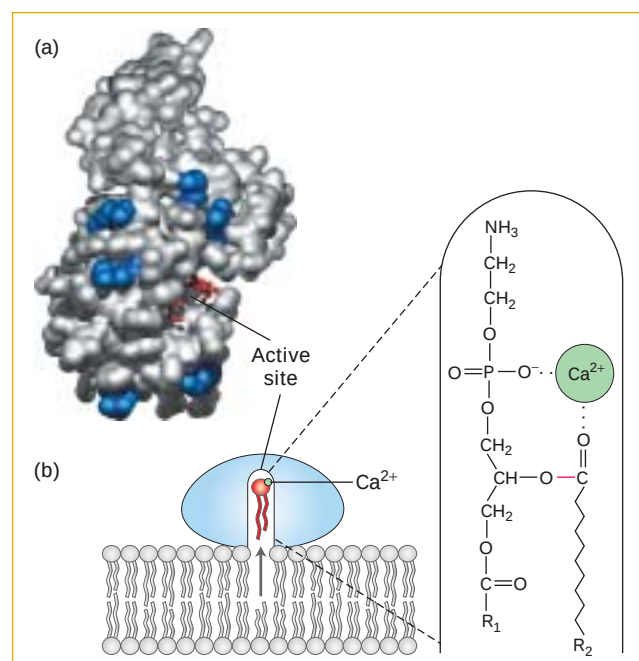
* PIP₂, PIP₃, and PI-3P = phosphatidylinositol derivatives with additional phosphate groups on the inositol ring (see Figure 14-26); PH = pleckstrin homology; PS = phosphatidylserine.

[†] These proteins have roles in linking the actin cytoskeleton to the plasma membrane.

mechanism by which peripheral proteins were bound to membranes. The results of more recent research indicate that protein–lipid interactions are equally important in localizing peripheral proteins to cellular membranes (see Figure 5-11).

Analyses of genome sequences have revealed several widely distributed lipid-binding motifs in proteins (Table 5-3). For instance, the *pleckstrin homology (PH) domain*, which binds two types of phosphorylated phosphatidylinositols, is the eleventh most common protein domain encoded in the human genome. This domain was initially recognized in pleckstrin, a protein found in platelets. The high frequency of the PH domain indicates that proteins localized to membrane surfaces carry out many important functions. Other common lipid-binding motifs include the C2 domain, the ankyrin-repeat domain, and the FERM domain. Originally discovered in protein kinase C, the C2 domain is a membrane-targeting domain for various kinases, phosphatases, and phospholipases.

The phospholipases are representative of those water-soluble enzymes that associate with the polar head groups of membrane phospholipids to carry out their catalytic functions. As noted earlier, phospholipases hydrolyze various bonds in the head groups of phospholipids (see Figure 5-9). These enzymes have an important role in the degradation of damaged or aged cell membranes and are active molecules in many snake venoms. The mechanism of action of phospholipase A₂ illustrates how such water-soluble enzymes can reversibly interact with membranes and catalyze reactions at the interface of an aqueous solution and lipid surface. When this enzyme is in aqueous solution, its Ca²⁺-containing active site is buried in a channel lined with hydrophobic amino acids. The enzyme binds with greatest affinity to bilayers composed of negatively charged phospholipids (e.g., phosphatidylethanolamine). This finding suggests that a rim of positively charged lysine and arginine residues around the entrance catalytic channel is particularly important in interfacial binding (Figure 5-17a). Binding



▲ FIGURE 5-17 Interfacial binding surface and mechanism of action of phospholipase A₂. (a) A structural model of the enzyme showing the surface that interacts with a membrane.

This interfacial binding surface contains a rim of positively charged arginine and lysine residues shown in blue surrounding the cavity of the catalytic active site in which a substrate lipid (red stick structure) is bound. (b) Diagram of catalysis by phospholipase A₂. When docked on a model lipid membrane, positively charged residues of the interfacial binding site bind to negatively charged polar groups at the membrane surface. This binding triggers a small conformational change, opening a channel lined with hydrophobic amino acids that leads from the bilayer to the catalytic site. As a phospholipid moves into the channel, an enzyme-bound Ca²⁺ ion (green) binds to the head group, positioning the ester bond to be cleaved next to the catalytic site. [Part (a) adapted from M. H. Gelb et al., 1999, *Curr. Opin. Struc. Biol.* **9**:428. Part (b), see D. Blow, 1991, *Nature* **351**:444.]

induces a small conformational change in phospholipase A₂ that fixes the protein to the phospholipid heads and opens the hydrophobic channel. As a phospholipid molecule diffuses from the bilayer into the channel, the enzyme-bound Ca²⁺ binds to the phosphate in the head group, thereby positioning the ester bond to be cleaved next to the catalytic site (Figure 5-17b).

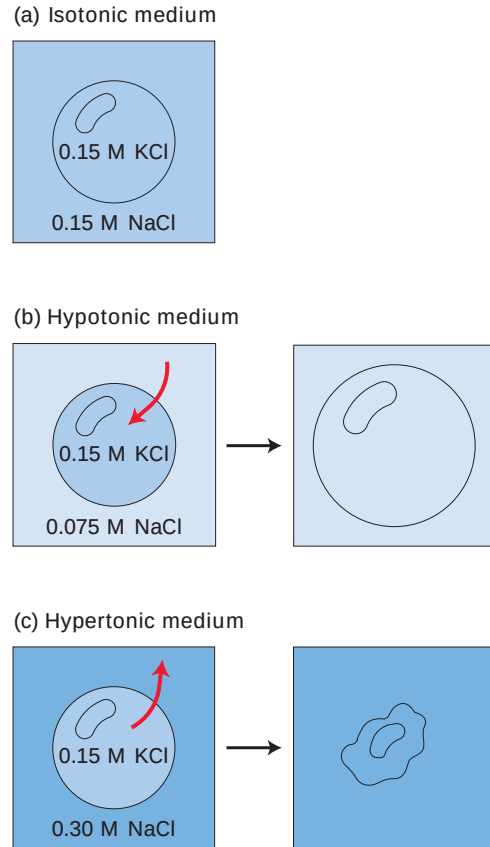
The Plasma Membrane Has Many Common Functions in All Cells

Although the lipid composition of a membrane largely determines its physical characteristics, its complement of proteins is primarily responsible for a membrane's functional properties. We have alluded to many functions of the plasma membrane in the preceding discussion and briefly consider its major functions here.

In all cells, the plasma membrane acts as a permeability barrier that prevents the entry of unwanted materials from the extracellular milieu and the exit of needed metabolites. Specific **membrane transport proteins** in the plasma membrane permit the passage of nutrients into the cell and metabolic wastes out of it; others function to maintain the proper ionic composition and pH (≈ 7.2) of the cytosol. The structure and function of proteins that make the plasma membrane selectively permeable to different molecules are discussed in Chapter 7.

The plasma membrane is highly permeable to water but poorly permeable to salts and small molecules such as sugars and amino acids. Owing to **osmosis**, water moves across such a semipermeable membrane from a solution of low solute (high water) concentration to one of high solute (low water) concentration until the total solute concentrations and thus the water concentrations on both sides are equal. Figure 5-18 illustrates the effect on animal cells of different external ion concentrations. When most animal cells are placed in an **isotonic** solution (i.e., one with total concentration of solutes equal to that of the cell interior), there is no net movement of water into or out of cells. However, when cells are placed in a **hypotonic** solution (i.e., one with a lower solute concentration than that of the cell interior), water flows into the cells, causing them to swell. Conversely, in a **hypertonic** solution (i.e., one with a higher solute concentration than that of the cell interior), water flows out of cells, causing them to shrink. Under normal *in vivo* conditions, ion channels in the plasma membrane control the movement of ions into and out of cells so that there is no net movement of water and the usual cell volume is maintained.

Unlike animal cells, bacterial, fungal, and plant cells are surrounded by a rigid cell wall and lack the extracellular matrix found in animal tissues. The plasma membrane is intimately engaged in the assembly of cell walls, which in plants are built primarily of **cellulose**. The cell wall prevents the swelling or shrinking of a cell that would otherwise occur when it is placed in a hypotonic or hyper-



▲ FIGURE 5-18 Effect of external ion concentration on water flow across the plasma membrane of an animal cell.

Sodium, potassium, and chloride ions do not move freely across the plasma membrane, but water channels (aquaporins) in the membrane permit the flow of water in the direction dictated by the ion concentration of the surrounding medium. (a) When the medium is isotonic, there is no net flux of water into or out of the cell. (b) When the medium is hypotonic, water flows into the cell (red arrow) until the ion concentration inside and outside the cell is the same. Because of the influx of water, the cell volume increases. (c) When the medium is hypertonic, water flows out of the cell until the ion concentration inside and outside the cell is the same. Because water is lost, the cell volume decreases.

tonic medium, respectively. For this reason, cells surrounded by a wall can grow in media having an osmotic strength much less than that of the cytosol. The properties, function, and formation of the plant cell wall are covered in Chapter 6.

In addition to these universal functions, the plasma membrane has other crucial roles in multicellular organisms. Few of the cells in multicellular plants and animals exist as isolated entities; rather, groups of cells with related specializations combine to form tissues. In animal cells, specialized areas of the plasma membrane contain proteins and glycolipids that form specific junctions between cells to strengthen tissues and to allow the exchange of metabolites

between cells. Certain plasma-membrane proteins anchor cells to components of the extracellular matrix, the mixture of fibrous proteins and polysaccharides that provides a bedding on which most sheets of epithelial cells or small glands lie. We examine both of these membrane functions in Chapter 6. Still other proteins in the plasma membrane act as anchoring points for many of the cytoskeletal fibers that permeate the cytosol, imparting shape and strength to cells (see Section 5.4).

The plasma membranes of many types of eukaryotic cells also contain receptor proteins that bind specific signaling molecules (e.g., hormones, growth factors, neurotransmitters), leading to various cellular responses. These proteins, which are critical for cell development and functioning, are described in several later chapters. Finally, peripheral cytosolic proteins that are recruited to the membrane surface function as enzymes, intracellular signal transducers, and structural proteins for stabilizing the membrane.

Like the plasma membrane, the membrane surrounding each organelle in eukaryotic cells contains a unique set of proteins essential for its proper functioning. In the next section, we provide a brief overview of the main eukaryotic organelles.

KEY CONCEPTS OF SECTION 5.2

Biomembranes: Protein Components and Basic Functions

- Biological membranes usually contain both integral (transmembrane) and peripheral membrane proteins, which do not enter the hydrophobic core of the bilayer (see Figure 5-11).
- Most integral membrane proteins contain one or more membrane-spanning hydrophobic α helices and hydrophilic domains that extend from the cytosolic and extracellular faces of the membrane (see Figure 5-12).
- The porins, unlike other integral proteins, contain membrane-spanning β sheets that form a barrel-like channel through the bilayer.
- Long-chain lipids attached to certain amino acids anchor some proteins to one or the other membrane leaflet (see Figure 5-15).
- Some peripheral proteins associate with the membrane by interactions with integral proteins. Lipid-binding motifs in other peripheral proteins interact with the polar head groups of membrane phospholipids (see Table 5-3).
- The binding of a water-soluble enzyme (e.g., a phospholipase, kinase, or phosphatase) to a membrane surface brings the enzyme close to its substrate and in some cases activates it. Such interfacial binding is due to the attraction between positive charges on basic residues in the protein and negative charges on phospholipid head groups in the bilayer.

5.3 Organelles of the Eukaryotic Cell

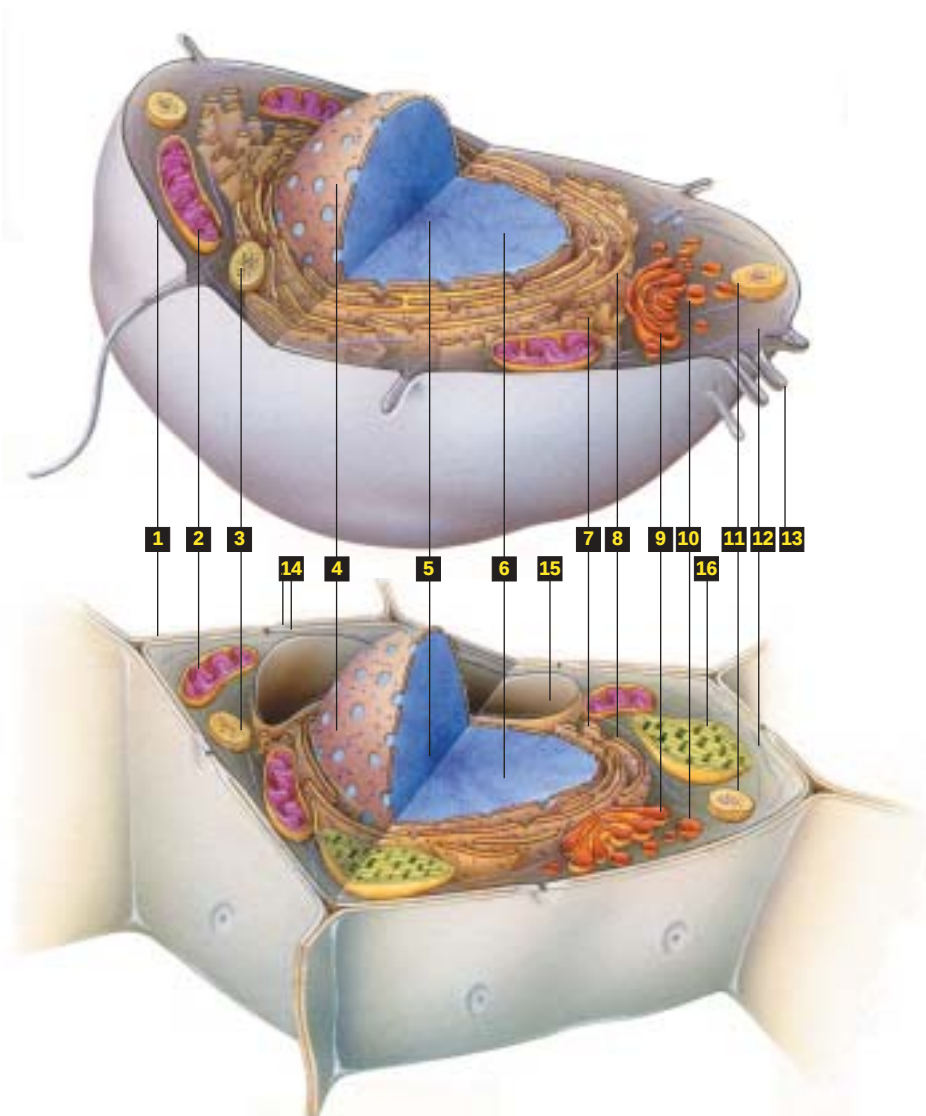
The cell is in a dynamic flux. In the light microscope, a live cell exhibits myriad movements ranging from the translocation of chromosomes and vesicles to the changes in shape associated with cell crawling and swimming. Investigation of intracellular structures begins with micrographs of fixed, sectioned cells in which all cell movements are frozen. Such static pictures of the cell reveal the organization of the cytoplasm into compartments and the stereotypic location of each type of organelle within the cell. In this section, we describe the basic structures and functions of the major organelles in animal and plant cells (Figure 5-19). Plant and fungal cells contain most of the organelles found in an animal cell but lack lysosomes. Instead, they contain a large central vacuole that subserves many of the functions of a lysosome. A plant cell also contains chloroplasts, and its membrane is strengthened by a rigid cell wall. Unique proteins in the interior and membranes of each type of organelle largely determine its specific functional characteristics, which are examined in more detail in later chapters. Those organelles bounded by a single membrane are covered first, followed by the three types that have a double membrane—the nucleus, mitochondrion, and chloroplast.

Endosomes Take Up Soluble Macromolecules from the Cell Exterior

Although transport proteins in the plasma membrane mediate the movement of ions and small molecules across the lipid bilayer, proteins and some other soluble macromolecules in the extracellular milieu are internalized by **endocytosis**. In this process, a segment of the plasma membrane invaginates into a “coated pit,” whose cytosolic face is lined by a specific set of proteins including **clathrin**. The pit pinches from the membrane into a small membrane-bounded vesicle that contains extracellular material and is delivered to an early **endosome**, a sorting station of membrane-limited tubules and vesicles (Figure 5-20a, b). From this compartment, some membrane proteins are recycled back to the plasma membrane; other membrane proteins are transported to a late endosome where further sorting takes place. The endocytic pathway ends when a late endosome delivers its membrane and internal contents to lysosomes for degradation. The entire endocytic pathway is described in some detail in Chapter 17.

Lysosomes Are Acidic Organelles That Contain a Battery of Degradative Enzymes

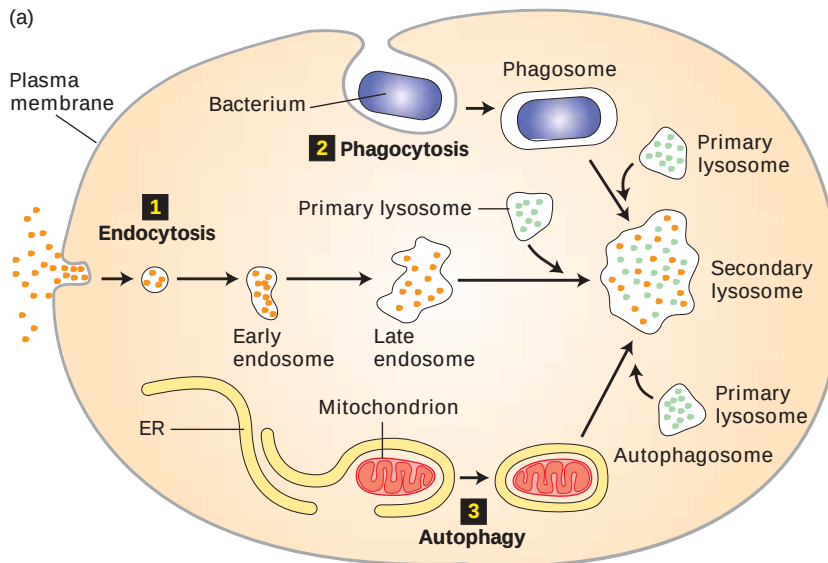
Lysosomes provide an excellent example of the ability of intracellular membranes to form closed compartments in which the composition of the lumen (the aqueous interior of the compartment) differs substantially from that of the surrounding cytosol. Found exclusively in animal cells,



- 1** Plasma membrane controls movement of molecules in and out of the cell and functions in cell-cell signaling and cell adhesion.
- 2** Mitochondria, which are surrounded by a double membrane, generate ATP by oxidation of glucose and fatty acids.
- 3** Lysosomes, which have an acidic lumen, degrade material internalized by the cell and worn-out cellular membranes and organelles.
- 4** Nuclear envelope, a double membrane, encloses the contents of the nucleus; the outer nuclear membrane is continuous with the rough ER.
- 5** Nucleolus is a nuclear subcompartment where most of the cell's rRNA is synthesized.
- 6** Nucleus is filled with chromatin composed of DNA and proteins; in dividing cells is site of mRNA and tRNA synthesis.
- 7** Smooth endoplasmic reticulum (ER) synthesizes lipids and detoxifies certain hydrophobic compounds.
- 8** Rough endoplasmic reticulum (ER) functions in the synthesis, processing, and sorting of secreted proteins, lysosomal proteins, and certain membrane.
- 9** Golgi complex processes and sorts secreted proteins, lysosomal proteins, and membrane proteins synthesized on the rough ER.
- 10** Secretory vesicles store secreted proteins and fuse with the plasma membrane to release their contents.
- 11** Peroxisomes detoxify various molecules and also break down fatty acids to produce acetyl groups for biosynthesis.
- 12** Cytoskeletal fibers form networks and bundles that support cellular membranes, help organize organelles, and participate in cell movement.
- 13** Microvilli increase surface area for absorption of nutrients from surrounding medium.
- 14** Cell wall, composed largely of cellulose, helps maintain the cell's shape and provides protection against mechanical stress.
- 15** Vacuole stores water, ions, and nutrients, degrades macromolecules, and functions in cell elongation during growth.
- 16** Chloroplasts, which carry out photosynthesis, are surrounded by a double membrane and contain a network of internal membrane-bounded sacs.

▲ **FIGURE 5-19 Schematic overview of a “typical” animal cell and plant cell and their major substructures.** Not every cell will contain all the organelles, granules, and fibrous

structures shown here, and other substructures can be present in some. Cells also differ considerably in shape and in the prominence of various organelles and substructures.



▲ **FIGURE 5-20 Cellular structures that participate in delivering materials to lysosomes.** (a) Schematic overview of three pathways by which materials are moved to lysosomes. Soluble macromolecules are taken into the cell by invagination of coated pits in the plasma membrane and delivered to lysosomes through the endocytic pathway (**1**). Whole cells and other large, insoluble particles move from the cell surface to lysosomes through the phagocytic pathway (**2**). Worn-out organelles and bulk cytoplasm are delivered to lysosomes through the autophagic pathway (**3**). Within the acidic lumen of lysosomes,

hydrolytic enzymes degrade proteins, nucleic acids, and other large molecules. (b) An electron micrograph of a section of a cultured mammalian cell that had taken up small gold particles coated with the egg protein ovalbumin. Gold-labeled ovalbumin (black spots) is found in early endosomes (EE) and late endosomes (LE), but very little is present in autophagosomes (AV). (c) Electron micrograph of a section of a rat liver cell showing a secondary lysosome containing fragments of a mitochondrion (M) and a peroxisome (P). [Part (b) from T. E. Tjelle et al., 1996, *J. Cell Sci.* **109**:2905. Part (c) courtesy of D. Friend.]

lysosomes are responsible for degrading certain components that have become obsolete for the cell or organism. The process by which an aged organelle is degraded in a lysosome is called *autophagy* (“eating oneself”). Materials taken into a cell by endocytosis or **phagocytosis** also may be degraded in lysosomes (see Figure 5-20a). In phagocytosis, large, insoluble particles (e.g., bacteria) are enveloped by the plasma membrane and internalized.

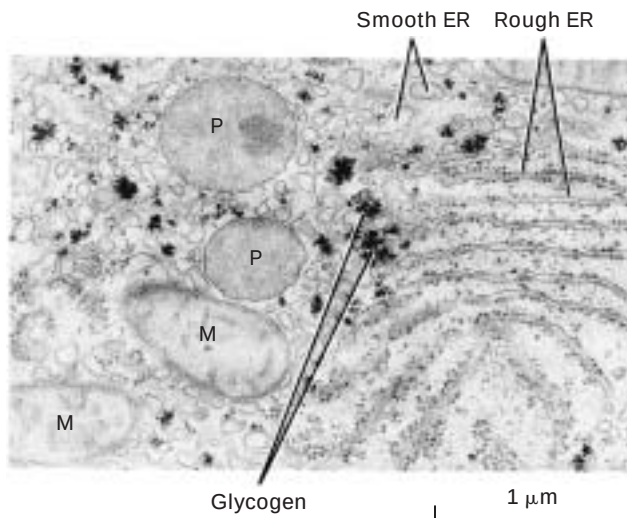
Lysosomes contain a group of enzymes that degrade polymers into their monomeric subunits. For example, nucleases degrade RNA and DNA into their mononucleotide building blocks; proteases degrade a variety of proteins and peptides; phosphatases remove phosphate groups from mononucleotides, phospholipids, and other compounds; still other enzymes degrade complex polysaccharides and glycolipids into smaller units. All the lysosomal enzymes work most efficiently at acid pH values and collectively are termed *acid hydrolases*. Two types of transport proteins in the lysosomal membrane work together to pump H^+ and Cl^- ions (HCl) from the cytosol across the membrane, thereby acidifying the lumen (see Figure 7-10b). The acid pH helps to denature proteins, making them accessible to the action of the lysosomal hydrolases, which themselves are resistant to acid denaturation. Lysosomal enzymes are poorly active at the neutral pH of cells and most extracellular fluids. Thus, if a

lysosome releases its enzymes into the cytosol, where the pH is between 7.0 and 7.3, they cause little degradation of cytosolic components. Cytosolic and nuclear proteins generally are not degraded in lysosomes but rather in proteasomes, large multiprotein complexes in the cytosol (see Figure 3-13).

Lysosomes vary in size and shape, and several hundred may be present in a typical animal cell. In effect, they function as sites where various materials to be degraded collect. *Primary lysosomes* are roughly spherical and do not contain obvious particulate or membrane debris. *Secondary lysosomes*, which are larger and irregularly shaped, appear to result from the fusion of primary lysosomes with other membrane-bounded organelles and vesicles. They contain particles or membranes in the process of being digested (Figure 5-20c).



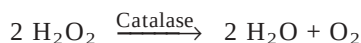
Tay-Sachs disease is caused by a defect in one enzyme catalyzing a step in the lysosomal breakdown of gangliosides. The resulting accumulation of these glycolipids, especially in nerve cells, has devastating consequences. The symptoms of this inherited disease are usually evident before the age of 1. Affected children commonly become demented and blind by age 2 and die before their third birthday. Nerve cells from such children are greatly enlarged with swollen lipid-filled lysosomes. ■



▲ **FIGURE 5-21 Electron micrograph showing various organelles in a rat liver cell.** Two peroxisomes (P) lie in close proximity to mitochondria (M) and the rough and smooth endoplasmic reticulum (ER). Also visible are accumulations of glycogen, a polysaccharide that is the primary glucose-storage molecule in animals. [Courtesy of P. Lazarow.]

Peroxisomes Degrade Fatty Acids and Toxic Compounds

All animal cells (except erythrocytes) and many plant cells contain **peroxisomes**, a class of roughly spherical organelles, 0.2–1.0 μm in diameter (Figure 5-21). Peroxisomes contain several *oxidases*—enzymes that use molecular oxygen to oxidize organic substances, in the process forming hydrogen peroxide (H₂O₂), a corrosive substance. Peroxisomes also contain copious amounts of the enzyme *catalase*, which degrades hydrogen peroxide to yield water and oxygen:



In contrast with the oxidation of fatty acids in mitochondria, which produces CO₂ and is coupled to the generation of ATP, peroxisomal oxidation of fatty acids yields acetyl groups and is not linked to ATP formation (see Figure 8-11). The energy released during peroxisomal oxidation is converted into heat, and the acetyl groups are transported into the cytosol, where they are used in the synthesis of cholesterol and other metabolites. In most eukaryotic cells, the peroxisome is the principal organelle in which fatty acids are oxidized, thereby generating precursors for important biosynthetic pathways. Particularly in liver and kidney cells, various toxic molecules that enter the bloodstream also are degraded in peroxisomes, producing harmless products.



In the human genetic disease *X-linked adrenoleukodystrophy (ADL)*, peroxisomal oxidation of very long chain fatty acids is defective. The *ADL* gene encodes the peroxisomal membrane protein that trans-

ports into peroxisomes an enzyme required for the oxidation of these fatty acids. Persons with the severe form of ADL are unaffected until midchildhood, when severe neurological disorders appear, followed by death within a few years. ■



Plant seeds contain *glyoxisomes*, small organelles that oxidize stored lipids as a source of carbon and energy for growth. They are similar to peroxisomes and contain many of the same types of enzymes as well as additional ones used to convert fatty acids into glucose precursors. ■

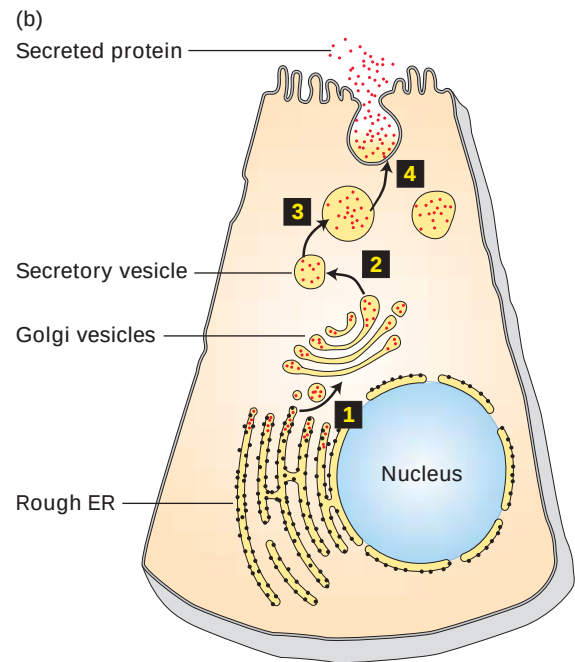
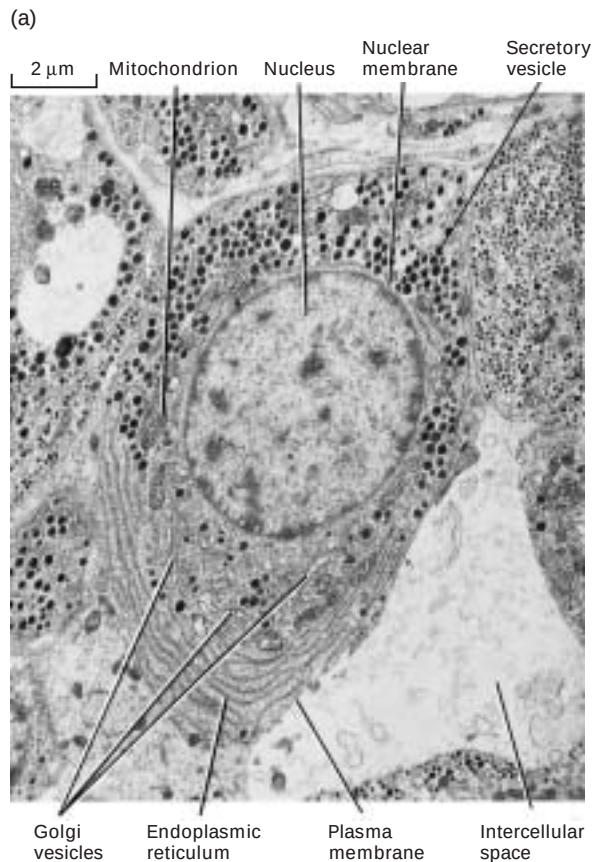
The Endoplasmic Reticulum Is a Network of Interconnected Internal Membranes

Generally, the largest membrane in a eukaryotic cell encloses the **endoplasmic reticulum (ER)**—an extensive network of closed, flattened membrane-bounded sacs called **cisternae** (see Figure 5-19). The endoplasmic reticulum has a number of functions in the cell but is particularly important in the synthesis of lipids, membrane proteins, and secreted proteins. The *smooth endoplasmic reticulum* is smooth because it lacks ribosomes. In contrast, the cytosolic face of the *rough endoplasmic reticulum* is studded with ribosomes.

The Smooth Endoplasmic Reticulum The synthesis of fatty acids and phospholipids takes place in the smooth ER. Although many cells have very little smooth ER, this organelle is abundant in hepatocytes. Enzymes in the smooth ER of the liver also modify or detoxify hydrophobic chemicals such as pesticides and carcinogens by chemically converting them into more water-soluble, conjugated products that can be excreted from the body. High doses of such compounds result in a large proliferation of the smooth ER in liver cells.

The Rough Endoplasmic Reticulum **Ribosomes** bound to the rough ER synthesize certain membrane and organelle proteins and virtually all proteins to be secreted from the cell (Chapter 16). A ribosome that fabricates such a protein is bound to the rough ER by the nascent polypeptide chain of the protein. As the growing polypeptide emerges from the ribosome, it passes through the rough ER membrane, with the help of specific proteins in the membrane. Newly made membrane proteins remain associated with the rough ER membrane, and proteins to be secreted accumulate in the lumen of the organelle.

All eukaryotic cells contain a discernible amount of rough ER because it is needed for the synthesis of plasma-membrane proteins and proteins of the extracellular matrix. Rough ER is particularly abundant in specialized cells that produce an abundance of specific proteins to be secreted. For example, plasma cells produce antibodies, pancreatic acinar cells synthesize digestive enzymes, and cells in the pancreatic islets of Langerhans produce the polypeptide hormones insulin and glucagon. In these secretory cells and others, a large part of the cytosol is filled with rough ER and secretory vesicles (Figure 5-22).



▲ **FIGURE 5-22 Characteristic features of cells specialized to secrete large amounts of particular proteins (e.g., hormones, antibodies).** (a) Electron micrograph of a thin section of a hormone-secreting cell from the rat pituitary. One end of the cell (*top*) is filled with abundant rough ER and Golgi sacs, where polypeptide hormones are synthesized and packaged. At the opposite end of the cell (*bottom*) are numerous secretory vesicles, which contain recently made hormones eventually to be secreted. (b) Diagram of a typical secretory cell tracing the pathway followed by a protein (small red dots) to be secreted. Immediately after their synthesis on ribosomes (blue dots) of the

rough ER, secreted proteins are found in the lumen of the rough ER. Transport vesicles bud off and carry these proteins to the Golgi complex (**1**), where the proteins are concentrated and packaged into immature secretory vesicles (**2**). These vesicles then coalesce to form larger mature secretory vesicles that lose water to the cytosol, leaving an almost crystalline mixture of secreted proteins in the lumen (**3**). After these vesicles accumulate under the apical surface, they fuse with the plasma membrane and release their contents (exocytosis) in response to appropriate hormonal or nerve stimulation (**4**). [Part (a) courtesy of Biophoto Associates.]

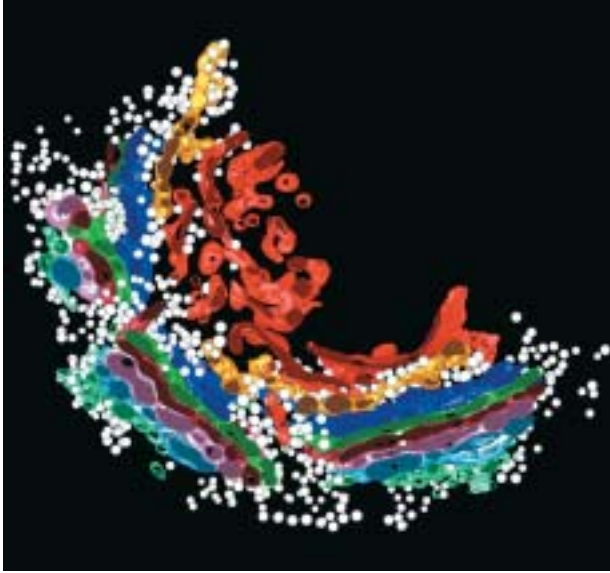
The Golgi Complex Processes and Sorts Secreted and Membrane Proteins

Several minutes after proteins are synthesized in the rough ER, most of them leave the organelle within small membrane-bounded transport vesicles. These vesicles, which bud from regions of the rough ER not coated with ribosomes, carry the proteins to another membrane-limited organelle, the **Golgi complex** (see Figure 5-22).

Three-dimensional reconstructions from serial sections of a Golgi complex reveal this organelle to be a series of flattened membrane vesicles or sacs (cisternae), surrounded by a number of more or less spherical membrane-limited vesicles (Figure 5-23). The stack of Golgi cisternae has three de-

finer regions—the *cis*, the *medial*, and the *trans*. Transport vesicles from the rough ER fuse with the *cis* region of the Golgi complex, where they deposit their protein contents. As detailed in Chapter 17, these proteins then progress from the *cis* to the *medial* to the *trans* region. Within each region are different enzymes that modify proteins to be secreted and membrane proteins differently, depending on their structures and their final destinations.

After proteins to be secreted and membrane proteins are modified in the Golgi complex, they are transported out of the complex by a second set of vesicles, which seem to bud from the *trans* side of the Golgi complex. Some vesicles carry membrane proteins destined for the plasma membrane or soluble proteins to be released from the cell surface; others



▲ **FIGURE 5-23 Model of the Golgi complex based on three-dimensional reconstruction of electron microscopy images.** Transport vesicles (white spheres) that have budded off the rough ER fuse with the *cis* membranes (light blue) of the Golgi complex. By mechanisms described in Chapter 17, proteins move from the *cis* region to the *medial* region and finally to the *trans* region of the Golgi complex. Eventually, vesicles bud off the *trans*-Golgi membranes (orange and red); some move to the cell surface and others move to lysosomes. The Golgi complex, like the rough endoplasmic reticulum, is especially prominent in secretory cells. [From B. J. Marsh et al., 2001, *Proc Nat'l. Acad. Sci USA* **98**:2399.]

carry soluble or membrane proteins to lysosomes or other organelles. How intracellular transport vesicles “know” with which membranes to fuse and where to deliver their contents is also discussed in Chapter 17.

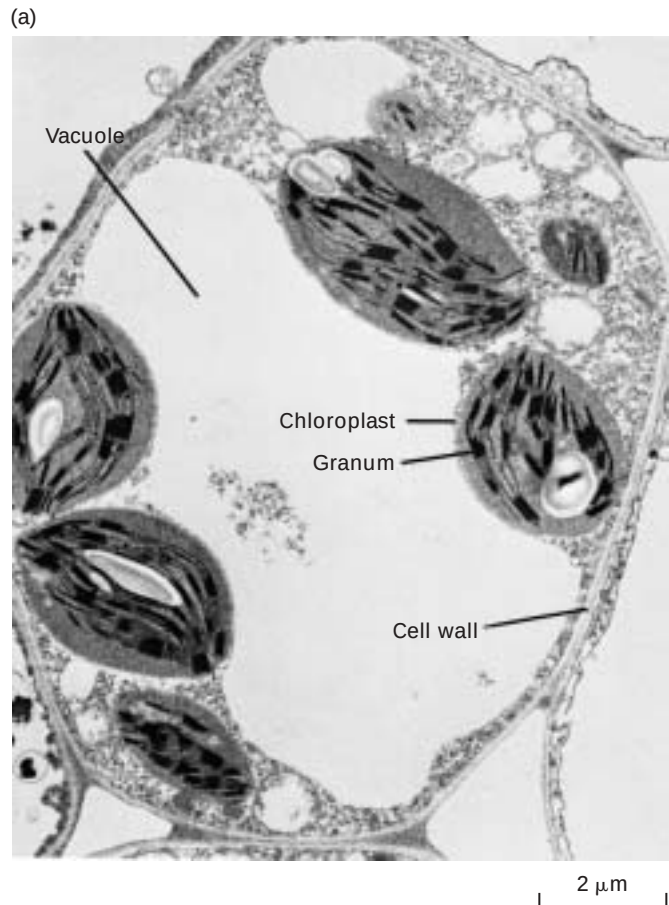
Plant Vacuoles Store Small Molecules and Enable a Cell to Elongate Rapidly



Most plant cells contain at least one membrane-limited internal vacuole. The number and size of vacuoles depend on both the type of cell and its stage of development; a single vacuole may occupy as much as 80 percent of a mature plant cell (Figure 5-24). A variety of transport proteins in the vacuolar membrane allow plant cells to accumulate and store water, ions, and nutrients (e.g., sucrose, amino acids) within vacuoles (Chapter 7). Like a lysosome, the lumen of a vacuole contains a battery of degradative enzymes and has an acidic pH, which is maintained by similar transport proteins in the vacuolar membrane. Thus plant vacuoles may also have a degradative

function similar to that of lysosomes in animal cells. Similar storage vacuoles are found in green algae and many microorganisms such as fungi.

Like most cellular membranes, the vacuolar membrane is permeable to water but is poorly permeable to the small molecules stored within it. Because the solute concentration is much higher in the vacuole lumen than in the cytosol or extracellular fluids, water tends to move by osmotic flow into vacuoles, just as it moves into cells placed in a hypotonic medium (see Figure 5-18). This influx of water causes both the vacuole to expand and water to move into the cell, creating hydrostatic pressure, or *turgor*, inside the cell. This pressure is balanced by the mechanical resistance of the cellulose-containing cell walls that surround plant cells. Most plant cells have a turgor of 5–20 atmospheres (atm); their cell walls must be strong enough to react to this pressure in a controlled way. Unlike animal cells, plant cells can elongate extremely rapidly, at rates of 20–75 $\mu\text{m}/\text{h}$. This elongation,



▲ **FIGURE 5-24 Electron micrograph of a thin section of a leaf cell.** In this cell, a single large vacuole occupies much of the cell volume. Parts of five chloroplasts and the cell wall also are visible. Note the internal subcompartments in the chloroplasts.

[Courtesy of Biophoto Associates/Myron C. Ledbetter/Brookhaven National Laboratory.]

which usually accompanies plant growth, occurs when a segment of the somewhat elastic cell wall stretches under the pressure created by water taken into the vacuole. ■

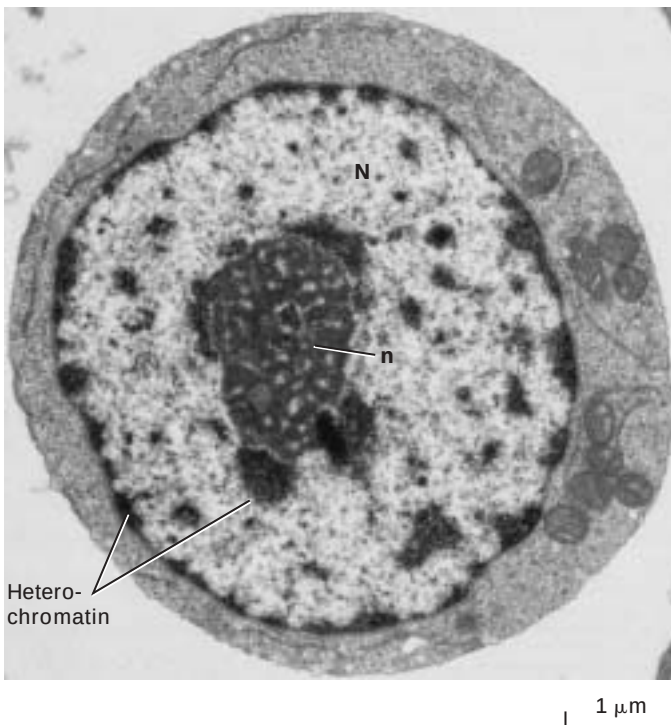
The Nucleus Contains the DNA Genome, RNA Synthetic Apparatus, and a Fibrous Matrix

The **nucleus**, the largest organelle in animal cells, is surrounded by two membranes, each one a phospholipid bilayer containing many different types of proteins. The inner nuclear membrane defines the nucleus itself. In most cells, the outer nuclear membrane is continuous with the rough endoplasmic reticulum, and the space between the inner and outer nuclear membranes is continuous with the lumen of the rough endoplasmic reticulum (see Figure 5-19). The two nuclear membranes appear to fuse at **nuclear pores**, the ringlike complexes composed of specific membrane proteins through which material moves between the nucleus and the cytosol. The structure of nuclear pores and the regulated transport of material through them are detailed in Chapter 12.

In a growing or differentiating cell, the nucleus is metabolically active, replicating DNA and synthesizing rRNA,

tRNA, and mRNA. Within the nucleus mRNA binds to specific proteins, forming ribonucleoprotein particles. Most of the cell's ribosomal RNA is synthesized in the **nucleolus**, a subcompartment of the nucleus that is not bounded by a phospholipid membrane (Figure 5-25). Some ribosomal proteins are added to ribosomal RNAs within the nucleolus as well. The finished or partly finished ribosomal subunits, as well as tRNAs and mRNA-containing particles, pass through a nuclear pore into the cytosol for use in protein synthesis (Chapter 4). In mature erythrocytes from nonmammalian vertebrates and other types of “resting” cells, the nucleus is inactive or dormant and minimal synthesis of DNA and RNA takes place.

How nuclear DNA is packaged into chromosomes is described in Chapter 10. In a nucleus that is not dividing, the chromosomes are dispersed and not dense enough to be observed in the light microscope. Only during cell division are individual chromosomes visible by light microscopy. In the electron microscope, the nonnucleolar regions of the nucleus, called the *nucleoplasm*, can be seen to have dark- and light-staining areas. The dark areas, which are often closely associated with the nuclear membrane, contain condensed concentrated DNA, called **heterochromatin** (see Figure 5-25). Fibrous proteins called **lamins** form a two-dimensional network along the inner surface of the inner membrane, giving it shape and apparently binding DNA to it. The breakdown of this network occurs early in cell division, as we detail in Chapter 21.



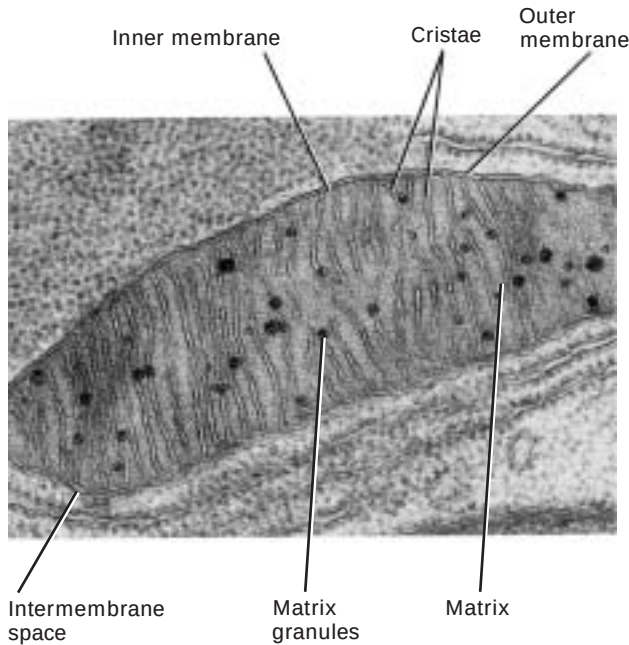
▲ **FIGURE 5-25 Electron micrograph of a thin section of a bone marrow stem cell.** The nucleolus (n) is a subcompartment of the nucleus (N) and is not surrounded by a membrane. Most ribosomal RNA is produced in the nucleolus. Darkly staining areas in the nucleus outside the nucleolus are regions of heterochromatin. [From P. C. Cross and K. L. Mercer, 1993, *Cell and Tissue Ultrastructure*, W. H. Freeman and Company, p. 165.]

Mitochondria Are the Principal Sites of ATP Production in Aerobic Cells

Most eukaryotic cells contain many **mitochondria**, which occupy up to 25 percent of the volume of the cytoplasm. These complex organelles, the main sites of ATP production during aerobic metabolism, are generally exceeded in size only by the nucleus, vacuoles, and chloroplasts.

The two membranes that bound a mitochondrion differ in composition and function. The outer membrane, composed of about half lipid and half protein, contains porins (see Figure 5-14) that render the membrane permeable to molecules having molecular weights as high as 10,000. In this respect, the outer membrane is similar to the outer membrane of gram-negative bacteria. The inner membrane, which is much less permeable, is about 20 percent lipid and 80 percent protein—a higher proportion of protein than exists in other cellular membranes. The surface area of the inner membrane is greatly increased by a large number of infoldings, or *cristae*, that protrude into the *matrix*, or central space (Figure 5-26).

In nonphotosynthetic cells, the principal fuels for ATP synthesis are fatty acids and glucose. The complete aerobic degradation of glucose to CO₂ and H₂O is coupled to the synthesis of as many as 30 molecules of ATP. In eukaryotic cells, the initial stages of glucose degradation take place in



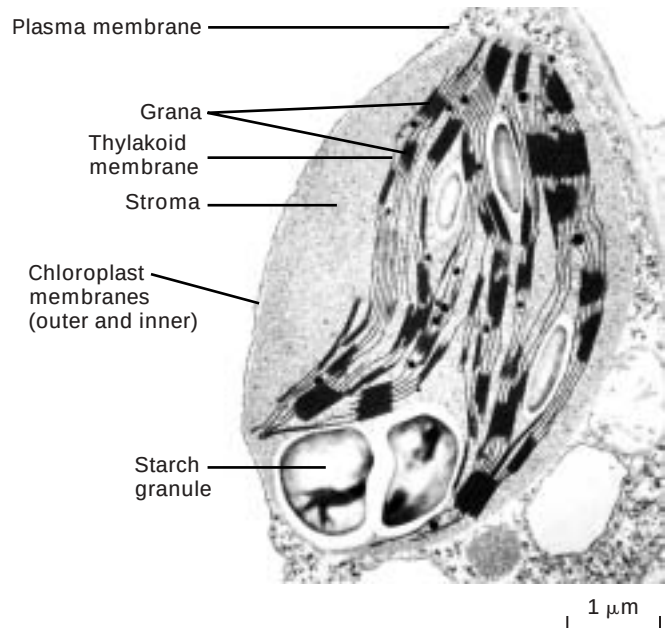
▲ **FIGURE 5-26 Electron micrograph of a mitochondrion.** Most ATP production in nonphotosynthetic cells takes place in mitochondria. The inner membrane, which surrounds the matrix space, has many infoldings, called cristae. Small calcium-containing matrix granules also are evident. [From D. W. Fawcett, 1981, *The Cell*, 2d ed., Saunders, p. 421]

the cytosol, where 2 ATP molecules per glucose molecule are generated. The terminal stages of oxidation and the coupled synthesis of ATP are carried out by enzymes in the mitochondrial matrix and inner membrane (Chapter 8). As many as 28 ATP molecules per glucose molecule are generated in mitochondria. Similarly, virtually all the ATP formed in the oxidation of fatty acids to CO_2 is generated in mitochondria. Thus mitochondria can be regarded as the “power plants” of the cell.

Chloroplasts Contain Internal Compartments in Which Photosynthesis Takes Place



Except for vacuoles, **chloroplasts** are the largest and the most characteristic organelles in the cells of plants and green algae. They can be as long as $10\ \mu\text{m}$ and are typically $0.5\text{--}2\ \mu\text{m}$ thick, but they vary in size and shape in different cells, especially among the algae. In addition to the double membrane that bounds a chloroplast, this organelle also contains an extensive internal system of interconnected membrane-limited sacs called **thylakoids**, which are flattened to form disks (Figure 5-27). Thylakoids often form stacks called *grana* and are embedded in a matrix, the *stroma*. The thylakoid membranes contain green pigments



▲ **FIGURE 5-27 Electron micrograph of a plant chloroplast.** The internal membrane vesicles (thylakoids) are fused into stacks (grana), which reside in a matrix (the stroma). All the chlorophyll in the cell is contained in the thylakoid membranes, where the light-induced production of ATP takes place during photosynthesis. [Courtesy of Biophoto Associates/M. C. Ledbetter/Brookhaven National Laboratory.]

(chlorophylls) and other pigments that absorb light, as well as enzymes that generate ATP during photosynthesis. Some of the ATP is used to convert CO_2 into three-carbon intermediates by enzymes located in the stroma; the intermediates are then exported to the cytosol and converted into sugars. ■

The molecular mechanisms by which ATP is formed in mitochondria and chloroplasts are very similar, as explained in Chapter 8. Chloroplasts and mitochondria have other features in common: both often migrate from place to place within cells, and they contain their own DNA, which encodes some of the key organellar proteins (Chapter 10). The proteins encoded by mitochondrial or chloroplast DNA are synthesized on ribosomes within the organelles. However, most of the proteins in each organelle are encoded in nuclear DNA and are synthesized in the cytosol; these proteins are then incorporated into the organelles by processes described in Chapter 16.

KEY CONCEPTS OF SECTION 5.3

Organelles of the Eukaryotic Cell

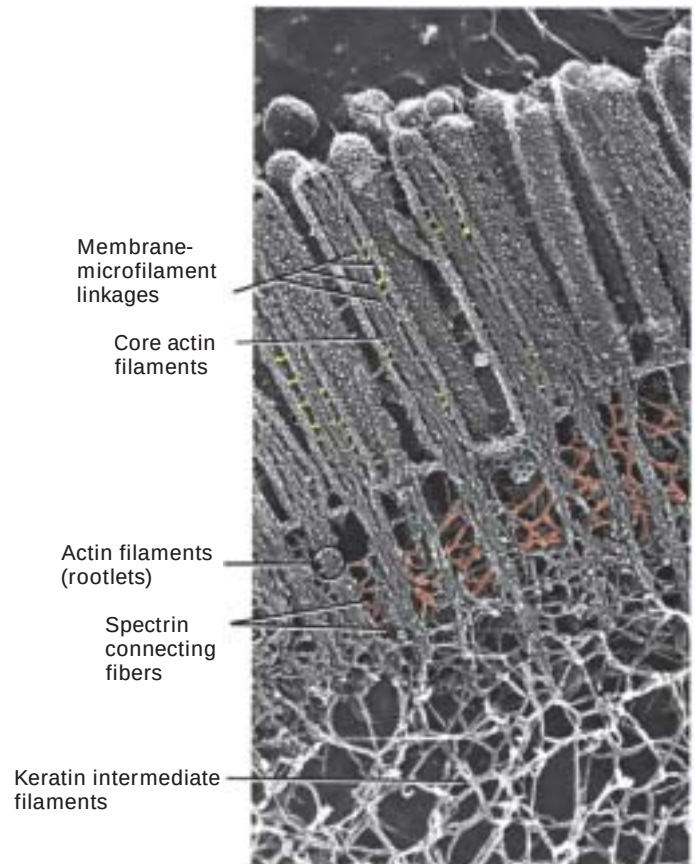
- All eukaryotic cells contain a nucleus and numerous other organelles in their cytosols (see Figure 5-19).

- The nucleus, mitochondrion, and chloroplast are bounded by two bilayer membranes separated by an intermembrane space. All other organelles are surrounded by a single membrane.
- Endosomes internalize plasma-membrane proteins and soluble materials from the extracellular medium, and they sort them back to the membranes or to lysosomes for degradation.
- Lysosomes have an acidic interior and contain various hydrolases that degrade worn-out or unneeded cellular components and some ingested materials (see Figure 5-20).
- Peroxisomes are small organelles containing enzymes that oxidize various organic compounds without the production of ATP. By-products of oxidation are used in biosynthetic reactions.
- Secreted proteins and membrane proteins are synthesized on the rough endoplasmic reticulum, a network of flattened membrane-bounded sacs studded with ribosomes.
- Proteins synthesized on the rough ER first move to the Golgi complex, where they are processed and sorted for transport to the cell surface or other destination (see Figure 5-22).
- Plant cells contain one or more large vacuoles, which are storage sites for ions and nutrients. Osmotic flow of water into vacuoles generates turgor pressure that pushes the plasma membrane against the cell wall.
- The nucleus houses the genome of a cell. The inner and outer nuclear membranes are fused at numerous nuclear pores, through which materials pass between the nucleus and the cytosol. The outer nuclear membrane is continuous with that of the rough endoplasmic reticulum.
- Mitochondria have a highly permeable outer membrane and a protein-enriched inner membrane that is extensively folded. Enzymes in the inner mitochondrial membrane and central matrix carry out the terminal stages of sugar and lipid oxidation coupled to ATP synthesis.
- Chloroplasts contain a complex system of thylakoid membranes in their interiors. These membranes contain the pigments and enzymes that absorb light and produce ATP during photosynthesis.

5.4 The Cytoskeleton: Components and Structural Functions

The cytosol is a major site of cellular metabolism and contains a large number of different enzymes. Proteins constitute about 20–30 percent of the cytosol by weight, and from a quarter to half of the total protein within cells is in the cytosol. Estimates of the protein concentration in the cytosol range from 200 to 400 mg/ml. Because of the high concentration of cytosolic proteins, complexes of proteins can form even if the energy that stabilizes them is weak. Many inves-

tigators believe that the cytosol is highly organized, with most soluble proteins either bound to filaments or otherwise localized in specific regions. In an electron micrograph of a typical animal cell, soluble proteins packing the cell interior conceal much of the internal structure. If a cell is pretreated with a nonionic detergent (e.g., Triton X-100), which permeabilizes the membrane, soluble cytosolic proteins diffuse away. In micrographs of detergent-extracted animal cells, two types of structures stand out—membrane-limited organelles and the filaments of the cytoskeleton, which fill the cytosol (Figure 5-28).



▲ **FIGURE 5-28 Electron micrograph of the apical part of a detergent-extracted intestinal epithelial cell.** Microvilli, fingerlike projections of the plasma membrane, cover the apical surface of an intestinal epithelial cell. A bundle of microfilaments in the core of each microvillus stabilizes the structure. The plasma membrane surrounding a microvillus is attached to the sides of the bundle by evenly spaced membrane-microfilament linkages (yellow). The bundle continues into the cell as a short rootlet. The rootlets of multiple microvilli are cross-braced by connecting fibers (red) composed of an intestinal isoform of spectrin. This fibrous actin-binding protein is found in a narrow band just below the plasma membrane in many animal cells. The bases of the rootlets are attached to keratin intermediate filaments. These numerous connections anchor the rootlets in a meshwork of filaments and thereby support the upright orientation of the microvilli. [Courtesy of N. Hirokawa.]

In this section, we introduce the protein filaments that compose the cytoskeleton and then describe how they support the plasma and nuclear membranes and organize the contents of the cell. Later chapters will deal with the dynamic properties of the cytoskeleton—its assembly and disassembly and its role in cellular movements.

Three Types of Filaments Compose the Cytoskeleton

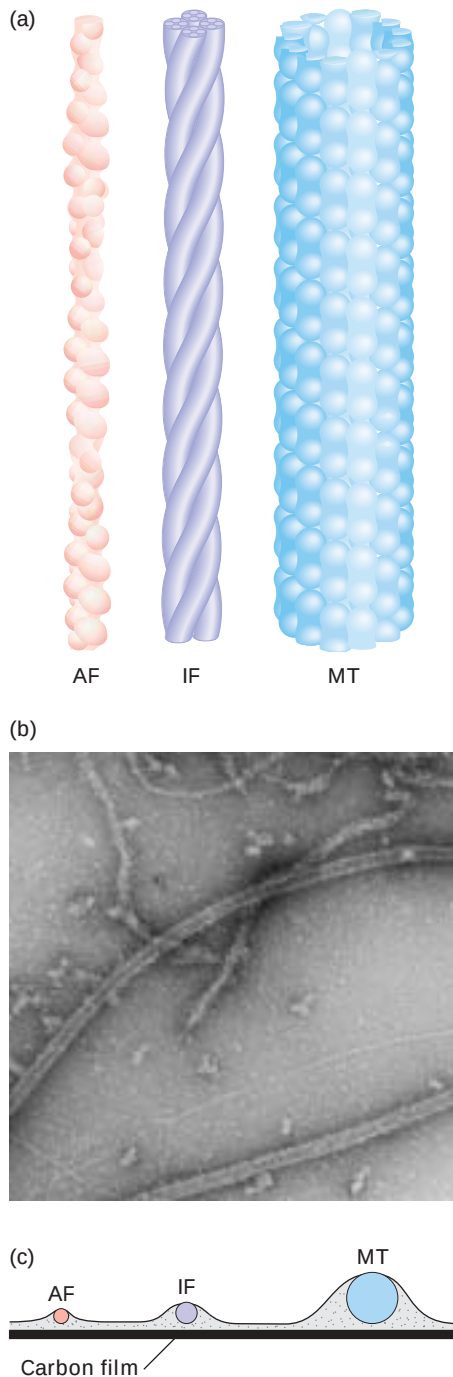
The cytosol of a eukaryotic cell contains three types of filaments that can be distinguished on the bases of their diameter, type of subunit, and subunit arrangement (Figure 5-29). **Actin filaments**, also called **microfilaments**, are 8–9 nm in diameter and have a twisted two-stranded structure. **Microtubules** are hollow tubelike structures, 24 nm in diameter, whose walls are formed by adjacent protofilaments. **Intermediate filaments (IFs)** have the structure of a 10-nm-diameter rope.

Each type of cytoskeletal filament is a polymer of protein subunits (Table 5-4). Monomeric **actin** subunits assemble into microfilaments; dimeric subunits composed of **α-** and **β-tubulin** polymerize into microtubules. Unlike microfilaments and microtubules, which are assembled from one or two proteins, intermediate filaments are assembled from a large diverse family of proteins. The most common intermediate filaments, found in the nucleus, are composed of *lamins*. Intermediate filaments constructed from other proteins are expressed preferentially in certain tissues: for example, *keratin*-containing filaments in epithelial cells, *desmin*-containing filaments in muscle cells, and *vimentin*-containing filaments in mesenchymal cells.

► **FIGURE 5-29 Comparison of the three types of filaments that form the cytoskeleton.** (a) Diagram of the basic structures of an actin filament (AF), intermediate filament (IF), and microtubule (MT). The beadlike structure of an actin filament shows the packing of actin subunits. Intermediate filament subunits pack to form ropes in which the individual subunits are difficult to distinguish. The walls of microtubules are formed from protofilaments of tubulin subunits. (b) Micrograph of a mixture of actin filaments, microtubules, and vimentin intermediate filaments showing the differences in their shape, size, and flexibility. Purified preparations of actin, tubulin, and vimentin subunits were separately polymerized in a test tube to form the corresponding filaments. A mixture of the filaments was applied to a carbon film on a microscope grid and then rinsed with a dilute solution of uranyl acetate (UC), which surrounds but does not penetrate the protein (c). Because uranyl acetate is a heavy metal that easily scatters electrons, areas of the microscope grid occupied by protein produce a “negative” image in metal film when projected onto a photographic plate, as seen in part (b). [Part (b) courtesy of G. Waller and P. Matsudaira.]

Most eukaryotic cells contain all three types of cytoskeletal filaments, often concentrated in distinct locations. For example, in the absorptive epithelial cells that line the lumen of the intestine, actin microfilaments are abundant in the apical region, where they are associated with cell–cell junctions and support a dense carpet of microvilli (Figure 5-30a). Actin filaments are also present in a narrow zone adjacent to the plasma membrane in the lateral regions of these cells. Keratin intermediate filaments,

TABLE 5-4 Protein Subunits in Cytoskeletal Filaments			
Protein Subunits	MW	Expression	Function
MICROFILAMENTS			
Actin	42,000	Fungi, plant, animal	Structural support, motility
MreB	36,000	Rod-shaped bacteria	Width control
MICROTUBULES			
Tubulin (α and β)	58,000	Fungi, plant, animal	Structural support, motility, cell polarity
FtsZ	58,000	Bacteria	Cell division
INTERMEDIATE FILAMENTS			
Lamins	Various	Plant, animal	Support for nuclear membrane
Desmin, keratin, vimentin, others	Various	Animal	Cell adhesion
OTHER			
MSP	50,000	Nematode sperm	Motility

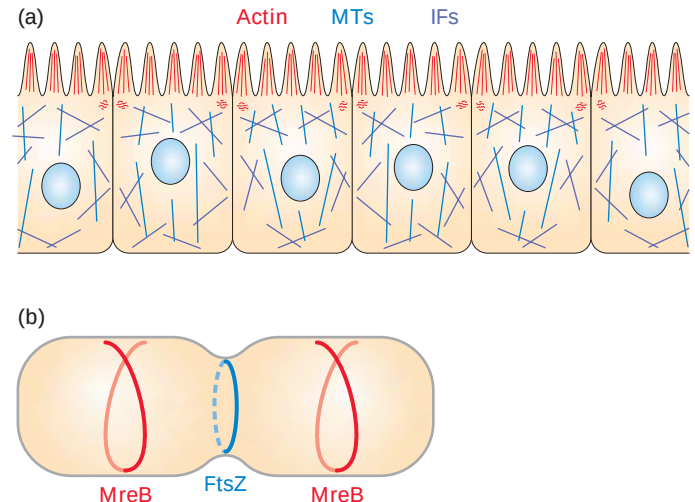


forming a meshwork, connect microvilli and are tethered to junctions between cells. Lamin intermediate filaments support the inner nuclear membrane. Finally, microtubules, aligned with the long axis of the cell, are in close proximity to major cell organelles such as the endoplasmic reticulum, Golgi complex, and vesicles.

The cytoskeleton has been highly conserved in evolution. A comparison of gene sequences shows only a small percentage of differences in sequence between yeast actin and tubulin and human actin and tubulin. This structural

conservation is explained by the variety of critical functions that depend on the cytoskeleton. A mutation in a cytoskeleton protein subunit could disrupt the assembly of filaments and their binding to other proteins. Analyses of gene sequences and protein structures have identified bacterial homologs of actin and tubulin. The absence of IF-like proteins in bacteria and unicellular eukaryotes is evidence that intermediate filaments appeared later in the evolution of the cytoskeletal system. The first IF protein to arise was most likely a nuclear lamin from which cytosolic IF proteins later evolved.

The simple bacterial cytoskeleton controls cell length, width, and the site of cell division. The FtsZ protein, a bacterial homolog of tubulin, is localized around the neck of dividing bacterial cells, suggesting that FtsZ participates in cell division (Figure 5-30b). The results of biochemical experiments with purified FtsZ demonstrate that it can polymerize into protofilaments, but these protofilaments do not assemble into intact microtubules. Another bacterial protein, MreB, has been found to be similar to actin in atomic structure and filament structure—strong evidence that actin evolved from MreB. Clues to the function of MreB include its localization in a filament that girdles rod-shaped bacterial cells, its absence from spherical bacteria, and the finding that mutant cells lacking MreB become wider but not longer. These observations suggest MreB controls the width of rod-shaped bacteria.



▲ FIGURE 5-30 Schematic depiction of the distribution of cytoskeletal filaments in eukaryotic cells and bacterial cells.

(a) In absorptive epithelial cells, actin filaments (red) are concentrated in the apical region and in a narrow band in the basolateral region. Microtubules (blue) are oriented with the long axis of the cell, and intermediate filaments (green) are concentrated along the cell periphery especially at specialized junctions with neighboring cells and lining the nuclear membrane. (b) In a rod-shaped bacterial cell, filaments of MreB, the bacterial actin homolog, ring the cell and constrict its width. The bacterial tubulin homolog, FtsZ, forms filaments at the site of cell division.

Cytoskeletal Filaments Are Organized into Bundles and Networks

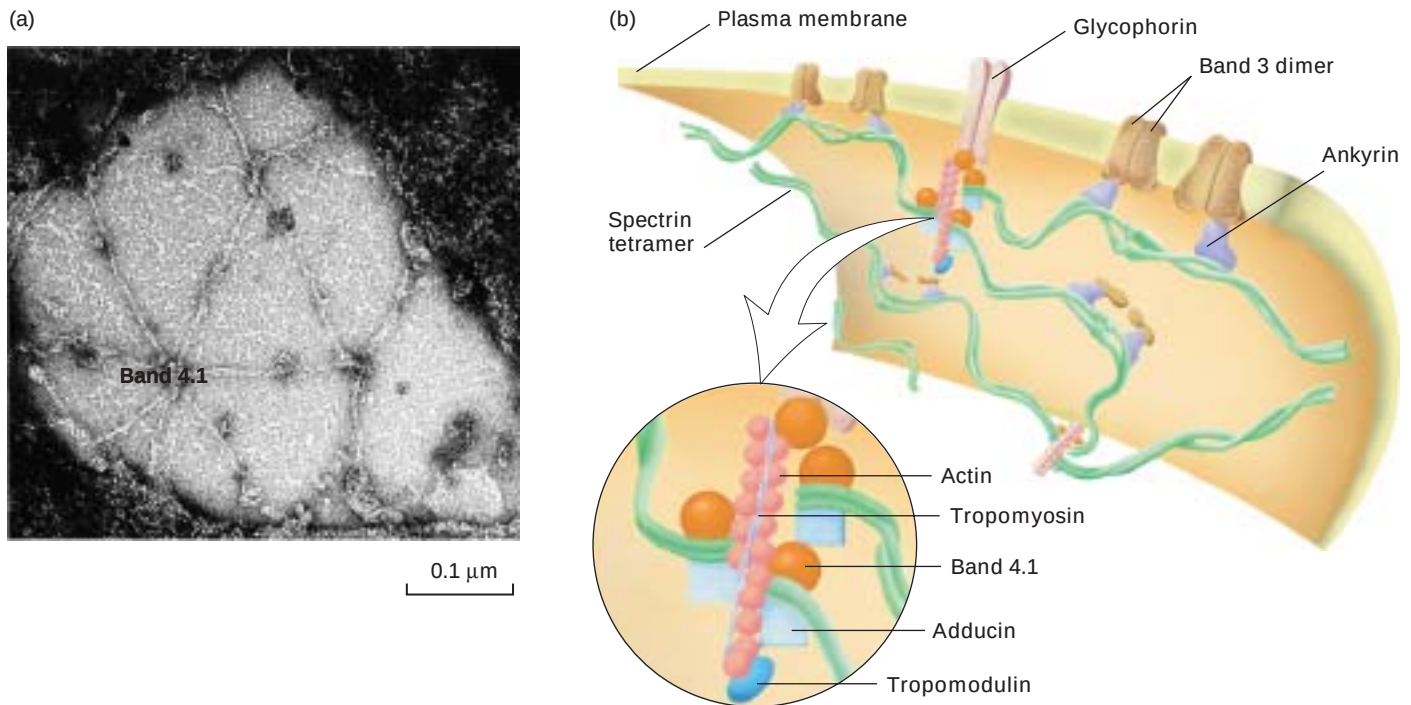
On first looking at micrographs of a cell, one is struck by the dense, seemingly disorganized mat of filaments present in the cytosol. However, a keen eye will start to pick out areas—generally where the membrane protrudes from the cell surface or where a cell adheres to the surface or another cell—in which the filaments are concentrated into bundles. From these bundles, the filaments continue into the cell interior, where they fan out and become part of a network of filaments. These two structures, *bundles* and *networks*, are the most common arrangements of cytoskeletal filaments in a cell.

Structurally, bundles differ from networks mainly in the organization of the filaments. In bundles, the filaments are closely packed in parallel arrays. In a network, the filaments crisscross, often at right angles, and are loosely packed. Networks can be further subdivided. One type, associated with the nuclear and plasma membranes, is planar (two-dimensional), like a net or a web; the other type, present within the cell, is three-dimensional, giving the cytosol gel-like properties. In all bundles and networks, the filaments are held together by various cross-linking proteins.

We will consider various cytoskeletal cross-linking proteins and their functions in Chapters 19 and 20.

Microfilaments and Membrane-Binding Proteins Form a Skeleton Underlying the Plasma Membrane

The distinctive shape of a cell depends on the organization of actin filaments and proteins that connect microfilaments to the membrane. These proteins, called *membrane–microfilament binding proteins*, act as spot welds that tack the actin cytoskeleton framework to the overlying membrane. When attached to a bundle of filaments, the membrane acquires the fingerlike shape of a microvillus or similar projection (see Figure 5-28). When attached to a planar network of filaments, the membrane is held flat like the red blood cell membrane. The simplest membrane–cytoskeleton connections entail the binding of integral membrane proteins directly to actin filaments. More common are complex linkages that connect actin filaments to integral membrane proteins through peripheral membrane proteins that function as adapter proteins. Such linkages between the cytoskeleton and certain plasma-membrane proteins are considered in Chapter 6.



▲ **FIGURE 5-31 Cortical cytoskeleton supporting the plasma membrane in human erythrocytes.** (a) Electron micrograph of the erythrocyte membrane showing the spoke-and-hub organization of the cytoskeleton. The long spokes are composed mainly of spectrin and can be seen to intersect at the hubs, or membrane-attachment sites. The darker spots along the spokes are ankyrin molecules, which cross-link spectrin to

integral membrane proteins. (b) Diagram of the erythrocyte cytoskeleton showing the various components. See text for discussion. [Part (a) from T. J. Byers and D. Branton, 1985, *Proc. Nat'l. Acad. Sci. USA* **82**:6153. Courtesy of D. Branton. Part (b) adapted from S. E. Lux, 1979, *Nature* **281**:426, and E. J. Luna and A. L. Hitt, 1992, *Science* **258**:955.]

The richest area of actin filaments in many cells lies in the *cortex*, a narrow zone just beneath the plasma membrane. In this region, most actin filaments are arranged in a network that excludes most organelles from the cortical cytoplasm. Perhaps the simplest cytoskeleton is the two-dimensional network of actin filaments adjacent to the erythrocyte plasma membrane. In more complicated cortical cytoskeletons, such as those in platelets, epithelial cells, and muscle, actin filaments are part of a three-dimensional network that fills the cytosol and anchors the cell to the substratum.

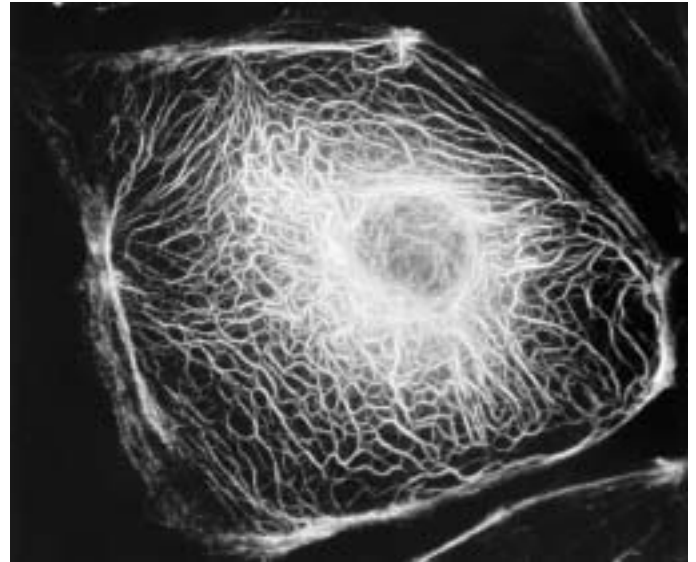
A red blood cell must squeeze through narrow blood capillaries without rupturing its membrane. The strength and flexibility of the erythrocyte plasma membrane depend on a dense cytoskeletal network that underlies the entire membrane and is attached to it at many points. The primary component of the erythrocyte cytoskeleton is *spectrin*, a 200-nm-long fibrous protein. The entire cytoskeleton is arranged in a spoke-and-hub network (Figure 5-31a). Each spoke is composed of a single spectrin molecule, which extends from two hubs and cross-links them. Each hub comprises a short (14-subunit) actin filament plus adducin, tropomyosin, and tropomodulin (Figure 5-31b, *inset*). The last two proteins strengthen the network by preventing the actin filament from depolymerizing. Six or seven spokes radiate from each hub, suggesting that six or seven spectrin molecules are bound to the same actin filament.

To ensure that the erythrocyte retains its characteristic shape, the spectrin-actin cytoskeleton is firmly attached to the overlying erythrocyte plasma membrane by two peripheral membrane proteins, each of which binds to a specific integral membrane protein and to membrane phospholipids. *Ankyrin* connects the center of spectrin to band 3 protein, an anion-transport protein in the membrane. *Band 4.1 protein*, a component of the hub, binds to the integral membrane protein glycophorin, whose structure was discussed previously (see Figure 5-12). Both ankyrin and band 4.1 protein also contain lipid-binding motifs, which help bind them to the membrane (see Table 5-3). The dual binding by ankyrin and band 4.1 ensures that the membrane is connected to both the spokes and the hubs of the spectrin-actin cytoskeleton (see Figure 5-31b).

Intermediate Filaments Support the Nuclear Membrane and Help Connect Cells into Tissues

Intermediate filaments typically crisscross the cytosol, forming an internal framework that stretches from the nuclear envelope to the plasma membrane (Figure 5-32). A network of intermediate filaments is located adjacent to some cellular membranes, where it provides mechanical support. For example, lamin A and lamin C filaments form an orthogonal lattice that is associated with lamin B. The entire supporting structure, called the **nuclear lamina**, is anchored to the inner nuclear membrane by prenyl anchors on lamin B.

At the plasma membrane, intermediate filaments are attached by adapter proteins to specialized cell junctions called

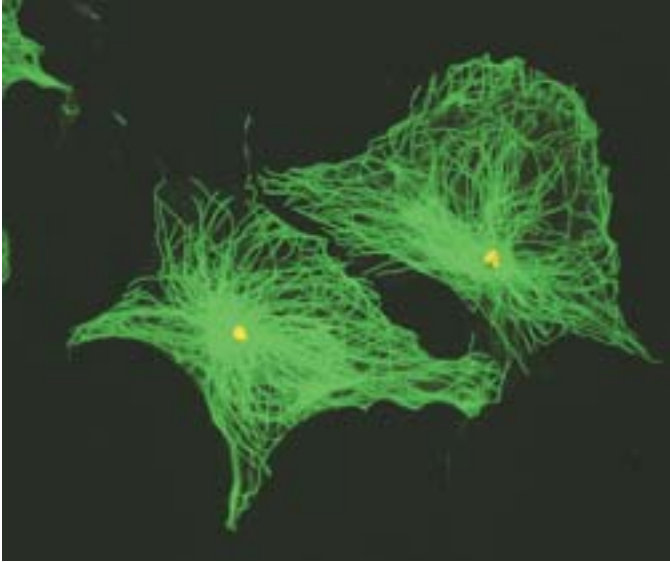


▲ **FIGURE 5-32 Fluorescence micrograph of a PtK2 fibroblast cell stained to reveal keratin intermediate filaments.** A network of filaments crisscrosses the cell from the nucleus to the plasma membrane. At the plasma membrane, the filaments are linked by adapter proteins to two types of anchoring junctions: desmosomes between adjacent cells and hemidesmosomes between the cell and the matrix. [Courtesy of R. D. Goldman.]

desmosomes and *hemidesmosomes*, which mediate cell–cell adhesion and cell–matrix adhesion, respectively, particularly in epithelial tissues. In this way, intermediate filaments in one cell are indirectly connected to intermediate filaments in a neighboring cell or to the extracellular matrix. Because of the important role of cell junctions in cell adhesion and the stability of tissues, we consider their structure and relation to cytoskeletal filaments in detail in Chapter 6.

Microtubules Radiate from Centrosomes and Organize Certain Subcellular Structures

Like microfilaments and intermediate filaments, microtubules are not randomly distributed in cells. Rather, microtubules radiate from the **centrosome**, which is the primary **microtubule-organizing center (MTOC)** in animal cells (Figure 5-33). As detailed in Chapter 20, the two ends of a microtubule differ in their dynamic properties and are commonly designated as the (+) and (–) ends. For this reason, microtubules can have two distinct orientations relative to one another and to other cell structures. In many nondividing animal cells, the MTOC is located at the center of the cell near the nucleus, and the radiating microtubules are all oriented with their (+) ends directed toward the cell periphery. Although most interphase animal cells contain a single perinuclear MTOC, epithelial cells and plant cells contain hundreds of MTOCs. Both of these cell types exhibit distinct



▲ **FIGURE 5-33 Fluorescence micrograph of a Chinese hamster ovary cell stained to reveal microtubules and the MTOC.** The microtubules (green), detected with an antibody to tubulin, are seen to radiate from a central point, the microtubule-organizing center (MTOC), near the nucleus. The MTOC (yellow) is detected with an antibody to a protein localized to the centrosome. [Courtesy of R. Kuriyama.]

functional or structural properties or both in different regions of the cell. The functional and structural **polarity** of these cells is linked to the orientation of microtubules within them.

Findings from studies discussed in Chapter 20 show that the association of microtubules with the endoplasmic reticulum and other membrane-bounded organelles may be critical to the location and organization of these organelles within the cell. For instance, if microtubules are destroyed by drugs such as nocodazole or colcemid, the ER loses its networklike organization. Microtubules are also critical to the formation of the mitotic apparatus—the elaborate, transient structure that captures and subsequently separates replicated chromosomes in cell division.

KEY CONCEPTS OF SECTION 5.4

The Cytoskeleton: Components and Structural Functions

- The cytosol is the internal aqueous medium of a cell exclusive of all organelles and the cytoskeleton. It contains numerous soluble enzymes responsible for much of the cell's metabolic activity.
- Three major types of protein filaments—actin filaments, microtubules, and intermediate filaments—make up the cytoskeleton (see Figure 5-29).

- Microfilaments are assembled from monomeric actin subunits; microtubules, from α,β -tubulin subunits; and intermediate filaments, from lamin subunits and other tissue-specific proteins.
- In all animal and plant cells, the cytoskeleton provides structural stability for the cell and contributes to cell movement. Some bacteria have a primitive cytoskeleton.
- Actin bundles form the core of microvilli and other fingerlike projections of the plasma membrane.
- Cortical spectrin-actin networks are attached to the cell membrane by bivalent membrane-microfilament binding proteins such as ankyrin and band 4.1 (see Figure 5-31).
- Intermediate filaments are assembled into networks and bundles by various intermediate filament-binding proteins, which also cross-link intermediate filaments to the plasma and nuclear membranes, microtubules, and microfilaments.
- In some animal cells, microtubules radiate out from a single microtubule-organizing center lying at the cell center (see Figure 5-33). Intact microtubules appear to be necessary for endoplasmic reticulum and Golgi membranes to form into organized structures.

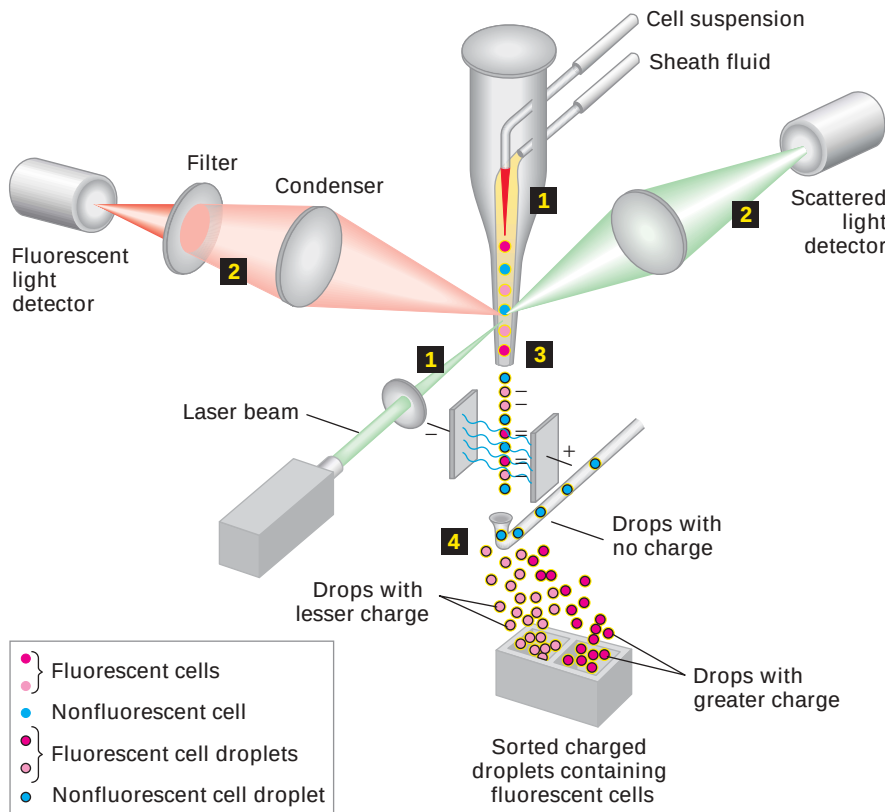
5.5 Purification of Cells and Their Parts

Many studies on cell structure and function require samples of a particular type of cell or subcellular organelle. Most animal and plant tissues, however, contain a mixture of cell types; likewise, most cells are filled with a variety of organelles. In this section, we describe several commonly used techniques for separating different cell types and organelles. The purification of membrane proteins presents some unique problems also considered here.

Flow Cytometry Separates Different Cell Types

Some cell types differ sufficiently in density that they can be separated on the basis of this physical property. White blood cells (leukocytes) and red blood cells (erythrocytes), for instance, have very different densities because erythrocytes have no nucleus; thus these cells can be separated by equilibrium density centrifugation (described shortly). Because most cell types cannot be differentiated so easily, other techniques such as flow cytometry must be used to separate them.

A flow cytometer identifies different cells by measuring the light that they scatter and the fluorescence that they emit as they flow through a laser beam; thus it can sort out cells of a particular type from a mixture. Indeed, a *fluorescence-activated cell sorter (FACS)*, an instrument based on flow cytometry, can select one cell from thousands of other cells (Figure 5-34). For example, if an antibody specific to a certain cell-surface molecule is linked to a fluorescent dye, any



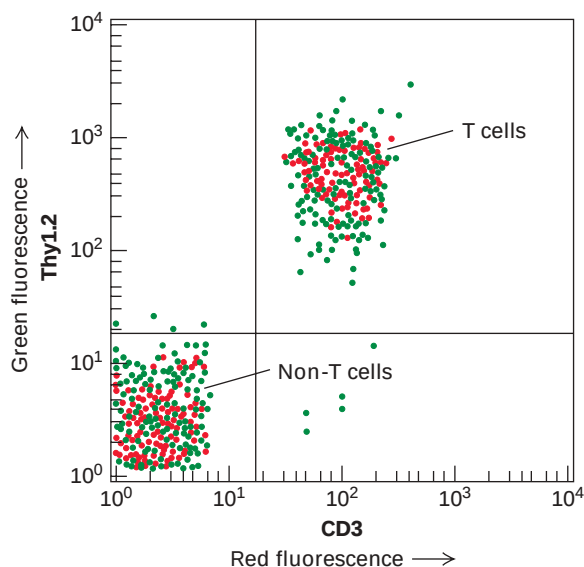
◀ **EXPERIMENTAL FIGURE 5-34**
Fluorescence-activated cell sorter (FACS)
separates cells that are labeled
differentially with a fluorescent reagent.

Step **1**: A concentrated suspension of labeled cells is mixed with a buffer (the sheath fluid) so that the cells pass single-file through a laser light beam. Step **2**: Both the fluorescent light emitted and the light scattered by each cell are measured; from measurements of the scattered light, the size and shape of the cell can be determined. Step **3**: The suspension is then forced through a nozzle, which forms tiny droplets containing at most a single cell. At the time of formation, each droplet is given a negative electric charge proportional to the amount of fluorescence of its cell. Step **4**: Droplets with no charge and those with different electric charges are separated by an electric field and collected. It takes only milliseconds to sort each droplet, and so as many as 10 million cells per hour can pass through the machine. In this way, cells that have desired properties can be separated and then grown. [Adapted from D. R. Parks and L. A. Herzenberg, 1982, *Meth. Cell Biol.* **26**:283.]

cell bearing this molecule will bind the antibody and will then be separated from other cells when it fluoresces in the FACS. Having been sorted from other cells, the selected cells can be grown in culture.

The FACS procedure is commonly used to purify the different types of white blood cells, each of which bears on its surface one or more distinctive proteins and will thus bind monoclonal antibodies specific for that protein. Only the T

cells of the immune system, for instance, have both CD3 and Thy1.2 proteins on their surfaces. The presence of these surface proteins allows T cells to be separated easily from other types of blood cells or spleen cells (Figure 5-35). In a variation of the use of monoclonal antibodies for separating cells, small magnetic beads are coated with a monoclonal antibody specific for a surface protein such as CD3 or Thy1.2. Only cells with these proteins will stick to the beads and can be



◀ **EXPERIMENTAL FIGURE 5-35**
T cells bound to
fluorescence-tagged antibodies to two cell-surface proteins
are separated from other white blood cells by FACS.

Spleen cells from a mouse reacted with a fluorescence-tagged monoclonal antibody (green) specific for the CD3 cell-surface protein and with a fluorescence-tagged monoclonal antibody (red) specific for a second cell-surface protein, Thy1.2. As cells were passed through a FACS machine, the intensity of the green and red fluorescence emitted by each cell was recorded. This plot of the red fluorescence (vertical axis) versus green fluorescence (horizontal axis) for thousands of cells shows that about half of the cells—the T cells—express both CD3 and Thy1.2 proteins on their surfaces (upper-right quadrant). The remaining cells, which exhibit low fluorescence (lower-left quadrant), express only background levels of these proteins and are other types of white blood cells. Note the logarithmic scale on both axes. [Courtesy of Chengcheng Zhang.]

recovered from the preparation by adhesion to a small magnet on the side of the test tube.

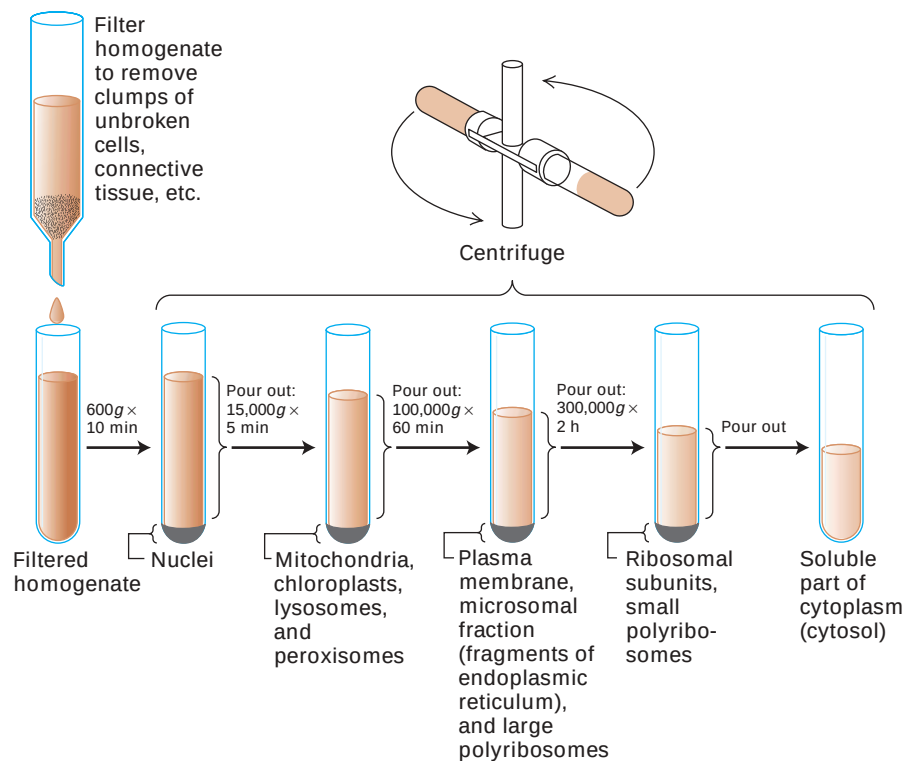
Other uses of flow cytometry include the measurement of a cell's DNA and RNA content and the determination of its general shape and size. The FACS can make simultaneous measurements of the size of a cell (from the amount of scattered light) and the amount of DNA that it contains (from the amount of fluorescence emitted from a DNA-binding dye).

Disruption of Cells Releases Their Organelles and Other Contents

The initial step in purifying subcellular structures is to rupture the plasma membrane and the cell wall, if present. First, the cells are suspended in a solution of appropriate pH and salt content, usually isotonic sucrose (0.25 M) or a combination of salts similar in composition to those in the cell's interior. Many cells can then be broken by stirring the cell suspension in a high-speed blender or by exposing it to ultrahigh-frequency sound (*sonication*). Plasma membranes can also be sheared by special pressurized tissue homogeniz-

ers in which the cells are forced through a very narrow space between the plunger and the vessel wall. As noted earlier, water flows into cells when they are placed in a hypotonic solution (see Figure 5-18). This osmotic flow causes cells to swell, weakening the plasma membrane and facilitating its rupture. Generally, the cell solution is kept at 0 °C to best preserve enzymes and other constituents after their release from the stabilizing forces of the cell.

Disrupting the cell produces a mix of suspended cellular components, the homogenate, from which the desired organelles can be retrieved. Homogenization of the cell and dilution of the cytosol cause the depolymerization of actin microfilaments and microtubules, releasing their monomeric subunits, and shear intermediate filaments into short fragments. Thus other procedures, described in Chapters 19 and 20, are used to study these important constituents. Because rat liver contains an abundance of a single cell type, this tissue has been used in many classic studies of cell organelles. However, the same isolation principles apply to virtually all cells and tissues, and modifications of these cell-fractionation techniques can be used to separate and purify any desired components.



▲ EXPERIMENTAL FIGURE 5-36 Differential centrifugation is a common first step in fractionating a cell homogenate.

The homogenate resulting from disrupting cells is usually filtered to remove unbroken cells and then centrifuged at a fairly low speed to selectively pellet the nucleus—the largest organelle. The undeposited material (the supernatant) is next centrifuged at a higher speed to sediment the mitochondria, chloroplasts, lysosomes, and peroxisomes. Subsequent centrifugation in the

ultracentrifuge at $100,000g$ for 60 minutes results in deposition of the plasma membrane, fragments of the endoplasmic reticulum, and large polyribosomes. The recovery of ribosomal subunits, small polyribosomes, and particles such as complexes of enzymes requires additional centrifugation at still higher speeds. Only the cytosol—the soluble aqueous part of the cytoplasm—remains in the supernatant after centrifugation at $300,000g$ for 2 hours.

Centrifugation Can Separate Many Types of Organelles

In Chapter 3, we considered the principles of centrifugation and the uses of centrifugation techniques for separating proteins and nucleic acids. Similar approaches are used for separating and purifying the various organelles, which differ in both size and density and thus undergo sedimentation at different rates.

Most cell-fractionation procedures begin with *differential centrifugation* of a filtered cell homogenate at increasingly higher speeds (Figure 5-36). After centrifugation at each speed for an appropriate time, the supernatant is poured off and centrifuged at higher speed. The pelleted fractions obtained by differential centrifugation generally contain a mixture of organelles, although nuclei and viral particles can sometimes be purified completely by this procedure. An impure organelle fraction obtained by differential centrifugation can be further purified by *equilibrium density-gradient centrifugation*, which separates cellular components according to their density. After the fraction is resuspended, it is layered on top of a solution that con-

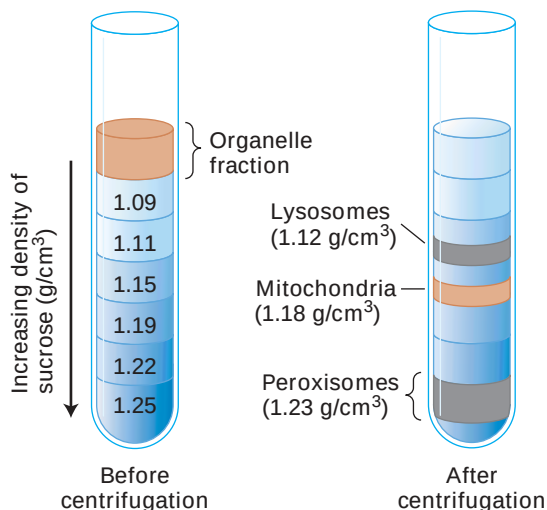
tains a gradient of a dense nonionic substance (e.g., sucrose or glycerol). The tube is centrifuged at a high speed (about 40,000 rpm) for several hours, allowing each particle to migrate to an equilibrium position where the density of the surrounding liquid is equal to the density of the particle (Figure 5-37).

Because each organelle has unique morphological features, the purity of organelle preparations can be assessed by examination in an electron microscope. Alternatively, organelle-specific marker molecules can be quantified. For example, the protein cytochrome *c* is present only in mitochondria; so the presence of this protein in a fraction of lysosomes would indicate its contamination by mitochondria. Similarly, catalase is present only in peroxisomes; acid phosphatase, only in lysosomes; and ribosomes, only in the rough endoplasmic reticulum or the cytosol.

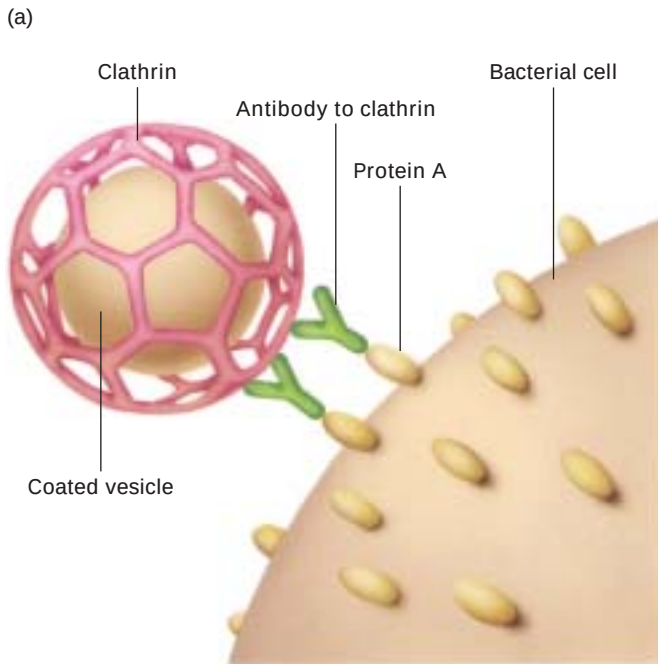
Organelle-Specific Antibodies Are Useful in Preparing Highly Purified Organelles

Cell fractions remaining after differential and equilibrium density-gradient centrifugation may still contain more than one type of organelle. Monoclonal antibodies for various organelle-specific membrane proteins are a powerful tool for further purifying such fractions. One example is the purification of coated vesicles whose outer surface is covered with clathrin (Figure 5-38). An antibody to clathrin, bound to a bacterial carrier, can selectively bind these vesicles in a crude preparation of membranes, and the whole antibody complex can then be isolated by low-speed centrifugation. A related technique uses tiny metallic beads coated with specific antibodies. Organelles that bind to the antibodies, and are thus linked to the metallic beads, are recovered from the preparation by adhesion to a small magnet on the side of the test tube.

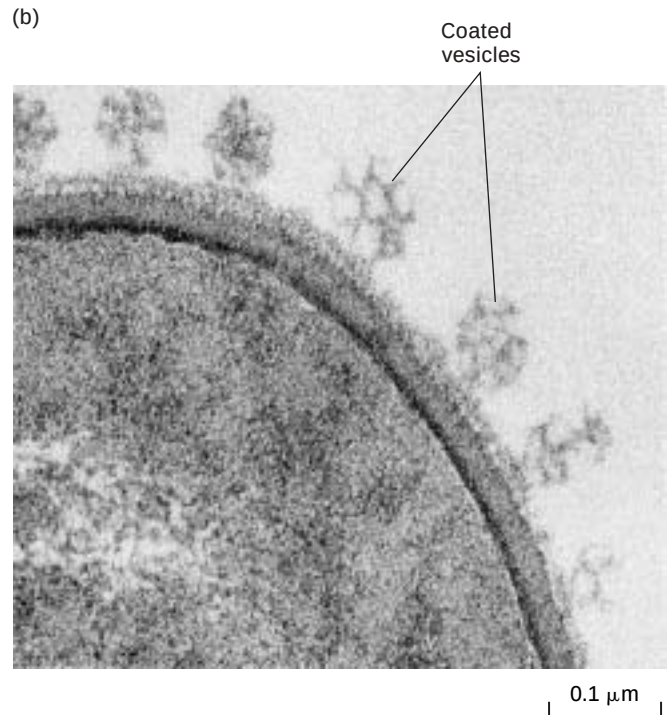
All cells contain a dozen or more different types of small membrane-limited vesicles of about the same size (50–100 nm in diameter) and density. Because of their similar size and density, these vesicles are difficult to separate from one another by centrifugation techniques. Immunological techniques are particularly useful for purifying specific classes of such vesicles. Fat and muscle cells, for instance, contain a particular glucose transporter (GLUT4) that is localized to the membrane of a specific kind of vesicle. When insulin is added to the cells, these vesicles fuse with the cell-surface membrane and increase the number of glucose transporters able to take up glucose from the blood. As will be seen in Chapter 15, this process is critical to maintaining the appropriate concentration of sugar in the blood. The GLUT4-containing vesicles can be purified by using an antibody that binds to a segment of the GLUT4 protein that faces the cytosol. Likewise, the various transport vesicles discussed in Chapter 17 are characterized by unique surface proteins that permit their separation with the aid of specific antibodies.



▲ EXPERIMENTAL FIGURE 5-37 A mixed organelle fraction can be further separated by equilibrium density-gradient centrifugation. In this example, material in the pellet from centrifugation at 15,000g (see Figure 5-36) is resuspended and layered on a gradient of increasingly more dense sucrose solutions in a centrifuge tube. During centrifugation for several hours, each organelle migrates to its appropriate equilibrium density and remains there. To obtain a good separation of lysosomes from mitochondria, the liver is perfused with a solution containing a small amount of detergent before the tissue is disrupted. During this perfusion period, detergent is taken into the cells by endocytosis and transferred to the lysosomes, making them less dense than they would normally be and permitting a “clean” separation of lysosomes from mitochondria.



▲ **EXPERIMENTAL FIGURE 5-38 Small vesicles can be purified by binding of antibody specific for a vesicle surface protein and linkage to bacterial cells.** In this example, a suspension of membranes from rat liver is incubated with an antibody specific for clathrin, a protein that coats the outer surface of certain cytosolic vesicles. To this mixture is added a suspension of *Staphylococcus aureus* bacteria whose surface membrane contains protein A, which binds to the Fc constant



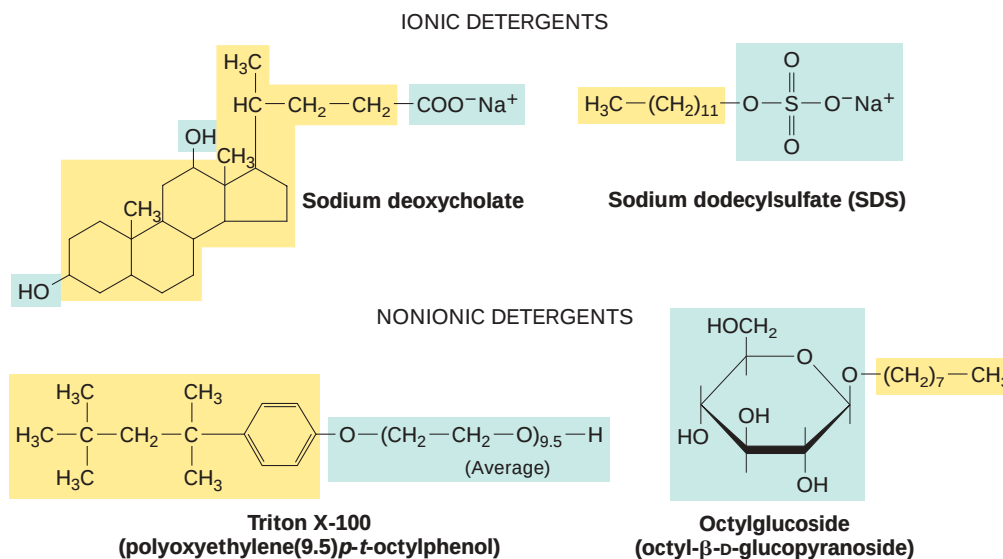
region of antibodies. (a) Interaction of protein A with antibodies bound to clathrin-coated vesicles links the vesicles to the bacterial cells. The vesicle–bacteria complexes can then be recovered by low-speed centrifugation. (b) A thin-section electron micrograph reveals clathrin-coated vesicles bound to an *S. aureus* cell. [See E. Merisko et al., 1982, *J. Cell Biol.* **93**:846. Micrograph courtesy of G. Palade.]

Proteins Can Be Removed from Membranes by Detergents or High-Salt Solutions

Detergents are amphipathic molecules that disrupt membranes by intercalating into phospholipid bilayers and solubilizing lipids and proteins. The hydrophobic part of a detergent molecule is attracted to hydrocarbons and mingles with them readily; the hydrophilic part is strongly attracted to water. Some detergents are natural products, but most are synthetic molecules developed for cleaning and for dispersing mixtures of oil and water (Figure 5-39). Ionic detergents, such as sodium deoxycholate and sodium dodecylsulfate (SDS), contain a charged group; nonionic detergents, such as Triton X-100 and octylglucoside, lack a charged group. At very low concentrations, detergents dissolve in pure water as isolated molecules. As the concentration increases, the molecules begin to form **micelles**—small, spherical aggregates in which hydrophilic parts of the molecules face outward and the hydrophobic parts cluster in the center (see Figure 2-20). The *critical micelle concentration (CMC)* at which micelles form is characteristic of each detergent and is a function of the structures of its hydrophobic and hydrophilic parts.

Ionic detergents bind to the exposed hydrophobic regions of membrane proteins as well as to the hydrophobic cores of water-soluble proteins. Because of their charge, these detergents also disrupt ionic and hydrogen bonds. At high concentrations, for example, sodium dodecylsulfate completely denatures proteins by binding to every side chain, a property that is exploited in SDS gel electrophoresis (see Figure 3-32). *Nonionic detergents* do not denature proteins and are thus useful in extracting proteins from membranes before purifying them. These detergents act in different ways at different concentrations. At high concentrations (above the CMC), they solubilize biological membranes by forming mixed micelles of detergent, phospholipid, and integral membrane proteins (Figure 5-40). At low concentrations (below the CMC), these detergents bind to the hydrophobic regions of most integral membrane proteins, making them soluble in aqueous solution.

Treatment of cultured cells with a buffered salt solution containing a nonionic detergent such as Triton X-100 extracts water-soluble proteins as well as integral membrane proteins. As noted earlier, the exoplasmic and cytosolic domains of integral membrane proteins are generally hydrophilic and sol-



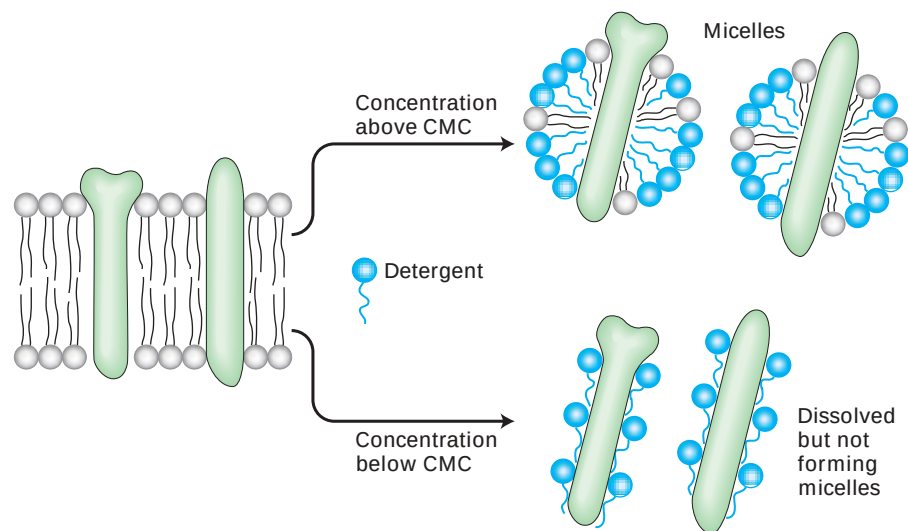
▲ **FIGURE 5-39 Structures of four common detergents.** The hydrophobic part of each molecule is shown in yellow; the hydrophilic part, in blue. The bile salt sodium deoxycholate is a natural product; the others are synthetic. Although ionic

detergents commonly cause denaturation of proteins, nonionic detergents do not and are thus useful in solubilizing integral membrane proteins.

uble in water. The membrane-spanning domains, however, are rich in hydrophobic and uncharged residues (see Figure 5-12). When separated from membranes, these exposed hydrophobic segments tend to interact with one another, causing the protein molecules to aggregate and precipitate from aqueous solutions. The hydrophobic parts of nonionic detergent molecules preferentially bind to the hydrophobic seg-

ments of transmembrane proteins, preventing protein aggregation and allowing the proteins to remain in the aqueous solution. Detergent-solubilized transmembrane proteins can then be purified by affinity chromatography and other techniques used in purifying water-soluble proteins (Chapter 3).

As discussed previously, most peripheral proteins are bound to specific transmembrane proteins or membrane



▲ **FIGURE 5-40 Solubilization of integral membrane proteins by nonionic detergents.** At a concentration higher than its critical micelle concentration (CMC), a detergent solubilizes lipids and integral membrane proteins, forming mixed micelles containing detergent, protein, and lipid molecules. At

concentrations below the CMC, nonionic detergents (e.g., octylglucoside, Triton X-100) can dissolve membrane proteins without forming micelles by coating the membrane-spanning regions.

phospholipids by ionic or other weak interactions. Generally, peripheral proteins can be removed from the membrane by solutions of high ionic strength (high salt concentrations), which disrupt ionic bonds, or by chemicals that bind divalent cations such as Mg^{2+} . Unlike integral proteins, most peripheral proteins are soluble in aqueous solution and need not be solubilized by nonionic detergents.

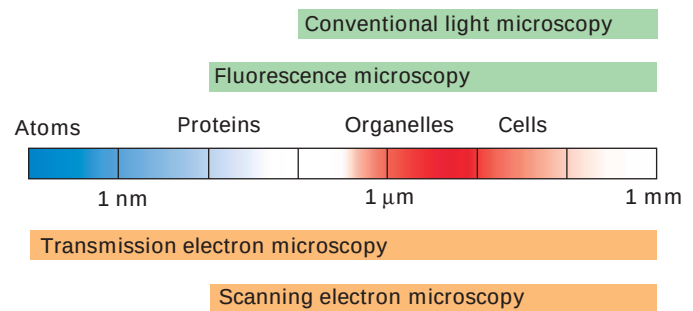
KEY CONCEPTS OF SECTION 5.5

Purification of Cells and Their Parts

- Flow cytometry can identify different cells on the basis of the light that they scatter and the fluorescence that they emit. The fluorescence-activated cell sorter (FACS) is useful in separating different types of cells (see Figures 5-34 and 5-35).
- Disruption of cells by vigorous homogenization, sonication, or other techniques releases their organelles. Swelling of cells in a hypotonic solution weakens the plasma membrane, making it easier to rupture.
- Sequential differential centrifugation of a cell homogenate yields fractions of partly purified organelles that differ in mass and density (see Figure 5-36).
- Equilibrium density-gradient centrifugation, which separates cellular components according to their densities, can further purify cell fractions obtained by differential centrifugation.
- Immunological techniques, using antibodies against organelle-specific membrane proteins, are particularly useful in purifying organelles and vesicles of similar sizes and densities.
- Transmembrane proteins are selectively solubilized and purified with the use of nonionic detergents.

5.6 Visualizing Cell Architecture

In the 1830s, Matthias Schleiden and Theodore Schwann proposed that individual cells constitute the fundamental unit of life. This first formulation of the cell theory was based on observations made with rather primitive light microscopes. Modern cell biologists have many more-powerful tools for revealing cell architecture. For example, variations of standard light microscopy permit scientists to view objects that were undetectable several decades ago. Electron microscopy, which can reveal extremely small objects, has yielded much information about subcellular particles and the organization of plant and animal tissues. Each technique is most suitable for detecting and imaging particular structural features of the cell (Figure 5-41). Digital recording systems and appropriate computer algorithms represent another advance in visualizing cell architecture that has spread widely in the past decade. Digital systems not only



▲ FIGURE 5-41 The range in sizes of objects imaged by different microscopy techniques. The smallest object that can be imaged by a particular technique is limited by the resolving power of the equipment and other factors.

can provide microscopic images of improved quality but also permit three-dimensional reconstructions of cell components from two-dimensional images.

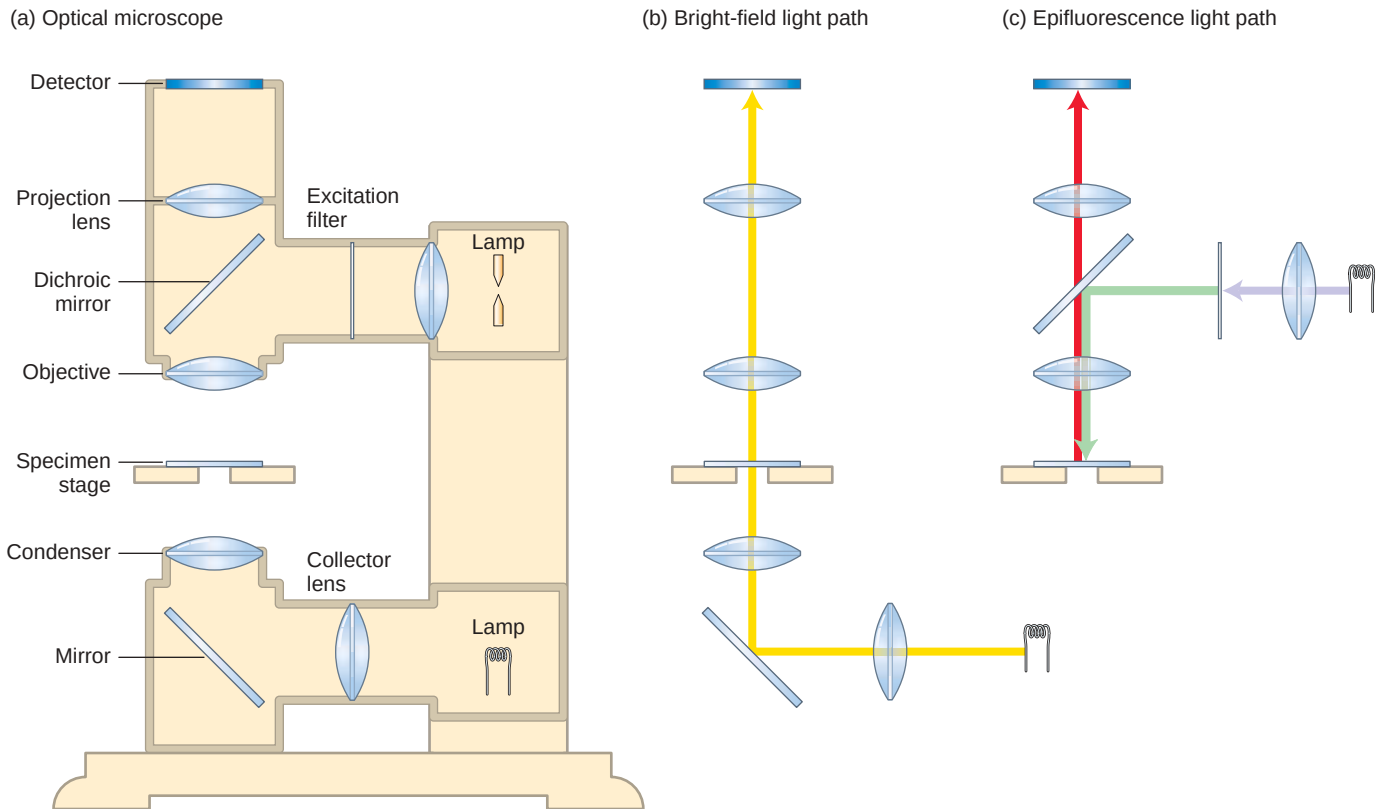
A Microscope Detects, Magnifies, and Resolves Small Objects

All microscopes produce a magnified image of a small object, but the nature of the images depends on the type of microscope employed and on the way in which the specimen is prepared. The compound microscope, used in conventional *bright-field light microscopy*, contains several lenses that magnify the image of a specimen under study (Figure 5-42a, b). The total magnification is a product of the magnification of the individual lenses: if the objective lens magnifies 100-fold (a 100× lens, the maximum usually employed) and the projection lens, or eyepiece, magnifies 10-fold, the final magnification recorded by the human eye or on film will be 1000-fold.

However, the most important property of any microscope is not its magnification but its resolving power, or **resolution**—the ability to distinguish between two very closely positioned objects. Merely enlarging the image of a specimen accomplishes nothing if the image is blurred. The resolution of a microscope lens is numerically equivalent to D , the minimum distance between two distinguishable objects. The smaller the value of D , the better the resolution. The value of D is given by the equation

$$D = \frac{0.61\lambda}{N \sin \alpha} \quad (5-1)$$

where α is the angular aperture, or half-angle, of the cone of light entering the objective lens from the specimen; N is the refractive index of the medium between the specimen and the objective lens (i.e., the relative velocity of light in the medium compared with the velocity in air); and λ is the wavelength of the incident light. Resolution is improved by using shorter wavelengths of light (decreasing the value of



▲ **EXPERIMENTAL FIGURE 5-42 Optical microscopes are commonly configured for both bright-field (transmitted) and epifluorescence microscopy.** (a) In a typical light microscope, the specimen is usually mounted on a transparent glass slide and positioned on the movable specimen stage. The two imaging methods require separate illumination systems but use the same light gathering and detection systems. (b) In bright-field light microscopy, light from a tungsten lamp is focused on the

specimen by a condenser lens below the stage; the light travels the pathway shown. (c) In epifluorescence microscopy, ultraviolet light from a mercury lamp positioned above the stage is focused on the specimen by the objective lens. Filters in the light path select a particular wavelength of ultraviolet light for illumination and are matched to capture the wavelength of the emitted light by the specimen.

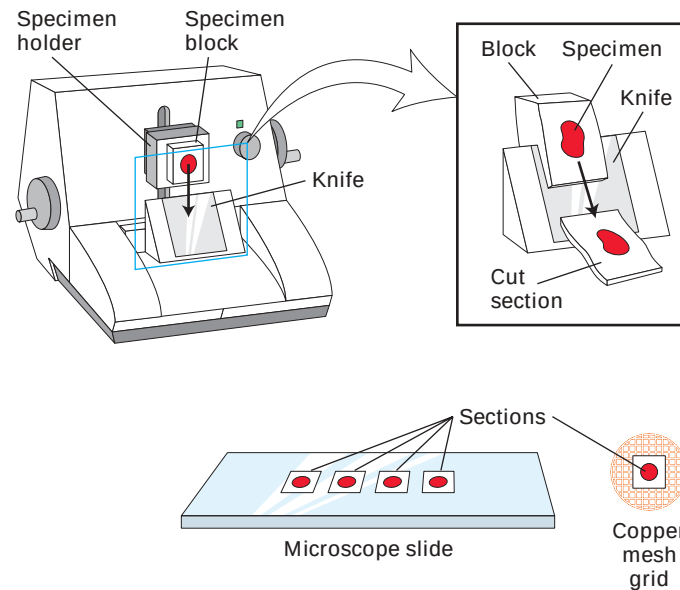
λ) or gathering more light (increasing either N or α). Note that the magnification is not part of this equation.

Owing to limitations on the values of α , λ , and N , the *limit of resolution* of a light microscope using visible light is about $0.2\ \mu\text{m}$ (200 nm). No matter how many times the image is magnified, the microscope can never resolve objects that are less than $\approx 0.2\ \mu\text{m}$ apart or reveal details smaller than $\approx 0.2\ \mu\text{m}$ in size. Despite this limit on resolution, the light microscope can be used to track the location of a small bead of known size to a precision of only a few nanometers. If we know the precise size and shape of an object—say, a 5-nm sphere of gold—and if we use a video camera to record the microscopic image as a digital image, then a computer can calculate the position of the center of the object to within a few nanometers. This technique has been used to measure nanometer-size steps as molecules and vesicles move along cytoskeletal filaments (see Figures 19-17, 19-18, and 20-18).

Samples for Microscopy Must Be Fixed, Sectioned, and Stained to Image Subcellular Details

Live cells and tissues lack compounds that absorb light and are thus nearly invisible in a light microscope. Although such specimens can be visualized by special techniques to be discussed shortly, these methods do not reveal the fine details of structure and require cells to be housed in special glass-faced chambers, called culture chambers, that can be mounted on a microscope stage. For these reasons, cells are often fixed, sectioned, and stained to reveal subcellular structures.

Specimens for light and electron microscopy are commonly fixed with a solution containing chemicals that cross-link most proteins and nucleic acids. Formaldehyde, a common fixative, cross-links amino groups on adjacent molecules; these covalent bonds stabilize protein–protein and protein–nucleic acid interactions and render the molecules



▲ **EXPERIMENTAL FIGURE 5-43 Tissues for microscopy are commonly fixed, embedded in a solid medium, and cut into thin sections.** A fixed tissue is dehydrated by soaking in a series of alcohol-water solutions, ending with an organic solvent compatible with the embedding medium. To embed the tissue for sectioning, the tissue is placed in liquid paraffin for light microscopy or in liquid plastic for electron microscopy; after the

block containing the specimen has hardened, it is mounted on the arm of a microtome and slices are cut with a knife. Typical sections cut for electron microscopy 50–100 nm thick; sections cut for light microscopy are 0.5–50 μm thick. The sections are collected either on microscope slides (light microscopy) or copper mesh grids (electron microscopy) and stained with an appropriate agent.

insoluble and stable for subsequent procedures. After fixation, a sample is usually embedded in paraffin or plastic and cut into sections 0.5–50 μm thick (Figure 5-43). Alternatively, the sample can be frozen without prior fixation and then sectioned; such treatment preserves the activity of enzymes for later detection by cytochemical reagents.

A final step in preparing a specimen for light microscopy is to stain it so as to visualize the main structural features of the cell or tissue. Many chemical stains bind to molecules that have specific features. For example, *hematoxylin* binds to basic amino acids (lysine and arginine) on many different kinds of proteins, whereas *eosin* binds to acidic molecules (such as DNA and side chains of aspartate and glutamate). Because of their different binding properties, these dyes stain various cell types sufficiently differently that they are distinguishable visually. If an enzyme catalyzes a reaction that produces a colored or otherwise visible precipitate from a colorless precursor, the enzyme may be detected in cell sections by their colored reaction products. Such staining techniques, although once quite common, have been largely replaced by other techniques for visualizing particular proteins or structures as discussed next.

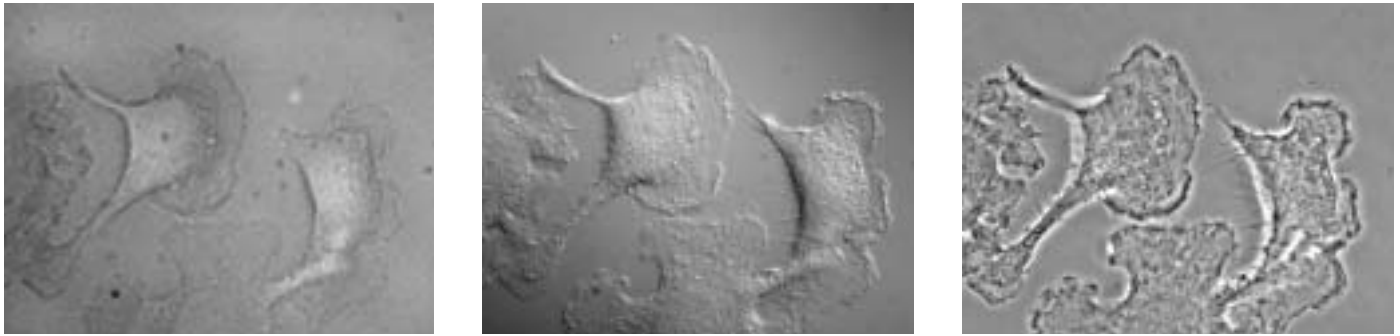
Phase-Contrast and Differential Interference Contrast Microscopy Visualize Unstained Living Cells

Two common methods for imaging live cells and unstained tissues generate contrast by taking advantage of differences

in the refractive index and thickness of cellular materials. These methods, called *phase-contrast microscopy* and *differential interference contrast (DIC) microscopy* (or Nomarski interference microscopy), produce images that differ in appearance and reveal different features of cell architecture. Figure 5-44 compares images of live, cultured cells obtained with these two methods and standard bright-field microscopy.

In phase-contrast images, the entire object and subcellular structures are highlighted by interference rings—concentric halos of dark and light bands. This artifact is inherent in the method, which generates contrast by interference between diffracted and undiffracted light by the specimen. Because the interference rings around an object obscure many details, this technique is suitable for observing only single cells or thin cell layers but not thick tissues. It is particularly useful for examining the location and movement of larger organelles in live cells.

DIC microscopy is based on interference between polarized light and is the method of choice for visualizing extremely small details and thick objects. Contrast is generated by differences in the index of refraction of the object and its surrounding medium. In DIC images, objects appear to cast a shadow to one side. The “shadow” primarily represents a difference in the refractive index of a specimen rather than its topography. DIC microscopy easily defines the outlines of large organelles, such as the nucleus and vacuole. In addition to having a “relief”-like appearance, a DIC image is a thin *optical section*, or slice, through the object. Thus details of the nucleus



▲ **EXPERIMENTAL FIGURE 5-44** Live cells can be visualized by microscopy techniques that generate contrast by interference. These micrographs show live, cultured macrophage cells viewed by bright-field microscopy (*left*), phase-contrast microscopy (*middle*), and differential interference contrast (DIC) microscopy (*right*). In a phase-contrast image, cells

are surrounded by alternating dark and light bands; in-focus and out-of-focus details are simultaneously imaged in a phase-contrast microscope. In a DIC image, cells appear in pseudorelief. Because only a narrow in-focus region is imaged, a DIC image is an optical slice through the object. [Courtesy of N. Watson and J. Evans.]

in thick specimens (e.g., an intact *Caenorhabditis elegans* roundworm) can be observed in a series of such optical sections, and the three-dimensional structure of the object can be reconstructed by combining the individual DIC images.

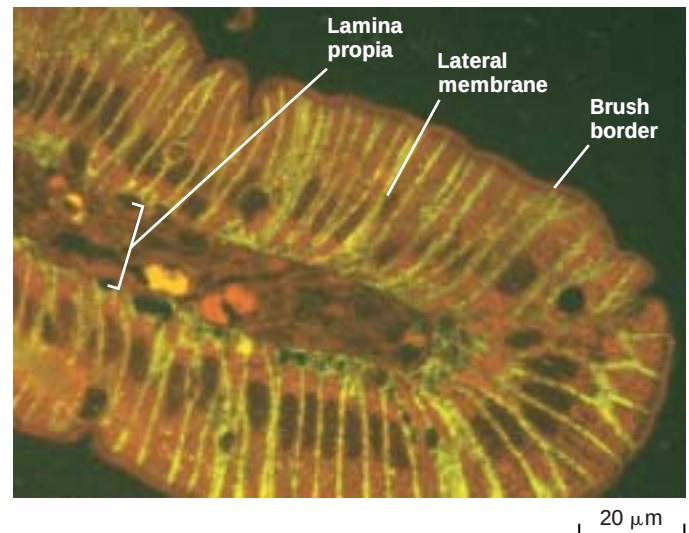
Fluorescence Microscopy Can Localize and Quantify Specific Molecules in Fixed and Live Cells

Perhaps the most versatile and powerful technique for localizing proteins within a cell by light microscopy is **fluorescent staining** of cells and observation by *fluorescence microscopy*. A chemical is said to be fluorescent if it absorbs light at one wavelength (the excitation wavelength) and emits light (fluoresces) at a specific and longer wavelength. Most fluorescent dyes, or **fluorochromes**, emit visible light, but some (such as Cy5 and Cy7) emit infrared light. In modern fluorescence microscopes, only fluorescent light emitted by the sample is used to form an image; light of the exciting wavelength induces the fluorescence but is then not allowed to pass the filters placed between the objective lens and the eye or camera (see Figure 5-42a, c).

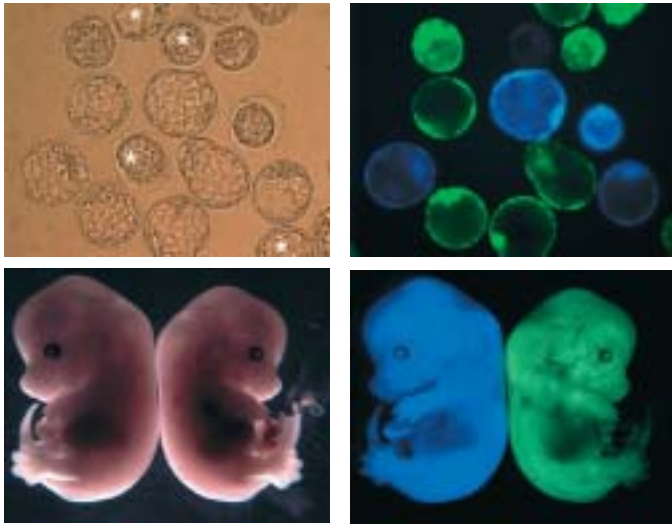
Immunological Detection of Specific Proteins in Fixed Cells

The common chemical dyes just mentioned stain nucleic acids or broad classes of proteins. However, investigators often want to detect the presence and location of specific proteins. A widely used method for this purpose employs specific antibodies covalently linked to fluorochromes. Commonly used fluorochromes include rhodamine and Texas red, which emit red light; Cy3, which emits orange light; and fluorescein, which emits green light. These fluorochromes can be chemically coupled to purified antibodies specific for almost any desired macromolecule. When a fluorochrome–antibody complex is added to a permeabilized cell or tissue section, the complex will bind to the corresponding antigens, which then

light up when illuminated by the exciting wavelength, a technique called *immunofluorescence microscopy* (Figure 5-45). Staining a specimen with two or three dyes that fluoresce at different wavelengths allows multiple proteins to be localized within a cell (see Figure 5-33).



▲ **EXPERIMENTAL FIGURE 5-45** One or more specific proteins can be localized in fixed tissue sections by immunofluorescence microscopy. A section of the rat intestinal wall was stained with Evans blue, which generates a nonspecific red fluorescence, and with a yellow green–fluorescing antibody specific for GLUT2, a glucose transport protein. As evident from this fluorescence micrograph, GLUT2 is present in the basal and lateral sides of the intestinal cells but is absent from the brush border, composed of closely packed microvilli on the apical surface facing the intestinal lumen. Capillaries run through the lamina propria, a loose connective tissue beneath the epithelial layer. [See B. Thorens et al., 1990, *Am. J. Physiol.* **259**:C279; courtesy of B. Thorens.]



▲ **EXPERIMENTAL FIGURE 5-46 Expression of fluorescent proteins in early and late mouse embryos is detected by emitted blue and yellow light.** The genes encoding blue fluorescent protein (ECFP) and yellow fluorescent protein (EYFP) were introduced into mouse embryonic stem cells, which then were grown into early-stage embryos (*top*) and late-stage embryos (*bottom*). These bright-field (*left*) and fluorescence (*right*) micrographs reveal that all but four of the early-stage embryos display a blue or yellow fluorescence, indicating expression of the introduced ECFP and EYFP genes. Of the two late-stage embryos shown, one expressed the ECFP gene (*left*) and one expressed the EYFP gene (*right*). [From A.-K. Hadjantonakis et al., 2002, *BMC Biotechnol.* 2:11.]

Expression of Fluorescent Proteins in Live Cells A naturally fluorescent protein found in the jellyfish *Aequorea victoria* can be exploited to visualize live cells and specific proteins within them. This 238-residue protein, called green fluorescent protein (GFP), contains a serine, tyrosine, and glycine sequence whose side chains have spontaneously cyclized to form a green-fluorescing chromophore. With the use of recombinant DNA techniques discussed in Chapter 9, the GFP gene can be introduced into living cultured cells or into specific cells of an entire animal. Cells containing the introduced gene will express GFP and thus emit a green fluorescence when irradiated; this GFP fluorescence can be used to localize the cells within a tissue. Figure 5-46 illustrates the results of this approach, in which a variant of GFP that emits blue fluorescence was used.

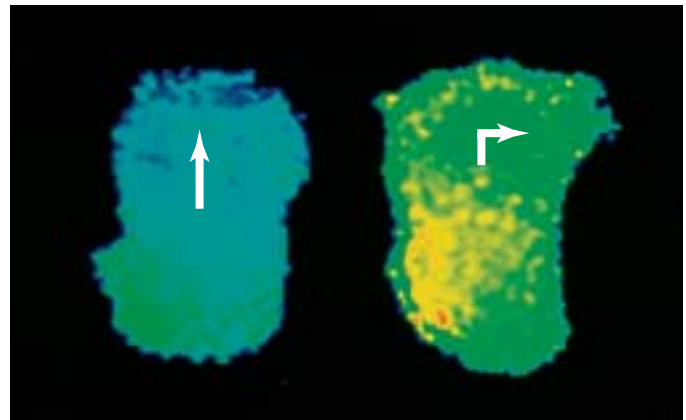
In a particularly useful application of GFP, a cellular protein of interest is “tagged” with GFP to localize it. In this technique, the gene for GFP is fused to the gene for a particular cellular protein, producing a recombinant DNA encoding one long chimeric protein that contains the entirety of both proteins. Cells in which this recombinant DNA has been introduced will synthesize the chimeric protein whose green fluorescence reveals the subcellular location of the protein of interest. This GFP-tagging technique, for example, has been

used to visualize the expression and distribution of specific proteins that mediate cell–cell adhesion (see Figure 6-8).

In some cases, a purified protein chemically linked to a fluorescent dye can be microinjected into cells and followed by fluorescence microscopy. For example, findings from careful biochemical studies have established that purified actin “tagged” with a fluorochemical is indistinguishable in function from its normal counterpart. When the tagged protein is microinjected into a cultured cell, the endogenous cellular and injected tagged actin monomers copolymerize into normal long actin fibers. This technique can also be used to study individual microtubules within a cell.

Determination of Intracellular Ca^{2+} and H^{+} Levels with Ion-Sensitive Fluorescent Dyes Fluorochemicals whose fluorescence depends on the concentration of Ca^{2+} or H^{+} have proved useful in measuring the concentration of these ions within live cells. As discussed in later chapters, intracellular Ca^{2+} and H^{+} concentrations have pronounced effects on many cellular processes. For instance, many hormones or other stimuli cause a rise in cytosolic Ca^{2+} from the resting level of about 10^{-7} M to 10^{-6} M, which induces various cellular responses including the contraction of muscle.

The fluorescent dye *fura-2*, which is sensitive to Ca^{2+} , contains five carboxylate groups that form ester linkages with ethanol. The resulting *fura-2* ester is lipophilic and can



▲ **EXPERIMENTAL FIGURE 5-47 Fura-2, a Ca^{2+} -sensitive fluorochemical, can be used to monitor the relative cytosolic Ca^{2+} concentrations in different regions of live cells.** (*Left*) In a moving leukocyte, a Ca^{2+} gradient is established. The highest levels (green) are at the rear of the cell, where cortical contractions take place, and the lowest levels (blue) are at the cell front, where actin undergoes polymerization. (*Right*) When a pipette filled with chemotactic molecules placed to the side of the cell induces the cell to turn, the Ca^{2+} concentration momentarily increases throughout the cytoplasm and a new gradient is established. The gradient is oriented such that the region of lowest Ca^{2+} (blue) lies in the direction that the cell will turn, whereas a region of high Ca^{2+} (yellow) always forms at the site that will become the rear of the cell. [From R. A. Brundage et al., 1991, *Science* 254:703; courtesy of F. Fay.]

diffuse from the medium across the plasma membrane into cells. Within the cytosol, esterases hydrolyze fura-2 ester, yielding fura-2, whose free carboxylate groups render the molecule nonlipophilic, and so it cannot cross cellular membranes and remains in the cytosol. Inside cells, each fura-2 molecule can bind a single Ca^{2+} ion but no other cellular cation. This binding, which is proportional to the cytosolic Ca^{2+} concentration over a certain range, increases the fluorescence of fura-2 at one particular wavelength. At a second wavelength, the fluorescence of fura-2 is the same whether or not Ca^{2+} is bound and provides a measure of the total amount of fura-2 in a region of the cell. By examining cells continuously in the fluorescence microscope and measuring rapid changes in the ratio of fura-2 fluorescence at these two wavelengths, one can quantify rapid changes in the fraction of fura-2 that has a bound Ca^{2+} ion and thus in the concentration of cytosolic Ca^{2+} (Figure 5-47).

Similarly to fura-2, fluorescent dyes (e.g., SNARF-1) that are sensitive to the H^+ concentration can be used to monitor the cytosolic pH of living cells.

Confocal Scanning and Deconvolution Microscopy Provide Sharp Images of Three-Dimensional Objects

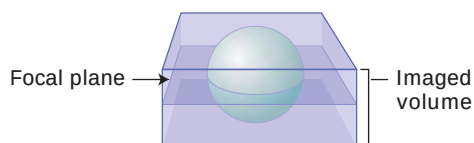
Conventional fluorescence microscopy has two major limitations. First, the physical process of cutting a section destroys material, and so in consecutive (serial) sectioning a

small part of a cell's structure is lost. Second, the fluorescent light emitted by a sample comes from molecules above and below the plane of focus; thus the observer sees a blurred image caused by the superposition of fluorescent images from molecules at many depths in the cell. The blurring effect makes it difficult to determine the actual three-dimensional molecular arrangement (Figure 5-48a). Two powerful refinements of fluorescence microscopy produce much sharper images by reducing the image-degrading effects of out-of-focus light.

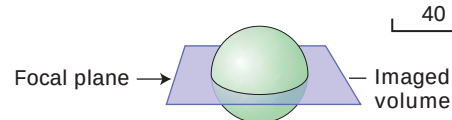
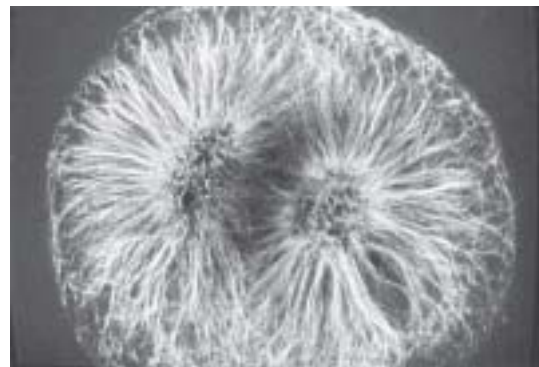
In *confocal scanning microscopy*, exciting light from a focused laser beam illuminates only a single small part of a sample for an instant and then rapidly moves to different spots in the sample focal plane. The emitted fluorescent light passes through a pinhole that rejects out-of-focus light, thereby producing a sharp image. Because light in focus with the image is collected by the pinhole, the scanned area is an optical section through the specimen. The intensity of light from these in-focus areas is recorded by a photomultiplier tube, and the image is stored in a computer (Figure 5-48b).

Deconvolution microscopy achieves the same image-sharpening effect as confocal scanning microscopy but through a different process. In this method, images from consecutive focal planes of the specimen are collected. A separate focal series of images from a test slide of subresolution size (i.e., $0.2\ \mu\text{m}$ diameter) bead are also collected. Each bead represents a pinpoint of light that becomes an object blurred by the imperfect optics of the microscope. Deconvolution

(a) Conventional fluorescence microscopy

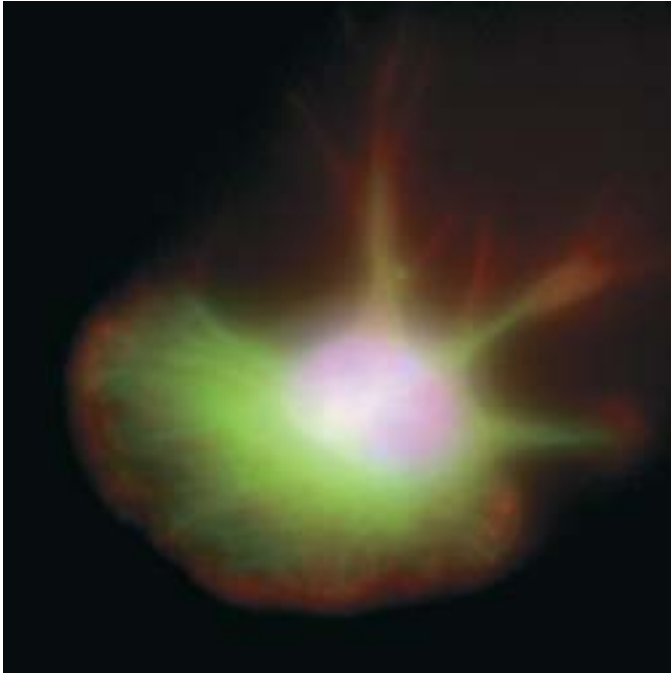


(b) Confocal fluorescence microscopy

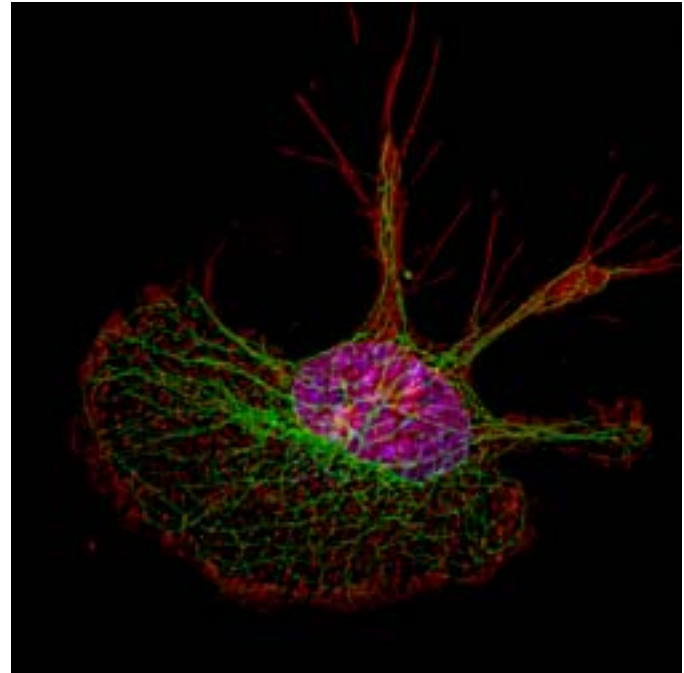


▲ **EXPERIMENTAL FIGURE 5-48 Confocal microscopy produces an in-focus optical section through thick cells.** A mitotic fertilized egg from a sea urchin (*Psammechinus*) was lysed with a detergent, exposed to an anti-tubulin antibody, and then exposed to a fluorescein-tagged antibody that binds to the first antibody. (a) When viewed by conventional fluorescence microscopy, the mitotic spindle is blurred. This blurring occurs

because background fluorescence is detected from tubulin above and below the focal plane as depicted in the sketch. (b) The confocal microscopic image is sharp, particularly in the center of the mitotic spindle. In this case, fluorescence is detected only from molecules in the focal plane, generating a very thin optical section. [Micrographs from J. G. White et al., 1987, *J. Cell Biol.* **104**:41]



▲ **EXPERIMENTAL FIGURE 5-49 Deconvolution fluorescence microscopy yields high-resolution optical sections that can be reconstructed into one three-dimensional image.** A macrophage cell was stained with fluorochrome-labeled reagents specific for DNA (blue), microtubules (green), and actin microfilaments (red). The series of fluorescent images obtained at consecutive focal planes (optical



sections) through the cell were recombined in three dimensions. (a) In this three-dimensional reconstruction of the raw images, the DNA, microtubules, and actin appear as diffuse zones in the cell. (b) After application of the deconvolution algorithm to the images, the fibrillar organization of microtubules and the localization of actin to adhesions become readily visible in the reconstruction. [Courtesy of J. Evans.]

reverses the degradation of the image by using the blurred beads as a reference object. The out-of-focus light is mathematically reassigned with the aid of deconvolution algorithms. Images restored by deconvolution display impressive detail without any blurring (Figure 5-49). Astronomers use deconvolution algorithms to sharpen images of distant stars.

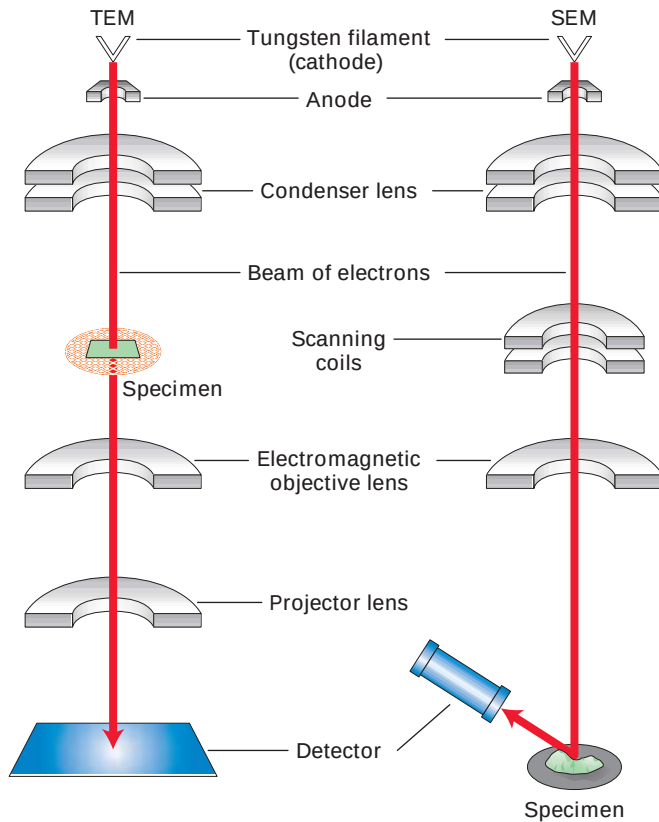
Resolution of Transmission Electron Microscopy Is Vastly Greater Than That of Light Microscopy

The fundamental principles of electron microscopy are similar to those of light microscopy; the major difference is that electromagnetic lenses, rather than optical lenses, focus a high-velocity electron beam instead of visible light. In the *transmission electron microscope (TEM)*, electrons are emitted from a filament and accelerated in an electric field. A condenser lens focuses the electron beam onto the sample; objective and projector lenses focus the electrons that pass through the specimen and project them onto a viewing screen or other detector (Figure 5-50, *left*). Because electrons are absorbed by atoms in air, the entire tube between the electron source and the detector is maintained under an ultrahigh vacuum.

The short wavelength of electrons means that the limit of resolution for the transmission electron microscope is the-

oretically 0.005 nm (less than the diameter of a single atom), or 40,000 times better than the resolution of the light microscope and 2 million times better than that of the unaided human eye. However, the effective resolution of the transmission electron microscope in the study of biological systems is considerably less than this ideal. Under optimal conditions, a resolution of 0.10 nm can be obtained with transmission electron microscopes, about 2000 times better than the best resolution of light microscopes. Several examples of cells and subcellular structures imaged by TEM are included in Section 5.3.

Because TEM requires very thin, fixed sections (about 50 nm), only a small part of a cell can be observed in any one section. Sectioned specimens are prepared in a manner similar to that for light microscopy, by using a knife capable of producing sections 50–100 nm in thickness (see Figure 5-43). The generation of the image depends on differential scattering of the incident electrons by molecules in the preparation. Without staining, the beam of electrons passes through a specimen uniformly, and so the entire sample appears uniformly bright with little differentiation of components. To obtain useful images by TEM, sections are commonly stained with heavy metals such as gold or osmium. Metal-stained areas appear dark on a micrograph because the metals scatter (diffract) most of the incident

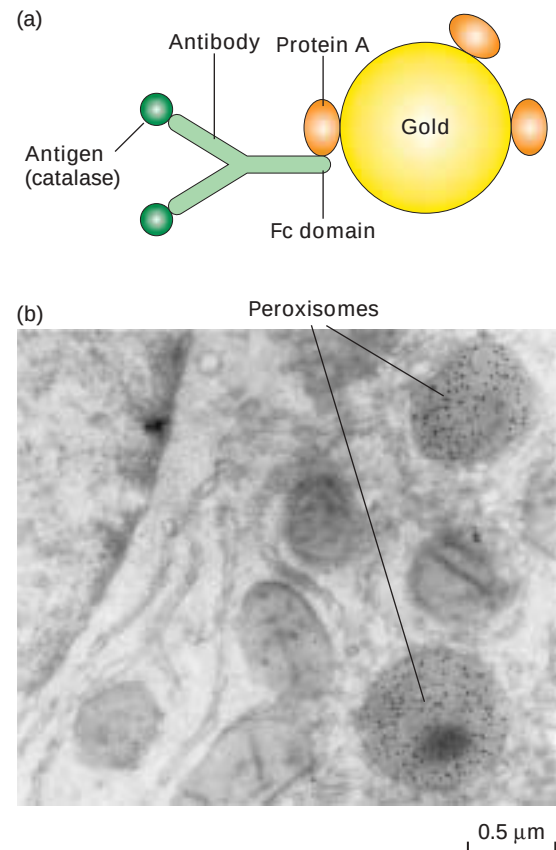


▲ EXPERIMENTAL FIGURE 5-50 In electron microscopy, images are formed from electrons that pass through a specimen or are released from a metal-coated specimen. In a transmission electron microscope (TEM), electrons are extracted from a heated filament, accelerated by an electric field, and focused on the specimen by a magnetic condenser lens. Electrons that pass through the specimen are focused by a series of magnetic objective and projector lenses to form a magnified image of the specimen on a detector, which may be a fluorescent viewing screen, photographic film, or a charged-couple-device (CCD) camera. In a scanning electron microscope (SEM), electrons are focused by condenser and objective lenses on a metal-coated specimen. Scanning coils move the beam across the specimen, and electrons from the metal are collected by a photomultiplier tube detector. In both types of microscopes, because electrons are easily scattered by air molecules, the entire column is maintained at a very high vacuum.

electrons; scattered electrons are not focused by the electromagnetic lenses and do not contribute to the image. Areas that take up less stain appear lighter. Osmium tetroxide preferentially stains certain cellular components, such as membranes (see Figure 5-2a). Specific proteins can be detected in thin sections by the use of electron-dense gold particles coated with protein A, a bacterial protein that binds antibody molecules nonspecifically (Figure 5-51).

Standard electron microscopy cannot be used to study live cells because they are generally too vulnerable to the required conditions and preparatory techniques. In particular, the absence of water causes macromolecules to become de-

natured and nonfunctional. However, the technique of *cryo-electron microscopy* allows examination of hydrated, unfixed, and unstained biological specimens directly in a transmission electron microscope. In this technique, an aqueous suspension of a sample is applied in an extremely thin film to a grid. After it has been frozen in liquid nitrogen and maintained in this state by means of a special mount, the sample is observed in the electron microscope. The very low temperature (-196°C) keeps water from evaporating, even in a vacuum, and the sample can be observed in detail in its native, hydrated state without fixing or heavy metal

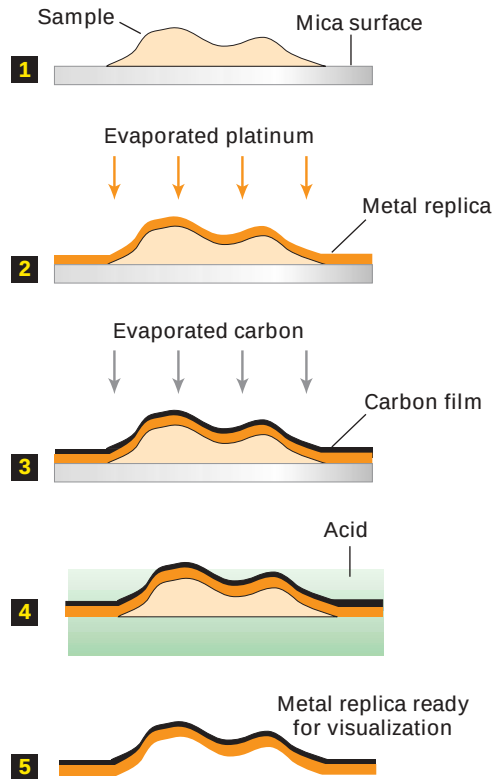


▲ EXPERIMENTAL FIGURE 5-51 Gold particles coated with protein A are used to detect an antibody-bound protein by transmission electron microscopy. (a) First antibodies are allowed to interact with their specific antigen (e.g., catalase) in a section of fixed tissue. Then the section is treated with a complex of protein A from the bacterium *S. aureus* and electron-dense gold particles. Binding of this complex to the Fc domains of the antibody molecules makes the location of the target protein, catalase in this case, visible in the electron microscope. (b) A slice of liver tissue was fixed with glutaraldehyde, sectioned, and then treated as described in part (a) to localize catalase. The gold particles (black dots) indicating the presence of catalase are located exclusively in peroxisomes. [From H. J. Geuze et al., 1981, *J. Cell Biol.* **89**:653. Reproduced from the *Journal of Cell Biology* by copyright permission of The Rockefeller University Press.]

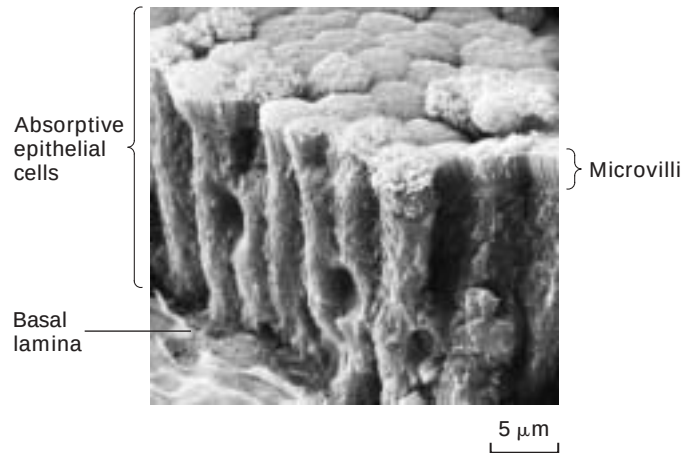
staining. By computer-based averaging of hundreds of images, a three-dimensional model almost to atomic resolution can be generated. For example, this method has been used to generate models of ribosomes (see Figure 4-27), the muscle calcium pump discussed in Chapter 7, and other large proteins that are difficult to crystallize.

Electron Microscopy of Metal-Coated Specimens Can Reveal Surface Features of Cells and Their Components

Transmission electron microscopy is also used to obtain information about the shapes of purified viruses, fibers, enzymes, and other subcellular particles by using a technique, called *metal shadowing*, in which a thin layer of metal, such



▲ **EXPERIMENTAL FIGURE 5-52 Metal shadowing makes surface details on very small particles visible by transmission electron microscopy.** The sample is spread on a mica surface and then dried in a vacuum evaporator (**1**). A filament of a heavy metal, such as platinum or gold, is heated electrically so that the metal evaporates and some of it falls over the sample grid in a very thin film (**2**). To stabilize the replica, the specimen is then coated with a carbon film evaporated from an overhead electrode (**3**). The biological material is then dissolved by acid (**4**), leaving a metal replica of the sample (**5**), which is viewed in a TEM. In electron micrographs of such preparations, the carbon-coated areas appear light—the reverse of micrographs of simple metal-stained preparations in which the areas of heaviest metal staining appear the darkest.



▲ **EXPERIMENTAL FIGURE 5-53 Scanning electron microscopy (SEM) produces a three-dimensional image of the surface of an unsectioned specimen.** Shown here is an SEM image of the epithelium lining the lumen of the intestine. Abundant fingerlike microvilli extend from the lumen-facing surface of each cell. The basal lamina beneath the epithelium helps support and anchor it to the underlying connective tissue (Chapter 6). Compare this image of intestinal cells with those in Figure 5-28, a transmission electron micrograph, and in Figure 5-45, a fluorescence micrograph. [From R. Kessel and R. Kardon, 1979, *Tissues and Organs, A Text-Atlas of Scanning Electron Microscopy*, W. H. Freeman and Company, p. 176.]

as platinum, is evaporated on a fixed and sectioned or rapidly frozen biological sample (Figure 5-52). Acid treatment dissolves away the cell, leaving a metal replica that is viewed in a transmission electron microscope.

Alternatively, the *scanning electron microscope* allows investigators to view the surfaces of unsectioned metal-coated specimens. An intense electron beam inside the microscope scans rapidly over the sample. Molecules in the coating are excited and release secondary electrons that are focused onto a scintillation detector; the resulting signal is displayed on a cathode-ray tube (see Figure 5-50, *right*). Because the number of secondary electrons produced by any one point on the sample depends on the angle of the electron beam in relation to the surface, the scanning electron micrograph has a three-dimensional appearance (Figure 5-53). The resolving power of scanning electron microscopes, which is limited by the thickness of the metal coating, is only about 10 nm, much less than that of transmission instruments.

Three-Dimensional Models Can Be Constructed from Microscopy Images

In the past decade, digital cameras have largely replaced optical cameras to record microscopy images. Digital images can be stored in a computer and manipulated by conventional photographic software as well as specialized algorithms. As mentioned earlier, the deconvolution algorithm

can sharpen an image by restoring out-of-focus photons to their origin—an example of a computational method that improves the quality of the image. The details in stored digital images also can be quantified, and objects in images can be reconstructed in three dimensions. For example, the three-dimensional model of an object can be calculated by tomographic methods from a collection of images that cover different views of the object. In light microscopy, a stack of optical sections collected with either a confocal or a deconvolution microscope can be recombined into one three-dimensional image (see Figure 5-49). If a TEM specimen is tilted through various degrees, the resulting images also can be recombined to generate a three-dimensional view of the object (see Figure 5-23).

KEY CONCEPTS OF SECTION 5.6

Visualizing Cell Architecture

- The limit of resolution of a light microscope is about 200 nm; of a scanning electron microscope, about 10 nm; and of a transmission electron microscope, about 0.1 nm.
- Because cells and tissues are almost transparent, various types of stains and optical techniques are used to generate sufficient contrast for imaging.
- Phase-contrast and differential interference contrast (DIC) microscopy are used to view the details of live, unstained cells and to monitor cell movement.
- In immunofluorescence microscopy, specific proteins and organelles in fixed cells are stained with fluorescence-labeled monoclonal antibodies. Multiple proteins can be localized in the same sample by staining with antibodies labeled with different fluorochromes.
- When proteins tagged with naturally occurring green fluorescent protein (GFP) or its variants are expressed in live cells, they can be visualized in a fluorescence microscope.
- With the use of dyes whose fluorescence is proportional to the concentration of Ca^{2+} or H^+ ions, fluorescence microscopy can measure the local concentration of Ca^{2+} ions and intracellular pH in living cells.
- Confocal microscopy and deconvolution microscopy use different methods to optically section a specimen, thereby reducing the blurring due to out-of-focus fluorescence light. Both methods provide much sharper images, particularly of thick specimens, than does standard fluorescence microscopy.
- Specimens for electron microscopy generally must be fixed, sectioned, dehydrated, and then stained with electron-dense heavy metals.
- Surface details of objects can be revealed by transmission electron microscopy of metal-coated specimens. Scanning electron microscopy of metal-coated unsectioned cells or tissues produces images that appear to be three-dimensional.

PERSPECTIVES FOR THE FUTURE

Advances in bioengineering will make major contributions not only to our understanding of cell and tissue function but also to the quality of human health. In a glass slide consisting of microfabricated wells and channels, for example, reagents can be introduced and exposed to selected parts of individual cells; the responses of the cells can then be detected by light microscopy and analyzed by powerful image-processing software. These types of studies will lead to discovery of new drugs, detection of subtle phenotypes of mutant cells (e.g., tumor cells), and development of comprehensive models of cellular processes. Bioengineers also are fabricating artificial tissues based on a synthetic three-dimensional architecture incorporating layers of different cells. Eventually such artificial tissues will provide replacements for defective tissues in sick, injured, or aging individuals.

Microscopy will continue to be a major tool in cell biology, providing images that relate to both the chemistry (i.e., interactions among proteins) and the mechanics (i.e., movements) involved in various cell processes. The forces causing molecular and cellular movements will be directly detected by fluorescent sensors in cells and the extracellular matrix. Improvements to high-resolution imaging methods will permit studies of single molecules in live cells, something that is currently possible only *in vitro*. Finally, cells will be studied in more natural contexts, not on glass coverslips but in 3D gels of extracellular matrix molecules. To aid in the imaging, the use of more fluorescent labels and tags will allow visualization of five or six different types of molecules simultaneously. With more labeled proteins, the complex interactions among proteins and organelles will become better understood.

Finally, the electron microscope will become the dominant instrument for studying protein machines *in vitro* and *in situ*. Tomographic methods applied to single cells and molecules combined with automated reconstruction methods will generate models of protein-based structures that cannot be determined by x-ray crystallography. High resolution three-dimensional models of molecules in cells will help explain the intricate biochemical interactions among proteins.

KEY TERMS

actin filaments 174	endosome 165
apical 153	exoplasmic face 150
basolateral 153	fluorescent
chloroplast 172	staining 187
cytoskeleton 147	glycolipid 151
cytosol 147	Golgi complex 169
cytosolic face 150	GPI anchor 161
endoplasmic	immunofluorescence
reticulum (ER) 168	microscopy 187

integral membrane protein 157	peripheral membrane protein 157
intermediate filament 174	peroxisome 168
lipid raft 156	phosphoglyceride 150
lumen 147	phospholipid bilayer 149
lysosome 165	pleckstrin homology (PH) domain 163
microfilament 174	porin 160
microtubule 174	prenyl anchor 160
mitochondrion 171	resolution 184
nuclear lamina 177	sphingolipid 151
nucleolus 171	

REVIEW THE CONCEPTS

1. When viewed by electron microscopy, the lipid bilayer is often described as looking like a railroad track. Explain how the structure of the bilayer creates this image.

2. Biomembranes contain many different types of lipid molecules. What are the three main types of lipid molecules found in biomembranes? How are the three types similar, and how are they different?

3. Lipid bilayers are considered to be two-dimensional fluids; what does this mean? What drives the movement of lipid molecules and proteins within the bilayer? How can such movement be measured? What factors affect the degree of membrane fluidity?

4. Explain the following statement: The structure of all biomembranes depends on the chemical properties of phospholipids, whereas the function of each specific biomembrane depends on the specific proteins associated with that membrane.

5. Name the three groups into which membrane-associated proteins may be classified. Explain the mechanism by which each group associates with a biomembrane.

6. Although both faces of a biomembrane are composed of the same general types of macromolecules, principally lipids and proteins, the two faces of the bilayer are not identical. What accounts for the asymmetry between the two faces?

7. One of the defining features of eukaryotic cells is the presence of organelles. What are the major organelles of eukaryotic cells, and what is the function of each? What is the cytosol? What cellular processes occur within the cytosol?

8. Cell organelles such as mitochondria, chloroplasts, and the Golgi apparatus each have unique structures. How is the structure of each organelle related to its function?

9. Much of what we know about cellular function depends on experiments utilizing specific cells and specific parts (e.g., organelles) of cells. What techniques do scientists commonly

use to isolate cells and organelles from complex mixtures, and how do these techniques work?

10. Isolation of some membrane proteins requires the use of detergents; isolation of others can be accomplished with the use of high-salt solutions. What types of membrane proteins require detergents as part of the isolation procedure? What types of membrane proteins may be isolated with high-salt solutions? Describe how the chemical properties of detergents and high salt facilitate the isolation process of each type of membrane protein.

11. Three systems of cytoskeletal filaments exist in most eukaryotic cells. Compare them in terms of composition, function, and structure.

12. Individual cytoskeletal filaments are typically organized into more complex structures within the cytosol. What two general types of structures do individual filaments combine to form in the cytosol? How are these structures created and maintained?

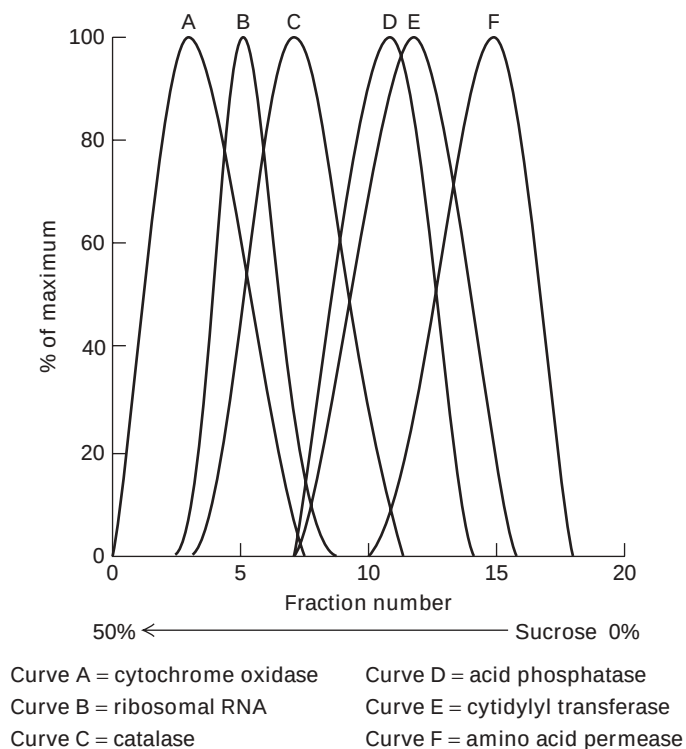
13. Both light and electron microscopy are commonly used to visualize cells, cell structures, and the location of specific molecules. Explain why a scientist may choose one or the other microscopy technique for use in research.

14. Why are chemical stains required for visualizing cells and tissues with the basic light microscope? What advantage does fluorescent microscopy provide in comparison to the chemical dyes used to stain specimens for light microscopy? What advantages do confocal scanning microscopy and deconvolution microscopy provide in comparison to conventional fluorescence microscopy?

15. In certain electron microscopy methods, the specimen is not directly imaged. How do these methods provide information about cellular structure, and what types of structures do they visualize?

ANALYZE THE DATA

Mouse liver cells were homogenized and the homogenate subjected to equilibrium density-gradient centrifugation with sucrose gradients. Fractions obtained from these gradients were assayed for *marker molecules* (i.e., molecules that are limited to specific organelles). The results of these assays are shown in the figure. The marker molecules have the following functions: Cytochrome oxidase is an enzyme involved in the process by which ATP is formed in the complete aerobic degradation of glucose or fatty acids; ribosomal RNA forms part of the protein-synthesizing ribosomes; catalase catalyzes decomposition of hydrogen peroxide; acid phosphatase hydrolyzes monophosphoric esters at acid pH; cytidylyl transferase is involved in phospholipid biosynthesis; and amino acid permease aids in transport of amino acids across membranes.



a. Name the marker molecule and give the number of the fraction that is *most* enriched for each of the following: lysosomes; peroxisomes; mitochondria; plasma membrane; rough endoplasmic reticulum; smooth endoplasmic reticulum.

b. Is the rough endoplasmic reticulum more or less dense than the smooth endoplasmic reticulum? Why?

c. Describe an alternative approach by which you could identify which fraction was enriched for which organelle.

d. How would addition of a detergent to the homogenate affect the equilibrium density-gradient results?

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