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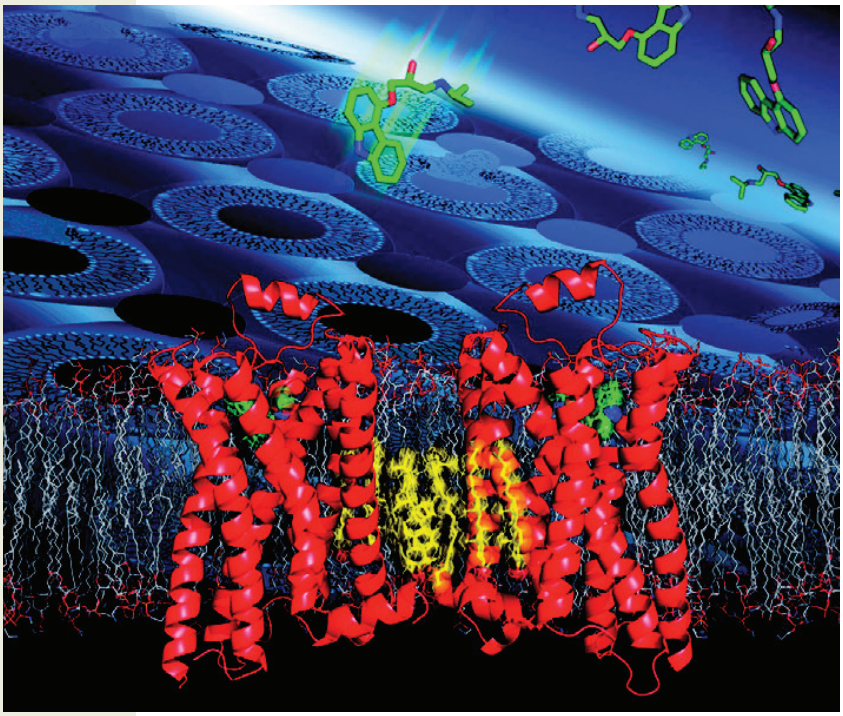
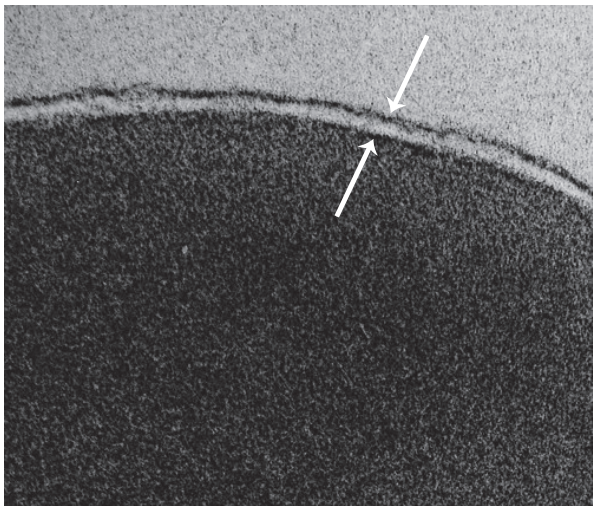


The Structure and Function of the Plasma Membrane

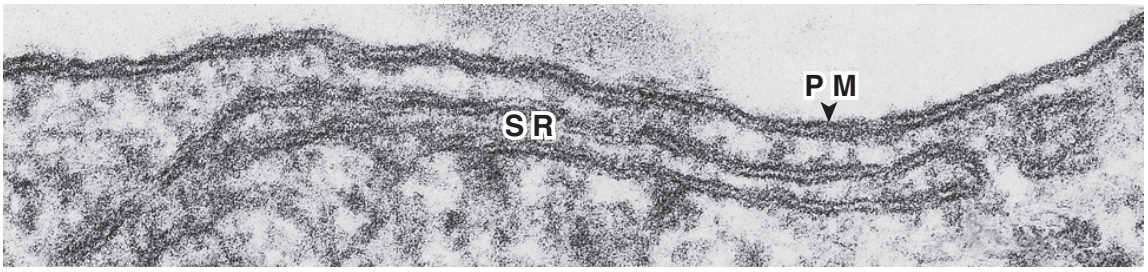
The outer walls of a house or car provide a strong, inflexible barrier that protects its human inhabitants from an unpredictable and harsh external world. You might expect the outer boundary of a living cell to be constructed of an equally tough and impenetrable barrier because it must also protect its delicate internal contents from a nonliving, and often inhospitable, environment. Yet cells are separated from the external world by a thin, fragile structure called the **plasma membrane** that is only 5 to 10 nm wide. It would require about five thousand plasma membranes stacked one on top of the other to equal the thickness of a single page of this book.

Because it is so thin, no hint of the plasma membrane is detected when a section of a cell is examined under a light microscope. In fact, it wasn't until the late 1950s that techniques for preparing and staining tissue had progressed to the point where the plasma membrane could be resolved in the electron

Three-dimensional, X-ray crystallographic structure of a β_2-adrenergic receptor (β_2-AR), which is a member of the G protein-coupled receptor (GPCR) superfamily. These integral membrane proteins are characterized as containing seven transmembrane helices. The β_2-AR is a resident of the plasma membrane of a variety of cells, where it normally binds the ligand epinephrine and mediates such responses as increased heart rate and relaxation of smooth muscle cells. Until recently, GPCRs had been very difficult to crystallize so that high-resolution structures of these important proteins have been lacking. This situation is now rapidly changing as the result of recent advances in crystallization technology. The image shown here depicts two β_2-ARs, which were crystallized in the presence of cholesterol and palmitic acid (yellow) and a receptor-binding ligand (green). The crystals used to obtain this image were selected from more than 15,000 trials. (From Vadim Cherezov et al., courtesy of Raymond C. Stevens, Science 318:1258, 2007; © 2007, reprinted with permission from AAAS.)



(a) 50 nm



(b) 0.1 µm

Figure 4.1 **The trilaminar appearance of membranes.** (*a*) Electron micrograph showing the three-layered (trilaminar) structure of the plasma membrane of an erythrocyte after staining the tissue with the heavy metal osmium. Osmium binds preferentially to the polar head groups of the lipid bilayer, producing the trilaminar pattern. The arrows denote the inner and outer edges of the membrane. (*b*) The outer edge of a differentiated muscle cell grown in culture showing the similar trilaminar structure of both the plasma membrane (PM) and the membrane of the sarcoplasmic reticulum (SR), a calcium-storing compartment of the cytoplasm. (A: Courtesy of J. D. Robertson; B: from Andrew R. Marks et al., J. Cell Biol. 114:305, 1991; reproduced with permission of The Rockefeller University Press.)

microscope. These early electron micrographs, such as those taken by J. D. Robertson of Duke University (Figure 4.1*a*), portrayed the plasma membrane as a three-layered structure, consisting of darkly staining inner and outer layers and lightly staining middle layer. All membranes that were examined closely—whether they were plasma, nuclear, or cytoplasmic membranes (Figure 4.1*b*), or taken from plants, animals, or microorganisms—showed this same ultrastructure. In addition to providing a visual image of this critically important cellular structure, these electron micrographs touched off a vigorous debate as to the molecular composition of the various layers of a membrane, an argument that went to the very heart of the subject of membrane structure and function. As we will see shortly, cell membranes contain a lipid bilayer, and the two dark-staining layers in the electron micrographs of Figure 4.1 correspond primarily to the inner and outer polar surfaces of the bilayer. We will return to the structure of membranes below, but first we will survey some of the major functions of membranes in the life of a cell (Figure 4.2).

4.1 | An Overview of Membrane Functions

1. ***Compartmentalization.*** Membranes are continuous, unbroken sheets and, as such, inevitably enclose compartments. The plasma membrane encloses the contents of the entire cell, whereas the nuclear and cytoplasmic membranes enclose diverse intracellular spaces. The various membrane-bounded compartments of a cell possess markedly different contents. Membrane compartmentalization allows specialized activities to proceed without external interference and enables cellular activities to be regulated independently of one another.
2. ***Scaffold for biochemical activities.*** Membranes not only enclose compartments but are also a distinct compartment themselves. As long as reactants are present in solution, their relative positions cannot be stabilized and their interactions are dependent on random collisions. Because of their construction, membranes provide the cell with an extensive framework or scaffolding within which components can be ordered for effective interaction.
3. ***Providing a selectively permeable barrier.*** Membranes prevent the unrestricted exchange of molecules from one side to the other. At the same time, membranes provide the means of communication between the compartments they separate. The plasma membrane, which encircles a

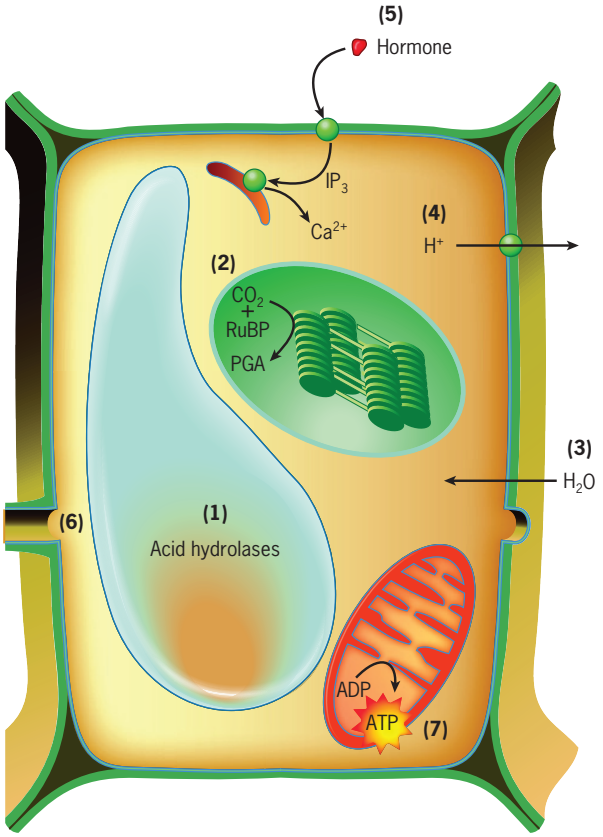


Figure 4.2 **A summary of membrane functions in a plant cell.** (1) An example of membrane compartmentalization in which hydrolytic enzymes (acid hydrolases) are sequestered within the membrane-bounded vacuole. (2) An example of the role of cytoplasmic membranes as a site of enzyme localization. The fixation of CO_2 by the plant cell is catalyzed by an enzyme that is associated with the outer surface of the thylakoid membranes of the chloroplasts. (3) An example of the role of membranes as a selectively permeable barrier. Water molecules are able to penetrate rapidly through the plasma membrane, causing the plant cell to fill out the available space and exert pressure against its cell wall. (4) An example of solute transport. Hydrogen ions, which are produced by various metabolic processes in the cytoplasm, are pumped out of plant cells into the extracellular space by a transport protein located in the plasma membrane. (5) An example of the involvement of a membrane in the transfer of information from one side to another (signal transduction). In this case, a hormone (e.g., abscisic acid) binds to the outer surface of the plasma membrane and triggers the release of a chemical message (such as IP_3) into the cytoplasm. In this case, IP_3 causes release of Ca^{2+} ions from a cytoplasmic warehouse. (6) An example of the role of membranes in cell–cell communication. Openings between adjoining plant cells, called plasmodesmata, allow materials to move directly from the cytoplasm of one cell into its neighbors. (7) An example of the role of membranes in energy transduction. The conversion of ADP to ATP occurs in close association with the inner membrane of the mitochondrion.

cell, can be compared to a moat around a castle: both serve as a general barrier, yet both have gated "bridges" that promote the movement of select elements into and out of the enclosed living space.

4. ***Transporting solutes.*** The plasma membrane contains the machinery for physically transporting substances from one side of the membrane to another, often from a region where the solute is present at low concentration into a region where that solute is present at much higher concentration. The membrane's transport machinery allows a cell to accumulate substances, such as sugars and amino acids, that are necessary to fuel its metabolism and build its macromolecules. The plasma membrane is also able to transport specific ions, thereby establishing ionic gradients across itself. This capability is especially critical for nerve and muscle cells.

5. ***Responding to external stimuli.*** The plasma membrane plays a critical role in the response of a cell to external stimuli, a process known as **signal transduction**. Membranes possess **receptors** that combine with specific molecules (**ligands**) or respond to other types of stimuli such as light or mechanical tension. Different types of cells have membranes with different receptors and are, therefore, capable of recognizing and responding to different environmental stimuli. The interaction of a plasma membrane receptor with an external stimulus may cause the membrane to generate a signal that stimulates or inhibits internal activities. For example, signals generated at the plasma membrane may tell a cell to manufacture more glycogen, to prepare for cell division, to move toward a higher concentration of a particular compound, to release calcium from internal stores, or possibly to commit suicide.

6. ***Intercellular interaction.*** Situated at the outer edge of every living cell, the plasma membrane of multicellular organisms mediates the interactions between a cell and its neighbors. The plasma membrane allows cells to recognize and signal one another, to adhere when appropriate, and to exchange materials and information. Proteins within the plasma membrane may also facilitate the interaction between extracellular materials and the intracellular cytoskeleton.

7. ***Energy transduction.*** Membranes are intimately involved in the processes by which one type of energy is converted to another type (energy transduction). The most fundamental energy transduction occurs during photosynthesis when energy in sunlight is absorbed by membrane-bound pigments, converted into chemical energy, and stored in carbohydrates. Membranes are also involved in the transfer of chemical energy from carbohydrates and fats to ATP. In eukaryotes, the machinery for these energy conversions is contained within membranes of chloroplasts and mitochondria.

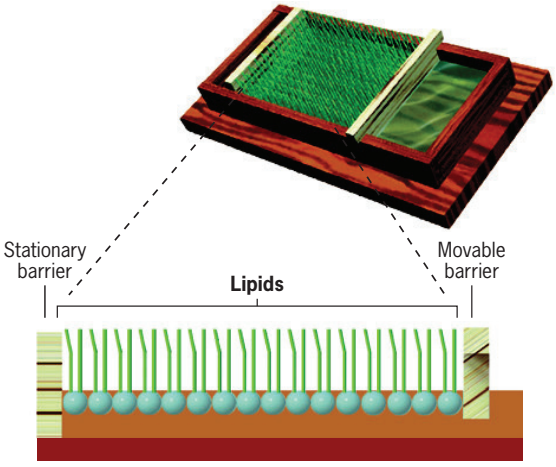
We will concentrate in this chapter on the structure and functions of the plasma membrane, but remember that the principles discussed here are common to all cell membranes. Specialized aspects of the structure and functions of mitochondrial, chloroplast, cytoplasmic, and nuclear membranes will be discussed in Chapters 5, 6, 8, and 12, respectively.

4.2 | A Brief History of Studies on Plasma Membrane Structure

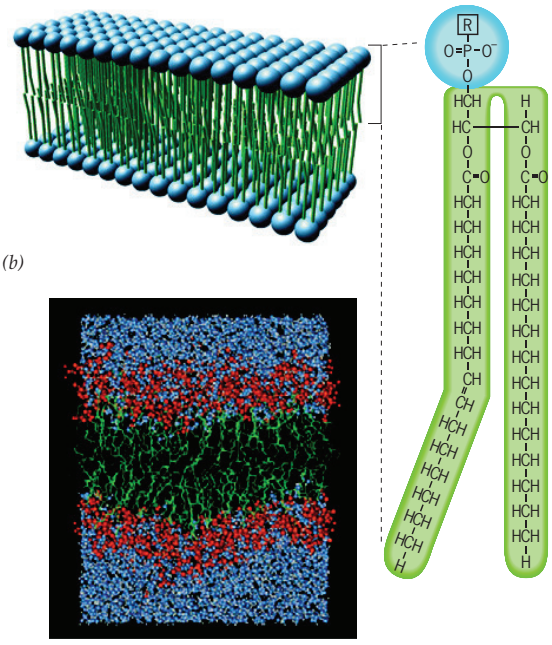
The first insights into the chemical nature of the outer boundary layer of a cell were obtained by Ernst Overton of the University of Zürich during the 1890s. Overton knew that nonpolar solutes dissolved more readily in nonpolar solvents than in polar solvents, and that polar solutes had the opposite solubility. Overton reasoned that a substance entering a cell from the medium would first have to dissolve in the outer boundary layer of that cell. To test the permeability of the outer boundary layer, Overton placed plant root hairs into hundreds of different solutions containing a diverse array of solutes. He discovered that the more lipid-soluble the solute, the more rapidly it would enter the root hair cells (see p. 149). He concluded that the dissolving power of the outer boundary layer of the cell matched that of a fatty oil.

The first proposal that cellular membranes might contain a lipid bilayer was made in 1925 by two Dutch scientists, E. Gorter and F. Grendel. These researchers extracted the lipid from human red blood cells and measured the amount of surface area the lipid would cover when spread over the surface of water (Figure 4.3*a*). Since mature mammalian red blood cells lack both nuclei and cytoplasmic organelles, the plasma membrane is the only lipid-containing structure in the cell. Consequently, all of the lipids extracted from the cells can be assumed to have resided in the cells' plasma membranes. The ratio of the surface area of water covered by the extracted lipid to the surface area calculated for the red blood cells from which the lipid was extracted varied between 1.8 to 1 and 2.2 to 1. Gorter and Grendel speculated that the actual ratio was 2:1 and concluded that the plasma membrane contained a bimolecular layer of lipids, that is, a **lipid bilayer** (Figure 4.3*b*). They also suggested that the polar groups of each molecular layer (or *leaflet*) were directed outward toward the aqueous environment, as shown in Figure 4.3*b*,*c*. This would be the thermodynamically favored arrangement, because the polar head groups of the lipids could interact with surrounding water molecules, just as the hydrophobic fatty acyl chains would be protected from contact with the aqueous environment (Figure 4.3*c*). Thus, the polar head groups would face the cytoplasm on one edge and the blood plasma on the other. Even though Gorter and Grendel made several experimental errors (which fortuitously canceled one another out), they still arrived at the correct conclusion that membranes contain a lipid bilayer.

In the 1920s and 1930s, cell physiologists obtained evidence that there must be more to the structure of membranes than simply a lipid bilayer. It was found, for example, that lipid



(*a*)



(*b*)

(*c*)

Figure 4.3 **The plasma membrane contains a lipid bilayer.** (*a*) Calculating the surface area of a lipid preparation. When a sample of phospholipids is dissolved in an organic solvent, such as hexane, and spread over an aqueous surface, the phospholipid molecules form a layer over the water that is a single molecule thick: a monomolecular layer. The molecules in the layer are oriented with their hydrophilic groups bonded to the surface of the water and their hydrophobic chains directed into the air. To estimate the surface area the lipids would cover if they were part of a membrane, the lipid molecules can be compressed into the smallest possible area by means of movable barriers. Using this type of apparatus, which is called a Langmuir trough after its inventor, Gorter and Grendel concluded that red blood cells contained enough lipid to form a layer over their surface that was two molecules thick: a bilayer. (*b*) As Gorter and Grendel first proposed, the core of a membrane contains a bimolecular layer of phospholipids oriented with their water-soluble head groups facing the outer surfaces and their hydrophobic fatty acid tails facing the interior. The structures of the head groups are given in Figure 4.6*a*. (*c*) Simulation of a fully hydrated lipid bilayer composed of the phospholipid phosphatidylcholine. Phospholipid head groups are orange, water molecules are blue and white, fatty acid chains are green. (C: From S.-W. Chiu, Trends in Biochem. Sci. 22:341, © 1997, with permission from Elsevier.)

solubility was not the sole determining factor as to whether or not a substance could penetrate the plasma membrane. Similarly, the surface tensions of membranes were calculated to be much lower than those of pure lipid structures. This decrease in surface tension could be explained by the presence of protein in the membrane. In 1935, Hugh Davson and James Danielli proposed that the plasma membrane was composed of a lipid bilayer that was lined on both its inner and outer surface by a layer of globular proteins. They revised their model in the early 1950s to account for the selective permeability of the membranes they had studied. In the revised version (Figure 4.4*a*), Davson and Danielli suggested that, in addition to the outer and inner protein layers, the lipid bilayer was also penetrated by protein-lined pores, which could provide conduits for polar solutes and ions to enter and exit the cell.

Experiments conducted in the late 1960s led to a new concept of membrane structure, as detailed in the fluid-mosaic model proposed in 1972 by S. Jonathan Singer and Garth Nicolson of the University of California, San Diego (Figure 4.4*b*). In the **fluid-mosaic model**, which has served as

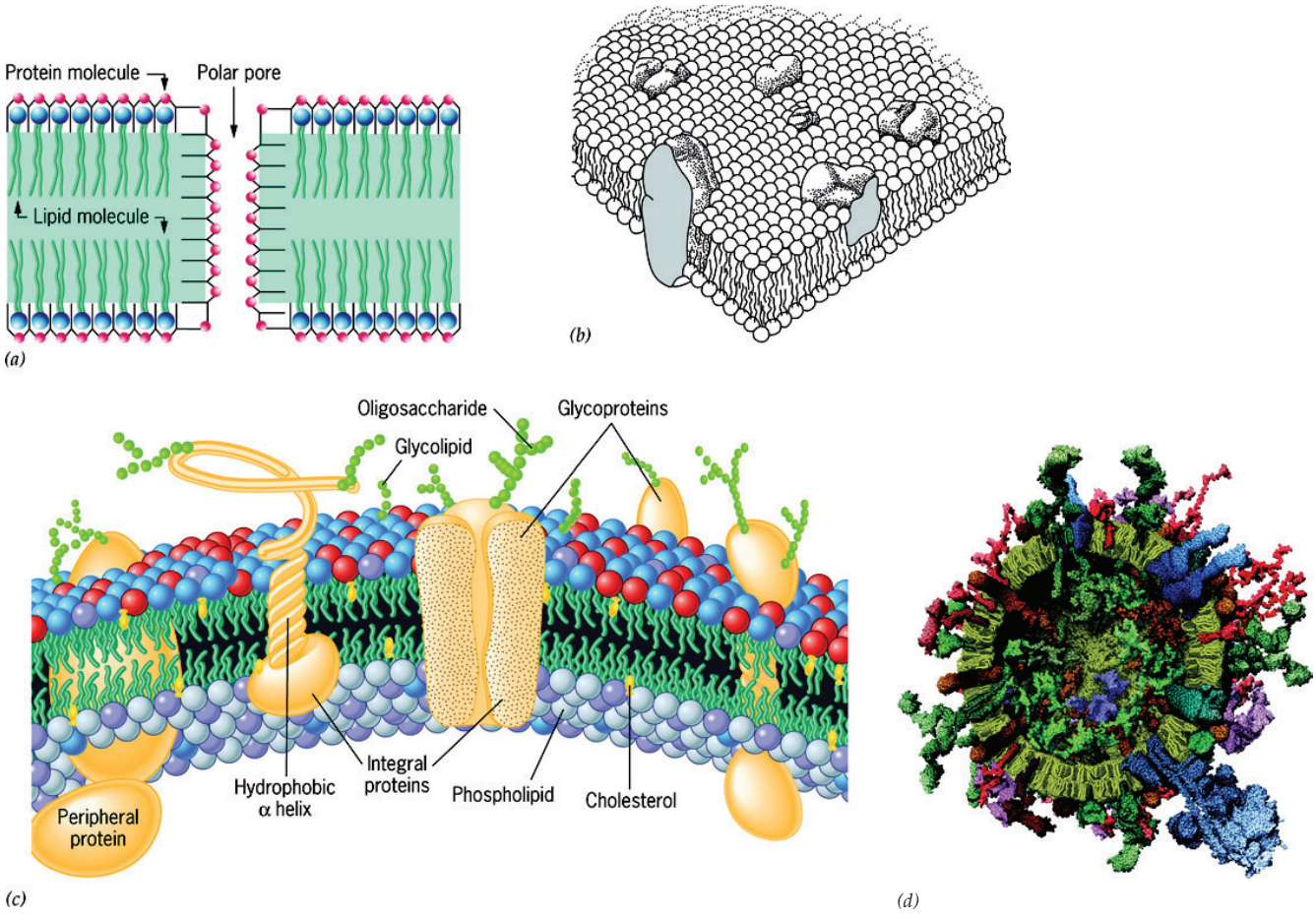


Figure 4.4 **A brief history of the structure of the plasma membrane.** (*a*) A revised 1954 version of the Davson-Danielli model showing the lipid bilayer, which is lined on both surfaces by a monomolecular layer of proteins that extends through the membrane to form protein-lined pores. (*b*) The fluid-mosaic model of membrane structure as initially proposed by Singer and Nicolson in 1972. Unlike previous models, the proteins penetrate the lipid bilayer. Although the original model shown here depicted a protein that was only partially embedded in the bilayer, lipid-penetrating proteins that have been studied span the entire bilayer. (*c*) A current representation of the plasma membrane showing the same basic organization as that proposed by Singer and Nicolson. The external surface of most membrane proteins, as well as a small percentage of the phospholipids, contain short chains of sugars, making them glycoproteins and glycolipids. Those portions of the polypeptide chains that extend through the lipid bilayer typically occur as α helices composed of hydrophobic amino acids. The two leaflets of the bilayer contain different types of lipids as indicated by the differently colored head groups. (*d*) Molecular model of the membrane of a synaptic vesicle constructed using known structures of the various proteins along with information on their relative numbers obtained from the analysis of purified synaptic vesicles. The high protein density of the membrane is apparent. Most of the proteins in this membrane are required for the interaction of the vesicle with the plasma membrane. The large blue protein at the lower right pumps H^+ ions into the vesicle. (A: From J. F. Danielli, Collston Papers 7:8, 1954A: reprinted with permission from S. J. Singer and G. L. Nicolson, Science 175:720, 1972; copyright 1972 reprinted with permission from AAAS. B: reprinted with permission from S. J. Singer and G. L. Nicolson, Science 175:720, 1972; copyright 1972, AAAS; D: from Shigeo Takamori, et al., courtesy of Reinhard Jahn, Cell 127:841, 2006, reprinted with permission from Elsevier.)

the "central dogma" of membrane biology for more than three decades, the lipid bilayer remains the core of the membrane, but attention is focused on the physical state of the lipid. Unlike previous models, the bilayer of a fluid-mosaic membrane is present in a fluid state, and individual lipid molecules can move laterally within the plane of the membrane.

The structure and arrangement of membrane proteins in the fluid-mosaic model differ from those of previous models in that they occur as a "mosaic" of discontinuous particles that penetrate the lipid sheet (Figure 4.4*b*). Most importantly, the fluid-mosaic model presents cellular membranes as dynamic structures in which the components are mobile and capable of coming together to engage in various types of transient or semipermanent interactions. In the following sections, we will examine some of the evidence used to formulate and support this dynamic portrait of membrane structure and look at some of the recent data that bring the model up to date (Figure 4.4*c*,*d*).

REVIEW

1. Describe some of the important roles of membranes in the life of a eukaryotic cell. What do you think might be the effect of a membrane that was incapable of performing one or another of these roles?
2. Summarize some of the major steps leading to the current model of membrane structure. How does each new model retain certain basic principles of earlier models?

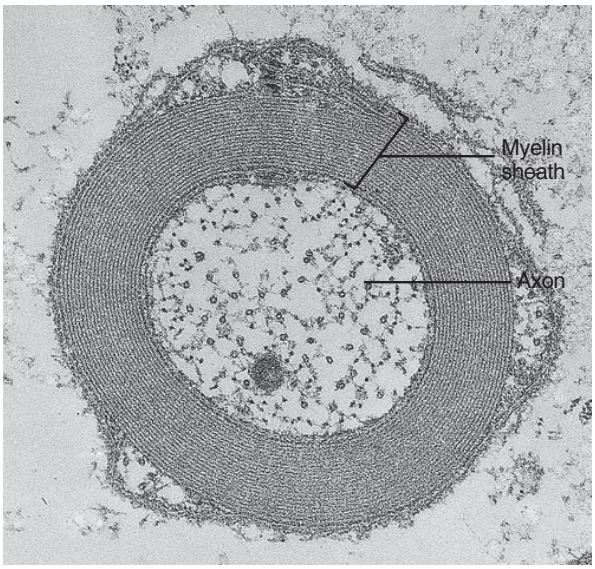


Figure 4.5 **The myelin sheath.** Electron micrograph of a nerve cell axon surrounded by a myelin sheath consisting of concentric layers of plasma membrane that have an extremely low protein/lipid ratio. The myelin sheath insulates the nerve cell from the surrounding environment, which increases the velocity at which impulses can travel along the axon (discussed on page 167). The perfect spacing between the layers is maintained by interlocking protein molecules (called P_0) that project from each membrane. (From Leonard Napolitano, Francis LeBaron, and Joseph Scaletti, J. Cell Biol. 34:820, 1967; reproduced with permission of The Rockefeller University Press.)

4.3 | The Chemical Composition of Membranes

Membranes are lipid–protein assemblies in which the components are held together in a thin sheet by noncovalent bonds. As noted above, the core of the membrane consists of a sheet of lipids arranged in a bimolecular layer (Figure 4.3*b*,*c*). The lipid bilayer serves primarily as a structural backbone of the membrane and provides the barrier that prevents random movements of water-soluble materials into and out of the cell. The proteins of the membrane, on the other hand, carry out most of the specific functions summarized in Figure 4.2. Each type of differentiated cell contains a unique complement of membrane proteins, which contributes to the specialized activities of that cell type (see Figure 4.32*d* for an example).

The ratio of lipid to protein in a membrane varies, depending on the type of cellular membrane (plasma vs. endoplasmic reticulum vs. Golgi), the type of organism (bacterium vs. plant vs. animal), and the type of cell (cartilage vs. muscle vs. liver). For example, the inner mitochondrial membrane has a very high ratio of protein/lipid in comparison to the red blood cell plasma membrane, which is high in comparison to the membranes of the myelin sheath that form a multilayered wrapping around a nerve cell (Figure 4.5). To a large degree, these differences can be correlated with the basic functions of these membranes. The inner mitochondrial membrane contains the protein carriers of the electron-transport chain, and relative to other membranes, lipid is diminished. In contrast, the myelin sheath acts primarily as electrical insulation for the nerve cell it encloses, a function that is best carried out by a thick lipid layer of high electrical resistance with a minimal content of protein. Membranes also contain carbohydrates, which are attached to the lipids and proteins as indicated in Figure 4.4*c*.

Membrane Lipids

Membranes contain a wide diversity of lipids, all of which are **amphipathic**; that is, they contain both hydrophilic and hydrophobic regions. There are three main types of membrane lipids: phosphoglycerides, sphingolipids, and cholesterol.

Phosphoglycerides Most membrane lipids contain a phosphate group, which makes them **phospholipids**. Because most membrane phospholipids are built on a glycerol backbone, they are called **phosphoglycerides** (Figure 4.6*a*). Unlike triglycerides, which have three fatty acids (page 47) and are not amphipathic, membrane glycerides are *diglycerides*—only two of the hydroxyl groups of the glycerol are esterified to fatty acids; the third is esterified to a hydrophilic phosphate group. Without any additional substitutions beyond the phosphate

and the two fatty acyl chains, the molecule is called *phosphatidic acid*, which is virtually absent in most membranes. Instead, membrane phosphoglycerides have an additional group linked to the phosphate, most commonly either choline (forming *phosphatidylcholine*, PC), ethanolamine (forming *phosphatidylethanolamine*, PE), serine (forming *phosphatidylserine*, PS), or inositol (forming *phosphatidylinositol*, PI). Each of these groups is small and hydrophilic and, together with the negatively charged phosphate to which it is attached, forms a highly water-soluble domain at one end of the molecule, called the **head group**. At physiologic pH, the head groups of PS and PI have an overall negative charge, whereas those of PC and PE are neutral. In contrast, the fatty acyl chains are hydrophobic, unbranched hydrocarbons approximately 16 to 22 carbons in length (Figure 4.6). A membrane fatty acid may be fully saturated (i.e., lack double bonds), monounsaturated (i.e., possess one double bond), or polyunsaturated (i.e., possess more than one double bond). Phosphoglycerides often contain one unsaturated and one saturated fatty acyl chain. Recent interest has focused on the apparent health benefits of two highly unsaturated fatty acids (EPA and DHA) found at high concentration in fish oil. EPA and DHA contain five and six double bonds, respectively, and are incorporated primarily into PE and PC molecules of certain membranes, most notably in the brain and retina. EPA and DHA are described as omega-3 fatty acids because their last double bond is situated 3 carbons from the omega (CH_3) end of the fatty acyl chain. With fatty acid chains at one end of the molecule and a polar head group at the other end, all of the phosphoglycerides exhibit a distinct amphipathic character.

Sphingolipids A less abundant class of membrane lipids, called **sphingolipids**, are derivatives of sphingosine, an amino alcohol that contains a long hydrocarbon chain (Figure 4.6*b*). Sphingolipids consist of sphingosine linked to a fatty acid (R of Figure 4.6*b*) by its amino group. This molecule is a *ceramide*. The various sphingosine-based lipids have additional groups esterified to the terminal alcohol of the sphingosine moiety. If the substitution is phosphorylcholine, the molecule is *sphingomyelin*, which is the only phospholipid of the membrane that is not built with a glycerol backbone. If the substitution is a carbohydrate, the molecule is a **glycolipid**. If the carbohydrate is a simple sugar, the glycolipid is called a *cerebroside*; if it is a small cluster of sugars that includes sialic acid, the glycolipid is called a *ganglioside*. Hundreds of different gangliosides have been identified by differences in their carbohydrate chains. Since all sphingolipids have two long, hydrophobic hydrocarbon chains at one end and a hydrophilic

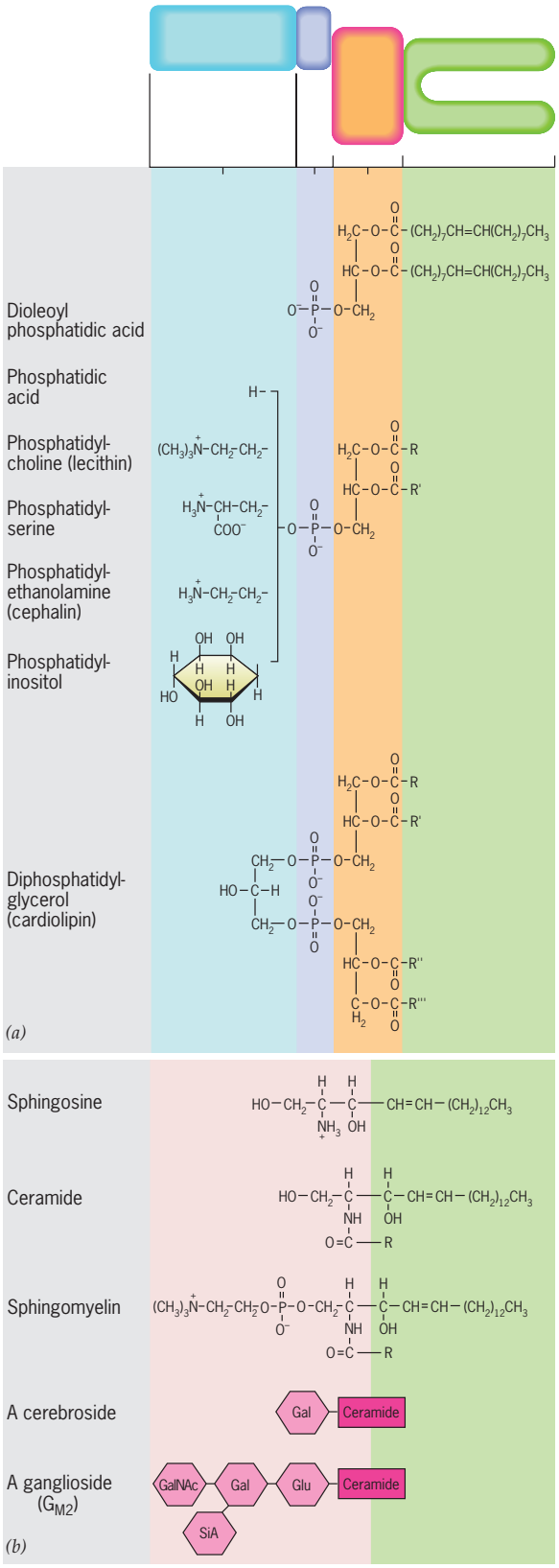


Figure 4.6 **The chemical structures of membrane lipids.** (*a*) The structures of phosphoglycerides (see also Figure 2.22). (*b*) The structures of sphingolipids. Sphingomyelin is a phospholipid; cerebrosides and gangliosides are glycolipids. A third membrane lipid is cholesterol, which is shown in the next figure. (R = fatty acyl chain). [The green portion of each lipid, which represents the hydrophobic tail(s) of the molecule, is actually much longer than the hydrophilic head group (see Figure 4.23).]

region at the other, they are also amphipathic and basically similar in overall structure to the phosphoglycerides. The fatty acyl chains of sphingolipids, however, tend to be longer and more highly saturated than those of phosphoglycerides.

Glycolipids are interesting membrane components. Relatively little is known about them, yet tantalizing hints have emerged to suggest they play crucial roles in cell function. The nervous system is particularly rich in glycolipids. The myelin sheath pictured in Figure 4.5 contains a high content of a particular glycolipid, called galactocerebroside (shown in Figure 4.6*b*), which is formed when a galactose is added to ceramide. Mice lacking the enzyme that carries out this reaction exhibit severe muscular tremors and eventual paralysis. Similarly, humans who are unable to synthesize a particular ganglioside (G_{M3}) suffer from a serious neurological disease characterized by severe seizures and blindness. Glycolipids also play a role in certain infectious diseases; the toxins that cause cholera and botulism both enter their target cell by first binding to cell-surface gangliosides, as does the influenza virus.

Cholesterol Another lipid component of certain membranes is the sterol **cholesterol** (see Figure 2.21), which in certain animal cells may constitute up to 50 percent of the lipid molecules in the plasma membrane. Plant cells contain cholesterol-like sterols, but biologists disagree as to whether or not they completely lack cholesterol. Cholesterol molecules are oriented with their small hydrophilic hydroxyl group toward the membrane surface and the remainder of the molecule embedded in the lipid bilayer (Figure 4.7). The hydrophobic rings of a cholesterol molecule are flat and rigid, and they interfere with the movements of the fatty acid tails of the phospholipids (page 138).

The Nature and Importance of the Lipid Bilayer Each type of cellular membrane has its own characteristic lipid composition, differing from one another in the types of lipids, the nature of the head groups, and the particular species of fatty acyl chain(s). Because of this structural variability, it is estimated that some biological membranes contain hundreds of chemically distinct species of phospholipids, which can be catalogued by mass spectrometry. The biological significance of this remarkable diversity of lipid species remains the subject of interest and speculation.

The percentages of some of the major types of lipids of a variety of membranes are given in Table 4.1. The lipids of a membrane are more than simple structural elements; they can have important effects on the biological properties of a membrane. Lipid composition can determine the physical state of the membrane (page 138) and influence the activity of particular membrane proteins. Membrane lipids also provide the precursors for highly active chemical messengers that regulate cellular function (Section 15.3).

Various types of measurements indicate that the combined fatty acyl chains of both leaflets of the lipid bilayer span a width of about 30 Å and that each row of head groups (with its adjacent shell of water molecules) adds another 15 Å. Thus, the entire lipid bilayer is only about 60 Å (6 nm) thick. The presence in membranes of this thin film of amphipathic

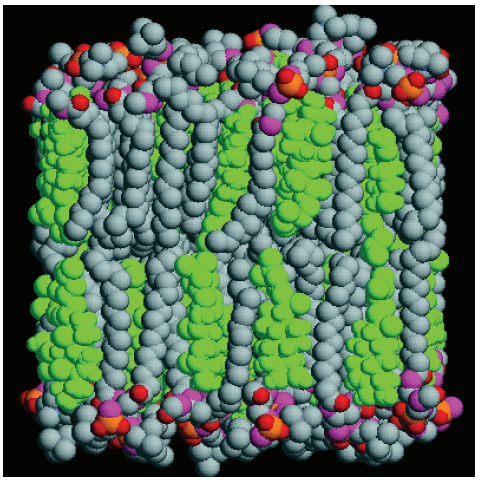


Figure 4.7 **The cholesterol molecules** (shown in green) of a lipid bilayer are oriented with their small hydrophilic end facing the external surface of the bilayer and the bulk of their structure packed in among the fatty acid tails of the phospholipids. The placement of cholesterol molecules interferes with the flexibility of the lipid hydrocarbon chains, which tends to stiffen the bilayer while maintaining its overall fluidity. Unlike other lipids of the membrane, cholesterol is often rather evenly distributed between the two layers (leaflets). (FROM H. L. SCOTT, CURR. OPIN. STRUCT. BIOL. 12:499, 2002, FIGURE 3; © 2002, WITH PERMISSION FROM ELSEVIER.)

lipid molecules has remarkable consequences for cell structure and function. Because of thermodynamic considerations, the hydrocarbon chains of the lipid bilayer are never exposed to the surrounding aqueous solution. Consequently, membranes are never seen to have a free edge; they are always continuous, unbroken structures. As a result, membranes form extensive interconnected networks within the cell. Because of the flexibility of the lipid bilayer, membranes are deformable and their overall shape can change, as occurs during locomotion

Table 4.1 ***Lipid Compositions of Some Biological Membranes****

Lipid	Human erythrocyte	Human myelin	Beef heart mitochondria	*E. coli*
Phosphatidic acid	1.5	0.5	0	0
Phosphatidylcholine	19	10	39	0
Phosphatidyl-ethanolamine	18	20	27	65
Phosphatidylgycerol	0	0	0	18
Phosphatidylserine	8.5	8.5	0.5	0
Cardiolipin	0	0	22.5	12
Sphingomyelin	17.5	8.5	0	0
Glycolipids	10	26	0	0
Cholesterol	25	26	3	0

*The values given are weight percent of total lipid.
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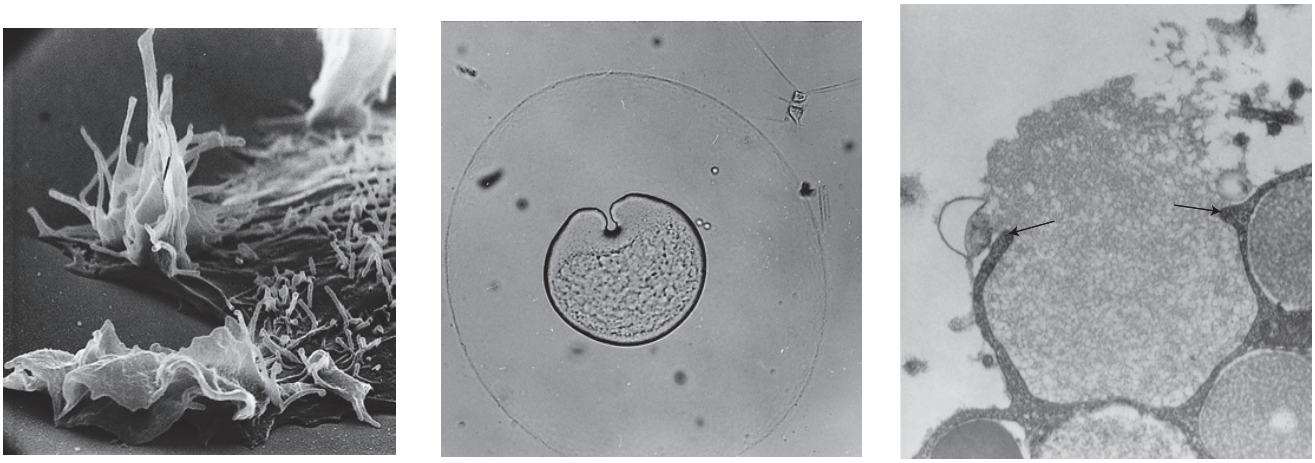


(a) (b) (c)

Figure 4.8 **The dynamic properties of the plasma membrane.** (*a*) The leading edge of a moving cell often contains sites where the plasma membrane displays undulating ruffles. (*b*) Division of a cell is accompanied by the deformation of the plasma membrane as it is pulled toward the center of the cell. Unlike most dividing cells, the cleavage furrow of this dividing ctenophore egg begins at one pole and moves unidirectionally through the egg. (*c*) Membranes are capable of fusing with other membranes. This electron micrograph shows a secretory granule discharging its contents after fusion with the overlying plasma membrane (arrows). (A: COURTESY OF JEAN PAUL REVEL; B: COURTESY OF GARY FREEMAN; C: COURTESY OF SUSAN JO BURWEN.)

(Figure 4.8*a*) or cell division (Figure 4.8*b*). The lipid bilayer is thought to facilitate the regulated fusion or budding of membranes. For example, the events of secretion, in which cytoplasmic vesicles fuse to the plasma membrane (Figure 4.8*c*), or of fertilization, where two cells fuse to form a single cell, involve processes in which two separate membranes come together to become one continuous sheet (see Figure 8.32). The importance of the lipid bilayer in maintaining the proper internal composition of a cell, in separating electric charges across the plasma membrane, and in many other cellular activities will be apparent throughout this and subsequent chapters.

Another important feature of the lipid bilayer is its ability to self-assemble, which can be demonstrated more easily within a test tube than a living cell. If, for example, a small amount of phosphatidylcholine is dispersed in an aqueous solution, the phospholipid molecules assemble spontaneously to form the walls of fluid-filled spherical vesicles, called **liposomes**. The walls of these liposomes consist of a continuous lipid bilayer that is organized in the same manner as that of the lipid bilayer of a natural membrane. Liposomes have proven invaluable in membrane research. Membrane proteins can be inserted into liposomes and their function studied in a much simpler environment than that of a natural membrane. Liposomes have also been developed as vehicles to deliver drugs or DNA molecules within the body. The drugs or DNA can be linked to the wall of the liposome or contained at high concentration within its lumen (Figure 4.9). In these studies, the walls of the liposomes are constructed to contain specific proteins (such as antibodies or hormones) that allow the liposomes to bind selectively to the surfaces of particular target cells where the drug or DNA is intended to go. Most of the early clinical studies with liposomes met with failure because the injected vesicles were rapidly removed by phagocytic cells of the immune system. This obstacle has been overcome with the development of so-called stealth liposomes (e.g., Caelyx) that contain an outer coating of a synthetic polymer that protects the liposomes from immune destruction (Figure 4.9).

The Asymmetry of Membrane Lipids

The lipid bilayer consists of two distinct leaflets that have a distinctly different lipid composition. One line of experiments that has led to this conclusion takes advantage of the fact that lipid-digesting enzymes cannot penetrate the plasma membrane and, consequently, are only able to digest lipids that reside in the outer leaflet of the bilayer. If intact human red blood cells are treated with a lipid-digesting phospholipase, approximately 80 percent of the phosphatidylcholine (PC) of the membrane is hydrolyzed, but only about 20 percent of the membrane's phosphatidylethanolamine (PE) and less than 10 percent of its phosphatidylserine (PS) are attacked. These data indicate that, compared to the inner leaflet, the outer leaflet has a relatively high concentration of PC (and sphingomyelin) and a low concentration of PE and PS (Figure 4.10). It follows that the lipid bilayer can be thought of as composed of two more-or-less stable, independent monolayers having different physical and chemical properties.

The different classes of lipids in Figure 4.10 exhibit different properties. All the glycolipids of the plasma membrane are in the outer leaflet where they often serve as receptors for extracellular ligands. Phosphatidylethanolamine, which is concentrated in the inner leaflet, tends to promote the curvature of

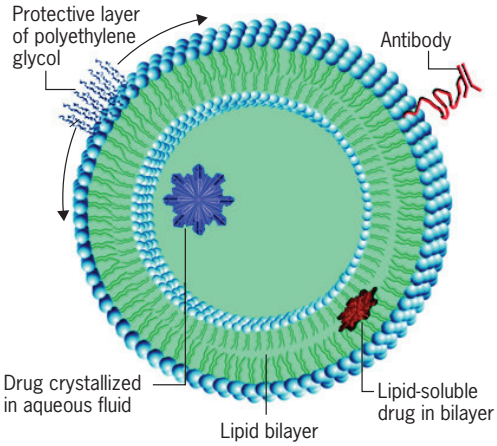


Figure 4.9 **Liposomes.** A schematic diagram of a stealth liposome containing a hydrophilic polymer (such as polyethylene glycol) to protect it from destruction by immune cells, antibody molecules that target it to specific body tissues, a water-soluble drug enclosed in the fluid-filled interior chamber, and a lipid-soluble drug in the bilayer.

the membrane, which is important in membrane budding and fusion. Phosphatidylserine, which is concentrated in the inner leaflet, has a net negative charge at physiologic pH, which makes it a candidate for binding positively charged lysine and arginine residues, such as those adjacent to the membrane-spanning α helix of glycophorin A in Figure 4.18. The appearance of PS on the outer surface of aging lymphocytes marks the cells for destruction by macrophages, whereas its appearance on the outer surface of platelets leads to blood coagulation. Phosphatidylinositol (PI), which is concentrated in the inner leaflet, can be phosphorylated at different sites on the inositol ring, which converts the lipid into a phosphoinositide. As discussed in Chapter 15, phosphoinositides play a key role in the transfer of stimuli from the plasma membrane

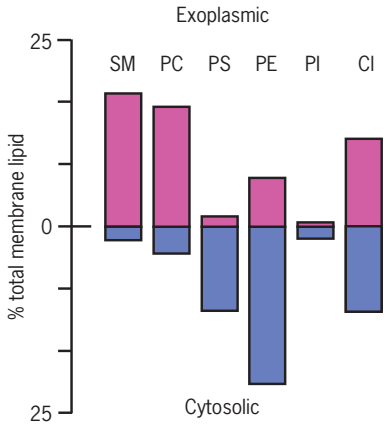


Figure 4.10 **The asymmetric distribution of phospholipids (and cholesterol) in the plasma membrane of human erythrocytes.** (SM, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; Cl, cholesterol.)

to the cytoplasm (Section 15.3) and the recruitment of proteins to the cytosolic face of the plasma membrane.

Membrane Carbohydrates

The plasma membranes of eukaryotic cells also contain carbohydrate. Depending on the species and cell type, the carbohydrate content of the plasma membrane ranges between 2 and 10 percent by weight. More than 90 percent of the membrane's carbohydrate is covalently linked to proteins to form glycoproteins; the remaining carbohydrate is covalently linked to lipids to form glycolipids, which were discussed on page 126. As indicated in Figure 4.4*c*, all of the carbohydrate of the plasma membrane faces outward into the extracellular space.[1] The carbohydrate of internal cellular membranes also faces away from the cytosol (the basis for this orientation is illustrated in Figure 8.14).

The modification of proteins was discussed briefly on page 54. The addition of carbohydrate, or **glycosylation**, is the most complex of these modifications. The carbohydrate of glycoproteins is present as short, branched hydrophilic **oligosaccharides**, typically having fewer than about 15 sugars per chain. In contrast to most high-molecular-weight carbohydrates (such as glycogen, starch, or cellulose), which are polymers of a single sugar, the oligosaccharides attached to membrane proteins and lipids can display extensive variability in composition and structure. Even the same protein can display different chains of sugars in different cells and tissues. Oligosaccharides may be attached to several different amino acids by two major types of linkages (Figure 4.11). These

[1]It can be noted that even though phosphatidylinositol contains a sugar group (Figure 4.6), it is not considered to be part of the carbohydrate portion of the membrane in this discussion.

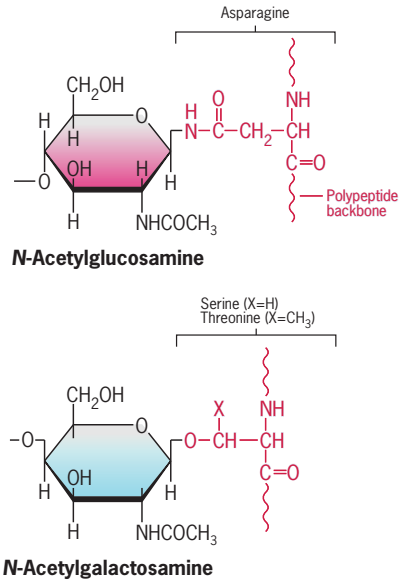


Figure 4.11 **Two types of linkages that join sugars to a polypeptide chain.** The *N*-glycosidic linkage between asparagine and *N*-acetylglucosamine is more common than the *O*-glycosidic linkage between serine or threonine and *N*-acetylgalactosamine.

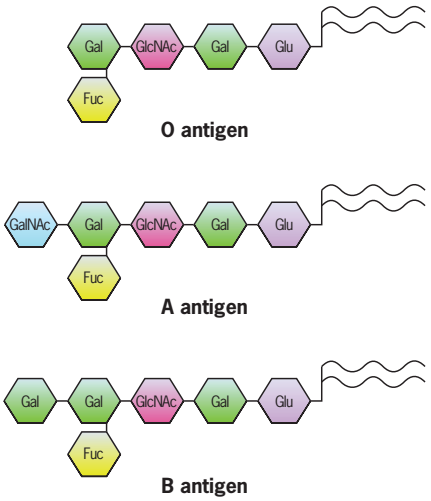


Figure 4.12 **Blood-group antigens.** Whether a person has type A, B, AB, or O blood is determined by a short chain of sugars covalently attached to membrane lipids and proteins of the red blood cell membrane. The oligosaccharides attached to membrane lipids (forming a ganglioside) that produce the A, B, and O blood types are shown here. A person with type AB blood has gangliosides with both the A and B structure. (Gal, galactose; GlcNAc, *N*-acetylglucosamine; Glu, glucose; Fuc, fucose; GalNAc, *N*-acetylgalactosamine.)

carbohydrate projections play an important role in mediating the interactions of a cell with its environment (Chapter 7) and sorting of membrane proteins to different cellular compartments (Chapter 8). The carbohydrates of the glycolipids of the red blood cell plasma membrane determine whether a person's blood type is A, B, AB, or O (Figure 4.12). A person having blood type A has an enzyme that adds an *N*-acetylgalactosamine to the end of the chain, whereas a person with type B blood has an enzyme that adds galactose to the chain terminus. These two enzymes are encoded by alternate versions of the same gene, yet they recognize different substrates. People with AB blood type possess both enzymes, whereas people with O blood type lack enzymes capable of attaching either terminal sugar. The function of the ABO blood-group antigens remains a mystery.

REVIEW

1. Draw the basic structure of the major types of lipids found in cellular membranes. How do sphingolipids differ from glycerolipids? Which lipids are phospholipids? Which are glycolipids? How are these lipids organized into a bilayer? How is the bilayer important for membrane activities?
2. What is a liposome? How are liposomes used in medical therapies?
3. What is an oligosaccharide? How are they linked to membrane proteins? How are they related to human blood types?

4.4 | The Structure and Functions of Membrane Proteins

Depending on the cell type and the particular organelle within that cell, a membrane may contain hundreds of different proteins. Each membrane protein has a defined orientation relative to the cytoplasm, so that the properties of one surface of a membrane are very different from those of the other surface (as in Figure 4.4*d*). This asymmetry is referred to as membrane "sidedness." In the plasma membrane, for example, those parts of membrane proteins that interact with other cells or with extracellular substances are exposed to the extracellular space, whereas those parts of membrane proteins that interact with cytoplasmic molecules are exposed to the cytosol. Membrane proteins can be grouped into three distinct classes distinguished by the intimacy of their relationship to the lipid bilayer (Figure 4.13). These are

1. **Integral proteins** that penetrate the lipid bilayer. Integral proteins are **transmembrane proteins**; that is, they pass entirely through the lipid bilayer and thus have domains that protrude from both the extracellular and cytoplasmic sides of the membrane. Some integral proteins have only one membrane-spanning segment, whereas others are multispanning. Genome-sequencing studies suggest that integral proteins constitute 25–30 percent of all encoded proteins and roughly 60 percent of all current drug targets.
2. **Peripheral proteins** that are located entirely outside of the lipid bilayer, on either the cytoplasmic or extracellular side, yet are associated with the surface of the membrane by noncovalent bonds.
3. **Lipid-anchored proteins** that are located outside the lipid bilayer, on either the extracellular or cytoplasmic surface, but are covalently linked to a lipid molecule that is situated within the bilayer.

Integral Membrane Proteins

Most integral membrane proteins function in the following capacities: as receptors that bind specific substances at the membrane surface, as channels or transporters involved in the movement of ions and solutes across the membrane, or as agents that transfer electrons during the processes of photosynthesis and respiration. Like the phospholipids of the bilayer, integral membrane proteins are also amphipathic, having both hydrophilic and hydrophobic portions. As discussed below, those portions of an integral membrane protein that reside within the lipid bilayer—the transmembrane domains—tend to have a hydrophobic character. Amino acid residues in transmembrane domains form van der Waals interactions with the fatty acyl chains of the bilayer, which seals the protein into the lipid "wall" of the membrane. As a result, the permeability barrier of the membrane is preserved, the protein is anchored within the bilayer, and the protein is brought into direct contact with surrounding lipid molecules (Figure 4.14*a*). According to current consensus, most of the lipid molecules that make contact with a transmembrane

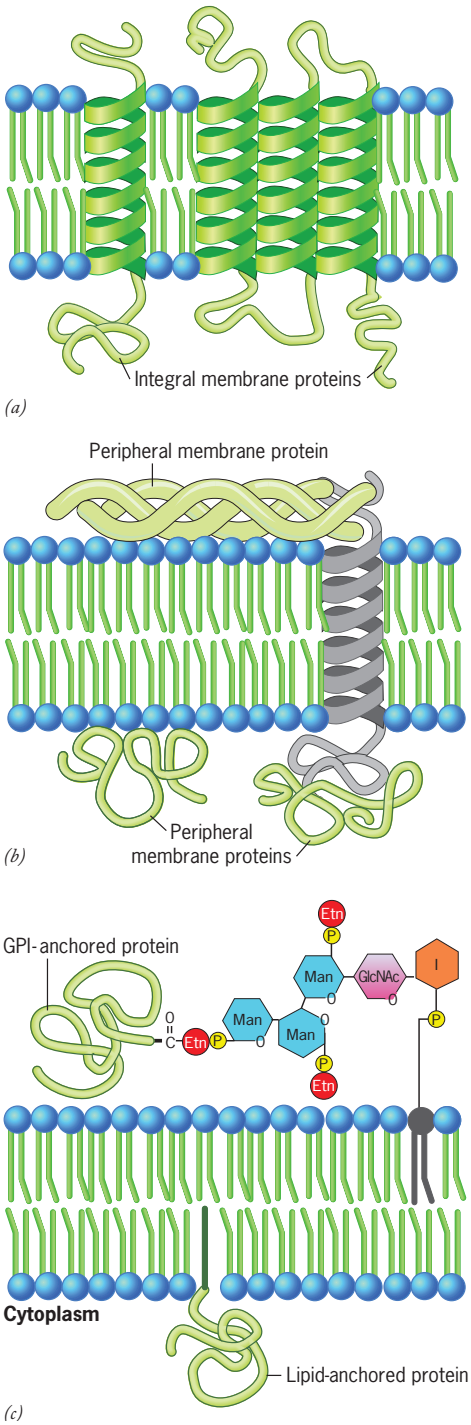


Figure 4.13 **Three classes of membrane protein.** (*a*) Integral proteins typically contain one or more transmembrane helices (see Figure 5.4 for an exception). (*b*) Peripheral proteins are noncovalently bonded to the polar head groups of the lipid bilayer and/or to an integral membrane protein. (*c*) Lipid-anchored proteins are covalently bonded to a lipid group that resides within the membrane. The lipid can be phosphatidylinositol, a fatty acid, or a prenyl group (a long-chain hydrocarbon built from five-carbon isoprenoid units). I, inositol; GlcNAc, *N*-acetylglucosamine; Man, mannose; Etn, ethanolamine; GPI, glycosylphosphatidylinositol.

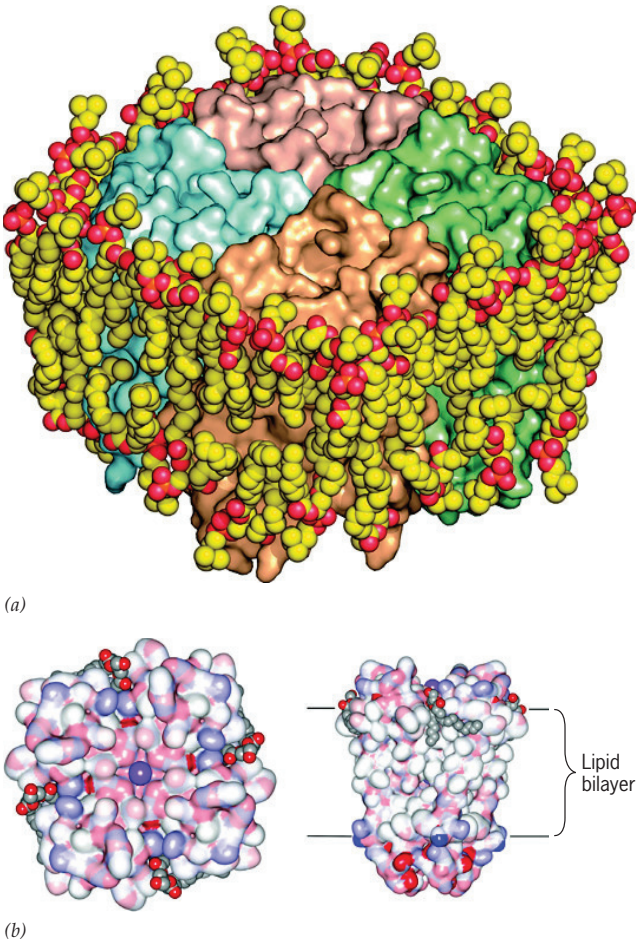


Figure 4.14 **The interactions between membrane proteins and lipids.** (*a*) Aquaporin is a membrane protein containing four subunits (colored differently in the illustration) with each subunit containing an aqueous channel. Analysis of the protein's structure revealed the presence of a surrounding layer of bound lipid molecules. In this case, these lipid molecules are not likely to play a role in the function of aquaporin because the protein retains its function as a water channel in bilayers containing nonnative lipids. (*b*) Two views of another tetrameric membrane protein, in this case the bacterial K^+ channel, KcsA. Anionic phosphatidylglycerol molecules (red/gray) are seen in each crevice between the subunits and are thought to be required for normal channel function. A K^+ ion (purple sphere) is seen in transit through the pore. (A: From Carola Hunte and Sebastian Richers, Curr. Opin. Struct. Biol. 18:407, © 2008; B: From Anthony G. Lee, Trends Biochem. Sci. 36:497, 2011, © both with permission from Elsevier Science.)

domain, such as those shown in Figure 4.14*a*, are simply passive bystanders and are rapidly exchanged with other lipid molecules in the bilayer. There is increasing evidence, however, that certain sites on the surface of many membrane proteins do form important functional interactions with specific lipid molecules. An example of this is shown in Figure 14.4*b*, where anionic lipid molecules are seen to bind in the crevice at the interfaces between the subunits of a tetrameric KcsA K^+

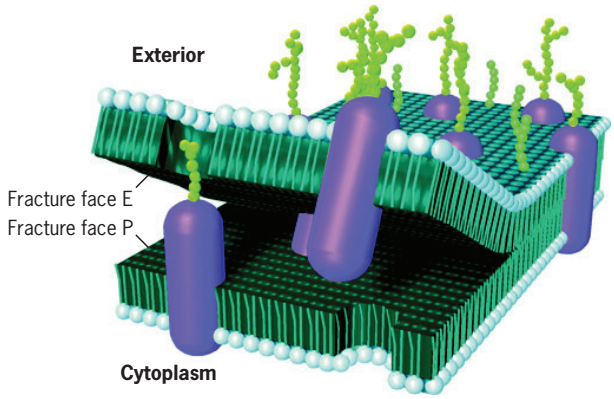
channel. The channel does not open normally in a bilayer that lacks these specific lipid molecules.

Those portions of an integral membrane protein that project into either the cytoplasm or extracellular space tend to be more like the globular proteins discussed in Section 2.5. These nonembedded domains tend to have hydrophilic surfaces that interact with water-soluble substances (low-molecular-weight substrates, hormones, and other proteins) at the edge of the membrane. In some membrane proteins, the transmembrane domains are essentially devoid of water molecules, whereas others allow the aqueous solvent to penetrate deep into the protein's membrane-embedded regions. Several large families of membrane proteins contain an interior channel that provides an aqueous passageway through the lipid bilayer. The linings of these channels typically contain key hydrophilic residues at strategic locations. As will be discussed later, integral proteins need not be fixed structures but may be able to move laterally within the membrane.

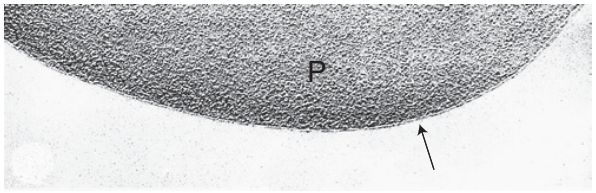
Distribution of Integral Proteins: Freeze-Fracture Analysis The concept that proteins penetrate through membranes, rather than simply remaining external to the bilayer, was derived primarily from the results of a technique called **freeze-fracture replication** (see Section 18.2). In this procedure, tissue is frozen solid and then struck with a knife blade, which fractures the block into two pieces. As this occurs, the fracture plane often takes a path between the two leaflets of the lipid bilayer (Figure 4.15*a*). Once the membranes are split in this manner, metals are deposited on their exposed surfaces to form a shadowed *replica*, which is viewed in the electron microscope (see Figure 18.17). As shown in Figure 4.15*b*, the replica resembles a road strewn with pebbles, which are called *membrane-associated particles*. Since the fracture plane passes through the center of the bilayer, most of these particles correspond to integral membrane proteins that extend at least halfway through the lipid core. When the fracture plane reaches a given particle, it goes around it rather than cracking it in half. Consequently, each protein (particle) separates with one half of the plasma membrane (Figure 4.15*c*), leaving a corresponding pit in the other half (see Figure 7.30*c*). One of the great values of the freeze-fracturing technique is that it allows an investigation of the microheterogeneity of the membrane. Localized differences in parts of the membrane stand out in these replicas and can be identified (as illustrated by the replica of a gap junction shown in Figure 7.32*d*). Biochemical analyses, in contrast, average out such differences.

Studying the Structure and Properties of Integral Membrane Proteins

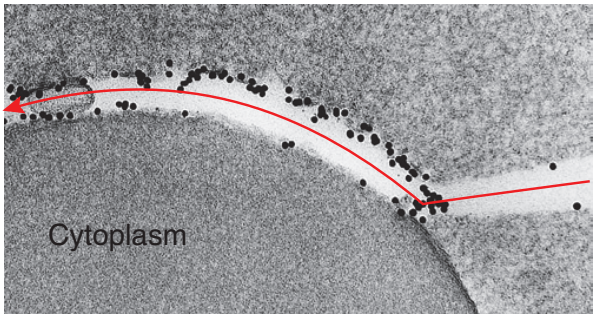
Because of their hydrophobic transmembrane domains, integral membrane proteins are difficult to isolate in a soluble form. Removal of these proteins from the membrane normally



(a)



(b) 0.2 µm



(c) 0.3 µm

Figure 4.15 **Freeze fracture: a technique for investigating cell membrane structure.** (*a*) When a block of frozen tissue is struck by a knife blade, a fracture plane runs through the tissue, often following a path that leads it through the middle of the lipid bilayer. The fracture plane goes around the proteins rather than cracking them in half, and they segregate with one of the two halves of the bilayer. The exposed faces within the center of the bilayer can then be covered with a metal deposit to form a metallic replica. These exposed faces are referred to as the E, or ectoplasmic face, and the P, or protoplasmic face. (*b*) Replica of a freeze-fractured human erythrocyte. The P fracture face is seen to be covered with particles approximately 8 nm in diameter. A small ridge (arrow) marks the junction of the particulate face with the surrounding ice. (*c*) This micrograph shows the surface of an erythrocyte that was frozen and then fractured, but rather than preparing a replica, the cell was thawed, fixed, and labeled with a marker for the carbohydrate groups that project from the external surface of the integral protein glycophorin (Figure 4.18). Thin sections of the labeled, fractured cell reveal that glycophorin molecules (black particles) have preferentially segregated with the outer half of the membrane. The red line shows the path of the fracture plane. (B: From Thomas W. Tillack and Vincent T. Marchesi, J. Cell Biol. 45:649, 1970; C: from Pedro Pinto da Silva and Maria R. Torrisi, J. Cell Biol. 93:467, 1982; B,C: Reproduced with permission of The Rockefeller University Press.)

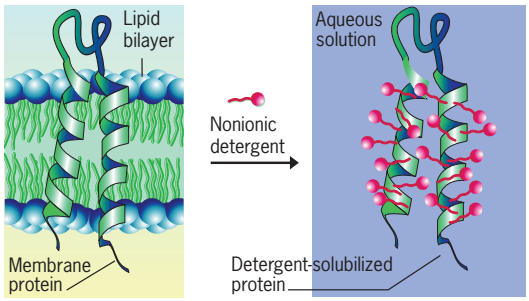
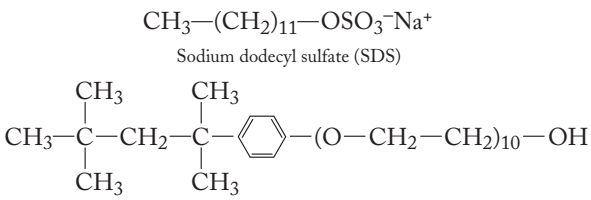


Figure 4.16 **Solubilization of membrane proteins with detergents.** The nonpolar ends of the detergent molecules associate with the nonpolar residues of the protein that had previously been in contact with the fatty acyl chains of the lipid bilayer. In contrast, the polar ends of the detergent molecules interact with the surrounding water molecules, keeping the protein in solution. Nonionic detergents, as shown here, solubilize membrane proteins without disrupting their structure.

requires the use of a detergent, such as the ionic (charged) detergent SDS (which denatures proteins) or the nonionic (uncharged) detergent Triton X-100 (which generally does not alter a protein's tertiary structure).



$CH_3—(CH_2)_{11}—OSO_3^-Na^+$

Sodium dodecyl sulfate (SDS)

Triton X-100

Like membrane lipids, detergents are amphipathic, being composed of a polar end and a nonpolar hydrocarbon chain (see Figure 2.20). As a consequence of their structure, detergents can substitute for phospholipids in stabilizing integral proteins while rendering them soluble in aqueous solution (Figure 4.16). Once the proteins have been solubilized by the detergent, various analyses can be carried out to determine the protein's amino acid composition, molecular mass, amino acid sequence, and so forth.

Researchers have had great difficulty obtaining crystals of most integral membrane proteins for use in X-ray crystallography. In fact, fewer than 1 percent of the known high-resolution protein structures represent integral membrane proteins (see http://blanco.biomol.uci.edu/MemPro_resources.html for a discussion of principles of protein structure and http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html for an updated gallery).[2] Furthermore, most of these structures represent prokaryotic versions of a particular protein, which are often smaller than their eukaryotic homologues and easier to obtain in large quantity. Once the structure of one member of a membrane protein family is determined, researchers can usually apply a strategy called *homology modeling* to learn about the structure and activity of other members of the family. For example, solution of the structure of the bacterial K^+ channel KcsA (shown in Figure 4.39) provided a wealth of data that could be applied to the structure and mechanism of action of the much more complex eukaryotic K^+ channels (Figure 4.42).

One of the first membrane proteins whose entire three-dimensional structure was determined by X-ray crystallography is shown in Figure 4.17. This protein—the bacterial photosynthetic reaction center—consists of three subunits containing 11 membrane-spanning α helices. Most membrane proteins have not been as amenable to crystallization as the photosynthetic reaction center. Among the obstacles, many membrane proteins (1) are present at low numbers per cell, (2) are unstable in the detergent-containing solutions required for their extraction, (3) are prone to aggregation, and (4) are heavily modified by glycosylation and cannot be expressed as recombinant proteins in other types of cells. Some of the technical difficulties in preparing membrane protein crystals have been overcome using new methodologies and laborious efforts. In one study, for example researchers were able to obtain high-quality crystals of a bacterial transporter after testing and refining more than 95,000 different conditions for crystallization. In some cases, mutant versions of a membrane protein are found to be better suited to forming the ordered arrays of molecules that make up a crystal lattice. In many other cases, crystallization has been achieved by covalently linking the membrane protein to other molecules, often small soluble proteins. These added elements stabilize

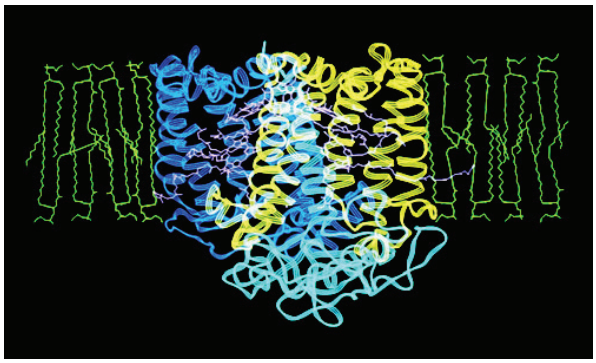


Figure 4.17 **An integral protein as it resides within the plasma membrane.** Tertiary structure of the photosynthetic reaction center of a bacterium as determined by X-ray crystallography. The protein contains three different membrane-spanning polypeptides, shown in yellow, light blue, and dark blue. The helical nature of each of the transmembrane segments is evident. (FROM G. FEHER, J. P. ALLEN, M. Y. OKAMURA, AND D. C. REES, NATURE 339:113, © 1989; REPRINTED BY PERMISSION FROM MACMILLAN PUBLISHERS LTD.)

[2]Many integral membrane proteins have a substantial portion that is present in the cytoplasm or extracellular space. In many cases, this soluble portion has been cleaved from its transmembrane domain, crystallized, and its tertiary structure determined. While this approach provides valuable data about the protein, it fails to provide information about the protein's orientation within the membrane. Another crystallographic approach to the study of membrane proteins, which uses the electron microscope rather than X-ray diffraction, is discussed in the Experimental Pathways on page 173.

the structure of the target protein and increase the number of sites on the surface of the protein that can participate in intermolecular contacts required for lattice formation. For example, the crystal structure of the β_2-adrenergic receptor shown in the chapter opening image on page 120 was obtained by inserting a T4-lysozyme molecule (derived from a T4 bacteriophage) into one of the intracellular loops of the receptor. Crystallization of the β_2-adrenergic receptor was also facilitated by the presence of a specific ligand bound to the receptor and the use of cholesterol in the lipid-phase medium in which the crystals were grown. Despite increasing success in protein crystallization, researchers still rely largely on indirect approaches for determining the three-dimensional organization of most membrane proteins. We will examine some of these approaches in the following paragraphs.

Identifying Transmembrane Domains A great deal can be learned about the structure of a membrane protein and its orientation within the lipid bilayer from a computer-based (computational) analysis of its amino acid sequence, which is readily deduced from the nucleotide sequence of an isolated gene. The first question one might ask is: Which segments of the polypeptide chain are actually embedded in the lipid bilayer? Those segments of a protein embedded within the membrane, which are described as the **transmembrane domains**, have a simple structure; they consist of a string of about 20 predominantly nonpolar amino acids that span the core of the lipid bilayer as an α helix.[3] The chemical structure of a single transmembrane helix is shown in Figure 4.18, which depicts the two-dimensional structure of glycophorin A, the major integral protein of the erythrocyte plasma membrane. Of the 20 amino acids that make up the lone α helix of a glycophorin monomer (amino acids 73 to 92 of Figure 4.18), all but three have hydrophobic side chains (or an H atom in the case of the glycine residues). The exceptions are serine and threonine, which are noncharged, polar residues (Figure 2.26). Figure 4.19*a* shows a portion of a transmembrane helix with a threonine residue, not unlike those of glycophorin A. The hydroxyl group of the residue's side chain can form a hydrogen bond with one of the oxygen atoms of the peptide backbone. Fully charged residues may also appear in transmembrane helices, but they tend to be near one of the ends of the helix and to be accommodated in ways that allow them to fit into their hydrophobic environment. This is illustrated in the model transmembrane helices shown in Figures 4.19*b* and *c*. Each of the helices in these figures contains a pair of charged residues whose side chains are able to reach out and interact with the innermost polar regions of the membrane, even if it requires distorting the helix to do so. Figure 4.19*d* shows two tyrosine residues with their hydrophobic aromatic side chains; each aromatic ring is oriented parallel with the hydrocarbon chains of the bilayer with which it has become integrated.

Knowing the amino acid sequence of an integral membrane protein, we can usually identify the transmembrane segments using a *hydropathy plot*, in which each site along a polypeptide is assigned a value that provides a measure of the *hydrophobicity* of the amino acid at that site as well as that of its neighbors. This approach provides a "running average" of the hydrophobicity of short sections of the polypeptide, and guarantees that one or a few polar amino acids in a sequence do not alter the profile of the entire stretch. Hydrophobicity of

[3]It was noted on page 55 that the α helix is a favored conformation because it allows for a maximum number of hydrogen bonds to be formed between atoms of the polypeptide backbone, thereby creating a highly stable (low-energy) configuration. This is particularly important for a membrane-spanning polypeptide that is surrounded by fatty acyl chains and, thus, cannot form hydrogen bonds with an aqueous solvent. Transmembrane helices are at least 20 amino acids in length, because this is the minimum stretch of polypeptide capable of spanning the hydrocarbon core of a lipid bilayer of 30 Å width. A few integral membrane proteins have been found to contain loops or helices that penetrate but do not span the bilayer. An example is the P helix in Figure 4.39.

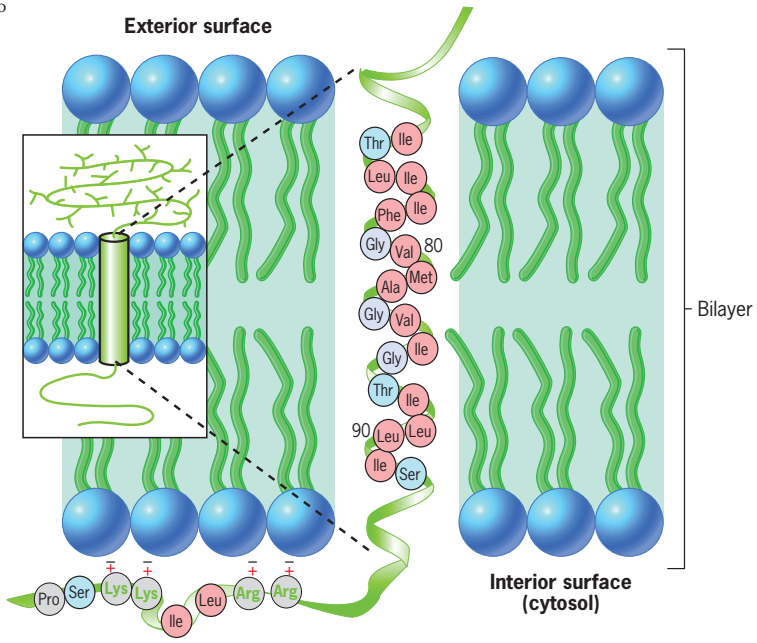
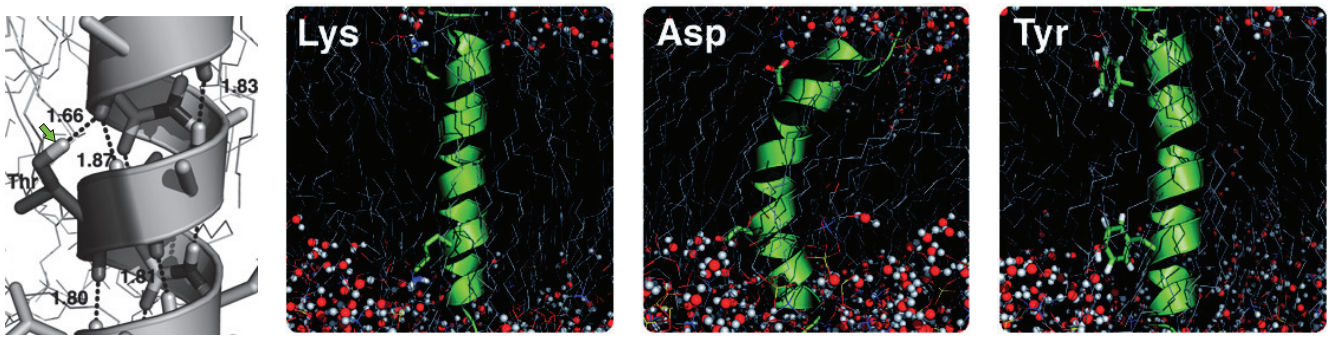


Figure 4.18 **Glycophorin A, an integral protein with a single transmembrane domain.** The single α helix that passes through the membrane consists predominantly of hydrophobic residues (brown-colored circles). The four positively charged amino acid residues of the cytoplasmic domain of the membrane protein form ionic bonds with negatively charged lipid head groups. Carbohydrates are attached to a number of amino acid residues on the outer surface of the protein (shown in the inset). All but one of the 16 oligosaccharides are small *O*-linked chains (the exception is a larger oligosaccharide linked to the asparagine residue at position 26). Glycophorin molecules are present as homodimers within the erythrocyte membrane (Figure 4.32*d*). The two helices of the dimer cross over one another in the region between residues 79 and 83. This Gly-X-X-X-Gly sequence is commonly found where transmembrane helices come into close proximity.



(a) (b) (c) (d)

Figure 4.19 **Accommodating various amino acid residues within transmembrane helices.** (*a*) In this portrait of a small portion of a transmembrane helix, the hydroxyl group of the threonine side chain (arrow) is able to form a (shared) hydrogen bond with a backbone oxygen within the lipid bilayer. Hydrogen bonds are indicated by the dashed lines and their distances are shown in angstroms. (*b*) The side chains of the two lysine residues of this transmembrane helix are sufficiently long and flexible to form bonds with the head groups and water molecules of the polar face of the lipid bilayer. (*c*) The side chains of the two aspartic acid residues of this transmembrane helix can also reach the polar face of the bilayer but introduce distortion in the helix to do so. (*d*) The aromatic side chains of the two tyrosine residues of this transmembrane helix are oriented perpendicular to the axis of the membrane and parallel to the fatty acyl chains with which they interact. (From Anna C. V. Johansson and Erik Lindahl, Biophys. J. 91:4459, 4453, 2006, © 2006, with permission from Elsevier.)

amino acids can be determined using various criteria, such as their lipid solubility or the energy that would be required to transfer them from a nonpolar medium into an aqueous medium. A hydropathy plot for glycophorin A is shown in Figure 4.20. Transmembrane segments are usually identified as a jagged peak that extends well into the hydrophobic side of the spectrum. A reliable prediction concerning the orientation of the transmembrane segment within the bilayer can usually be made by examining the flanking amino acid residues. In most cases, as illustrated by glycophorin in Figure 4.18, those parts of the polypeptide at the cytoplasmic flank of a transmembrane segment tend to be more positively charged than those at the extracellular flank.

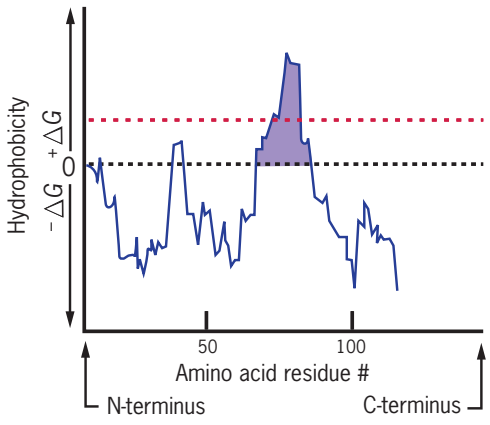
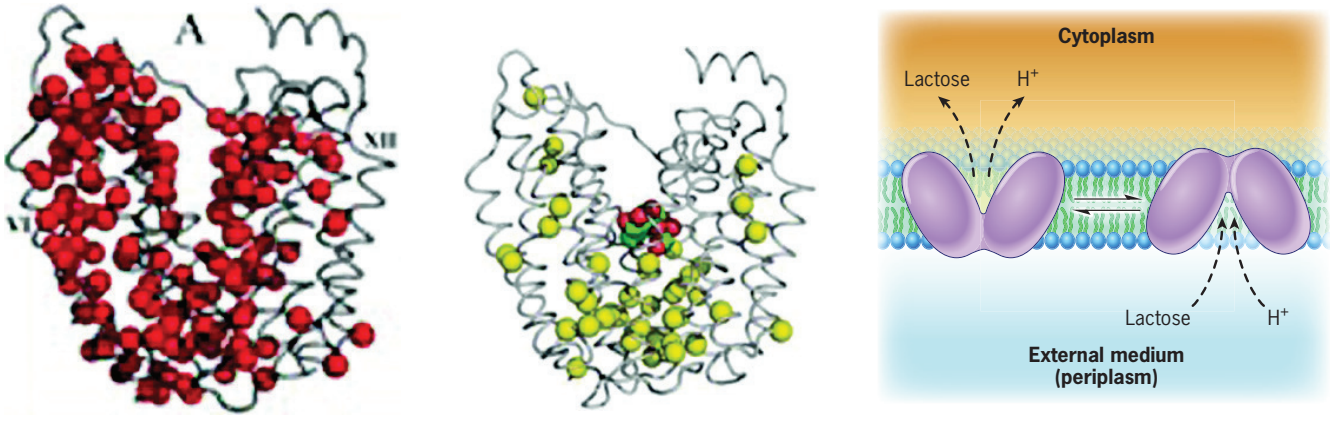


Figure 4.20 **Hydropathy plot for glycophorin A, a single membrane-spanning protein.** Hydrophobicity is measured by the free energy required to transfer each segment of the polypeptide from a nonpolar solvent to an aqueous medium. Values above the 0 line are energy-requiring ($+\Delta G$s), indicating they consist of stretches of amino acids that have predominantly nonpolar side chains. Peaks that project above the red-colored line are interpreted as a transmembrane domain.

Not all integral membrane proteins contain transmembrane α helices. A number of membrane proteins contain a relatively large channel positioned within a circle of membrane-spanning β strands organized into a barrel as illustrated in Figure 5.4. To date, aqueous channels constructed of β barrels have only been found in the outer membranes of bacteria, mitochondria, and chloroplasts.

Experimental Approaches to Identifying Conformational Changes within an Integral Membrane Protein Suppose you have isolated a gene for an integral membrane protein and, based on its nucleotide sequence, determined that it contains 12 apparent membrane-spanning α helices. You might want to know which sites within these transmembrane domains are accessible to the aqueous environment and how this changes as the protein carries out its function. This type of information is particularly important when working with a membrane channel or transporter that functions in the movement of hydrophilic substances across the membrane. Although these determinations are difficult to make without detailed structural models, considerable insight can be gained by site-directed mutagenesis, that is, by introducing specific changes into the gene that encodes the protein (Section 18.17). An experiment of this type, which was conducted on lactose permease, a sugar-transporting protein in bacterial cell membranes, is shown in Figure 4.21. In this experiment, researchers prepared different versions of the permease in which nearly all of the individual amino acids of the protein were replaced, one at a time, by a cysteine residue. Each mutated membrane protein was then incubated with a water-soluble reagent (NEM) that is capable of adding an alkyl group to the cysteine side chain—as long as it has access to that side chain. The incubations with NEM were carried out



(a) (b) (c)

Figure 4.21 **An experiment employing site-directed mutagenesis to learn about dynamic changes in the conformation of a membrane protein as it carries out its activity.** The experimental strategy of the experiment and its results are discussed in the text. The cytoplasmic surface of the protein (lactose permease) is at the top. (*a*) The red spheres indicate the residues of the membrane protein that reacted with the alkylating agent NEM in the absence of a sugar to be transported. (*b*) The gold spheres denote the residues that are much more accessible to the alkylating agent when the protein is incubated with the sugar. The gold spheres in *b* are seen to cluster in a portion of the protein near the external medium (the periplasm in bacteria). The authors concluded that the gold spheres correspond to residues that line an outward-facing cavity, i.e., one that is open to the external medium. The results support a model of alternating access to the medium as depicted in part *c*. (Note the structure that is depicted in parts *a*–*b* is that of the inward-facing conformation as determined by X-ray crystallography. The structure of the outward-facing conformation has not been directly determined, which is why these types of labeling experiments remain important.) (A–B: From H. Ronald Kaback, et al., PNAS 104:492, 2007, © 2007 National Academy of Sciences, U. S. A.) C: Reprinted from Current Opinion in Structural Biology 14:414, 2004 Fig. 1 by H. R. Kaback, with permission from Elsevier.

under two different conditions: either in the presence of the sugar to be transported or in its absence. The image in Figure 4.21*a* shows those residues (depicted as red spheres) of the protein that become alkylated by NEM *in the absence of the sugar*. Many of these accessible residues are thought to line an inward-facing hydrophilic cavity in the permease that is open to the cytoplasm. Those cysteine residues that are not labeled, most notably on helices VI and XII, are presumed to either face the fatty acyl chains of the phospholipids or to be located in tightly packed regions of the molecule, thus making them inaccessible to NEM.

The image in Figure 4.21*b* shows those residues (depicted as gold spheres) that show a significant *increase* in reactivity to NEM when the permease is incubated with the sugar to be transported. The difference in NEM reactivity of the residues between the protein in the absence of the sugar (*a*) and the presence of the sugar (*b*) indicates that different parts of the protein are accessible to the aqueous medium under these two conditions. The results, in other words, suggest that addition of the sugar induces a conformational change in the permease. Careful analysis of the positions of the residues that become labeled in these two conformations supports the model shown in Figure 4.21*c*. According to this model of alternating access, the sugar-binding site is open to the cytoplasm of the bacterial cell in one conformation and is open to the extracellular space in the other conformation. Alternation between the two conformations brings about transport of the sugar across the membrane. This type of ion-driven transport system is discussed further on page 159.

Determining spatial relationships between specific amino acids in a membrane protein is another approach to learning about dynamic events that occur as a protein carries out its function. One way to learn about the distance between selected residues in a protein is to introduce chemical groups whose properties are sensitive to the distance that separates them. *Nitroxides* are chemical groups that contain an unpaired electron, which produces a characteristic spectrum when monitored by a technique called *electron paramagnetic resonance* (*EPR*) *spectroscopy*. A nitroxide group can be introduced at any site in a protein by first mutating that site to a cysteine and then attaching the nitroxide to the —SH group of the cysteine residue. Figure 4.22 shows how this technique was used to discover the conformational changes that occur in a membrane protein as its channel is opened in response to changes in the pH of the medium. The protein in question, a bacterial K^+ channel, is a tetramer composed of four identical subunits. The cytoplasmic opening to the channel is bounded by four transmembrane helices, one from each subunit of the protein. Figure 4.22*a* shows the EPR spectra that were obtained when a nitroxide was introduced near the cytoplasmic end of each transmembrane helix. The red line shows the spectrum obtained at pH 6.5 when the channel is in the closed state, and the blue line shows the spectrum at pH 3.5 when the channel is open. The shape of each line depends on the proximity of the nitroxides to one another. The spectrum is broader at pH 6.5 because the nitroxide groups on the four subunits are closer together at this pH, which decreases the intensity of their EPR signals. These results indicate that the

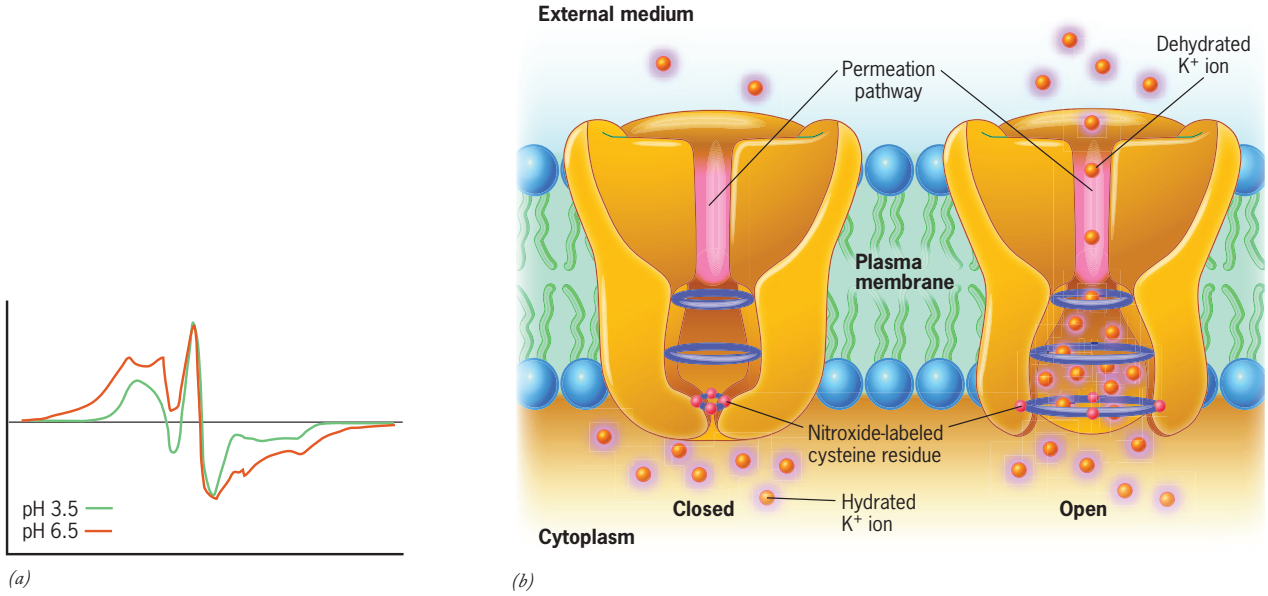


Figure 4.22 **Use of EPR spectroscopy to monitor changes in conformation of a bacterial K^+ ion channel as it opens and closes.** (*a*) EPR spectra from nitroxides that have been attached to cysteine residues near the cytoplasmic end of the four transmembrane helices that line the channel. The cysteine residue in each helix replaces a glycine residue that is normally at that position. The shapes of the spectra depend on the distances between unpaired electrons in the nitroxides on different subunits. (Nitroxides are described as "spin-labels," and this technique is known as site-directed spin labeling.) (*b*) A highly schematic model of the ion channel in the open and closed states based on the data from part *a*. Opening of the channel is accompanied by the movement of the four nitroxide groups apart from one another. (A: Reprinted by permission from Macmillan Publishers Ltd: from E. Perozo et al., Nature Struct. Biol. 5:468, 1998.)

activation of the channel is accompanied by an increased separation between the labeled residues of the four subunits (Figure 4.22*b*). An increase in diameter of the channel opening allows ions in the cytoplasm to reach the actual permeation pathway (shown in red) within the channel, which allows only the passage of K^+ ions (discussed on page 153). An alternate technique, called FRET, that can also be used to measure changes in the distance between labeled groups within a protein, is illustrated in Figure 18.8.

Peripheral Membrane Proteins

Peripheral proteins are associated with the membrane by weak electrostatic bonds (refer to Figure 4.13*b*). Peripheral proteins can usually be solubilized by extraction with high-concentration salt solutions that weaken the electrostatic bonds holding peripheral proteins to a membrane. In actual fact, the distinction between integral and peripheral proteins is blurred because many integral membrane proteins consist of several polypeptides, some that penetrate the lipid bilayer and others that remain on the periphery.

The best studied peripheral proteins are located on the internal (cytosolic) surface of the plasma membrane, where they form a fibrillar network that acts as a membrane "skeleton" (see Figure 4.32*d*). These proteins provide mechanical support for the membrane and function as an anchor for integral membrane proteins. Other peripheral proteins on the internal plasma membrane surface function as enzymes, specialized coats (see Figure 8.24), or factors that transmit transmembrane signals (see Figure 15.19). Peripheral proteins typically have a dynamic relationship with the membrane, being recruited to the membrane or released from the membrane depending on prevailing conditions.

Lipid-Anchored Membrane Proteins

Several types of lipid-anchored membrane proteins can be distinguished. Numerous proteins present on the external face of the plasma membrane are bound to the membrane by a small, complex oligosaccharide linked to a molecule of phosphatidylinositol that is embedded in the outer leaflet of the lipid bilayer (refer to Figure 4.13*c*). Peripheral membrane proteins containing this type of glycosyl-phosphatidylinositol linkage are called **GPI-anchored proteins**. They were discovered when it was shown that certain membrane proteins could be released by a phospholipase that specifically recognized and cleaved inositol-containing phospholipids. The normal cellular scrapie protein PrP^C (page 66) is a GPI-linked molecule, as are various receptors, enzymes, and cell-adhesion proteins. A rare type of anemia, paroxysmal nocturnal hemoglobinuria, results from a deficiency in GPI synthesis that makes red blood cells susceptible to lysis.

Another group of proteins present on the *cytoplasmic* side of the plasma membrane is anchored to the membrane by one

or more long hydrocarbon chains embedded in the inner leaflet of the lipid bilayer (refer to Figure 4.13*c* and accompanying legend). At least two proteins associated with the plasma membrane in this way (Src and Ras) have been implicated in the transformation of a normal cell to a malignant state.

REVIEW

1. Why are detergents necessary to solubilize membrane proteins? How might one determine the diversity of integral proteins that reside in a purified membrane fraction?
2. How can one determine: (1) the location of transmembrane segments in the amino acid sequence or (2) the relative locations of transmembrane helices with access to the external medium?
3. What is meant by the statement that the proteins of a membrane are distributed asymmetrically? Is this also true of the membrane's carbohydrate?
4. Describe the properties of the three classes of membrane proteins (integral, peripheral, and lipid-anchored), how they differ from one another, and how they vary among themselves.

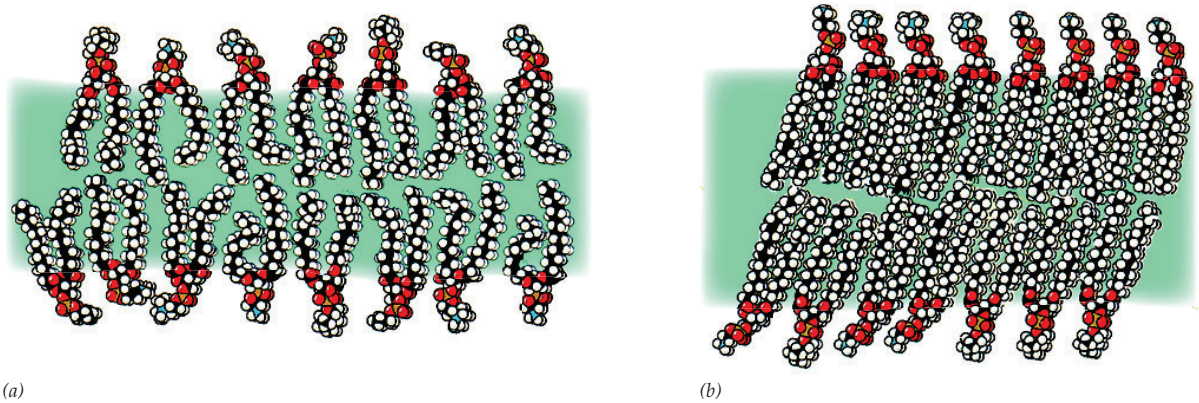
4.5 | Membrane Lipids and Membrane Fluidity

The physical state of the lipid of a membrane is described by its fluidity (or viscosity).[4] Consider a simple artificial bilayer composed of phosphatidylcholine and phosphatidylethanolamine, whose fatty acids are largely unsaturated. If the temperature of the bilayer is kept relatively warm (e.g., 37°C), the lipid exists in a relatively fluid state (Figure 4.23*a*). At this temperature, the lipid bilayer is best described as a two-dimensional liquid crystal. As in a crystal, the molecules still retain a specified orientation; in this case, the long axes of the molecules tend toward a parallel arrangement, yet individual phospholipids can rotate around their axis or move laterally within the plane of the bilayer. If the temperature is slowly lowered, a point is reached where the bilayer distinctly changes (Figure 4.23*b*). The lipid is converted from a liquid crystalline phase to a frozen crystalline gel in which the movement of the phospholipid fatty acid chains is greatly restricted. The temperature at which this change occurs is called the **transition temperature**.

The transition temperature of a particular bilayer depends on the ability of the lipid molecules to be packed together, which depends in turn on the particular lipids of which it is constructed. Saturated fatty acids have the shape of a straight, flexible rod. *Cis*-unsaturated fatty acids, on the other hand, have crooks in the chain at the sites of a double bond (Figures 2.19 and 4.23). Consequently, phospholipids with saturated chains pack together more tightly than those containing unsaturated chains. The greater the degree of unsaturation of the fatty acids of the bilayer, the *lower* the temperature before the bilayer gels. The introduction of one double bond in a molecule of stearic acid lowers the melting temperature almost 60°C (Table 4.2).[5] Another factor that influences bilayer fluidity is fatty acid chain length. The shorter the fatty acyl chains of a phospholipid, the lower its melting temperature. The physical state of the membrane is also affected by cholesterol. Because of their orientation within the bilayer (Figure 4.7), cholesterol molecules disrupt the close packing of fatty

[4]Fluidity and viscosity are inversely related; fluidity is a measure of the ease of flow, and viscosity is a measure of the resistance to flow.

[5]The effect of fatty acid saturation on melting temperature is illustrated by familiar food products. Vegetable oils remain a liquid in the refrigerator, whereas margarine is a solid. Vegetable oils contain polyunsaturated fatty acids, whereas the fatty acids of margarine have been saturated by a chemical process that hydrogenates the double bonds of the vegetable oils used as the starting material.



(*a*) (*b*)

Figure 4.23 **The structure of the lipid bilayer depends on the temperature.** The bilayer shown here is composed of two phospholipids: phosphatidylcholine and phosphatidylethanolamine. (*a*) Above the transition temperature, the lipid molecules and their hydrophobic tails are free to move in certain directions, even though they retain a considerable degree of order. (*b*) Below the transition temperature, the movement of the molecules is greatly restricted, and the entire bilayer can be described as a crystalline gel. (A–B: R. N. ROBERTSON, THE LIVELY MEMBRANES, CAMBRIDGE UNIV. PRESS; 1983, REPRINTED WITH PERMISSION OF CAMBRIDGE UNIV. PRESS.)

Table 4.2 Melting Points of the Common 18-Carbon Fatty Acids

Fatty acid	*cis* Double bonds	M.p.(°C)
Stearic acid	0	70
Oleic acid	1	13
Linoleic acid	2	−9
Linolenic acid	3	−17
Eicosapentanoic acid (EPA)*	5	−54

*EPA has 20 carbons.

acyl chains and interfere with their mobility. The presence of cholesterol tends to abolish sharp transition temperatures and creates a condition of intermediate fluidity. In physiologic terms, cholesterol tends to increase the durability while decreasing the permeability of a membrane.

The Importance of Membrane Fluidity

What effect does the physical state of the lipid bilayer have on the biological properties of the membrane? Membrane fluidity provides a perfect compromise between a rigid, ordered structure in which mobility would be absent and a completely fluid, nonviscous liquid in which the components of the membrane could not be oriented and structural organization and mechanical support would be lacking. In addition, fluidity allows for interactions to take place within the membrane. For example, membrane fluidity makes it possible for clusters of membrane proteins to assemble at particular sites within the membrane and form specialized structures, such as intercellular junctions, light-capturing photosynthetic complexes, and synapses. Because of membrane fluidity, molecules that interact can come together, carry out the necessary reaction, and move apart.

Fluidity also plays a key role in membrane assembly, a subject discussed in Chapter 8. Membranes arise only from preexisting membranes, and their growth is accomplished by the insertion of lipids and proteins into the fluid matrix of the membranous sheet. Many of the most basic cellular processes, including cell movement, cell growth, cell division, formation of intercellular junctions, secretion, and endocytosis, depend on the movement of membrane components and would probably not be possible if membranes were rigid, nonfluid structures.

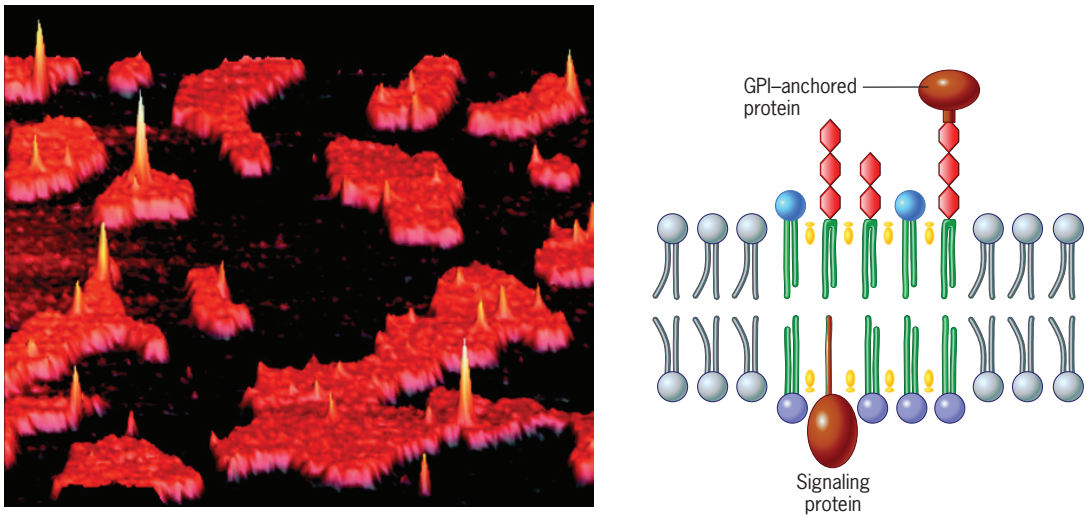
Maintaining Membrane Fluidity

The internal temperature of most organisms (other than birds and mammals) fluctuates with the temperature of the external environment. Since it is essential for many activities that the membranes of a cell remain in a fluid state, cells respond to changing conditions by altering the types of phospholipids of which they are made. Maintenance of membrane fluidity is an example of homeostasis at the cellular level and can be demonstrated in various ways. For example, if the temperature of a culture of cells is lowered, the cells respond metabolically. The initial "emergency" response is mediated by enzymes that remodel membranes, making the cell more cold resistant. Remodeling is accomplished by (1) desaturating single bonds in fatty acyl chains to form double bonds, and (2) reshuffling the chains between different phospholipid molecules to produce ones that contain two unsaturated fatty acids, which greatly lowers the melting temperature of the bilayer. Desaturation of single bonds to form double bonds is catalyzed by enzymes called *desaturases*. Reshuffling is accomplished by *phospholipases*, which split the fatty acid from the glycerol backbone, and *acyltransferases*, which transfer fatty acids between phospholipids. In addition, the cell changes the types of phospholipids being synthesized in favor of ones containing more unsaturated fatty acids. As a result of the activities of these various enzymes, the physical properties of a cell's membranes are matched to the prevailing environmental conditions. Maintenance of fluid membranes by adjustments in fatty acyl composition has been demonstrated in a variety of organisms, including hibernating mammals, pond-dwelling fish whose body temperature changes markedly from day to night, cold-resistant plants, and bacteria living in hot springs. Prokaryotic cells that live at very high temperatures have plasma membranes that contain highly unusual lipids.

Lipid Rafts

Every so often an issue emerges that splits the community of cell biologists into believers and nonbelievers. The issue of lipid rafts falls into this category. When membrane lipids are extracted from cells and used to prepare *artificial* lipid bilayers, cholesterol and sphingolipids tend to self-assemble into microdomains that are more gelated and highly ordered than surrounding regions consisting primarily of phosphoglycerides. Because of their distinctive physical properties, such microdomains tend to float within the more fluid and disordered environment of the artificial bilayer (Figure 4.24*a*). As a result, these patches of cholesterol and sphingolipid are referred to as **lipid rafts**. When added to these artificial bilayers, certain proteins tend to become concentrated in the lipid rafts, whereas others tend to remain outside their boundaries. GPI-anchored proteins show a particular fondness for the ordered regions of the bilayer (Figure 4.24*a*).

The controversy arises over whether similar types of sphingolipid- and cholesterol-rich lipid rafts, such as that illustrated in Figure 4.24*b*, exist within living cells. Most of the evidence in favor of lipid rafts is derived from studies that employ unnatural treatments, such as detergent extraction or cholesterol depletion, which makes the results difficult to interpret. Attempts to demonstrate the presence of lipid rafts in living cells have generally been unsuccessful, which can either mean that such rafts do not exist or they are so small (5 to 25 nm diameter) and short-lived as to be difficult to detect with current techniques. The concept of lipid rafts is very appealing because it provides a means to introduce order into a seemingly random sea of lipid molecules. Lipid rafts are postulated to serve as floating platforms that concentrate particular proteins, thereby organizing the membrane into functional compartments (Figure 4.24*b*). For example, lipid rafts are thought



(a) (b)

Figure 4.24 **Lipid rafts.** (*a*) Image of the upper surface of an artificial lipid bilayer containing phosphatidylcholine, which appears as the black background, and sphingomyelin molecules, which organize themselves spontaneously into the orange-colored rafts. The yellow peaks show the positions of a GPI-anchored protein, which is almost exclusively raft-associated. This image is provided by an atomic force microscope, which measures the height of various parts of the specimen at the molecular level. (*b*) Schematic model of a lipid raft within a cell. The outer leaflet of the raft consists primarily of cholesterol (yellow) and sphingolipids (red head groups). Phosphatidylcholine molecules (blue head groups) with long saturated fatty acids also tend to concentrate in this region. GPI-anchored proteins are thought to become concentrated in lipid rafts. The lipids in the outer leaflet of the raft have an organizing effect on the lipids of the inner leaflet. As a result, the inner-leaflet raft lipids consist primarily of cholesterol and glycerophospholipids with long saturated fatty acyl tails. The inner leaflet tends to concentrate lipid-anchored proteins, such as Src kinase, that are involved in cell signaling. (The controversy over the existence of lipid rafts is discussed in *Nature Revs. Mol. Cell Biol.* 11:688, 2010 and *Science* 334:1046, 2011.) (A: From D. E. Saslowsky, et al., J. Biol. Chem. 277, cover of #30, July 26, 2002; Courtesy of J. Michael Edwardson © 2002 The American Society for Biochemistry and Molecular Biology.)

to provide a favorable local environment for cell-surface receptors to interact with other membrane proteins that transmit signals from the extracellular space to the cell interior.

REVIEW

1. What is the importance of fatty acid unsaturation for membrane fluidity? Of enzymes that are able to desaturate fatty acids?
2. What is meant by a membrane's transition temperature, and how is it affected by the degree of saturation or length of fatty acyl chains? How are these properties important in the formation of lipid rafts?
3. Why is membrane fluidity important to a cell?
4. How can the two sides of a lipid bilayer have different ionic charges?

4.6 | The Dynamic Nature of the Plasma Membrane

It is apparent from the previous discussion that the lipid bilayer can exist in a relatively fluid state. As a result, a phospholipid can move laterally within the same leaflet with considerable ease. The mobility of individual lipid molecules within the bilayer of the plasma membrane can be directly observed under the microscope by linking the polar heads of the lipids to gold particles or fluorescent compounds (see Figure 4.29). It is estimated that a phospholipid can diffuse from one end of a bacterium to the other end in a second or two. In contrast, it takes a phospholipid molecule a matter of hours to days to move across to the other leaflet. Thus, of all the possible motions that a phospholipid can make, its flip-flop to the other side of the membrane is the most restricted (Figure 4.25). This finding is not surprising. For flip-flop to occur, the hydrophilic head group of the lipid must pass through the internal hydrophobic sheet of the membrane, which is thermodynamically unfavorable. However, cells contain enzymes that actively move certain phospholipids from one leaflet to the other. These enzymes play a role in establishing lipid asymmetry and may also reverse the slow rate of passive transmembrane movement.

Because lipids provide the matrix in which integral proteins of a membrane are embedded, the physical state of the lipid is an important determinant of the mobility of integral proteins. The demonstration that integral proteins can move within the plane of the membrane was a cornerstone in the formulation of the fluid-mosaic model. The dynamic properties of membrane proteins have been revealed in several ways.

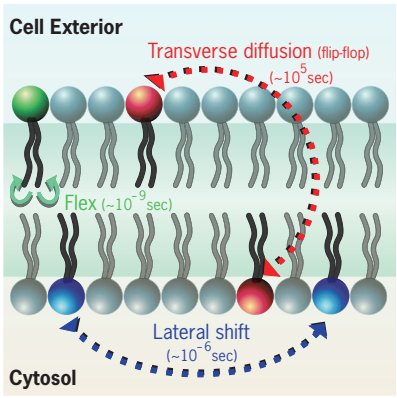
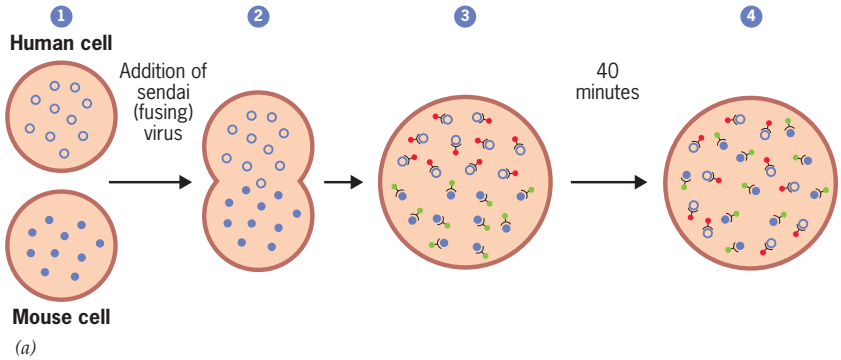


Figure 4.25 **The possible movements of phospholipids in a membrane.** The types of movements in which membrane phospholipids can engage and the approximate time scales over which they occur. Whereas phospholipids move from one leaflet to another at a very slow rate, they diffuse laterally within a leaflet rapidly. Lipids lacking polar groups, such as cholesterol, can move across the bilayer quite rapidly.

The Diffusion of Membrane Proteins after Cell Fusion

Cell fusion is a technique whereby two different types of cells, or cells from two different species, can be fused to produce one cell with a common cytoplasm and a single, continuous plasma membrane. Cells are induced to fuse with one another by making the outer surface of the cells "sticky" so that their plasma membranes adhere to one another. Cells can be induced to fuse by addition of certain inactivated viruses that attach to the surface membrane, by adding the compound polyethylene glycol, or by a mild electric shock. Cell fusion has played an important role in cell biology and is currently used in an invaluable technique to prepare specific antibodies (Section 18.18).

The first experiments to demonstrate that membrane proteins could move within the plane of the membrane utilized cell fusion, and they were reported in 1970 by Larry Frye and Michael Edidin of Johns Hopkins University. In their experiments, mouse and human cells were fused, and the locations of specific proteins of the plasma membrane were followed once the two membranes had become continuous. To follow the distribution of either the mouse membrane proteins or the human membrane proteins at various times after fusion, antibodies against one or the other type of protein were prepared and covalently linked to fluorescent dyes. The antibodies against the mouse proteins were complexed with a dye that fluoresces green and the antibodies against human proteins with one that fluoresces red. When the antibodies were added to fused cells, they bound to the human or mouse proteins and could be located under a fluorescence light microscope (Figure 4.26*a*). At the time of fusion, the plasma membrane appeared half human and half mouse; that is, the two protein types remained segregated in their own hemisphere (step 3, Figure 4.26*a*,*b*). As the time after fusion increased, the membrane proteins were seen to move laterally within the membrane into the opposite hemisphere. By about 40 minutes, each species' proteins were uniformly distributed around the entire hybrid cell membrane (step 4, Figure 4.26*a*). If the same experiment was performed at lower temperature, the viscosity of the lipid bilayer increased, and the mobility of the membrane proteins decreased. These early cell fusion experiments gave the impression that integral membrane proteins were capable of virtually unrestricted movements. As we will see shortly, subsequent studies made it apparent that membrane dynamics was a much more complex subject than first envisioned.



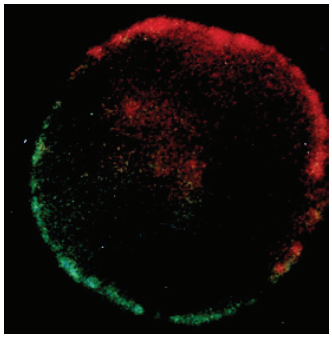


Figure 4.26 **The use of cell fusion to reveal mobility of membrane proteins.** (*a*) Outline of the experiment in which human and mouse cells were fused (steps 1–2) and the distribution of the proteins on the surface of each cell were followed in the hybrids over time (steps 3–4). Mouse membrane proteins are indicated by solid circles, human membrane proteins by open circles. Locations of human and mouse proteins in the plasma membranes of the hybrid cells were monitored by interaction with fluorescent red and fluorescent green antibodies, respectively. (*b*) Micrograph showing a fused cell in which mouse and human proteins are still in their respective hemispheres (equivalent to the hybrid in step 3 of part *a*). (B: From L. D. Frye and Michael Edidin, J. Cell Science 7:328, 334, 1970; By permission of The Company of Biologists, Ltd. Courtesy of Michael Edidin, Johns Hopkins University. http://jcs.biologists.org/content/7/2/319.full.pdf+html?sid=d93ae648-abca-4f5d-90a6-a6d9726d7d30)

Restrictions on Protein and Lipid Mobility

Several techniques allow researchers to follow the movements of molecules in the membranes of living cells using the light microscope. In a technique called **fluorescence recovery after photobleaching (FRAP)**, which is illustrated in Figure 4.27*a*, integral membrane components in cultured cells are first labeled by linkage to a fluorescent dye. A particular membrane protein can be labeled using a specific probe, such as a fluorescent antibody. Once labeled, cells are placed under the microscope and irradiated by a sharply focused laser beam that bleaches the fluorescent molecules in its path, leaving a circular spot (typically about 1 μm diameter) on the surface of the cell that is largely devoid of fluorescence. If the labeled proteins in the membrane are mobile, then the random movements of these molecules should produce a gradual reappearance of fluorescence in the irradiated circle. The rate of fluorescence recovery (Figure 4.27*b*) provides a direct measure of the rate of diffusion (expressed as a diffusion coefficient, *D*) of the mobile molecules. The extent of fluorescence recovery (expressed as a percentage of the original intensity) provides a measure of the percentage of the labeled molecules that are free to diffuse.

Early studies utilizing FRAP suggested that (1) membrane proteins moved much more slowly in a plasma membrane than they would in a pure lipid bilayer and (2) a significant fraction of membrane proteins (30 to 70 percent) were not free to diffuse back into the irradiated circle. But the FRAP technique has its drawbacks. FRAP can only follow the average movement of a relatively large number of labeled molecules (hundreds to thousands) as they diffuse over a relatively large distance (e.g., 1 μm). As a result, researchers using FRAP cannot distinguish between proteins that are truly immobile and ones that can only diffuse over a limited distance in the time allowed. To get around these limitations, alternate techniques have been developed that allow researchers to observe the movements of individual protein molecules over very short distances and to determine how they might be restrained.

In **single-particle tracking (SPT)**, individual membrane protein molecules are labeled, usually with antibody-coated gold particles (approximately 40 nm in diameter), and the movements of the labeled molecules are followed by computer-enhanced video microscopy (Section 18.1). The results of these studies often depend on the particular protein being investigated. For example,

- Some membrane proteins move randomly throughout the membrane (Figure 4.28, protein A), though generally at rates considerably less than would be measured in an artificial lipid bilayer. (If protein mobility were based strictly on physical parameters such as lipid viscosity and protein size, one would expect proteins to migrate with diffusion coefficients of approximately 10^{-8} to 10^{-9} cm^2/sec rather than 10^{-10} to 10^{-12} cm^2/sec, as is observed for molecules of this group.) The reasons for the reduced diffusion coefficient have been debated.
- Some membrane proteins fail to move and are considered to be immobilized (Figure 4.28, protein B).
- In some cases, a protein is found to move in a highly directed (i.e., nonrandom) manner toward one part of the cell or another (Figure 4.28, protein C). For example, a

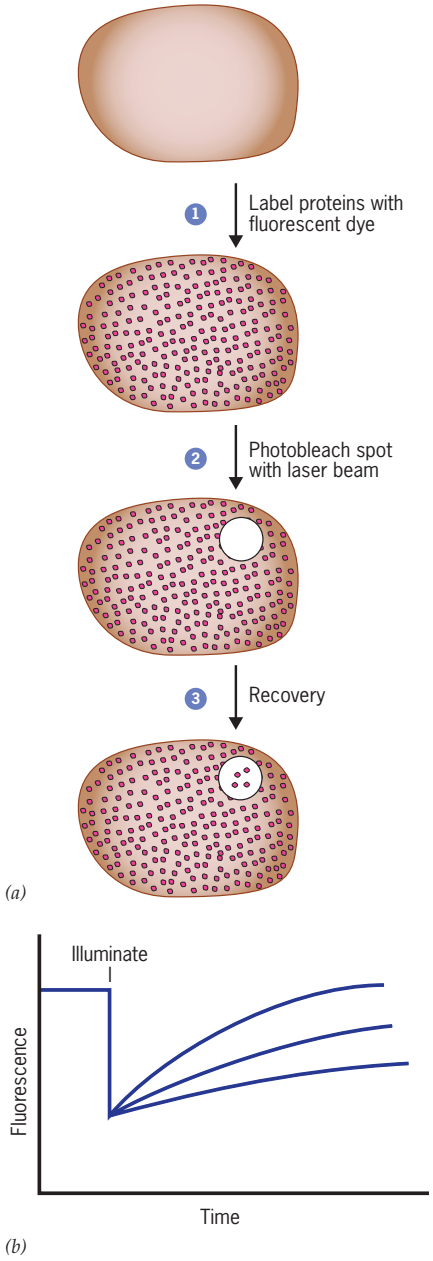


Figure 4.27 **Measuring the diffusion rates of membrane proteins by fluorescence recovery after photobleaching (FRAP).** (*a*) In this technique, a particular component of the membrane is first labeled with a fluorescent dye (step 1). A small region of the surface is then irradiated to bleach the dye molecules (step 2), and the recovery of fluorescence in the bleached region is followed over time (step 3). (*b*) The rate of fluorescence recovery within the illuminated spot can vary depending on the protein(s) being followed. The rate of recovery is related to the diffusion coefficient of the fluorescently labeled protein.

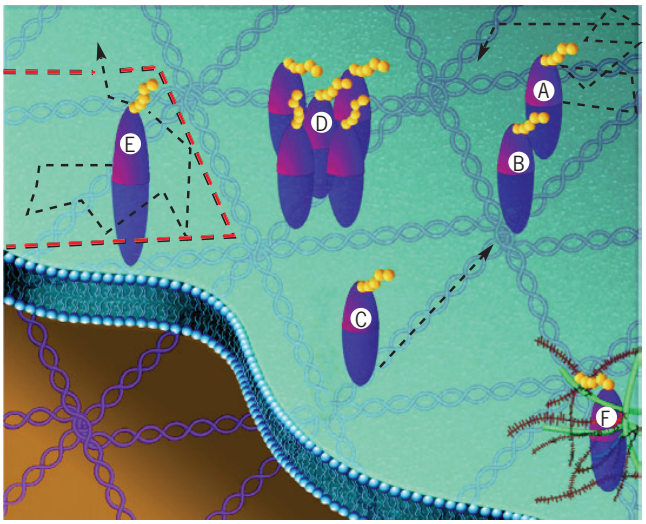


Figure 4.28 **Patterns of movement of integral membrane proteins.** Depending on the cell type and the conditions, integral membrane proteins can exhibit several different types of mobility. Protein A is capable of diffusing randomly throughout the membrane, though its rate of movement may be limited; protein B is immobilized as the result of its interaction with the underlying membrane skeleton; protein C is being moved in a particular direction as the result of its interaction with a motor protein at the cytoplasmic surface of the membrane; movement of protein D is restricted by other integral proteins of the membrane; movement of protein E is restricted by fences formed by proteins of the membrane skeleton, but it can hop into adjacent compartments through transient openings in a fence; movement of protein F is restrained by extracellular materials.

particular membrane protein may tend to move toward the leading or the trailing edge of a moving cell.

- In most studies, the largest fraction of protein species exhibit random (Brownian) movement within the membrane at rates consistent with free diffusion (diffusion coefficients about 5×10^{-9} cm^2/sec), but the molecules are unable to migrate freely more than a few tenths of a micrometer. Long-range diffusion occurs but at slower rates, apparently because of the presence of a system of barriers. These barriers are discussed in the following sections.

Control of Membrane Protein Mobility It is apparent that plasma membrane proteins are not totally free to drift around randomly on the lipid "sea." Instead, this class of proteins is subjected to various influences that affect their mobility and, in doing so, promote intramembrane organization. Some membranes are crowded with proteins, so that the random movements of one molecule can be impeded by its neighbors (Figure 4.28, protein D). The strongest influences on an integral membrane protein are thought to be exerted from just beneath the membrane on its cytoplasmic face. The plasma membranes of many cells possess a fibrillar network, or "membrane skeleton," consisting of peripheral proteins situated on the cytoplasmic surface of the membrane. A certain proportion of a membrane's integral protein molecules are either tethered to the membrane skeleton (Figure 4.28, protein B) or otherwise restricted by it.

Information concerning the presence of membrane barriers has been obtained using an innovative technique that allows investigators to trap integral proteins and drag them through the plasma membrane with a known force. This technique, which uses an apparatus referred to as *optical tweezers*, takes advantage of the tiny optical forces that are generated by a focused laser beam. The integral proteins to be studied are tagged with antibody-coated beads, which serve as handles that can be gripped by the laser beam. It is generally found that optical tweezers can drag an integral protein for a limited distance before the protein encounters a barrier that causes it to be released from the laser's grip. As it is released, the protein typically springs backward, suggesting that the barriers are elastic structures.

One approach to studying factors that affect membrane protein mobility is to genetically modify cells so that they produce altered membrane proteins. Integral proteins whose cytoplasmic portions have been genetically deleted often move much greater *distances* than their intact counterparts, indicating that barriers reside on the cytoplasmic side of the membrane. These findings suggest that the membrane's underlying skeleton forms a network of "fences" around portions of the membrane, creating compartments that restrict the distance an integral protein can travel (Figure 4.28, protein E). Proteins move across the boundaries from one compartment to another through breaks in the fences. Such openings are thought to appear and disappear along with the dynamic disassembly and reassembly of parts of the meshwork. Membrane compartments may keep specific combinations of proteins in close enough proximity to facilitate their interaction.

Integral proteins lacking that portion that would normally project into the extracellular space typically move at a much faster *rate* than the wild-type version of the protein. This finding suggests that the movement of a transmembrane protein through the bilayer is slowed by extracellular materials that can entangle the external portion of the protein molecule (Figure 4.28, protein F).

Membrane Lipid Mobility Proteins are huge molecules, so it isn't surprising that their movement within the lipid bilayer might be restricted. Phospholipids, by comparison, are small molecules that make up the very fabric of the lipid bilayer. One might expect their movements to be completely unfettered, yet a number of studies have suggested that phospholipid diffusion is also restricted. When individual phospholipid molecules of a plasma membrane are tagged and followed under the microscope using ultra high-speed cameras, they are seen to be confined for very brief periods and then hop from one confined area to another. Figure 4.29*a* shows the path taken by an individual phospholipid within the plasma membrane over a period of 56 milliseconds. Computer analysis indicates that the phospholipid diffuses freely within one

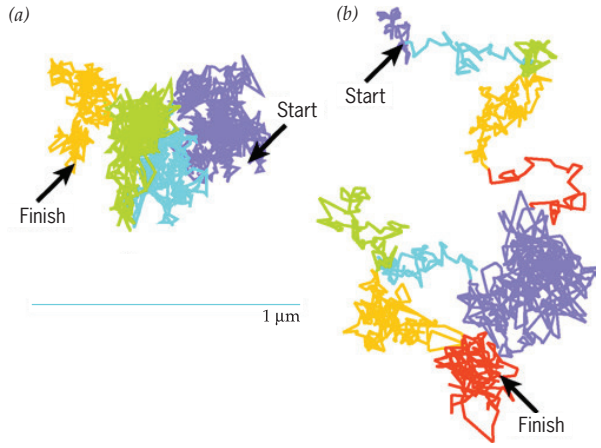


Figure 4.29 **Experimental demonstration that diffusion of phospholipids within the plasma membrane is confined.** (*a*) The track of a single labeled unsaturated phospholipid is followed for 56 ms as it diffuses within the plasma membrane of a rat fibroblast. Phospholipids diffuse freely within a confined compartment before hopping into a neighboring compartment. The rate of diffusion within a compartment is as rapid as that expected by unhindered Brownian movement. However, the overall rate of diffusion of the phospholipid appears slowed because the molecule must hop a barrier to continue its movement. The movement of the phospholipid within each compartment is represented by a single color. (*b*) The same experiment shown in *a* is carried out for 33 ms in an artificial bilayer, which lacks the "picket fences" present in a cellular membrane. The much more open, extended trajectory of the phospholipid can now be explained by simple, unconfined Brownian movement. For the sake of comparison, fake compartments were assigned in *b* and indicated by different colors. (From Takahiro Fujiwara et al., courtesy of Akihiro Kusumi, Nagoya, J. Cell Biol. 157:1073, 2002; Reproduced with permission of The Rockefeller University Press.)

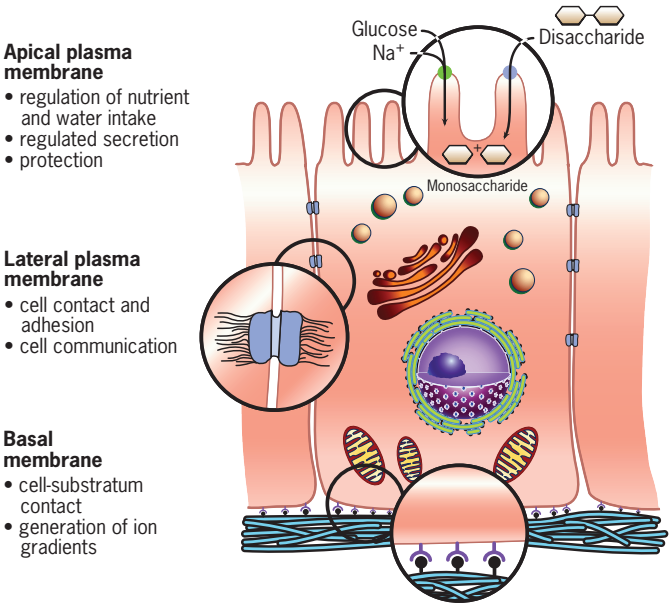


Figure 4.30 **Differentiated functions of the plasma membrane of an epithelial cell.** The apical surface of this intestinal epithelial cell contains integral proteins that function in ion transport and hydrolysis of disaccharides, such as sucrose and lactose; the lateral surface contains integral proteins that function in intercellular interaction; and the basal surface contains integral proteins that function in the association of the cell with the underlying basement membrane.

compartment (shaded in purple) before it jumps the "fence" into a neighboring compartment (shaded in blue) and then over another fence into an adjacent compartment (shaded in green), and so forth. Treatment of the membrane with agents that disrupt the underlying membrane skeleton removes some of the fences that restrict phospholipid diffusion. But if the membrane skeleton lies beneath the lipid bilayer, how can it interfere with phospholipid movement? The authors of these studies speculate that the fences are constructed of rows of integral membrane proteins whose cytoplasmic domains are attached to the membrane skeleton. This is not unlike the confinement of horses or cows by a picket fence whose posts are embedded in the underlying soil.

Membrane Domains and Cell Polarity For the most part, studies of membrane dynamics, such as those discussed above, are carried out on the relatively homogeneous plasma membrane situated at the upper or lower surface of a cell residing on a culture dish. Most membranes, however, exhibit distinct variations in protein composition and mobility, especially in cells whose various surfaces display distinct functions. For example, the epithelial cells that line the intestinal wall or make up the microscopic tubules of the kidney are highly polarized cells whose different surfaces carry out different functions (Figure 4.30). Studies indicate that the apical plasma membrane, which selectively absorbs substances from the lumen, possesses different enzymes than the lateral plasma membrane, which interacts with neighboring epithelial cells, or the basal membrane, which adheres to an underlying extracellular substrate (a *basement membrane*). In other examples, the receptors for neurotransmitter substances are concentrated into regions of the plasma membrane located within synapses (see Figure 4.57), and receptors for low-density lipoproteins are concentrated into patches of the plasma membrane specialized to facilitate their internalization (see Figure 8.38).

Of all the various types of mammalian cells, sperm may have the most highly differentiated structure. A mature sperm can be divided into head, midpiece, and tail, each having its own specialized functions. Although divided into a number of distinct parts, a sperm is covered by a *continuous* plasma membrane which, as revealed by numerous techniques, consists of a mosaic of different types of localized domains. For example, when sperm are treated with a variety of antibodies, each antibody combines with the surface of the cell in a unique topographic pattern that reflects the distribution within the plasma membrane of the particular protein antigen recognized by that antibody (Figure 4.31).

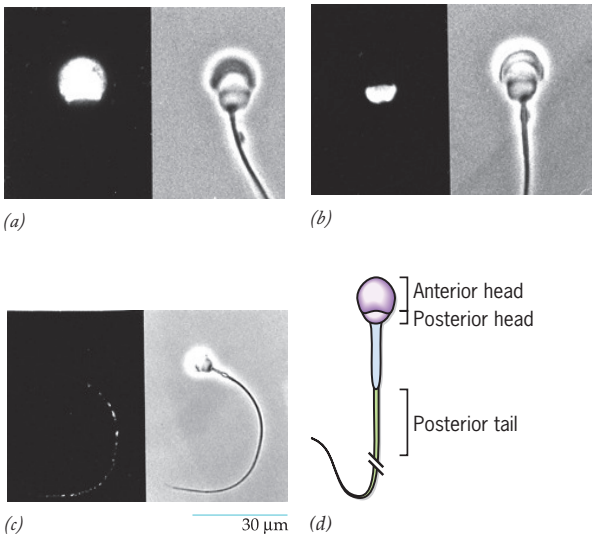


Figure 4.31 **Differentiation of the mammalian sperm plasma membrane as revealed by fluorescent antibodies.** (*a–c*) Three pairs of micrographs, each showing the distribution of a particular protein at the cell surface as revealed by a bound fluorescent antibody. The three proteins are localized in different parts of the continuous sperm membrane. Each pair of photographs shows the fluoresence pattern of the bound antibody and a phase contrast micrograph of the same cell. (*d*) Diagram summarizing the distribution of the proteins. (A–C: FROM DIANA G. MYLES, PAUL PRIMAKOFF, AND ANTHONY R. BELLVÉ, CELL 23:434, 1981 © WITH PERMISSION FROM ELSEVIER.)

The Red Blood Cell: An Example of Plasma Membrane Structure

Of all the diverse types of membranes, the plasma membrane of the human erythrocyte (red blood cell) is the most studied and best understood (Figure 4.32). There are several reasons for the popularity of this membrane. The cells are inexpensive to obtain and readily available in huge numbers from whole blood. They are already present as single cells and need not be dissociated from a complex tissue. The cells are simple by comparison with other cell types, lacking nuclear and cytoplasmic membranes that inevitably contaminate plasma membrane preparations from other cells. In addition, purified, *intact* erythrocyte plasma membranes can be obtained simply by placing the cells in a dilute (hypotonic) salt solution. The cells respond to this osmotic shock by taking up water and swelling, a phenomenon termed *hemolysis*. As the surface area of each cell increases, the cell becomes leaky, and the contents, composed almost totally of dissolved hemoglobin, flow out of the cell leaving behind a plasma membrane "ghost" (Figure 4.32*b*).

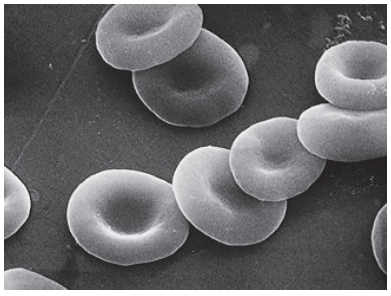
Once erythrocyte plasma membranes are isolated, the proteins can be solubilized and separated from one another (fractionated), providing a better idea of the diversity of proteins within the membrane. Fractionation of membrane proteins can be accomplished using polyacrylamide gel electrophoresis (PAGE) in the presence of the ionic detergent sodium dodecyl sulfate (SDS). (The technique of SDS–PAGE is discussed in Section 18.7.) The SDS keeps the integral proteins soluble and, in addition, adds a large number of negative charges to the proteins with which it associates. Because the number of charged SDS molecules per unit weight of protein tends to be relatively constant, the molecules separate from one another according to their molecular weight. The largest proteins move most slowly through the molecular sieve of the gel. The major proteins of the erythrocyte membrane are separated into about a dozen conspicuous bands by SDS–PAGE (Figure 4.32*c*). Among the proteins are a variety of enzymes (including glyceraldehyde 3-phosphate dehydrogenase, one of the enzymes of glycolysis), transport proteins (for ions and sugars), and skeletal proteins (e.g., spectrin).

Integral Proteins of the Erythrocyte Membrane A model of the erythrocyte plasma membrane showing its major proteins is seen in Figure 4.32*d*. The most abundant integral proteins of this membrane are a pair of carbohydrate-containing, membrane-spanning proteins, called band 3 and glycophorin A. The high density of these proteins within the membrane is evident in the freeze-fracture micrographs of Figure 4.15. Band 3, which gets its name from its position in an electrophoretic gel (Figure 4.32*c*), is present as a dimer composed of two identical subunits (a *homodimer*). Each subunit spans the membrane at least a dozen times and contains a relatively small amount of carbohydrate (6–8 percent of the molecule's weight). Band 3 protein serves as a channel for the passive exchange of anions across the membrane. As blood circulates through the tissues, carbon dioxide becomes dissolved in the fluid of the bloodstream (the plasma) and undergoes the following reaction:

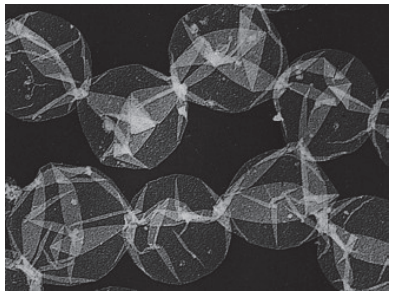
$$H_2O + CO_2 \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$$

The bicarbonate ions (HCO_3^-) enter the erythrocyte in exchange for chloride ions, which leave the cell. In the lungs, where carbon dioxide is released, the reaction is reversed and bicarbonate ions leave the erythrocyte in exchange for chloride ions. The reciprocal movement of HCO_3^- and Cl^- occurs through a channel in the center of each band 3 dimer.

Glycophorin A was the first membrane protein to have its amino acid sequence determined. The arrangement of the polypeptide chain of glycophorin A within the plasma membrane is shown in Figure 4.18. (Other related glycophorins, B, C, D, and E, are also present in the membrane at much lower concentrations.) Like band 3, glycophorin A is also present in the membrane as a dimer. Unlike band 3, each glycophorin A subunit spans the membrane only once, and it contains a bushy carbohydrate cover consisting of 16 oligosaccharide chains that together make up about 60 percent of the molecule's weight. It is thought that the primary function of glycophorin derives from the large number of negative charges borne on sialic acid, the sugar residue at the end of each carbohydrate chain. Because of these charges, red blood cells repel each other, which prevents the cells from clumping as they circulate through the body's tiny vessels. It is noteworthy that persons who lack both glycophorin A and B in their red blood cells show no ill-effects from their absence. At the same time,



(a) 7 μm



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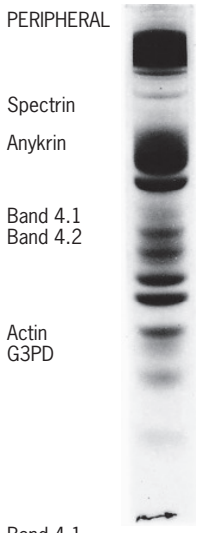

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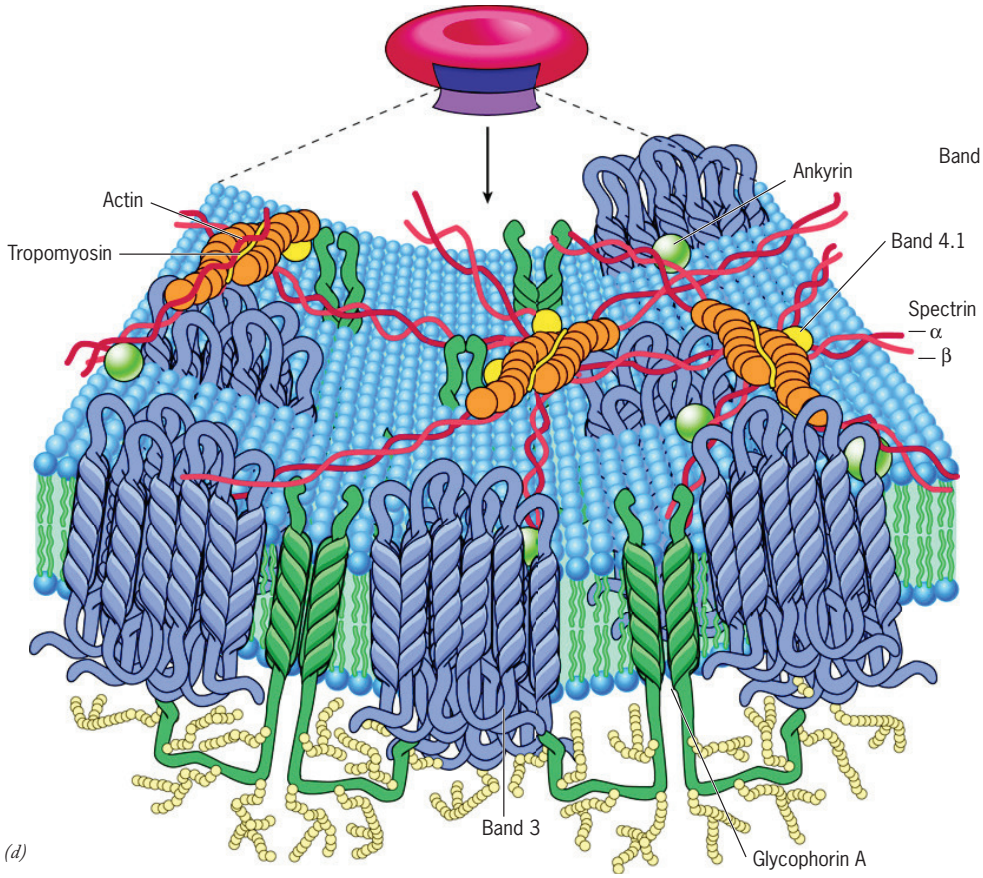
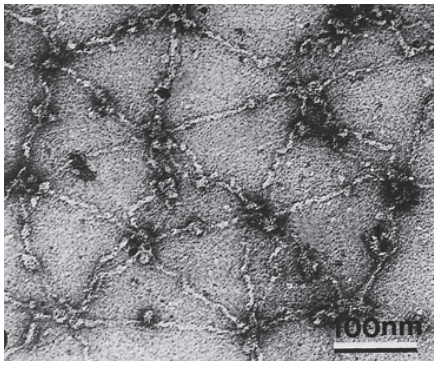


(d)



(e)

Figure 4.32 **The plasma membrane of the human erythrocyte.** (*a*) Scanning electron micrograph of human erythrocytes. (*b*) Micrograph showing plasma membrane ghosts, which were isolated by allowing erythrocytes to swell and hemolyze as described in the text. (*c*) The results of SDS–polyacrylamide gel electrophoresis (SDS–PAGE) used to fractionate the proteins of the erythrocyte membrane, which are identified at the sides of the gel. (*d*) A model of the erythrocyte plasma membrane as viewed from the internal surface, showing the integral proteins embedded in the lipid bilayer and the arrangement of peripheral proteins that make up the membrane's internal skeleton. The band 3 dimer shown here is simplified. The band 4.1 protein stabilizes actin–spectrin complexes. (*e*) Electron micrograph showing the arrangement of the proteins of the inner membrane skeleton. (A: Courtesy François M. M. Morel, Richard F. Baker, and Harold Wayland; B: courtesy of Joseph F. Hoffman; C: from V. T. Marchesi, H. Furthmayr, and M. Tomita, Annu. Rev. Biochem. vol. 45; © 1976 by Annual Reviews Inc.; D: after D. Voet and J. G. Voet, Biochemistry, 2D ed.; copyright © 1995, John Wiley & Sons, Inc.; E: from Shih-Chun Liu, Laura H. Derick, and Jiri Palek, J. Cell Biol. 104:527, 1987; A, E: reproduced with permission of The Rockefeller University Press.)

the band 3 proteins in these individuals are more heavily glycosylated, which apparently compensates for the otherwise missing negative charges required to prevent cell–cell interaction. Glycophorin also happens to be the receptor utilized by the protozoan that causes malaria, providing a path for entry into the blood cell. Consequently, individuals whose erythrocytes lack glycophorin A and B are thought to be protected from acquiring malaria. Differences in glycophorin amino acid sequence determine whether a person has an MM, MN, or NN blood type.

The Erythrocyte Membrane Skeleton The peripheral proteins of the erythrocyte plasma membrane are located on its internal surface and constitute a fibrillar membrane skeleton (Figure 4.32*d*,*e*) that plays a major role in determining the biconcave shape of the erythrocyte. As discussed on page 143, the membrane skeleton can establish domains within the membrane that enclose particular groups of membrane proteins and may greatly restrict the movement of these proteins. The major component of the skeleton is an elongated fibrous protein, called *spectrin*. Spectrin is a heterodimer approximately 100 nm long, consisting of an α and β subunit that curl around one another. Two such dimeric molecules are linked at their head ends to form a 200-nm-long filament that is both flexible and elastic. Spectrin is attached to the internal surface of the membrane by means of noncovalent bonds to another peripheral protein, *ankyrin* (the green spheres of Figure 4.32*d*), which in turn is linked noncovalently to the cytoplasmic domain of a band 3 molecule. As is evident in Figures 4.32*d* and *e*, spectrin filaments are organized into hexagonal or pentagonal arrays. This two-dimensional network is constructed by linking both ends of each spectrin filament to a cluster of proteins that include a short filament of *actin* and *tropomyosin*, proteins typically involved in contractile activities. A number of genetic diseases (*hemolytic anemias*) characterized by fragile, abnormally shaped erythrocytes have been traced to mutations in ankyrin or spectrin.

If the peripheral proteins are removed from erythrocyte ghosts, the membrane becomes fragmented into small vesicles, indicating that the inner protein network is required to maintain the integrity of the membrane. Erythrocytes are circulating cells that are squeezed under pressure through microscopic capillaries whose diameter is considerably less than that of the erythrocytes themselves. To traverse these narrow passageways, and to do so day after day, the red blood cell must be highly deformable, durable, and capable of withstanding shearing forces that tend to pull it apart. The spectrin–actin network gives the cell the strength, elasticity, and pliability necessary to carry out its demanding function.

When first discovered, the membrane skeleton of the erythrocyte was thought to be a unique structure suited to the unique shape and mechanical needs of this cell type. However, as other cells were examined, similar types of membrane skeletons containing members of the spectrin and ankyrin families have been revealed, indicating that inner membrane skeletons are widespread. Dystrophin, for example, is a member of the spectrin family that is found in the membrane skeleton of muscle cells. Mutations in dystrophin are responsible for causing muscular dystrophy, a devastating disease that cripples and kills children. As in the case of cystic fibrosis (page 163), the most debilitating mutations are ones that lead to a complete absence of the protein in the cell. The plasma membranes of muscle cells lacking dystrophin are apparently destroyed as a consequence of the mechanical stress exerted on them as the muscle contracts. As a result, the muscle cells die and eventually are no longer replaced.

REVIEW

1. Describe two techniques to measure the rates of diffusion of a specific membrane protein.
2. Compare and contrast the types of protein mobility depicted in Figure 4.28.
3. Discuss two major functions of the integral and peripheral proteins of the erythrocyte membrane.
4. Compare the rate of lateral diffusion of a lipid with that of flip-flop. What is the reason for the difference?

4.7 | The Movement of Substances Across Cell Membranes

Because the contents of a cell are completely surrounded by its plasma membrane, all communication between the cell and the extracellular medium must be mediated by this structure. In a sense, the plasma membrane has a dual function. On one hand, it must retain the dissolved materials of the cell so that they do not simply leak out into the environment, while on the other hand, it must allow the necessary exchange of materials into and out of the cell. The lipid bilayer of the membrane is ideally suited to prevent the loss of charged and polar solutes from a cell. Consequently, some special provision must be made to allow the movement of nutrients, ions, waste products, and other compounds, in and out of the cell. There are basically two means for the movement of substances through a membrane: passively by diffusion or actively by an energy-coupled transport process. Both types of movements lead to the net flux of a particular ion or compound. The term *net flux* indicates that the movement of the substance into the cell (*influx*) and out of the cell (*efflux*) is not balanced, but that one exceeds the other.

Several different processes are known by which substances move across membranes: simple diffusion through the lipid bilayer; simple diffusion through an aqueous, protein-lined channel; diffusion that is facilitated by a protein transporter; and active transport, which requires an energy-driven protein "pump" capable of moving substances against a concentration gradient (Figure 4.33). We will consider each in turn, but first we will discuss the energetics of solute movement.

The Energetics of Solute Movement

Diffusion is a spontaneous process in which a substance moves from a region of high concentration to a region of low concentration, eventually eliminating the concentration

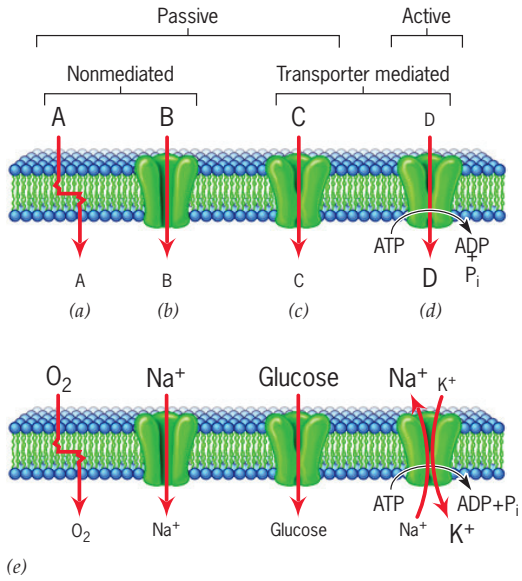


Figure 4.33 **Four basic mechanisms by which solute molecules move across membranes.** The relative sizes of the letters indicate the directions of the concentration gradients. (*a*) Simple diffusion through the bilayer, which always proceeds from high to low concentration. (*b*) Simple diffusion through an aqueous channel formed within an integral membrane protein or a cluster of such proteins. As in *a*, movement is always down a concentration gradient. (*c*) Facilitated diffusion in which solute molecules bind specifically to a membrane protein carrier (a facilitative transporter). As in *a* and *b*, movement is always from high to low concentration. (*d*) Active transport by means of a protein transporter with a specific binding site that undergoes a change in affinity driven with energy released by an exergonic process, such as ATP hydrolysis. Movement occurs against a concentration gradient. (*e*) Examples of each type of mechanism as it occurs in the membrane of an erythrocyte.

difference between the two regions. As discussed on page 88, diffusion depends on the random thermal motion of solutes and is an exergonic process driven by an increase in entropy. We will restrict the following discussion to diffusion of substances across membranes.

The free-energy change when an uncharged solute (a nonelectrolyte) diffuses across a membrane depends on the magnitude of the concentration gradient, that is, the difference in concentration on each side of the membrane. The following relationship describes the movement of a nonelectrolyte *into* the cell:

$$\Delta G = RT \ln \frac{[C_i]}{[C_o]}$$

$$\Delta G = 2.303\, RT \log_{10} \frac{[C_i]}{[C_o]}$$

where ΔG is the free-energy change (Section 3.1), R is the gas constant, T is the absolute temperature, and $[C_i]/[C_o]$ is the ratio of the concentration of the solute on the inside (i) and outside (o) surfaces of the membrane. At 25°C,

$$\Delta G = 1.4 \text{ kcal/mol} \cdot \log_{10} \frac{[C_i]}{[C_o]}$$

If the ratio of $[C_i]/[C_o]$ is less than 1.0, then the log of the ratio is negative, ΔG is negative, and the net influx of solute is thermodynamically favored (exergonic). If, for example, the external concentration of solute is 10 times the internal concentration, $\Delta G = -1.4$ kcal/mole. Thus, the maintenance of a tenfold concentration gradient represents a storage of 1.4 kcal/mol. As solute moves into the cell, the concentration gradient decreases, the stored energy is dissipated, and ΔG decreases until, at equilibrium, ΔG is zero. (To calculate ΔG for movement of a solute out of the cell, the term for concentration ratios becomes $[C_o]/[C_i]$).

If the solute is an electrolyte (a charged species), the overall charge difference between the two compartments must also be considered. As a result of the mutual repulsion of ions of like charges, it is thermodynamically unfavorable for an electrolyte to move across a membrane from one compartment into another compartment having a net charge of the same sign. Conversely, if the charge of the electrolyte is opposite in sign to the compartment into which it is moving, the process is thermodynamically favored. The greater the difference in charge (the potential difference or voltage) between the two compartments, the greater the difference in free energy. Thus, the tendency of an electrolyte to diffuse between two compartments depends on two gradients: a chemical gradient, determined by the concentration difference of the substance between the two compartments, and the electric potential gradient, determined by the difference in charge. Together these differences are combined to form an **electrochemical gradient**. The free-energy change for the diffusion of an electrolyte into the cell is

$$\Delta G = RT \ln \frac{[C_i]}{[C_o]} + zF\Delta E_m$$

where z is the charge of the solute, F is the Faraday constant (23.06 kcal/V · equivalent, where an equivalent is the amount of the electrolyte having one mole of charge), and ΔE_m is the potential difference (in volts) between the two compartments. We saw in the previous example that a tenfold difference in concentration of a nonelectrolyte across a membrane at 25°C generates a ΔG of −1.4 kcal/mol. Suppose the concentration gradient consisted of Na^+ ions, which were present at tenfold higher concentration outside the cell than in the cytoplasm. Because the voltage across the membrane of a cell is typically about −70 mV (page 165), the free-energy change for the movement of a mole of Na^+ ions into the cell under these conditions would be

$$\Delta G = -1.4 \text{ kcal/mol} + zF\Delta E_m$$

$$\Delta G = -1.4 \text{ kcal/mol} + (1)(23.06 \text{ kcal/V} \cdot \text{mol})\ (-0.07 \text{ V})$$

$$= -3.1 \text{ kcal/mol}$$

Thus under these conditions, the concentration difference and the electric potential make similar contributions to the storage of free energy across the membrane.

The interplay between concentration and potential differences is seen in the diffusion of potassium ions (K^+) out of a cell. The efflux of the ion is favored by the K^+ concentration gradient, which has a higher K^+ concentration inside the cell, but hindered by the electrical gradient that its diffusion creates, which leaves a higher negative charge inside the cell. We will discuss this subject further when we consider the topic of membrane potentials and nerve impulses in Section 4.8.

Diffusion of Substances through Membranes

Two qualifications must be met before a nonelectrolyte can diffuse passively across a plasma membrane. The substance must be present at higher concentration on one side of the membrane than the other, and the membrane must be permeable to the substance. A membrane may be permeable to a given solute either (1) because that solute can pass directly through the lipid bilayer, or (2) because that solute can traverse an aqueous pore that spans the membrane. Let us begin by considering the former route in which a substance must dissolve in the lipid bilayer on its way through the membrane.

Discussion of simple diffusion leads us to consider the polarity of a solute. One simple measure of the polarity (or nonpolarity) of a substance is its **partition coefficient**, which is the ratio of its solubility in a nonpolar solvent, such as octanol or a vegetable oil, to that in water under conditions where the nonpolar solvent and water are mixed together. Figure 4.34 shows the relationship between partition coefficient and membrane permeability of a variety of chemicals and drugs. It is evident that the greater the lipid solubility, the faster the penetration.

Another factor determining the rate of penetration of a compound through a membrane is its size. If two molecules have approximately equivalent partition coefficients, the smaller molecule tends to penetrate the lipid bilayer of a membrane more rapidly than the larger one. Very small, uncharged molecules penetrate very rapidly through cellular membranes. Consequently, membranes are highly permeable to small inorganic molecules, such as O_2, CO_2, NO, and H_2O, which are thought to slip between adjacent phospholipids. In contrast, larger polar molecules, such as sugars, amino acids, and phosphorylated intermediates, exhibit poor membrane penetrability. As a result, the lipid bilayer of the plasma membrane provides an effective barrier that keeps these essential metabolites from diffusing out of the cell. Some of these molecules (e.g., sugars and amino acids) must enter cells from the bloodstream, but they cannot do so by simple diffusion. Instead, special mechanisms must be available to mediate their penetration through the plasma membrane. The use of such mechanisms allows a cell to regulate the movement of substances across its surface barrier. We will return to this feature of membranes later.

The Diffusion of Water through Membranes Water molecules move much more rapidly through a cell membrane than do dissolved ions or small polar organic solutes, which are essentially nonpenetrating. Because of this difference in the penetrability of water versus solutes, membranes are said to be **semipermeable**. Water moves readily through a semipermeable membrane from a region of lower *solute* concentration to a region of higher *solute* concentration. This process is called **osmosis**, and it is readily demonstrated by placing a cell into a solution containing a nonpenetrating solute at a concentration different than that present within the cell itself.

When two compartments of different solute concentration are separated by a semipermeable membrane, the compartment of higher solute concentration is said to be **hypertonic** (or **hyperosmotic**) relative to the compartment of lower solute concentration, which is described as being **hypotonic** (or **hypoosmotic**). When a cell is placed into a hypotonic solution, the cell rapidly gains water by osmosis and swells (Figure 4.35*a*). Conversely, a cell placed into a hypertonic solution rapidly loses water by osmosis and shrinks (Figure 4.35*b*). These simple observations show that a cell's volume is controlled by the difference between the solute concentration inside the cell and that in the extracellular medium. The swelling and shrinking of cells in slightly hypotonic and

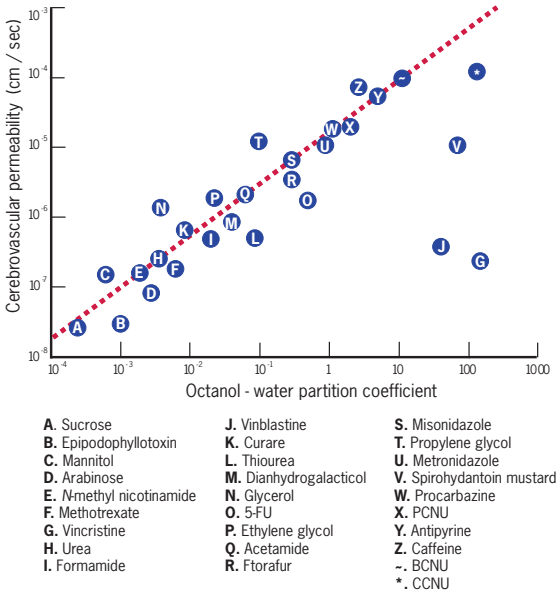


Figure 4.34 **The relationship between partition coefficient and membrane permeability.** In this case, measurements were made of the penetration of a variety of chemicals and drugs across the plasma membranes of the cells that line the capillaries of the brain. Substances penetrate by passage through the lipid bilayer of these cells. The partition coefficient is expressed as the ratio of solubility of a solute in octanol to its solubility in water. Permeability is expressed as penetrance (P) in cm/sec. For all but a few compounds, such as vinblastine and vincristine, penetrance is directly proportional to lipid solubility. (From N. J. Abbott and I. A. Romero, Molec. Med. Today, 2:110, 1996; Copyright 1996, with permission from Elsevier Science.)

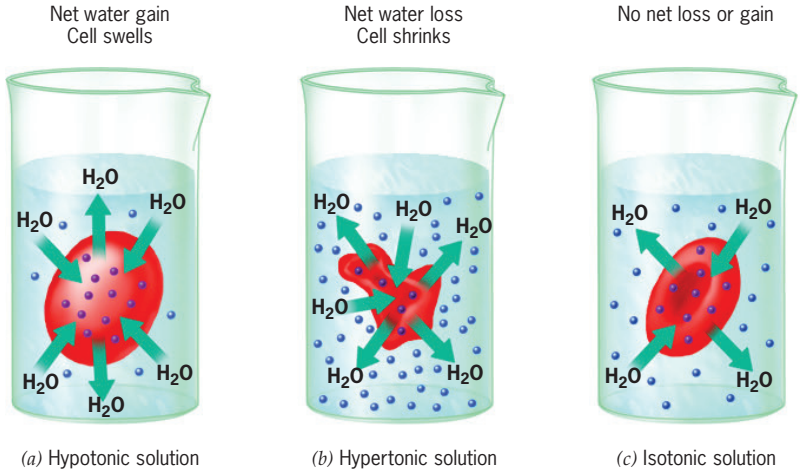


Figure 4.35 **The effects of differences in the concentration of solutes on opposite sides of the plasma membrane.** (*a*) A cell placed in a hypotonic solution (one having a lower solute concentration than the cell) swells because of a net gain of water by osmosis. (*b*) A cell in a hypertonic solution shrinks because of a net loss of water by osmosis. (*c*) A cell placed in an isotonic solution maintains a constant volume because the inward flux of water is equal to the outward flux.

hypertonic media are usually only temporary events. Within a few minutes, the cells recover and return to their original volume. In a hypotonic medium, recovery occurs as the cells lose ions, thereby reducing their internal osmotic pressure. In a hypertonic medium, recovery occurs as the cells gain ions from the medium. Once the internal solute concentration (which includes a high concentration of dissolved proteins) equals the external solute concentration, the internal and external fluids are **isotonic** (or **isosmotic**), and no net movement of water into or out of the cells occurs (Figure 4.35*c*).

Osmosis is an important factor in a multitude of bodily functions. Your digestive tract, for example, secretes several liters of fluid daily, which is reabsorbed osmotically by the cells that line your intestine. If this fluid weren't reabsorbed, as happens in cases of extreme diarrhea, you would face the prospect of rapid dehydration. Plants utilize osmosis in different ways. Unlike animal cells, which are generally isotonic with the medium in which they are bathed, plant cells are generally hypertonic compared to their fluid environment. As a result, there is a tendency for water to enter the cell, causing it to develop an internal (*turgor*) pressure that pushes against its surrounding wall (Figure 4.36*a*). Turgor pressure provides support for nonwoody plants and for the nonwoody parts of trees, such as the leaves. If a plant cell is placed into a hypertonic medium, its volume shrinks as the plasma membrane pulls away from the surrounding cell wall, a process called **plasmolysis** (Figure 4.36*b*). The loss of water due to plasmolysis causes plants to lose their support and wilt.

Not all cells are equally permeable to water. In fact, many cells are much more permeable to water than can be explained by simple diffusion through the lipid bilayer. In the early 1990s, Peter Agre and colleagues at Johns Hopkins University were attempting to isolate and purify the membrane proteins responsible for the Rh antigen on the surface of red blood cells. During this pursuit, they identified a protein they thought might be the long-sought water channel of the erythrocyte membrane. To test their hypothesis, they engineered frog oocytes to incorporate the newly discovered protein into their plasma membranes and then placed the oocytes in a

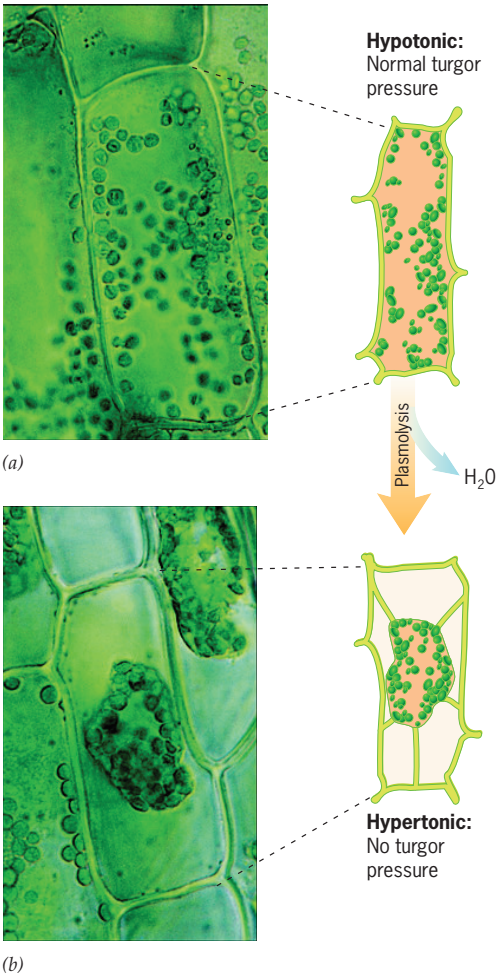
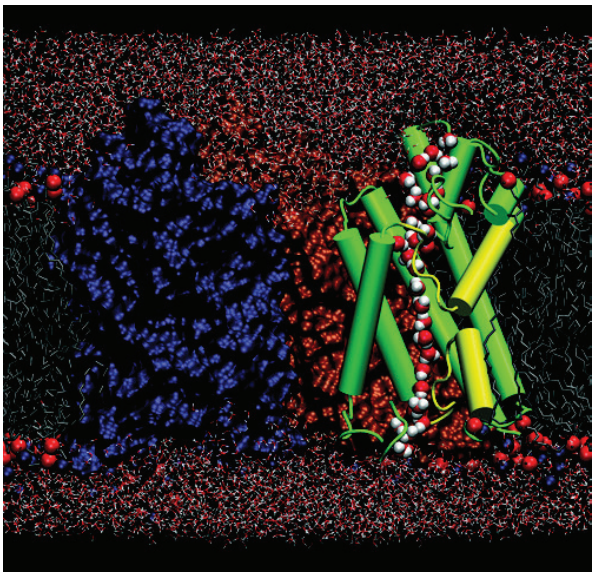
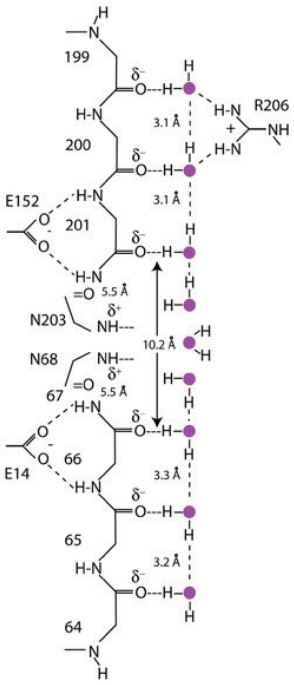


Figure 4.36 **The effects of osmosis on a plant cell.** (*a*) Aquatic plants living in freshwater are surrounded by a hypotonic environment. Water therefore tends to flow into the cells, creating turgor pressure. (*b*) If the plant is placed in a hypertonic solution, such as seawater, the cell loses water, and the plasma membrane pulls away from the cell wall. (Ed Reschke.)



(a)



(b)

Figure 4.37 **Passage of water molecules through an aquaporin channel.** (*a*) Snapshot from a molecular dynamics simulation of a stream of water molecules (red and white spheres) passing in single file through the channel in one of the subunits of an aquaporin molecule residing within a membrane. (*b*) A model describing the mechanism by which water molecules pass through an aquaporin channel with the simultaneous exclusion of protons. Nine water molecules are shown to be lined up in single file along the wall of the channel. Each water molecule is depicted as a red circular O atom with two associated Hs. In this model, the four water molecules at the top and bottom of the channel are oriented, as the result of their interaction with the carbonyl (C═O) groups of the protein backbone (page 51), with their H atoms pointed away from the center of the channel. These water molecules are able to form hydrogen bonds (dashed lines) with their neighbors. In contrast, the single water molecule in the center of the channel is oriented in a position that prevents it from forming hydrogen bonds with other water molecules, which has the effect of interrupting the flow of protons through the channel. Animations of aquaporin channels can be found at www.nobelprize.org/nobel_prizes/chemistry/laureates/2003/animations.html (A: From Benoit Roux and Klaus Schulten, Structure 12:1344, 2004, with permission from Elsevier; B: From R. M. Stroud et al., Curr. Opin. Struct. Biol. 13:428, © 2003, with permission from Elsevier.)

hypotonic medium. Just as predicted, the oocytes swelled due to the influx of water and eventually burst. The team had discovered a family of small integral proteins, called *aquaporins*, that allow the passive movement of water from one side of the plasma membrane to the other. Each aquaporin subunit (in the four-subunit protein) contains a central channel that is lined primarily by hydrophobic amino acid residues and is highly specific for water molecules. A billion or so water molecules can pass—in single file—through each channel every second. At the same time, H^+ ions, which normally hop along a chain of water molecules, are not able to penetrate these open pores. The apparent mechanism by which these channels are able to exclude protons has been suggested by a combination of X-ray crystallographic studies, which has revealed the structure of the protein, and molecular dynamics simulations (page 60), which has put this protein structure into operation. A model based on such simulations is shown in Figure 4.37*a*. Very near its narrowest point, the wall of an aquaporin channel contains a pair of precisely positioned positive charges (residues N203 and N68 in Figure 4.37*b*) that attract the oxygen atom of each water molecule as it speeds through the constriction in the protein. This interaction reorients the central water molecule in a position that prevents it from maintaining the hydrogen bonds that normally link it to its neighboring water molecules. This removes the bridge that would normally allow protons to move from one water molecule to the next.

Aquaporins are particularly prominent in cells, such as those of a kidney tubule or plant root, where the passage of water plays a crucial role in the tissue's physiologic activities. The hormone vasopressin, which stimulates water retention by the collecting ducts of the kidney, acts by way of one of these proteins (AQP2). Some cases of the inherited disorder *congenital nephrogenic diabetes insipidus* arise from mutations in this aquaporin channel. Persons suffering from this disease excrete large quantities of urine because their kidneys do not respond to vasopressin.

The Diffusion of Ions through Membranes The lipid bilayer that constitutes the core of biological membranes is highly impermeable to charged substances, including small ions such as Na^+, K^+, Ca^{2+}, and Cl^-. Yet the rapid movement (**conductance**) of these ions across membranes plays a critical role in a multitude of cellular activities, including formation and propagation of a nerve impulse, secretion of substances into the extracellular space, muscle contraction, regulation of cell volume, and the opening of stomatal pores on plant leaves.

In 1955, Alan Hodgkin and Richard Keynes of Cambridge University first proposed that cell membranes contain **ion channels**, that is, openings in the membrane that are permeable to specific ions. During the late 1960s and 1970s, Bertil Hille of the University of Washington and Clay

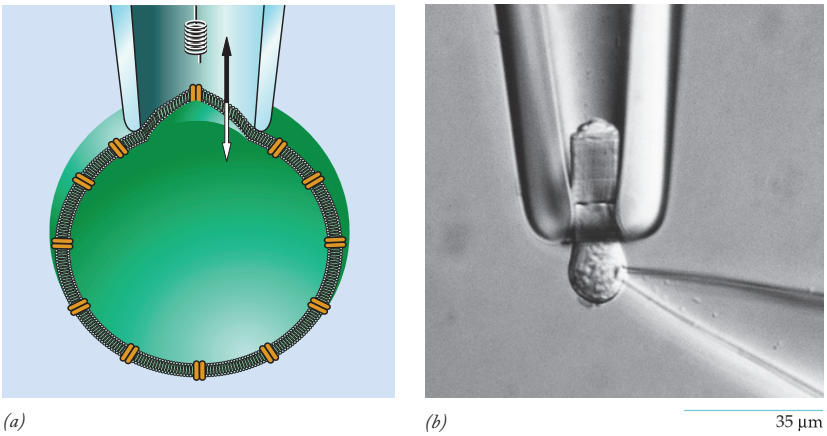


Figure 4.38 **Measuring ion channel conductance by patch-clamp recording.** (*a*) In this technique, a highly polished glass micropipette is placed against a portion of the outer surface of a cell, and suction is applied to seal the rim of the pipette against the plasma membrane. Because the pipette is wired as an electrode (a *microelectrode*), a voltage can be applied across the patch of membrane enclosed by the pipette, and the responding flow of ions through the membrane channels can be measured. As indicated in the figure, the micropipette can enclose a patch of membrane containing a single ion channel, which allows investigators to monitor the opening and closing of a single gated channel, as well as its conductance at different applied voltages. (*b*) The micrograph shows patch-clamp recordings being made from a single photoreceptor cell of the retina of a salamander. One portion of the cell is drawn into a glass micropipette by suction, while a second micropipette-electrode (lower right) is sealed against a small patch of the plasma membrane on another portion of the cell. (B: From T. D. Lamb, H. R. Matthews, and V. Torre, J. Physiology 372:319, 1986. © 1986, reproduced with permission of John Wiley & Sons.)

Armstrong of the University of Pennsylvania began to obtain evidence for the existence of such channels. The final "proof" emerged through the work of Bert Sakmann and Erwin Neher at the Max-Planck Institute in Germany in the late 1970s and early 1980s who developed techniques to monitor the ionic current passing through a single ion channel. This is accomplished using very fine micropipette-electrodes made of polished glass that are placed on the outer cell surface and sealed to the membrane by suction. The voltage across the membrane can be maintained (*clamped*) at any particular value, and the current originating in the small patch of membrane surrounded by the pipette can be measured (Figure 4.38). These landmark studies marked the first successful investigations into the activities of individual protein molecules. Today, biologists have identified a bewildering variety of ion channels, each formed by integral membrane proteins that enclose a central aqueous pore. As might be predicted, mutations in the genes encoding ion channels can lead to many serious diseases (see Table 1 of the Human Perspective, page 162).

Most ion channels are highly selective in allowing only one particular type of ion to pass through the pore. As with the passive diffusion of other types of solutes across membranes, the diffusion of ions through a channel is always downhill, that is, from a state of higher energy to a state of lower energy. Most of the ion channels that have been identified can exist in either an open or a closed conformation; such channels are said to be **gated**. The opening and closing of the gates are subject to complex physiologic regulation and can be induced by a variety of factors depending on the particular channel. Three major categories of gated channels are distinguished:

1. **Voltage-gated channels** whose conformational state depends on the difference in ionic charge on the two sides of the membrane.
2. **Ligand-gated channels** whose conformational state depends on the binding of a specific molecule (the ligand), which is usually not the solute that passes through the channel. Some ligand-gated channels are opened (or closed) following the binding of a molecule to the outer surface of the channel; others are opened (or closed) following the binding of a ligand to the inner surface of the channel. For example, neurotransmitters, such as acetylcholine, act on the outer surface of certain cation channels, while cyclic nucleotides, such as cAMP, act on the inner surface of certain calcium ion channels.
3. **Mechano-gated channels** whose conformational state depends on mechanical forces (e.g., stretch tension) that are applied to the membrane. Members of one family of cation channels, for example, are opened by the movements of stereocilia (see Figure 9.54) on the hair cells of the inner ear in response to sound or motions of the head.

We will focus in the following discussion on the structure and function of voltage-gated potassium ion channels because these are the best understood.

In 1998, Roderick MacKinnon and his colleagues at Rockefeller University provided the first atomic-resolution image of an ion channel protein, in this case, a bacterial K^+ ion channel called KcsA. The relationship between structure and function is evident everywhere in the biological world, but it would be difficult to find a better example than that of the K^+ ion channel depicted in Figure 4.39. As we will see shortly, the formulation of this structure led directly to an understanding of the mechanism by which these remarkable molecular machines are able to select overwhelmingly for K^+ ions over Na^+ ions, yet at the same time allow an incredibly rapid conductance of K^+ ions through the membrane. We will also see that the mechanisms of ion selectivity and conductance in this bacterial channel are virtually identical to those operating in the much larger mammalian channels. Evidently, the basic challenges in operating an ion channel were solved relatively early in evolution, although many refinements appeared over the following one or two billion years.

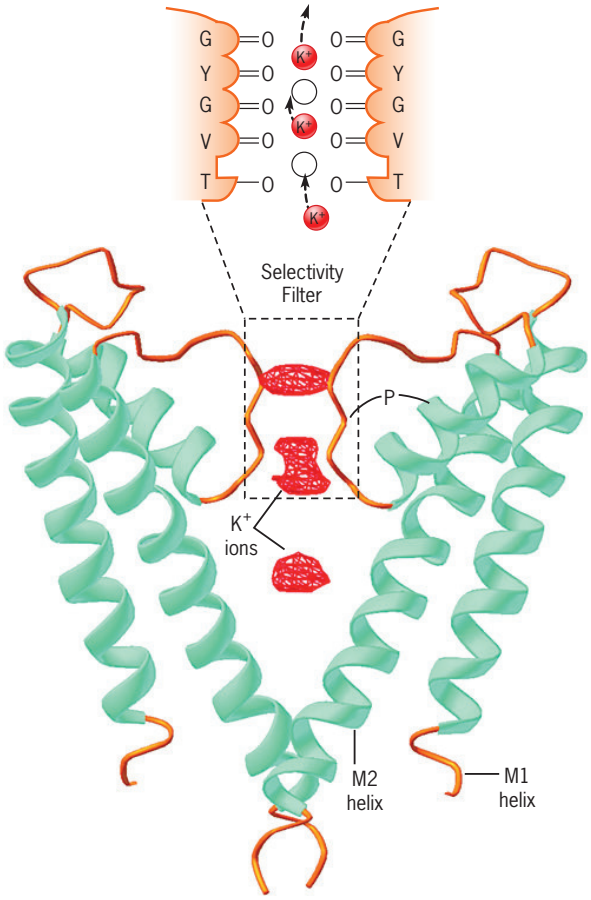


Figure 4.39 **Three-dimensional structure of the bacterial KcsA channel and the selection of K^+ ions.** This K^+ ion channel consists of four subunits, two of which are shown here. Each subunit is comprised of M1 and M2 helices joined by a P (pore) segment consisting of a short helix and a nonhelical portion that lines the channel through which the ions pass. A portion of each P segment contains a conserved pentapeptide (GYGVT) whose residues line the selectivity filter that screens for K^+ ions. The oxygen atoms of the carbonyl groups of these residues project into the channel where they can interact selectively with K^+ ions (indicated by the red mesh objects) within the filter. As indicated in the top inset, the selectivity filter contains four rings of carbonyl O atoms and one ring of threonyl O atoms; each of these five rings contains four O atoms, one donated by each subunit. The diameter of the rings is just large enough so that eight O atoms can coordinate a single K^+ ion, replacing its normal water of hydration. Although four K^+ binding sites are shown, only two are occupied at one time. (From Roderick MacKinnon, Nature Med. 5:1108, © 1999, reprinted by permission from Macmillan Publishers Ltd.)

The KcsA channel consists of four subunits, two of which are shown in Figure 4.39. Each subunit of Figure 4.39 is seen to contain two membrane-spanning helices (M1 and M2) and a pore region (P) at the extracellular end of the channel. P consists of a short pore helix that extends approximately one-third the width of the channel and a nonhelical loop (colored light brown in Figure 4.39) that forms the lining of a narrow *selectivity filter*, so named because of its role in allowing only the passage of K^+ ions.

The lining of the selectivity filter contains a highly conserved pentapeptide—Gly-Tyr-Gly-Val-Thr (or GYGVT in single-letter nomenclature). The X-ray crystal structure of the KcsA channel shows that the backbone carbonyl (C═O) groups from the conserved pentapeptide (see the backbone structure on page 51) create five successive rings of oxygen atoms (four rings are made up of carbonyl oxygens from the polypeptide backbone, and one ring consists of oxygen atoms from the threonine side chain). Each ring contains four oxygen atoms (one from each subunit) and has a diameter of approximately 3 Å, which is slightly larger than the 2.7 Å diameter of a K^+ ion that has lost its normal shell of hydration. Consequently, the electronegative O atoms that line the selectivity filter can substitute for the shell of water molecules that are displaced as each K^+ ion enters the pore. In this model, the selectivity filter contains four potential K^+ ion binding sites. As indicated in the top inset of Figure 4.39, a K^+ ion bound at any of these four sites would occupy the center of a "box" having four O atoms in a plane above the ion and four O atoms in a plane below the atom. As a result, each K^+ ion in one of these sites could coordinate with eight O atoms of the selectivity filter. Whereas the selectivity filter is a precise fit for a dehydrated K^+ ion, it is much larger than the diameter of a dehydrated Na^+ ion (1.9 Å). Consequently, a Na^+ ion cannot interact optimally with the eight oxygen atoms necessary to stabilize it in the pore. As a result, the smaller Na^+ ions cannot overcome the higher energy barrier required to penetrate the pore.

Although there are four potential K^+ ion binding sites, only two are occupied at any given time. Potassium ions are thought to move, two at a time—from sites 1 and 3 to sites 2 and 4—as indicated in the top inset of Figure 4.39. The entry of a third K^+ ion into the selectivity filter creates an electrostatic repulsion that ejects the ion bound at the opposite end of the line. Studies indicate that there is virtually no energy barrier for an ion to move from one binding site to the next, which accounts for the extremely rapid flow of ions across the membrane. Taken together, these conclusions concerning K^+ ion selectivity and conductance provide a superb example of how much can be learned about biological function through an understanding of molecular structure.

The KcsA channel depicted in Figure 4.39 has a gate, just like eukaryotic channels. The opening of the gate of the KcsA channel in response to very low pH was illustrated in Figure 4.22. The structure of KcsA shown in Figure 4.39 is actually the closed conformation of the protein (despite the fact that it contains ions in its channel). It has not been possible to crystallize the KcsA channel in its open conformation, but the structure of a homologous prokaryotic K^+ channel (called MthK) in the open conformation has been crystallized and its structure determined. Comparison of the open structure of MthK and the closed structure of the homologous protein KcsA strongly suggested that gating of these molecules is accomplished by conformational changes of the cytoplasmic ends of the inner (M2) helices. In the closed conformation, as

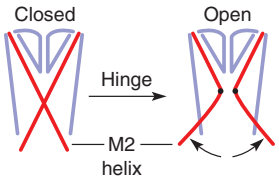


Figure 4.40 **Schematic illustration of the hinge-bending model for the opening of the bacterial KcsA channel.** The M2 helices from each subunit bend outward at a specific glycine residue, which opens the gate at the intracellular end of the channel to K^+ ions. (Reprintd by permission from Macmillan Publisher Ltd: from B. L. Kelly and A. Gross, Nature Struct. Biol. 10:280, 2003; copyright 2003.)

seen in Figure 4.39 and Figure 4.40, left drawing, the M2 helices are straight and cross over one another to form a "helix bundle" that seals the cytoplasmic face of the pore. In the model shown in Figure 4.40, the channel opens when the M2 helices bend at a specific hinge point where a glycine residue is located.

Now that we have seen how these prokaryotic K^+ channels operate, we are in a better position to understand the structure and function of the more complex eukaryotic versions, which are thought to perform in a similar manner. Genes that encode a variety of distinct voltage-gated K^+ (or Kv) channels have been isolated and the molecular anatomy of their proteins scrutinized. The Kv channels of plants play an important role in salt and water balance and in regulation of cell volume. The Kv channels of animals are best known for their role in muscle and nerve function, which is explored at the end of the chapter. Eukaryotic Kv channel subunits contain six membrane-associated helices, named S1–S6, which are shown two-dimensionally in Figure 4.41. These six helices can be grouped into two functionally distinct domains:

1. a ***pore domain***, which has the same basic architecture as that of the entire bacterial channel illustrated in Figure 4.39 and contains the selectivity filter that permits the selective passage of K^+ ions. Helices M1 and M2 and the P segment of the KcsA channel of Figure 4.39 are homologous to helices S5 and S6 and the P segment of the voltage-gated eukaryotic channel illustrated in Figure 4.41. Like the four M2 helices of KcsA, the four S6 helices line much of the pore, and their configuration determines whether the gate to the channel is open or closed.
2. a ***voltage-sensing domain*** consisting of helices S1–S4 that senses the voltage across the plasma membrane (as discussed below).

The three-dimensional crystal structure of a complete eukaryotic Kv channel purified from rat brain is shown in Figure 4.42. Determination of this structure was made possible by use of a mixture of detergent and lipid throughout the purification and crystallization process. The presence of negatively charged phospholipids is thought to be important in maintaining the native structure of the membrane protein and promoting its function as a voltage-gated channel. Like the KcsA channel, a single eukaryotic Kv channel consists of four homologous subunits arranged symmetrically around the central ion-conducting pore. The selectivity filter, and thus the presumed mechanism of K^+ ion selection, is virtually identical in the prokaryotic KcsA and eukaryotic Kv proteins. The gate leading into a Kv channel is formed by the inner ends of the S6 helices and is thought to open and close in a manner roughly similar to that of the M2 helices of the bacterial channel (shown in Figure 4.40). The protein depicted in Figure 4.42 represents the open state of the channel.[6]

The S4 helix, which contains several positively charged amino acid residues spaced along the polypeptide chain (inset of Figure 4.41), acts as the key element of the voltage sensor. The voltage-sensing domain is seen to be connected to the

[6]Recent studies strongly suggest that K^+ channels are gated by two distinct mechanisms: one involving the opening and closing of the inner helix bundle as described here and another involving the selectivity filter, which is not discussed (see *PNAS* 107:7623, 2010 and *Nature* 466:203, 2010).

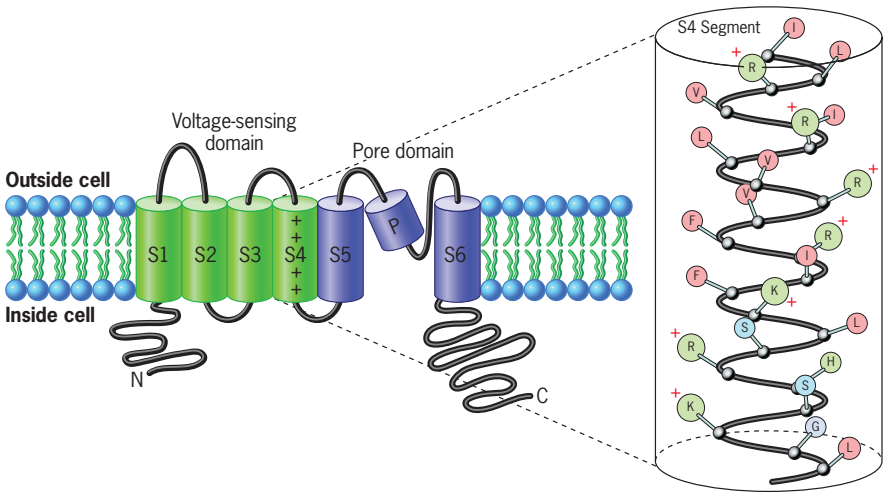
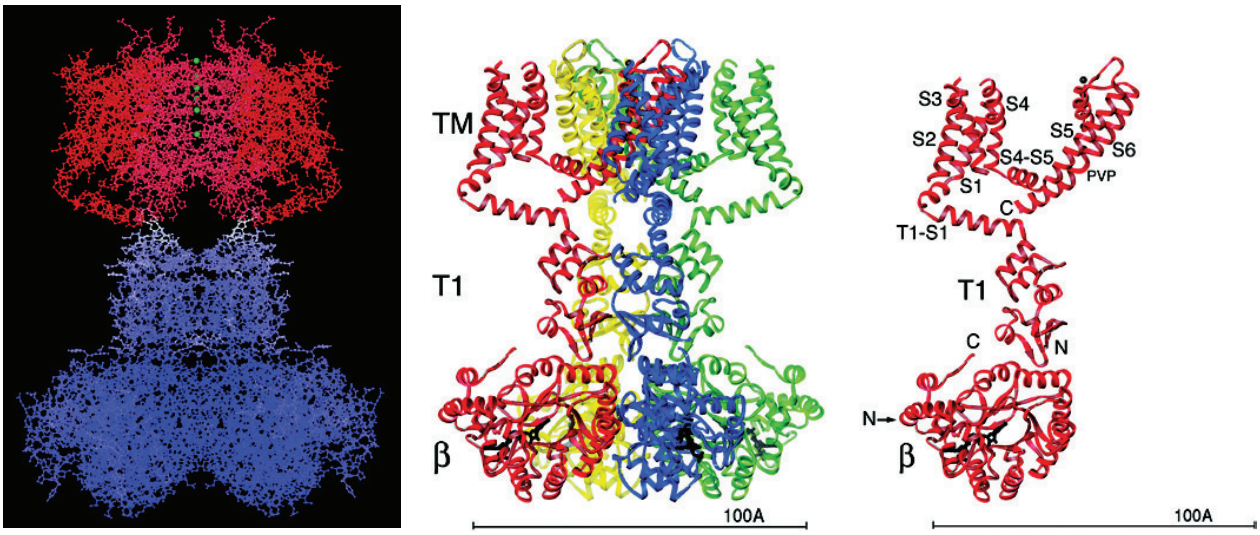


Figure 4.41 **The structure of one subunit of a eukaryotic, voltage-gated K^+ channel.** A two-dimensional portrait of a K^+ channel subunit showing its six transmembrane helices and a portion of the polypeptide (called the pore helix or P) that dips into the protein to form part of the channel's wall. The inset shows the sequence of amino acids of the positively charged S4 helix of the *Drosophila* K^+ *Shaker* ion channel, which serves as a voltage sensor. The positively charged side chains are situated at every third residue along the otherwise hydrophobic helix. This member of the Kv family is called a *Shaker* channel because flies with certain mutations in the protein shake vigorously when anesthesized with ether. The *Shaker* channel was the first K^+ channel to be identified and cloned in 1987.



(a) *(b)* *(c)*

Figure 4.42 **Three-dimensional structure of a voltage-gated mammalian K^+ channel.** (*a*) The crystal structure of the entire tetrameric Kv1.2 channel, a member of the *Shaker* family of K^+ ion channels found in nerve cells of the brain. The transmembrane portion is shown in red, and the cytoplasmic portion in blue. The potassium ion binding sites are indicated in green. (*b*) Ribbon drawing of the same channel shown in *a*, with the four subunits that make up the channel shown in different colors. If you focus on the red subunit, you can see (1) the spatial separation between the voltage-sensing and pore domains of the subunit and (2) the manner in which the voltage-sensing domain from each subunit is present on the outer edge of the pore domain of a neighboring subunit. The cytoplasmic portion of this particular channel consists of a T1 domain, which is part of the channel polypeptide itself, and a separate β polypeptide. (*c*) Ribbon drawing of a single subunit showing the spatial orientation of the six membrane-spanning helices (S1–S6) and also the presence of the S4–S5 linker helix, which connects the voltage-sensing and pore domains. This linker transmits the signal from the S4 voltage sensor that opens the channel. The inner surface of the channel below the pore domain is lined by the S6 helix (roughly similar to the M2 helix of the bacterial channel shown in Figure 4.39). The channel shown here is present in the open configuration with the S6 helices curved outward (compare to Figure 4.40) at the site marked PVP (standing for Pro-Val-Pro, which is likely the amino acid sequence of the "hinge"). (From Stephen B. Long et al., Science 309:867, 899, 2005, courtesy of Roderick MacKinnon; © 2005, reprinted with permission from AAAS.)

pore domain by a short linker helix denoted as S4–S5 in the model in Figure 4.42. Under resting conditions, the negative potential across the membrane (page 165) keeps the gate closed. A change in the potential to a more positive value (a depolarization, page 166) exerts an electric force on the S4 helix. This force is thought to cause the transmembrane S4 helix to move in such a way that its positively charged residues shift from a position where they were exposed to the cytoplasm to a new position where they are exposed to the outside of the cell. Voltage sensing is a dynamic process whose mechanism cannot be resolved by a single static view of the protein such as that shown in Figure 4.42. In fact, several competing models describing the mechanism of action of the voltage sensor are currently debated. However it occurs, the movement of the S4 helix in response to membrane depolarization initiates a series of conformational changes within the protein that opens the gate at the cytoplasmic end of the channel.

Once opened, more than ten million potassium ions can pass through the channel per second, which is nearly the rate that would occur by free diffusion in solution. Because of the large ion flux, the opening of a relatively small number of K^+ channels has significant impact on the electrical properties of the membrane. After the channel is open for a few milliseconds, the movement of K^+ ions is "automatically" stopped by a process known as inactivation. To understand channel inactivation, we have to consider an additional portion of a Kv channel besides the two transmembrane domains discussed above.

Eukaryotic Kv channels typically contain a large cytoplasmic structure whose composition varies among different channels. As indicated in Figure 4.43*a* inactivation of the channel is accomplished by movement of a small inactivation peptide that dangles from the cytoplasmic portion of the protein. The inactivation peptide is thought to gain access to the cytoplasmic mouth of the pore by snaking its way through one of four "side windows" indicated in the figure. When one of these dangling peptides moves up into the mouth of the pore (Figure 4.43*a*), the passage of ions is blocked, and the channel is inactivated. At a subsequent stage of the cycle, the inactivation peptide is released, and the gate to the channel is closed. It follows from this discussion that the potassium channel can exist in three different states—open, inactivated, and closed—which are illustrated schematically in Figure 4.43*b*.

Potassium channels come in many different varieties. It is remarkable that *C. elegans*, a nematode worm whose body consists of only about 1000 cells, contains approximately 80

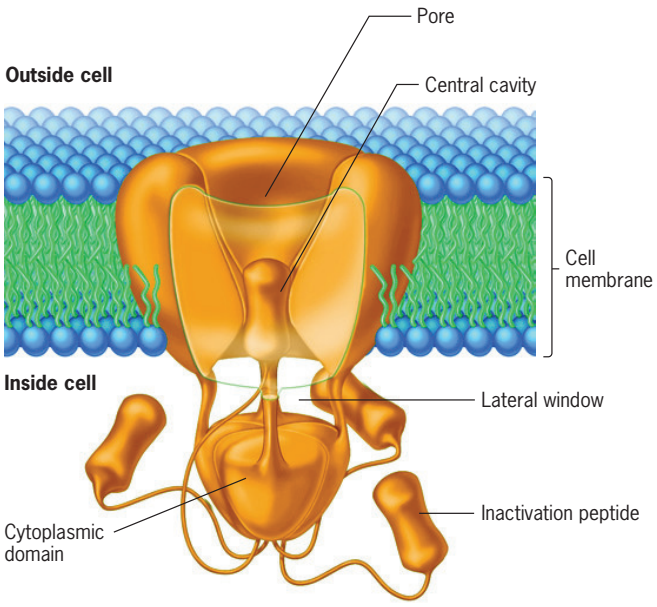


Figure 4.43 **Conformational states of a voltage-gated K^+ ion channel.** (*a*) Three-dimensional model of a eukaryotic K^+ ion channel. Inactivation of channel activity occurs as one of the inactivation peptides, which dangle from the cytoplasmic portion of the complex, fits into the cytoplasmic opening of the channel. (*b*) Schematic representation of a view into a K^+ ion channel, perpendicular to the membrane from the cytoplasmic side, showing the channel in the closed (resting), open, and inactivated state. (B: Reprinted from Neuron, vol. 20, C. M. Armstrong and B. Hille, Voltage-Gated Ion Channels and Electrical Excitability, p. 377; copyright 1998, with permission from Elsevier Science.)

different genes that encode K^+ channels. It is evident that a single cell—whether in a nematode, human, or plant—is likely to possess a variety of different K^+ channels that open and close in response to different voltages. In addition, the voltage required to open or close a particular K^+ channel can vary depending on whether or not the channel protein is phosphorylated, which in turn is regulated by hormones and other factors. It is apparent that ion channel function is under the control of a diverse and complex set of regulatory agents. The structure and function of a very different type of ion channel, the ligand-gated nicotinic acetylcholine receptor, is the subject of the Experimental Pathways section at the end of this chapter.

Facilitated Diffusion

Substances always diffuse across a membrane from a region of higher concentration on one side to a region of lower concentration on the other side, but they do not always diffuse through the lipid bilayer or through a channel. In many cases, the diffusing substance first binds selectively to a membrane-spanning protein, called a **facilitative transporter,** that facilitates the diffusion process. The binding of the solute to the facilitative transporter on one side of the membrane is thought to trigger a conformational change in the protein, exposing the solute to the other surface of the membrane, from where it can diffuse down its concentration gradient. This mechanism is illustrated in Figure 4.44. Because they operate passively, that is, without being coupled to an energy-releasing system, facilitated transporters can mediate the movement of solutes equally well in both directions. The direction of net flux depends on the relative concentration of the substance on the two sides of the membrane.

Facilitated diffusion, as this process is called, is similar in many ways to an enzyme-catalyzed reaction. Like enzymes, facilitative transporters are specific for the molecules they transport, discriminating, for example, between D and L stereoisomers (page 44). In addition, both enzymes and

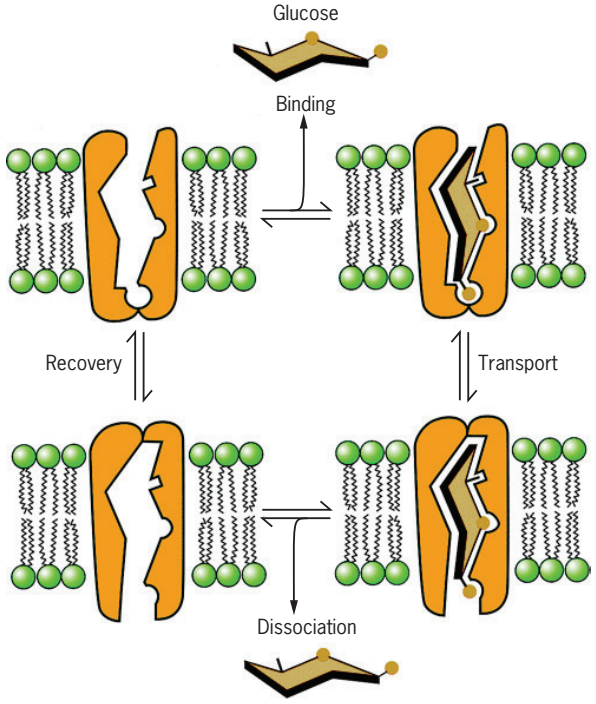


Figure 4.44 **Facilitated diffusion.** A schematic model for the facilitated diffusion of glucose depicts the alternating conformation of a carrier that exposes the glucose binding site to either the inside or outside of the membrane. (Reprinted from S. A. Baldwin and G. E. Lienhard, Trends Biochem. Sci. 6:210, 1981. Used with permission from Elsevier, Ltd.)

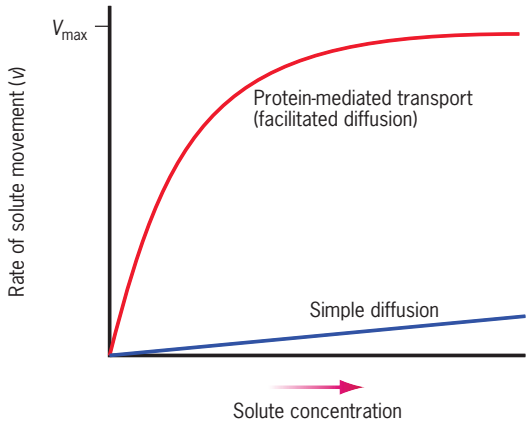


Figure 4.45 **The kinetics of facilitated diffusion as compared to that of simple physical diffusion.**

transporters exhibit saturation-type kinetics (Figure 4.45). Unlike ion channels, which can conduct millions of ions per second, most facilitative transporters can move only hundreds to thousands of solute molecules per second across the membrane. Another important feature of facilitative transporters is that, like enzymes and ion channels, their activity can be regulated. Facilitated diffusion is particularly important in mediating the entry and exit of polar solutes, such as sugars and amino acids, that do not penetrate the lipid bilayer. This is illustrated in the following section.

The Glucose Transporter: An Example of Facilitated Diffusion Glucose is the body's primary source of direct energy, and most mammalian cells contain a membrane protein that facilitates the diffusion of glucose from the bloodstream into the cell (as depicted in Figures 4.44 and 4.49). A gradient favoring the continued diffusion of glucose into the cell is maintained by phosphorylating the sugar after it enters the cytoplasm, thus lowering the intracellular glucose concentration. Humans have at least five related proteins (isoforms) that act as facilitative glucose transporters. These isoforms, termed GLUT1 to GLUT5, are distinguished by the tissues in which they are located, as well as their kinetic and regulatory characteristics.

Insulin is a hormone produced by endocrine cells of the pancreas and plays a key role in maintaining proper blood sugar levels. An increase in blood glucose levels triggers the secretion of insulin, which stimulates the uptake of glucose into various target cells, most notably skeletal muscle and fat cells (adipocytes). Insulin-responsive cells share a common isoform of the facilitative glucose transporter, specifically GLUT4. When insulin levels are low, these cells contain relatively few glucose transporters on their plasma membrane. Instead, the transporters are present within the membranes of cytoplasmic vesicles. Rising insulin levels act on target cells to stimulate the fusion of the cytoplasmic vesicles to the plasma membrane, which moves transporters to the cell surface where they can bring glucose into the cell (see Figure 15.26).

Active Transport

Life cannot exist under equilibrium conditions (page 93). Nowhere is this more apparent than in the imbalance of ions across the plasma membrane. The differences in concentration of the major ions between the outside and inside of a typical mammalian cell are shown in Table 4.3. The ability of a cell to generate such steep concentration gradients across its plasma membrane cannot occur by either simple or facilitated diffusion. Rather, these gradients must be generated by **active transport**.

Like facilitated diffusion, active transport depends on integral membrane proteins that selectively bind a particular solute and move it across the membrane in a process driven by changes in the protein's conformation. Unlike facilitated diffusion, however, movement of a solute against a gradient requires the coupled input of energy. Consequently, the endergonic movement of ions or other solutes across the membrane against a concentration gradient is coupled to an exergonic process, such as the hydrolysis of ATP, the absorbance of light, the transport of electrons, or the flow of other substances down their gradients. Proteins that carry out active transport are often referred to as "pumps."

Primary Active Transport: Coupling Transport to ATP Hydrolysis In 1957, Jens Skou, a Danish physiologist, discovered an ATP-hydrolyzing enzyme in the nerve cells of a crab that was only active in the presence of both Na^+ and K^+ ions. Skou proposed, and correctly so, that this enzyme, which was responsible for ATP hydrolysis, was the same protein that was active in transporting the two ions; the enzyme was called the Na^+/K^+-ATPase, or the *sodium–potassium pump*.

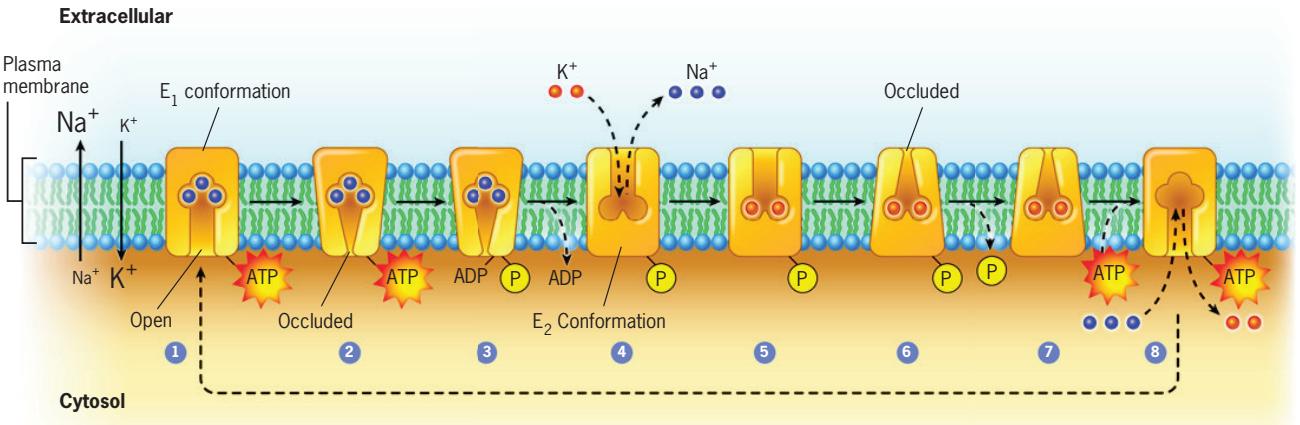
Unlike the protein-mediated movement of a facilitated diffusion system, which will carry the substance equally well in either direction, active transport drives the movement of ions in only one direction. It is the Na^+/K^+-ATPase that is responsible for the large excess of Na^+ ions outside of the cell and the large excess of K^+ ions inside the cell. The positive charges carried by these two cations are balanced by negative charges carried by various anions so that the extracellular and intracellular compartments are, for the most part, electrically neutral. Cl^- ions are present at greater concentration outside of cells, where they balance the extracellular Na^+ ions. The abundance of intracellular K^+ ions is balanced primarily by excess negative charges carried by proteins and nucleic acids.

Table 4.3 ***Ion Concentrations Inside and Outside of a Typical Mammalian Cell***

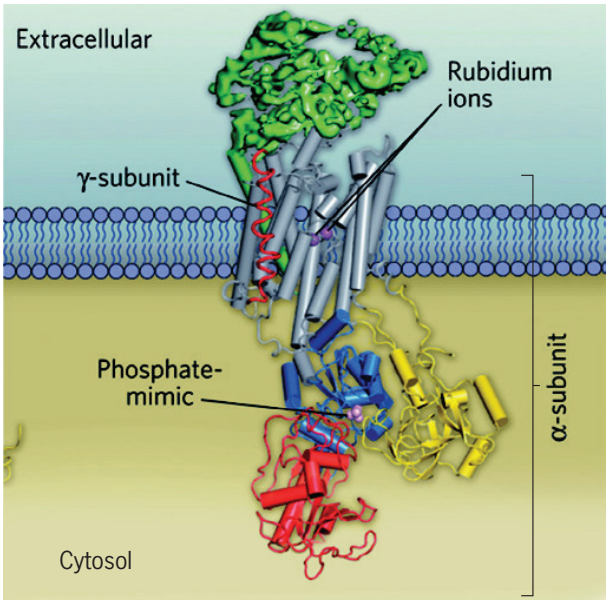
	Extracellular concentration	Intracellular concentration	Ionic gradient
Na^+	150 mM	10 mM	15×
K^+	5 mM	140 mM	28×
Cl^-	120 mM	10 mM	12×
Ca^{2+}	10^{-3} M	10^{-7} M	10,000×
H^+	$10^{-7.4}$ M (pH of 7.4)	$10^{-7.2}$ M (pH of 7.2)	Nearly 2×

The ion concentrations for the squid axon are given on page 177.

FIGURE IN FOCUS



(a)



(b)

?

Figure 4.46 **The Na^+/K^+-ATPase.** (*a*) Simplified schematic model of the transport cycle as described in the text. Note that the actual Na^+/K^+-ATPase is composed of at least two different membrane-spanning subunits: a larger α subunit, which carries out the transport activity, and a smaller β subunit, which functions primarily in the maturation and assembly of the pump within the membrane. A third (γ) subunit may also be present. (*b*) A model of the E_2 conformation of the protein based on an X-ray crystallographic study. The cation binding sites are located deep within the transmembrane domain, which consists of 10 membrane-spanning helices. The two rubidium ions are located where the potassium ions would normally be bound. (B: FROM AYAKO TAKEUCHI, COURTESY OF DAVID C. GADSBY, NATURE 450:958, 2007, © 2007 REPRINTED BY PERMISSION FROM MACMILLAN PUBLISHERS LTD.)

The ratio of $Na^+:K^+$ pumped by the Na^+/K^+-ATPase is not 1:1, but 3:2 (see Figure 4.46). In other words, for each ATP hydrolyzed, three sodium ions are pumped out as two potassium ions are pumped in. Because of this pumping ratio, the Na^+/K^+-ATPase is *electrogenic*, which means that it contributes directly to the separation of charge across the membrane. The Na^+/K^+-ATPase is an example of a *P-type* ion pump. The "P" stands for phosphorylation, indicating that, during the pumping cycle, the hydrolysis of ATP leads to the transfer of the released phosphate group to an aspartic acid residue of the transport protein. As denoted by the name of the protein, this reaction is catalyzed by the pump itself.

Consider the activity of the protein. It must pick up sodium or potassium ions from a region of low concentration, which means that the protein must have a relatively high affinity for the ions. Then the protein must release the ions on the other side of the membrane into a much greater concentration of each ion. To do this, the affinity of the protein for that ion must decrease. Thus, the affinity for each ion on the two sides of the membrane must be different. This is achieved by ATP hydrolysis and the subsequent release of ADP, which induces a large conformational change within the protein molecule. The change in protein structure—between the E_1 and E_2 conformations—also serves to alternately expose the ion binding sites to the opposite side of the membrane, as discussed in the following paragraph.

A proposed scheme for the pumping cycle of the Na^+/K^+-ATPase is shown in Figure 4.46*a*, which is based on recent X-ray crystallographic structures of P-type ion pumps. In step 1, Figure 4.46*a*, the pump is in the E_1 conformation, and the ion binding sites are accessible to the inside of the cell. In this step, the

protein has bound three Na^+ ions and an ATP. In step 2, a gate within the protein has closed, shifting the pump into an occluded E_1 state in which the Na^+ ions can no longer flow back into the cytosol. Hydrolysis of the bound ATP (step 2→3) and release of ADP (step 3→4) cause the change from the E_1 to the E_2 conformation. In doing so, the binding site becomes accessible to the extracellular compartment, and the protein loses its affinity for Na^+ ions, which are then released outside the cell. Once the three Na^+ ions have been released, the protein picks up two K^+ions (step 5). The closing of another gate within the protein shifts the pump into an occluded state (step 6), preventing back flow of the K^+ ions into the extracellular space. Occlusion is followed by dephosphorylation (step 6→7) and ATP binding (step 8), which induces the return of the protein to the original E_1 conformation. In this state, the binding site is open to the internal surface of the membrane and has lost its affinity for K^+ ions, leading to the release of these ions into the cell. The cycle is then repeated. A model of the crystal structure of the Na^+/K^+-ATPase structure is shown in Figure 4.46*b*. Because they require complex conformational changes, these active transport pumps move ions across membranes at rates that are several orders of magnitude lower than their flow through ion channels.

The sodium–potassium pump is found only in animal cells. This protein is thought to have evolved in primitive animals as the primary means to maintain cell volume and as the mechanism to generate the steep Na^+ and K^+ gradients that play such a key role in the formation of impulses in nerve and muscle cells. These same ionic gradients are used in nonexcitable cells to power the movement of other solutes as discussed below. The importance of the sodium–potassium pump becomes evident when one considers that it consumes approximately one-third of the energy produced by most animal cells and two-thirds of the energy produced by nerve cells. Digitalis, a steroid obtained from the foxglove plant that has been used for 200 years as a treatment for congestive heart disease, binds to the Na^+/K^+-ATPase. Digitalis strengthens the heart's contraction by inhibiting the Na^+/K^+ pump, which leads to a chain of events that increases Ca^{2+} availability inside the muscle cells of the heart.

Other Primary Ion Transport Systems The best studied P-type pump is the Ca^{2+}-ATPase whose three-dimensional structure has been determined at several stages of the pumping cycle. The calcium pump is present in the membranes of the endoplasmic reticulum, where it actively transports calcium ions out of the cytosol into the lumen of this organelle. Plant cells have a H^+-transporting, P-type, plasma membrane pump. In plants, this proton pump plays a key role in the secondary transport of solutes (discussed later), in the control of cytosolic pH, and possibly in control of cell growth by means of acidification of the plant cell wall.

The epithelial lining of the stomach also contains a P-type pump, the H^+/K^+-ATPase, which secretes a solution of concentrated acid (up to 0.16 N HCl) into the stomach chamber. In the resting state, these pump molecules are situated in cytoplasmic membranes of the parietal cells of the stomach lining and are nonfunctional (Figure 4.47). When food enters the stomach, a hormonal message is transmitted to the parietal cells that causes the pump-containing membranes to move to the apical cell surface, where they fuse with the plasma membrane and begin secreting acid (Figure 4.47). In addition to functioning in digestion, stomach acid can also lead to heartburn. Prilosec and related drugs are widely used to prevent heartburn by inhibiting the stomach's H^+/K^+-ATPase. Other acid-blocking heartburn medications (e.g., Zantac, Pepcid, and Tagamet) do not inhibit the H^+/K^+-ATPase directly, but block a receptor on the surface

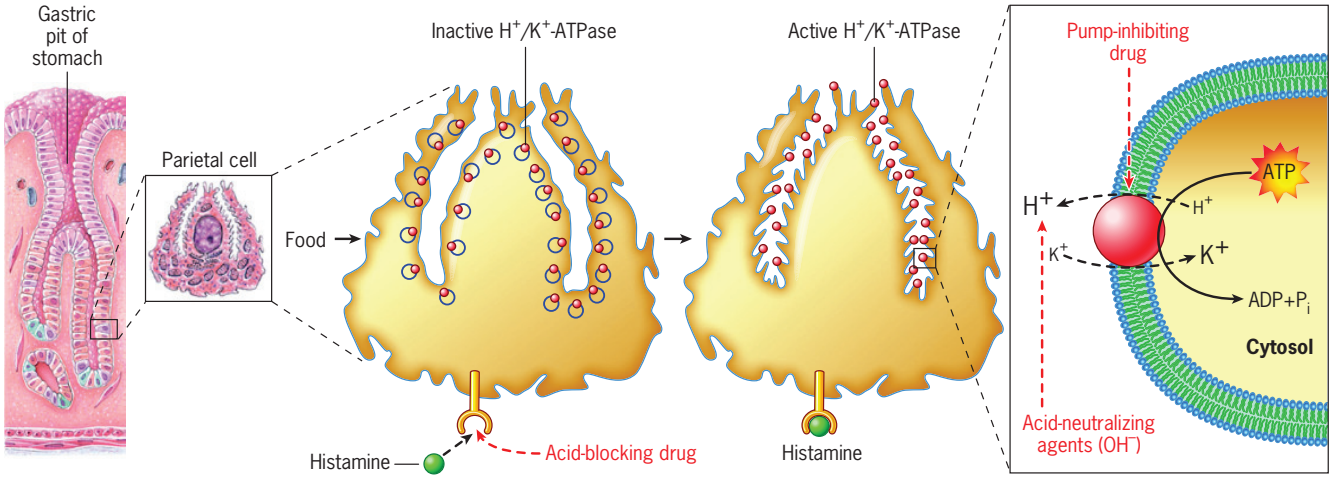


Figure 4.47 **Control of acid secretion in the stomach.** In the resting state, the H^+/K^+-ATPase molecules are present in the walls of cytoplasmic vesicles. Food entering the stomach triggers a cascade of hormone-stimulated reactions in the stomach wall leading to the release of histamine, which binds to a receptor on the surface of the acid-secreting parietal cells. Binding of histamine to its receptor stimulates a response that causes the H^+/K^+-ATPase-containing vesicles to fuse to the plasma membrane, forming deep folds, or canaliculi. Once at the surface, the transport protein is activated and pumps protons into the stomach cavity against a concentration gradient (indicated by the size of the letters). The heartburn drugs Prilosec, Nexium, and Prevacid block acid secretion by directly inhibiting the H^+/K^+-ATPase, whereas several other acid-blocking medications interfere with activation of the parietal cells. Acid-neutralizing medications provide basic anions that combine with the secreted protons.

of the parietal cells, thereby stopping the cells from becoming activated by the hormone.

Unlike P-type pumps, *V-type* pumps utilize the energy of ATP without forming a phosphorylated protein intermediate. V-type pumps actively transport hydrogen ions across the walls of cytoplasmic organelles and vacuoles (hence the designation V-type). They occur in the membranes that line lysosomes, secretory granules, and plant cell vacuoles where they maintain the low pH of the contents. V-type pumps have also been found in the plasma membranes of a variety of cells. For example, a V-type pump in the plasma membranes of kidney tubules helps maintain the body's acid–base balance by secreting protons into the forming urine. V-type pumps are large multi-subunit complexes (shown in Figure 4.4*d*) and similar in structure to that of the ATP synthase shown in Figure 5.23.

Another diverse group of proteins that actively transport ions is the *ATP-binding cassette* (*ABC*) *transporters*, so called because all of the members of this superfamily share a homologous ATP-binding domain. The best studied ABC transporter is described in the accompanying Human Perspective.

Using Light Energy to Actively Transport Ions *Halobacterium salinarium* (or *H. halobium*) is an archaebacterium that lives in extremely salty environments, such as that found in the Great Salt Lake. When grown under anaerobic conditions, the plasma membranes of these prokaryotes take on a purple color due to the presence of one particular protein, *bacteriorhodopsin,* which acts a light-driven proton pump. As shown in Figure 4.48, bacteriorhodopsin contains retinal, the same prosthetic group present in rhodopsin, the light-absorbing protein of the rods of the vertebrate retina. The absorption of light energy by the retinal group induces a series of conformational changes in the protein that cause a proton to move from the retinal group, through a channel in the protein, to the cell exterior (Figure 4.48). The proton donated by the photoexcited retinal is replaced by another proton transferred to the protein from the cytoplasm. In effect, this process results in the translocation of protons from the cytoplasm to the external environment, thereby generating a steep H^+ gradient across the plasma membrane. This gradient is subsequently used by an ATP-synthesizing enzyme to phosphorylate ADP, as described in the next chapter.

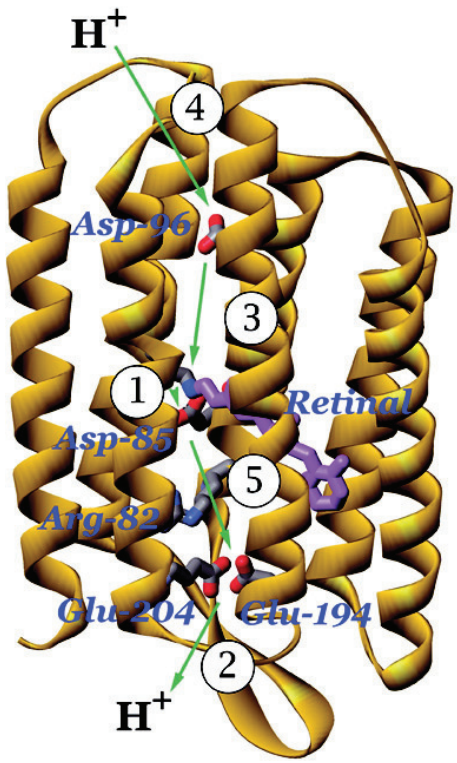


Figure 4.48 **Bacteriorhodopsin: a light-driven proton pump.** The protein contains seven membrane-spanning helices and a centrally located retinal group (shown in purple), which serves as the light-absorbing element (chromophore). Absorption of a photon of light causes a change in the electronic structure of retinal, leading to the transfer of a proton from the —NH^+ group to a closely associated, negatively charged aspartic acid residue (#85) (step 1). The proton is then released to the extracellular side of the membrane (step 2) by a relay system consisting of several amino acid residues (Asp82, Glu204, and Glu194). The spaces between these residues are filled with hydrogen-bonded water molecules that help shuttle protons along the pathway. The deprotonated retinal is returned to its original state (step 3) when it accepts a proton from an undissociated aspartic acid residue (Asp96) located near the cytoplasmic side of the membrane. Asp96 is then reprotonated by a H^+ from the cytoplasm (step 4). Asp85 is deprotonated (step 5) prior to receiving a proton from retinal in the next pumping cycle. As a result of these events, protons move from the cytoplasm to the cell exterior through a central channel in the protein. (From Hartmut Luecke et al., courtesy of Janos K. Lanyi, Science 286:255, 1999; © 1999, reprinted with permission from AAAS.)

Secondary Active Transport (or Cotransport): Coupling Transport to Existing Ion Gradients The establishment of concentration gradients, such as those of Na^+, K^+, and H^+, provides a means by which free energy can be stored in a cell. The potential energy stored in ionic gradients is utilized by a cell in various ways to perform work, including the transport of other solutes. Consider the physiologic activity of the intestine. Within its lumen, enzymes hydrolyze high-molecular-weight polysaccharides into simple sugars, which are absorbed by the epithelial cells that line the intestine. The movement of glucose across the apical plasma membrane of the epithelial cells, against a concentration gradient, occurs by **cotransport** with sodium ions, as illustrated in Figure 4.49. The Na^+ concentration is kept very low within the cells by the action of a *primary* active transport system (the Na^+/K^+-ATPase), located in the basal and lateral plasma membrane, which pumps sodium ions out of the cell against a concentration gradient. The tendency for sodium ions to diffuse back across the apical plasma membrane down their concentration gradient is "tapped" by the epithelial cells to drive the cotransport of glucose molecules into the cell *against* a concentration gradient. The glucose molecules are said to be driven by *secondary active transport.* In this case, the transport protein, called a *Na^+/glucose cotransporter*, moves two sodium ions and one glucose molecule with each cycle. Once inside, the glucose molecules diffuse through the cell and are moved across the basal membrane by facilitated diffusion (page 157).

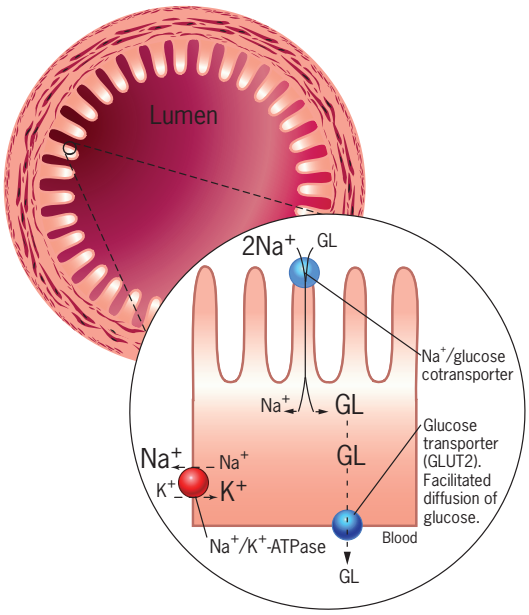


Figure 4.49 **Secondary transport: the use of energy stored in an ionic gradient.** The Na^+/K^+-ATPase residing in the plasma membrane of the lateral surface maintains a very low cytosolic concentration of Na^+. The Na^+ gradient across the plasma membrane represents a storage of energy that can be tapped to accomplish work, such as the transport of glucose by a Na^+/glucose cotransporter located in the apical plasma membrane. Once transported across the apical surface into the cell, the glucose molecules diffuse to the basal surface where they are carried by a glucose facilitative transporter out of the cell and into the bloodstream. The relative size of the letters indicates the directions of the respective concentration gradients. Two Na^+ ions are transported for each glucose molecule; the 2:1 Na^+/glucose provides a much greater driving force for moving glucose into the cell than a 1:1 ratio.

To appreciate the power of an ion gradient in accumulating other types of solutes in cells, we can briefly consider the energetics of the Na^+/glucose cotransporter. Recall from page 148 that the free-energy change for the movement of a mole of Na^+ ions into the cell is equal to −3.1 kcal/mol, and thus 6.2 kcal for two moles of Na^+ ions, which would be available to transport one mole of glucose uphill into the cell. Recall also from page 148 that the equation for the movement of a nonelectrolyte, such as glucose, across the membrane is

$$\Delta G = RT \ln \frac{[C_i]}{[C_o]}$$

$$\Delta G = 2.303\, RT \log_{10} \frac{[C_i]}{[C_o]}$$

Using this equation, we can calculate how steep a concentration gradient of glucose (X) that this cotransporter can generate. At 25°C,

$$-6.2 \text{ kcal/mol} = 1.4 \text{ kcal/mol} \cdot \log_{10} X$$

$$\log_{10} X = -4.43$$

$$X = \frac{1}{23{,}000}$$

This calculation indicates that the Na^+/glucose cotransporter is capable of transporting glucose into a cell against a concentration gradient greater than 20,000-fold.

Plant cells rely on secondary active transport systems to take up a variety of nutrients, including sucrose, amino acids, and nitrate. In plants, uptake of these compounds is coupled to the downhill, inward movement of H^+ ions rather than Na^+ ions. The secondary active transport of glucose into the epithelial cells of the intestine and the transport of sucrose into a plant cell are examples of *symport*, in which the two transported species (Na^+ and glucose or H^+ and sucrose) move in the same direction. Numerous cotransporters have been isolated that engage in ***antiport***, in which the two transported species move in opposite directions. For example, cells often maintain a proper cytoplasmic pH by coupling the inward, downhill movement of Na^+ with the outward movement of H^+. Cotransporters that mediate antiport are usually called *exchangers*. The three-dimensional structures of a number of secondary transporters have been solved in recent years, and, like the Na^+/K^+-ATPase, they exhibit a transport cycle in which the protein's binding sites gain alternating access to the cytoplasm and the extracellular space. A proposed model of the transport cycle of one of the major families of secondary transporters is shown in Figure 4.50.

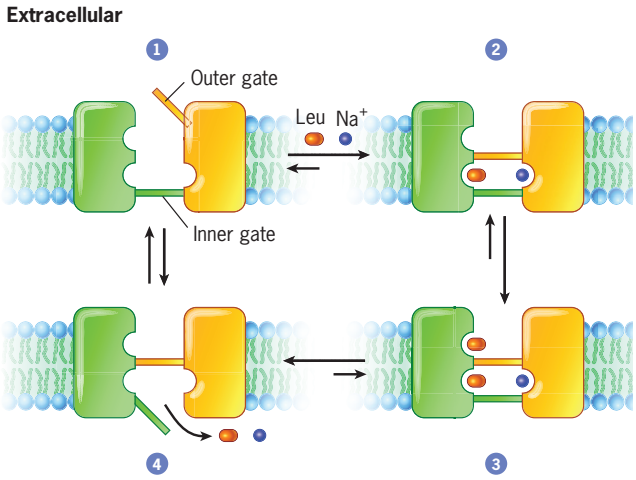


Figure 4.50 **A schematic model of the transport cycle of a secondary transporter.** Four different conformational states during the transport cycle of a bacterial symporter of the LeuT family are shown. The protein actively transports the amino acid leucine into the cell using the established Na^+ ion gradient as its source of energy. In step 1, the outer gate in the protein is open, whch allows both Na^+ and leucine to reach their binding sites from the extracellular space. In step, 2, the outer gate closes, occluding the substrates within the protein. In step 3, a second leucine molecule binds to another site just outside the outer gate. In step 4, the inner gate opens and the substrates are released into the cytoplasm. The protein returns to its original state when the inner gate is closed and the outer gate is opened. (REPRINTED BY PERMISSION FROM MACMILLAN PUBLISHERS LTD: NATURE, 465:171, 2010 BY N. K. KARPOWICH AND DA-NENG WANG (NYU SCHOOL OF MEDICINE)

THE HUMAN PERSPECTIVE

Defects in Ion Channels and Transporters as a Cause of Inherited Disease

Several severe, inherited disorders have been traced to mutations in genes that encode ion channel proteins (Table 1). Most of the disorders listed in Table 1 affect the movement of ions across the plasma membranes of excitable cells (i.e., muscle, nerve, and sensory cells), reducing the ability of these cells to develop or transmit impulses (page 167). In contrast, cystic fibrosis, the best studied and most common inherited ion channel disorder, results from a defect in the ion channels of epithelial cells.

On average, 1 out of every 25 persons of Northern European descent carries one copy of the mutant gene that can cause cystic fibrosis. Because they show no symptoms of the mutant gene, most heterozygotes are unaware that they are carriers. Consequently, approximately 1 out of every 2500 infants in this Caucasian population ($1/25 \times 1/25 \times 1/4$) is homozygous recessive at this locus and born with cystic fibrosis (CF). Although cystic fibrosis affects various organs, including the intestine, pancreas, sweat glands, and reproductive tract, the respiratory tract usually exhibits the most severe effects. Victims of CF produce a thickened, sticky mucus that is very hard to propel out of the airways. Afflicted individuals typically suffer from chronic lung infections and inflammation, which progressively destroy pulmonary function.

The gene responsible for cystic fibrosis was isolated in 1989. Once the sequence of the CF gene was determined and the amino acid sequence of the corresponding polypeptide was deduced, it was apparent that the polypeptide was a member of the ABC transporter superfamily. The protein was named *cystic fibrosis transmembrane conductance regulator* (*CFTR*), an ambiguous term that reflected the fact that researchers weren't sure of its precise function. The question was thought to be answered after the protein was purified, incorporated into artificial lipid bilayers, and shown to act as a cyclic AMP-regulated chloride channel, not a transporter. But subsequent studies have added numerous complications to the story as it has been shown that, in addition to functioning as a chloride channel, CFTR also (1) conducts bicarbonate (HCO_3^-) ions, (2) suppresses the activity of an epithelial Na^+ ion channel (ENaC), and (3) stimulates the activity of a family of epithelial chloride/bicarbonate exchangers. As the role of CFTR has become more complex, it has become difficult to establish precisely how a defect in this protein leads to the development of chronic lung infections. While there is considerable debate, many researchers would agree with the following statements.

Because the movement of water out of epithelial cells by osmosis follows the movement of salts, abnormalities in the flux of Cl^-, HCO_3^-, and/or Na^+ caused by CFTR deficiency leads to a decrease in the fluid that bathes the epithelial cells of the airways (Figure 1). A reduction in volume of the surface liquid, and a resulting increase in viscosity of the secreted mucus, impair the function of the cilia that push mucus and bacteria out of the respiratory tract. Many CF patients are helped by inhaling a mist of hypertonic saline, which helps to draw more water into the airways and reduce the viscosity of the mucus. Clinical trials are also being carried out on compounds that have the potential to increase the volume of surface fluid by altering the movements of ions in and out of the epithelial cells (Figure 1). These compounds include Na^+-channel inhibitors (e.g., GS-9411) with the potential to reduce Na^+ ion absorption from the airway fluid into the epithelium and activators of Cl^- channels (other than CFTR) (e.g., Moli1901) with the potential to increase Cl^- ion conductance from the epithelium into the airway fluid.

In the past decade, researchers have identified more than 1000 different mutations that give rise to cystic fibrosis. However, approximately 70 percent of the alleles responsible for cystic fibrosis in the United States contain the same genetic alteration (designated ΔF508)—they are all missing three base pairs of DNA that encode a phenylalanine at position 508, within one of the cytoplasmic domains of the CFTR polypeptide. Subsequent research has revealed

Table 1

Inherited disorder	Type of channel	Gene	Clinical consequences
Familial hemiplegic migraine (FHM)	Ca^{2+}	*CACNL1A4*	Migraine headaches
Episodic ataxia type-2 (EA-2)	Ca^{2+}	*CACNL1A4*	Ataxia (lack of balance and coordination)
Hypokalemic periodic paralysis	Ca^{2+}	*CACNL1A3*	Periodic myotonia (muscle stiffness) and paralysis
Episodic ataxia type-1	K^+	*KCNA1*	Ataxia
Benign familial neonatal convulsions	K^+	*KCNQ2*	Epileptic convulsions
Nonsyndromic dominant deafness	K^+	*KCNQ4*	Deafness
Long QT syndrome	K^+	*HERG*	Dizziness, sudden death from ventricular fibrillation
	Na^+	*KCNQ1, or SCN5A*	
Hyperkalemic periodic paralysis	Na^+	*SCN4A*	Periodic myotonia and paralysis
Liddle Syndrome	Na^+	*B-ENaC*	Hypertension (high blood pressure)
Myasthenia gravis	Na^+	*nAChR*	Muscle weakness
Dent's disease	Cl^-	*CLCN5*	Kidney stones
Myotonia congenita	Cl^-	*CLC-1*	Periodic myotonia
Bartter's syndrome type IV	Cl^-	*CLC-Kb*	Kidney dysfunction, deafness
Cystic fibrosis	Cl^-	*CFTR*	Lung congestion and infections
Cardiac arrhythmias	Na^+ K^+ Ca^{2+}	many different genes	Irregular or rapid heartbeat

See *Nature Cell Biol.* 6:1040, 2004, or *Nature* 440:444, 2006, for a more complete list.

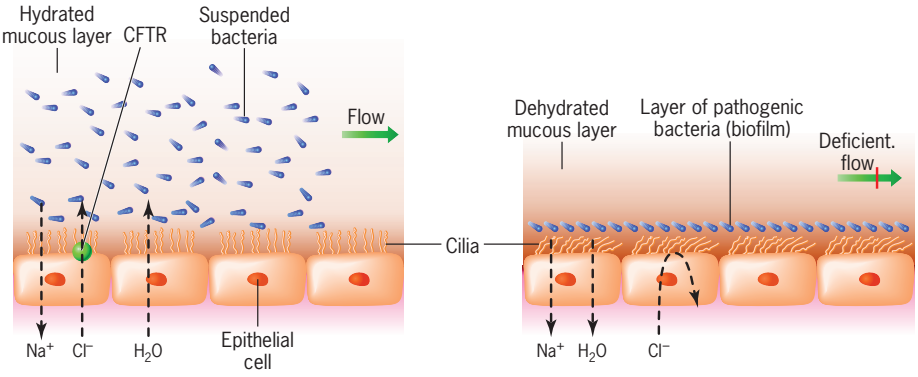


Figure 1 **An explanation for the debilitating effects on lung function from the absence of the CFTR protein.** In the airway epithelium of a normal individual, water flows out of the epithelial cells in response to the outward movement of ions, thus hydrating the surface mucous layer. The hydrated mucous layer, with its trapped bacteria, is readily moved out of the airways. In the airway epithelium of a person with cystic fibrosis, the abnormal movement of ions causes water to flow in the opposite direction, thus dehydrating the mucous layer. As a result, trapped bacteria cannot be moved out of the airways, which allows them to proliferate as a biofilm (page 13) and cause chronic infections.

that CFTR polypeptides lacking this particular amino acid fail to be processed normally within the membranes of the endoplasmic reticulum and, in fact, never reach the surface of epithelial cells. As a result, CF patients who are homozygous for the ΔF508 allele completely lack the CFTR channel in their plasma membranes and have a severe form of the disease. When cells from these patients are grown in culture at lower temperature, the mutant protein is transported to the plasma membrane where it functions quite well. This finding has prompted a number of drug companies to screen for small molecules that can bind to these mutant CFTR molecules, preventing their destruction in the cytoplasm and allowing them to reach the cell surface. One of these candidate drugs, VX-809, is in phase II clinical trials. VX-809 is given in combination with another drug, Kalydeco, which can keep the ion channel open if it arrives at the cell surface. Kalydeco has recently been approved as a solo treatment for the approximately 4 percent of CF patients whose CFTR protein carries a single amino acid substitution, G551D. While this mutation does not interfere with the protein reaching the cell surface, it does prevent the ion channel from opening properly.

According to one estimate, the ΔF508 mutation had to have originated more than 50,000 years ago to have reached such a high frequency in the population. The fact that the CF gene has reached this frequency suggests that heterozygotes may receive some selective advantage over those lacking a copy of the defective gene. It has been proposed that CF heterozygotes may be protected from the effects of cholera, a disease that is characterized by excessive fluid secretion by the wall of the intestine. One difficulty with this proposal is that there is no record of cholera epidemics in Europe until the 1820s. An alternate proposal suggests that heterozygotes are protected from typhoid fever because the bacterium responsible for this disease adheres poorly to the wall of an intestine having a reduced number of CFTR molecules.

Ever since the isolation of the gene responsible for CF, the development of a cure by gene therapy—that is, by replacement of the defective gene with a normal version—has been a major goal of CF researchers. Cystic fibrosis is a good candidate for gene therapy because the worst symptoms of the disease result from the defective activities of epithelial cells that line the airways and, therefore, are accessible to agents that can be delivered by inhalation of an aerosol. Clinical trials have been conducted using several different types of delivery systems. In one group of trials, the normal *CFTR* gene was incorporated into the DNA of a defective adenovirus, a type of virus that normally causes upper respiratory tract infections. The recombinant virus particles were then allowed to infect the cells of the airway, delivering the normal gene to the genetically deficient cells. The primary disadvantage in using adenovirus is that the viral DNA (along with the normal *CFTR* gene) does not become integrated into the chromosomes of the infected host cell so that the virus must be readministered frequently. As a result, the procedure often induces an immune response within the patient that eliminates the virus and leads to lung inflammation. Researchers are hesitant to employ viruses that integrate their genomes for fear of initiating the formation of cancers. In other trials, the DNA encoding the normal *CFTR* gene has been linked to positively charged liposomes (page 128) that can fuse with the plasma membranes of the airway cells, delivering their DNA contents into the cytoplasm. Lipid-based delivery has an advantage over viruses in being less likely to stimulate a destructive immune response following repeated treatments, but has the disadvantage of being less effective in achieving genetic modification of target cells. To date, none of the clinical trials of gene therapy has resulted in significant improvement of either physiologic processes or disease symptoms. The development of more effective DNA delivery systems, which are capable of genetically altering a greater percentage of airway cells, will be required if a treatment for CF based on gene therapy is to be achieved.

REVIEW

1. Compare and contrast the four basically different ways that a substance can move across the plasma membrane (as indicated in Figure 4.33).
2. Contrast the energetic difference between the diffusion of an electrolyte versus a nonelectrolyte across the membrane.
3. Describe the relationship between partition coefficient and molecular size with regard to membrane permeability.
4. Explain the effects of putting a cell into a hypotonic, hypertonic, or isotonic medium.

5. Describe two ways in which energy is utilized to move ions and solutes against a concentration gradient.
6. How does the Na^+/K^+-ATPase illustrate the asymmetry of the plasma membrane?
7. What is the role of phosphorylation in the mechanism of action of the Na^+/K^+-ATPase?
8. What is the structural relation between the parts of the prokaryotic KcsA K^+ channel and the eukaryotic voltage-regulated K^+ channel? Which part of the channel is involved in ion selectivity, which part in channel gating, and which part in channel inactivation? How does each of these processes (ion selectivity, gating, and inactivation) occur?
9. Because of its smaller size, one would expect Na^+ ions to be able to penetrate any pore large enough for a K^+ ion. How does the K^+ channel select for this specific ion?

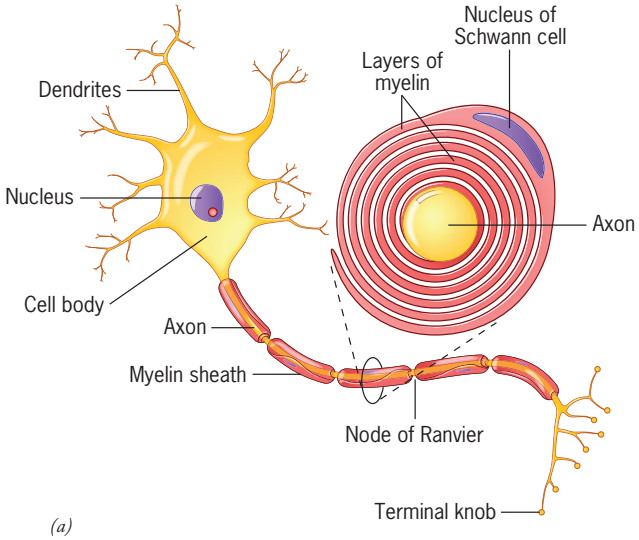
4.8 | Membrane Potentials and Nerve Impulses

All organisms respond to external stimulation, a property referred to as *irritability*. Even a single-celled ameba, if poked with a fine glass needle, responds by withdrawing its pseudopodia, rounding up, and moving off in another direction. Irritability in an ameba depends on the same basic properties of membranes that lead to the formation and propagation of nerve impulses, which is the subject of the remainder of the chapter.

Nerve cells (or *neurons*) are specialized for the collection, conduction, and transmission of information, which is coded in the form of fast-moving electrical impulses. The basic parts of a typical neuron are illustrated in Figure 4.51. The nucleus of the neuron is located within an expanded region called the *cell body*, which is the metabolic center of the cell and the site where most of its material contents are manufactured. Extending from the cell bodies of most neurons are a number of fine extensions, called **dendrites**, which receive *incoming* information from external sources, typically other neurons. Also emerging from the cell body is a single, more prominent extension, the **axon**, which conducts *outgoing* impulses away from the cell body and toward the target cell(s). Although some axons may be only a few micrometers in length, others extend for many meters in the body of a large vertebrate, such as a giraffe or whale. Most axons split near their ends into smaller processes, each ending in a *terminal knob*—a specialized site where impulses are transmitted from neuron to target cell. Many neurons in the brain end in thousands of terminal knobs, allowing these brain cells to communicate with thousands of potential targets. As discussed on page 167, most neurons in the vertebrate body are wrapped in a lipid-rich **myelin sheath**, whose function is described below.

The Resting Potential

A voltage (or electric potential difference) between two points, such as the inside and outside of the plasma membrane, results when there is an excess of positive ions at one point and an excess of negative ions at the other point. Voltages across plasma



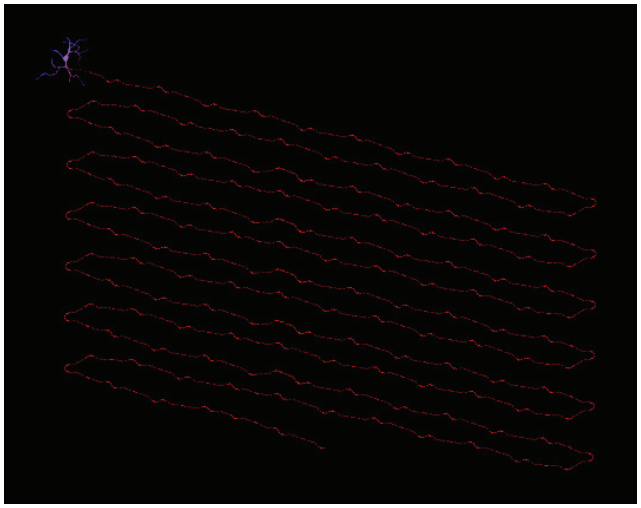


Figure 4.51 **The structure of a nerve cell.** (*a*) Schematic drawing of a simple neuron with a myelinated axon. As the inset shows, the myelin sheath comprises individual Schwann cells that have wrapped themselves around the axon. The sites where the axon lacks myelin wrapping are called nodes of Ranvier. (*Note:* Myelin-forming cells within the central nervous system are called oligodendrocytes rather than Schwann cells.) (*b*) A composite micrograph of a single rat hippocampal neuron with cell body and dendrites (purple) and an axon 1 cm in length (red). Motor nerve cells in larger mammals can be 100 times this length. (B: From Carlos F. Ibáñez, Trends Cell Biol. 17:520, 2007, © 2007, with permission from Elsevier.)

membranes can be measured by inserting one fine glass electrode (or *microelectrode*) into the cytoplasm of a cell, placing another electrode in the extracellular fluid outside the cell, and connecting the electrodes to a voltmeter, an instrument that measures a difference in charge between two points (Figure 4.52). When this experiment was first carried out on a giant axon of the squid, a potential difference of approximately 70 millivolts (mV) was recorded, the inside being negative with respect to the outside (indicated with a minus sign, −70 mV). The presence of a **membrane potential** is not unique to nerve cells; such potentials are present in all types of cells, the magnitude varying between about −15 and −100 mV. When a nerve or muscle cell is in an unexcited state, the membrane potential is referred to as the **resting potential** because it is subject to dramatic change, as discussed in the following section.

The magnitude and direction of the voltage across the plasma membrane are determined by the differences in concentrations of ions on either side of the membrane and their relative permeabilities. As described earlier in the chapter, the Na^+/K^+-ATPase pumps Na^+ out of the cell and K^+ into the cell, thereby establishing steep gradients of these two ions across the plasma membrane. Because of these gradients, you might expect that potassium ions would leak out of the cell and sodium ions would leak inward through their respective ion channels. However, the vast majority of the ion channels in the plasma membrane of a *resting* nerve cell are closed.

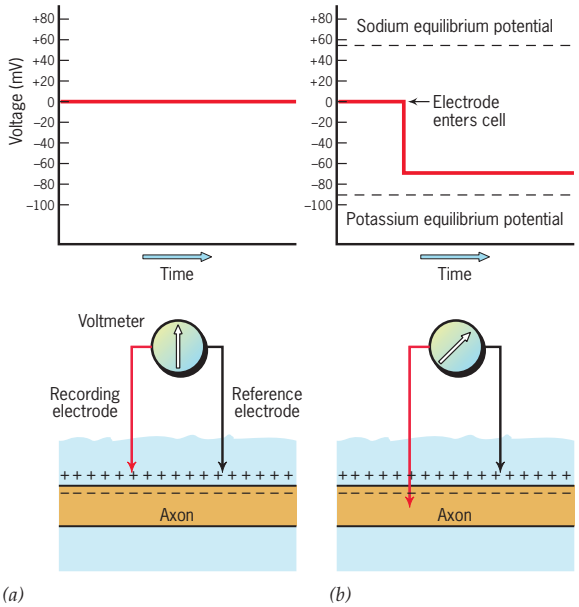


Figure 4.52 **Measuring a membrane's resting potential.** A potential is measured when a difference in charge is detected between the reference and recording electrodes. In (*a*), both electrodes are on the outside of the cell, and no potential difference (voltage) is measured. As one electrode penetrates the plasma membrane of the axon in (*b*), the potential immediately drops to −70 mV (inside negative), which approaches the equilibrium potential for potassium ions, that is, the potential that would result if the membrane were impermeable to all ions except potassium.

Those that are open are selective for K^+; they are often referred to as *K^+ leak channels*. K^+ leak channels are members of the K2P family of K^+ channels, which lack the S4 voltage sensor (page 155) and fail to respond to changes in voltage.

Because K^+ ions are the only charged species with significant permeability in a resting nerve cell, their outflow through the membrane leaves an excess of negative charges on the cytoplasmic side of the membrane. Although the concentration gradient across the membrane favors continued efflux of K^+, the electrical gradient resulting from the excess negative charge on the inside of the membrane favors the retention of K^+ ions inside the cell. When these two opposing forces are balanced, the system is at equilibrium, and there is no further *net* movement of K^+ ions across the membrane. Using the following equation, which is called the Nernst equation, one can calculate the membrane potential (V_m) that would be measured at equilibrium if the plasma membrane of a nerve cell were permeable only to K^+ ions.[7] In this case, V_m would be equal to the potassium equilibrium potential (E_K):

$$E_K = 2.303 \frac{RT}{zF} \cdot \log_{10} \frac{[K_o^+]}{[K_i^+]}$$

For a squid giant axon, the internal $[K_i^+]$ is approximately 350 mM, while the external $[K_o^+]$ is approximately 10 mM; thus at 25°C (298 K) and $z = +1$ (for the univalent K^+ ion),

$$E_K = 59 \log_{10} 0.028 = -91 \text{ mV}$$

A similar calculation of the Na^+ equilibrium potential (E_{Na}) would produce a value of approximately +55 mV. Because measurements of the voltage across the resting nerve membrane are similar in sign and magnitude (−70 mV) to the potassium equilibrium potential just calculated, the movement of potassium ions across the membrane is considered the most important factor in determining the resting potential. The difference between the calculated K^+ equilibrium potential (−91 mV) and the measured resting potential (−70 mV, Figure 4.52) is due to a slight permeability of the membrane to Na^+ through a recently described Na^+ leak channel.

The Action Potential

Our present understanding of membrane potentials and nerve impulses rests on a body of research carried out on the giant axons of the squid in the late 1940s and early 1950s by a group of British physiologists, most notably Alan Hodgkin, Andrew Huxley, and Bernard Katz. These axons, which are approximately 1 mm in diameter, carry impulses at high speeds, enabling the squid to escape rapidly from predators. If the membrane of a resting squid axon is stimulated by poking it with a fine needle or jolting it with a very small electric current, some of its sodium channels open, allowing a limited number of sodium ions to diffuse into the cell. This opportunity for positively charged ions to move into the cell reduces the membrane

[7]The Nernst equation is derived from the equation provided on page 148, by setting ΔG at zero, which is the case when the movement of the ions is at equilibrium. Walther Nernst was a German physical chemist who won the 1920 Nobel Prize.

potential, making it less negative. Because the positive change in membrane voltage causes a *decrease* in the polarity between the two sides of the membrane, it is called a **depolarization**.

If the stimulus causes the membrane to depolarize by only a few millivolts, say from −70 to −60 mV, the membrane rapidly returns to its resting potential as soon as the stimulus has ceased (Figure 4.53*a*, left box). If, however, the stimulus depolarizes the membrane beyond a certain point, called the **threshold**, which occurs at about −50 mV, then a new series of events is launched. The change in voltage causes the voltage-gated sodium channels to open. As a result, sodium ions diffuse freely into the cell (Figure 4.53*a*, middle box) down both their concentration and electric gradients. The increased permeability of the membrane to Na^+ ions and the corresponding movement of positive charge into the cell causes the membrane to reverse potential briefly (Figure 4.53*b*), becoming positive at about +40 mV, which approaches the equilibrium potential for Na^+ (Figure 4.52).

After approximately 1 msec, the sodium channels spontaneously inactivate, blocking further influx of Na^+ ions. According to the prevailing view, inactivation results from the random diffusion of an inactivation peptide into the opening of the channel pore in a manner similar to that described for K^+ channels on page 155. Meanwhile, the change in membrane potential caused by Na^+ influx triggers the opening of the voltage-gated potassium channels (Figure 4.53*a*, right box) discussed on page 154. As a result, potassium ions diffuse freely out of the cell down their steep concentration gradient. The decreased permeability of the membrane to Na^+ and the increased permeability to K^+ cause the membrane potential to swing back to a negative value of about −80 mV, approaching that of the K^+ equilibrium potential (Figure 4.52). The large negative membrane potential causes the voltage-gated potassium channels to close (see Figure 4.43*b*), which returns the membrane to its resting state. Collectively, these changes in membrane potential are called an **action potential** (Figure 4.53*b*). The entire series of changes during an action potential takes only about 5 msec in the squid axon and less than 1 msec in a myelinated mammalian nerve cell. Following an action potential, the membrane enters a brief *refractory period* during which it cannot be restimulated. The refractory period occurs because the sodium channels that were inactivated during the initial stage of the action potential must close before they can be reopened in response to another stimulus. As depicted in Figure 4.43, the transformation of the ion channel from the inactivated to the closed conformation can only occur after the inactivating peptide has swung out of the opening of the pore.

Although the action potential changes membrane voltage dramatically, only a minute percentage of the ions on the two sides of the membrane are involved in any given action potential. The striking changes in membrane potential seen in Figure 4.53*b* are not caused by changes in Na^+ and K^+ ion *concentra-*

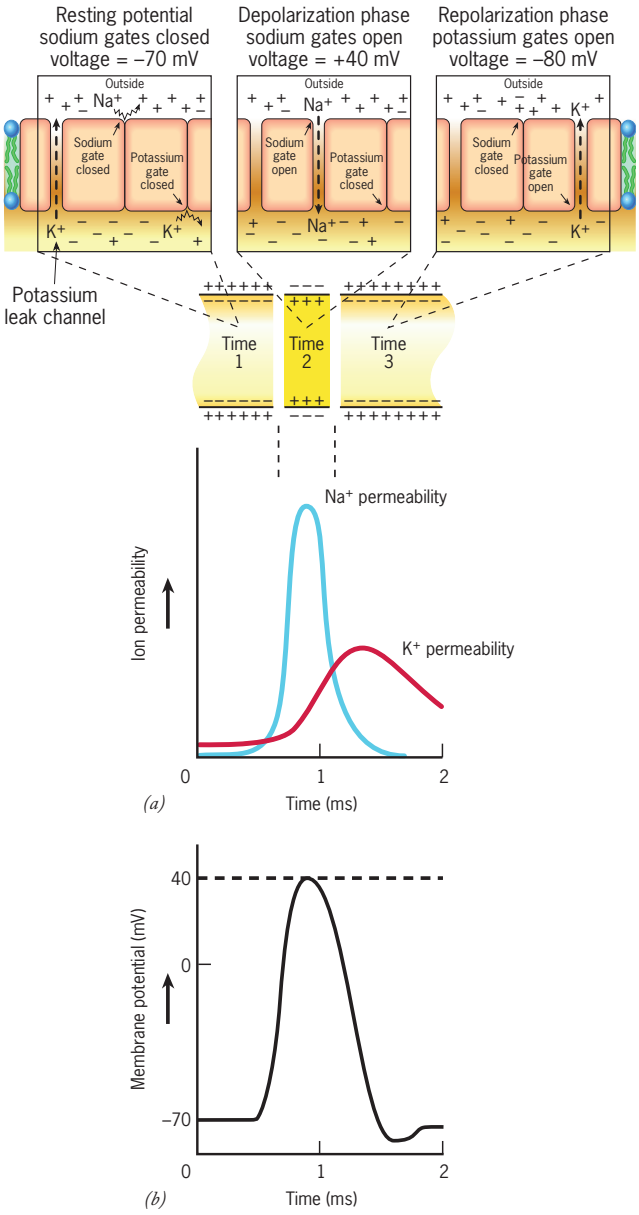


Figure 4.53 **Formation of an action potential.** (*a*) Time 1, upper left box: The membrane in this region of the nerve cell exhibits the resting potential, in which only the K^+ leak channels are open and the membrane voltage is approximately −70 mV. Time 2, upper middle box, shows the depolarization phase: The membrane has depolarized beyond the threshold value, opening the voltage-regulated sodium gates, leading to an influx of Na^+ ions (indicated in the permeability change in the lower graph). The increased Na^+ permeability causes the membrane voltage to temporarily reverse itself, reaching a value of approximately +40 mV in the squid giant axon (time 2). It is this reversal of membrane potential that constitutes the action potential. Time 3, upper right box, shows the repolarization phase: Within a tiny fraction of a second, the sodium gates are inactivated and the potassium gates open, allowing potassium ions to diffuse across the membrane (lower part of the drawing) and establish an even more negative potential at that location (−80 mV) than that of the resting potential. Almost as soon as they open, the potassium gates close, leaving the potassium leak channels as the primary path of ion movement across the membrane and reestablishing the resting potential. (*b*) A summary of the voltage changes that occur during an action potential, as described in part *a*.

tions on the two sides of the membrane (such changes are insignificant). Rather, they are caused by the movements of charge in one direction or the other that result from the fleeting changes in permeability to these ions. Those Na^+ and K^+ ions that do change places across the membrane during an action potential are eventually pumped back by the Na^+/K^+-ATPase. Even if the Na^+/K^+-ATPase is inhibited, a neuron can often continue to fire thousands of impulses before the ionic gradients established by the pump's activity are dissipated.

Once the membrane of a neuron is depolarized to the threshold value, a full-blown action potential is triggered without further stimulation. This feature of nerve cell function is known as the *all-or-none law.* There is no in-between; subthreshold depolarization is incapable of triggering an action potential, whereas threshold depolarization automatically elicits a maximum response. It is also noteworthy that an action potential is not an energy-requiring process, but one that results from the flow of ions down their respective electrochemical gradients. Energy is required by the Na^+/K^+-ATPase to generate the steep ionic gradients across the plasma membrane, but once that is accomplished, the various ions are poised to flow through the membrane as soon as their ion channels are opened.

The movements of ions across the plasma membrane of nerve cells form the basis for neural communication. Certain *local* anesthetics, such as procaine and novocaine, act by closing ion channels in the membranes of sensory cells and neurons. As long as these ion channels remain closed, the affected cells are unable to generate action potentials and thus unable to inform the brain of events occurring at the skin or teeth.

Propagation of Action Potentials as an Impulse

Up to this point, we have restricted the discussion to events occurring at a particular site on the nerve cell membrane where experimental depolarization has triggered an action potential. Once an action potential has been initiated, it does not remain localized at a particular site but is *propagated* as a **nerve impulse** down the length of the cell to the nerve terminals.

Nerve impulses are propagated along a membrane because an action potential at one site has an effect on the adjacent site. The large depolarization that accompanies an action potential creates a difference in charge along the inner and outer surfaces of the plasma membrane (Figure 4.54). As a result, positive ions move toward the site of depolarization on the outer surface of the membrane and away from that site on the inner surface (Figure 4.54). This local flow of current causes the membrane in the region just ahead of the action potential to become depolarized. Because the depolarization accompanying the action potential is very large, the membrane in the adjacent region is readily depolarized to a level greater than the threshold value, which opens the sodium channels in this adjacent region, generating another action potential. Thus, once triggered, a succession of action potentials passes down the entire length of the neuron without any loss of intensity, arriving at its target cell with the same strength it had at its point of origin.

Because all impulses traveling along a neuron exhibit the same strength, stronger stimuli cannot produce "bigger" impulses than weaker stimuli. Yet, we are clearly capable of detecting differences in the strength of a stimulus. The ability to make sensory discriminations depends on several factors. For example, a stronger stimulus, such as scalding water, activates more nerve cells than does a weaker stimulus, such as warm water. It also activates "high-threshold" neurons that would remain at rest if the stimulus were weaker. Stimulus strength is also encoded in the pattern and frequency by which action potentials are launched down a particular neuron. In most cases, the stronger the stimulus, the greater the number of impulses generated.

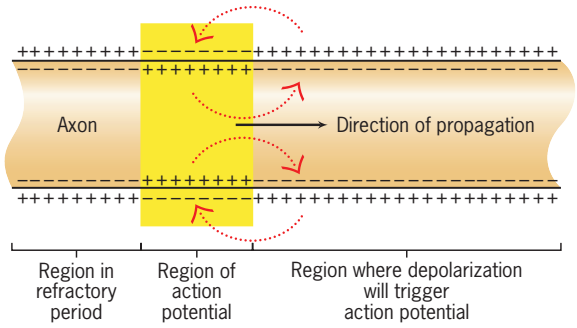


Figure 4.54 **Propagation of an impulse results from the local flow of ions.** An action potential at one site on the membrane depolarizes an adjacent region of the membrane, triggering an action potential at the second site. The action potential can only flow in the forward direction because the portion of the membrane that has just experienced an action potential remains in a refractory period.

Speed Is of the Essence The greater the diameter of an axon, the less the resistance to local current flow and the more rapidly an action potential at one site can activate adjacent regions of the membrane. Some invertebrates, such as squid and tube worms, have evolved giant axons that facilitate the animal's escape from danger. There is, however, a limit to this evolutionary approach. Because the speed of conduction increases with the square root of the increase in diameter, an axon that is 480 μm in diameter can conduct an action potential only four times faster than one that is 30 μm in diameter.

During the evolution of vertebrates, an increase in conduction velocity was achieved when the axon became wrapped in a myelin sheath (see Figures 4.5 and 4.51). Because it is composed of many layers of lipid-containing membranes, the myelin sheath is ideally suited to prevent the passage of ions across the plasma membrane. In addition, nearly all of the Na^+ ion channels of a myelinated neuron reside in the unwrapped gaps, or *nodes of Ranvier*, between adjacent Schwann cells or oligodendrocytes that make up the sheath (see Figure 4.51). Consequently, the nodes of Ranvier are the only sites where action potentials can be generated. An action potential at one node triggers an action potential at the next node (Figure 4.55), causing the impulse to jump from node to node without having to activate the intervening membrane. Propagation of an impulse by this mechanism is called **saltatory conduction**. Impulses are conducted along a myelinated axon at speeds up to 120 meters per second, which is more than

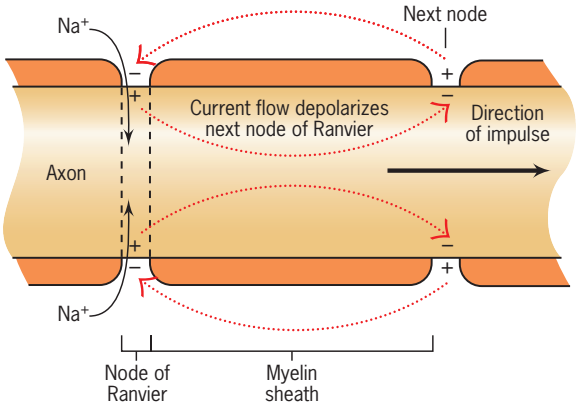


Figure 4.55 **Saltatory conduction.** During saltatory conduction, only the membrane in the nodal region of the axon becomes depolarized and capable of forming an action potential. This is accomplished as current flows directly from an activated node to the next resting node along the axon.

20 times faster than the speed that impulses travel in an unmyelinated neuron of the same diameter.

The importance of myelination is dramatically illustrated by multiple sclerosis (MS), a disease associated with deterioration of the myelin sheath that surrounds axons in various parts of the nervous system. Manifestations of the disease usually begin in young adulthood; patients experience weakness in their hands, difficulty in walking, and problems with their vision.

Neurotransmission: Jumping the Synaptic Cleft

Neurons are linked with their target cells at specialized junctions called **synapses**. Careful examination of a synapse reveals that the two cells do not make direct contact but are separated from each other by a narrow gap of about 20 to 50 nm. This gap is called the **synaptic cleft**. A **presynaptic cell** (a receptor cell or a neuron) conducts impulses toward a

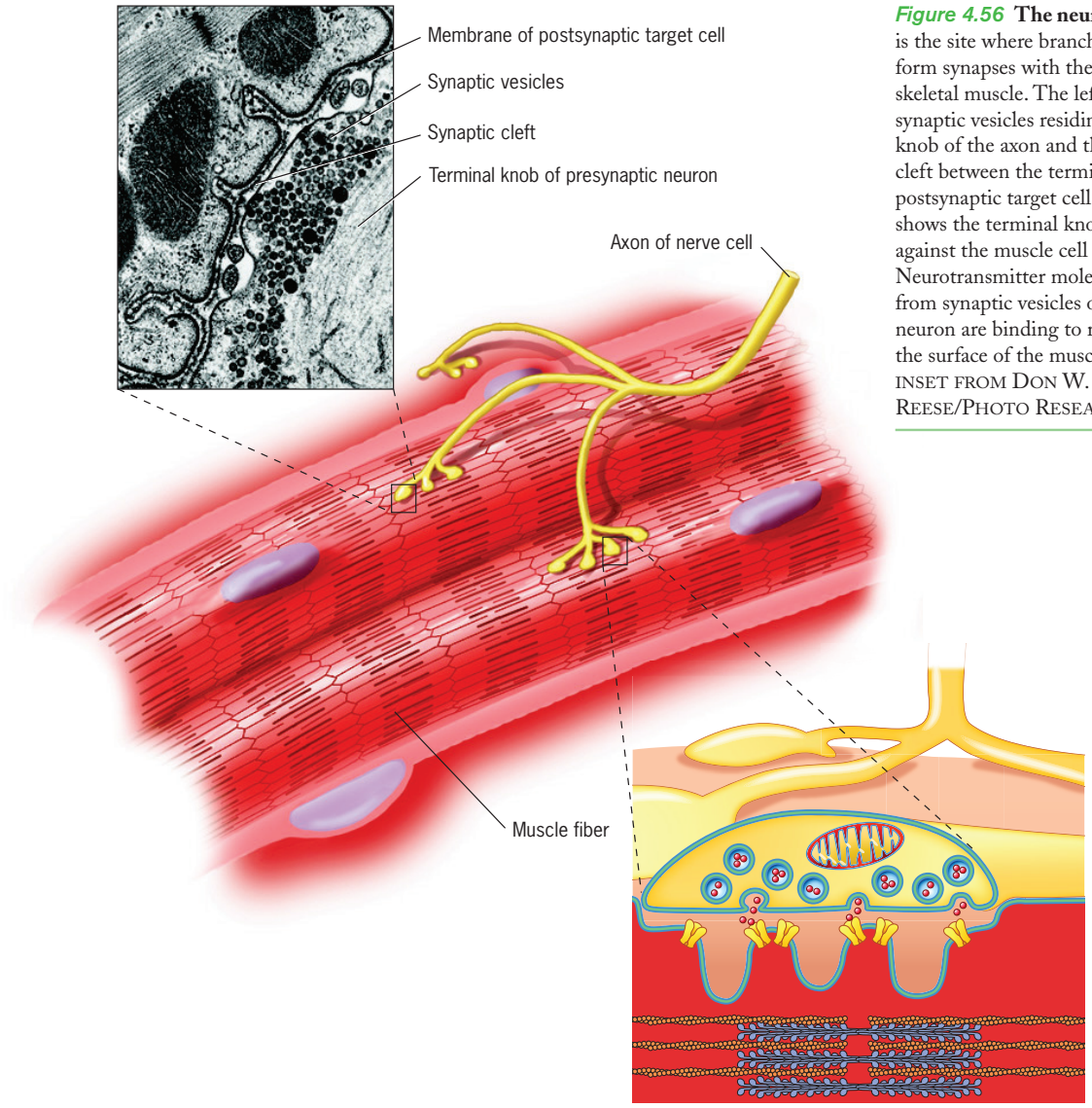
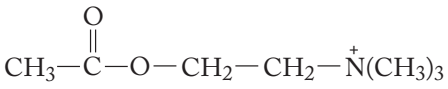


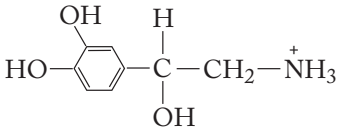
Figure 4.56 **The neuromuscular junction** is the site where branches from a motor axon form synapses with the muscle fibers of a skeletal muscle. The left inset shows the synaptic vesicles residing within the terminal knob of the axon and the narrow synaptic cleft between the terminal knob and the postsynaptic target cell. The right inset shows the terminal knob pressed closely against the muscle cell plasma membrane. Neurotransmitter molecules (red) released from synaptic vesicles of the presynaptic neuron are binding to receptors (yellow) on the surface of the muscle cell (blue). (LEFT INSET FROM DON W. FAWCETT/T. REESE/PHOTO RESEARCHERS, INC.)

synapse, and a **postsynaptic cell** (a neuron, muscle, or gland cell) always lies on the receiving side of a synapse. Figure 4.56 shows a number of synapses between the terminal branches of an axon and a skeletal muscle cell; synapses of this type are called **neuromuscular junctions**.

How does an impulse in a presynaptic neuron jump across the synaptic cleft and affect the postsynaptic cell? Studies carried out decades ago indicated that a chemical substance is involved in the transmission of an impulse from one cell to another (page 171). The very tips (terminal knobs) of the branches of an axon appear in the electron microscope to contain large numbers of **synaptic vesicles** (Figure 4.56, left inset) that serve as storage sites for the chemical transmitters that act on postsynaptic cells. Two of the best studied **neurotransmitters** are acetylcholine and norepinephrine,



Acetylcholine (ACh)



Norepinephrine

which transmit impulses to the body's skeletal and cardiac muscles.

The sequence of events during synaptic transmission can be summarized as follows (Figure 4.57). When an impulse reaches a terminal knob (step 1, Figure 4.57), the accompanying depolarization induces the opening of a number of voltage-gated Ca^{2+} channels in the plasma membrane of this part of the presynaptic nerve cell (step 2, Figure 4.57). Calcium ions are normally present at very low concentration within the neuron (about 100 nM), as in all cells. When the gates open, calcium ions diffuse from the extracellular fluid into the terminal knob of the neuron, causing the $[Ca^{2+}]$ to rise more than a thousandfold within localized microdomains near the channels. The elevated $[Ca^{2+}]$ triggers the rapid fusion of one or a few nearby synaptic vesicles with the plasma membrane, causing the release of neurotransmitter molecules into the synaptic cleft (step 3, Figure 4.57).

Once released from the synaptic vesicles, the neurotransmitter molecules diffuse across the narrow gap and bind selectively to receptor molecules that are concentrated directly across the cleft in the postsynaptic plasma membrane (step 4, Figure 4.57). A neurotransmitter molecule can have one of two opposite effects depending on the type of receptor on the target cell membrane to which it binds:[8]

1. The bound transmitter can trigger the opening of cation-selective channels in the membrane, leading primarily to an

[8]It is important to note that this discussion ignores an important class of neurotransmitter receptors that are not ion channels and thus do not *directly* affect membrane voltage. This other group of receptors are members of a class of proteins called GPCRs, which are discussed at length in Section 15.3. When a neurotransmitter binds to one of these receptors, it can initiate a variety of responses, which often includes the opening of ion channels by an indirect mechanism.

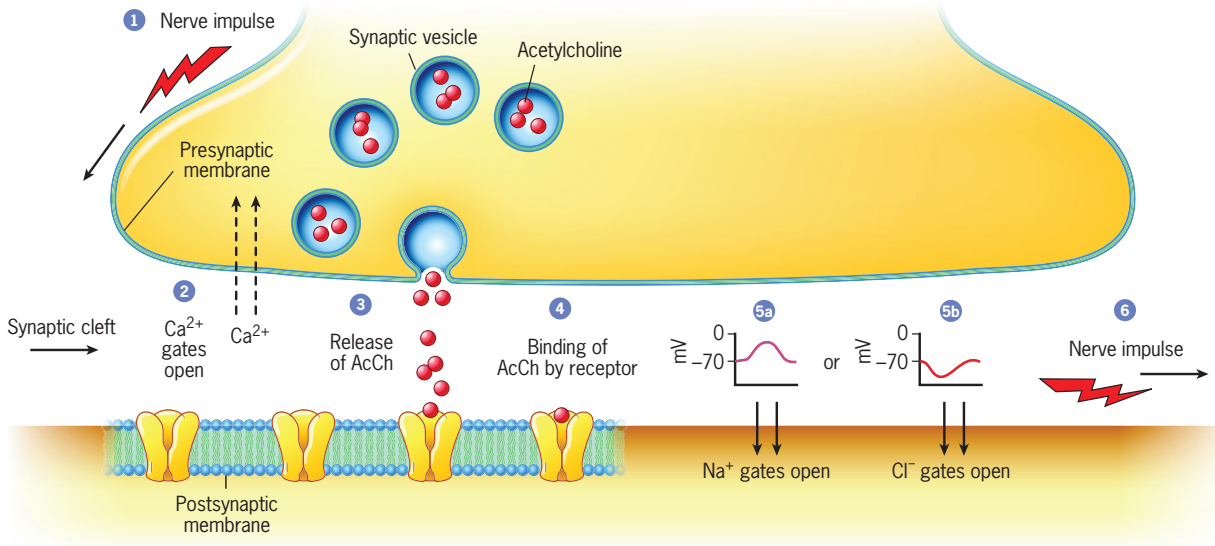


Figure 4.57 **The sequence of events during synaptic transmission with acetylcholine as the neurotransmitter.** During steps 1–4, a nerve impulse reaches the terminal knob of the axon, calcium gates open leading to an influx of Ca^{2+}, and acetylcholine is released from synaptic vesicles and binds to receptors on the postsynaptic membrane. If the binding of the neurotransmitter molecules causes a depolarization of the postsynaptic membrane (as in 5a), a nerve impulse may be generated there (6). If, however, the binding of neurotransmitter causes a hyperpolarization of the postsynaptic membrane (5b), the target cell is inhibited, making it more difficult for an impulse to be generated in the target cell by other excitatory stimulation. The breakdown of the neurotransmitter by acetylcholinesterase is not shown.

influx of sodium ions and a less negative (more positive) membrane potential. This depolarization of the postsynaptic membrane *excites* the cell, making the cell more likely to respond to this or subsequent stimuli by generating an action potential of its own (Figure 4.57, steps 5a and 6).

2. The bound transmitter can trigger the opening of anion-selective channels, leading mainly to an influx of chloride ions, and a more negative (hyperpolarized) membrane potential. Hyperpolarization of the postsynaptic membrane makes it less likely the cell will generate an action potential because greater sodium influx is subsequently required to reach the membrane's threshold (Figure 4.57, step 5b).

Most nerve cells in the brain receive both excitatory and inhibitory signals from many different presynaptic neurons. It is the summation of these opposing influences that determine whether or not an impulse will be generated in the postsynaptic neuron.

All terminal knobs of a given neuron release the same neurotransmitter(s). However, a given neurotransmitter may have a stimulatory effect on one particular postsynaptic membrane and an inhibitory effect on another. Acetylcholine, for example, inhibits contractility of the heart but stimulates contractility of skeletal muscle. Within the brain, glutamate serves as the primary excitatory neurotransmitter and gamma-aminobutyric acid (GABA) as the primary inhibitory neurotransmitter. A number of general anesthetics, as well as Valium and its derivatives, act by binding to the GABA receptor and enhancing the activity of the brain's primary "off" switch. As will be evident in the following section, many different drugs affecting disorders, such as anxiety, depression, insomnia, and schizophrenia, exert their action at the synapse.

Actions of Drugs on Synapses It is important that a neurotransmitter has only a short half-life following its release from a presynaptic neuron; otherwise the effect of the neurotransmitter would be extended, and the postsynaptic neuron would not recover. A neurotransmitter is eliminated from the synapse in two ways: by enzymes that destroy neurotransmitter molecules in the synaptic cleft and by proteins that transport neurotransmitter molecules back to the presynaptic terminals—a process called *reuptake*. Because of the destruction or reuptake of neurotransmitter molecules, the effect of each impulse lasts no more than a few milliseconds.

Interfering with the destruction or reuptake of neurotransmitters can have dramatic physiologic and behavioral effects. Acetylcholinesterase is an enzyme localized within the synaptic cleft that hydrolyzes acetylcholine. If this enzyme is inhibited by exposure to the nerve gas DFP, for example, the skeletal muscles of the body undergo violent contraction due to the continued presence of high concentrations of acetylcholine. Milder inhibitors of acetylcholinesterase are used to treat the symptoms of Alzheimer's disease, which is characterized by the loss of acetylcholine-releasing neurons.

Many drugs act by inhibiting the transporters that sweep neurotransmitters out of the synaptic cleft. A number of widely prescribed antidepressants, including Prozac and Zoloft, inhibit the reuptake of serotonin, a neurotransmitter implicated in mood disorders. Cocaine, on the other hand, interferes with the reuptake of the neurotransmitter dopamine that is released by certain nerve cells in a portion of the brain known as the limbic system. The limbic system contains the brain's "pleasure" or "reward" centers. The sustained presence of dopamine in the synaptic clefts of the limbic system produces a short-lived feeling of euphoria, as well as a strong desire to repeat the activity. Mice that have been genetically engineered to lack the dopamine transporter (DAT)—the protein responsible for dopamine reuptake—show behavior similar to that of normal mice that have been given cocaine or amphetamines. Administration of cocaine or amphetamines has no additional behavioral effects on animals lacking the *DAT* gene. Numerous other drugs act on presynaptic dopamine-releasing or postsynaptic dopamine-responding neurons. Amphetamines are thought to stimulate the excessive release of dopamine from presynaptic terminals and also interfere with the reuptake of the neurotransmitter molecules from the synaptic cleft. A number of antipsychotic drugs bind to certain subtypes of the dopamine receptor on postsynaptic neurons and block their stimulation by dopamine.

The active compound in marijuana (Δ^9-tetrahydrocannabinol) acts by a totally different mechanism. It binds to cannabinoid (CB1) receptors located on the *presynaptic* terminals of certain neurons of the brain, which reduces the likelihood that these neurons will release neurotransmitters. CB1 receptors normally interact with compounds produced in the body called *endocannabinoids*. Endocannabinoids are produced by postsynaptic neurons following depolarization. These substances diffuse "backwards" across the synaptic cleft to the presynaptic membrane, where they bind to CB1 receptors, suppressing synaptic transmission. CB1 receptors are located in many areas of the brain, including the hippocampus, cerebellum, and hypothalamus, which explains the effects of marijuana on memory, motor coordination, and appetite, respectively. If marijuana increases appetite by binding to CB1 receptors, it follows that blocking these receptors might decrease appetite. This line of reasoning led to the development of a CB1-blocking weight-loss drug called Acomplia, which has been pulled from the market because of side effects.

Synaptic Plasticity Synapses are more than simply connecting sites between adjacent neurons; they are key determinants in the routing of impulses through the nervous system. The human brain is thought to contain at least one hundred trillion synapses. These synapses act like gates stationed along the various pathways, allowing some pieces of information to pass from one neuron to another, while holding back other pieces or rerouting them in another direction. While synapses are often perceived as fixed, unchanging structures, they can display a remarkable dynamic quality known as "synaptic plasticity." Synaptic plasticity is particularly important during infancy and childhood, when the neuronal circuitry of the brain achieves it mature configuration.

Synaptic plasticity is most readily observed in studies on neurons from the hippocampus, a portion of the brain that is vitally important in learning and short-term memory. When hippocampal neurons are repeatedly stimulated over a short period of time, the synapses that connect these neurons to their neigh-

bors become "strengthened" by a process known as long-term potentiation (LTP), which may last for days, weeks, or even longer. Research into LTP has focused on the NMDA receptor, which is one of several receptor types that bind the excitatory neurotransmitter glutamate. When glutamate binds to a postsynaptic NMDA receptor, it opens an internal cation channel within the receptor that allows the influx of Ca^{2+} ions into the postsynaptic neuron, triggering a cascade of biochemical changes that lead to synaptic strengthening. Synapses that have undergone LTP are able to transmit weaker stimuli and evoke stronger responses in postsynaptic cells. These changes are thought to play a major role as newly learned information or memories are encoded in the neural circuits of the brain. When laboratory animals are treated with drugs that inhibit LTP, such as those that interfere with the activity of the NMDA receptor, their ability to learn new information is greatly reduced.

There are numerous other reasons the study of synapses is so important. For example, a number of diseases of the nervous system, including myasthenia gravis, Parkinson's disease, schizophrenia, and even depression, are thought to have their roots in synaptic dysfunction.

REVIEW

1. What is a resting potential? How is it established as a result of ion flow?
2. What is an action potential? What are the steps that lead to its various phases?
3. How is an action potential propagated along an axon? What is saltatory conduction, and how does such a process occur?
4. What is the role of the myelin sheath in conduction of an impulse?
5. Describe the steps between the time an impulse reaches the terminal knob of a presynaptic neuron and an action potential is initiated in a postsynaptic cell.
6. Contrast the roles of ion pumps and channels in establishing and using ion gradients, particularly as it applies to nerve cells.

EXPERIMENTAL PATHWAYS

The Acetylcholine Receptor

In 1843, at the age of 30, Claude Bernard moved from a small French town, where he had been a pharmacist and an aspiring playwright, to Paris, where he planned to pursue his literary career. Instead, Bernard enrolled in medical school and went on to become the foremost physiologist of the nineteenth century. Among his many interests was the mechanism by which nerves stimulate the contraction of skeletal muscles. His studies included the use of curare, a highly toxic drug isolated from tropical plants and utilized for centuries by native South American hunters to make poisonous darts. Bernard found that curare paralyzed a skeletal muscle without interfering with either the ability of nerves to carry impulses to that muscle or the ability of the muscle to contract on direct stimulation. Bernard concluded that curare somehow acted on the region of contact between the nerve and muscle.

This conclusion was confirmed and extended by John Langley, a physiologist at Cambridge University. Langley was studying the ability of nicotine, another substance derived from plants, to stimulate the contraction of isolated frog skeletal muscles and the effect of curare in inhibiting nicotine action. In 1906, Langley concluded that "the nervous impulse should not pass from nerve to muscle by an electric discharge, but by the secretion of a special substance on the end of the nerve."[1] Langley proposed that this "chemical transmitter" was binding to a "receptive substance" on the surface of the muscle cells, the same site that bound nicotine and curare. These proved to be farsighted proposals.

Langley's suggestion that the stimulus from nerve to muscle was transmitted by a chemical substance was confirmed in 1921 in an ingenious experiment by the Austrian-born physiologist, Otto Loewi, the design of which came to Loewi during a dream. The heart rate of a vertebrate is regulated by input from two opposing (antagonistic) nerves. Loewi isolated a frog's heart with both nerves intact. When he stimulated the inhibitory (*vagus*) nerve, a chemical was released from the heart preparation into a salt solution, which was allowed to drain into the medium bathing a second isolated heart. The rate of the second heart slowed dramatically, as though its own inhibitory nerve had been activated.[2] Loewi called the substance responsible for inhibiting the frog's heart "Vagusstoff." Within a few years, Loewi had shown that the chemical and physiologic properties of Vagusstoff were identical to acetylcholine, and he concluded that acetylcholine (ACh) was the substance released by the tips of the nerve cells that made up the vagus nerve.

In 1937, David Nachmansohn, a neurophysiologist at the Sorbonne, was visiting the World's Fair in Paris where he observed several living electric fish of the species *Torpedo marmarota* that were on display. These rays have electric organs that deliver strong shocks (40–60 volts) capable of killing potential prey. At the time, Nachmansohn was studying the enzyme acetylcholinesterase, which acts to destroy ACh after its release from the tips of motor nerves. Nachmansohn was aware that the electric organs of these fish were derived from modified skeletal muscle tissue (Figure 1), and he asked if he could have a couple of the fish for study once the fair had ended. The results of the first test showed the electric organ was an extraordinarily rich source of acetylcholinesterase.[3] It was also a very rich source of the nicotinic acetylcholine receptor (nAChR),* the receptor present on the postsynaptic membranes of skeletal muscle cells

*The receptor is described as nicotinic because it can be activated by nicotine as well as by acetylcholine. This contrasts with muscarinic acetylcholine receptors of the parasympathetic nerve synapses, which can be activated by muscarine, but not nicotine, and are inhibited by atropine, but not curare. Smokers' bodies become accustomed to high levels of nicotine, and they experience symptoms of withdrawal when they stop smoking because the postsynaptic neurons that possess nAChRs are no longer stimulated at their usual level. The drug Chantix, which is marketed as an aid to stop smoking, acts by binding to the most common version of brain nAChR (one with $\alpha 4\beta 2$ subunits). Once bound, the Chantix molecule partially stimulates the receptor while preventing binding of nicotine.