CHAPTER **ELEVEN**

11

Membrane Structure

THE LIPID BILAYER

MEMBRANE PROTEINS

A living cell is a self-reproducing system of molecules held inside a container. That container is the **plasma membrane**—a protein-studded, fatty film so thin that it cannot be seen directly in the light microscope. Every cell on Earth uses such a membrane to separate and protect its chemical components from the outside environment. Without membranes, there would be no cells, and thus no life.

The structure of the plasma membrane is simple: it consists of a two-ply sheet of lipid molecules about 5 nm—or 50 atoms—thick, into which proteins have been inserted. Its properties, however, are unlike those of any sheet of material we are familiar with in the everyday world. Although it serves as a barrier to prevent the contents of the cell from escaping and mixing with the surrounding medium (**Figure 11–1**), the plasma membrane does much more than that. If a cell is to survive and grow, nutrients must pass inward across the plasma membrane, and waste products must pass out. To facilitate this exchange, the membrane is penetrated by highly selective channels and transporters—proteins that allow specific,

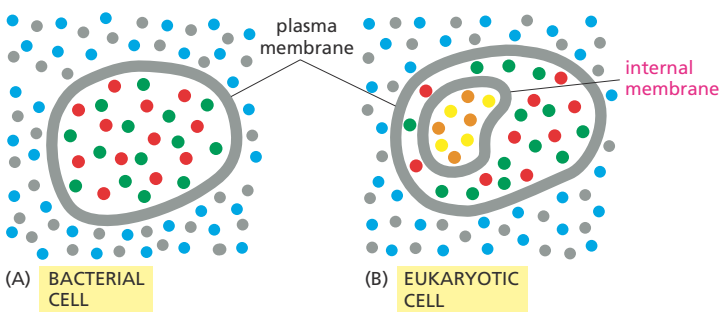


Figure 11–1 Cell membranes act as selective barriers. The plasma membrane separates a cell from its surroundings, enabling the molecular composition of a cell to differ from that of its environment. (A) In some bacteria, the plasma membrane is the only membrane. (B) Eukaryotic cells also have internal membranes that enclose individual organelles. All cell membranes prevent molecules on one side from freely mixing with those on the other, as schematically indicated by the colored dots.

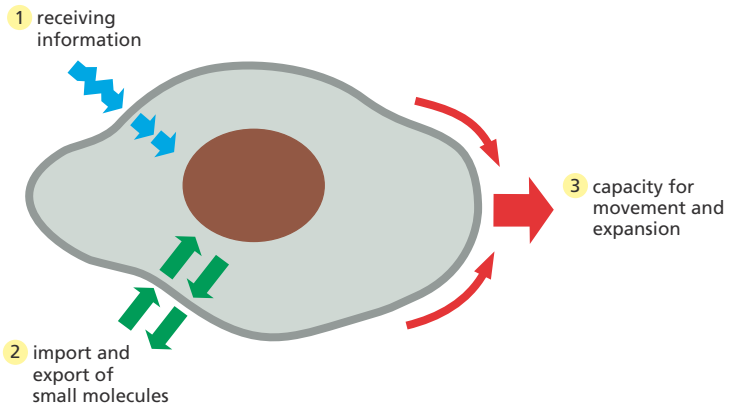


Figure 11–2 The plasma membrane is involved in cell communication, import and export of molecules, and cell growth and motility. (1) Receptor proteins in the plasma membrane enable the cell to receive signals from the environment; (2) transport proteins in the membrane enable the import and export of small molecules; (3) the flexibility of the membrane and its capacity for expansion allow the cell to grow, change shape, and move.

small molecules and ions to be imported and exported. Other proteins in the membrane act as sensors, or receptors, that enable the cell to receive information about changes in its environment and respond to them in appropriate ways. The mechanical properties of the plasma membrane are equally remarkable. When a cell grows or changes shape, so does its membrane: it enlarges in area by adding new membrane without ever losing its continuity, and it can deform without tearing (**Figure 11–2**). If the membrane is pierced, it neither collapses like a balloon nor remains torn; instead, it quickly reseals.

As shown in Figure 11–1, the simplest bacteria have only a single membrane—the plasma membrane—whereas eukaryotic cells also contain internal membranes that enclose intracellular compartments. The internal membranes form various organelles, including the endoplasmic reticulum, Golgi apparatus, and mitochondria (**Figure 11–3**). Although these internal membranes are constructed on the same principles as the plasma membrane, there are subtle differences in their composition, especially in their resident proteins.

Regardless of their location, all cell membranes are composed of lipids and proteins and share a common general structure (**Figure 11–4**). The lipids are arranged in two closely apposed sheets, forming a *lipid bilayer* (see Figure 11–4B and C). This lipid bilayer serves as a permeability barrier to most water-soluble molecules. The proteins carry out the other functions of the membrane and give different membranes their individual characteristics.

In this chapter, we consider the structure of biological membranes and the organization of their two main constituents: lipids and proteins. Although we focus mainly on the plasma membrane, most of the concepts we discuss also apply to internal membranes. The functions of cell membranes, including their role in cell communication, the transport of small molecules, and energy generation, are considered in later chapters.

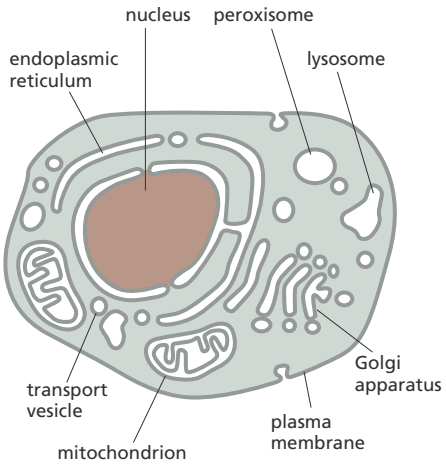


Figure 11–3 Internal membranes form many different compartments in a eukaryotic cell. Some of the main membrane-enclosed organelles in a typical animal cell are shown here. Note that the nucleus and mitochondria are each enclosed by two membranes.

THE LIPID BILAYER

Because cells are filled with—and surrounded by—water, the structure of cell membranes is determined by the way membrane lipids behave in a watery (aqueous) environment. In this section, we take a closer look at the **lipid bilayer**, which constitutes the fundamental structure of all cell membranes. We consider how lipid bilayers form, how they are maintained, and how their properties establish the general properties of all cell membranes.

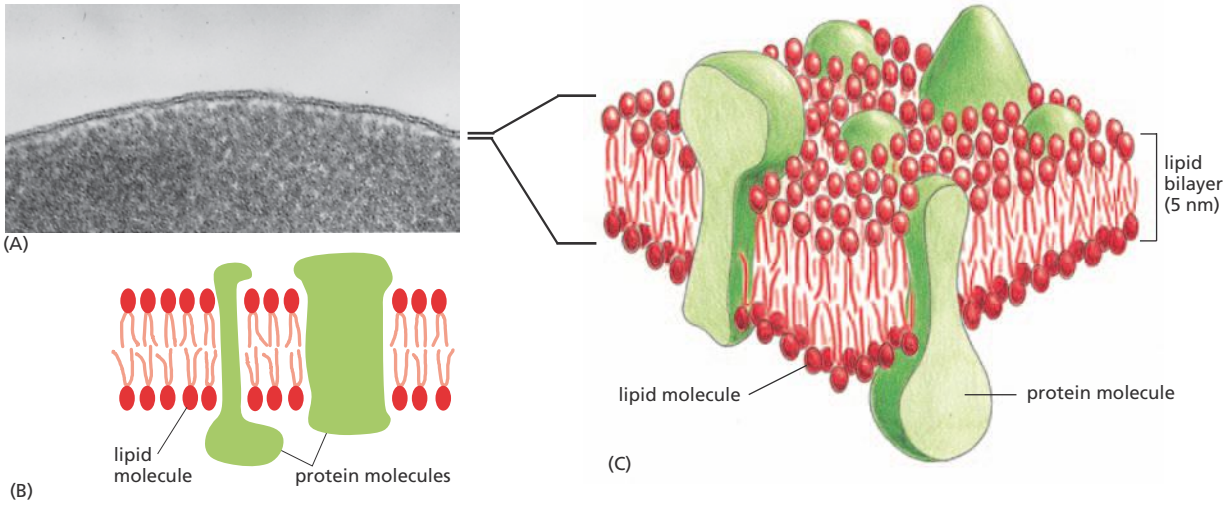


Figure 11–4 A cell membrane can be viewed in a number of ways. (A) An electron micrograph of a plasma membrane of a human red blood cell seen in cross section. (B and C) Schematic drawings showing two-dimensional and three-dimensional views of a cell membrane. (A, courtesy of Daniel S. Friend.)

Membrane Lipids Form Bilayers in Water

The lipids in cell membranes combine two very different properties in a single molecule: each lipid has a hydrophilic ("water-loving") head and a hydrophobic ("water-fearing") tail. The most abundant lipids in cell membranes are the **phospholipids**, which have a phosphate-containing, hydrophilic head linked to a pair of hydrophobic tails (**Figure 11–5**). **Phosphatidylcholine**, for example, has the small molecule choline attached to a phosphate group as its hydrophilic head (**Figure 11–6**).

Molecules with both hydrophilic and hydrophobic parts are termed **amphipathic**, a property shared by other types of membrane lipids, including the cholesterol, which is found in animal cell membranes and the glycolipids, which have sugars as part of their hydrophilic head (**Figure 11–7**). Having both hydrophilic and hydrophobic parts plays a crucial part in driving these lipid molecules to assemble into bilayers in an aqueous environment.

As discussed in Chapter 2 (see Panel 2–2, pp. 68–69), hydrophilic molecules dissolve readily in water because they contain either charged groups or uncharged polar groups that can form either electrostatic attractions or hydrogen bonds with water molecules (**Figure 11–8**). Hydrophobic molecules, by contrast, are insoluble in water because all—or almost all—of their atoms are uncharged and nonpolar; they therefore cannot form favorable interactions with water molecules. Instead, they force adjacent water molecules to reorganize into a cagelike structure around them (**Figure 11–9**). Because this cagelike structure is more highly ordered than the rest of the water, its formation requires free energy. This energy cost is minimized when the hydrophobic molecules cluster together, limiting their contacts with the surrounding water molecules. Thus purely hydrophobic molecules, like the fats found in animal fat cells and the oils found in plant seeds (**Figure 11–10A**), coalesce into a single large drop when dispersed in water.

Amphipathic molecules, such as phospholipids (**Figure 11–10B**), are subject to two conflicting forces: the hydrophilic head is attracted to water, while the hydrophobic tails shun water and seek to aggregate with other hydrophobic molecules. This conflict is beautifully resolved by the

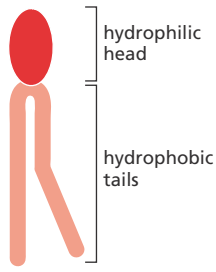


Figure 11–5 A typical membrane lipid molecule has a hydrophilic head and two hydrophobic tails.

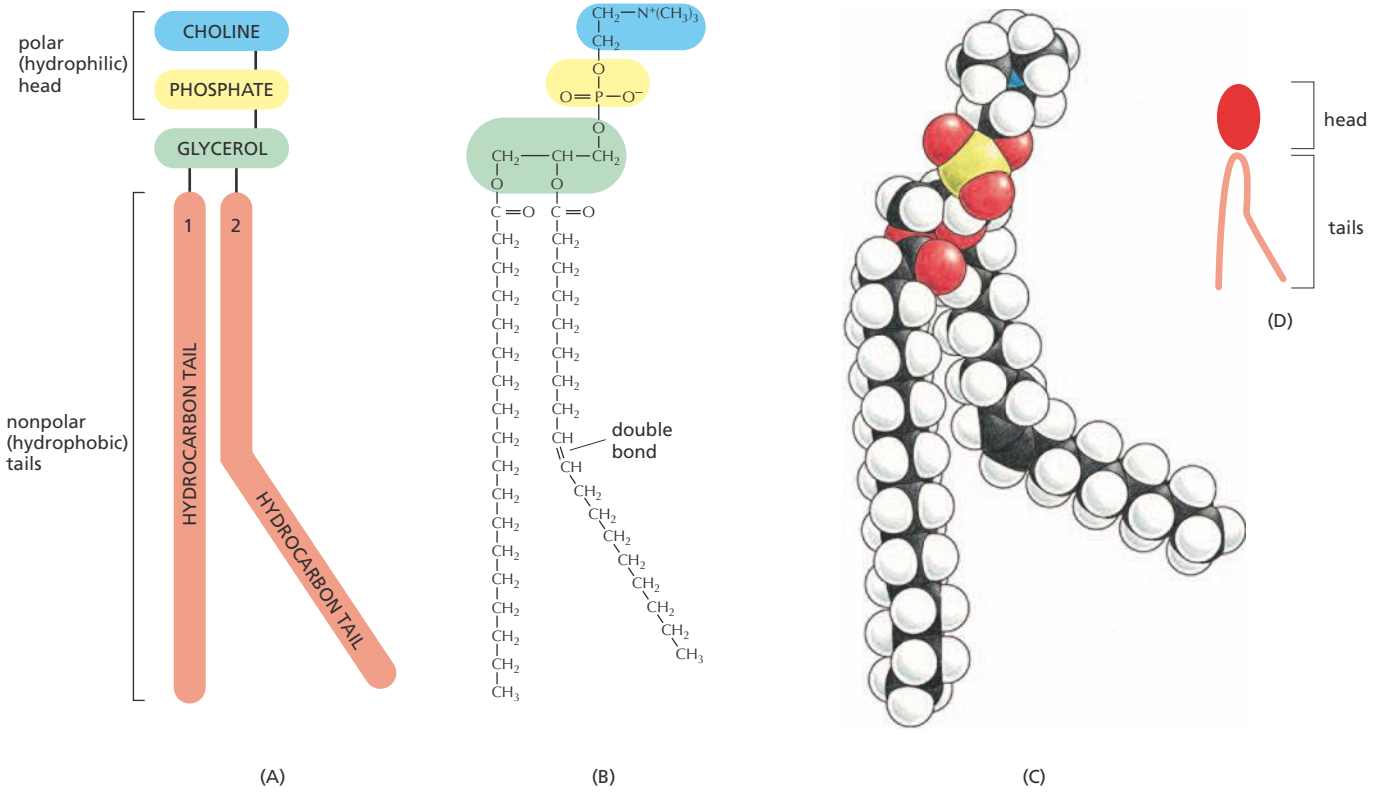


Figure 11–6 Phosphatidylcholine is the most common phospholipid in cell membranes. It is represented schematically in (A), as a chemical formula in (B), as a space-filling model in (C), and as a symbol in (D). This particular phospholipid is built from five parts: the hydrophilic head, which consists of *choline* linked to a *phosphate group*; two *hydrocarbon chains*, which form the hydrophobic tails; and a molecule of glycerol, which links the head to the tails. Each of the hydrophobic tails is a *fatty acid*—a hydrocarbon chain with a –COOH group at one end—which has been attached to glycerol via this group. A kink in one of the hydrocarbon chains occurs where there is a double bond between two carbon atoms. (The "phosphatidyl" part of the name of a phospholipid refers to the phosphate–glycerol–fatty acid portion of the molecule.)

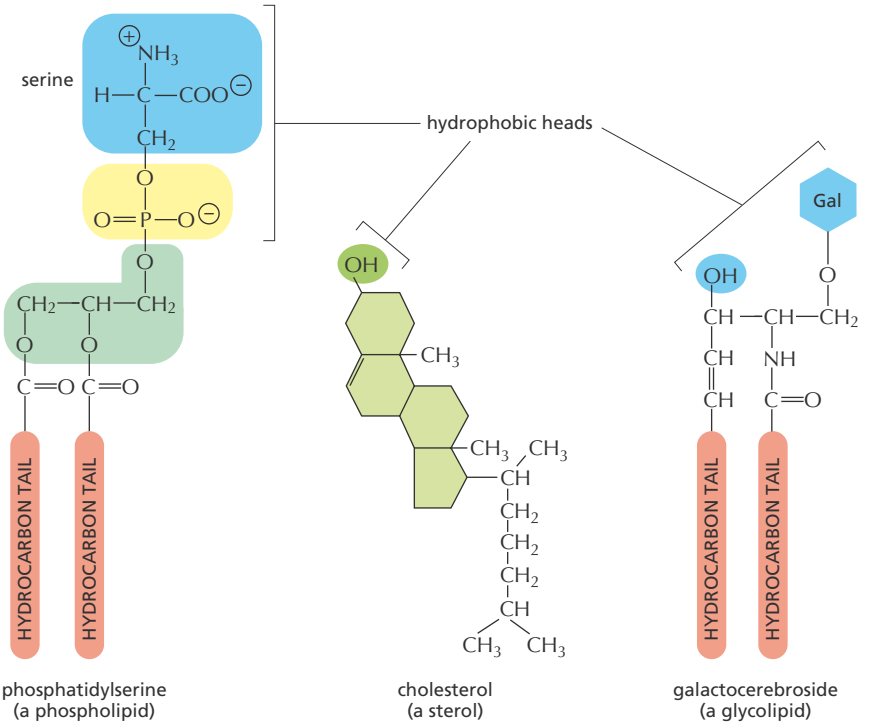


Figure 11–7 Different types of membrane lipids are all amphipathic. Each of the three types shown here has a hydrophilic head and one or two hydrophobic tails. The hydrophilic head (shaded *blue* and *yellow*) is serine phosphate in phosphatidylserine, an –OH group in cholesterol, and a sugar (galactose) plus an –OH group in galactocerebroside. See also Panel 2–4, pp. 72–73.

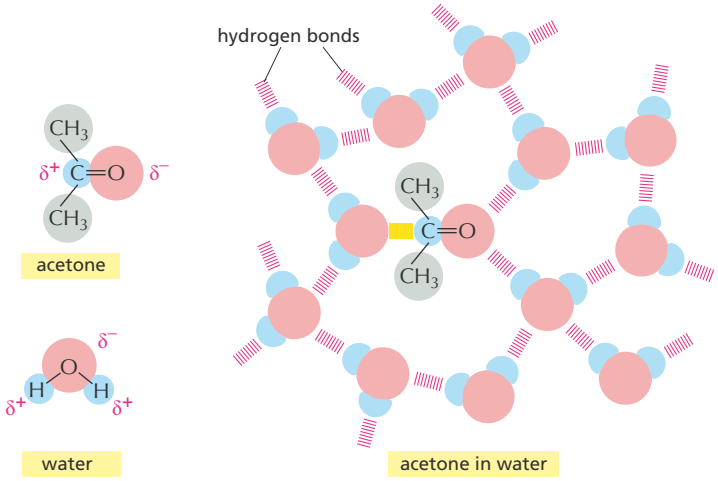


Figure 11–8 A hydrophilic molecule attracts water molecules. Both acetone and water are polar molecules: thus acetone readily dissolves in water. Polar atoms are shown in *red* and *blue*, with δ^- indicating a partial negative charge, and δ^+ indicating a partial positive charge. Hydrogen bonds (*red*) and an electrostatic attraction (*yellow*) form between acetone and the surrounding water molecules. Nonpolar groups are shown in *gray*.

formation of a lipid bilayer—an arrangement that satisfies all parties and is energetically most favorable. The hydrophilic heads face water on both surfaces of the bilayer; but the hydrophobic tails are all shielded from the water, as they lie next to one another in the interior, like the filling in a sandwich (**Figure 11–11**).

The same forces that drive the amphipathic molecules to form a bilayer help to make the bilayer self-sealing. Any tear in the sheet will create a free edge that is exposed to water. Because this situation is energetically unfavorable, the molecules of the bilayer will spontaneously rearrange to eliminate the free edge. If the tear is small, this spontaneous rearrangement will exclude the water molecules and lead to repair of the bilayer, restoring a single continuous sheet. If the tear is large, the sheet may begin to fold in on itself and break up into separate closed vesicles. In either case, the overriding principle is that free edges are quickly eliminated.

The prohibition on free edges has a profound consequence: the only way a finite amphipathic sheet can avoid having free edges is to bend and seal,

QUESTION 11–1

Water molecules are said "to reorganize into a cagelike structure" around hydrophobic compounds (e.g., see Figure 11–9). This seems paradoxical because water molecules do not interact with the hydrophobic compound. So how could they "know" about its presence and change their behavior to interact differently with one another? Discuss this argument and, in doing so, develop a clear concept of what is meant by a "cagelike" structure. How does it compare to ice? Why would this cagelike structure be energetically unfavorable?

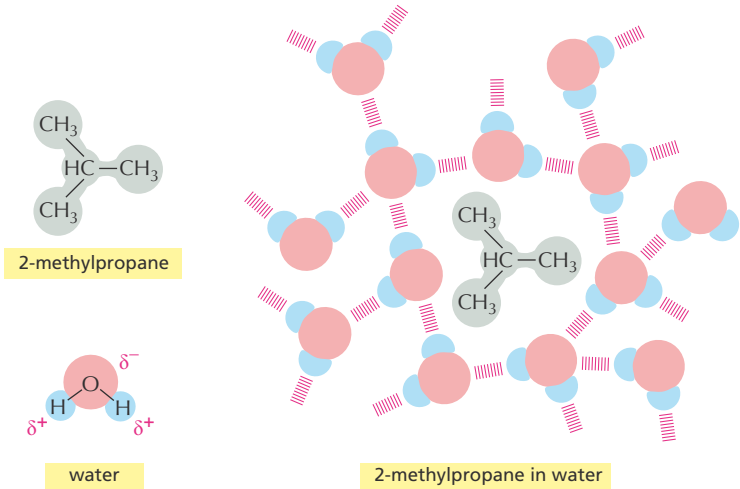
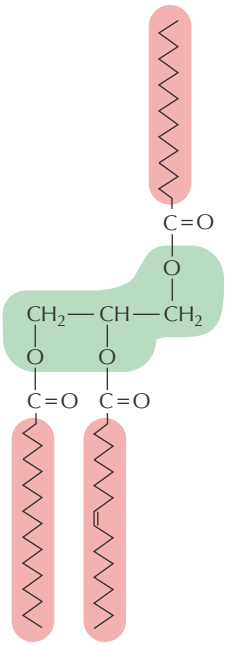


Figure 11–9 A hydrophobic molecule tends to avoid water. Because the 2-methylpropane molecule is entirely hydrophobic, it cannot form favorable interactions with water. This causes the adjacent water molecules to reorganize into a cagelike structure around it, in order to maximize their hydrogen bonds with each other.



triacylglycerol

Figure 11–10 Fat molecules are hydrophobic, unlike phospholipids. Triacylglycerols, which are the main constituents of animal fats and plant oils, are entirely hydrophobic. Here, the third hydrophobic tail of the triacylglycerol molecule in (A) is drawn facing upward for comparison with the phospholipid (see Figure 11–6), although normally it is depicted facing down (see Panel 2–4, pp. 72–73).

forming a boundary around a closed space (**Figure 11–12**). Therefore, amphipathic molecules such as phospholipids necessarily assemble into self-sealing containers that define closed compartments. This remarkable behavior, fundamental to the creation of a living cell, is simply a result of the property that each molecule is hydrophilic at one end and hydrophobic at the other.

The Lipid Bilayer Is a Flexible Two-dimensional Fluid

The aqueous environment inside and outside a cell prevents membrane lipids from escaping from the bilayer, but nothing stops these molecules from moving about and changing places with one another within the plane of the bilayer. The membrane therefore behaves as a two-dimensional fluid, a fact that is crucial for membrane function and integrity (**Movie 11.1**).

The lipid bilayer is also flexible—that is, it is able to bend. Like fluidity, flexibility is important for membrane function, and it sets a lower limit of about 25 nm to the size of vesicle that cell membranes can form.

The fluidity of lipid bilayers can be studied using synthetic lipid bilayers, which are easily produced by the spontaneous aggregation of amphipathic lipid molecules in water. Pure phospholipids, for example, will form closed spherical vesicles, called liposomes, when added to water; they vary in size from about 25 nm to 1 mm in diameter (**Figure 11–13**).

Such simple synthetic bilayers allow the movements of the lipid molecules to be measured. These measurements reveal that some types of movement are rare, while others are frequent and rapid. Thus, in synthetic lipid bilayers, phospholipid molecules very rarely tumble from one half of the bilayer, or monolayer, to the other. Without proteins to facilitate the process, it is estimated that this event, called "flip-flop," occurs less than once a month for any individual lipid molecule under conditions

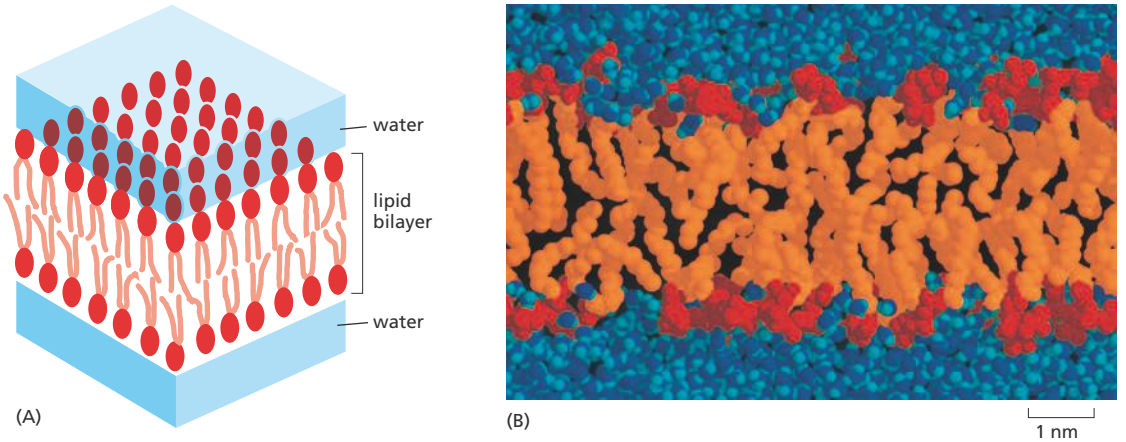


Figure 11–11 Amphipathic phospholipids form a bilayer in water. (A) Schematic drawing of a phospholipid bilayer in water. (B) Computer simulation showing the phospholipid molecules (*red* heads and *orange* tails) and the surrounding water molecules (*blue*) in a cross section of a lipid bilayer. (B, adapted from *Science* 262:223–228, 1993, with permission from the AAAS; courtesy of R. Venable and R. Pastor.)

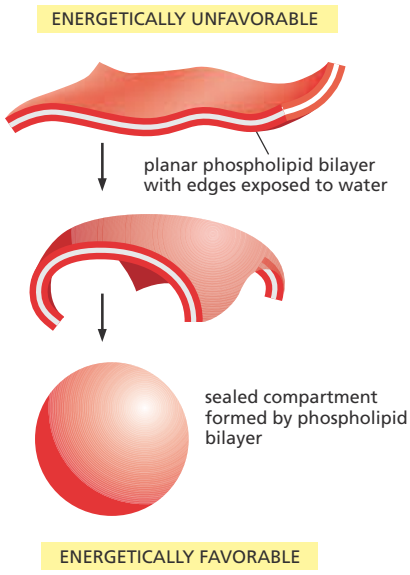


Figure 11–12 Phospholipid bilayers spontaneously close in on themselves to form sealed compartments. The closed structure is stable because it avoids the exposure of the hydrophobic hydrocarbon tails to water, which would be energetically unfavorable.

similar to those in a cell. On the other hand, as the result of random thermal motions, lipid molecules continuously exchange places with their neighbors in the same monolayer. This exchange leads to rapid lateral diffusion of lipid molecules within the plane of each monolayer, so that, for example, a lipid in an artificial bilayer may diffuse a length equal to that of an entire bacterial cell (~2 μm) in about one second.

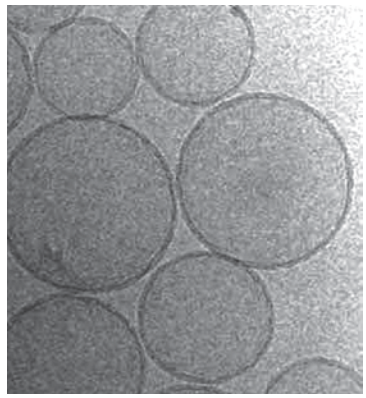
Similar studies show that individual lipid molecules not only flex their hydrocarbon tails, but they also rotate rapidly about their long axis—some reaching speeds of 500 revolutions per second. Studies of whole cells—and isolated cell membranes—indicate that lipid molecules in cell membranes undergo the same movements as they do in synthetic bilayers. The movements of membrane phospholipid molecules are summarized in **Figure 11–14**.

The Fluidity of a Lipid Bilayer Depends on Its Composition

The fluidity of a cell membrane—the ease with which its lipid molecules move within the plane of the bilayer—is important for membrane function and has to be maintained within certain limits. Just how fluid a lipid bilayer is at a given temperature depends on its phospholipid composition and, in particular, on the nature of the hydrocarbon tails: the closer and more regular the packing of the tails, the more viscous and less fluid the bilayer will be. Two major properties of hydrocarbon tails affect how tightly they pack in the bilayer: their length and the number of double bonds they contain.

A shorter chain length reduces the tendency of the hydrocarbon tails to interact with one another and therefore increases the fluidity of the bilayer. The hydrocarbon tails of membrane phospholipids vary in length between 14 and 24 carbon atoms, with 18–20 atoms being most usual. Most phospholipids contain one hydrocarbon tail that has one or more double bonds between adjacent carbon atoms, and a second tail with single bonds only (see Figure 11–6). The chain that harbors a double bond does not contain the maximum number of hydrogen atoms that could, in principle, be attached to its carbon backbone; it is thus said to be **unsaturated** with respect to hydrogen. The hydrocarbon tail with no double bonds has a full complement of hydrogen atoms and is said to be **saturated**. Each double bond in an unsaturated tail creates a small kink in the tail (see Figure 11–6), which makes it more difficult for the tails to pack against one another. For this reason, lipid bilayers that contain a large proportion of unsaturated hydrocarbon tails are more fluid than those with lower proportions.

In bacterial and yeast cells, which have to adapt to varying temperatures, both the lengths and the unsaturation of the hydrocarbon tails in the bilayer are constantly adjusted to maintain the membrane at a relatively constant fluidity: at higher temperatures, for example, the cell makes



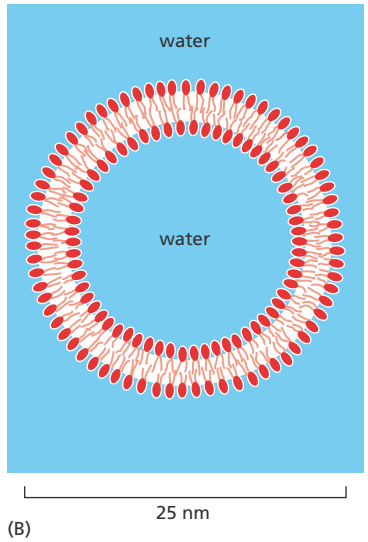


Figure 11–13 Pure phospholipids can form closed, spherical liposomes. (A) An electron micrograph of phospholipid vesicles (liposomes) showing the bilayer structure of the membrane. (B) A drawing of a small, spherical liposome seen in cross section. (A, courtesy of Jean Lepault.)

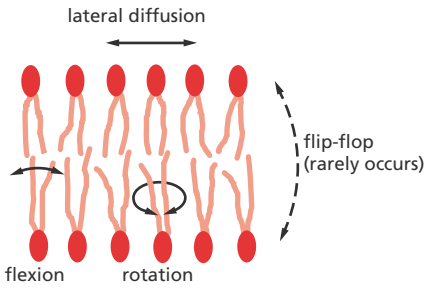


Figure 11–14 Membrane phospholipids are motile. The drawing shows the types of movement that phospholipid molecules undergo in a lipid bilayer. Because of these movements, the bilayer behaves as a two-dimensional fluid, in which the individual lipid molecules are able to move in their own monolayer. Note that lipid molecules do not move spontaneously from one monolayer to the other.

membrane lipids with tails that are longer and that contain fewer double bonds. A similar trick is used in the manufacture of margarine from vegetable oils. The fats produced by plants are generally unsaturated and therefore liquid at room temperature, unlike animal fats such as butter or lard, which are generally saturated and therefore solid at room temperature. Margarine is made of hydrogenated vegetable oils; their double bonds have been removed by the addition of hydrogen, so that they are more solid and butterlike at room temperature.

In animal cells, membrane fluidity is modulated by the inclusion of the sterol **cholesterol**. This molecule is present in especially large amounts in the plasma membrane, where it constitutes approximately 20% of the lipids in the membrane by weight. Because cholesterol molecules are short and rigid, they fill the spaces between neighboring phospholipid molecules left by the kinks in their unsaturated hydrocarbon tails (**Figure 11–15**). In this way, cholesterol tends to stiffen the bilayer, making it less flexible, as well as less permeable. The chemical properties of membrane lipids—and how they affect membrane fluidity—are reviewed in **Movie 11.2**.

For all cells, membrane fluidity is important for many reasons. It enables many membrane proteins to diffuse rapidly in the plane of the bilayer and to interact with one another, as is crucial, for example, in cell signaling (discussed in Chapter 16). It permits membrane lipids and proteins to diffuse from sites where they are inserted into the bilayer after their synthesis to other regions of the cell. It ensures that membrane molecules are distributed evenly between daughter cells when a cell divides. And, under appropriate conditions, it allows membranes to fuse with one another and mix their molecules (discussed in Chapter 15). If biological membranes were not fluid, it is hard to imagine how cells could live, grow, and reproduce.

QUESTION 11–2

Five students in your class always sit together in the front row. This could be because (A) they really like each other or (B) nobody else in your class wants to sit next to them. Which explanation holds for the assembly of a lipid bilayer? Explain. Suppose, instead, that the other explanation held for lipid molecules. How would the properties of the lipid bilayer be different?

Membrane Assembly Begins in the ER

In eukaryotic cells, new phospholipids are manufactured by enzymes bound to the cytosolic surface of the *endoplasmic reticulum* (*ER*; see Figure 11–3). Using free fatty acids as substrates (see Panel 2–4, pp. 72–73), the enzymes deposit the newly made phospholipids exclusively in the cytosolic half of the bilayer.

Despite this preferential treatment, cell membranes manage to grow evenly. So how do new phospholipids make it to the opposite monolayer?

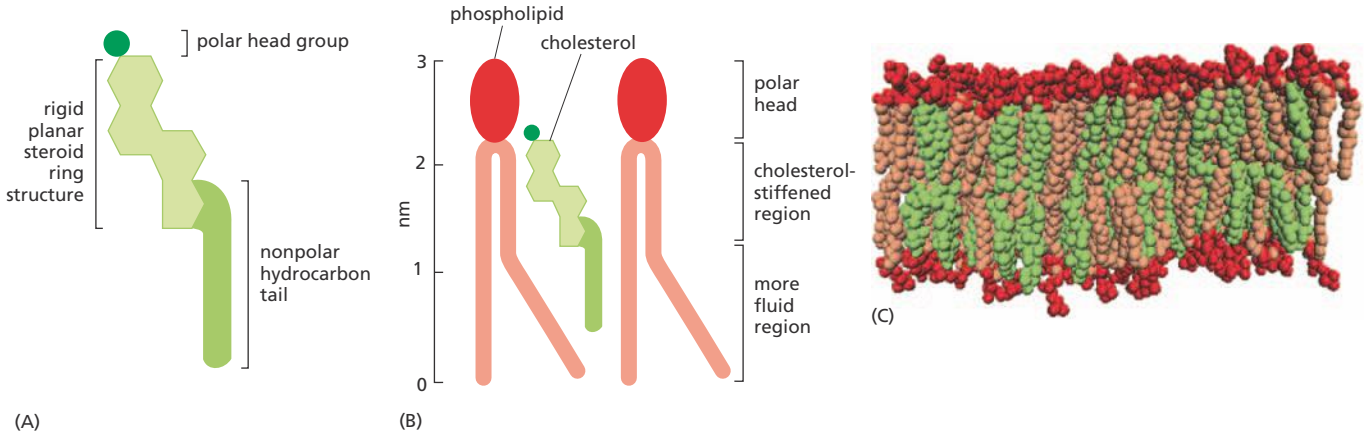


Figure 11–15 Cholesterol tends to stiffen cell membranes. (A) The shape of a cholesterol molecule. (B) How cholesterol fits into the gaps between phospholipid molecules in a lipid bilayer. (C) Space-filling model of the bilayer, with cholesterol molecules in *green*. The chemical formula of cholesterol is shown in Figure 11–7. (C, from H.L. Scott, *Curr. Opin. Struct. Biol.* 12: 499, 2002.)

As we saw in Figure 11–14, the transfer of lipids from one monolayer to the other rarely occur spontaneously. Instead, they are catalyzed by enzymes called *scramblases*, which remove randomly selected phospholipids from one half of the lipid bilayer and insert them in the other. As a result of this scrambling, newly made phospholipids are redistributed equally between each monolayer of the ER membrane (**Figure 11–16A**).

Some of this newly assembled membrane will remain in the ER; the rest will be used to supply fresh membrane to other compartments in the cell. Bits of membrane are continually pinching off the ER to form small, spherical vesicles that then fuse with other membranes, such as those of the Golgi apparatus. Additional vesicles bubble from the Golgi to become incorporated into the plasma membrane. We discuss this dynamic process of membrane transport in detail in Chapter 15.

Certain Phospholipids Are Confined to One Side of the Membrane

Most cell membranes are asymmetrical: the two halves of the bilayer often include strikingly different sets of phospholipids. But if membranes emerge from the ER with an evenly scrambled set of phospholipids, where does this asymmetry arise? It begins in the Golgi apparatus. The Golgi membrane contains another family of phospholipid-handling enzyme, called *flippases*. These enzymes remove specific phospholipids from the side of the bilayer facing the exterior space and flip them into the monolayer that faces the cytosol (**Figure 11–16B**).

The action of these flippases—and similar enzymes in the plasma membrane—initiates and maintains the asymmetric arrangement of phospholipids that is characteristic of the membranes of animal cells. This asymmetry is preserved as membranes bud from one organelle and fuse with another—or with the plasma membrane. This means that all

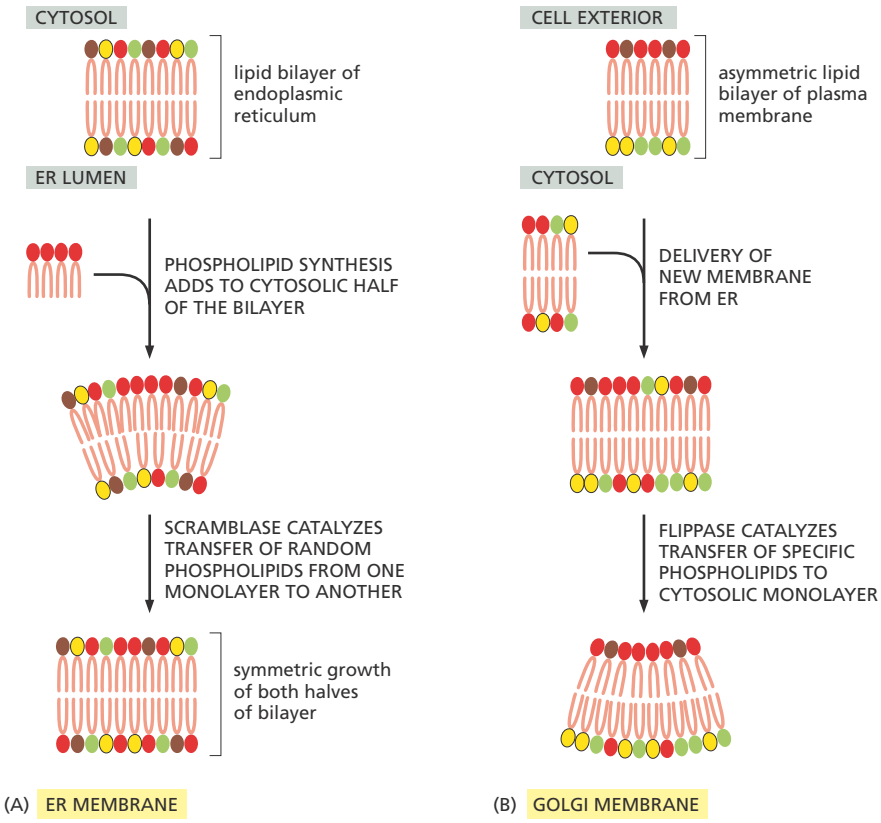


Figure 11–16 Newly synthesized phospholipids are added to the cytosolic side of the ER membrane and then redistributed by enzymes that transfer them from one half of the lipid bilayer to the other. (A) Biosynthetic enzymes bound to the cytosolic monolayer of the ER membrane (not shown) produce new phospholipids from free fatty acids and insert them into the cytosolic monlayer. Enzymes called scramblases then randomly transfer phospholipid molecules from one monolayer to the other, allowing the membrane to grow as a bilayer. (B) When membranes leave the ER and are incorporated in the Golgi, they encounter enzymes called flippases, which selectively remove phosphatidylserine (*light green*) and phosphatidylethanolamine (*yellow*) from the noncytosolic monolayer and flip them to the cytosolic side. This transfer leaves phosphatidylcholine (*red*) and sphingomyelin (*brown*) concentrated in the noncytosolic monolayer. The resulting curvature of the membrane may actually help drive subsequent vesicle budding.

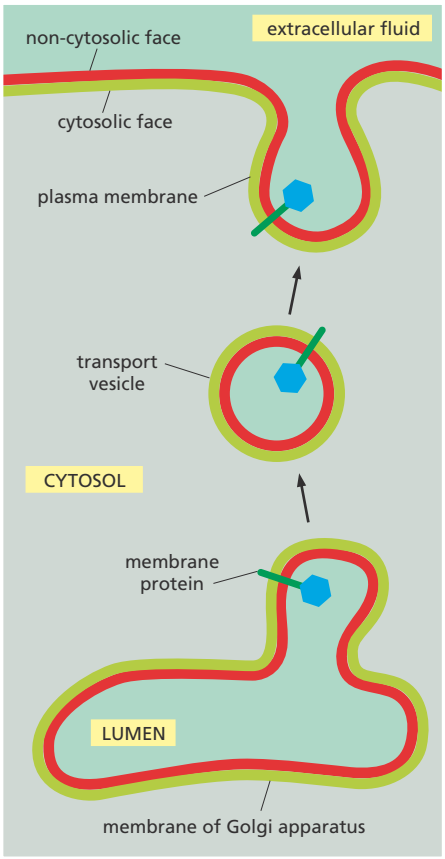


Figure 11–17 Membranes retain their orientation during transfer between cell compartments. Membranes are transported by a process of vesicle budding and fusing. Here, a vesicle is shown budding from the Golgi apparatus and fusing with the plasma membrane. Note that the orientations of both the membrane lipids and proteins are preserved during the process: the original cytosolic surface of the lipid bilayer (*green*) remains facing the cytosol, and the noncytosolic surface (*red*) continues to face away from the cytosol, toward the lumen of the Golgi or transport vesicle—or toward the extracellular compartment. Similarly, the glycoprotein shown here remains in the same orientation, with its attached sugar facing the noncytosolic side.

cell membranes have distinct "inside" and "outside" faces: the cytosolic monolayer always faces the cytosol, while the noncytosolic monolayer is exposed to either the cell exterior—in the case of the plasma membrane—or to the interior space (*lumen*) of an organelle. This conservation of orientation applies not only to the phospholipids that make up the membrane, but to any proteins that might be inserted in the membrane (**Figure 11–17**). For membrane proteins, this positioning is very important, as their orientation within the lipid bilayer is often crucial for their function (see Figure 11–19).

Among lipids, those that show the most dramatically lopsided distribution in cell membranes are the glycolipids, which are located mainly in the plasma membrane, and only in the noncytosolic half of the bilayer (**Figure 11–18**). Their sugar groups face the cell exterior, where they form part of a continuous coat of carbohydrate that surrounds and protects animal cells. Glycolipid molecules acquire their sugar groups in the Golgi apparatus, where the enzymes that engineer this chemical modification are confined. These enzymes are oriented such that sugars are added only to lipid molecules in the noncytosolic half of the bilayer. Once a glycolipid molecule has been created in this way, it remains trapped in this monolayer, as there are no flippases that transfer glycolipids to the cytosolic side. Thus, when a glycolipid molecule is finally delivered to the plasma membrane, it displays its sugars to the exterior of the cell.

Other lipid molecules show different types of asymmetric distributions, which relate to their specific functions. For example, the inositol phospholipids—a minor component of the plasma membrane—have a special

QUESTION 11–3

It seems paradoxical that a lipid bilayer can be fluid yet asymmetrical. Explain.

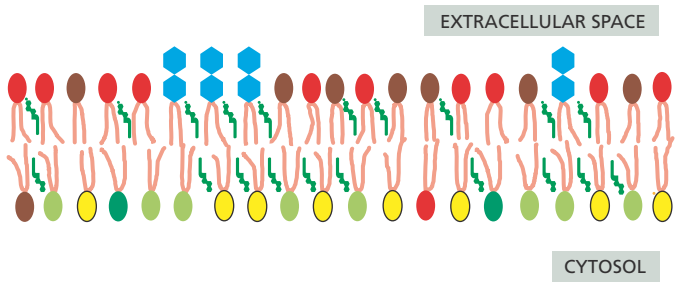


Figure 11–18 Phospholipids and glycolipids are distributed asymmetrically in the lipid bilayer of a eukaryotic plasma membrane. Phosphatidylcholine (*red*) and sphingomyelin (*brown*) are concentrated in the noncytosolic monolayer, whereas phosphatidylserine (*light green*), and phosphatidylethanolamine (*yellow*) are found mainly on the cytosolic side. In addition to these phospholipids, phosphatidylinositols (*dark green*), a minor constituent of the plasma membrane, are shown in the cytosolic monolayer, where they participate in cell signaling. Glycolipids are drawn with hexagonal *blue* head groups to represent sugars; these are found exclusively in the noncytosolic monolayer of the membrane. Within the bilayer, cholesterol (*green*) is distributed almost equally in both monolayers.

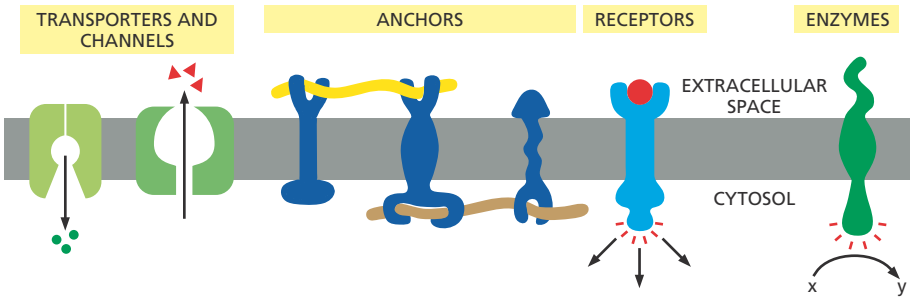


Figure 11–19 Plasma membrane proteins have a variety of functions.

role in relaying signals from the cell surface to the cell interior (discussed in Chapter 16); thus they are concentrated in the cytosolic half of the lipid bilayer.

MEMBRANE PROTEINS

Although the lipid bilayer provides the basic structure of all cell membranes and serves as a permeability barrier to the hydrophilic molecules on either side of it, most membrane functions are carried out by **membrane proteins**. In animals, proteins constitute about 50% of the mass of most plasma membranes, the remainder being lipid plus the relatively small amounts of carbohydrate found on some of the lipids (glycolipids) and many of the proteins (glycoproteins). Because lipid molecules are much smaller than proteins, however, a cell membrane typically contains about 50 times more lipid molecules than protein molecules (see Figure 11–4C).

Membrane proteins serve many functions. Some transport particular nutrients, metabolites, and ions across the lipid bilayer. Others anchor the membrane to macromolecules on either side. Still others function as receptors that detect chemical signals in the cell's environment and relay them into the cell interior, or work as enzymes to catalyze specific reactions at the membrane (**Figure 11–19** and **Table 11–1**). Each type of cell membrane contains a different set of proteins, reflecting the specialized functions of the particular membrane. In this section, we discuss the structure of membrane proteins and how they associate with the lipid bilayer.

TABLE 11–1 SOME EXAMPLES OF PLASMA MEMBRANE PROTEINS AND THEIR FUNCTIONS

Functional Class	Protein Example	Specific Function
Transporters	Na^+ pump	actively pumps Na^+ out of cells and K^+ in (discussed in Chapter 12)
Ion channels	K^+ leak channel	allows K^+ ions to leave cells, thereby having a major influence on cell excitability (discussed in Chapter 12)
Anchors	integrins	link intracellular actin filaments to extracellular matrix proteins (discussed in Chapter 20)
Receptors	platelet-derived growth factor (PDGF) receptor	binds extracellular PDGF and, as a consequence, generates intracellular signals that cause the cell to grow and divide (discussed in Chapters 16 and 18)
Enzymes	adenylyl cyclase	catalyzes the production of the small intracellular signaling molecule cyclic AMP in response to extracellular signals (discussed in Chapter 16)

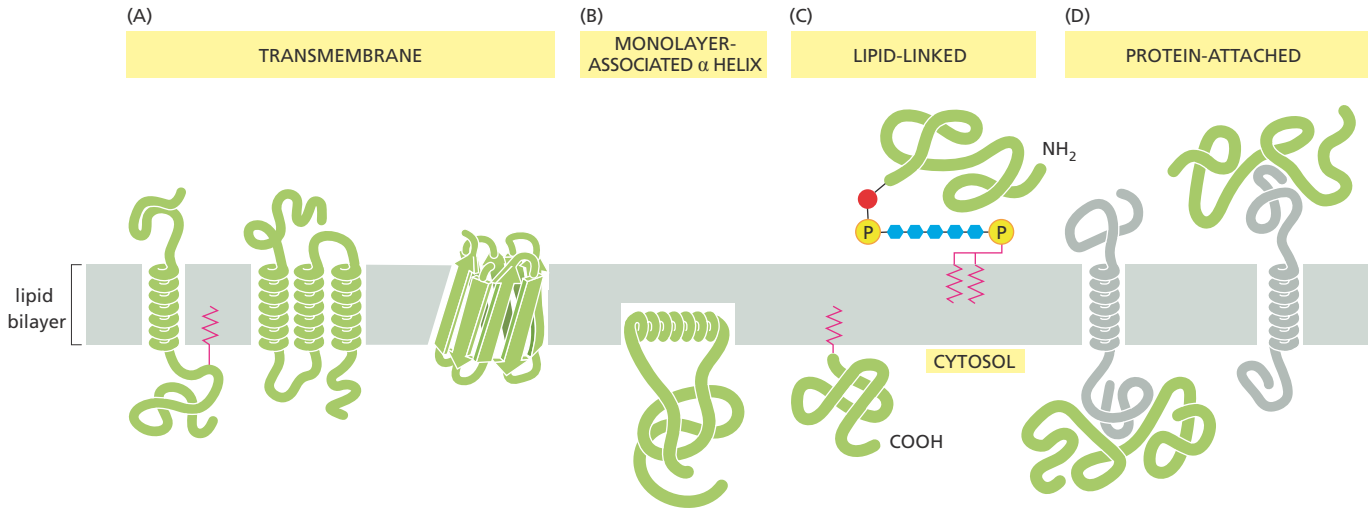


Figure 11–20 Membrane proteins can associate with the lipid bilayer in different ways. (A) Transmembrane proteins extend across the bilayer as a single α helix, as multiple α helices, or as a rolled-up β sheet (called a β barrel). (B) Some membrane proteins are anchored to the cytosolic half of the lipid bilayer by an amphipathic α helix. (C) Others are linked to either side of the bilayer solely by a covalently attached lipid molecule (*red* zigzag lines). (D) Many proteins are attached to the membrane only by relatively weak, noncovalent interactions with other membrane proteins. All except (D) are *integral membrane proteins*.

Membrane Proteins Associate with the Lipid Bilayer in Different Ways

Proteins can be associated with the lipid bilayer of a cell membrane in any one of the ways illustrated in **Figure 11–20**.

1. Many membrane proteins extend through the bilayer, with part of their mass on either side (Figure 11–20A). Like their lipid neighbors, these *transmembrane proteins* are amphipathic, having both hydrophobic and hydrophilic regions. Their hydrophobic regions lie in the interior of the bilayer, nestled against the hydrophobic tails of the lipid molecules. Their hydrophilic regions are exposed to the aqueous environment on either side of the membrane.
2. Other membrane proteins are located almost entirely in the cytosol and are associated with the cytosolic half of the lipid bilayer by an amphipathic α helix exposed on the surface of the protein (Figure 11–20B).
3. Some proteins lie entirely outside the bilayer, on one side or the other, attached to the membrane only by one or more covalently attached lipid groups (Figure 11–20C).
4. Yet other proteins are bound indirectly to one or the other face of the membrane, held in place only by their interactions with other membrane proteins (Figure 11–20D).

Proteins that are directly attached to the lipid bilayer—whether they are transmembrane, associated with the lipid monolayer, or lipid-linked—can be removed only by disrupting the bilayer with detergents, as discussed shortly. Such proteins are known as *integral membrane proteins*. The remaining membrane proteins are known as *peripheral membrane proteins*; they can be released from the membrane by more gentle extraction procedures that interfere with protein–protein interactions but leave the lipid bilayer intact.

A Polypeptide Chain Usually Crosses the Lipid Bilayer as an α Helix

All membrane proteins have a unique orientation in the lipid bilayer, which is essential for their function. For a transmembrane receptor protein, for example, the part of the protein that receives a signal from the environment must be on the outside of the cell, whereas the part that passes along the signal must be in the cytosol (see Figure 11–19). This orientation is a consequence of the way in which membrane proteins are synthesized (discussed in Chapter 15). The portions of a transmembrane protein located on either side of the lipid bilayer are connected by specialized membrane-spanning segments of the polypeptide chain (see Figure 11–20A). These segments, which run through the hydrophobic environment of the interior of the lipid bilayer, are composed largely of amino acids with hydrophobic side chains. Because these side chains cannot form favorable interactions with water molecules, they prefer to interact with the hydrophobic tails of the lipid molecules, where no water is present.

In contrast to the hydrophobic side chains, however, the peptide bonds that join the successive amino acids in a protein are normally polar, making the polypeptide backbone hydrophilic (**Figure 11–21**). Because water is absent from the interior of the bilayer, atoms forming the backbone are driven to form hydrogen bonds with one another. Hydrogen-bonding is maximized if the polypeptide chain forms a regular α helix, and so the great majority of the membrane-spanning segments of polypeptide chains traverse the bilayer as α helices (see Figure 4–13). In these membrane-spanning α helices, the hydrophobic side chains are exposed on the outside of the helix, where they contact the hydrophobic lipid tails, while atoms in the polypeptide backbone form hydrogen bonds with one another on the inside of the helix (**Figure 11–22**).

In many transmembrane proteins, the polypeptide chain crosses the membrane only once (see Figure 11–20A). Many of these *single-pass* transmembrane proteins are receptors for extracellular signals. Other transmembrane proteins function as channels, forming aqueous pores across the lipid bilayer to allow small, water-soluble molecules to cross the membrane. Such channels cannot be formed by proteins with a single transmembrane α helix. Instead, they usually consist of a series of α helices that cross the bilayer a number of times (see Figure 11–20A). In many of these *multipass* transmembrane proteins, one or more of the membrane-spanning regions are amphipathic—formed from α helices that contain both hydrophobic and hydrophilic amino acid side chains. These amino acids tend to be arranged so that the hydrophobic side chains fall on one side of the helix, while the hydrophilic side chains are concentrated on the other side. In the hydrophobic environment of the lipid bilayer, α helices of this sort pack side by side in a ring, with the hydrophobic side chains exposed to the lipids of the membrane and the hydrophilic side chains forming the lining of a hydrophilic pore through the lipid bilayer (**Figure 11–23**). How such channels function in the selective transport of small, water-soluble molecules, especially inorganic ions, is discussed in Chapter 12.

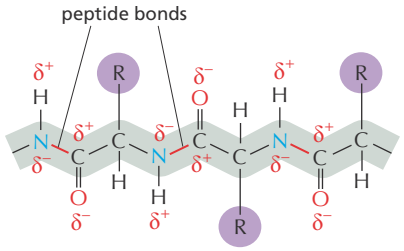


Figure 11–21 The backbone of a polypeptide chain is hydrophilic. The atoms on either side of a peptide bond (*red line*) are polar and carry partial positive or negative charges (δ^+ or δ^-). These charges allow these atoms to hydrogen-bond with one another when the polypeptide folds into an α helix that spans the lipid bilayer (see Figure 11–22).

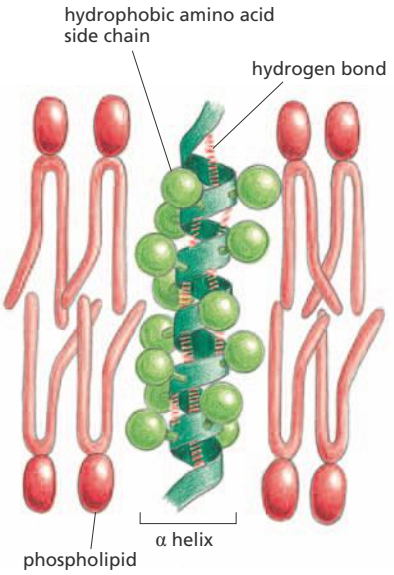


Figure 11–22 A transmembrane polypeptide chain usually crosses the lipid bilayer as an α helix. In this segment of a transmembrane protein, the hydrophobic side chains (*light green*) of the amino acids forming the α helix contact the hydrophobic hydrocarbon tails of the phospholipid molecules, while the hydrophilic parts of the polypeptide backbone form hydrogen bonds with one another in the interior of the helix. An α helix containing about 20 amino acids is required to completely traverse a cell membrane.

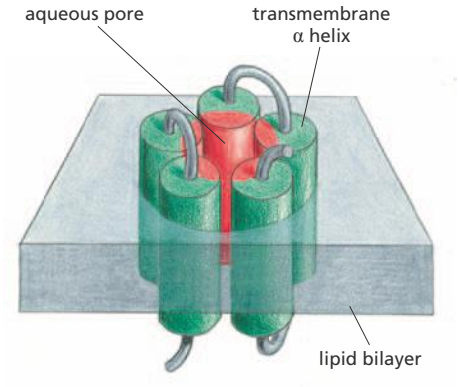


Figure 11–23 A transmembrane hydrophilic pore can be formed by multiple amphipathic α helices. In this example, five such transmembrane α helices form a water-filled channel across the lipid bilayer. The hydrophobic amino acid side chains (*green*) on one side of each helix contact the hydrophobic lipid tails, while the hydrophilic side chains (*red*) on the opposite side of the helices form a water-filled pore.

Although the α helix is by far the most common form in which a polypeptide chain crosses a lipid bilayer, the polypeptide chain of some transmembrane proteins crosses the lipid bilayer as a β sheet that is rolled into a cylinder, forming a keglike structure called a β barrel (see Figure 11–20A). As expected, the amino acid side chains that face the inside of the barrel, and therefore line the aqueous channel, are mostly hydrophilic, while those on the outside of the barrel, which contact the hydrophobic core of the lipid bilayer, are exclusively hydrophobic. The most striking example of a β-barrel structure is found in the *porin* proteins, which form large, water-filled pores in mitochondrial and bacterial outer membranes (**Figure 11–24**). Mitochondria and some bacteria are surrounded by a double membrane, and porins allow the passage of small nutrients, metabolites, and inorganic ions across their outer membranes, while preventing unwanted larger molecules from crossing.

QUESTION 11–4

Explain why the polypeptide chain of most transmembrane proteins crosses the lipid bilayer as an α helix or a β barrel.

Membrane Proteins Can Be Solubilized in Detergents

To understand a protein fully, one needs to know its structure in detail. For membrane proteins, this presents special problems. Most biochemical procedures are designed for studying molecules in aqueous solution. Membrane proteins, however, are built to operate in an environment that is partly aqueous and partly fatty, and taking them out of this environment and purifying them while preserving their essential structure is no easy task.

Before an individual protein can be studied in detail, it must be separated from all the other cell proteins. For most membrane proteins, the first step in this separation process involves solubilizing the membrane with agents that destroy the lipid bilayer by disrupting hydrophobic associations. The most widely used disruptive agents are **detergents** (**Movie 11.3**). These small, amphipathic, lipidlike molecules differ from membrane phospholipids in that they have only a single hydrophobic tail (**Figure 11–25**). Because they have one tail, detergent molecules are shaped like cones; in water, they thus tend to aggregate into small clusters called *micelles*, rather than forming a bilayer as do the phospholipids, which—with their two tails—are more cylindrical in shape.

When mixed in great excess with membranes, the hydrophobic ends of detergent molecules interact with the membrane-spanning hydrophobic regions of the transmembrane proteins, as well as with the hydrophobic

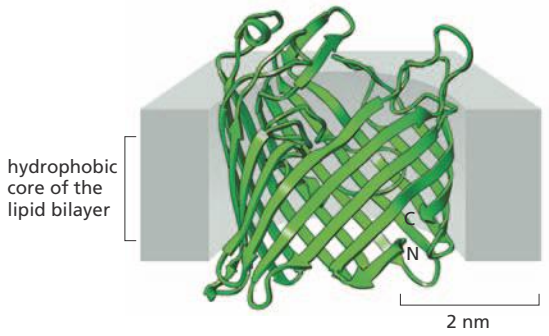
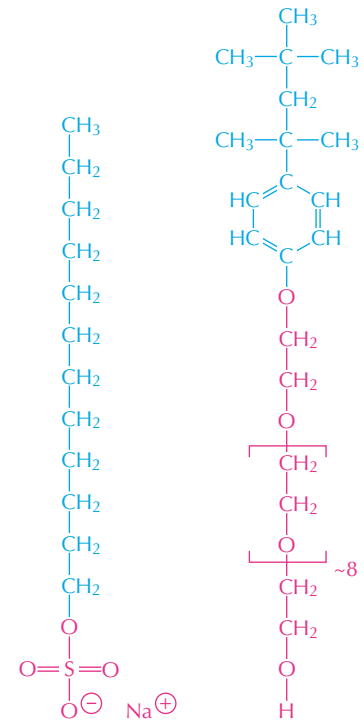


Figure 11–24 Porin proteins form water-filled channels in the outer membrane of a bacterium. The protein illustrated is from *E. coli*, and it consists of a 16-stranded β sheet curved around on itself to form a transmembrane water-filled channel. The three-dimensional structure was determined by X-ray crystallography. Although not shown in the drawing, three porin proteins associate to form a trimer, which has three separate channels.



sodium dodecyl sulfate (SDS)

Triton X-100

Figure 11–25 SDS and Triton X-100 are two commonly used detergents. Sodium dodecyl sulfate (SDS) is a strong ionic detergent—that is, it has an ionized (charged) group at its hydrophilic end. Triton X-100 is a mild nonionic detergent—that is, it has a nonionized but polar structure at its hydrophilic end. The hydrophobic portion of each detergent is shown in *blue*, and the hydrophilic portion in *red*. The bracketed portion of Triton X-100 is repeated about eight times. Strong ionic detergents like SDS not only displace lipid molecules from proteins but also unfold the proteins (see Panel 4–5, p. 167).

tails of the phospholipid molecules, thereby disrupting the lipid bilayer and separating the proteins from most of the phospholipids. Because the other end of the detergent molecule is hydrophilic, these interactions bring the membrane proteins into solution as protein–detergent complexes; at the same time, the detergent solubilizes the phospholipids (**Figure 11–26**). The protein–detergent complexes can then be separated from one another and from the lipid–detergent complexes for further analysis.

We Know the Complete Structure of Relatively Few Membrane Proteins

For many years, much of what we knew about the structure of membrane proteins was learned by indirect means. The standard method for determining a protein's three-dimensional structure directly is X-ray crystallography (see Figure 4–52), but this requires ordered crystalline arrays of the molecule. Because membrane proteins have to be purified in detergent micelles that are often heterogeneous in size, they are harder to crystallize than the soluble proteins that inhabit the cell cytosol or extracellular fluids. Nevertheless, with recent advances in protein preparation and X-ray crystallography, the structures of an increasing number of membrane proteins have now been determined to high resolution.

One example is bacteriorhodopsin, the structure of which first revealed exactly how α helices cross the lipid bilayer. **Bacteriorhodopsin** is a small protein (about 250 amino acids) found in large amounts in the plasma membrane of an archaean, called *Halobacterium halobium*, that lives in salt marshes. Bacteriorhodopsin acts as a membrane transport protein that pumps H^+ (protons) out of the cell. Pumping requires energy, and bacteriorhodopsin gets its energy directly from sunlight. Each bacteriorhodopsin molecule contains a single light-absorbing nonprotein

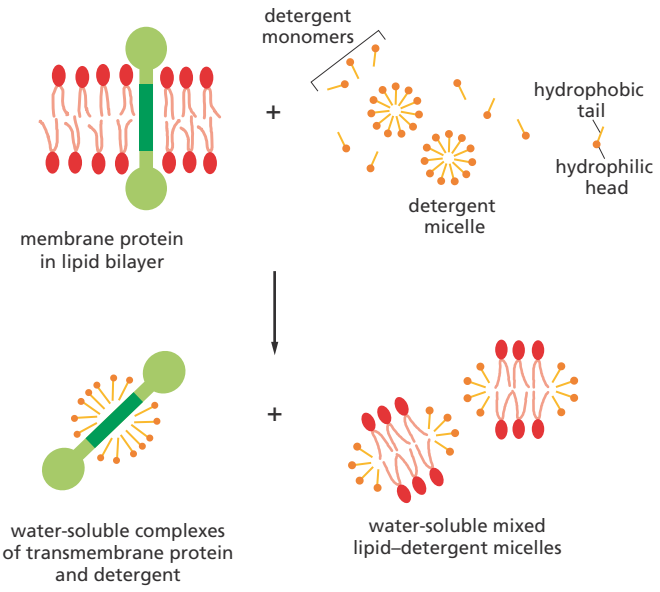


Figure 11–26 Membrane proteins can be solubilized by a mild detergent such as Triton X-100. The detergent molecules (*gold*) are shown as both monomers and micelles, the form in which detergent molecules tend to aggregate in water. The detergent disrupts the lipid bilayer and brings the proteins into solution as protein–detergent complexes. As illustrated, the phospholipids in the membrane are also solubilized by the detergents, forming lipid–detergent micelles.

QUESTION 11–5

For the two detergents shown in Figure 11–25, explain why the red portions of the molecules are hydrophilic and the blue portions hydrophobic. Draw a short stretch of a polypeptide chain made up of three amino acids with hydrophobic side chains (see Panel 2–5, pp. 74–75) and apply a similar color scheme.

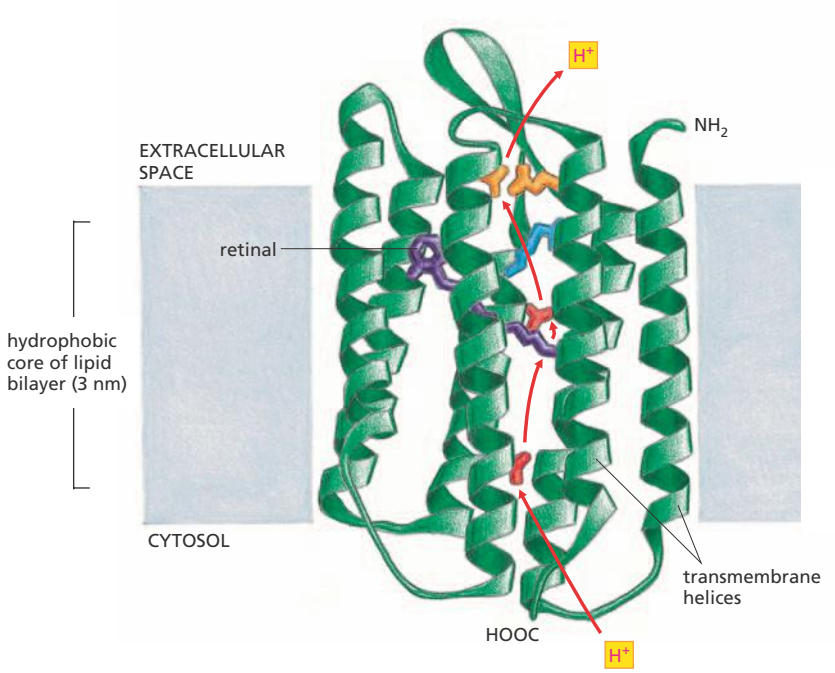


Figure 11–27 Bacteriorhodopsin acts as a proton pump. The polypeptide chain crosses the lipid bilayer as seven α helices. The location of the retinal (*purple*) and the probable pathway taken by protons during the light-activated pumping cycle (*red* arrows) are highlighted. Strategically placed polar amino acid side chains, shown in *red, yellow*, and *blue*, guide the movement of the proton across the bilayer, allowing the proton to avoid contact with the lipid environment. The proton-transfer steps are shown in Movie 11.4. Retinal is also used to detect light in our own eyes, where it is attached to a protein with a structure very similar to bacteriorhodopsin. (Adapted from H. Luecke et al., *Science* 286:255–260, 1999. With permission from the AAAS.)

molecule, called *retinal*, that gives the protein—and the bacterium—a deep purple color. This small hydrophobic molecule is covalently attached to one of bacteriorhodopsin's seven transmembrane α helices (**Figure 11–27**). When retinal absorbs a photon of light, it changes shape, and in doing so, it causes the protein embedded in the lipid bilayer to undergo a series of small conformational changes. These changes result in the transfer of one H^+ from the retinal to the outside of the bacterium (see Figure 11–27). The retinal is then regenerated by taking up a H^+ from the cytosol, returning the protein to its original conformation so that it can repeat the cycle. The overall outcome is the movement of one H^+ from inside to outside the cell.

In the presence of sunlight, thousands of bacteriorhodopsin molecules pump H^+ out of the cell, generating a concentration gradient of H^+ across the plasma membrane. The cell uses this proton gradient to store energy and convert it into ATP, as we discuss in detail in Chapter 14. Bacteriorhodopsin is a pump protein, a class of transmembrane protein that actively moves small organic molecules and inorganic ions into and out of cells (see Figure 11–19). We will meet other pump proteins in Chapter 12.

The Plasma Membrane Is Reinforced by the Underlying Cell Cortex

A cell membrane by itself is extremely thin and fragile. It would require nearly 10,000 cell membranes laid on top of one another to achieve the thickness of this paper. Most cell membranes are therefore strengthened and supported by a framework of proteins, attached to the membrane via transmembrane proteins. For plants, yeasts, and bacteria, the cell's shape and mechanical properties are conferred by a rigid cell wall—a meshwork of proteins, sugars, and other macromolecules that encases the plasma membrane. By contrast, the plasma membrane of animal cells is stabilized by a meshwork of fibrous proteins, called the cell cortex, that is attached to the underside of the membrane.

The cortex of human red blood cells is a relatively simple and regular structure and has been especially well studied. These cells are small and have a distinctive flattened shape (**Figure 11–28**). The main component of their cortex is the dimeric protein spectrin, a long, thin, flexible rod

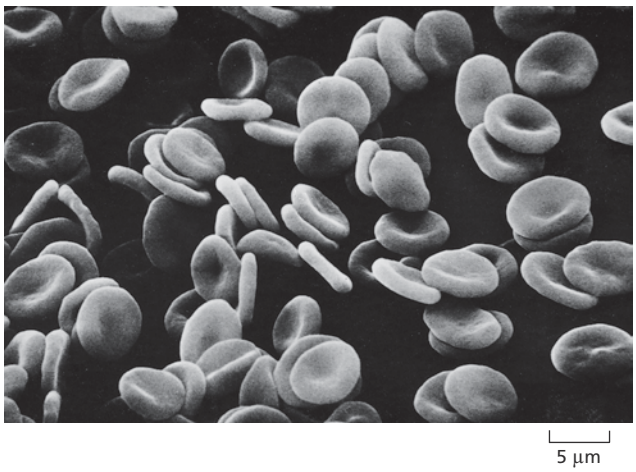


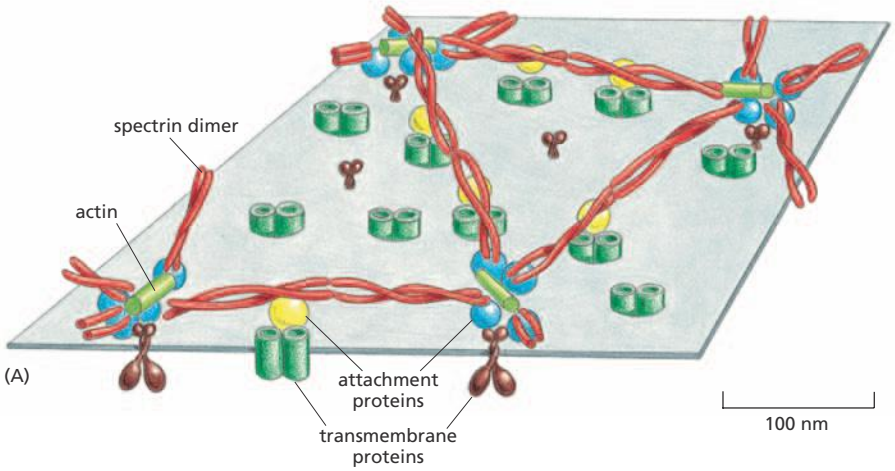
Figure 11–28 Human red blood cells have a characteristic flattened biconcave shape, as seen in this scanning electron micrograph. These cells lack a nucleus and other intracellular organelles. (Courtesy of Bernadette Chailley.)

QUESTION 11–6

Look carefully at the transmembrane proteins shown in Figure 11–29. What can you say about their mobility in the membrane?

about 100 nm in length. It forms a meshwork that provides support for the plasma membrane and maintains the cell's biconcave shape. The spectrin meshwork is connected to the membrane through intracellular attachment proteins that link the spectrin to specific transmembrane proteins (**Figure 11–29** and **Movie 11.5**). The importance of this meshwork is seen in mice and humans that have genetic abnormalities in spectrin structure. These individuals are anemic: they have fewer red blood cells than normal. The red cells they do have are spherical instead of flattened and are abnormally fragile.

Proteins similar to spectrin and to its associated attachment proteins are present in the cortex of most animal cells. But the cortex in these cells is especially rich in actin and the motor protein *myosin*, and it is much more complex than that of red blood cells. While red blood cells need their cortex mainly to provide mechanical strength as they are pumped through blood vessels, other cells also need their cortex to allow them to selectively take up materials from their environment, to change their shape actively, and to move, as we discuss in Chapter 17. In addition, cells use their cortex to restrain the diffusion of proteins within the plasma membrane, as we see next.



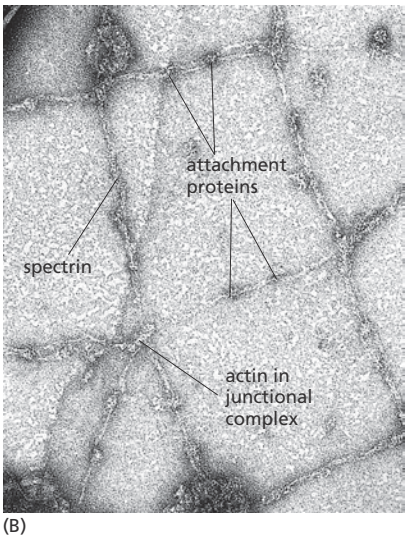


Figure 11–29 A spectrin meshwork forms the cell cortex in human red blood cells. (A) Spectrin dimers are linked end-to-end to form longer tetramers. The spectrin tetramers, together with a smaller number of actin molecules, are linked together into a mesh. This network is attached to the plasma membrane by the binding of at least two types of attachment proteins (shown here in *yellow* and *blue*) to two kinds of transmembrane proteins (shown here in *green* and *brown*). (B) Electron micrograph showing the spectrin meshwork on the cytoplasmic side of a red blood cell membrane. The meshwork has been stretched out to show the details of its structure; in the normal cell, the meshwork shown would be much more crowded and would occupy only about one-tenth of this area. (B, courtesy of T. Byers and D. Branton, *Proc. Natl. Acad. Sci. USA* 82:6153–6157, 1985. With permission from the National Academy of Sciences.)

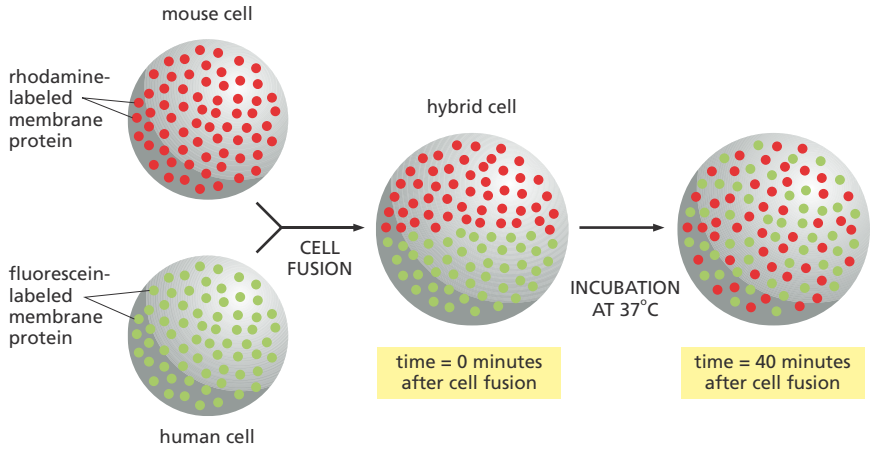


Figure 11–30 Formation of mouse–human hybrid cells shows that some plasma membrane proteins can move laterally in the lipid bilayer. When the mouse and human cells are first fused, their proteins are confined to their own halves of the newly formed hybrid-cell plasma membrane. Within a short time, however, they completely intermix. To monitor the movement of a selected sampling of these proteins, the cells are labeled with antibodies that bind to either human or mouse proteins; the antibodies are coupled to two different fluorescent tags—rhodamine (*red*) or fluorescein (*green*)—so they can be distinguished in a fluorescence microscope (see Panel 4–2, pp. 146–147). (Based on observations of L.D. Frye and M. Edidin, *J. Cell Sci.* 7:319–335, 1970. With permission from The Company of Biologists Ltd.)

A Cell Can Restrict the Movement of Its Membrane Proteins

Because a membrane is a two-dimensional fluid, many of its proteins, like its lipids, can move freely within the plane of the lipid bilayer. This lateral diffusion was initially demonstrated by experimentally fusing a mouse cell with a human cell to form a double-sized hybrid cell and then monitoring the distribution of certain mouse and human plasma membrane proteins. At first, the mouse and human proteins are confined to their own halves of the newly formed hybrid cell, but within half an hour or so the two sets of proteins become evenly mixed over the entire cell surface (**Figure 11–30**). We describe some other techniques for studying the movement of membrane proteins in **How We Know**, pp. 378–379.

The picture of a cell membrane as a sea of lipid in which all proteins float freely is too simple, however. Cells have ways of confining particular proteins to localized areas within the bilayer membrane, thereby creating functionally specialized regions, or **membrane domains**, on the cell or organelle surface.

As illustrated in **Figure 11–31**, plasma membrane proteins can be tethered to structures outside the cell—for example, to molecules in the extracellular matrix or on an adjacent cell (discussed in Chapter 20)—or to relatively immobile structures inside the cell, especially to the cell cortex (see Figure 11–29). Additionally, cells can create barriers that restrict particular membrane components to one membrane domain. In epithelial cells that line the gut, for example, it is important that transport proteins involved in the uptake of nutrients from the gut be confined to

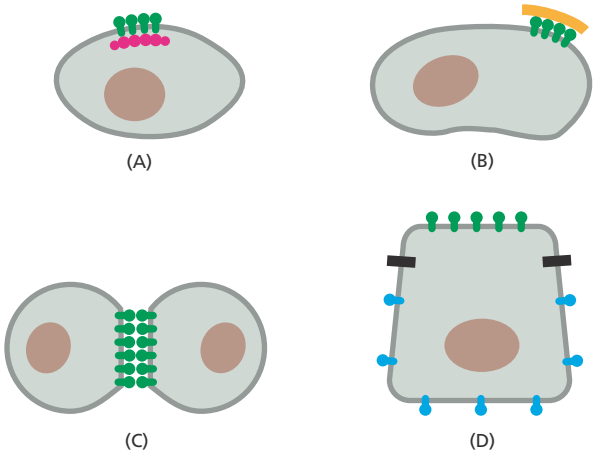


Figure 11–31 The lateral mobility of plasma membrane proteins can be restricted in several ways. Proteins can be tethered to the cell cortex inside the cell (A), to extracellular matrix molecules outside the cell (B), or to proteins on the surface of another cell (C). Diffusion barriers (shown as *black* bars) can restrict proteins to a particular membrane domain (D).

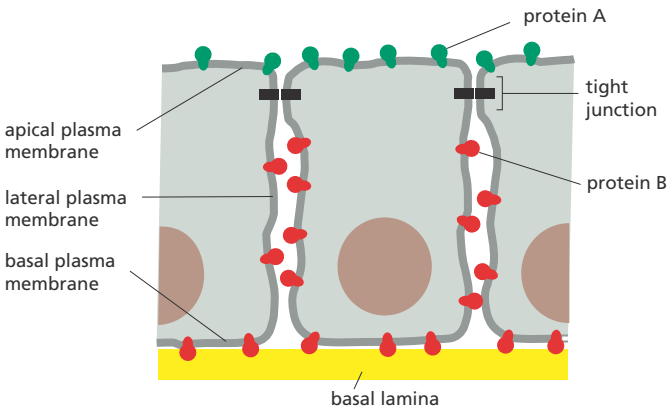


Figure 11–32 Membrane proteins are restricted to particular domains of the plasma membrane of epithelial cells in the gut. Protein A (in the apical membrane) and protein B (in the basal and lateral membranes) can diffuse laterally in their own membrane domains but are prevented from entering the other domain by a specialized cell junction called a tight junction. The basal lamina is a mat of extracellular matrix that supports all epithelial sheets (discussed in Chapter 20).

the *apical* surface of the cells (the surface that faces the gut contents) and that other transport proteins involved in the export of solutes out of the epithelial cell into the tissues and bloodstream be confined to the *basal* and *lateral* surfaces (see Figure 12–17). This asymmetric distribution of membrane proteins is maintained by a barrier formed along the line where the cell is sealed to adjacent epithelial cells by a so-called *tight junction* (**Figure 11–32**). At this site, specialized junctional proteins form a continuous belt around the cell where the cell contacts its neighbors, creating a seal between adjacent plasma membranes (see Figure 20–23). Membrane proteins cannot diffuse past the junction.

The Cell Surface Is Coated with Carbohydrate

We saw earlier that some of the lipids in the outer layer of the plasma membrane have sugars covalently attached to them. The same is true for most of the proteins in the plasma membrane. The great majority of these proteins have short chains of sugars, called oligosaccharides, linked to them; they are called *glycoproteins*. Other membrane proteins, the *proteoglycans*, contain one or more long polysaccharide chains. All of the carbohydrate on the glycoproteins, proteoglycans, and glycolipids is located on the outside of the plasma membrane, where it forms a sugar coating called the *carbohydrate layer* or **glycocalyx** (**Figure 11–33**).

This layer of carbohydrate helps protect the cell surface from mechanical damage. As the oligosaccharides and polysaccharides adsorb water, they also give the cell a slimy surface, which helps motile cells such as white blood cells squeeze through narrow spaces and prevents blood cells from sticking to one another or to the walls of blood vessels.

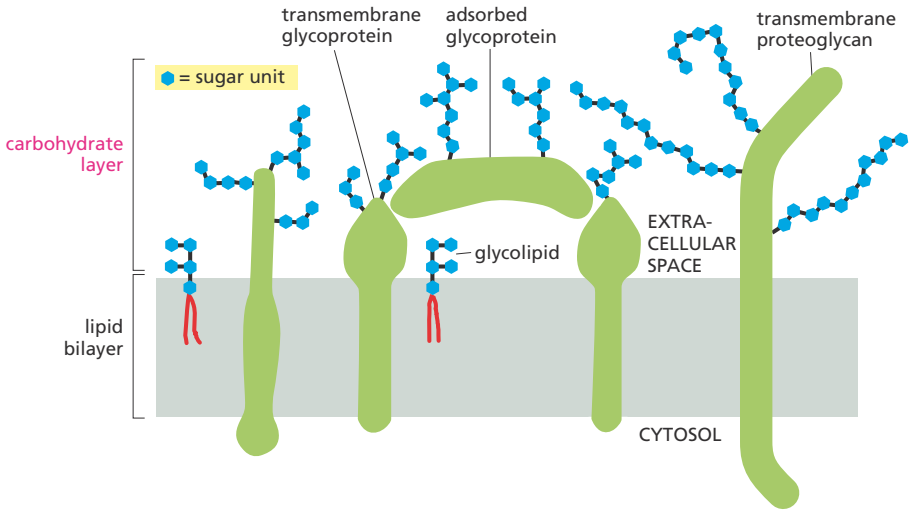


Figure 11–33 Eukaryotic cells are coated with sugars. The carbohydrate layer is made of the oligosaccharide side chains attached to membrane glycolipids and glycoproteins, and of the polysaccharide chains on membrane proteoglycans. As shown, glycoproteins that have been secreted by the cell and then adsorbed back onto its surface can also contribute. Note that all the carbohydrate is on the external (noncytosolic) surface of the plasma membrane.

MEASURING MEMBRANE FLOW

An essential feature of the lipid bilayer is its fluidity, which is crucial for cell membrane integrity and function. This property allows many membrane-embedded proteins to move laterally in the plane of the bilayer, so that they can engage in the various protein–protein interactions on which cells depend. The fluid nature of cell membranes is so central to their proper function that it may seem surprising that this property was not recognized until the early 1970s.

Given its importance for membrane structure and function, how do we measure and study the fluidity of cell membranes? The most common methods are visual: simply label some of the molecules native to the membrane and then watch them move. Such an approach first demonstrated the lateral movement of membrane proteins that had been tagged with labeled antibodies (see Figure 11–30). This experiment seemed to suggest that membrane proteins diffuse freely, without restriction, in an open sea of lipids. We now know that this image is not entirely accurate. To probe membrane fluidity more thoroughly, researchers had to invent more precise methods for tracking the movement of proteins within a membrane such as the plasma membrane of a living cell.

The FRAP attack

One such technique, called fluorescence recovery after photobleaching (FRAP), involves uniformly labeling the components of the cell membrane—its lipids or, more often, its proteins—with some sort of fluorescent marker. Labeling membrane proteins can be accomplished by incubating living cells with a fluorescent antibody or by covalently attaching a fluorescent protein such as green fluorescent protein (GFP) to a membrane protein of interest using recombinant DNA techniques (discussed in Chapter 10).

Once a protein has been labeled, a small patch of membrane is irradiated with an intense pulse of light from a sharply focused laser beam. This treatment irreversibly "bleaches" the fluorescence from the labeled proteins in that small patch of membrane, typically an area about 1 μm square. The fluorescence of this irradiated membrane is monitored in a fluorescence microscope, and the amount of time it takes for the neighboring, unbleached fluorescent proteins to migrate into the bleached region of the membrane is measured (**Figure 11–34**). The rate of this "fluorescence recovery" is a direct measure of the rate at which the protein molecules can diffuse within the membrane (**Movie 11.6**). Such experiments have revealed that, generally speaking, a cell membrane is about as viscous as olive oil.

One-by-one

One drawback to the FRAP approach is that the technique monitors the movement of fairly large populations of proteins—hundreds or thousands—across a relatively

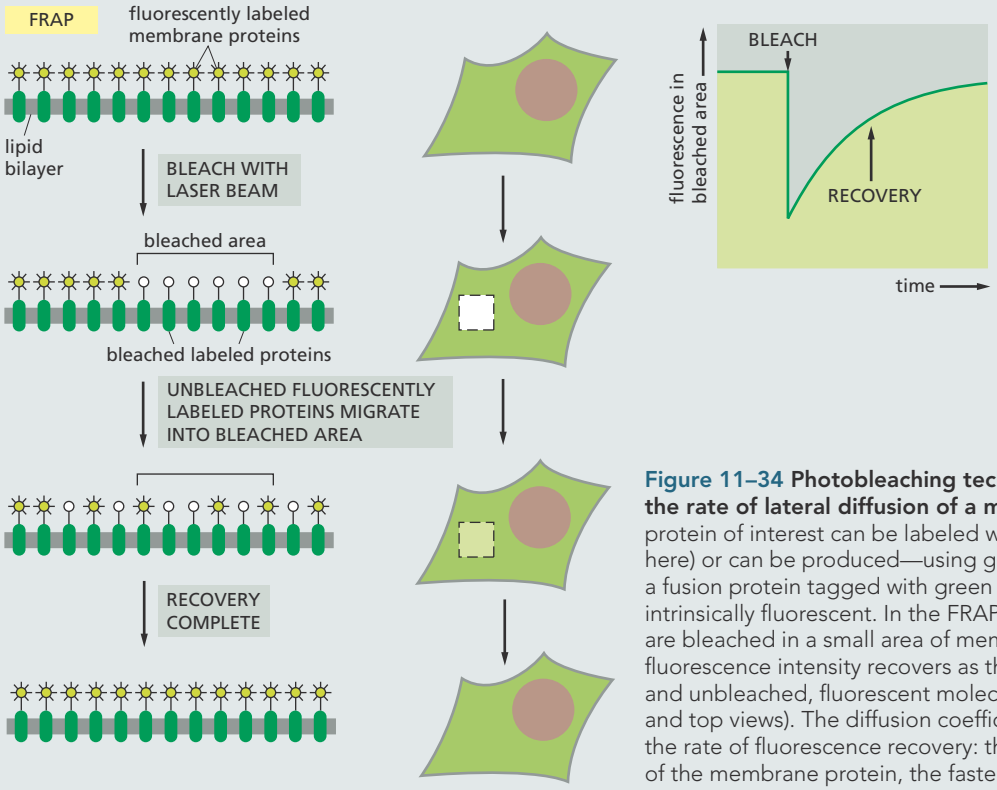


Figure 11–34 Photobleaching techniques can be used to measure the rate of lateral diffusion of a membrane protein. A specific protein of interest can be labeled with a fluorescent antibody (as shown here) or can be produced—using genetic engineering techniques—as a fusion protein tagged with green fluorescent protein (GFP), which is intrinsically fluorescent. In the FRAP technique, fluorescent molecules are bleached in a small area of membrane using a laser beam. The fluorescence intensity recovers as the bleached molecules diffuse away and unbleached, fluorescent molecules diffuse in (shown here in side and top views). The diffusion coefficient is calculated from a graph of the rate of fluorescence recovery: the greater the diffusion coefficient of the membrane protein, the faster the recovery.

large area of the membrane. With this technique it is impossible to track the motion of individual molecules. If the labeled proteins fail to migrate into the bleached zone over the course of a FRAP study, for example, is it because they are immobile, essentially anchored in one place in the membrane? Or, alternatively, are they restricted to movement within a very small region—fenced in by cytoskeletal proteins—and thus only appear motionless?

To get around this problem, researchers have developed methods for labeling and observing the movement of individual molecules or small clusters of molecules. One such technique, dubbed single-particle tracking (SPT) microscopy, relies on tagging protein molecules with antibody-coated gold nanoparticles. The gold particles look like tiny black dots when seen with a light microscope, and their movement, and thus the movement of individually tagged protein molecules, can be followed using video microscopy.

From the studies carried out to date, it appears that membrane proteins can display a variety of patterns of movement, from random diffusion to complete immobility (**Figure 11–35**). Some proteins rapidly switch between these different kinds of motion.

Freed from cells

In many cases, researchers wish to study the behavior of a particular type of membrane protein in a synthetic lipid bilayer, in the absence of other proteins that might restrain its movement or alter its activity. For such studies, membrane proteins can be isolated from cells and the protein of interest purified and reconstituted in artificial phospholipid vesicles (**Figure 11–36**). The lipids allow the purified protein to maintain its proper structure and function, so that its activity and behavior can be analyzed in detail.

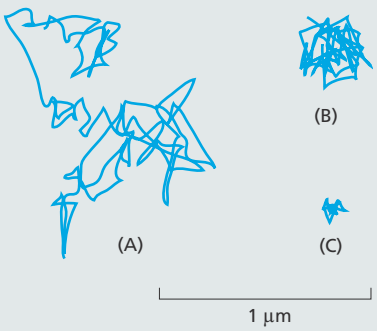


Figure 11–35 Proteins show different patterns of diffusion. Single-particle tracking studies reveal some of the pathways that real proteins follow on the surface of a living cell. Shown here are some trajectories representative of different kinds of proteins in the plasma membrane. (A) Tracks made by a protein that is free to diffuse randomly in the lipid bilayer. (B) Tracks made by a protein that is corralled within a small membrane domain by other proteins. (C) Tracks made by a protein that is tethered to the cytoskeleton and hence is essentially immobile. The movement of the proteins is monitored over a period of seconds.

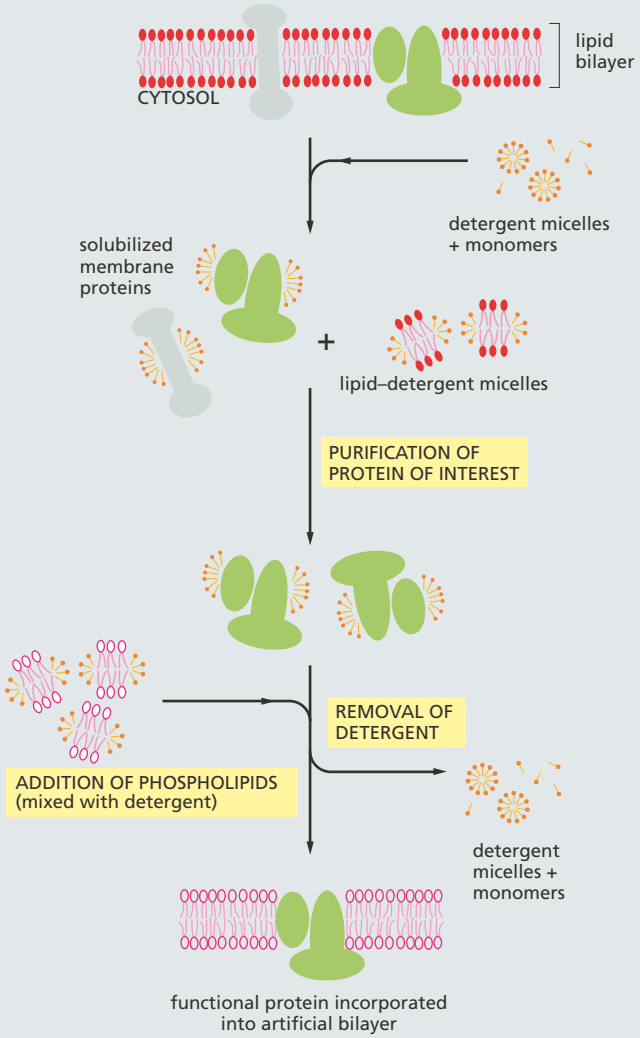


Figure 11–36 Mild detergents can be used to solubilize and reconstitute functional membrane proteins.

It is apparent from such studies that membrane proteins diffuse more freely and rapidly in artificial lipid bilayers than in cell membranes. The fact that most proteins show reduced mobility in a cell membrane makes sense, as these membranes are crowded with many types of proteins and contain a greater variety of lipids than an artificial lipid bilayer. Furthermore, many membrane proteins in a cell are tethered to proteins in the extracellular matrix, or anchored to the cell cortex just under the plasma membrane, or both (as illustrated in Figure 11–31).

Taken together, such studies have revolutionized our understanding of membrane proteins and of the architecture and organization of cell membranes.

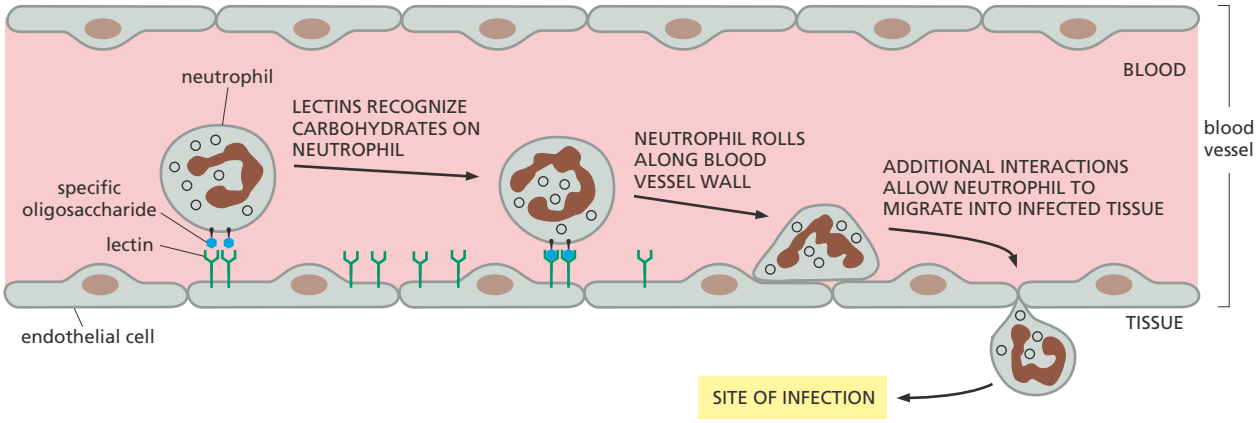


Figure 11–37 The recognition of the cell-surface carbohydrate on neutrophils is the first stage of their migration out of the blood at sites of infection. Specialized transmembrane proteins (called lectins) are made by the endothelial cells lining the blood vessel in response to chemical signals emanating from a site of infection. These proteins recognize particular sugar groups carried by glycolipids and glycoproteins on the surface of neutrophils (a type of white blood cell) circulating in the blood. The neutrophils consequently stick to the endothelial cells that line the blood vessel wall. This association is not very strong, but it leads to another, much stronger protein–protein interaction (not shown) that helps the neutrophil slip between the endothelial cells, so it can migrate out of the bloodstream and into the tissue at the site of infection (**Movie 11.7**).

Cell-surface carbohydrates do more than just protect and lubricate the cell, however. They have an important role in cell–cell recognition and adhesion. Just as many proteins will recognize a particular site on another protein, proteins called *lectins* are specialized to bind to particular oligosaccharide side chains. The oligosaccharide side chains of glycoproteins and glycolipids, although short (typically fewer than 15 sugar units), are enormously diverse. Unlike proteins, in which the amino acids are all joined together in a linear chain by identical peptide bonds, sugars can be joined together in many different arrangements, often forming elaborate branched structures (see Panel 2–3, pp. 70–71). Using a variety of covalent linkages, even three different sugars can form hundreds of different trisaccharides.

The carbohydrate layer on the surface of cells in a multicellular organism serves as a kind of distinctive clothing, like a police officer's uniform. It is characteristic of each cell type and is recognized by other cell types that interact with it. Specific oligosaccharides in the carbohydrate layer are involved, for example, in the recognition of an egg by a sperm (discussed in Chapter 19). Similarly, in the early stages of a bacterial infection, the carbohydrate on the surface of white blood cells called neutrophils is recognized by a lectin on the cells lining the blood vessels at the site of infection; this recognition causes the neutrophils to adhere to the blood vessel wall and then migrate from the bloodstream into the infected tissue, where they help destroy the invading bacteria (**Figure 11–37**).

ESSENTIAL CONCEPTS

- **Cell membranes enable cells to create barriers that confine particular molecules to specific compartments. They consist of a continuous double layer—a bilayer—of lipid molecules in which proteins are embedded.**
- **The lipid bilayer provides the basic structure and barrier function of all cell membranes.**
- **Membrane lipid molecules are amphipathic, having both hydrophobic and hydrophilic regions. This property promotes their spontaneous assembly into bilayers when placed in water, forming closed compartments that reseal if torn.**
- **There are three major classes of membrane lipid molecules: phospholipids, sterols, and glycolipids.**
- **The lipid bilayer is fluid, and individual lipid molecules are able to diffuse within their own monolayer; they do not, however, spontaneously flip from one monolayer to the other.**

- The two lipid monolayers of a cell membrane have different lipid compositions, reflecting the different functions of the two faces of the membrane.
- Cells that live at different temperatures maintain their membrane fluidity by modifying the lipid composition of their membranes.
- Membrane proteins are responsible for most of the functions of cell membranes, including the transport of small, water-soluble molecules across the lipid bilayer.
- Transmembrane proteins extend across the lipid bilayer, usually as one or more α helices but sometimes as a β sheet rolled into the form of a barrel.
- Other membrane proteins do not extend across the lipid bilayer but are attached to one or the other side of the membrane, either by noncovalent association with other membrane proteins, by covalent attachment of lipids, or by association of an exposed amphipathic α helix with a single lipid monolayer.
- Most cell membranes are supported by an attached framework of proteins. An especially important example is the meshwork of fibrous proteins that forms the cell cortex underneath the plasma membrane.
- Although many membrane proteins can diffuse rapidly in the plane of the membrane, cells have ways of confining proteins to specific membrane domains. They can also immobilize particular membrane proteins by attaching them to intracellular or extracellular macromolecules.
- Many of the proteins and some of the lipids exposed on the surface of cells have attached sugar chains, which form a carbohydrate layer that helps protect and lubricate the cell surface, while also being involved in specific cell–cell recognition.

KEY TERMS

amphipathic
bacteriorhodopsin
cholesterol
detergent
glycocalyx
lipid bilayer
membrane domain
membrane protein
phosphatidylcholine
phospholipid
plasma membrane
saturated
unsaturated

QUESTIONS

QUESTION 11–7

Describe the different methods that cells use to restrict proteins to specific regions of the plasma membrane. Is a membrane with many of its proteins restricted still fluid?

QUESTION 11–8

Which of the following statements are correct? Explain your answers.

A. Lipids in a lipid bilayer spin rapidly around their long axis.

B. Lipids in a lipid bilayer rapidly exchange positions with one another in their own monolayer.

C. Lipids in a lipid bilayer do not flip-flop readily from one lipid monolayer to the other.

D. Hydrogen bonds that form between lipid head groups and water molecules are continually broken and re-formed.

E. Glycolipids move between different membrane-enclosed compartments during their synthesis but remain restricted to one side of the lipid bilayer.

F. Margarine contains more saturated lipids than the vegetable oil from which it is made.

G. Some membrane proteins are enzymes.

H. The sugar layer that surrounds all cells makes cells more slippery.

QUESTION 11–9

What is meant by the term "two-dimensional fluid"?

QUESTION 11–10

The structure of a lipid bilayer is determined by the particular properties of its lipid molecules. What would happen if

A. Phospholipids had only one hydrocarbon tail instead of two?

B. The hydrocarbon tails were shorter than normal, say, about 10 carbon atoms long?

C. All of the hydrocarbon tails were saturated?

D. All of the hydrocarbon tails were unsaturated?

E. The bilayer contained a mixture of two kinds of phospholipid molecules, one with two saturated hydrocarbon tails and the other with two unsaturated hydrocarbon tails?

F. Each phospholipid molecule were covalently linked through the end carbon atom of one of its hydrocarbon tails to a phospholipid tail in the opposite monolayer?

QUESTION 11–11

What are the differences between a phospholipid molecule and a detergent molecule? How would the structure of a phospholipid molecule need to change to make it a detergent?

QUESTION 11–12

A. Membrane lipid molecules exchange places with their lipid neighbors every 10^{-7} second. A lipid molecule diffuses from one end of a 2-μm-long bacterial cell to the other in about 1 second. Are these two numbers in agreement (assume that the diameter of a lipid head group is about 0.5 nm)? If not, can you think of a reason for the difference?

B. To get an appreciation for the great speed of molecular diffusion, assume that a lipid head group is about the size of a ping-pong ball (4 cm in diameter) and that the floor of your living room (6 m × 6 m) is covered wall-to-wall with these balls. If two neighboring balls exchanged positions once every 10^{-7} second, what would their speed be in kilometers per hour? How long would it take for a ball to move from one side of the room to the opposite side?

QUESTION 11–13

Why does a red blood cell plasma membrane need transmembrane proteins?

QUESTION 11–14

Consider a transmembrane protein that forms a hydrophilic pore across the plasma membrane of a eukaryotic cell, allowing Na^+ to enter the cell when it is activated upon binding a specific ligand on its extracellular side. It is made of five similar transmembrane subunits, each containing a membrane-spanning α helix with hydrophilic amino acid side chains on one surface of the helix and hydrophobic amino acid side chains on the opposite surface. Considering the function of the protein as a channel for Na^+ ions to enter the cell, propose a possible arrangement of the five membrane-spanning α helices in the membrane.

QUESTION 11–15

In the membrane of a human red blood cell, the ratio of the mass of protein (average molecular weight 50,000) to phospholipid (molecular weight 800) to cholesterol (molecular weight 386) is about 2:1:1. How many lipid molecules are there for every protein molecule?

QUESTION 11–16

Draw a schematic diagram that shows a close-up view of two plasma membranes as they come together during cell fusion, as shown in Figure 11–30. Show membrane proteins in both cells that were labeled from the outside by the binding of differently colored fluorescent antibody molecules. Indicate in your drawing the fates of these color tags as the cells fuse. Will they remain on the outside of the hybrid cell after cell fusion and still be there after the mixing of membrane proteins that occurs during the incubation at 37°C? How would the experimental outcome be different if the incubation were done at 0°C?

QUESTION 11–17

Compare the hydrophobic forces that hold a membrane protein in the lipid bilayer with those that help proteins fold into a unique three-dimensional structure.

QUESTION 11–18

Predict which one of the following organisms will have the highest percentage of unsaturated phospholipids in its membranes. Explain your answer.

A. Antarctic fish

B. Desert snake

C. Human being

D. Polar bear

E. Thermophilic bacterium that lives in hot springs at 100°C.

QUESTION 11–19

Which of the three 20-amino-acid sequences listed below in the single-letter amino acid code is the most likely candidate to form a transmembrane region (α helix) of a transmembrane protein? Explain your answer.

A. I T L I Y F G N M S S V T Q T I L L I S

B. L L L I F F G V M A L V I V V I L L I A

C. L L K K F F R D M A A V H E T I L E E S

CHAPTER **TWELVE** 12

Transport Across Cell Membranes

To survive and grow, cells must be able to exchange molecules with their environment. They must import nutrients such as sugars and amino acids and eliminate metabolic waste products. They must also regulate the concentrations of a variety of inorganic ions in their cytosol and organelles. A few molecules, such as CO_2 and O_2, can simply diffuse across the lipid bilayer of the plasma membrane. But the vast majority cannot. Instead, their transfer depends on specialized **membrane transport proteins** that span the lipid bilayer, providing private passageways across the membrane for select substances (**Figure 12–1**).

In this chapter, we consider how cell membranes control the traffic of inorganic ions and small, water-soluble molecules into and out of the cell and its membrane-enclosed organelles. Cells can also selectively transfer macromolecules such as proteins across their membranes, but this transport requires more elaborate machinery and is discussed in Chapter 15.

We begin by outlining some of the general principles that guide the passage of ions and small molecules through cell membranes. We then examine, in turn, the two main classes of membrane proteins that mediate this transfer: transporters and channels. *Transporters* shift small organic molecules or inorganic ions from one side of the membrane to the other by changing shape. *Channels*, in contrast, form tiny hydrophilic pores across the membrane through which such substances can pass by diffusion. Most channels only permit passage of inorganic ions and are therefore called *ion channels*. Because these ions are electrically charged, their movements can create a powerful electric force—or voltage—across the membrane. In the final part of the chapter, we discuss how these voltage differences enable nerve cells to communicate—and ultimately to shape our behavior.

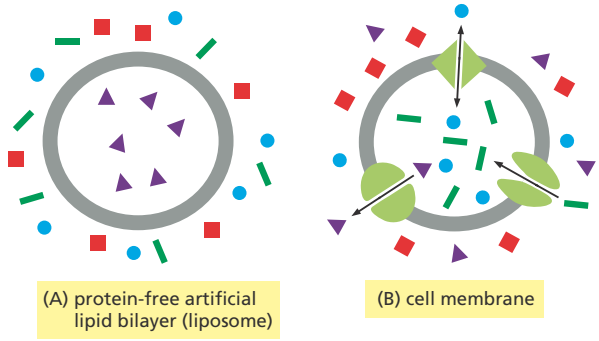


Figure 12–1 Cell membranes contain specialized membrane transport proteins that facilitate the passage of selected small water-soluble molecules. (A) Protein-free, artificial lipid bilayers such as liposomes (see Figure 11–13) are impermeable to most water-soluble molecules. (B) Cell membranes, by contrast, contain transport proteins, each of which transfers a particular type of molecule. This selective transport can include the active pumping of specific molecules either out of (*purple triangles*) or into (*green bars*) the cell. The combined action of different transport proteins allows a specific set of solutes to build up inside a membrane-enclosed compartment, such as the cytosol or an organelle.

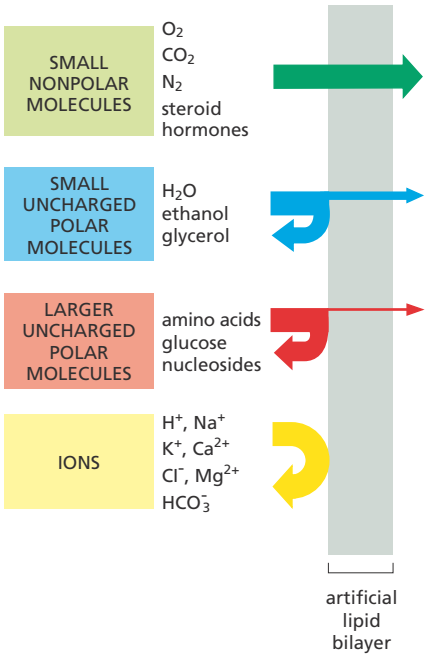


Figure 12–2 The rate at which a molecule crosses a protein-free artificial lipid bilayer by simple diffusion depends on its size and solubility. The smaller the molecule and, more importantly, the fewer its favorable interactions with water (that is, the less polar it is), the more rapidly the molecule diffuses across the bilayer. Note that many of the organic molecules that a cell uses as nutrients (shaded in *red*) are too large and polar to pass through an artificial lipid bilayer that does not contain the appropriate membrane-transport proteins.

PRINCIPLES OF TRANSMEMBRANE TRANSPORT

As we saw in Chapter 11, the hydrophobic interior of the lipid bilayer creates a barrier to the passage of most hydrophilic molecules, including all ions. These molecules are as reluctant to enter a fatty environment as hydrophobic molecules are reluctant to enter water. But cells and organelles must also allow the passage of many hydrophilic, water-soluble molecules, such as inorganic ions, sugars, amino acids, nucleotides, and other cell metabolites. These molecules cross lipid bilayers far too slowly by *simple diffusion*, so their passage across cell membranes must be accelerated by specialized membrane transport proteins—a process called *facilitated transport*. In this section, we review the basic principles of such facilitated transmembrane transport and introduce the various types of membrane transport proteins that mediate this movement. We also discuss why the transport of inorganic ions, in particular, is of such fundamental importance for all cells.

Lipid Bilayers Are Impermeable to Ions and Most Uncharged Polar Molecules

Given enough time, virtually any molecule will diffuse across a lipid bilayer. The rate at which it diffuses, however, varies enormously depending on the size of the molecule and its solubility properties. In general, the smaller the molecule and the more hydrophobic, or nonpolar, it is, the more rapidly it will diffuse across the membrane.

Of course, many of the molecules that are of interest to cells are polar and water-soluble. These *solutes*—substances that, in this case, are dissolved in water—are unable to cross the lipid bilayer without the aid of membrane transport proteins. The relative ease with which a variety of solutes can cross cell membranes is shown in **Figure 12–2**.

1. *Small nonpolar molecules*, such as molecular oxygen (O_2, molecular mass 32 daltons) and carbon dioxide (CO_2, 44 daltons), dissolve readily in lipid bilayers and therefore rapidly diffuse across them; indeed, cells depend on this permeability to gases for the cell respiration processes discussed in Chapter 14.
2. *Uncharged polar molecules* (molecules with an uneven distribution of electric charge) also diffuse readily across a bilayer if they are small enough. Water (H_2O, 18 daltons) and ethanol (46 daltons), for example, cross at a measureable rate, while glycerol (92 daltons) crosses less rapidly. Larger uncharged polar molecules such as glucose (180 daltons) cross hardly at all.
3. In contrast, lipid bilayers are highly impermeable to all *charged molecules,* including all inorganic ions, no matter how small. These molecules' charges and their strong electrical attraction to water

molecules inhibit their entry into the inner, hydrocarbon phase of the bilayer. Thus synthetic lipid bilayers are a billion (10^9) times more permeable to water than they are to even small ions such as Na^+ or K^+.

The Ion Concentrations Inside a Cell Are Very Different from Those Outside

Because cell membranes are impermeable to inorganic ions, living cells are able to maintain internal ion concentrations that are very different from the concentrations of ions in the media that surrounds them. These differences in ion concentration are crucial for a cell's survival and function. Among the most important inorganic ions for cells are Na^+, K^+, Ca^{2+}, Cl^-, and H^+ (protons). The movement of these ions across cell membranes plays an essential part in many biological processes, but is perhaps most striking in the production of ATP by all cells, and in communication by nerve cells (to be discussed later).

Na^+ is the most plentiful positively charged ion (cation) outside the cell, whereas K^+ is the most abundant inside (**Table 12–1**). For a cell to avoid being torn apart by electrical forces, the quantity of positive charge inside the cell must be balanced by an almost exactly equal quantity of negative charge, and the same is true for the charge in the surrounding fluid. The high concentration of Na^+ outside the cell is electrically balanced chiefly by extracellular Cl^-, whereas the high concentration of K^+ inside is balanced by a variety of negatively charged organic and inorganic ions (anions) including nucleic acids, proteins, and many cell metabolites (see Table 12–1).

Differences in the Concentration of Inorganic Ions Across a Cell Membrane Create a Membrane Potential

Although the electrical charges inside and outside the cell are generally kept in balance, tiny excesses of positive or negative charge, concentrated in the neighborhood of the plasma membrane, do occur. Such electrical imbalances generate a voltage difference across the membrane called the **membrane potential**.

TABLE 12–1 A COMPARISON OF ION CONCENTRATIONS INSIDE AND OUTSIDE A TYPICAL MAMMALIAN CELL

Component	Intracellular Concentration (mM)	Extracellular Concentration (mM)
Cations		
Na^+	5–15	145
K^+	140	5
Mg^{2+}	0.5*	1–2
Ca^{2+}	10^{-4}*	1–2
H^+	7×10^{-5} ($10^{-7.2}$ M or pH 7.2)	4×10^{-5} ($10^{-7.4}$ M or pH 7.4)
Anions**		
Cl^-	5–15	110

*The concentrations of Mg^{2+} and Ca^{2+} given are for the free ions. There is a total of about 20 mM Mg^{2+} and 1–2 mM Ca^{2+} in cells, but these ions are mostly bound to proteins and other organic molecules and, for Ca^{2+}, stored within various organelles.
**In addition to Cl^-, a cell contains many other anions not listed in this table. In fact, most cell constituents are negatively charged (HCO_3^-, PO_4^{3-}, proteins, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.).

When a cell is "unstimulated," the exchange of anions and cations across the membrane will be precisely balanced. In such steady-state conditions, the voltage difference across the cell membrane—called the *resting membrane potential*—holds steady. But it is not zero. In animal cells, for example, the resting membrane potential can be anywhere between –20 and –200 millivolts (mV), depending on the organism and cell type. The value is expressed as a negative number because the interior of the cell is more negatively charged than the exterior. This membrane potential allows cells to power the transport of certain metabolites and provides those cells that are excitable with a means to communicate with their neighbors.

It is the activity of membrane transport proteins embedded in the bilayer that enables cells to establish and maintain their membrane potential, as we discuss next.

Cells Contain Two Classes of Membrane Transport Proteins: Transporters and Channels

Membrane transport proteins occur in many forms and are present in all cell membranes. Each provides a private portal across the membrane for a particular small, water-soluble molecule—an ion, sugar, or amino acid, for example. Most of these proteins allow passage of only select members of a particular molecular class: some permit transit of Na^+ but not K^+, others K^+ but not Na^+, and so on. Each type of cell membrane has its own characteristic set of transport proteins, which determines exactly which solutes can pass into and out of the cell or an organelle.

As discussed in Chapter 11, most membrane transport proteins have polypeptide chains that traverse the lipid bilayer multiple times—that is, they are multipass transmembrane proteins (see Figure 11–23). By criss-crossing back and forth across the bilayer, the polypeptide chain forms a continuous protein-lined pathway that allows selected small, hydrophilic molecules to cross the membrane without coming into direct contact with the hydrophobic interior of the lipid bilayer.

There are two main classes of membrane transport proteins: transporters and channels. These proteins differ in the way they discriminate between solutes, transporting some but not others (**Figure 12–3**). *Channels* discriminate mainly on the basis of size and electric charge: when the channel is open, any ion or molecule that is small enough and carries the appropriate charge can pass through. A *transporter*, on the other hand, transfers only those molecules or ions that fit into specific binding sites on the protein. Transporters bind their solutes with great specificity, in the same way an enzyme binds its substrate, and it is this requirement for specific binding that gives transporters their selectivity.

Solutes Cross Membranes by Either Passive or Active Transport

Transporters and channels allow small hydrophilic molecules to cross the cell membrane, but what controls whether these solutes move into the

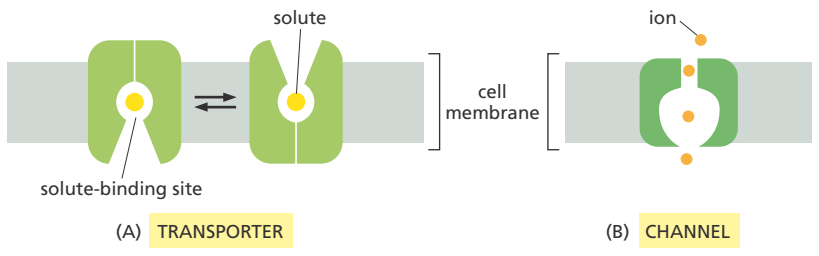


Figure 12–3 Inorganic ions and small, polar organic molecules can cross a cell membrane through either a transporter or a channel. (A) A transporter undergoes a series of conformational changes to transfer small solutes across the lipid bilayer. (B) A channel, when open, forms a pore across the bilayer through which specific inorganic ions or, in some cases, polar organic molecules can diffuse. As would be expected, channels transfer solutes at a much greater rate than transporters.

Ion channels can exist in either an open or a closed conformation, and they transport only in the open conformation, which is shown here. Channel opening and closing is usually controlled by an external stimulus or by conditions within the cell.

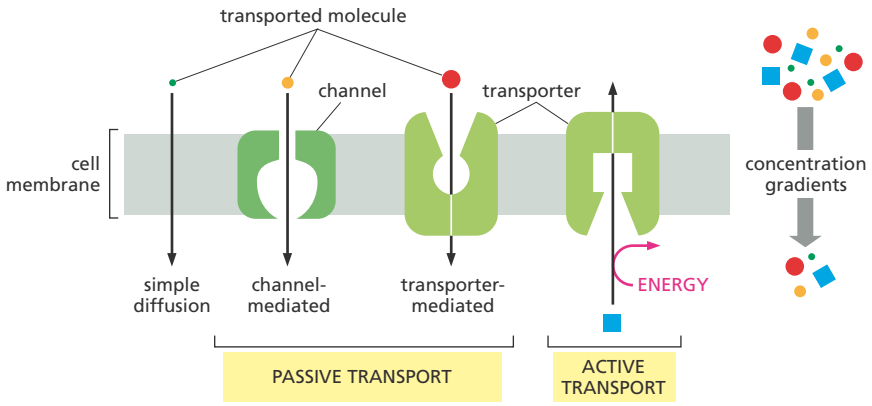


Figure 12–4 Solutes cross cell membranes by either passive or active transport. Some small nonpolar molecules such as CO_2 (see Figure 12–2) can move passively down their concentration gradient across the lipid bilayer by simple diffusion, without the help of a transport protein. Most solutes, however, require the assistance of a channel or transporter. Passive transport, which allows molecules to move down their concentration gradients, occurs spontaneously; whereas active transport against a concentration gradient requires an input of energy. Only transporters can carry out active transport.

cell or organelle—or out of it? In many cases, the direction of transport depends only on the relative concentrations of the solute on either side of the membrane. Molecules will spontaneously flow "downhill" from a region of high concentration to a region of low concentration, provided a pathway exists. Such movements are called passive, because they need no additional driving force. If, for example, a solute is present at a higher concentration outside the cell than inside, and an appropriate channel or transporter is present in the plasma membrane, the solute will move into the cell by **passive transport**, without expenditure of energy by the transport protein. This is because even though the solute moves in both directions across the membrane, more solute will move in than out until the two concentrations equilibrate. All channels and many transporters act as conduits for such passive transport.

To move a solute against its concentration gradient, a membrane transport protein must do work: it has to drive the flow "uphill" by coupling it to some other process that provides an input of energy (as discussed in Chapter 3 for enzyme-catalyzed reactions). The movement of a solute against its concentration gradient in this way is termed **active transport**, and it is carried out by special types of transporters called *pumps*, which harness an energy source to power the transport process (**Figure 12–4**). As discussed later, this energy can come from ATP hydrolysis, a transmembrane ion gradient, or sunlight.

Both the Concentration Gradient and Membrane Potential Influence the Passive Transport of Charged Solutes

For an uncharged molecule, the direction of passive transport is determined solely by its concentration gradient, as we have implied above. But for electrically charged molecules, whether inorganic ions or small organic molecules, an additional force comes into play. As mentioned earlier, most cell membranes have a voltage across them—a difference in charge referred to as a membrane potential. The membrane potential exerts a force on any molecule that carries an electric charge. The cytosolic side of the plasma membrane is usually at a negative potential relative to the extracellular side, so the membrane potential tends to pull positively charged solutes into the cell and drive negatively charged ones out.

At the same time, a charged solute will also tend to move down its concentration gradient. The net force driving a charged solute across a cell membrane is therefore a composite of two forces, one due to the concentration gradient and the other due to the membrane potential. This net driving force, called the solute's **electrochemical gradient**, determines

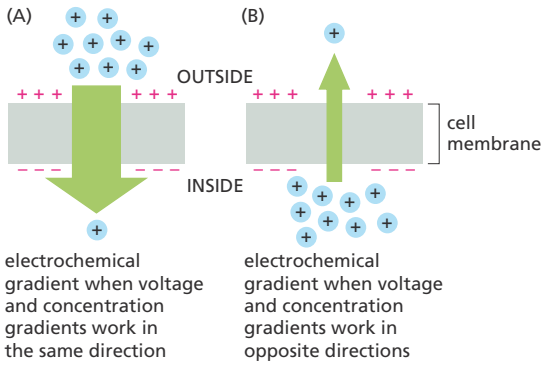


Figure 12–5 An electrochemical gradient has two components. The net driving force (the electrochemical gradient) tending to move a charged solute (ion) across a cell membrane is the sum of a force from the concentration gradient of the solute and a force from the membrane potential. The membrane potential is represented here by the + and – signs on opposite sides of the membrane. The width of the *green* arrow represents the magnitude of the electrochemical gradient for a positively charged solute in two different situations. In (A), the concentration gradient and membrane potential work together to increase the driving force for movement of the solute. In (B), the membrane potential acts against the concentration gradient, decreasing the electrochemical driving force.

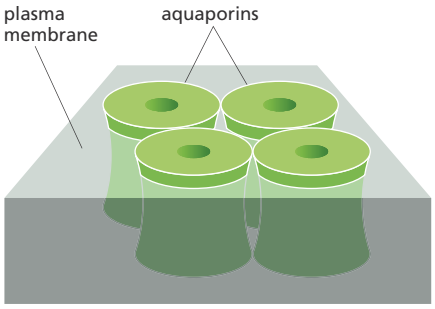
the direction that each solute will flow across the membrane by passive transport. For some ions, the voltage and concentration gradients work in the same direction, creating a relatively steep electrochemical gradient (**Figure 12–5A**). This is the case for Na^+, which is positively charged and at a higher concentration outside cells than inside (see Table 12–1). Na^+ therefore tends to enter cells if given an opportunity. If, however, the voltage and concentration gradients have opposing effects, the resulting electrochemical gradient can be small (**Figure 12–5B**). This is the case for K^+, which is present at a much higher concentration inside cells than outside. Because of its small electrochemical gradient across the resting plasma membrane, there is little net movement of K^+ across the membrane even when K^+ channels are open.

Water Moves Passively Across Cell Membranes Down Its Concentration Gradient—a Process Called Osmosis

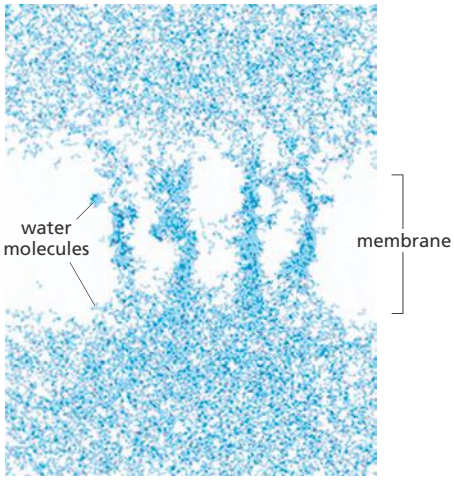
Cells are mostly water (generally about 70% by weight), and so the movement of water across cell membranes is crucially important for living things. Because water molecules are small and uncharged, they can diffuse directly across the lipid bilayer—although slowly (see Figure 12–2). However, some cells also contain specialized channel proteins called *aquaporins* in their plasma membrane, which greatly facilitate this flow (**Figure 12–6** and **Movie 12.1**).

But which way does water tend to flow? As we saw in Table 12–1, cells contain a high concentration of solutes, including many charged molecules and ions. Thus the total concentration of solute particles inside the cell—also called its *osmolarity*—generally exceeds solute concentration outside the cell. The resulting osmotic gradient tends to "pull" water into the cell. This movement of water down its concentration gradient—from an area of low solute concentration (high water concentration) to an area of high solute concentration (low water concentration)—is called **osmosis**.

Osmosis, if it occurs without constraint, can make a cell swell. Different cells cope with this osmotic challenge in different ways. Most animal



(A)



(B)

Figure 12–6 Water molecules diffuse rapidly through aquaporin channels in the plasma membrane of some cells. (A) Shaped like an hourglass, each aquaporin channel forms a pore across the bilayer, allowing the selective passage of water molecules. Shown here is an aquaporin tetramer, the biologically active form of the protein. (B) In this snapshot, taken from a real-time, molecular dynamics simulation, four columns of water molecules can be seen passing though the pores of an aquaporin tetramer (not shown). The space where the membrane would be located is indicated. (B, adapted from B. de Groot and H. Grubmüller, *Science* 294:2353–2357, 2001.)

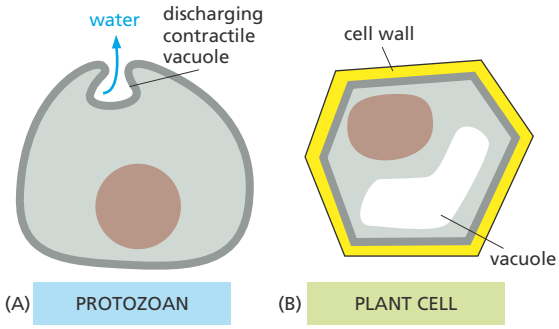


Figure 12–7 Cells use different tactics to avoid osmotic swelling. (A) A fresh water amoeba avoids swelling by periodically ejecting the water that moves into the cell and accumulates in contractile vacuoles. The contractile vacuole first accumulates solutes, which cause water to follow by osmosis; it then pumps most of the solutes back into the cytosol before emptying its contents at the cell surface. (B) The plant cell's tough cell wall prevents swelling.

cells have a gel-like cytoplasm (see Figure 1–25) that resists osmotic swelling. Some fresh water protozoans, such as amoebae, eliminate excess water using contractile vacuoles that periodically discharge their contents to the exterior (**Figure 12–7A**). Plant cells are prevented from swelling by their tough cell walls and so can tolerate a large osmotic difference across their plasma membrane (**Figure 12–7B**); indeed, plant cells make use of osmotic swelling pressure, or *turgor pressure*, to keep their cell walls tense, so that the stems of the plant are rigid and its leaves are extended. If turgor pressure is lost, plants wilt.

TRANSPORTERS AND THEIR FUNCTIONS

Transporters are responsible for the movement of most small, water-soluble, organic molecules and some inorganic ions across cell membranes. Each transporter is highly selective, often transferring just one type of molecule. To guide and propel the complex traffic of solutes into and out of the cell, and between the cytosol and the different membrane-enclosed organelles, each cell membrane contains a characteristic set of different transporters appropriate to that particular membrane. For example, the plasma membrane contains transporters that import nutrients such as sugars, amino acids, and nucleotides; the lysosome membrane contains an H^+ transporter that imports H^+ to acidify the lysosome interior and other transporters that move digestion products out of the lysosome into the cytosol; the inner membrane of mitochondria contains transporters for importing the pyruvate that mitochondria use as fuel for generating ATP, as well as transporters for exporting ATP once it is synthesized (**Figure 12–8**).

In this section, we describe the general principles that govern the function of transporters, and we present a more detailed view of the molecular mechanisms that drive the movement of a few key solutes.

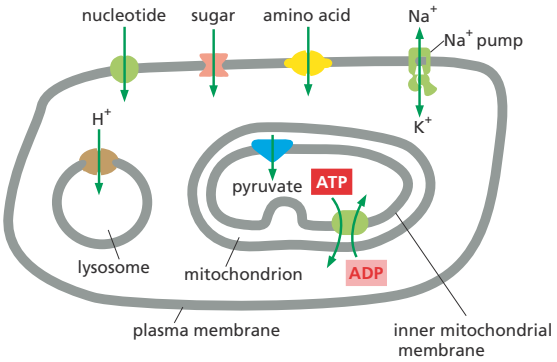


Figure 12–8 Each cell membrane has its own characteristic set of transporters. Only a few of these are indicated here.

Passive Transporters Move a Solute Along Its Electrochemical Gradient

An important example of a transporter that mediates passive transport is the *glucose transporter* in the plasma membrane of many mammalian cell types. The protein, which consists of a polypeptide chain that crosses the membrane at least 12 times, can adopt several conformations—and it switches reversibly and randomly between them. In one conformation, the transporter exposes binding sites for glucose to the exterior of the cell; in another, it exposes the sites to the cell interior.

Because glucose is uncharged, the chemical component of its electrochemical gradient is zero. Thus the direction in which it is transported is determined by its concentration gradient alone. When glucose is plentiful outside cells, as it is after a meal, the sugar binds to the transporter's externally displayed binding sites; when the protein switches conformation—spontaneously and at random—it carries the bound sugar inward and releases it into the cytosol, where the glucose concentration is low (**Figure 12–9**). Conversely, when blood glucose levels are low as they are when you are hungry—the hormone glucagon stimulates liver cells to produce large amounts of glucose by the breakdown of glycogen. As a result, the glucose concentration is higher inside liver cells than outside. This glucose binds to the internally displayed binding sites on the transporter. When the protein switches conformation in the opposite direction, the glucose is transported out of the cells, where it is made available for others to import. The net flow of glucose can thus go either way, according to the direction of the glucose concentration gradient across the plasma membrane: inward if glucose is more concentrated outside the cell than inside, and outward if the opposite is true.

Although passive transporters of this type play no part in determining the direction of transport, they are highly selective. For example, the binding sites in the glucose transporter bind only D-glucose and not its mirror image, L-glucose, which the cell cannot use for glycolysis.

QUESTION 12–1

A simple enzyme reaction can be described by the equation $E + S \leftrightarrow ES \leftrightarrow E + P$, where E is the enzyme, S the substrate, P the product, and ES the enzyme–substrate complex.

A. Write a corresponding equation describing the workings of a transporter (T) that mediates the transport of a solute (S) down its concentration gradient.

B. What does this equation tell you about the function of a transporter?

C. Why would this equation be an inappropriate description of channel function?

Pumps Actively Transport a Solute Against Its Electrochemical Gradient

Cells cannot rely solely on passive transport. An active transport of solutes against their electrochemical gradient is essential to maintain the appropriate intracellular ionic composition of cells and to import solutes that are at a lower concentration outside the cell than inside. For these purposes, cells depend on transmembrane **pumps**, which can carry out

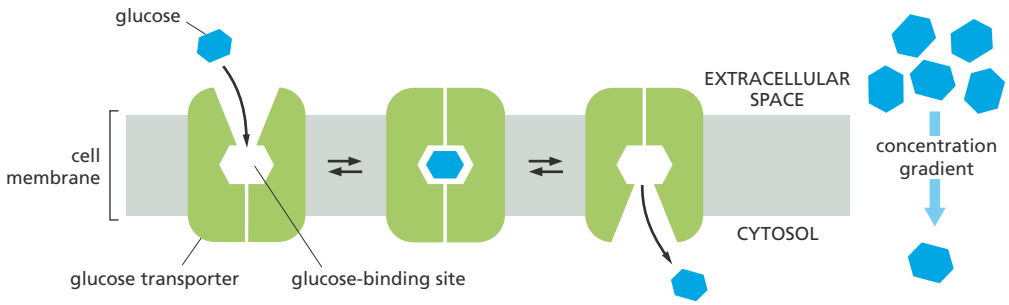


Figure 12–9 Conformational changes in a transporter mediate the passive transport of a solute such as glucose. The transporter is shown in three conformational states: in the outward–open state (*left*), the binding sites for solute are exposed on the outside; in the inward–open state (*right*), the sites are exposed on the inside of the bilayer, and in the occluded state (*center*), the sites are not accessible from either side. The transition between the states occurs randomly, is completely reversible, and—most importantly for the function of the transporter shown—does not depend on whether the solute-binding site is occupied. Therefore, if the solute concentration is higher on the outside of the bilayer, more solute will bind to the transporter in the outward–open conformation than in the inward–open conformation, and there will be a net transport of glucose down its concentration gradient.

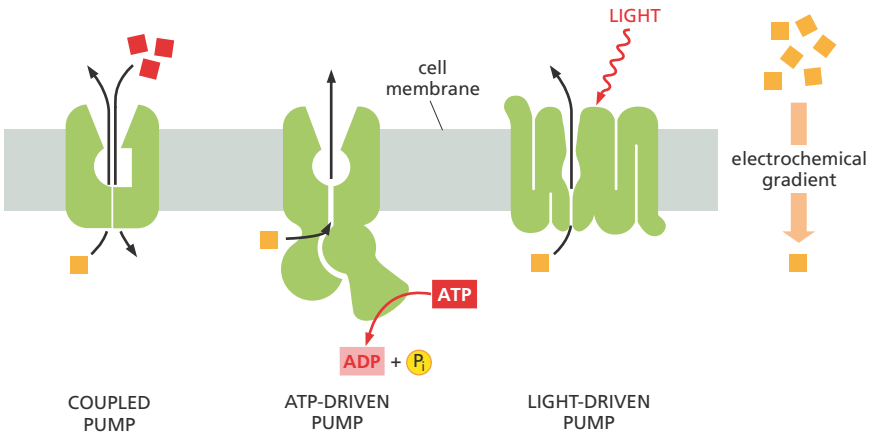


Figure 12–10 Pumps carry out active transport in three main ways. The actively transported generic molecule is shown in *yellow*, and the energy source is shown in *red*.

active transport in three main ways (**Figure 12–10**): (i) *ATP-driven pumps* hydrolyze ATP to drive uphill transport. (ii) *Coupled pumps* link the uphill transport of one solute across a membrane to the downhill transport of another. (iii) *Light-driven pumps*, which are found mainly in bacterial cells, use energy derived from sunlight to drive uphill transport, as discussed in Chapter 11 for bacteriorhodopsin (see Figure 11–27).

The different forms of active transport are often linked. Thus, in the plasma membrane of an animal cell, an ATP-driven Na^+ pump transports Na^+ out of the cell against its electrochemical gradient; this Na^+ can then flow back into the cell, down its electrochemical gradient. As the ion flows back in through various Na^+-coupled pumps, the influx of Na^+ provides the energy for the active transport of many other substances into the cell against their electrochemical gradients. If the Na^+ pump ceased operating, the Na^+ gradient would soon run down, and transport through Na^+-coupled pumps would come to a halt. For this reason, the ATP-driven Na^+ pump has a central role in the active transport of small molecules across the plasma membrane of animal cells. Plant cells, fungi, and many bacteria, use ATP-driven H^+ pumps in an analogous way: in pumping H^+ out of the cell, these proteins create an electrochemical gradient of H^+ across the plasma membrane that is subsequently harnessed for solute transport, as we discuss later.

The Na^+ Pump in Animal Cells Uses Energy Supplied by ATP to Expel Na^+ and Bring in K^+

The ATP-driven **Na^+ pump** plays such a central part in the energy economy of animal cells, that it typically accounts for 30% or more of their total ATP consumption. This pump uses the energy derived from ATP hydrolysis to transport Na^+ out of the cell as it carries K^+ in. The pump is therefore also known as the *Na^+-K^+ ATPase* or the *Na^+-K^+ pump*.

The energy from ATP hydrolysis induces a series of protein conformational changes that drive the Na^+/K^+ ion exchange. As part of the process, the phosphate group removed from ATP gets transferred to the pump itself (**Figure 12–11**).

The ion transport (Na^+ out, K^+ in) involves a reaction cycle, in which each step depends on the one before. If any of the individual steps is prevented from occurring, the entire cycle halts. The toxin, ouabain, for example, inhibits the pump by preventing the binding of extracellular K^+, arresting the cycle. The process is very efficient: the whole cycle takes only 10 milliseconds. Furthermore, the tight coupling between steps in the pumping cycle ensures that the pump operates only when the appropriate ions are available to be transported, thereby avoiding useless ATP hydrolysis.

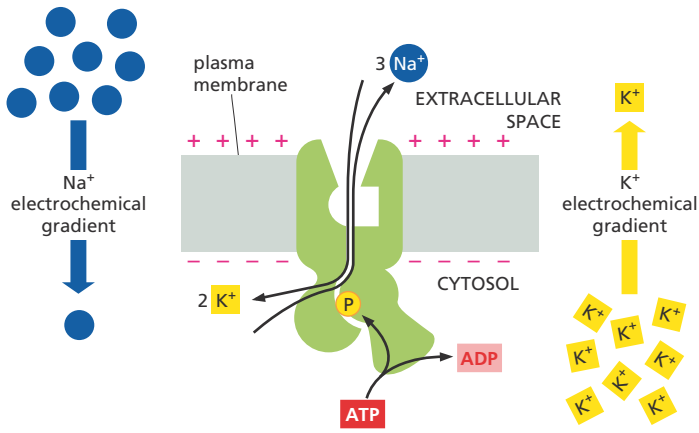


Figure 12–11 The Na^+ pump uses the energy of ATP hydrolysis to pump Na^+ out of animal cells and K^+ in. In this way, the pump helps keep the cytosolic concentrations of Na^+ low and K^+ high (Movie 12.2).

The Na^+ Pump Generates a Steep Concentration Gradient of Na^+ Across the Plasma Membrane

The Na^+ pump functions like a bilge pump in a leaky ship, ceaselessly expeling the Na^+ that is constantly entering the cell through other transporters and ion channels in the plasma membrane. In this way, the pump keeps the Na^+ concentration in the cytosol about 10–30 times lower than in the extracellular fluid and the K^+ concentration about 10–30 times higher (see Table 12–1, p. 385).

The steep concentration gradient of Na^+ across the plasma membrane acts together with the membrane potential to create a large Na^+ electrochemical gradient, which tends to pull Na^+ back into the cell (see Figure 12–5A). This high concentration of Na^+ outside the cell, on the uphill side of its electrochemical gradient, is like a large volume of water behind a high dam: it represents a very large store of energy (**Figure 12–12**). Even if one artificially halts the operation of the Na^+ pump with ouabain, this stored energy is sufficient to sustain for many minutes the various pumps in the plasma membrane that are driven by the downhill flow of Na^+, which we discuss shortly.

Figure 12–12 The high concentration of Na^+ outside the cell is like water behind a high dam. The water in the dam has potential energy, which can be used to drive energy-requiring processes. In the same way, an ion gradient across a membrane can be used to drive active processes in a cell, including the active transport of other molecules across the plasma membrane. Shown here is the Table Rock Dam in Branson, Missouri, USA. (Courtesy of K. Trimble.)

Ca^{2+} Pumps Keep the Cytosolic Ca^{2+} Concentration Low

Ca^{2+}, like Na^+, is also kept at a low concentration in the cytosol compared with its concentration in the extracellular fluid, but it is much less plentiful than Na^+, both inside and outside cells (see Table 12–1). The movement of Ca^{2+} across cell membranes is nonetheless crucial, because Ca^{2+} can bind tightly to a variety of proteins in the cell, altering their activities. An influx of Ca^{2+} into the cytosol through Ca^{2+} channels, for example, is used by different cells as an intracellular signal to trigger various cell processes, such as muscle contraction (discussed in Chapter 17), fertilization (discussed in Chapters 16 and 19), and nerve cell communication, discussed later.

The lower the background concentration of free Ca^{2+} in the cytosol, the more sensitive the cell is to an increase in cytosolic Ca^{2+}. Thus eukaryotic cells in general maintain a very low concentration of free Ca^{2+} in their cytosol (about 10^{-4} mM) in the face of a very much higher extracellular Ca^{2+} concentration (typically 1–2 mM). This huge concentration difference is achieved mainly by means of ATP-driven **Ca^{2+} pumps** in both the plasma membrane and the endoplasmic reticulum membrane, which actively pump Ca^{2+} out of the cytosol.

Ca^{2+} pumps are ATPases that work in much the same way as the Na^+ pump depicted in Figure 12–11. The main difference is that Ca^{2+} pumps

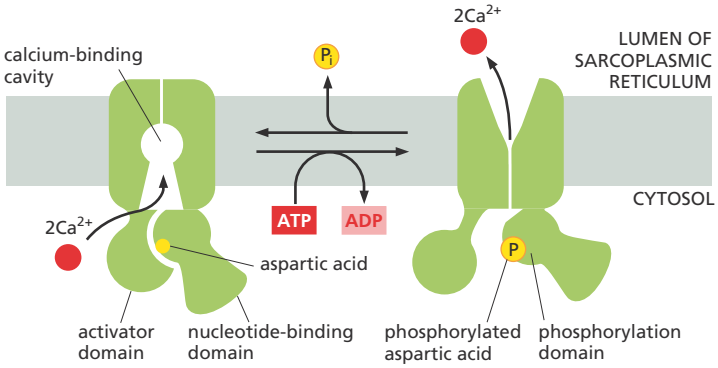


Figure 12–13 The Ca^{2+} pump in the sarcoplasmic reticulum was the first ATP-driven ion pump to have its three-dimensional structure determined by X-ray crystallography. When a muscle cell is stimulated, Ca^{2+} floods into the cytosol from the sarcoplasmic reticulum—a specialized form of endoplasmic reticulum. The influx of Ca^{2+} stimulates the cell to contract; to recover from the contraction, Ca^{2+} must be pumped back into the sarcoplasmic reticulum by this Ca^{2+} pump.

The Ca^{2+} pump uses ATP to phosphorylate itself, inducing a series of conformational changes that—when the pump is open to the lumen of the sarcoplasmic reticulum—eliminate the Ca^{2+}-binding sites, ejecting the two Ca^{2+} ions into the organelle.

return to their original conformation without a requirement for binding and transporting a second ion (**Figure 12–13**). The Na^+ and Ca^{2+} pumps have similar amino acid sequences and structures, indicating that they share a common evolutionary origin.

Coupled Pumps Exploit Solute Gradients to Mediate Active Transport

A gradient of any solute across a membrane, like the electrochemical Na^+ gradient generated by the Na^+ pump, can be used to drive the active transport of a second molecule. The downhill movement of the first solute down its gradient provides the energy to power the uphill transport of the second. The active transporters that work in this way are called **coupled pumps** (see Figure 12–10). They can couple the movement of one inorganic ion to that of another, the movement of an inorganic ion to that of a small organic molecule, or the movement of one small organic molecule to that of another. If the pump moves both solutes in the same direction across the membrane, it is called a *symport*. If it moves them in opposite directions, it is called an *antiport*. A transporter that ferries only one type of solute across the membrane (and is therefore not a coupled transporter) is called a *uniport* (**Figure 12–14**). The passive glucose transporter described earlier (see Figure 12–9) is an example of a uniport.

The Electrochemical Na^+ Gradient Drives Coupled Pumps in the Plasma Membrane of Animal Cells

Symports that make use of the inward flow of Na^+ down its steep electrochemical gradient have an especially important role in driving the import of other solutes into animal cells. The epithelial cells that line the gut, for example, pump glucose from the gut lumen across the gut epithelium and, ultimately, into the blood. If these cells had only the passive glucose uniport just mentioned, they would release glucose into the gut

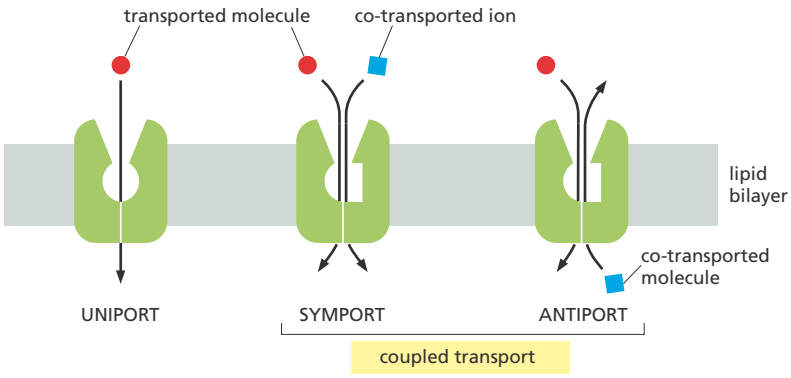


Figure 12–14 Transporters can function as uniports, symports, or antiports. Transporters that carry a single solute across the membrane are called uniports. Transporters that move multiple solutes are called coupled transporters. In coupled transport, the solutes can be transferred either in the same direction, by symports, or in the opposite direction, by antiports (**Movie 12.3**). Uniports, symports, and antiports can be used for either passive or active transport. Some coupled transporters, for example, act as pumps, coupling the uphill transport of one solute to the downhill transport of another.

after fasting as freely as they take it up from the gut after a feast (see Figure 12–9). But these epithelial cells also possess a *glucose–Na^+ symport*, which they can use to take up glucose from the gut lumen, even when the concentration of glucose is higher in the cell's cytosol than it is in the gut lumen. Because the electrochemical gradient for Na^+ is steep, when Na^+ moves into the cell down its gradient, glucose is, in a sense, "dragged" into the cell with it. Because the binding of Na^+ and glucose is cooperative—the binding of one enhances the binding of the other—if one of the two solutes is missing, the other fails to bind; therefore both molecules must be present for coupled transport to occur (**Figure 12–15**).

If the gut epithelial cells had only this symport, however, they could never release glucose for use by the other cells of the body. These cells, therefore, have two types of glucose transporters located at opposite ends of the cell. In the apical domain of the plasma membrane, which faces the gut lumen, they have the glucose–Na^+ symports. These take up glucose actively, creating a high glucose concentration in the cytosol. In the basal and lateral domains of the plasma membrane, the cells have the passive glucose uniports, which release the glucose down its concentration gradient for use by other tissues (**Figure 12–16**). As shown in the figure, the two types of glucose transporters are kept segregated in their proper domains of the plasma membrane by a diffusion barrier formed by a tight junction around the apex of the cell. This prevents mixing of membrane components between the two domains, as discussed in Chapter 11 (see Figure 11–32).

Cells in the lining of the gut and in many other organs, including the kidney, contain a variety of active symports in their plasma membrane that are similarly driven by the electrochemical gradient of Na^+; each of these coupled pumps specifically imports a small group of related sugars or amino acids into the cell. But Na^+-driven pumps that operate as antiports are also important for cells. For example, the *Na^+–H^+ exchanger* in the

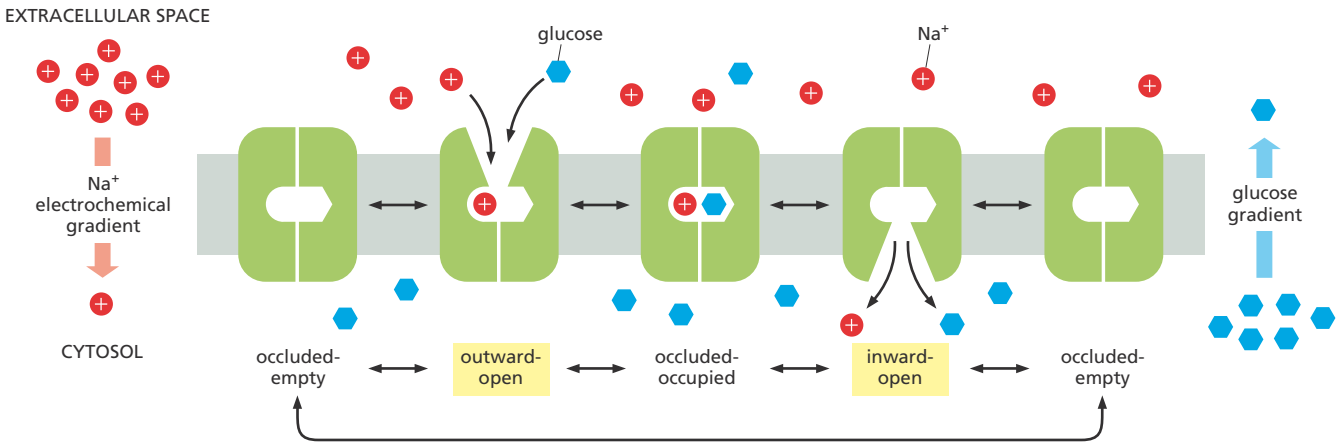


Figure 12–15 A glucose–Na^+ symport protein uses the electrochemical Na^+ gradient to drive the active import of glucose. The pump oscillates randomly between alternate states. In one state ("outward-open") the protein is open to the extracellular space; in another state ("inward-open") it is open to the cytosol. Although Na^+ and glucose can each bind to the pump in either of these "open" states, the pump can transition between them only through an "occluded" state. For their symport, the occluded state can only be reached when both glucose and Na^+ are bound ("occluded-occupied") or when neither is bound ("occluded-empty"). Because the Na^+ concentration is high in the extracellular space, the Na^+ binding site is readily occupied in the outward-open state, and the transporter will have to wait for a rare glucose molecule to bind. When that happens, the pump flips to the occluded-occupied state, trapping both solutes.

Because conformational transitions are reversible, one of two things can happen: the transporter could flip back to the outward-open state. In this case, the solutes would dissociate, and nothing would be gained. Alternatively, it could flip into the inward-open state, exposing the solute binding sites to the cytosol where the Na^+ concentration is very low. Thus sodium readily dissociates and then is pumped back out of the cell by the Na^+ pump (shown in Figure 12–11) to maintain the steep Na^+ gradient. The transporter is now trapped with a partially occupied binding site until the glucose molecule also dissociates. At this point, with no solute bound, it can transition into the "occluded-empty" state and from there back to the outward-open state to repeat the transport cycle.

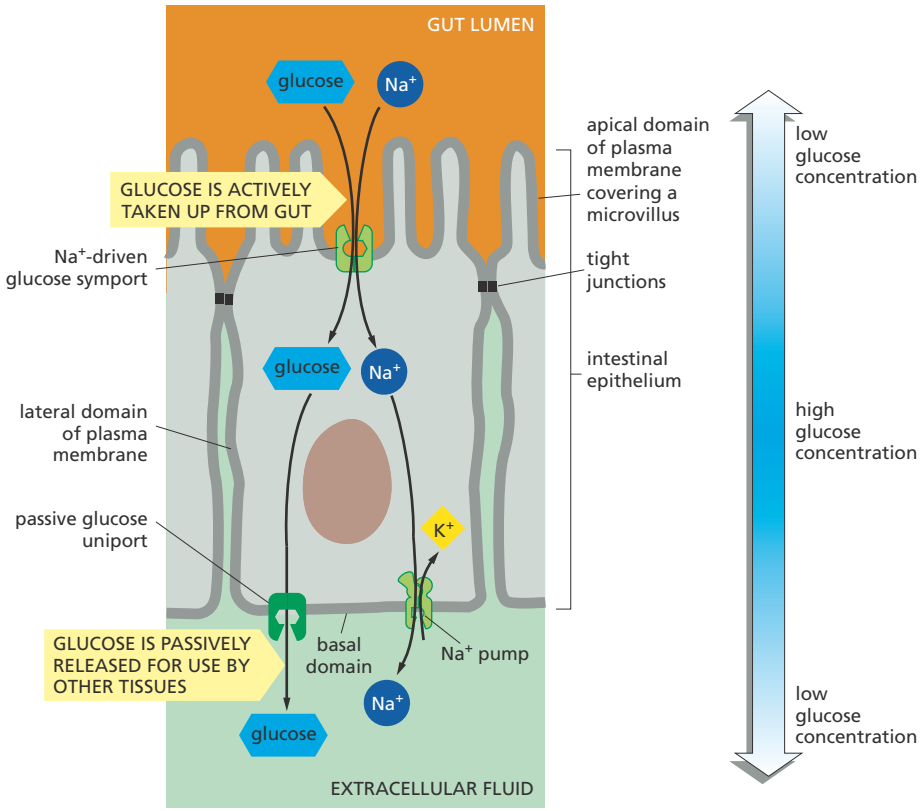


Figure 12–16 Two types of glucose transporters enable gut epithelial cells to transfer glucose across the epithelial lining of the gut. In addition, to keep the concentration of Na^+ in the cytosol low—and the Na^+ electrochemical gradient steep—Na^+ that enters the cell via the Na^+-driven glucose symport is pumped out by Na^+ pumps in the basal and lateral plasma membranes, as indicated. The diet provides ample Na^+ in the gut lumen to drive the Na^+-coupled glucose symport. The process is shown in **Movie 12.4**.

plasma membranes of many animal cells uses the downhill influx of Na^+ to pump H^+ out of the cell; it is one of the main devices that animal cells use to control the pH in their cytosol—preventing the cell interior from becoming too acidic.

Electrochemical H^+ Gradients Drive Coupled Pumps in Plants, Fungi, and Bacteria

Plant cells, bacteria, and fungi (including yeasts) do not have Na^+ pumps in their plasma membrane. Instead of an electrochemical Na^+ gradient, they rely mainly on an electrochemical gradient of H^+ to import solutes into the cell. The gradient is created by **H^+ pumps** in the plasma membrane that pump H^+ out of the cell, thus setting up an electrochemical proton gradient across this membrane and creating an acid pH in the medium surrounding the cell. The import of many sugars and amino acids into bacterial cells is then mediated by H^+ symports, which use the electrochemical H^+ gradient in much the same way that animal cells use the electrochemical Na^+ gradient to import these nutrients.

In some photosynthetic bacteria, the H^+ gradient is created by the activity of light-driven H^+ pumps such as bacteriorhodopsin (see Figure 11–27). In other bacteria, fungi, and plants, the H^+ gradient is generated by H^+ pumps in the plasma membrane that use the energy of ATP hydrolysis to pump H^+ out of the cell; these H^+ pumps resemble the Na^+ pumps and Ca^{2+} pumps in animal cells discussed earlier.

A different type of ATP-dependent H^+ pump is found in the membranes of some intracellular organelles, such as the lysosomes of animal cells and the central vacuole of plant and fungal cells. These pumps—which resemble the turbine-like enzyme that synthesizes ATP in mitochondria and chloroplasts (discussed in Chapter 14)—actively transport H^+ out of the cytosol into the organelle, thereby helping to keep the pH of the

QUESTION 12–2

A rise in the intracellular Ca^{2+} concentration causes muscle cells to contract. In addition to an ATP-driven Ca^{2+} pump, muscle cells that contract quickly and regularly, such as those of the heart, have an additional type of Ca^{2+} pump—an antiport that exchanges Ca^{2+} for extracellular Na^+ across the plasma membrane. The majority of the Ca^{2+} ions that have entered the cell during contraction are rapidly pumped back out of the cell by this antiport, thus allowing the cell to relax. Ouabain and digitalis are used for treating patients with heart disease because they make heart muscle cells contract more strongly. Both drugs function by partially inhibiting the Na^+ pump in the plasma membrane of these cells. Can you propose an explanation for the effects of the drugs in the patients? What will happen if too much of either drug is taken?

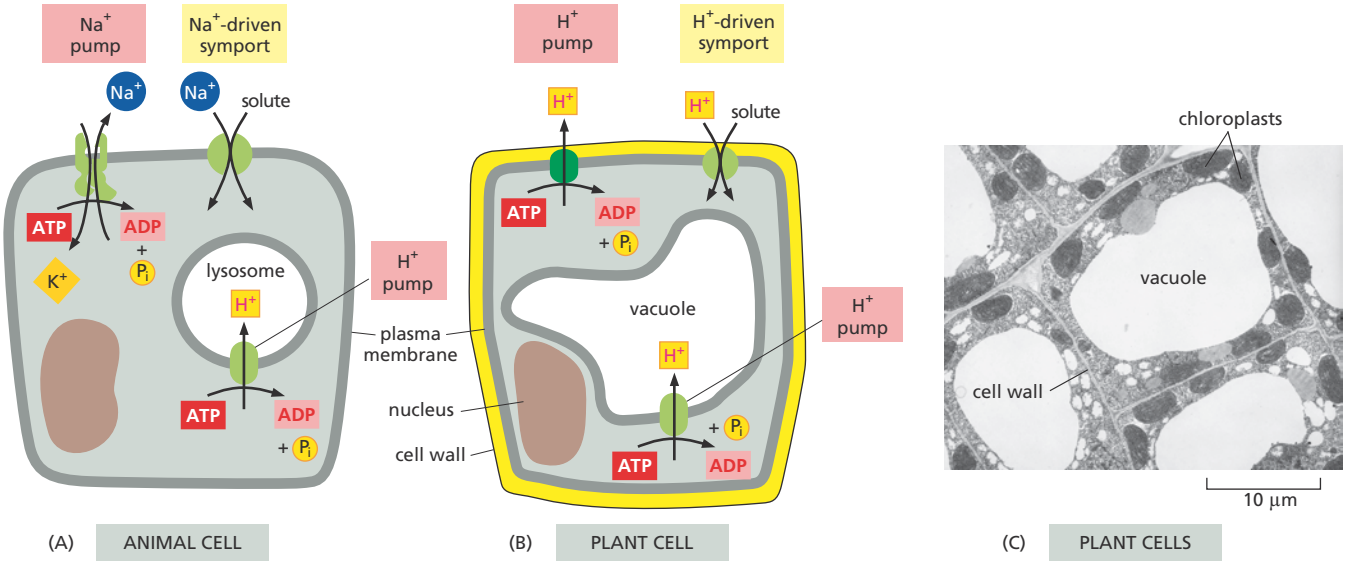


Figure 12–17 Animal and plant cells use a variety of transmembrane pumps to drive the active transport of solutes. (A) In animal cells, an electrochemical Na^+ gradient across the plasma membrane generated by the Na^+ pump, is used by symports to import various solutes. (B) In plant cells, an electrochemical gradient of H^+, set up by an H^+ pump, is often used for this purpose; a similar strategy is used by bacteria and fungi (not shown). The lysosomes in animal cells and the vacuoles in plant and fungal cells contain a similar H^+ pump in their membrane that pumps in H^+, helping to keep the internal environment of these organelles acidic. (C) An electron micrograph shows the vacuole in plant cells in a young tobacco leaf. (C, courtesy of J. Burgess.)

cytosol neutral and the pH of the interior of the organelle acidic. The acid environment in many organelles is crucial to their function, as we discuss in Chapter 15.

Some of the transmembrane pumps considered in this chapter are shown in **Figure 12–17** and are listed in **Table 12–2**.

ION CHANNELS AND THE MEMBRANE POTENTIAL

In principle, the simplest way to allow a small water-soluble molecule to cross from one side of a membrane to the other is to create a hydrophilic channel through which the molecule can pass. Channel proteins, or **channels**, perform this function in cell membranes, forming transmembrane pores that allow the passive movement of small water-soluble molecules into or out of the cell or organelle.

TABLE 12–2 SOME EXAMPLES OF TRANSMEMBRANE PUMPS

Transporter	Location	Energy Source	Function
Na^+-driven glucose pump (Na^+-glucose symport)	apical plasma membrane of kidney and intestinal cells	Na^+ gradient	active import of glucose
Na^+-H^+ exchanger	plasma membrane of animal cells	Na^+ gradient	active export of H^+ ions, pH regulation
Na^+ pump (Na^+-K^+ ATPase)	plasma membrane of most animal cells	ATP hydrolysis	active export of Na^+ and import of K^+
Ca^{2+} pump (Ca^{2+} ATPase)	plasma membrane of eukaryotic cells	ATP hydrolysis	active export of Ca^{2+}
Ca^{2+} pump (Ca^{2+} ATPase)	sarcoplasmic reticulum membrane of muscle cells and endoplasmic reticulum of most animal cells	ATP hydrolysis	active import of Ca^{2+} into sarcoplasmic reticulum
H^+ pump (H^+ ATPase)	plasma membrane of plant cells, fungi, and some bacteria	ATP hydrolysis	active export of H^+
H^+ pump (H^+ ATPase)	membranes of lysosomes in animal cells and of vacuoles in plant and fungal cells	ATP hydrolysis	active export of H^+ from cytosol into vacuole
Bacteriorhodopsin	plasma membrane of some bacteria	light	active export of H^+

A few channels form relatively large, aqueous pores: examples are the proteins that form *gap junctions* between two adjacent cells (see Figure 20–29) and the *porins* that form pores in the outer membrane of mitochondria and some bacteria (see Figure 11–24). But such large, permissive channels would lead to disastrous leaks if they directly connected the cytosol of a cell to the extracellular space. Thus most of the channels in the plasma membrane form narrow, highly selective pores. The *aquaporins* discussed earlier, for example, facilitate the flow of water across the plasma membrane of some prokaryotic and eukaryotic cells. These pores are structured in such a way that they allow the passive diffusion of uncharged water molecules, while prohibiting the movement of ions, including even the smallest ion, H^+.

The bulk of a cell's channels facilitate the passage of select inorganic ions. It is these *ion channels* we discuss in this section.

Ion Channels Are Ion-selective and Gated

Two important properties distinguish **ion channels** from simple holes in the membrane. First, they show *ion selectivity*, permitting some inorganic ions to pass but not others. Ion selectivity depends on the diameter and shape of the ion channel and on the distribution of the charged amino acids that line it. Each ion in aqueous solution is surrounded by a small shell of water molecules, most of which have to be shed for the ions to pass, in single file, through the selectivity filter in the narrowest part of the channel (**Figure 12–18**). An ion channel is narrow enough in places to force ions into contact with the channel wall so that only those ions of appropriate size and charge are able to pass (**Movie 12.5**).

The second important distinction between simple holes and ion channels is that ion channels are not continuously open. Ion transport would be of no value to the cell if the many thousands of ion channels in a cell membrane were open all the time and there were no means of controlling the flow of ions through them. Instead, ion channels open only briefly and then close again (**Figure 12–19**). As we discuss later, most ion channels are *gated*: a specific stimulus triggers them to switch between a closed and an open state by a change in their conformation.

Unlike a transporter, an open ion channel does not need to undergo conformational changes with each ion it passes, and so it has a large advantage over a transporter with respect to its maximum rate of

QUESTION 12–3

A transmembrane protein has the following properties: it has two binding sites, one for solute A and one for solute B. The protein can undergo a conformational change to switch between two states: either both binding sites are exposed exclusively on one side of the membrane or both binding sites are exposed exclusively on the other side of the membrane. The protein can switch between the two conformational states only if both binding sites are occupied or if both binding sites are empty, but cannot switch if only one binding site is occupied.

A. What kind of protein do these properties define?

B. Do you need to specify any additional properties to turn this protein into a symport that couples the movement of solute A up its concentration gradient to the movement of solute B down its electrochemical gradient?

C. Write a set of rules that defines an antiport.

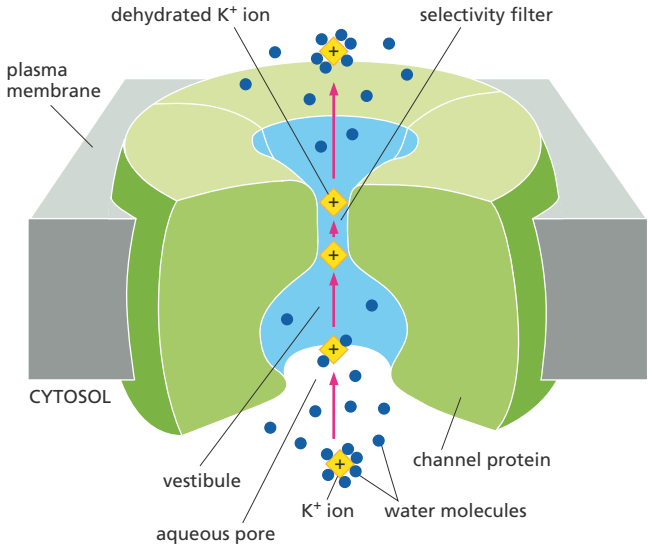


Figure 12–18 An ion channel has a selectivity filter that controls which inorganic ions it will allow to cross the membrane. Shown here is a portion of a bacterial K^+ channel. One of the four protein subunits has been omitted from the drawing to expose the interior structure of the pore (*blue*). From the cytosolic side, the pore opens into a vestibule that sits in the middle of the membrane. K^+ ions in the vestibule are still partially cloaked in their associated water molecules. The narrow selectivity filter, which connects the vestibule with the outside of the cell, is lined with polar groups (not shown) that form transient binding sites for the K^+ ions once the ions have shed their water shell. To observe this selectivity in action, see Movie 12.5.) (Adapted from D.A. Doyle et al., *Science* 280:69–77, 1998. With permission from the AAAS.)

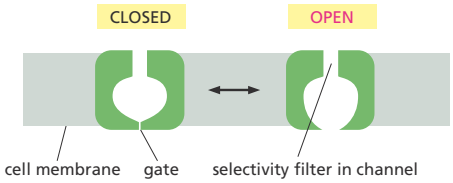


Figure 12–19 A typical ion channel fluctuates between closed and open conformations. The channel shown here in cross section forms a hydrophilic pore across the lipid bilayer only in the "open" conformation. As illustrated in Figure 12–18, the pore narrows to atomic dimensions in the selectivity filter, where the ion selectivity of the channel is largely determined.

transport. More than a million ions can pass through an open channel each second, which is 1000 times greater than the fastest rate of transfer known for any transporter. On the other hand, channels cannot couple the ion flow to an energy source to carry out active transport: most simply make the membrane transiently permeable to selected inorganic ions, mainly Na^+, K^+, Ca^{2+}, or Cl^-.

Thanks to active transport by pumps, the concentrations of most ions are far from equilibrium across a cell membrane. When an ion channel opens, therefore, ions usually flow through it, moving rapidly down their electrochemical gradients. This rapid shift of ions changes the membrane potential, as we discuss next.

Membrane Potential Is Governed by the Permeability of a Membrane to Specific Ions

Changes in membrane potential are the basis of electrical signaling in many types of cells, whether they are the nerve or muscle cells in animals, or the touch-sensitive cells of a carnivorous plant (**Figure 12–20**). Such electrical changes are mediated by alterations in the permeability of membranes to ions. In an animal cell that is in an unstimulated, or "resting," state, the negative charges on the organic molecules inside the cell are largely balanced by K^+, the predominant intracellular ion (see Table 12–1). K^+ is actively imported into the cell by the Na^+ pump, which generates a K^+ gradient across the plasma membrane. The plasma membrane, however, also contains a set of K^+ channels known as **K^+ leak channels**. These channels randomly flicker between open and closed states no matter what the conditions are inside or outside the cell; when they are open, they allow K^+ to move freely. In a resting cell, these are the main ion channels open in the plasma membrane, rendering the membrane much more permeable to K^+ than to other ions.

When the channels are open, K^+ has a tendency to flow out of the cell down its steep concentration gradient. This transfer of K^+ across the plasma membrane leaves behind unbalanced negative charges on the other side, creating a voltage difference, or membrane potential (**Figure 12–21**). Because this charge imbalance will oppose any further movement of K^+ out of the cell, an equilibrium condition is established in which the membrane potential keeping K^+ inside the cell is just strong enough to counteract the tendency of K^+ to move down its concentration gradient and out of the cell. In this state of equilibrium, the electrochemical gradient for K^+ is zero, even though there is still a much higher concentration of K^+ inside the cell than out (**Figure 12–22**).

The membrane potential in such steady-state conditions—in which the flow of positive and negative ions across the plasma membrane is

Figure 12–20 A Venus flytrap uses electrical signaling to capture its prey. The leaves snap shut in less than half a second when an insect moves on them. The response is triggered by touching any two of the three trigger hairs in succession in the center of each leaf. This mechanical stimulation opens ion channels in the plasma membrane and thereby sets off an electrical signal, which, by an unknown mechanism, leads to a rapid change in turgor pressure that closes the leaf. (Courtesy of Gabor Izso, Getty Images.)

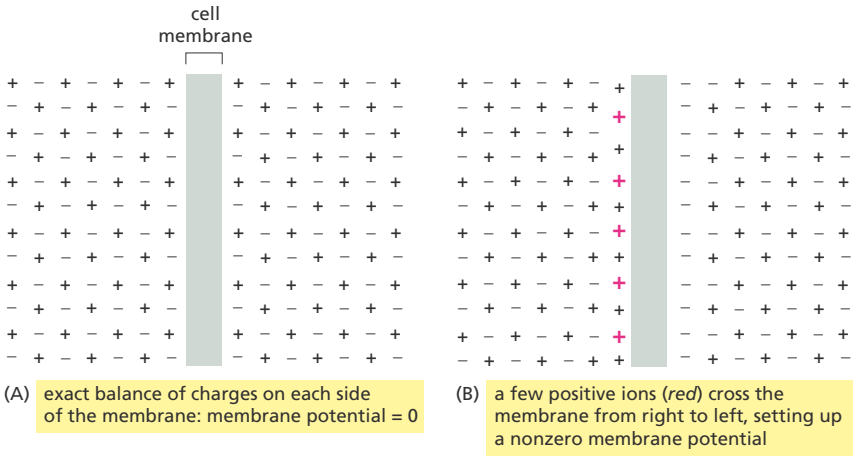


Figure 12–21 The distribution of ions on either side of a cell membrane gives rise to its membrane potential. The membrane potential results from a thin (<1 nm) layer of ions close to the membrane, held in place by their electrical attraction to oppositely charged ions on the other side of the membrane. (A) When there is an exact balance of charges on either side of the membrane, there is no membrane potential. (B) When ions of one type cross the membrane, they set up a charge difference between the two sides of the membrane that creates a membrane potential. The number of ions that must move across the membrane to set up a membrane potential is a tiny fraction of all those present on either side. In the case of the plasma membrane in animal cells, for example, 6000 K^+ ions crossing 1 μm^2 of membrane are enough to shift the membrane potential by about 100 mV; the number of K^+ ions in 1 μm^3 of cytosol is 70,000 times larger than this.

precisely balanced, so that no further difference in charge accumulates across the membrane—is called the **resting membrane potential**. A simple formula called the **Nernst equation** expresses this equilibrium quantitatively and makes it possible to calculate the theoretical resting membrane potential if the ion concentrations on either side of the membrane are known (**Figure 12–23**). In animal cells, the resting membrane potential—which varies between –20 to –200 mV—is chiefly a reflection of the electrochemical K^+ gradient across the plasma membrane, because, at rest, the plasma membrane is chiefly permeable to K^+, and K^+ is the main positive ion inside the cell.

When a cell is stimulated, other ion channels in the plasma membrane open, changing the membrane's permeability to those ions. Whether the

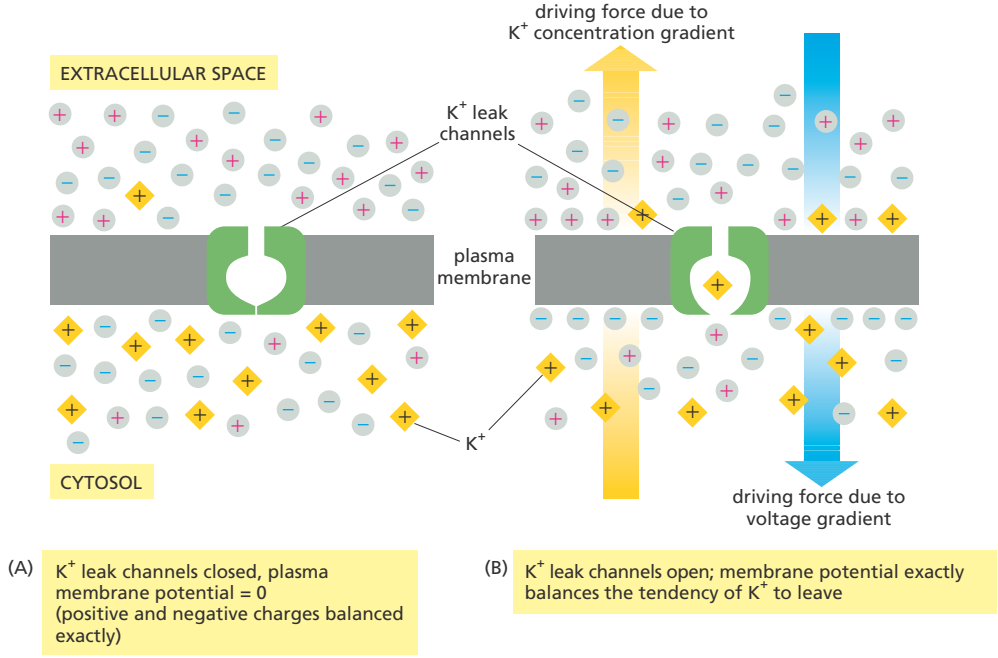


Figure 12–22 The K^+ concentration gradient and K^+ leak channels play major parts in generating the resting membrane potential across the plasma membrane in animal cells. (A) A hypothetical situation in which the K^+ leak channels are closed, and the membrane potential is zero. (B) As soon as the channels open, K^+ will tend to leave the cell, moving down its concentration gradient. Assuming the membrane contains no open channels permeable to other ions, K^+ will cross the membrane, but negative ions will be unable to follow. The resulting charge imbalance gives rise to a membrane potential that tends to drive K^+ back into the cell. At equilibrium, the effect of the K^+ concentration gradient is exactly balanced by the effect of the membrane potential, and there is no net movement of K^+ across the membrane.

The Na^+ pump also contributes to the resting potential—both by helping to establish the K^+ gradient and by pumping 3 Na^+ ions out of the cell for every 2 K^+ ions it pumps in (see Figure 12–11), thereby helping to keep the inside of the cell more negative than the outside (not shown here).

The force tending to drive an ion across a membrane is made up of two components: one due to the electrical membrane potential and one due to the concentration gradient of the ion. At equilibrium, the two forces are balanced and satisfy a simple mathematical relationship given by the

Nernst equation

$$V = 62 \log_{10} (C_o / C_i)$$

where V is the membrane potential in millivolts, and C_o and C_i are the outside and inside concentrations of the ion, respectively. This form of the equation assumes that the ion carries a single positive charge and that the temperature is 37°C.

Figure 12–23 The Nernst equation can be used to calculate the resting potential of a membrane. The relevant ion concentrations are those on either side of the membrane. From this equation, we see that each tenfold change in the ion concentration ratio (C_o/C_i) alters the membrane potential by 62 millivolts.

ions enter or leave the cell depends on the direction of their electrochemical gradients. Thus the membrane potential at any time depends on both the state of the membrane's ion channels and the ion concentrations on either side of the plasma membrane. Bulk changes in ion concentrations cannot occur quickly enough to drive the rapid changes in membrane potential that are associated with electrical signaling. Instead, it is the rapid opening and closing of ion channels, which occurs within milliseconds, that matters most for this type of cell signaling.

Ion Channels Randomly Snap Between Open and Closed States

Measuring changes in electrical current is the main method used to study ion movements and ion channels in living cells. Amazingly, electrical recording techniques can detect and measure the electric current flowing through a single channel molecule. The procedure developed for doing this is known as **patch-clamp recording**, and it provides a direct and surprising picture of how individual ion channels behave.

In patch-clamp recording, a fine glass tube is used as a *microelectrode* to isolate and make electrical contact with a small area of the membrane at the surface of the cell (**Figure 12–24**). The technique makes it

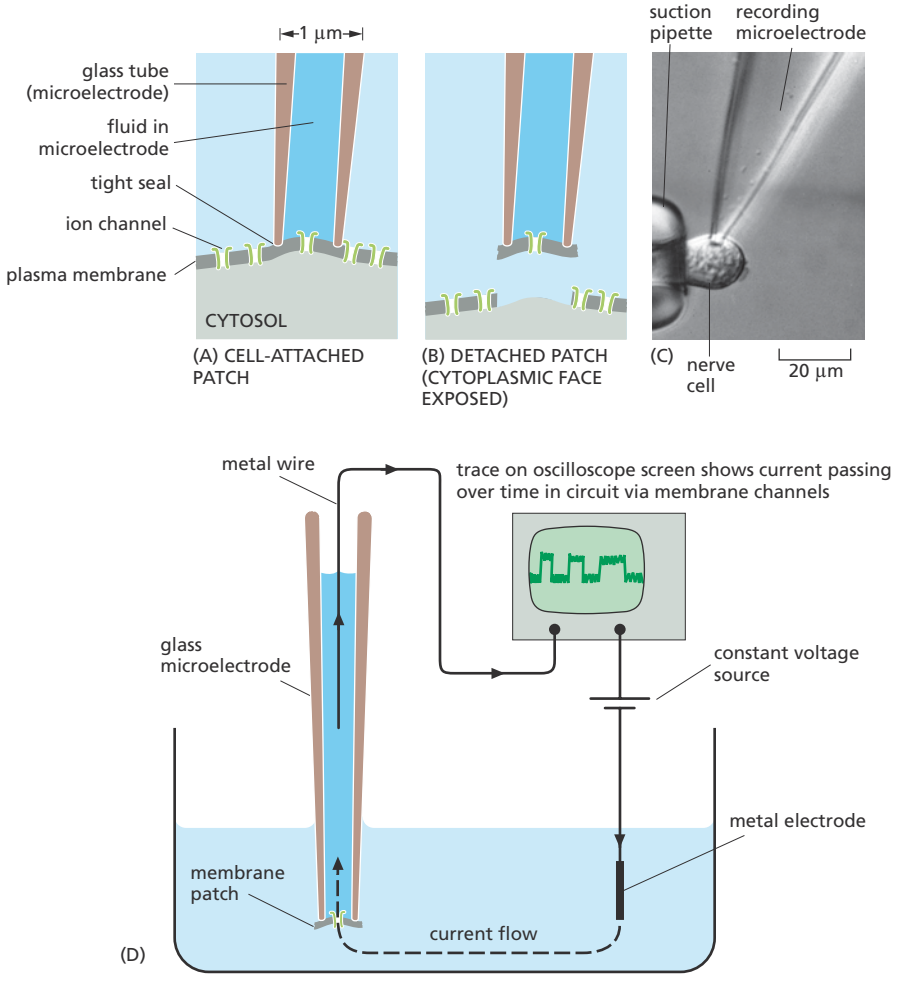


Figure 12–24 Patch-clamp recording is used to monitor ion channel activity. First, a microelectrode is made by heating a glass tube and pulling it to create an extremely fine tip with a diameter of no more than a few micrometers; the tube is then filled with an aqueous conducting solution, and the tip is pressed against the cell surface. (A) With gentle suction, a tight electrical seal is formed where the cell membrane contacts the mouth of the microelectrode. Because of the extremely tight seal, current can enter or leave the microelectrode only by passing through the ion channel or channels in the patch of membrane covering its tip. (B) To expose the cytosolic face of the membrane, the patch of membrane held in the microelectrode can be torn from the cell. The advantage of the detached patch is that it is easy to alter the composition of the solution on either side of the membrane to test the effect of various solutes on channel behavior. (C) A micrograph showing an isolated nerve cell held in a suction pipette (the tip of which is shown on the left), while a microelectrode is being used for patch-clamp recording. (D) The circuitry for patch-clamp recording. At the open end of the microelectrode, a metal wire is inserted. Current that enters the microelectrode through ion channels in the small patch of membrane covering its tip passes via the wire, through measuring instruments, back into the bath of medium surrounding the cell or the detached patch. (C, from T.D. Lamb, H.R. Mathews, and V. Torre, *J. Physiol.* 37:315–349, 1986. With permission from Blackwell Publishing.)

possible to record the activity of ion channels in all sorts of cell types—particularly in large nerve and muscle cells, which are famous for their electrical activities. By varying the concentrations of ions on either side of the patch, one can test which ions will go through the channels in the patch. With the appropriate electronic circuitry, the voltage across the membrane patch—that is, the membrane potential—can also be set and held "clamped" at any chosen value (hence the term "patch-clamp"). The ability to expose the membrane to different voltages makes it possible to see how changes in membrane potential affect the opening and closing of the ion channels in the membrane.

With a sufficiently small area of membrane in the patch, sometimes only a single ion channel will be present. Modern electrical instruments are sensitive enough to reveal the ion flow through a single channel, detected as a minute electric current (of the order of 10^{-12} ampere or 1 picoampere). Monitoring individual ion channels in this way revealed something surprising about the way they behave: even when conditions are held constant, the currents abruptly appear and disappear, as though an on/off switch were being jiggled randomly (**Figure 12–25**). This behavior indicates that the channel has moving parts and is snapping back and forth between open and closed conformations (see Figure 12–19) as the channel is knocked from one conformation to the other by the random thermal movements of the molecules in its environment. Patch-clamp recording was the first technique that could monitor such conformational changes, and the picture it paints—of a jerky piece of machinery subjected to constant external buffeting—is now known to apply also to other proteins with moving parts.

The activity of each ion channel is very much "all-or-none": when an ion channel is open, it is fully open; when it is closed, it is fully closed. That raises a fundamental question: If ion channels randomly snap between open and closed conformations even when conditions on each side of the membrane are held constant, how can their state be regulated by conditions inside or outside the cell? The answer is that, when the appropriate conditions change, the random behavior continues but with a greatly changed bias: if the altered conditions tend to open the channel, for example, the channel will now spend a much greater proportion of its time in the open conformation, although it will not remain open continuously (see Figure 12–25).

Different Types of Stimuli Influence the Opening and Closing of Ion Channels

There are more than a hundred types of ion channels, and even simple organisms can possess many different types. The nematode worm *C. elegans*, for example, has genes that encode 68 different but related K^+ channels alone. Ion channels differ from one another primarily with

Figure 12–25 The behavior of a single ion channel can be observed using the patch-clamp technique. The voltage (the membrane potential) across the isolated patch of membrane is held constant during the recording. In this example, the neurotransmitter acetylcholine is present, and the membrane patch from a muscle cell contains a single channel protein that is responsive to acetylcholine (discussed later, see Figure 12–41). As seen, this ion channel opens to allow passage of positive ions when acetylcholine binds to the exterior face of the channel. But even when acetylcholine is bound to the channel, as is the case during the three channel openings shown here, the channel does not remain open all the time. Instead, it flickers between open and closed states. Note that how long the channel remains open is variable. If acetylcholine were not present, the channel would only rarely open. (Courtesy of David Colquhoun.)

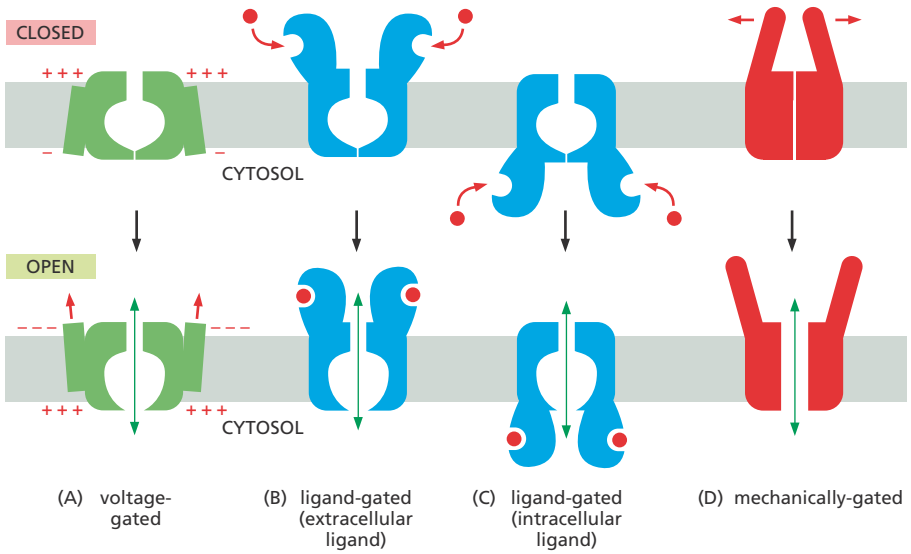


Figure 12–26 Different types of gated ion channels respond to different types of stimuli. Depending on the type of channel, the probability of gate opening is controlled by (A) a change in the voltage difference across the membrane, (B) the binding of a chemical ligand to the extracellular face of a channel, (C) ligand binding to the intracellular face of a channel, or (D) mechanical stress.

respect to their *ion selectivity*—the type of ions they allow to pass—and their *gating*—the conditions that influence their opening and closing. For a **voltage-gated channel**, the probability of being open is controlled by the membrane potential (**Figure 12–26A**). For a **ligand-gated channel**, opening is controlled by the binding of some molecule (the ligand) to the channel (**Figure 12–26B and C**). For a **mechanically-gated channel,** opening is controlled by a mechanical force applied to the channel (**Figure 12–26D**).

The *auditory hair* cells in the ear are an important example of cells that depend on mechanically-gated channels. Sound vibrations pull the channels open, causing ions to flow into the hair cells; this ion flow sets up an electrical signal that is transmitted from the hair cell to the auditory nerve, which conveys the signal to the brain (**Figure 12–27**).

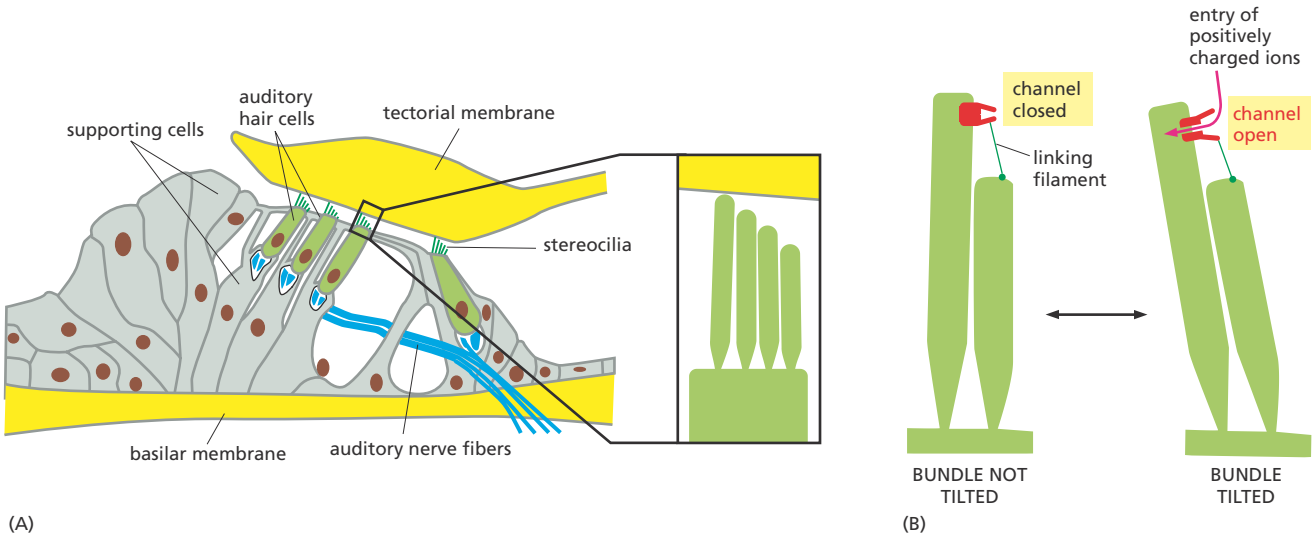


Figure 12–27 Mechanically-gated ion channels allow us to hear. (A) A section through the organ of Corti, which runs the length of the cochlea, the auditory portion of the inner ear. Each auditory hair cell has a tuft of spiky extensions called stereocilia projecting from its upper surface. The hair cells are embedded in an epithelial sheet of supporting cells, which is sandwiched between the *basilar membrane* below and the *tectorial membrane* above. (These are not lipid bilayer membranes but sheets of extracellular matrix.) (B) Sound vibrations cause the basilar membrane to vibrate up and down, causing the stereocilia to tilt. Each stereocilium in the staggered array of stereocilia on each hair cell is attached to the next shorter stereocilium by a fine filament. The tilting stretches the filaments, which pull open mechanically-gated ion channels in the stereocilium plasma membrane, allowing positively charged ions to enter from the surrounding fluid (**Movie 12.6**). The influx of ions activates the hair cells, which stimulate underlying nerve endings of the auditory nerve fibers that relay the auditory signal to the brain.

The hair-cell mechanism is astonishingly sensitive: the faintest sounds we can hear have been estimated to stretch the filaments by an average of about 0.04 nm, which is less than the diameter of a hydrogen ion (**Movie 12.7**).

Figure 12–28 Both mechanically-gated and voltage-gated ion channels underlie the leaf-closing response in the touch-sensitive plant, *Mimosa pudica*. (A) Resting leaf. (B and C) Successive responses to touch. A few seconds after the leaf is touched, the leaflets snap shut. The response involves the opening of mechanically-gated ion channels in touch-sensitive sensory cells, which then pass a signal to cells containing voltage-gated ion channels, generating an electric impulse. When the impulse reaches specialized hinge cells at the base of each leaflet, a rapid loss of water by these cells occurs, causing the leaflets to fold into a closed conformation suddenly and progressively down the leaf stalk.

Voltage-gated Ion Channels Respond to the Membrane Potential

Voltage-gated ion channels play a major role in propagating electrical signals along all nerve cell processes, such as those that relay signals from our brain to our toe muscles. But voltage-gated ion channels are present in many other cell types, too, including muscle cells, egg cells, protozoans, and even plant cells, where they enable electrical signals to travel from one part of the plant to another, as in the leaf-closing response of a *Mimosa pudica* plant (**Figure 12–28**).

Voltage-gated ion channels have domains called *voltage sensors* that are extremely sensitive to changes in the membrane potential: changes above a certain threshold value exert sufficient electrical force on these domains to encourage the channel to switch from its closed to its open conformation. As discussed earlier, a change in the membrane potential does not affect how wide the channel is open, but instead alters the probability that it will open (see Figure 12–25). Thus, in a large patch of membrane containing many molecules of the channel protein, one might find that on average 10% of them are open at any instant when the membrane is at one potential, whereas 90% are open after this potential changes.

When one type of voltage-gated ion channel opens, the membrane potential of the cell can change. This in turn can activate or inactivate other voltage-gated ion channels. This control circuit, from ion channels → membrane potential → ion channels, is fundamental to all electrical signaling in cells. To see how such a circuit can be used for electrical signaling, we now turn to nerve cells: they—more than any other cell type—have made a profession of electrical signaling, and they employ ion channels in very sophisticated ways.

ION CHANNELS AND NERVE CELL SIGNALING

The fundamental task of a nerve cell, or **neuron**, is to receive, integrate, and transmit signals. Neurons carry signals inward from sense organs, such as eyes and ears, to the *central nervous system*—the brain and spinal cord. In the central nervous system, neurons signal from one to another through networks of enormous complexity, allowing the brain and spinal cord to analyze, interpret, and respond to the signals coming in from the sense organs.

Every neuron consists of a *cell body*, which contains the nucleus and has a number of long, thin extensions radiating outward from it. Usually, a neuron has one long extension called an **axon**, which conducts electrical signals away from the cell body toward distant target cells; it also usually has several shorter, branching extensions called **dendrites**, which radiate from the cell body like antennae and provide an enlarged surface area to receive signals from the axons of other neurons (**Figure 12–29**). The

QUESTION 12–4

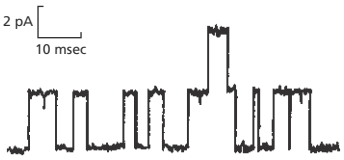


Figure Q12–4 (above) shows a recording from a patch-clamp experiment in which the electrical current passing across a patch of membrane is measured as a function of time. The membrane patch was plucked from the plasma membrane of a muscle cell by the technique shown in Figure 12–24 and contains molecules of the acetylcholine receptor, which is a ligand-gated cation channel that is opened by the binding of acetylcholine to the extracellular face of the channel. To obtain a recording, acetylcholine was added to the solution inside the microelectrode. (A) Describe what you can learn about the channels from this recording. (B) How would the recording differ if acetylcholine were (i) omitted or (ii) added to the solution outside the microelectrode only?

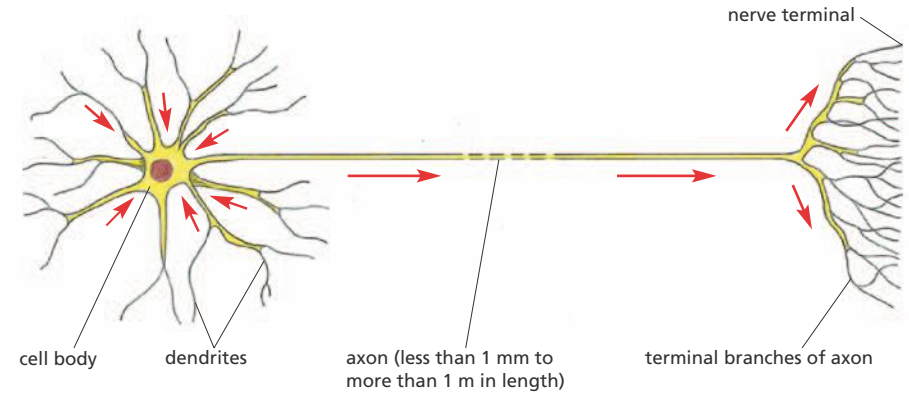


Figure 12–29 A typical neuron has a cell body, a single axon, and multiple dendrites. The axon conducts electrical signals away from the cell body toward its target cells, while the multiple dendrites receive signals from the axons of other neurons. The *red* arrows indicate the direction in which signals travel.

QUESTION 12–5

Using the Nernst equation and the ion concentrations given in Table 12–1 (p. 385), calculate the equilibrium membrane potential of K^+ and Na^+—that is, the membrane potential where there would be no net movement of the ion across the plasma membrane (assume that the concentration of intracellular Na^+ is 10 mM). What membrane potential would you predict in a resting animal cell? Explain your answer. What would happen if a large number of Na^+ channels suddenly opened, making the membrane much more permeable to Na^+ than to K^+? (Note that because few ions need to move across the membrane to change the charge distribution across the membrane drastically, you can safely assume that the ion concentrations on either side of the membrane do not change significantly.) What would you predict would happen next if the Na^+ channels closed again?

axon commonly divides at its far end into many branches, each of which ends in a **nerve terminal**, so that the neuron's message can be passed simultaneously to many target cells—muscle or gland cells or other neurons. Likewise, the branching of the dendrites can be extensive, in some cases, sufficient to receive as many as 100,000 inputs on a single neuron.

No matter what the meaning of the signal a neuron carries—whether it is visual information from the eye, a motor command to a muscle, or one step in a complex network of neural processing in the brain—the form of the signal is always the same: it consists of changes in the electrical potential across the neuron's plasma membrane.

Action Potentials Allow Rapid Long-Distance Communication Along Axons

A neuron is stimulated by a signal—typically from another neuron—delivered to a localized site on its surface. This signal initiates a change in the membrane potential at that site. To transmit the signal onward, this local change in membrane potential has to spread from this point, which is usually on a dendrite or the cell body, to the axon terminals, which relay the signal to the next cells in the pathway—forming a *neural circuit*. The distances required can be substantial: a signal that leaves a motor neuron in your spinal cord may have to travel a meter or more before it reaches a muscle in your foot.

The local change in membrane potential generated by a signal can spread passively along an axon or a dendrite to adjacent regions of the plasma membrane. Such a passively spread signal, however, rapidly becomes weaker with increasing distance from the source. Over short distances, this weakening is unimportant. But for long-distance communication, such *passive spread* is inadequate.

Neurons solve this long-distance communication problem by employing an active signaling mechanism. Here a local electrical stimulus of sufficient strength triggers an explosion of electrical activity in the plasma membrane that propagates rapidly along the membrane of the axon, continuously renewing itself all along the way. This traveling wave of electrical excitation, known as an **action potential**, or a *nerve impulse*, can carry a message, without the signal weakening, all the way from one end of a neuron to the other, at speeds of up to 100 meters per second.

The early research that established this mechanism of electrical signaling along axons was done on the giant axon of the squid (**Figure 12–30**). This axon has such a large diameter that it is possible to record its electrical activity from an electrode inserted directly into it (**How We Know**, pp. 406–407). From such studies, it was deduced how action potentials

are the direct consequence of the properties of voltage-gated ion channels in the axonal plasma membrane, as we now explain.

Action Potentials Are Mediated by Voltage-gated Cation Channels

When a neuron is stimulated, the membrane potential of the plasma membrane shifts to a less negative value (that is, toward zero). If this **depolarization** is sufficiently large, it will cause **voltage-gated Na^+ channels** in the membrane to open transiently at the site. As these channels flicker open, they allow a small amount of Na^+ to enter the cell down its steep electrochemical gradient. The influx of positive charge depolarizes the membrane further (that is, it makes the membrane potential even less negative), thereby opening additional voltage-gated Na^+ channels and causing still further depolarization. This process continues in an explosive, self-amplifying fashion until, within about a millisecond, the membrane potential in the local region of the neuron's plasma membrane has shifted from its resting value of about –60 mV to about +40 mV (**Figure 12–31**).

The voltage of +40 mV is close to the membrane potential at which the electrochemical driving force for movement of Na^+ across the membrane is zero—that is, at which the effects of the membrane potential and the concentration gradient for Na^+ are equal and opposite, so that Na^+ has no further tendency to enter or leave the cell. If the channels continued to respond to the altered membrane potential, the cell would get stuck with most of its voltage-gated Na^+ channels open.

The cell is saved from this fate because the Na^+ channels have an automatic inactivating mechanism—a kind of "timer" that causes them to rapidly adopt (within a millisecond or so) a special inactivated conformation, in which the channel is closed, even though the membrane is still depolarized. The Na^+ channels remain in this *inactivated state* until the membrane potential has returned to its initial negative value. A

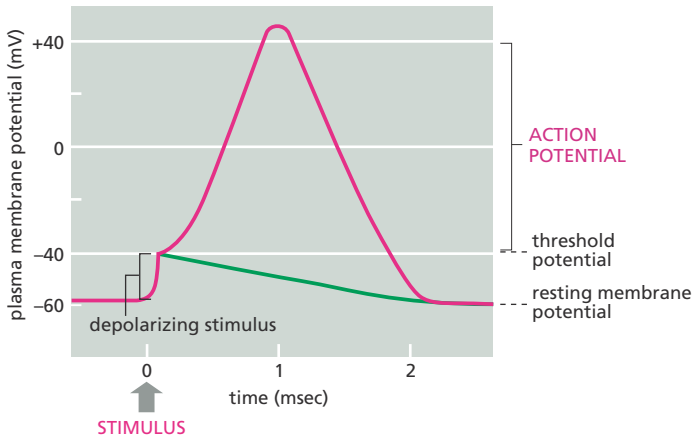


Figure 12–31 An action potential is triggered by a depolarization of a neuron's plasma membrane. The resting membrane potential in this neuron is –60 mV, and a stimulus that depolarizes the plasma membrane to about –40 mV (the threshold potential) is sufficient to open voltage-gated Na^+ channels in the membrane and thereby trigger an action potential. The membrane rapidly depolarizes further, and the membrane potential (*red* curve) swings past zero, reaching +40 mV before it returns to its resting negative value as the action potential terminates. The *green* curve shows how the membrane potential would simply have relaxed back to the resting value after the initial depolarizing stimulus if there had been no amplification by voltage-gated ion channels in the plasma membrane.

Figure 12–30 The squid *Loligo* has a nervous system that is adept at responding rapidly to threats in the animal's environment. Among the nerve cells that make up this escape system is one that possesses a "giant axon," with a very large diameter. Long before patch clamping allowed recordings from single ion channels in small cells (see Figure 12–24), the squid giant axon was routinely used to record and study action potentials.

SQUID REVEAL SECRETS OF MEMBRANE EXCITABILITY

Each spring, *Loligo pealei* migrate to the shallow waters off Cape Cod on the eastern coast of the United States. There they spawn, launching the next generation of squid. But more than just meeting and breeding, these animals provide neuroscientists summering at the Marine Biological Laboratory in Woods Hole, Massachusetts, with a golden opportunity to study the mechanism of electrical signaling along nerve axons.

Like most animals, squid survive by catching prey and escaping predators. Fast reflexes and an ability to accelerate rapidly and make sudden changes in swimming direction help them avoid danger while chasing down a decent meal. Squid derive their speed and agility from a specialized biological jet propulsion system: they draw water into their mantle cavity and then contract their muscular body wall to expel the collected water rapidly through a tubular siphon, thus propelling themselves through the water.

Controlling such quick and coordinated muscle contraction requires a nervous system that can convey signals with great speed down the length of the animal's body. Indeed, *Loligo pealei* possesses some of the largest nerve cell axons found in nature. Squid giant axons can reach 10 cm in length and are over 100 times the diameter of a mammalian axon—about the width of a pencil lead. Generally speaking, the larger the diameter of an axon, the more rapidly signals can travel along its length.

In the 1930s, scientists first started to take advantage of the squid giant axon for studying the electrophysiology of the nerve cell. Because of its relatively large size, an investigator can isolate an individual axon and insert an electrode into it to measure the axon's membrane potential and monitor its electrical activity. This experimental system allowed researchers to address a variety of questions, including which ions are important for establishing the resting membrane potential and for initiating and propagating an action potential, and how changes in the membrane potential control ion permeability.

Setup for action

Because the squid axon is so long and wide, an electrode made from a glass capillary tube containing a conducting solution can be thrust down the axis of the isolated axon so that its tip lies deep in the cytoplasm (**Figure 12–32A**). This setup allowed investigators to measure the voltage difference between the inside and the outside of the axon—that is, the membrane potential—as an action potential sweeps past the tip of the electrode (**Figure 12–32B**). The action potential itself was triggered by applying a brief electrical stimulus to one end of the axon. It didn't matter which end was stimulated, as the action potential could travel in either direction; it also didn't matter how big the stimulus was, as long as it exceeded a certain threshold (see Figure 12–31), indicating that an action potential is all or nothing.

Once researchers could reliably generate and measure an action potential, they could use the preparation to answer other questions about membrane excitability. For example, which ions are critical for an action potential? The three most plentiful ions, both inside and outside an axon, are Na^+, K^+, and Cl^-. Do they have

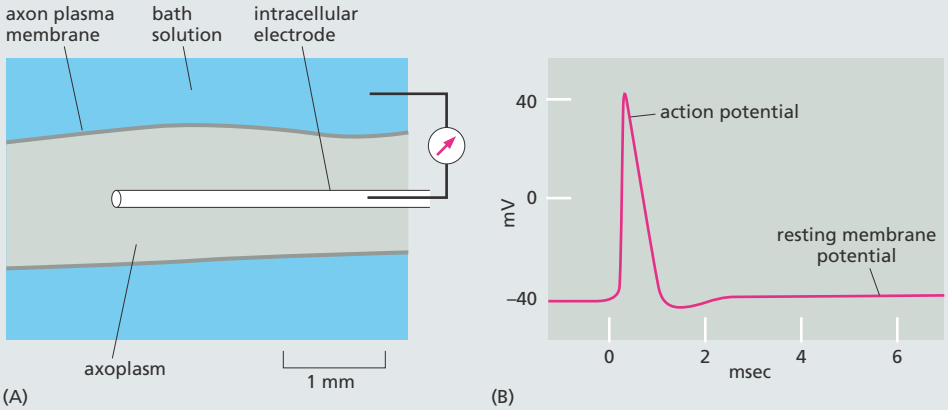


Figure 12–32 Scientists can study nerve cell excitability using an isolated axon from squid. An electrode can be inserted into the cytoplasm (axoplasm) of a squid giant axon (A) to measure the resting membrane potential and monitor action potentials induced when the axon is electrically stimulated (B).

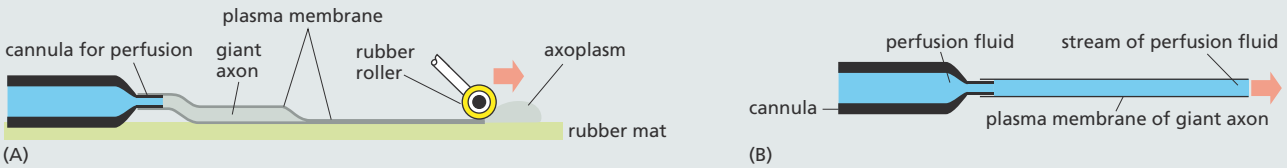


Figure 12–33 The cytoplasm in a squid axon can be removed and replaced with an artificial solution of pure ions. (A) The axon cytoplasm is extruded using a rubber roller. (B) A perfusion fluid containing the desired concentration of ions is pumped gently through the emptied-out axon.

equal importance when it comes to the action potential? Because the squid axon is so large and robust, it was possible to extrude the cytoplasm from the axon like toothpaste from a tube (**Figure 12–33A**). The emptied-out axon could then be reinflated by perfusing it with a pure solution of Na^+, K^+, or Cl^-, (**Figure 12–33B**). Thus, the ions inside the axon and in the bath solution (see Figure 12–32) could be varied independently. In this way, the researchers could show that the axon would generate a normal action potential if and only if the concentrations of Na^+ and K^+ approximated the natural concentrations found inside and outside the cell. Thus, they concluded that the cell components crucial to the action potential are the plasma membrane, Na^+ and K^+ ions, and the energy provided by the concentration gradients of these ions across the membrane; all other components, including other sources of metabolic energy, were presumably removed by the perfusion.

Channel traffic

Once Na^+ and K^+ had been singled out as critical for an action potential, the question then became: What does each of these ions contribute to the action potential? How permeable is the membrane to each, and how does the membrane permeability change as an action potential sweeps by? Again, the squid giant axon provided some answers. The concentrations of Na^+ and K^+ inside and outside the axon could be altered, and the effects of these changes on the membrane potential could be measured directly. From such studies, it was determined that, at rest, the membrane potential of an axon is close to the equilibrium potential for K^+: when the external concentration of K^+ was varied, the resting potential of the axon changed roughly in accordance with the Nernst equation (see Figure 12–23). They concluded that at rest, the membrane is chiefly permeable to K^+; we now know that K^+ leak channels provide the main pathway these ions can take through the resting plasma membrane.

The situation for Na^+ is very different. When the external concentration of Na^+ was varied, there was no effect on the resting potential of the axon. However, the height of the peak of the action potential varied with the concentration of Na^+ outside the axon (**Figure 12–34**). During the action potential, therefore, the membrane appeared to be chiefly permeable to Na^+, presumably as the result of the opening of Na^+ channels. In the aftermath of the action potential, the Na^+ permeability decreased and the membrane potential reverted to a negative value, which depended on the external concentration of K^+. As the membrane lost its permeability to Na^+, it became even more permeable to K^+ than before, presumably because additional K^+ channels opened, accelerating the resetting of the membrane potential to the resting state, and readying the membrane for the next action potential.

These studies on the squid giant axon made an enormous contribution to our understanding of nerve cell excitability, and the researchers who made these discoveries in the 1940s and 1950s—Alan Hodgkin and Andrew Huxley—received a Nobel Prize in 1963. However, it was years before the various ion channel proteins that they had hypothesized to exist would be biochemically identified. We now know the three-dimensional structures of many of these channel proteins, allowing us to marvel at the fundamental beauty of these molecular machines.

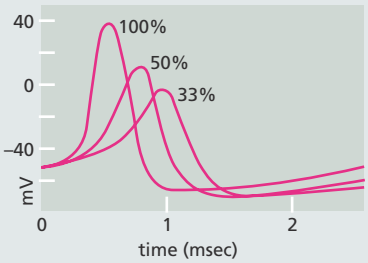


Figure 12–34 The shape of the action potential depends on the concentration of Na^+ outside the squid axon. Shown here are action potentials recorded when the external medium contains 100%, 50%, or 33% of the normal extracellular concentration of Na^+.

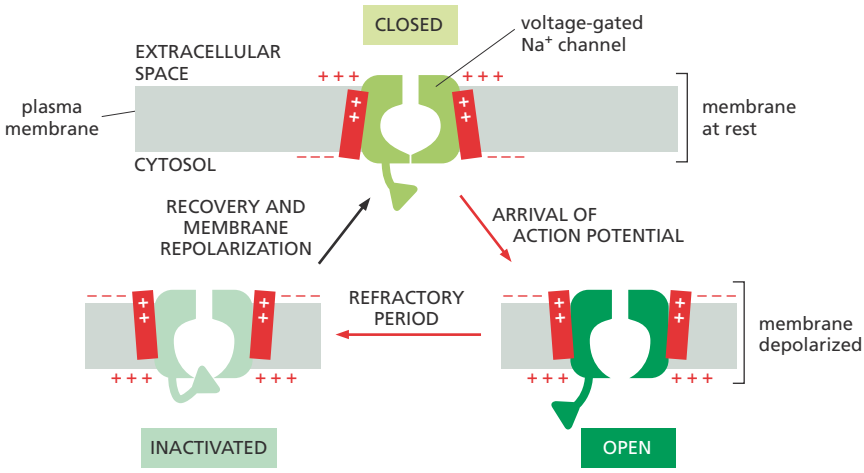


Figure 12–35 A voltage-gated Na^+ channel can flip from one conformation to another, depending on the membrane potential. When the membrane is at rest and highly polarized, positively charged amino acids in its voltage sensors (*red bars*) are oriented by the membrane potential in a way that keeps the channel in its closed conformation. When the membrane is depolarized, the voltage sensors shift, changing the channel's conformation so the channel has a high probability of opening. But in the depolarized membrane, the inactivated conformation is even more stable than the open conformation, and so, after a brief period spent in the open conformation, the channel becomes temporarily inactivated and cannot open. The *red* arrows indicate the sequence that follows a sudden depolarization, and the *black* arrow indicates the return to the original conformation after the membrane has repolarized.

schematic illustration of these three distinct states of the voltage-gated Na^+ channel—*closed, open,* and *inactivated*—is shown in **Figure 12–35**. How they contribute to the rise and fall of an action potential is shown in **Figure 12–36**.

During an action potential, Na^+ channels do not act alone. The depolarized axonal membrane is helped to return to its resting potential by the opening of *voltage-gated K^+ channels.* These also open in response to depolarization, but not as promptly as the Na^+ channels, and they stay open as long as the membrane remains depolarized. As the local depolarization reaches its peak, K^+ ions (carrying positive charge) therefore start to flow out of the cell through these newly opened K^+ channels down their electrochemical gradient, temporarily unhindered by the negative membrane potential that normally restrains them in the resting cell. The rapid outflow of K^+ through the voltage-gated K^+ channels brings the membrane back to its resting state much more quickly than could be achieved by K^+ outflow through the K^+ leak channels alone.

Once it begins, the self-amplifying depolarization of a small patch of plasma membrane quickly spreads outward: Na^+ flowing in through open Na^+ channels begins to depolarize the neighboring region of the membrane, which then goes through the same self-amplifying cycle. In this way, an action potential spreads outward as a traveling wave from the initial site of depolarization, eventually reaching the axon terminals (**Figure 12–37**).

Faced with the consequences of the Na^+ and K^+ fluxes caused by a passing action potential, Na^+ pumps in the axon plasma membrane labor

QUESTION 12–6

Explain as precisely as you can but in no more than 100 words the ionic basis of an action potential and how it is passed along an axon.

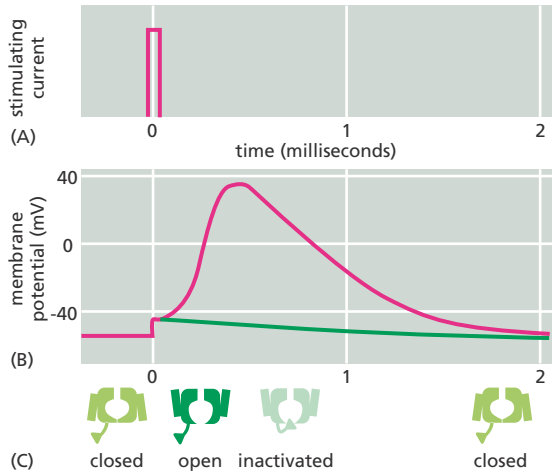


Figure 12–36 Voltage-gated Na^+ channels change their conformation during an action potential. In this example, the action potential is triggered by a brief pulse of electric current (A), which partially depolarizes the membrane, as shown in the plot of membrane potential versus time in (B). (B) The course of the action potential (*red* curve), which reflects the opening and subsequent inactivation of voltage-gated Na^+ channels, whose state is shown in (C). Even if restimulated, the plasma membrane cannot produce a second action potential until the Na^+ channels have returned from the inactivated to the closed conformation (see Figure 12–35). Until then, the membrane is resistant, or refractory, to stimulation.

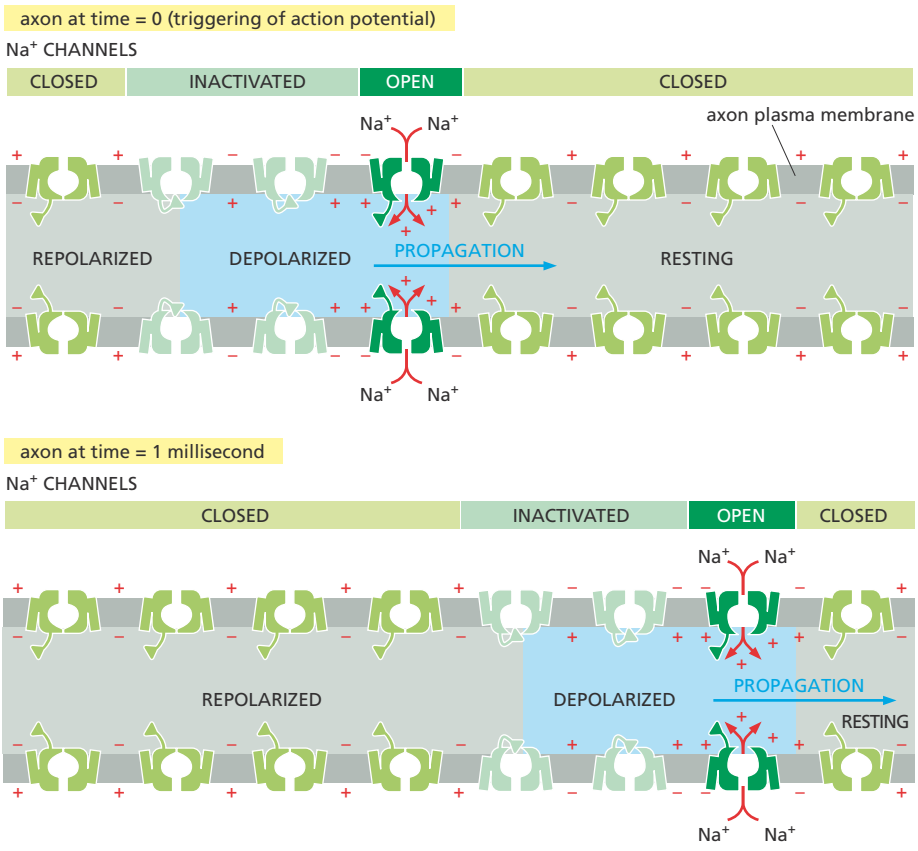


Figure 12–37 An action potential propagates along the length of an axon. The changes in the Na^+ channels and the consequent flow of Na^+ across the membrane (*red* arrows) alters the membrane potential and gives rise to the traveling action potential, as shown here and in **Movie 12.8**. The region of the axon with a depolarized membrane is shaded in *blue*. Note that an action potential can only travel forward from the site of depolarization. This is because Na^+-channel inactivation in the aftermath of an action potential prevents the advancing front of depolarization from spreading backward (see also Figure 12–36).

continuously to restore the ion gradients of the resting cell. It is remarkable that the human brain consumes 20% of the total energy generated from the metabolism of food, mostly to power this pump.

Voltage-gated Ca^{2+} Channels in Nerve Terminals Convert an Electrical Signal into a Chemical Signal

When an action potential reaches the nerve terminals at the end of an axon, the signal must somehow be relayed to the *target cells* that the terminals contact—usually neurons or muscle cells. The signal is transmitted to the target cells at specialized junctions known as **synapses**. At most synapses, the plasma membranes of the cells transmitting and receiving the message—the *presynaptic* and the *postsynaptic* cells, respectively—are separated from each other by a narrow *synaptic cleft* (typically 20 nm across), which the electrical signal cannot cross. To transmit the message across this gap, the electrical signal is converted into a chemical signal, in the form of a small, secreted signal molecule known as a **neurotransmitter**. Neurotransmitters are initially stored in the nerve terminals within membrane-enclosed **synaptic vesicles** (**Figure 12–38**).

When an action potential reaches the nerve terminal, some of the synaptic vesicles fuse with the plasma membrane, releasing their neurotransmitters into the synaptic cleft. This link between the arrival of an action potential and the secretion of neurotransmitter involves the activation of yet another type of voltage-gated cation channel. The depolarization of the nerve-terminal plasma membrane caused by the arrival of the action potential transiently opens *voltage-gated Ca^{2+} channels*, which are concentrated in the plasma membrane of the presynaptic nerve terminal. Because the Ca^{2+} concentration outside the terminal is more than 1000 times greater than the free Ca^{2+} concentration in its cytosol (see Table 12–1), Ca^{2+} rushes into the nerve terminal through the open channels.

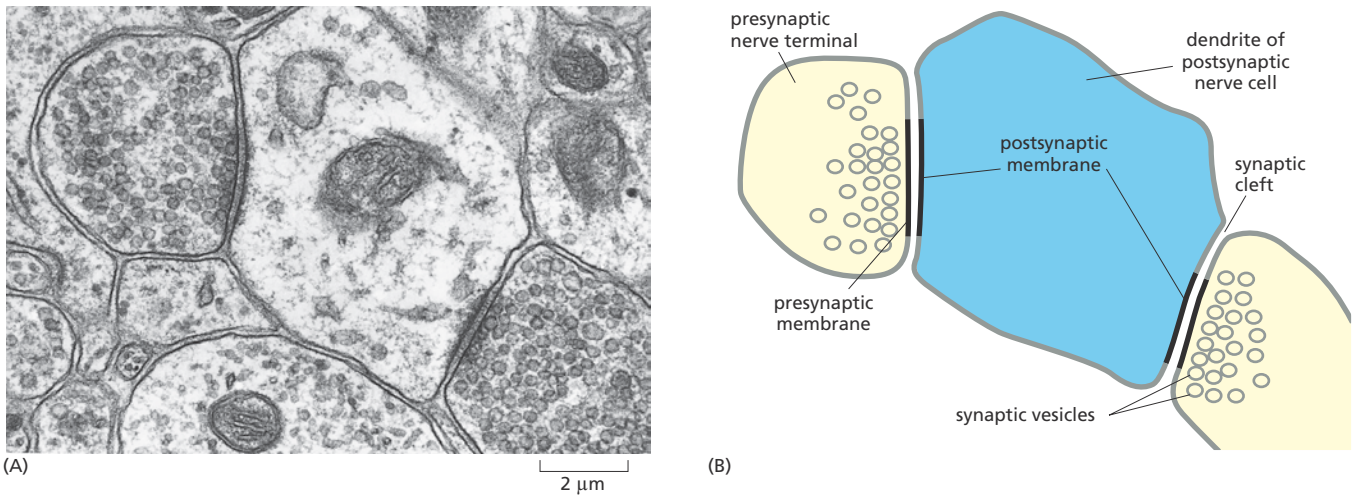


Figure 12–38 Neurons connect to their target cells at synapses. An electron micrograph (A) and drawing (B) of a cross section of two nerve terminals (*yellow*) forming synapses on a single nerve cell dendrite (*blue*) in the mammalian brain. Neurotransmitters carry the signal across the synaptic cleft that separates the presynaptic and postsynaptic cell. The neurotransmitter in the presynaptic terminal is contained within synaptic vesicles, which release it into the synaptic cleft. Note that both the presynaptic and postsynaptic membranes are thickened and highly specialized at the synapse. (A, courtesy of Cedric Raine.)

The resulting increase in Ca^{2+} concentration in the cytosol of the terminal immediately triggers the membrane fusion that releases the neurotransmitter. Thanks to these voltage-gated Ca^{2+} channels, the electrical signal has now been converted into a chemical signal that is secreted into the synaptic cleft (**Figure 12–39**).

Transmitter-gated Ion Channels in the Postsynaptic Membrane Convert the Chemical Signal Back into an Electrical Signal

The released neurotransmitter rapidly diffuses across the synaptic cleft and binds to *neurotransmitter receptors* concentrated in the postsynaptic plasma membrane of the target cell. The binding of neurotransmitter to its receptors causes a change in the membrane potential of the target cell, which—if large enough—triggers the cell to fire an action potential. The neurotransmitter is then quickly removed from the synaptic cleft—either by enzymes that destroy it, by pumping it back into the nerve terminals that released it, or by uptake into neighboring non-neuronal cells. This rapid removal of the neurotransmitter limits the duration and spread of the signal and ensures that, when the presynaptic cell falls quiet, the postsynaptic cell will do the same.

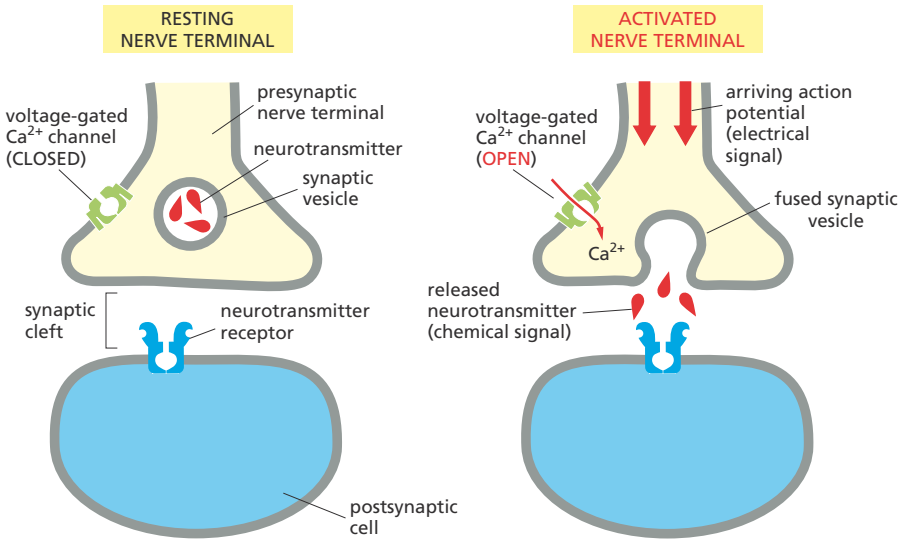


Figure 12–39 An electrical signal is converted into a secreted chemical signal at a nerve terminal. When an action potential reaches a nerve terminal, it opens voltage-gated Ca^{2+} channels in the plasma membrane, allowing Ca^{2+} to flow into the terminal. The increased Ca^{2+} in the nerve terminal stimulates the synaptic vesicles to fuse with the plasma membrane, releasing their neurotransmitter into the synaptic cleft—a process called exocytosis (discussed in Chapter 15).

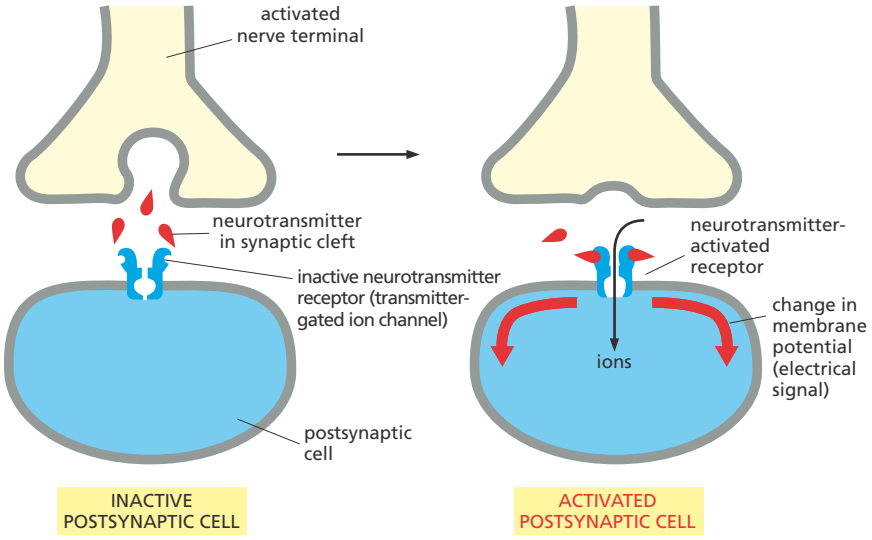


Figure 12–40 A chemical signal is converted into an electrical signal by postsynaptic transmitter-gated ion channels at a synapse. The released neurotransmitter binds to and opens the transmitter-gated ion channels in the plasma membrane of the postsynaptic cell. The resulting ion flows alter the membrane potential of the postsynaptic cell, thereby converting the chemical signal back into an electrical one (**Movie 12.9**).

Neurotransmitter receptors can be of various types; some mediate relatively slow effects in the target cell, whereas others trigger more rapid responses. Rapid responses—on a time scale of milliseconds—depend on receptors that are **transmitter-gated ion channels** (also called ion-channel-coupled receptors). These constitute a subclass of ligand-gated ion channels (see Figure 12–26B), and their function is to convert the chemical signal carried by a neurotransmitter back into an electrical signal. The channels open transiently in response to the binding of the neurotransmitter, thus changing the ion permeability of the postsynaptic membrane. This in turn causes a change in the membrane potential (**Figure 12–40**). If the change is big enough, it will depolarize the postsynaptic membrane and trigger an action potential in the postsynaptic cell.

A well-studied example of a transmitter-gated ion channel is found at the *neuromuscular junction*—the specialized synapse formed between a motor neuron and a skeletal muscle cell. In vertebrates, the neurotransmitter here is *acetylcholine*, and the transmitter-gated ion channel is an *acetylcholine receptor* (**Figure 12–41**). But not all neurotransmitters excite the postsynaptic cell, as we consider next.

Neurotransmitters Can Be Excitatory or Inhibitory

Neurotransmitters can either excite or inhibit a postsynaptic cell, and it is the character of the receptor that recognizes the neurotransmitter that determines how the postsynaptic cell will respond. The chief receptors for excitatory neurotransmitters, such as *acetylcholine* and *glutamate*, are ligand-gated cation channels. When a neurotransmitter binds, these channels open to allow an influx of Na^+, which depolarizes the plasma membrane and thus tends to activate the postsynaptic cell, encouraging it to fire an action potential. By contrast, the main receptors for inhibitory neurotransmitters, such as *γ-aminobutyric acid (GABA)* and *glycine*, are ligand-gated Cl^- channels. When neurotransmitters bind, these channels open, increasing the membrane permeability to Cl^-; this change in permeability inhibits the postsynaptic cell by making its plasma membrane harder to depolarize.

Toxins that bind to one of these excitatory or inhibitory neurotransmitter receptors can have dramatic effects on humans. *Curare*, for example, causes muscle paralysis by blocking excitatory acetylcholine receptors at the neuromuscular junction. This drug was used by South American

QUESTION 12–7

In the disease myasthenia gravis, the human body makes—by mistake—antibodies to its own acetylcholine receptor molecules. These antibodies bind to and inactivate acetylcholine receptors on the plasma membrane of muscle cells. The disease leads to a devastating progressive weakening of the people affected. Early on, they may have difficulty opening their eyelids, for example, and, in an animal model of the disease, rabbits have difficulty holding their ears up. As the disease progresses, most muscles weaken, and people with myasthenia gravis have difficulty speaking and swallowing. Eventually, impaired breathing can cause death. Explain which step of muscle function is affected.

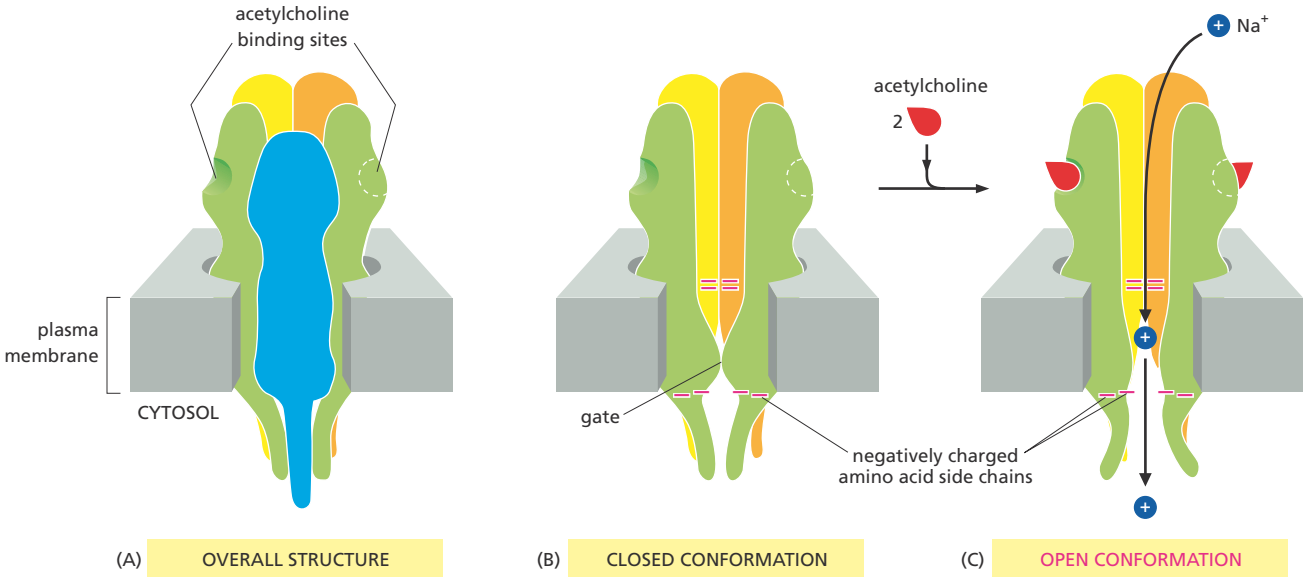


Figure 12–41 The acetylcholine receptor in the plasma membrane of vertebrate skeletal muscle cells opens when it binds the neurotransmitter acetylcholine. (A) This transmitter-gated ion channel is composed of five transmembrane protein subunits, two of which (*green*) are identical. The subunits combine to form a transmitter-gated aqueous pore across the lipid bilayer. The pore is lined by five transmembrane α helices, one contributed by each subunit. There are two acetylcholine-binding sites, one formed by parts of a *green* and *blue* subunit, the other by parts of a *green* and *orange* subunit, as shown. (B) The closed conformation. The *blue* subunit has been removed here and in (C) to show the interior of the pore. Negatively charged amino acid side chains at either end of the pore (indicated here by *red* minus signs) ensure that only positively charged ions, mainly Na^+ and K^+, can pass. But when acetylcholine is not bound and the channel is in its closed conformation, the pore is occluded (blocked) by hydrophobic amino acid side chains in the region called the gate. (C) The open conformation. When acetylcholine, released by a motor neuron, binds to both binding sites, the channel undergoes a conformational change; the hydrophobic side chains move apart and the gate opens, allowing Na^+ to flow across the membrane down its electrochemical gradient, depolarizing the membrane. Even with acetylcholine bound, the channel flickers randomly between the open and closed states (see Figure 12–25); without acetylcholine bound, it rarely opens.

Indians to make poison arrows and is still used by surgeons to relax muscles during an operation. By contrast, *strychnine*—a common ingredient in rat poisons—causes muscle spasms, convulsions, and death by blocking inhibitory glycine receptors on neurons in the brain and spinal cord.

The locations and functions of the ion channels discussed in this chapter are summarized in **Table 12–3**.

TABLE 12–3 SOME EXAMPLES OF ION CHANNELS

Ion Channel	Typical Location	Function
K^+ leak channel	plasma membrane of most animal cells	maintenance of resting membrane potential
Voltage-gated Na^+ channel	plasma membrane of nerve cell axon	generation of action potentials
Voltage-gated K^+ channel	plasma membrane of nerve cell axon	return of membrane to resting potential after initiation of an action potential
Voltage-gated Ca^{2+} channel	plasma membrane of nerve terminal	stimulation of neurotransmitter release
Acetylcholine receptor (acetylcholine-gated cation channel)	plasma membrane of muscle cell (at neuromuscular junction)	excitatory synaptic signaling
Glutamate receptors (glutamate-gated cation channels)	plasma membrane of many neurons (at synapses)	excitatory synaptic signaling
GABA receptor (GABA-gated Cl^- channel)	plasma membrane of many neurons (at synapses)	inhibitory synaptic signaling
Glycine receptor (glycine-gated Cl^- channel	plasma membrane of many neurons (at synapses)	inhibitory synaptic signaling
Mechanically-activated cation channel	auditory hair cell in inner ear	detection of sound vibrations

Most Psychoactive Drugs Affect Synaptic Signaling by Binding to Neurotransmitter Receptors

Many drugs used in the treatment of insomnia, anxiety, depression, and schizophrenia act by binding to transmitter-gated ion channels in the brain. Sedatives and tranquilizers such as barbiturates, Valium, Ambien, and Restoril, for example, bind to GABA-gated Cl^- channels. Their binding makes the channels easier to open by GABA, rendering the neuron more sensitive to GABA's inhibitory action. By contrast, the antidepressant Prozac blocks the Na^+-driven symport responsible for the reuptake of the excitatory neurotransmitter *serotonin*, increasing the amount of serotonin available in the synapses that use it. This drug has changed the lives of many people who suffer from depression—although why boosting serotonin can elevate mood is still unknown.

The number of distinct types of neurotransmitter receptors is very large, although they fall into a small number of families. There are, for example, many subtypes of acetylcholine, glutamate, GABA, glycine, and serotonin receptors; they are usually located on different neurons and often differ only subtly in their electrophysiological properties. With such a large variety of receptors, it may be possible to design a new generation of psychoactive drugs that will act more selectively on specific sets of neurons to mitigate the mental illnesses that devastate so many people's lives. One percent of the human population, for example, have schizophrenia, another 1% have bipolar disorder, about 1% have an autistic disorder, and many more suffer from anxiety or depressive disorders. Mutations in genes that affect synaptic function can greatly increase the risk of the most serious of these disorders. The fact that these disorders are so prevalent suggests that the complexity of synaptic signaling may make the brain especially vulnerable to genetic abnormalities. But complexity also provides some distinct advantages, as we discuss next.

QUESTION 12–8

When an inhibitory neurotransmitter such as GABA opens Cl^- channels in the plasma membrane of a postsynaptic neuron, why does this make it harder for an excitatory neurotransmitter to excite the neuron?

The Complexity of Synaptic Signaling Enables Us to Think, Act, Learn, and Remember

For a process so critical for animal survival, the mechanism that governs synaptic signaling seems unnecessarily cumbersome, as well as error-prone. For a signal to pass from one neuron to the next, the nerve terminal of the presynaptic cell must convert an electrical signal into a secreted chemical. This chemical signal must then diffuse across the synaptic cleft so that a postsynaptic cell can convert it back into an electric one. Why would evolution have favored such an apparently inefficient and vulnerable way to pass a signal between cells? It would seem more efficient and robust to have a direct electrical connection between them—or to do away with the synapse altogether and use a single continuous cell.

The value of synapses that rely on secreted chemical signals becomes clear when we consider how they function in the context of the nervous system—a huge network of neurons, interconnected by many branching circuits, performing complex computations, storing memories, and generating plans for action. To carry out these functions, neurons have to do more than merely generate and relay signals: they must also combine them, interpret them, and record them. Chemical synapses make these activities possible. A motor neuron in the spinal cord, for example, receives inputs from hundreds or thousands of other neurons that make synapses on it (**Figure 12–42**). Some of these signals tend to stimulate the neuron, while others inhibit it. The motor neuron has to combine all of the information it receives and react, either by stimulating a muscle to contract or by remaining quiet.

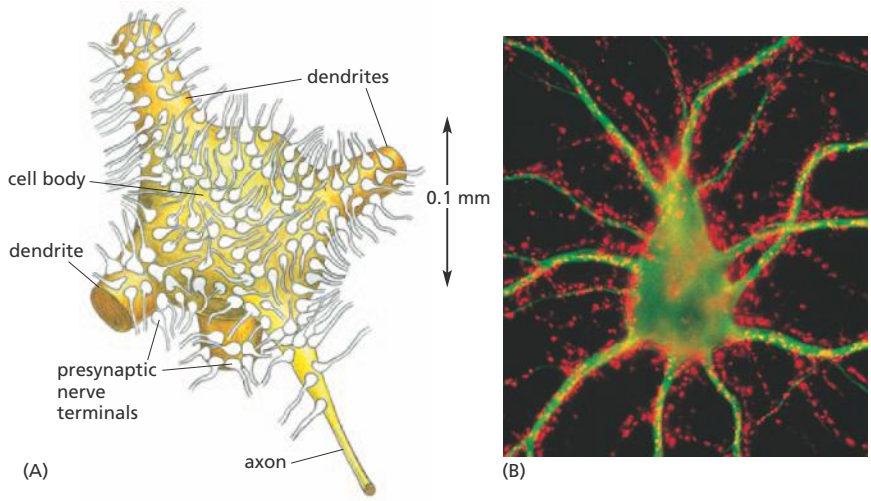


Figure 12–42 Thousands of synapses form on the cell body and dendrites of a motor neuron in the spinal cord. (A) Many thousands of nerve terminals synapse on this neuron, delivering signals from other parts of the animal to control the firing of action potentials along the neuron's axon. (B) A rat nerve cell in culture. Its cell body and dendrites (*green*) are stained with a fluorescent antibody that recognizes a cytoskeletal protein. Thousands of axon terminals (*red*) from other nerve cells (not visible) make synapses on the cell's surface; they are stained with a fluorescent antibody that recognizes a protein in synaptic vesicles, which are located in the terminals (see Figure 12–38). (B, courtesy of Olaf Mundigl and Pietro de Camilli.)

This task of computing an appropriate output from a babble of inputs is achieved by a complicated interplay between different types of ion channels in the neuron's plasma membrane. Each of the hundreds of types of neurons in your brain has its own characteristic set of receptors and ion channels that enables the cell to respond in a particular way to a certain set of inputs and thus to perform its specialized task.

In addition to integrating a variety of chemical inputs, a synapse can also adjust the magnitude of its response—reacting more vigorously (or less vigorously) to an incoming action potential—based on how heavily that synapse has been used in the past. This ability to adapt, called **synaptic plasticity**, is triggered by the entry of Ca^{2+} through special cation channels in the postsynaptic plasma membrane, which can lead to functional alterations on either side of the synapse—in the amount of neurotransmitter released from the axon terminal, the way the postsynaptic cell responds to the transmitter, or both. These synaptic changes can last hours, days, weeks, or longer, and they are thought to play an important part in learning and memory.

Synapses are thus critical components of the machinery that enables us to act, think, feel, speak, learn, and remember. Given that they operate in neuronal circuits that are so dauntingly complex, will it ever be possible to deeply understand the circuits that drive complex human behaviors? Although cracking this problem in humans is still far in the future, we now have increasingly powerful ways to study the neural circuits—and molecules—that underlie behavior in experimental animals. One of the most promising techniques makes use of a type of light-gated ion channel borrowed from unicellular algae, as we now discuss.

Optogenetics Uses Light-gated Ion Channels to Transiently Activate or Inactivate Neurons in Living Animals

Photosynthetic green algae use light-gated channels to sense and navigate toward sunlight. In response to blue light, one of these channels—called *channelrhodopsin*—allows Na^+ to flow into the cell. This depolarizes the plasma membrane and, ultimately, modulates the beating of the flagella the cell uses to swim. Although these channels are peculiar to unicellular green algae, they continue to function properly even when they are artificially transferred into other cell types, thereby rendering those cells responsive to light.

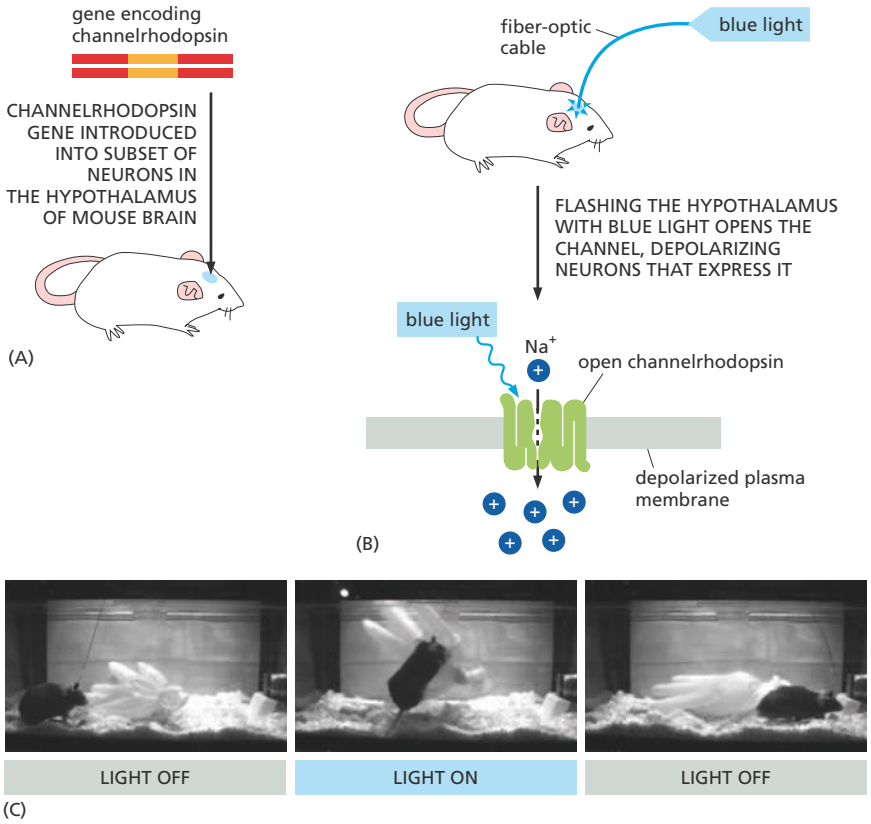


Figure 12–43 Light-gated ion channels can control the activity of specific neurons in a living animal. (A) In this experiment, the gene encoding channelrhodopsin was introduced into a subset of neurons in the mouse hypothalamus. (B) When the neurons are exposed to blue light using a tiny fiber-optic cable implanted into the animal's brain, channelrhodopsin opens, depolarizing and stimulating the channel-containing neurons. (C) When the light is switched on, the mouse immediately becomes aggressive; when the light is switched off, its behavior immediately returns to normal. (C, from D. Lin et al., *Nature* 470:221–226, 2011. With permission from Macmillan Publishers Ltd.)

Because nerve cells are activated by a depolarizing influx of Na^+ (see Figure 12–36), channelrhodopsin can be used to manipulate the activity of neurons and neural circuits. It has even been used to control the behavior of living animals. In one particularly stunning experiment, the channelrhodopsin gene was introduced into a select subpopulation of neurons in the mouse hypothalamus—a brain region involved in many functions, including aggression. When the channels were subsequently illuminated by a thin, optic fiber implanted in the animal's brain, the mouse launched an attack on any object in its path—including other mice or, in one comical instance, an inflated rubber glove. When the light was switched off, the neurons fell silent, and the mouse's behavior immediately returned to normal (**Figure 12–43** and **Movie 12.10**).

Because the approach uses light to control neurons into which channelrhodopsin—or any other light-gated channel—has been introduced by genetic engineering techniques (discussed in Chapter 10), the method has been dubbed **optogenetics**. This new tool is revolutionizing neurobiology, allowing investigators to dissect the neural circuits that govern even the most complex behaviors in a variety of experimental animals, from fruit flies to monkeys. But its implications extend beyond the laboratory. As genetic studies continue to identify genes associated with various human neurological and psychiatric disorders, the ability to exploit light-gated ion channels to study where and how these genes function in model organisms promises to greatly advance our understanding of the molecular and cellular basis of human behavior.

ESSENTIAL CONCEPTS

- **The lipid bilayer of cell membranes is highly permeable to small nonpolar molecules such as oxygen and carbon dioxide and, to a lesser extent, to very small polar molecules such as water. It is highly impermeable to most large water-soluble molecules and to all ions.**

- Transfer of nutrients, metabolites, and inorganic ions across cell membranes depends on membrane transport proteins.
- Cell membranes contain a variety of transport proteins that function either as transporters or channels, each responsible for the transfer of a particular type of solute.
- Channel proteins form pores across the lipid bilayer through which solutes can passively diffuse.
- Both transporters and channels can mediate passive transport, in which an uncharged solute moves spontaneously down its concentration gradient.
- For the passive transport of a charged solute, its electrochemical gradient determines its direction of movement, rather than its concentration alone.
- Transporters can act as pumps to mediate active transport, in which solutes are moved uphill against their concentration or electrochemical gradients; this process requires energy that is provided by ATP hydrolysis, a downhill flow of Na^+ or H^+ ions, or sunlight.
- Transporters transfer specific solutes across a membrane by undergoing conformational changes that expose the solute-binding site first on one side of the membrane and then on the other.
- The Na^+ pump in the plasma membrane of animal cells is an ATPase; it actively transports Na^+ out of the cell and K^+ in, maintaining a steep Na^+ gradient across the plasma membrane that is used to drive other active transport processes and to convey electrical signals.
- Ion channels allow inorganic ions of appropriate size and charge to cross the membrane. Most are gated and open transiently in response to a specific stimulus.
- Even when activated by a specific stimulus, ion channels do not remain continuously open: they flicker randomly between open and closed conformations. An activating stimulus increases the proportion of time that the channel spends in the open state.
- The membrane potential is determined by the unequal distribution of charged ions on the two sides of a cell membrane; it is altered when these ions flow through open ion channels in the membrane.
- In most animal cells, the negative value of the resting membrane potential across the plasma membrane depends mainly on the K^+ gradient and the operation of K^+-selective leak channels; at this resting potential, the driving force for the movement of K^+ across the membrane is almost zero.
- Neurons propagate electrical impulses in the form of action potentials, which can travel long distances along an axon without weakening. Action potentials are mediated by voltage-gated Na^+ channels that open in response to depolarization of the plasma membrane.
- Voltage-gated Ca^{2+} channels in a nerve terminal couple the arrival of an action potential to neurotransmitter release at a synapse. Transmitter-gated ion channels convert this chemical signal back into an electrical one in the postsynaptic target cell.
- Excitatory neurotransmitters open transmitter-gated cation channels that allow the influx of Na^+, which depolarizes the postsynaptic cell's plasma membrane and encourages the cell to fire an action potential. Inhibitory neurotransmitters open transmitter-gated Cl^- channels in the postsynaptic cell plasma membrane, making it harder for the membrane to depolarize and fire an action potential.
- Complex sets of nerve cells in the human brain exploit all of the above mechanisms to make human behaviors possible.

KEY TERMS

action potential
active transport
antiport
axon
Ca^{2+} pump (or Ca^{2+}-ATPase)
channel
coupled pumps
dendrite
depolarization
electrochemical gradient
H^+ pump (or H^+ ATPase)
ion channel
K^+ leak channels
ligand-gated channel
mechanically-gated channel
membrane potential
membrane transport protein
Na^+ pump (or Na^+-K^+ ATPase)
Nernst equation
nerve terminal
neuron
neurotransmitter
optogenetics
osmosis
passive transport
patch-clamp recording
pump
resting membrane potential
symport
synapse
synaptic plasticity
synaptic vesicle
transmitter-gated ion channel
voltage-gated channel
voltage-gated Na^+ channel

QUESTIONS

QUESTION 12–9

The diagram in Figure 12–9 shows a passive transporter that mediates the transfer of a solute down its concentration gradient across the membrane. How would you need to change the diagram to convert the transporter into a pump that moves the solute up its concentration gradient by hydrolyzing ATP? Explain the need for each of the steps in your new illustration.

QUESTION 12–10

Which of the following statements are correct? Explain your answers.

A. The plasma membrane is highly impermeable to all charged molecules.

B. Channels have specific binding pockets for the solute molecules they allow to pass.

C. Transporters allow solutes to cross a membrane at much faster rates than do channels.

D. Certain H^+ pumps are fueled by light energy.

E. The plasma membrane of many animal cells contains open K^+ channels, yet the K^+ concentration in the cytosol is much higher than outside the cell.

F. A symport would function as an antiport if its orientation in the membrane were reversed (i.e., if the portion of the molecule normally exposed to the cytosol faced the outside of the cell instead).

G. The membrane potential of an axon temporarily becomes more negative when an action potential excites it.

QUESTION 12–11

List the following compounds in order of increasing lipid bilayer permeability: RNA, Ca^{2+}, glucose, ethanol, N_2, water.

QUESTION 12–12

Name at least one similarity and at least one difference between the following (it may help to review the definitions of the terms using the Glossary):

A. Symport and antiport

B. Active transport and passive transport

C. Membrane potential and electrochemical gradient

D. Pump and transporter

E. Axon and telephone wire

F. Solute and ion

QUESTION 12–13

Discuss the following statement: "The differences between a channel and a transporter are like the differences between a bridge and a ferry."

QUESTION 12–14

The neurotransmitter acetylcholine is made in the cytosol and then transported into synaptic vesicles, where its concentration is more than 100-fold higher than in the cytosol. When synaptic vesicles are isolated from neurons, they can take up additional acetylcholine added to the solution in which they are suspended, but only when ATP is present. Na^+ ions are not required for the uptake, but, curiously, raising the pH of the solution in which the synaptic vesicles are suspended increases the rate of uptake.

Furthermore, transport is inhibited when drugs are added that make the membrane permeable to H^+ ions. Suggest a mechanism that is consistent with all of these observations.

QUESTION 12–15

The resting membrane potential of a typical animal cell is about –70 mV, and the thickness of a lipid bilayer is about 4.5 nm. What is the strength of the electric field across the membrane in V/cm? What do you suppose would happen if you applied this field strength to two metal electrodes separated by a 1-cm air gap?

QUESTION 12–16

Phospholipid bilayers form sealed spherical vesicles in water (discussed in Chapter 11). Assume you have constructed lipid vesicles that contain Na^+ pumps as the sole membrane protein, and assume for the sake of simplicity that each pump transports one Na^+ one way and one K^+ the other way in each pumping cycle. All the Na^+ pumps have the portion of the molecule that normally faces the cytosol oriented toward the outside of the vesicles. With the help of Figure 12–11, determine what would happen if:

A. Your vesicles were suspended in a solution containing both Na^+ and K^+ ions and had a solution with the same ionic composition inside them.

B. You add ATP to the suspension described in (A).

C. You add ATP, but the solution—outside as well as inside the vesicles—contains only Na^+ ions and no K^+ ions.

D. The concentrations of Na^+ and K^+ were as in (A), but half of the pump molecules embedded in the membrane of each vesicle were oriented the other way around so that the normally cytosolic portions of these molecules faced the inside of the vesicles. You then add ATP to the suspension.

E. You add ATP to the suspension described in (A), but in addition to Na^+ pumps, the membrane of your vesicles also contains K^+ leak channels.

QUESTION 12–17

Name the three ways in which an ion channel can be gated.

QUESTION 12–18

One thousand Ca^{2+} channels open in the plasma membrane of a cell that is 1000 μm^3 in size and has a cytosolic Ca^{2+} concentration of 100 nM. For how long would the channels need to stay open in order for the cytosolic Ca^{2+} concentration to rise to 5 μM? There is virtually unlimited Ca^{2+} available in the outside medium (the extracellular Ca^{2+} concentration in which most animal cells live is a few millimolar), and each channel passes 10^6 Ca^{2+} ions per second.

QUESTION 12–19

Amino acids are taken up by animal cells using a symport in the plasma membrane. What is the most likely ion whose electrochemical gradient drives the import? Is ATP consumed in the process? If so, how?

QUESTION 12–20

We will see in Chapter 15 that endosomes, which are membrane-enclosed intracellular organelles, need an acidic lumen in order to function. Acidification is achieved by an H^+ pump in the endosomal membrane, which also contains Cl^- channels. If the channels do not function properly (e.g., because of a mutation in the genes encoding the channel proteins), acidification is also impaired.

A. Can you explain how Cl^- channels might help acidification?

B. According to your explanation, would the Cl^- channels be absolutely required to lower the pH inside the endosome?

QUESTION 12–21

Some bacterial cells can grow on either ethanol (CH_3CH_2OH) or acetate (CH_3COO^-) as their only carbon source. Dr. Schwips measured the rate at which the two compounds traverse the bacterial plasma membrane but, due to excessive inhalation of one of the compounds (which one?), failed to label his data accurately.

A. Plot the data from the table below.

Concentration of Carbon Source (mM)	Rate of Transport (μmol/min)	
	Compound A	Compound B
0.1	2.0	18
0.3	6.0	46
1.0	20	100
3.0	60	150
10.0	200	182

B. Determine from your graph whether the data describing compound A correspond to the uptake of ethanol or acetate.

C. Determine the rates of transport for compounds A and B at 0.5 mM and 100 mM. (This part of the question requires that you be familiar with the principles of enzyme kinetics discussed in Chapter 3.)

Explain your answers.

QUESTION 12–22

Acetylcholine-gated cation channels do not discriminate between Na^+, K^+, and Ca^{2+} ions, allowing all to pass through them freely. So why is it that when acetylcholine binds to this protein in the plasma membrane of muscle cells, the channel opens and there is a large net influx of primarily Na^+ ions?

QUESTION 12–23

The ion channels that are regulated by binding of neurotransmitters, such as acetylcholine, glutamate, GABA, or glycine, have a similar overall structure. Yet, each class of these channels consists of a very diverse set of subtypes with different transmitter affinities, different channel conductances, and different rates of opening and closing. Do you suppose that such extreme diversity is a good or a bad thing from the standpoint of the pharmaceutical industry?