Membrane Transport of Small Molecules and the Electrical Properties of Membranes

CHAPTER 11

IN THIS CHAPTER

Because of its hydrophobic interior, the lipid bilayer of cell membranes restricts the passage of most polar molecules. This barrier function allows the cell to maintain concentrations of solutes in its cytosol that differ from those in the extracellular fluid and in each of the intracellular membrane-enclosed compartments. To benefit from this barrier, however, cells have had to evolve ways of transferring specific water-soluble molecules and ions across their membranes in order to ingest essential nutrients, excrete metabolic waste products, and regulate intracellular ion concentrations. Cells use specialized *membrane transport proteins* to accomplish this goal. The importance of such small molecule transport is reflected in the large number of genes in all organisms that code for the transmembrane transport proteins involved, which make up 15–30% of the membrane proteins in all cells. Some mammalian cells, such as nerve and kidney cells, devote up to two-thirds of their total metabolic energy consumption to such transport processes.

Cells can also transfer macromolecules and even large particles across their membranes, but the mechanisms involved in most of these cases differ from those used for transferring small molecules, and they are discussed in Chapters 12 and 13.

We begin this chapter by describing some general principles of how small water-soluble molecules traverse cell membranes. We then consider, in turn, the two main classes of membrane proteins that mediate this transmembrane traffic: *transporters*, which undergo sequential conformational changes to transport specific small molecules across membranes, and *channels*, which form narrow pores, allowing passive transmembrane movement, primarily of water and small inorganic ions. Transporters can be coupled to a source of energy to catalyze active transport, which together with selective passive permeability, creates large differences in the composition of the cytosol compared with that of either the extracellular fluid (**Table 11–1**) or the fluid within membrane-enclosed organelles. By generating inorganic ion-concentration differences across the lipid bilayer, cell membranes can store potential energy in the form of electrochemical gradients, which drive various transport processes, convey electrical signals in electrically excitable cells, and (in mitochondria, chloroplasts, and bacteria) make most of the cell's ATP. We focus our discussion mainly on transport across the plasma membrane, but similar mechanisms operate across the other membranes of the eukaryotic cell, as discussed in later chapters.

In the last part of the chapter, we concentrate mainly on the functions of ion channels in neurons (nerve cells). In these cells, channel proteins perform at their highest level of sophistication, enabling networks of neurons to carry out all the astonishing feats your brain is capable of.

PRINCIPLES OF MEMBRANE TRANSPORT

We begin this section by describing the permeability properties of protein-free, synthetic lipid bilayers. We then introduce some of the terms used to describe the various forms of membrane transport and some strategies for characterizing the proteins and processes involved.

TABLE 11–1 A Comparison of Inorganic Ion Concentrations Inside and Outside a Typical Mammalian Cell*

Component	Cytoplasmic 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 cell must contain equal quantities of positive and negative charges (that is, it must be electrically neutral). Thus, in addition to Cl^-, the cell contains many other anions not listed in this table; in fact, most cell constituents are negatively charged (HCO_3^-, PO_4^{3-}, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of Ca^{2+} and Mg^{2+} given are for the free ions: although there is a total of about 20 mM Mg^{2+} and 1–2 mM Ca^{2+} in cells, both ions are mostly bound to other substances (such as proteins, free nucleotides, RNA, etc.) and, for Ca^{2+}, stored within various organelles.

Protein-Free Lipid Bilayers Are Impermeable to Ions

Given enough time, virtually any molecule will diffuse across a protein-free lipid bilayer down its concentration gradient. The rate of diffusion, however, varies enormously, depending partly on the size of the molecule but mostly on its relative hydrophobicity (solubility in oil). In general, the smaller the molecule and the more hydrophobic, or nonpolar, it is, the more easily it will diffuse across a lipid bilayer. Small nonpolar molecules, such as O_2 and CO_2, readily dissolve in lipid bilayers and therefore diffuse rapidly across them. Small uncharged polar molecules, such as water or urea, also diffuse across a bilayer, albeit much more slowly (**Figure 11–1** and see Movie 10.3). By contrast, lipid bilayers are essentially impermeable to charged molecules (ions), no matter how small: the charge and high degree of hydration of such molecules prevents them from entering the hydrocarbon phase of the bilayer (**Figure 11–2**).

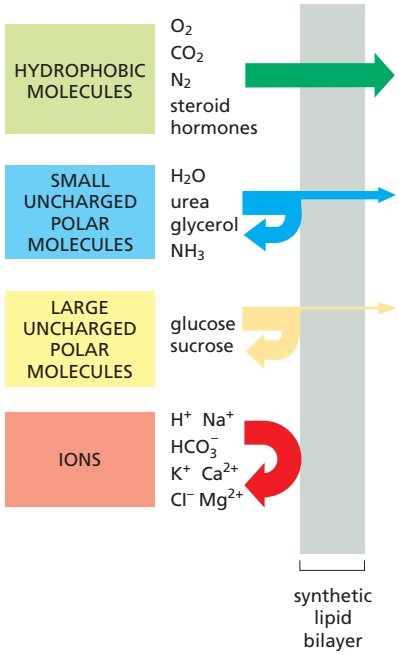


Figure 11–1 The relative permeability of a synthetic lipid bilayer to different classes of molecules. The smaller the molecule and, more importantly, the less strongly it associates with water, the more rapidly the molecule diffuses across the bilayer.

There Are Two Main Classes of Membrane Transport Proteins: Transporters and Channels

Like synthetic lipid bilayers, cell membranes allow small nonpolar molecules to permeate by diffusion. Cell membranes, however, also have to allow the passage of various polar molecules, such as ions, sugars, amino acids, nucleotides, water, and many cell metabolites that cross synthetic lipid bilayers only very slowly. Special **membrane transport proteins** transfer such solutes across cell membranes. These proteins occur in many forms and in all types of biological membranes. Each protein often transports only a specific molecular species or sometimes a class of molecules (such as ions, sugars, or amino acids). Studies in the 1950s found that bacteria with a single-gene mutation were unable to transport sugars across their plasma membrane, thereby demonstrating the specificity of membrane transport proteins. We now know that humans with similar mutations suffer from various inherited diseases that hinder the transport of a specific solute or solute class in the kidney, intestine, or other cell type. Individuals with the inherited disease *cystinuria*, for example, cannot transport certain amino acids (including cystine, the disulfide-linked dimer of cysteine) from either the urine or the intestine into the

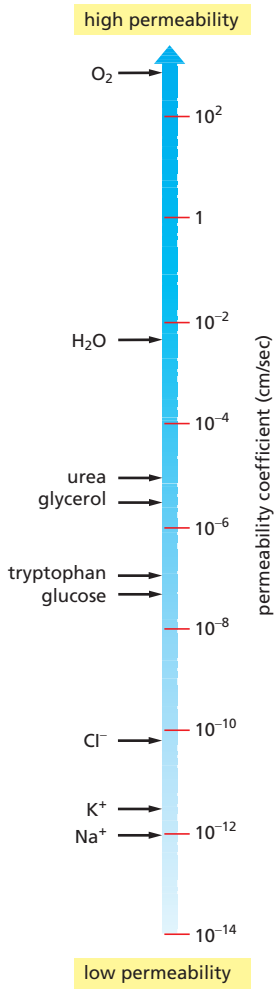


Figure 11–2 Permeability coefficients for the passage of various molecules through synthetic lipid bilayers. The rate of flow of a solute across the bilayer is directly proportional to the difference in its concentration on the two sides of the membrane. Multiplying this concentration difference (in mol/cm^3) by the permeability coefficient (in cm/sec) gives the flow of solute in moles per second per square centimeter of bilayer. A concentration difference of tryptophan of 10^{-4} mol/cm^3 (10^{-4} mol / 10^{-3} L = 0.1 M), for example, would cause a flow of 10^{-4} mol/cm^3 × 10^{-7} cm/sec = 10^{-11} mol/sec through 1 cm^2 of bilayer, or 6 × 10^4 molecules/sec through 1 μm^2 of bilayer.

blood; the resulting accumulation of cystine in the urine leads to the formation of cystine stones in the kidneys.

All membrane transport proteins that have been studied in detail are multipass transmembrane proteins—that is, their polypeptide chains traverse the lipid bilayer multiple times. By forming a protein-lined pathway across the membrane, these proteins enable specific hydrophilic solutes to cross the membrane without coming into direct contact with the hydrophobic interior of the lipid bilayer.

Transporters and channels are the two major classes of membrane transport proteins (Figure 11–3). **Transporters** (also called *carriers*, or *permeases*) bind the specific solute to be transported and undergo a series of conformational changes that alternately expose solute-binding sites on one side of the membrane and then on the other to transfer the solute across it. **Channels**, by contrast, interact with the solute to be transported much more weakly. They form continuous pores that extend across the lipid bilayer. When open, these pores allow specific solutes (such as inorganic ions of appropriate size and charge and in some cases small molecules, including water, glycerol, and ammonia) to pass through them and thereby cross the membrane. Not surprisingly, transport through channels occurs at a much faster rate than transport mediated by transporters. Although water can slowly diffuse across synthetic lipid bilayers, cells use dedicated channel proteins (called *water channels*, or *aquaporins*) that greatly increase the permeability of their membranes to water, as we discuss later.

Active Transport Is Mediated by Transporters Coupled to an Energy Source

All channels and many transporters allow solutes to cross the membrane only passively ("downhill"), a process called **passive transport**. In the case of transport of a single uncharged molecule, the difference in the concentration on the two sides of the membrane—its *concentration gradient*—drives passive transport and determines its direction (Figure 11–4A). If the solute carries a net charge, however, both its concentration gradient and the electrical potential difference across the membrane, the *membrane potential*, influence its transport. The concentration gradient and the electrical gradient combine to form a net driving force, the **electrochemical gradient**, for each charged solute (Figure 11–4B). We discuss electrochemical gradients in more detail later and in Chapter 14. In fact, almost all plasma membranes have an electrical potential (i.e., a voltage) across them, with the inside usually negative with respect to the outside. This potential favors the entry of positively charged ions into the cell but opposes the entry of negatively charged ions (see Figure 11–4B); it also opposes the efflux of positively charged ions.

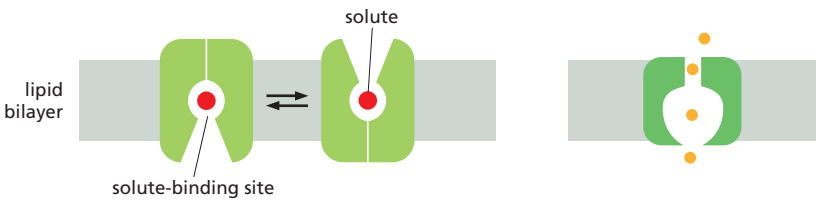


Figure 11–3 Transporters and channel proteins. (A) A transporter alternates between two conformations, so that the solute-binding site is sequentially accessible on one side of the bilayer and then on the other. (B) In contrast, a channel protein forms a pore across the bilayer through which specific solutes can passively diffuse.

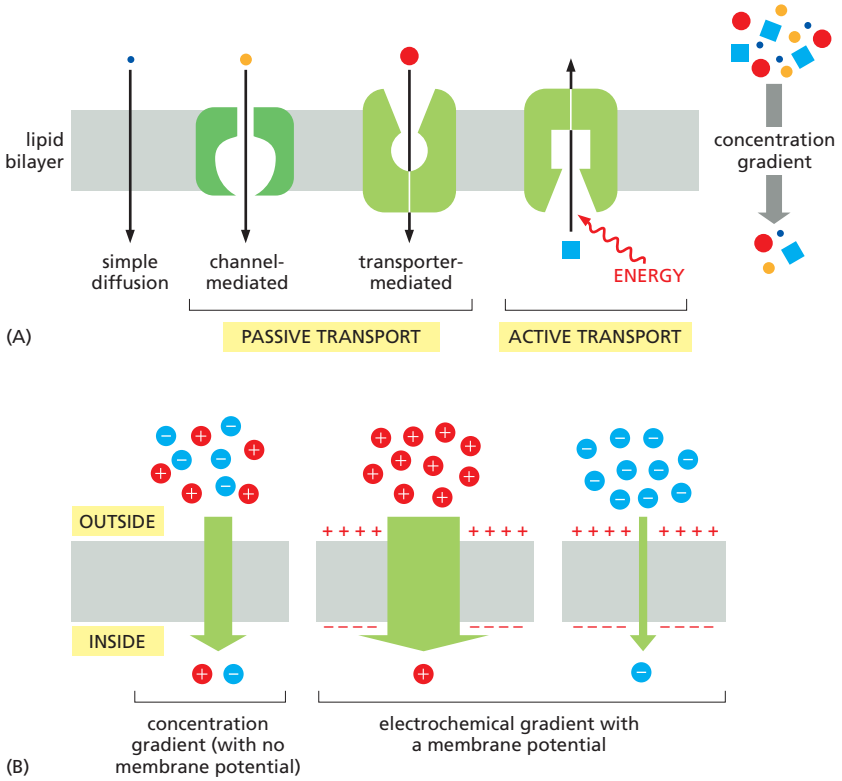


Figure 11–4 Different forms of membrane transport and the influence of the membrane. Passive transport down a concentration gradient (or an electrochemical gradient—see B below) occurs spontaneously, by diffusion, either through the lipid bilayer directly or through channels or passive transporters. By contrast, active transport requires an input of metabolic energy and is always mediated by transporters that pump the solute against its concentration or electrochemical gradient. (B) The electrochemical gradient of a charged solute (an ion) affects its transport. This gradient combines the membrane potential and the concentration gradient of the solute. The electrical and chemical gradients can work additively to increase the driving force on an ion across the membrane (*middle*) or can work against each other (*right*).

As shown in Figure 11–4A, in addition to passive transport, cells need to be able to actively pump certain solutes across the membrane "uphill," against their electrochemical gradients. Such **active transport** is mediated by transporters whose pumping activity is directional because it is tightly coupled to a source of metabolic energy, such as an ion gradient or ATP hydrolysis, as discussed later. Transmembrane movement of small molecules mediated by transporters can be either active or passive, whereas that mediated by channels is always passive (see Figure 11–4A).

Summary

Lipid bilayers are virtually impermeable to most polar molecules. To transport small water-soluble molecules into or out of cells or intracellular membrane-enclosed compartments, cell membranes contain various membrane transport proteins, each of which is responsible for transferring a particular solute or class of solutes across the membrane. There are two classes of membrane transport proteins—transporters and channels. Both form protein pathways across the lipid bilayer. Whereas transmembrane movement mediated by transporters can be either active or passive, solute flow through channel proteins is always passive. Both active and passive ion transport is influenced by the ion's concentration gradient and the membrane potential—that is, its electrochemical gradient.

TRANSPORTERS AND ACTIVE MEMBRANE TRANSPORT

The process by which a transporter transfers a solute molecule across the lipid bilayer resembles an enzyme–substrate reaction, and in many ways transporters behave like enzymes. By contrast to ordinary enzyme–substrate reactions, however, the transporter does not modify the transported solute but instead delivers it unchanged to the other side of the membrane.

Each type of transporter has one or more specific binding sites for its solute (substrate). It transfers the solute across the lipid bilayer by undergoing reversible

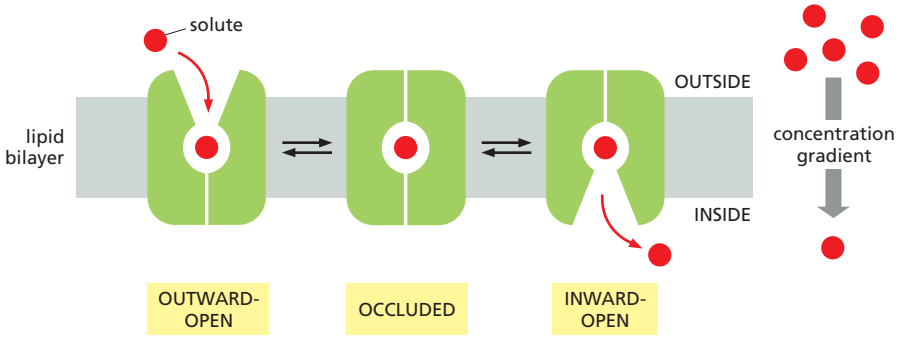


Figure 11–5 A model of how a conformational change in a transporter mediates the passive movement of a solute. The transporter is shown in three conformational states: in the outward-open state, the binding sites for solute are exposed on the outside; in the occluded state, the same sites are not accessible from either side; and in the inward-open state, the sites are exposed on the inside. The transitions between the states occur randomly. They are completely reversible and do 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 binds to the transporter in the outward-open conformation than in the inward-open conformation, and there is a net transport of solute down its concentration gradient (or, if the solute is an ion, down its electrochemical gradient).

conformational changes that alternately expose the solute-binding site first on one side of the membrane and then on the other—but never on both sides at the same time. The transition occurs through an intermediate state in which the solute is inaccessible, or occluded, from either side of the membrane (**Figure 11–5**). When the transporter is saturated (that is, when all solute-binding sites are occupied), the rate of transport is maximal. This rate, referred to as V_{max} (V for velocity), is characteristic of the specific carrier. V_{max} measures the rate at which the carrier can flip between its conformational states. In addition, each transporter has a characteristic affinity for its solute, reflected in the K_m of the reaction, which is equal to the concentration of solute when the transport rate is half its maximum value (**Figure 11–6**). As with enzymes, the binding of solute can be blocked by either competitive inhibitors (which compete for the same binding site and may or may not be transported) or noncompetitive inhibitors (which bind elsewhere and alter the structure of the transporter).

As we discuss shortly, it requires only a relatively minor modification of the model shown in Figure 11–5 to link a transporter to a source of energy in order to pump a solute uphill against its electrochemical gradient. Cells carry out such active transport in three main ways (**Figure 11–7**):

1. *Coupled transporters* harness the energy stored in concentration gradients to couple the uphill transport of one solute across the membrane to the downhill transport of another.
2. *ATP-driven pumps* couple uphill transport to the hydrolysis of ATP.
3. *Light- or redox-driven pumps*, which are known in bacteria, archaea, mitochondria, and chloroplasts, couple uphill transport to an input of energy from light, as with bacteriorhodopsin (discussed in Chapter 10), or from a redox reaction, as with cytochrome *c* oxidase (discussed in Chapter 14).

Amino acid sequence and three-dimensional structure comparisons suggest that, in many cases, there are strong similarities in structure between transporters that mediate active transport and those that mediate passive transport. Some bacterial transporters, for example, that use the energy stored in the H^+ gradient across the plasma membrane to drive the active uptake of various sugars are structurally similar to the transporters that mediate passive glucose transport into most animal cells. This suggests an evolutionary relationship between various transporters. Given the importance of small metabolites and sugars as energy sources, it is not surprising that the superfamily of transporters is an ancient one.

We begin our discussion of active membrane transport by considering a class of coupled transporters that are driven by ion concentration gradients. These proteins have a crucial role in the transport of small metabolites across membranes in all cells. We then discuss ATP-driven pumps, including the Na^+-K^+ pump that is found in the plasma membrane of most animal cells. Examples of the third class of active transport—light- or redox-driven pumps—are discussed in Chapter 14.

Active Transport Can Be Driven by Ion-Concentration Gradients

Some transporters simply passively mediate the movement of a single solute from one side of the membrane to the other at a rate determined by their V_{max} and

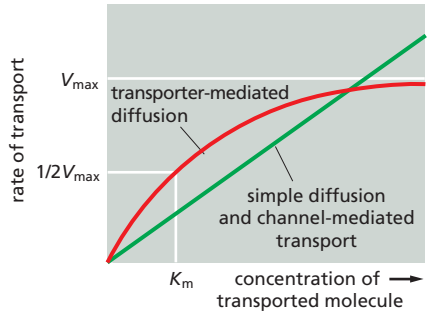


Figure 11–6 The kinetics of simple diffusion compared with transporter-mediated diffusion. Whereas the rate of diffusion and channel-mediated transport is directly proportional to the solute concentration (within the physical limits imposed by total surface area or total channels available), the rate of transporter-mediated diffusion reaches a maximum (V_{max}) when the transporter is saturated. The solute concentration when the transport rate is at half its maximal value approximates the binding constant (K_m) of the transporter for the solute and is analogous to the K_m of an enzyme for its substrate. The graph applies to a transporter moving a single solute; the kinetics of coupled transport of two or more solutes is more complex and exhibits cooperative behavior.

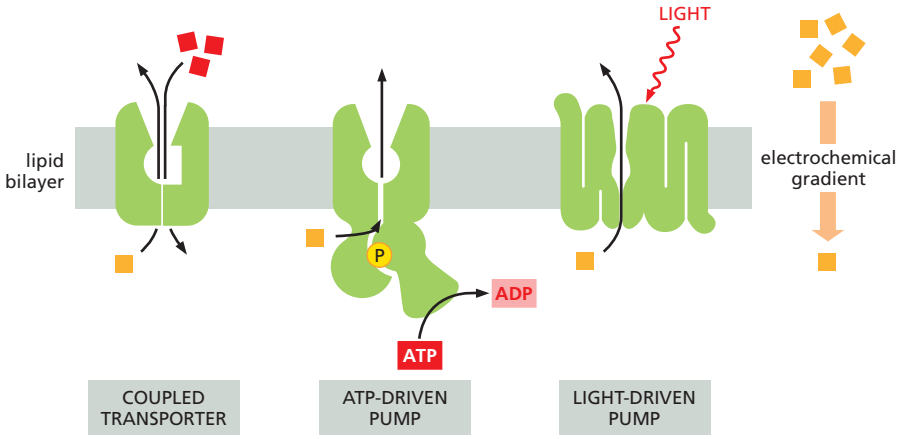


Figure 11–7 Three ways of driving active transport. The actively transported molecule is shown in *orange*, and the energy source is shown in *red*. Redox driven active transport is discussed in Chapter 14 (see Figures 14–18 and 14–19).

K_m; they are called **uniporters**. Others function as *coupled transporters*, in which the transfer of one solute strictly depends on the transport of a second. Coupled transport involves either the simultaneous transfer of a second solute in the same direction, performed by **symporters** (also called *co-transporters*), or the transfer of a second solute in the opposite direction, performed by **antiporters** (also called *exchangers*) (**Figure 11–8**).

The tight coupling between the transfer of two solutes allows the coupled transporters to harvest the energy stored in the electrochemical gradient of one solute, typically an inorganic ion, to transport the other. In this way, the free energy released during the movement of an inorganic ion down an electrochemical gradient is used as the driving force to pump other solutes uphill, against their electrochemical gradient. This strategy can work in either direction; some coupled transporters function as symporters, others as antiporters. In the plasma membrane of animal cells, Na^+ is the usual co-transported ion because its electrochemical gradient provides a large driving force for the active transport of a second molecule. The Na^+ that enters the cell during coupled transport is subsequently pumped out by an ATP-driven Na^+-K^+ pump in the plasma membrane (as we discuss later), which, by maintaining the Na^+ gradient, indirectly drives the coupled transport. Such ion-driven coupled transporters as just described are said to mediate *secondary active transport*. In contrast, ATP-driven pumps are said to mediate *primary active transport* because in these the free energy of ATP hydrolysis is used to directly drive the transport of a solute against its concentration gradient.

Intestinal and kidney epithelial cells contain a variety of symporters that are driven by the Na^+ gradient across the plasma membrane. Each Na^+-driven symporter is specific for importing a small group of related sugars or amino acids into the cell. Because the Na^+ tends to move into the cell down its electrochemical gradient, the sugar or amino acid is, in a sense, "dragged" into the cell with it. The greater the electrochemical gradient for Na^+, the more solute is pumped

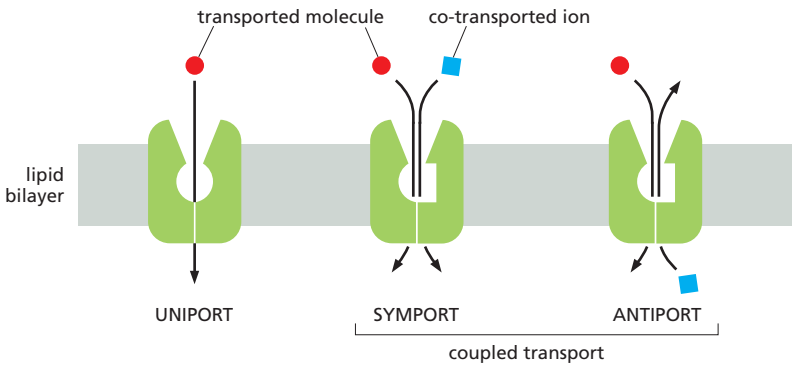


Figure 11–8 This schematic diagram shows transporters functioning as uniporters, symporters, and antiporters (Movie 11.1).

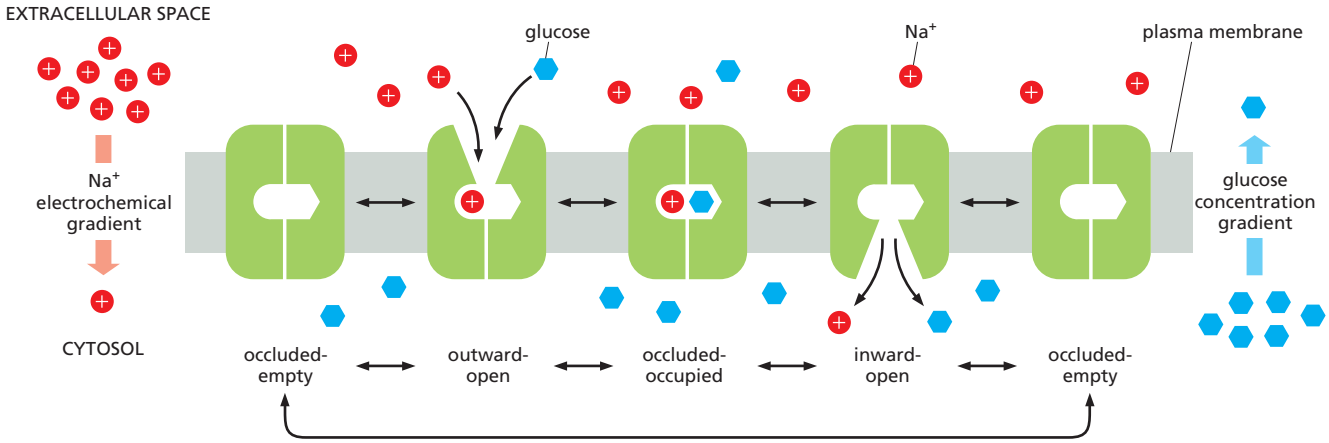


Figure 11–9 Mechanism of glucose transport fueled by a Na^+ gradient. As in the model shown in Figure 11–5, the transporter alternates between inward-open and outward-open states via an occluded intermediate state. Binding of Na^+ and glucose is cooperative—that is, the binding of either solute increases the protein's affinity for the other. Since the Na^+ concentration is much higher in the extracellular space than in the cytosol, glucose is more likely to bind to the transporter in the outward-facing state. The transition to the occluded state occurs only when both Na^+ and glucose are bound; their precise interactions in the solute-binding sites slightly stabilize the occluded state and thereby make this transition energetically favorable. Stochastic fluctuations caused by thermal energy drive the transporter randomly into the inward-open or outward-open conformation. If it opens outwardly, nothing is achieved, and the process starts all over. However, whenever it opens inwardly, Na^+ dissociates quickly in the low-Na^+-concentration environment of the cytosol. Glucose dissociation is likewise enhanced when Na^+ is lost, because of cooperativity in binding of the two solutes. The overall result is the net transport of both Na^+ and glucose into the cell. Because the occluded state is not formed when only one of the solutes is bound, the transporter switches conformation only when it is fully occupied or fully empty, thereby assuring strict coupling of the transport of Na^+ and glucose.

into the cell (**Figure 11–9**). Neurotransmitters (released by nerve cells to signal at synapses—as we discuss later) are taken up again by Na^+ symporters after their release. These neurotransmitter transporters are important drug targets: stimulants, such as cocaine and antidepressants, inhibit them and thereby prolong signaling by the neurotransmitters, which are not cleared efficiently.

Despite their great variety, transporters share structural features that can explain how they function and how they evolved. Transporters are typically built from bundles of 10 or more α helices that span the membrane. Solute- and ion-binding sites are located midway through the membrane, where some helices are broken or distorted and amino acid side chains and polypeptide backbone atoms form ion- and solute-binding sites. In the inward-open and outward-open conformations, these binding sites are accessible by passageways from one side of the membrane but not the other. In switching between the two conformations, the transporter protein transiently adopts an occluded conformation, in which both passageways are closed; this prevents the driving ion and the transported solute from crossing the membrane unaccompanied, which would deplete the cell's energy store to no purpose. Because only transporters with both types of binding sites appropriately filled change their conformation, tight coupling between ion and solute transport is assured.

Like enzymes, transporters can work in the reverse direction if ion and solute gradients are appropriately adjusted experimentally. This chemical symmetry is mirrored in their physical structure. Crystallographic analyses have revealed that transporters are built from *inverted repeats*: the packing of the transmembrane α helices in one half of the helix bundle is structurally similar to the packing in the other half, but the two halves are inverted in the membrane relative to each other. Transporters are therefore said to be pseudosymmetric, and the passageways that open and close on either side of the membrane have closely similar geometries, allowing alternating access to the ion- and solute-binding sites in the center (**Figure 11–10**). It is thought that the two halves evolved by gene duplication of a smaller ancestor protein.

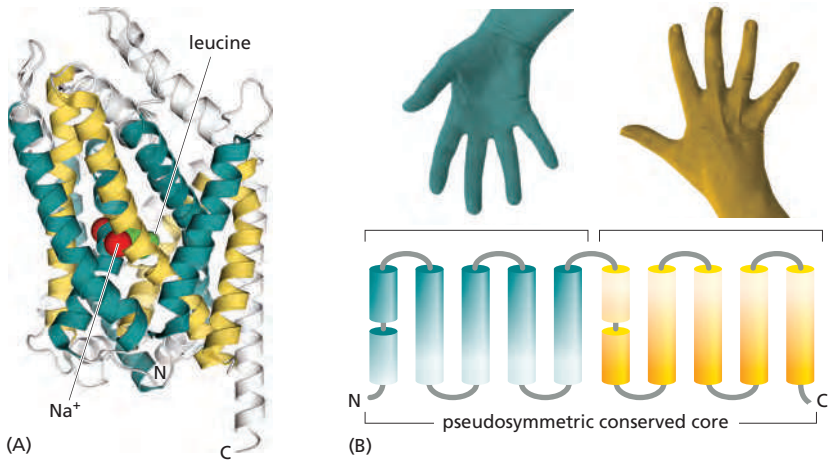


Figure 11–10 Transporters are built from inverted repeats. (A) LeuT, a bacterial leucine/Na^+ symporter related to human neurotransmitter transporters, such as the serotonin transporter, is shown. The core of the transporter is built from two bundles, each composed of five α helices (*blue* and *yellow*). The helices shown in *gray* differ among members of this transporter family and are thought to play regulatory roles, which are specific to a particular transporter. (B) Both core helix bundles are packed in a similar arrangement (shown as a hand, with the broken helix as the thumb), but the second bundle is inverted with respect to the first. The transporter's structural pseudosymmetry reflects its functional symmetry: the transporter can work in either direction, depending on the direction of the ion gradient. (Adapted from K.R. Vinothkumar and R. Henderson, *Q. Rev. Biophys*. 43:65–158, 2010. With permission from Cambridge University Press. PDB code: 3F3E.)

Some other types of important membrane transport proteins are also built from inverted repeats. Examples even include channel proteins such as the aquaporin water channel (discussed later) and the Sec61 channel through which nascent polypeptides move into the endoplasmic reticulum (discussed in Chapter 12). It is thought that these channels evolved from coupled transporters, in which the gating functions were lost, allowing them to open toward both sides of the membrane simultaneously to provide a continuous path across the membrane.

In bacteria, yeasts, and plants, as well as in many membrane-enclosed organelles of animal cells, most ion-driven active transport systems depend on H^+ rather than Na^+ gradients, reflecting the predominance of H^+ pumps in these membranes. An electrochemical H^+ gradient across the bacterial plasma membrane, for example, drives the inward active transport of many sugars and amino acids.

Transporters in the Plasma Membrane Regulate Cytosolic pH

Most proteins operate optimally at a particular pH. Lysosomal enzymes, for example, function best at the low pH (~5) found in lysosomes, whereas cytosolic enzymes function best at the close-to-neutral pH (~7.2) found in the cytosol. It is therefore crucial that cells control the pH of their intracellular compartments.

Most cells have one or more types of Na^+-driven antiporters in their plasma membrane that help to maintain the cytosolic pH at about 7.2. These transporters use the energy stored in the Na^+ gradient to pump out excess H^+, which either leaks in or is produced in the cell by acid-forming reactions. Two mechanisms are used: either H^+ is directly transported out of the cell or HCO_3^- is brought into the cell to neutralize H^+ in the cytosol (according to the reaction $HCO_3^- + H^+ \rightarrow H_2O + CO_2$). One of the antiporters that uses the first mechanism is a *Na^+–H^+ exchanger*, which couples an influx of Na^+ to an efflux of H^+. Another, which uses a combination of the two mechanisms, is a *Na^+-driven Cl^-–HCO_3^- exchanger* that couples an influx of Na^+ and HCO_3^- to an efflux of Cl^- and H^+ (so that $NaHCO_3$ comes in and HCl goes out). The Na^+-driven Cl^-–HCO_3^- exchanger is twice as effective as the Na^+–H^+ exchanger: it pumps out one H^+ and neutralizes another for each Na^+ that enters the cell. If HCO_3^- is available, as is usually the case, this antiporter is the most important transporter regulating the cytosolic pH. The pH inside the cell regulates both exchangers; when the pH in the cytosol falls, both exchangers increase their activity.

A *Na^+-independent Cl^-–HCO_3^- exchanger* adjusts the cytosolic pH in the reverse direction. Like the Na^+-dependent transporters, pH regulates the Na^+-independent Cl^-–HCO_3^- exchanger, but the exchanger's activity increases as the cytosol becomes too alkaline. The movement of HCO_3^- in this case is normally out of the cell, down its electrochemical gradient, which decreases the pH of the

cytosol. A Na^+-independent Cl^-–HCO_3^- exchanger in the membrane of red blood cells (called band 3 protein—see Figure 10–38) facilitates the quick discharge of CO_2 (as HCO_3^-) as the cells pass through capillaries in the lung.

The intracellular pH is not entirely regulated by transporters in the plasma membrane: ATP-driven H^+ pumps are used to control the pH of many intracellular compartments. As discussed in Chapter 13, H^+ pumps maintain the low pH in lysosomes, as well as in endosomes and secretory vesicles. These H^+ pumps use the energy of ATP hydrolysis to pump H^+ into these organelles from the cytosol.

An Asymmetric Distribution of Transporters in Epithelial Cells Underlies the Transcellular Transport of Solutes

In epithelial cells, such as those that absorb nutrients from the gut, transporters are distributed nonuniformly in the plasma membrane and thereby contribute to the **transcellular transport** of absorbed solutes. By the actions of the transporters in these cells, solutes are moved across the epithelial cell layer into the extracellular fluid from where they pass into the blood. As shown in **Figure 11–11**, Na^+-linked symporters located in the apical (absorptive) domain of the plasma membrane actively transport nutrients into the cell, building up substantial concentration gradients for these solutes across the plasma membrane. Uniporters in the basal and lateral (basolateral) domains allow the nutrients to leave the cell passively down these concentration gradients.

In many of these epithelial cells, the plasma membrane area is greatly increased by the formation of thousands of microvilli, which extend as thin, fingerlike projections from the apical surface of each cell. Such microvilli can increase the total absorptive area of a cell as much as 25-fold, thereby enhancing its transport capabilities.

As we have seen, ion gradients have a crucial role in driving many essential transport processes in cells. Ion pumps that use the energy of ATP hydrolysis establish and maintain these gradients, as we discuss next.

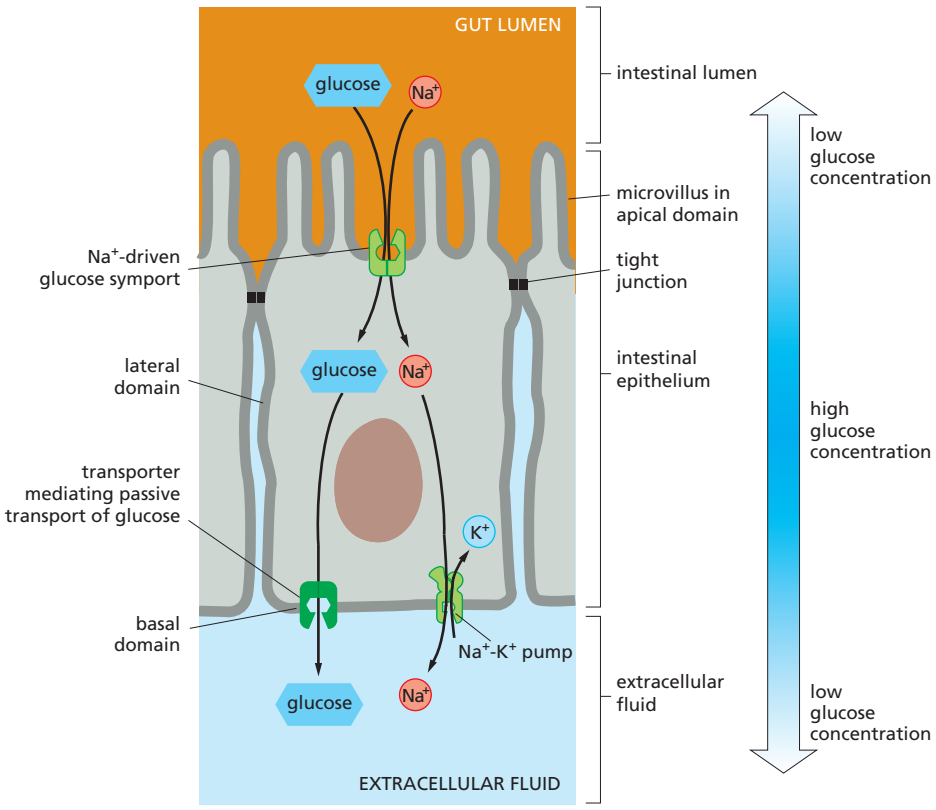
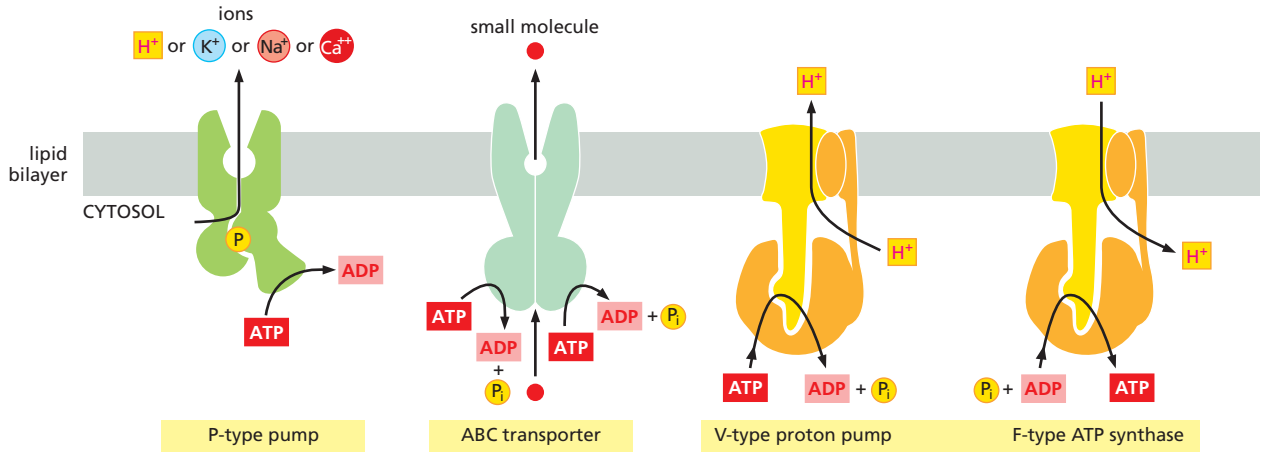


Figure 11–11 Transcellular transport. The transcellular transport of glucose across an intestinal epithelial cell depends on the nonuniform distribution of transporters in the cell's plasma membrane. The process shown here results in the transport of glucose from the intestinal lumen to the extracellular fluid (from where it passes into the blood). Glucose is pumped into the cell through the apical domain of the membrane by a Na^+-powered glucose symporter. Glucose passes out of the cell (down its concentration gradient) by passive movement through a glucose uniporter in the basal and lateral membrane domains. The Na^+ gradient driving the glucose symport is maintained by the Na^+-K^+ pump in the basal and lateral plasma membrane domains, which keeps the internal concentration of Na^+ low (**Movie 11.2**). Adjacent cells are connected by impermeable tight junctions, which have a dual function in the transport process illustrated: they prevent solutes from crossing the epithelium between cells, allowing a concentration gradient of glucose to be maintained across the cell sheet (see Figure 19–18). They also serve as diffusion barriers (fences) within the plasma membrane, which help confine the various transporters to their respective membrane domains (see Figure 10–34).



There Are Three Classes of ATP-Driven Pumps

ATP-driven pumps are often called *transport ATPases* because they hydrolyze ATP to ADP and phosphate and use the energy released to pump ions or other solutes across a membrane. There are three principal classes of ATP-driven pumps (**Figure 11–12**), and representatives of each are found in all prokaryotic and eukaryotic cells.

1. **P-type pumps** are structurally and functionally related multipass transmembrane proteins. They are called "P-type" because they phosphorylate themselves during the pumping cycle. This class includes many of the ion pumps that are responsible for setting up and maintaining gradients of Na^+, K^+, H^+, and Ca^{2+} across cell membranes.
2. **ABC transporters** (**A**TP-**B**inding **C**assette transporters) differ structurally from P-type ATPases and primarily pump small molecules across cell membranes.
3. **V-type pumps** are turbine-like protein machines, constructed from multiple different subunits. The V-type proton pump transfers H^+ into organelles such as lysosomes, synaptic vesicles, and plant or yeast vacuoles (V = vacuolar), to acidify the interior of these organelles (see Figure 13–37).

Structurally related to the V-type pumps is a distinct family of *F-type ATPases*, more commonly called *ATP synthases* because they normally work in reverse: instead of using ATP hydrolysis to drive H^+ transport, they use the H^+ gradient across the membrane to drive the synthesis of ATP from ADP and phosphate (see Figure 14–30). ATP synthases are found in the plasma membrane of bacteria, the inner membrane of mitochondria, and the thylakoid membrane of chloroplasts. The H^+ gradient is generated either during the electron-transport steps of oxidative phosphorylation (in aerobic bacteria and mitochondria), during photosynthesis (in chloroplasts), or by the light-driven H^+ pump (bacteriorhodopsin) in *Halobacterium*. We discuss some of these proteins in detail in Chapter 14.

For the remainder of this section, we focus on P-type pumps and ABC transporters.

A P-type ATPase Pumps Ca^{2+} into the Sarcoplasmic Reticulum in Muscle Cells

Eukaryotic cells maintain very low concentrations of free Ca^{2+} in their cytosol (~10^{-7} M) in the face of a very much higher extracellular Ca^{2+} concentration (~10^{-3} M). Therefore, even a small influx of Ca^{2+} significantly increases the concentration of free Ca^{2+} in the cytosol, and the flow of Ca^{2+} down its steep concentration gradient in response to extracellular signals is one means of transmitting these signals rapidly across the plasma membrane (discussed in Chapter 15). It is thus

Figure 11–12 Three types of ATP-driven pumps. Like any enzyme, all ATP-driven pumps can work in either direction, depending on the electrochemical gradients of their solutes and the ATP/ADP ratio. When the ATP/ADP ratio is high, they hydrolyze ATP; when the ATP/ADP ratio is low, they can synthesize ATP. The F-type ATPase in mitochondria normally works in this "reverse" mode to make most of the cell's ATP.

important that the cell maintains a steep Ca^{2+} gradient across its plasma membrane. Ca^{2+} transporters that actively pump Ca^{2+} out of the cell help maintain the gradient. One of these is a P-type Ca^{2+} ATPase; the other is an antiporter (called a *Na^+–Ca^{2+} exchanger*) that is driven by the Na^+ electrochemical gradient (discussed in Chapter 15).

The **Ca^{2+} pump**, or **Ca^{2+} ATPase**, in the *sarcoplasmic reticulum* (SR) membrane of skeletal muscle cells is a well-understood P-type transport ATPase. The SR is a specialized type of endoplasmic reticulum that forms a network of tubular sacs in the muscle cell cytoplasm, and it serves as an intracellular store of Ca^{2+}. When an action potential depolarizes the muscle cell plasma membrane, Ca^{2+} is released into the cytosol from the SR through *Ca^{2+}-release channels*, stimulating the muscle to contract (discussed in Chapters 15 and 16). The Ca^{2+} pump, which accounts for about 90% of the membrane protein of the SR, moves Ca^{2+} from the cytosol back into the SR. The endoplasmic reticulum of nonmuscle cells contains a similar Ca^{2+} pump, but in smaller quantities.

Enzymatic studies and analyses of the three-dimensional structures of transport intermediates of the SR Ca^{2+} pump and related pumps have revealed the molecular mechanism of P-type transport ATPases in great detail. They all have similar structures, containing 10 transmembrane α helices connected to three cytosolic domains (**Figure 11–13**). In the Ca^{2+} pump, amino acid side chains protruding from the transmembrane helices form two centrally positioned binding sites for Ca^{2+}. As shown in **Figure 11–14**, in the pump's ATP-bound nonphosphorylated state, these binding sites are accessible only from the cytosolic side of the SR membrane. Ca^{2+} binding triggers a series of conformational changes that close the passageway to the cytosol and activate a phosphotransfer reaction in which the terminal phosphate of the ATP is transferred to an aspartate that is highly conserved among all P-type ATPases. The ADP then dissociates and is replaced with a fresh ATP, causing another conformational change that opens a passageway to the SR lumen through which the two Ca^{2+} ions exit. They are replaced by two H^+ ions and a water molecule that stabilize the empty Ca^{2+}-binding sites and close the passageway to the SR lumen. Hydrolysis of the labile phosphoryl-aspartate bond returns the pump to the initial conformation, and the cycle starts again. The transient self-phosphorylation of the pump during its cycle is an essential characteristic of all P-type pumps.

The Plasma Membrane Na^+-K^+ Pump Establishes Na^+ and K^+ Gradients Across the Plasma Membrane

The concentration of K^+ is typically 10–30 times higher inside cells than outside, whereas the reverse is true of Na^+ (see Table 11–1, p. 598). A **Na^+-K^+ pump**, or **Na^+-K^+ ATPase**, found in the plasma membrane of virtually all animal cells maintains

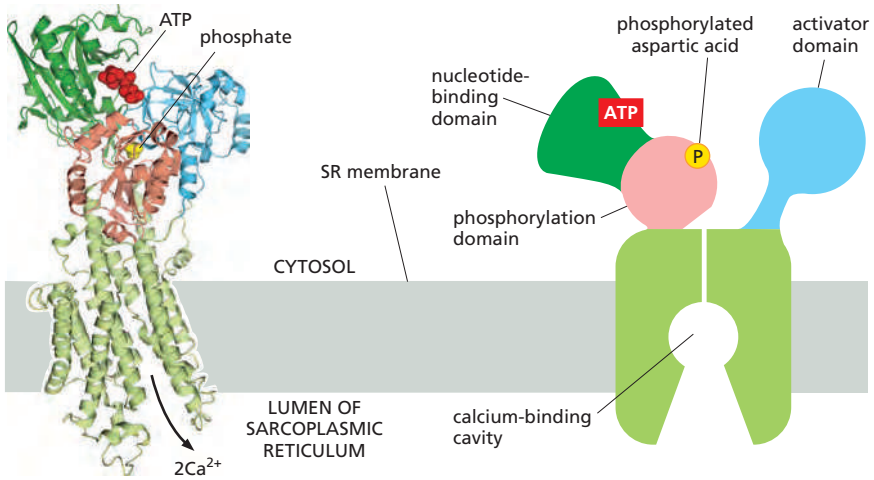


Figure 11–13 The structure of the sarcoplasmic reticulum Ca^{2+} pump. The ribbon model (*left*), derived from x-ray crystallographic analyses, shows the pump in its phosphorylated, ATP-bound state. The three globular cytosolic domains of the pump—the nucleotide-binding domain (*dark green*), the activator domain (*blue*), and the phosphorylation domain (*red*), also shown schematically on the *right*—change conformation dramatically during the pumping cycle. These changes in turn alter the arrangement of the transmembrane helices, which allows the Ca^{2+} to be released from its binding cavity into the SR lumen (**Movie 11.3**). (PDB code: 3B9B.)

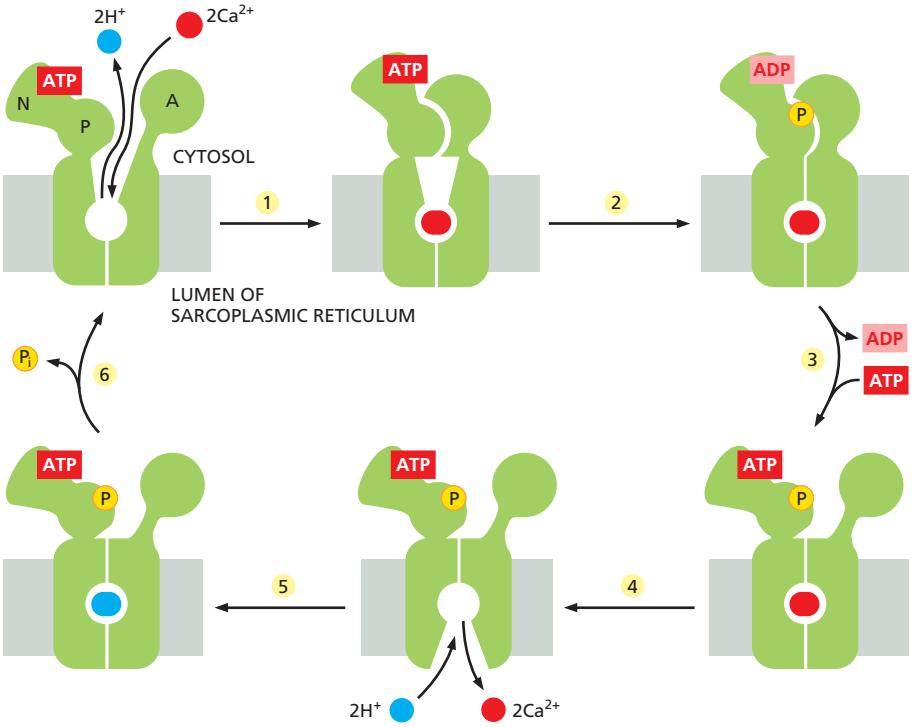


Figure 11–14 The pumping cycle of the sarcoplasmic reticulum Ca^{2+} pump. Ion pumping proceeds by a series of stepwise conformational changes in which movements of the pump's three cytosolic domains [the nucleotide-binding domain (N), the phosphorylation domain (P), and the activator domain (A)] are mechanically coupled to movements of the transmembrane α helices. Helix movement opens and closes passageways through which Ca^{2+} enters from the cytosol and binds to the two centrally located Ca^{2+} binding sites. The two Ca^{2+} then exit into the SR lumen and are replaced by two H^+, which are transported in the opposite direction. The Ca^{2+}-dependent phosphorylation and H^+-dependent dephosphorylation of aspartic acid are universally conserved steps in the reaction cycle of all P-type pumps: they cause the conformational transitions to occur in an orderly manner, enabling the proteins to do useful work. (Adapted from C. Toyoshima et al., *Nature* 432:361–368, 2004 and J.V. Møller et al., *Q. Rev. Biophys.* 43:501–566, 2010.)

these concentration differences. Like the Ca^{2+} pump, the Na^+-K^+ pump belongs to the family of P-type ATPases and operates as an ATP-driven antiporter, actively pumping Na^+ out of the cell against its steep electrochemical gradient and pumping K^+ in (Figure 11–15).

We mentioned earlier that the Na^+ gradient produced by the Na^+-K^+ pump drives the transport of most nutrients into animal cells and also has a crucial role in regulating cytosolic pH. A typical animal cell devotes almost one-third of its energy to fueling this pump, and the pump consumes even more energy in nerve cells and in cells that are dedicated to transport processes, such as those forming kidney tubules.

Since the Na^+-K^+ pump drives three positively charged ions out of the cell for every two it pumps in, it is *electrogenic*: it drives a net electric current across the membrane, tending to create an electrical potential, with the cell's inside being negative relative to the outside. This electrogenic effect of the pump, however, seldom directly contributes more than 10% to the membrane potential. The remaining 90%, as we discuss later, depends only indirectly on the Na^+-K^+ pump.

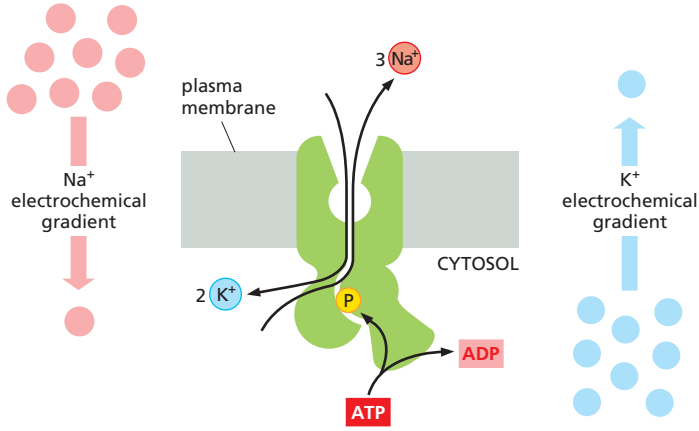


Figure 11–15 The function of the Na^+-K^+ pump. This P-type ATPase actively pumps Na^+ out of and K^+ into a cell against their electrochemical gradients. It is structurally closely related to the Ca^{2+} ATPase but differs in its selectivity for ions: for every molecule of ATP hydrolyzed by the pump, three Na^+ are pumped out and two K^+ are pumped in. As in the Ca^{2+} pump, an aspartate is phosphorylated and dephosphorylated during the pumping cycle (Movie 11.4).

ABC Transporters Constitute the Largest Family of Membrane Transport Proteins

The last type of transport ATPase that we discuss is the family of the ABC transporters, so named because each member contains two highly conserved ATPase domains, or **A**TP-**B**inding "**C**assettes," on the cytosolic side of the membrane. ATP binding brings together the two ATPase domains, and ATP hydrolysis leads to their dissociation (**Figure 11–16**). These movements of the cytosolic domains are transmitted to the transmembrane segments, driving cycles of conformational changes that alternately expose solute-binding sites on one side of the membrane and then on the other, as we have seen for other transporters. In this way, ABC transporters harvest the energy released upon ATP binding and hydrolysis to drive transport of solutes across the bilayer. The transport is directional toward inside or toward outside, depending on the particular conformational change in the solute binding site that is linked to ATP hydrolysis (see Figure 11–16).

ABC transporters constitute the largest family of membrane transport proteins and are of great clinical importance. The first of these proteins to be characterized was found in bacteria. We have already mentioned that the plasma membranes of all bacteria contain transporters that use the H^+ gradient across the membrane to actively transport a variety of nutrients into the cell. In addition, bacteria use ABC transporters to import certain small molecules. In bacteria such as *E. coli* that have double membranes (**Figure 11–17**), the ABC transporters are located in the inner membrane, and an auxiliary mechanism operates to capture the nutrients and deliver them to the transporters (**Figure 11–18**).

In *E. coli,* 78 genes (an amazing 5% of the bacterium's genes) encode ABC transporters, and animal genomes encode an even larger number. Although each transporter is thought to be specific for a particular molecule or class of molecules, the variety of substrates transported by this superfamily is great and includes inorganic ions, amino acids, mono- and polysaccharides, peptides, lipids, drugs, and, in some cases, even proteins that can be larger than the transporter itself.

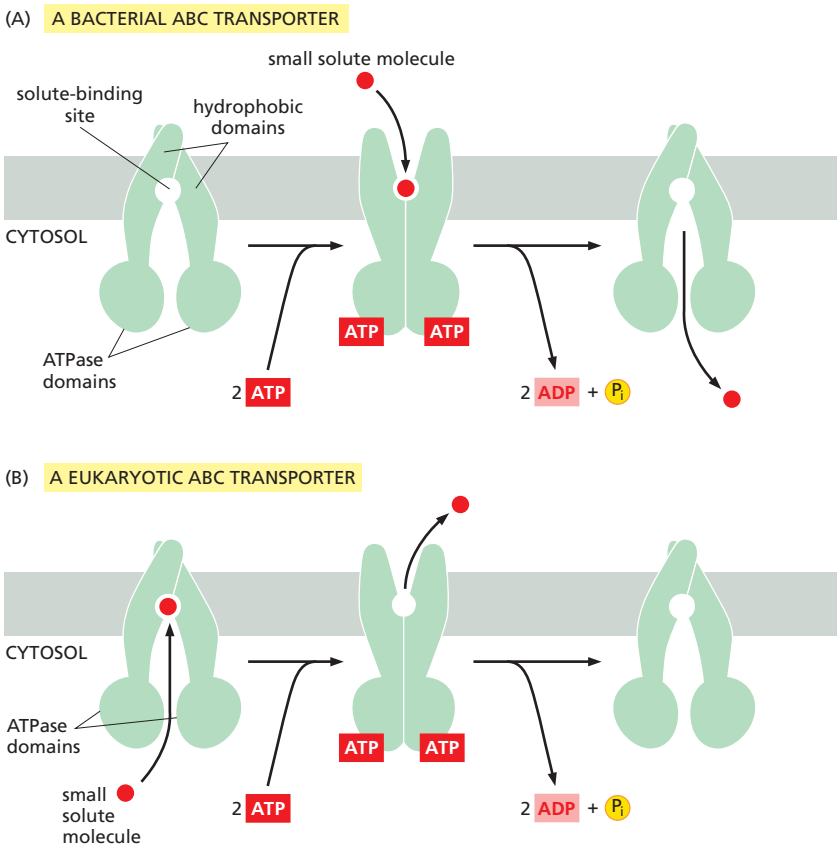


Figure 11–16 Small-molecule transport by typical ABC transporters. ABC transporters consist of multiple domains. Typically, two hydrophobic domains, each built of six membrane-spanning α helices, together form the translocation pathway and provide substrate specificity. Two ATPase domains protrude into the cytosol. In some cases, the two halves of the transporter are formed by a single polypeptide, whereas in other cases they are formed by two or more separate polypeptides that assemble into a similar structure. Without ATP bound, the transporter exposes a substrate-binding site on one side of the membrane. ATP binding induces a conformational change that exposes the substrate-binding site on the opposite side; ATP hydrolysis followed by ADP dissociation returns the transporter to its original conformation. Most individual ABC transporters are unidirectional. (A) Both importing and exporting ABC transporters are found in bacteria; an ABC importer is shown in this cartoon. The crystal structure of a bacterial ABC transporter is shown in Figure 3–76. (B) In eukaryotes, most ABC transporters export substances—either from the cytosol to the extracellular space or from the cytosol to a membrane-bound intracellular compartment such as the endoplasmic reticulum—or from the mitochondrial matrix to the cytosol.

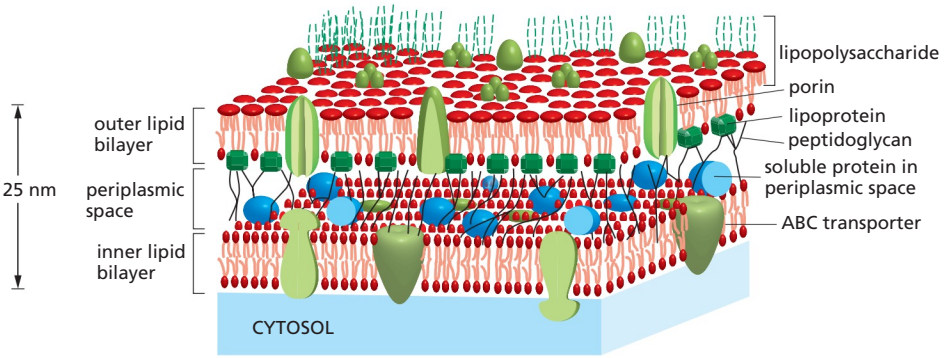


Figure 11–17 A small section of the double membrane of an *E. coli* bacterium. The inner membrane is the cell's plasma membrane. Between the inner and outer membranes is a highly porous, rigid peptidoglycan layer, composed of protein and polysaccharide that constitute the bacterial cell wall. It is attached to lipoprotein molecules in the outer membrane and fills the *periplasmic space* (only a little of the peptidoglycan layer is shown). This space also contains a variety of soluble protein molecules. The dashed threads (shown in *green*) at the top represent the polysaccharide chains of the special lipopolysaccharide molecules that form the external monolayer of the outer membrane; for clarity, only a few of these chains are shown. Bacteria with double membranes are called *Gram-negative* because they do not retain the dark blue dye used in Gram staining. Bacteria with single membranes (but thicker peptidoglycan cell walls), such as staphylococci and streptococci, retain the blue dye and are therefore called *Gram-positive*; their single membrane is analogous to the inner (plasma) membrane of Gram-negative bacteria.

The first eukaryotic ABC transporters identified were discovered because of their ability to pump hydrophobic drugs out of the cytosol. One of these transporters is the **multidrug resistance (MDR) protein**, also called P-glycoprotein. It is present at elevated levels in many human cancer cells and makes the cells simultaneously resistant to a variety of chemically unrelated cytotoxic drugs that are widely used in cancer chemotherapy. Treatment with any one of these drugs can result in the selective survival and overgrowth of those cancer cells that express an especially large amount of the MDR transporter. These cells pump drugs out of the cell very efficiently and are therefore relatively resistant to the drugs' toxic effects (**Movie 11.5**). Selection for cancer cells with resistance to one drug can thereby lead to resistance to a wide variety of anticancer drugs. Some studies indicate that up to 40% of human cancers develop multidrug resistance, making it a major hurdle in the battle against cancer.

A related and equally sinister phenomenon occurs in the protist *Plasmodium falciparum*, which causes malaria. More than 200 million people are infected worldwide with this parasite, which remains a major cause of human death, killing almost a million people every year. The development of resistance to the antimalarial drug *chloroquine* has hampered the control of malaria. The resistant *P. falciparum* have amplified a gene encoding an ABC transporter that pumps out the chloroquine.

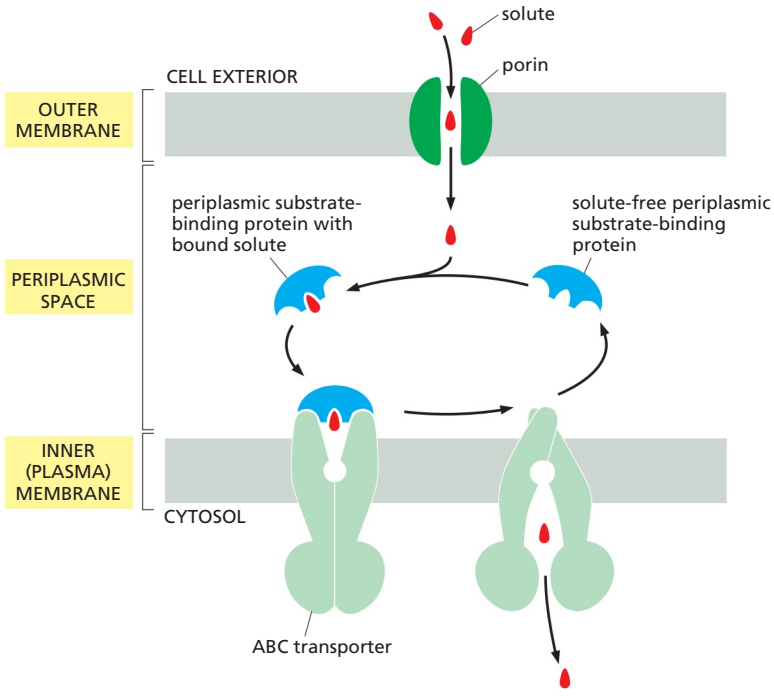


Figure 11–18 The auxiliary transport system associated with transport ATPases in bacteria with double membranes. The solute diffuses through channel proteins (porins) in the outer membrane and binds to a *periplasmic substrate-binding protein* that delivers it to the ABC transporter, which pumps it across the plasma membrane. The peptidoglycan is omitted for simplicity; its porous structure allows the substrate-binding proteins and water-soluble solutes to move through it by diffusion.

In most vertebrate cells, an ABC transporter in the endoplasmic reticulum (ER) membrane (named *transporter associated with antigen processing*, or *TAP transporter*) actively pumps a wide variety of peptides from the cytosol into the ER lumen. These peptides are produced by protein degradation in proteasomes (discussed in Chapter 6). They are carried from the ER to the cell surface, where they are displayed for scrutiny by cytotoxic T lymphocytes, which kill the cell if the peptides are derived from a virus or other microorganism lurking in the cytosol of an infected cell (discussed in Chapter 24).

Yet another member of the ABC transporter family is the *cystic fibrosis transmembrane conductance regulator* protein (CFTR), which was discovered through studies of the common genetic disease *cystic fibrosis.* This disease is caused by a mutation in the gene encoding CFTR, a Cl^- transport protein in the plasma membrane of epithelial cells. CFTR regulates ion concentrations in the extracellular fluid, especially in the lung. One in 27 Caucasians carries a gene encoding a mutant form of this protein; in 1 in 2900, both copies of the gene are mutated, causing the disease. In contrast to other ABC transporters, ATP binding and hydrolysis in the CFTR protein do not drive the transport process. Instead, they control the opening and closing of a continuous channel, which provides a passive conduit for Cl^- to move down its electrochemical gradient. Thus, some ABC proteins can function as transporters and others as gated channels.

Summary

Transporters bind specific solutes and transfer them across the lipid bilayer by undergoing conformational changes that alternately expose the solute-binding site on one side of the membrane and then on the other. Some transporters move a single solute "downhill," whereas others can act as pumps to move a solute "uphill" against its electrochemical gradient, using energy provided by ATP hydrolysis, by a downhill flow of another solute (such as Na^+ or H^+), or by light to drive the requisite series of conformational changes in an orderly manner. Transporters belong to a small number of protein families. Each family evolved from a common ancestral protein, and its members all operate by a similar mechanism. The family of P-type transport ATPases, which includes Ca^{2+} and Na^+-K^+ pumps, is an important example; each of these ATPases sequentially phosphorylates and dephosphorylates itself during the pumping cycle. The superfamily of ABC transporters is the largest family of membrane transport proteins and is especially important clinically. It includes proteins that are responsible for cystic fibrosis, for drug resistance in both cancer cells and malaria-causing parasites, and for pumping pathogen-derived peptides into the ER for cytotoxic lymphocytes to reorganize on the surface of infected cells.

CHANNELS AND THE ELECTRICAL PROPERTIES OF MEMBRANES

Unlike transporters, channels form pores across membranes. One class of channel proteins found in virtually all animals forms *gap junctions* between adjacent cells; each plasma membrane contributes equally to the formation of the channel, which connects the cytoplasm of the two cells. These channels are discussed in Chapter 19 and will not be considered further here. Both gap junctions and *porins*, the channels in the outer membranes of bacteria, mitochondria, and chloroplasts (discussed in Chapter 10), have relatively large and permissive pores, and it would be disastrous if they directly connected the inside of a cell to an extracellular space. Indeed, many bacterial toxins do exactly that to kill other cells (discussed in Chapter 24).

In contrast, most channels in the plasma membrane of animal and plant cells that connect the cytosol to the cell exterior necessarily have narrow, highly selective pores that can open and close rapidly. Because these proteins are concerned specifically with inorganic ion transport, they are referred to as **ion channels**. For transport efficiency, ion channels have an advantage over transporters, in that

they can pass up to 100 million ions through one open channel each second—a rate 10^5 times greater than the fastest rate of transport mediated by any known transporter. As discussed earlier, however, channels cannot be coupled to an energy source to perform active transport, so the transport they mediate is always passive (downhill). Thus, the function of ion channels is to allow specific inorganic ions—primarily Na^+, K^+, Ca^{2+}, or Cl^-—to diffuse rapidly down their electrochemical gradients across the lipid bilayer. In this section, we will see that the ability to control ion fluxes through these channels is essential for many cell functions. Nerve cells (neurons), in particular, have made a specialty of using ion channels, and we will consider how they use many different ion channels to receive, conduct, and transmit signals. Before we discuss ion channels, however, we briefly consider the aquaporin water channels that we mentioned earlier.

Aquaporins Are Permeable to Water But Impermeable to Ions

Because cells are mostly water (typically ~70% by weight), water movement across cell membranes is fundamentally important for life. Cells also contain a high concentration of solutes, including numerous negatively charged organic molecules that are confined inside the cell (the so-called *fixed anions*) and their accompanying cations that are required for charge balance. This creates an osmotic gradient, which mostly is balanced by an opposite osmotic gradient due to a high concentration of inorganic ions—chiefly Na^+ and Cl^-—in the extracellular fluid. The small remaining osmotic force tends to "pull" water into the cell, causing it to swell until the forces are balanced. Because all biological membranes are moderately permeable to water (see Figure 11–2), cell volume equilibrates in minutes or less in response to an osmotic gradient. For most animal cells, however, osmosis has only a minor role in regulating cell volume. This is because most of the cytoplasm is in a gel-like state and resists large changes in its volume in response to changes in osmolarity.

In addition to the direct diffusion of water across the lipid bilayer, some prokaryotic and eukaryotic cells have **water channels**, or **aquaporins**, embedded in their plasma membrane to allow water to move more rapidly. Aquaporins are particularly abundant in animal cells that must transport water at high rates, such as the epithelial cells of the kidney or exocrine cells that must transport or secrete large volumes of fluids, respectively (**Figure 11–19**).

Aquaporins must solve a problem that is opposite to that facing ion channels. To avoid disrupting ion gradients across membranes, they have to allow the rapid passage of water molecules while completely blocking the passage of ions. The three-dimensional structure of an aquaporin reveals how it achieves this remarkable selectivity. The channels have a narrow pore that allows water molecules to traverse the membrane in single file, following the path of carbonyl oxygens that line one side of the pore (**Figure 11–20**A and B). Hydrophobic amino acids line the other side of the pore. The pore is too narrow for any hydrated ion to enter, and the energy cost of dehydrating an ion would be enormous because the hydrophobic wall of the pore cannot interact with a dehydrated ion to compensate for the loss of water. This design readily explains why the aquaporins cannot conduct K^+,

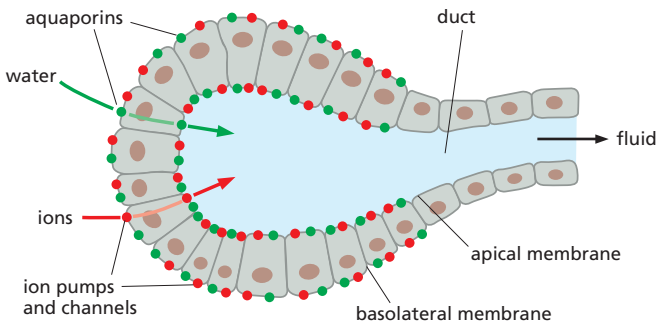


Figure 11–19 The role of aquaporins in fluid secretion. Cells lining the ducts of exocrine glands (as found, for example, in the pancreas and liver, and in mammary, sweat, and salivary glands) secrete large volumes of body fluids. These cells are organized into epithelial sheets in which their apical plasma membrane faces the lumen of the duct. Ion pumps and channels situated in the basolateral and apical plasma membrane move ions (mostly Na^+ and Cl^-) into the ductal lumen, creating an osmotic gradient between the surrounding tissue and the duct. Water molecules rapidly follow the osmotic gradient through aquaporins that are present in high concentrations in both the apical and basolateral membranes.

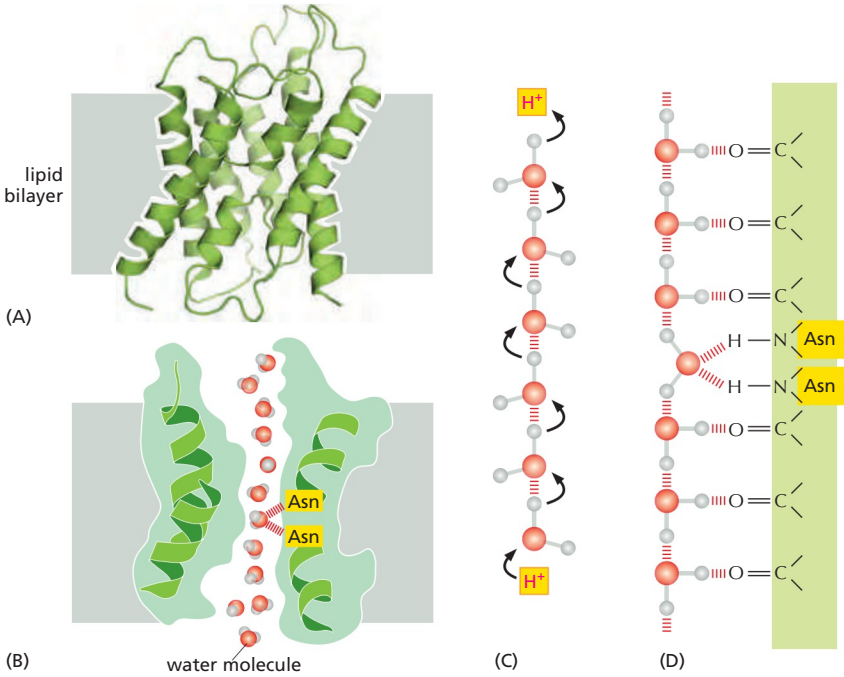


Figure 11–20 The structure of aquaporins. (A) A ribbon diagram of an aquaporin monomer. In the membrane, aquaporins form tetramers, with each monomer containing an aqueous pore in its center (not shown). Each individual aquaporin channel passes about 10^9 water molecules per second. (B) A longitudinal cross section through one aquaporin monomer, in the plane of the central pore. One face of the pore is lined with hydrophilic amino acids, which provide transient hydrogen bonds to water molecules; these bonds help line up the transiting water molecules in a single row and orient them as they traverse the pore. (C and D) A model explaining why aquaporins are impermeable to H^+. (C) In water, H^+ diffuses extremely rapidly by being relayed from one water molecule to the next. (D) Carbonyl groups (C=O) lining the hydrophilic face of the pore align water molecules, and two strategically placed asparagines in the center help tether a central water molecule such that both valences on its oxygen are occupied. This arrangement bipolarizes the entire line of water molecules, with each water molecule acting as a hydrogen-bond acceptor from its inner neighbor (**Movie 11.6**). (A and B, adapted from R.M. Stroud et al., *Curr. Opin. Struct. Biol.* 13:424–431, 2003. With permission from Elsevier.)

Na^+, Ca^{2+}, or Cl^- ions. These channels are also impermeable to H^+, which is mainly present in cells as H_3O^+. These hydronium ions diffuse through water extremely rapidly, using a molecular relay mechanism that requires the making and breaking of hydrogen bonds between adjacent water molecules (Figure 11–20C). Aquaporins contain two strategically placed asparagines, which bind to the oxygen atom of the central water molecule in the line of water molecules traversing the pore, imposing a bipolarity on the entire column of water molecules (Figure 11–20C and D). This makes it impossible for the "making and breaking" sequence of hydrogen bonds (shown in Figure 11–20C) to get past the central asparagine-bonded water molecule. Because both valences of this central oxygen are unavailable for hydrogen-bonding, the central water molecule cannot participate in an H^+ relay, and the pore is therefore impermeable to H^+.

We now turn to ion channels, the subject of the rest of the chapter.

Ion Channels Are Ion-Selective and Fluctuate Between Open and Closed States

Two important properties distinguish ion channels from aqueous pores. First, they show *ion selectivity*, permitting some inorganic ions to pass, but not others. This suggests that their pores must be narrow enough in places to force permeating ions into intimate contact with the walls of the channel so that only ions of appropriate size and charge can pass. The permeating ions have to shed most or all of their associated water molecules to pass, often in single file, through the narrowest part of the channel, which is called the *selectivity filter*; this limits their rate of passage (**Figure 11–21**). Thus, as the ion concentration increases, the flux of the ion through a channel increases proportionally but then levels off (saturates) at a maximum rate.

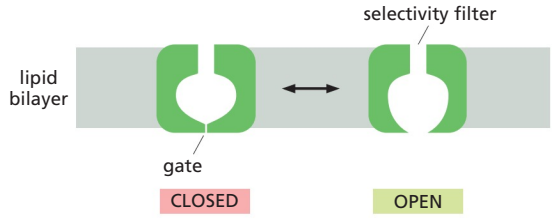


Figure 11–21 A typical ion channel, which fluctuates between closed and open conformations. The ion channel shown here in cross section forms a pore across the lipid bilayer only in the "open" conformational state. The pore narrows to atomic dimensions in one region (the selectivity filter), where the ion selectivity of the channel is largely determined. Another region of the channel forms the gate.

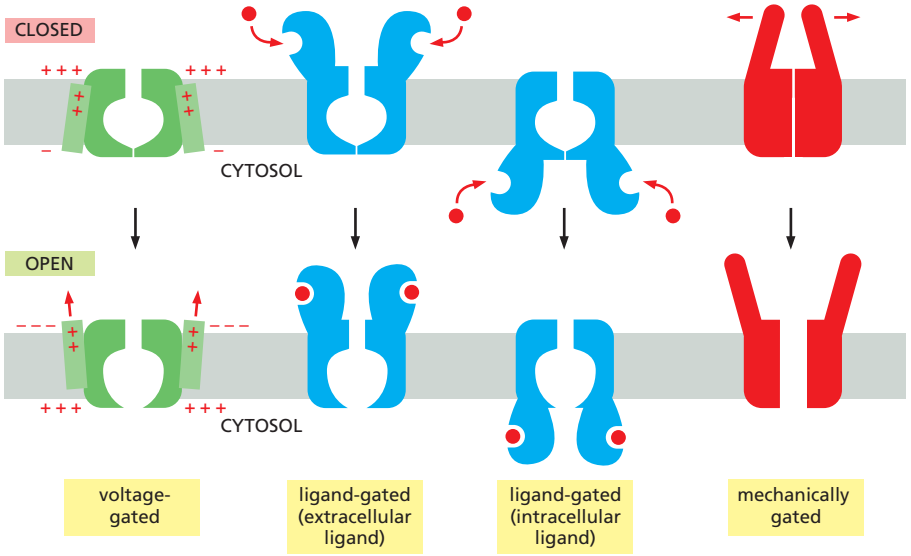


Figure 11–22 The gating of ion channels. This schematic drawing shows several kinds of stimuli that open ion channels. Mechanically gated channels often have cytoplasmic extensions (not shown) that link the channel to the cytoskeleton.

The second important distinction between ion channels and aqueous pores is that ion channels are not continuously open. Instead, they are *gated*, which allows them to open briefly and then close again. Moreover, with prolonged (chemical or electrical) stimulation, most ion channels go into a closed "desensitized," or "inactivated," state, in which they are refractory to further opening until the stimulus has been removed, as we discuss later. In most cases, the gate opens in response to a specific stimulus. As shown in **Figure 11–22**, the main types of stimuli that are known to cause ion channels to open are a change in the voltage across the membrane (*voltage-gated channels*), a mechanical stress (*mechanically gated channels*), or the binding of a ligand (*ligand-gated channels*). The ligand can be either an extracellular mediator—specifically, a neurotransmitter (*transmitter-gated channels*)—or an intracellular mediator such as an ion (*ion-gated channels*) or a nucleotide (*nucleotide-gated channels*). In addition, protein phosphorylation and dephosphorylation regulates the activity of many ion channels; this type of channel regulation is discussed, together with nucleotide-gated ion channels, in Chapter 15.

More than 100 types of ion channels have been identified thus far, and new ones are still being discovered, each characterized by the ions it conducts, the mechanism by which it is gated, and its abundance and localization in the cell and in specific cells. Ion channels are responsible for the electrical excitability of muscle cells, and they mediate most forms of electrical signaling in the nervous system. A single neuron typically contains 10 or more kinds of ion channels, located in different domains of its plasma membrane. But ion channels are not restricted to electrically excitable cells. They are present in all animal cells and are found in plant cells and microorganisms: they propagate the leaf-closing response of the mimosa plant, for example (**Movie 11.7**), and allow the single-celled *Paramecium* to reverse direction after a collision.

Ion channels that are permeable mainly to K^+ are found in the plasma membrane of almost all cells. An important subset of K^+ channels opens even in an unstimulated or "resting" cell, and hence these are called **K^+ leak channels**. Although this term applies to many different K^+ channels, depending on the cell type, they serve a common purpose: by making the plasma membrane much more permeable to K^+ than to other ions, they have a crucial role in maintaining the membrane potential across all plasma membranes, as we discuss next.

The Membrane Potential in Animal Cells Depends Mainly on K^+ Leak Channels and the K^+ Gradient Across the Plasma Membrane

A **membrane potential** arises when there is a difference in the electrical charge on the two sides of a membrane, due to a slight excess of positive ions over negative ones on one side and a slight deficit on the other. Such charge differences can result both from active electrogenic pumping (see p. 608) and from passive ion diffusion. As we discuss in Chapter 14, electrogenic H^+ pumps in the mitochondrial inner membrane generate most of the membrane potential across this membrane. Electrogenic pumps also generate most of the electrical potential across the plasma membrane in plants and fungi. In typical animal cells, however, passive ion movements make the largest contribution to the electrical potential across the plasma membrane.

As explained earlier, due to the action of the Na^+-K^+ pump, there is little Na^+ inside the cell, and other intracellular inorganic cations have to be plentiful enough to balance the charge carried by the cell's fixed anions—the negatively charged organic molecules that are confined inside the cell. This balancing role is performed largely by K^+, which is actively pumped into the cell by the Na^+-K^+ pump and can also move freely in or out through the *K^+ leak channels* in the plasma membrane. Because of the presence of these channels, K^+ comes almost to equilibrium, where an electrical force exerted by an excess of negative charges attracting K^+ into the cell balances the tendency of K^+ to leak out down its concentration gradient. The membrane potential (of the plasma membrane) is the manifestation of this electrical force, and we can calculate its equilibrium value from the steepness of the K^+ concentration gradient. The following argument may help to make this clear.

Suppose that initially there is no voltage gradient across the plasma membrane (the membrane potential is zero) but the concentration of K^+ is high inside the cell and low outside. K^+ will tend to leave the cell through the K^+ leak channels, driven by its concentration gradient. As K^+ begins to move out, each ion leaves behind an unbalanced negative charge, thereby creating an electrical field, or membrane potential, which will tend to oppose the further efflux of K^+. The net efflux of K^+ halts when the membrane potential reaches a value at which this electrical driving force on K^+ exactly balances the effect of its concentration gradient—that is, when the electrochemical gradient for K^+ is zero. Although Cl^- ions also equilibrate across the membrane, the membrane potential keeps most of these ions out of the cell because their charge is negative.

The equilibrium condition, in which there is no net flow of ions across the plasma membrane, defines the **resting membrane potential** for this idealized cell. A simple but very important formula, the **Nernst equation**, quantifies the equilibrium condition and, as explained in **Panel 11–1**, makes it possible to calculate the theoretical resting membrane potential if we know the ratio of internal and external ion concentrations. As the plasma membrane of a real cell is not exclusively permeable to K^+ and Cl^-, however, the actual resting membrane potential is usually not exactly equal to that predicted by the Nernst equation for K^+ or Cl^-.

The Resting Potential Decays Only Slowly When the Na^+-K^+ Pump Is Stopped

Movement of only a minute number of inorganic ions across the plasma membrane through ion channels suffices to set up the membrane potential. Thus, we can think of the membrane potential as arising from movements of charge that leave ion *concentrations* practically unaffected and result in only a very slight discrepancy in the number of positive and negative ions on the two sides of the membrane (**Figure 11–23**). Moreover, these movements of charge are generally rapid, taking only a few milliseconds or less.

Consider the change in the membrane potential in a real cell after the sudden inactivation of the Na^+-K^+ pump. A slight drop in the membrane potential occurs immediately. This is because the pump is electrogenic and, when active, makes a

THE NERNST EQUATION AND ION FLOW

The flow of any inorganic ion through a membrane channel is driven by the electrochemical gradient for that ion. This gradient represents the combination of two influences: the voltage gradient and the concentration gradient of the ion across the membrane. When these two influences just balance each other, the electrochemical gradient for the ion is zero, and there is no *net* flow of the ion through the channel. The voltage gradient (membrane potential) at which this equilibrium is reached is called the equilibrium potential for the ion. It can be calculated from an equation that will be derived below, called the Nernst equation.

The Nernst equation is $$V = \frac{RT}{zF} \ln \frac{C_o}{C_i}$$

where

- V = the equilibrium potential in volts (internal potential minus external potential)
- C_o and C_i = outside and inside concentrations of the ion, respectively
- R = the gas constant (8.3 J mol^{-1} K^{-1})
- T = the absolute temperature (K)
- F = Faraday's constant (9.6×10^4 J V^{-1} mol^{-1})
- z = the valence (charge) of the ion
- ln = logarithm to the base e

The Nernst equation is derived as follows:

A molecule in solution (a solute) tends to move from a region of high concentration to a region of low concentration simply due to the random movement of molecules, which results in their equilibrium. Consequently, movement down a concentration gradient is accompanied by a favorable free-energy change ($\Delta G < 0$), whereas movement up a concentration gradient is accompanied by an unfavorable free-energy change ($\Delta G > 0$). (Free energy is introduced in Chapter 2 and discussed in the context of redox reactions in Panel 14–1, p. 765.)

The free-energy change per mole of solute moved across the plasma membrane (ΔG_{conc}) is equal to $-RT \ln C_o / C_i$.

If the solute is an ion, moving it into a cell across a membrane whose inside is at a voltage V relative to the outside will cause an additional free-energy change (per mole of solute moved) of $\Delta G_{volt} = zFV$.

At the point where the concentration and voltage gradients just balance,

$$\Delta G_{conc} + \Delta G_{volt} = 0$$

and the ion distribution is at equilibrium across the membrane.

Thus,

$$zFV - RT \ln \frac{C_o}{C_i} = 0$$

and, therefore,

$$V = \frac{RT}{zF} \ln \frac{C_o}{C_i}$$

or, using the constant that converts natural logarithms to base 10,

$$V = 2.3 \frac{RT}{zF} \log_{10} \frac{C_o}{C_i}$$

For a univalent cation,

$$2.3 \frac{RT}{F} = 58 \text{ mV at } 20^\circ C \quad \text{and} \quad 61.5 \text{ mV at } 37^\circ C.$$

Thus, for such an ion at 37°C,

$$V = +61.5 \text{ mV for } C_o / C_i = 10,$$

whereas

$$V = 0 \text{ for } C_o / C_i = 1.$$

The K^+ equilibrium potential (V_K), for example, is

$$61.5 \log_{10}([K^+]_o / [K^+]_i) \text{ millivolts}$$

(–89 mV for a typical cell, where $[K^+]_o$ = 5 mM and $[K^+]_i$ = 140 mM).

At V_K, there is no net flow of K^+ across the membrane.

Similarly, when the membrane potential has a value of

$$61.5 \log_{10}([Na^+]_o / [Na^+]_i),$$

the Na^+ equilibrium potential (V_{Na}),

there is no net flow of Na^+.

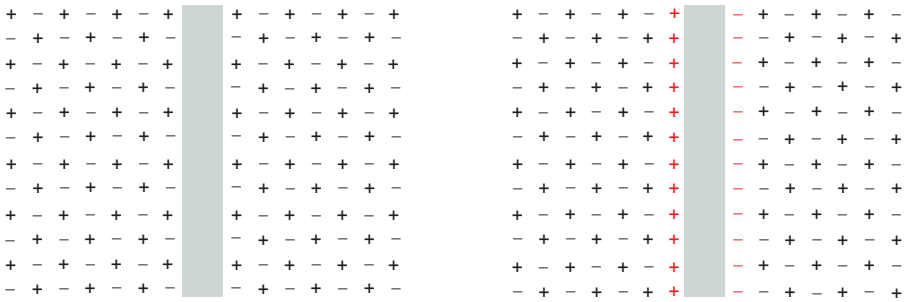
For any particular membrane potential, V_M, the net force tending to drive a particular type of ion out of the cell, is proportional to the difference between V_M and the equilibrium potential for the ion: hence,

for K^+ it is $V_M - V_K$

and for Na^+ it is $V_M - V_{Na}$.

When there is a voltage gradient across the membrane, the ions responsible for it—the positive ions on one side and the negative ions on the other—are concentrated in thin layers on either side of the membrane because of the attraction between positive and negative electric charges. The number of ions that go to form the layer of charge adjacent to the membrane is minute compared with the total number inside the cell. For example, the movement of 6000 Na^+ ions across 1 μm^2 of membrane will carry sufficient charge to shift the membrane potential by about 100 mV.

Because there are about 3×10^7 Na^+ ions in a typical cell (1 μm^3 of bulk cytoplasm), such a movement of charge will generally have a negligible effect on the ion concentration gradients across the membrane.



exact balance of charges on each side of the membrane; membrane potential = 0

a few of the positive ions *(red)* cross the membrane from right to left, leaving their negative counterions *(red)* behind; this sets up a nonzero membrane potential

Figure 11–23 The ionic basis of a membrane potential. A small flow of inorganic ions through an ion channel carries sufficient charge to cause a large change in the membrane potential. The ions that give rise to the membrane potential lie in a thin (< 1 nm) surface layer close to the membrane, held there by their electrical attraction to their oppositely charged counterparts (counterions) on the other side of the membrane. For a typical cell, 1 microcoulomb of charge (6×10^{12} monovalent ions) per square centimeter of membrane, transferred from one side of the membrane to the other, changes the membrane potential by roughly 1 V. This means, for example, that in a spherical cell of diameter 10 μm, the number of K^+ ions that have to flow out to alter the membrane potential by 100 mV is only about 1/100,000 of the total number of K^+ ions in the cytosol. This amount is so minute that the intracellular K^+ concentration remains virtually unchanged.

small direct contribution to the membrane potential by pumping out three Na^+ for every two K^+ that it pumps in (see Figure 11–15). However, switching off the pump does not abolish the major component of the resting potential, which is generated by the K^+ equilibrium mechanism just described. This component of the membrane potential persists as long as the Na^+ concentration inside the cell stays low and the K^+ ion concentration high—typically for many minutes. But the plasma membrane is somewhat permeable to all small ions, including Na^+. Therefore, without the Na^+-K^+ pump, the ion gradients set up by the pump will eventually run down, and the membrane potential established by diffusion through the K^+ leak channels will fall as well. As Na^+ enters, the cell eventually comes to a new resting state where Na^+, K^+, and Cl^- are all at equilibrium across the membrane. The membrane potential in this state is much less than it was in the normal cell with an active Na^+-K^+ pump.

The resting potential of an animal cell varies between –20 mV and –120 mV, depending on the organism and cell type. Although the K^+ gradient always has a major influence on this potential, the gradients of other ions (and the disequilibrating effects of ion pumps) also have a significant effect: the more permeable the membrane for a given ion, the more strongly the membrane potential tends to be driven toward the equilibrium value for that ion. Consequently, changes in a membrane's permeability to ions can cause significant changes in the membrane potential. This is one of the key principles relating the electrical excitability of cells to the activities of ion channels.

To understand how ion channels select their ions and how they open and close, we need to know their atomic structure. The first ion channel to be crystallized and studied by x-ray diffraction was a bacterial K^+ channel. The details of its structure revolutionized our understanding of ion channels.

The Three-Dimensional Structure of a Bacterial K^+ Channel Shows How an Ion Channel Can Work

Scientists were puzzled by the remarkable ability of ion channels to combine exquisite ion selectivity with a high conductance. K^+ leak channels, for example, conduct K^+ 10,000-fold faster than Na^+, yet the two ions are both featureless spheres and have similar diameters (0.133 nm and 0.095 nm, respectively). A single amino acid substitution in the pore of an animal cell K^+ channel can result in a loss of ion selectivity and cell death. We cannot explain the normal K^+ selectivity by pore size, because Na^+ is smaller than K^+. Moreover, the high conductance rate is incompatible with the channel's having selective, high-affinity K^+-binding sites, as the binding of K^+ ions to such sites would greatly slow their passage.

The puzzle was solved when the structure of a *bacterial K^+ channel* was determined by x-ray crystallography. The channel is made from four identical transmembrane subunits, which together form a central pore through the membrane. Each subunit contributes two transmembrane α helices, which are tilted outward in the membrane and together form a cone, with its wide end facing the outside of

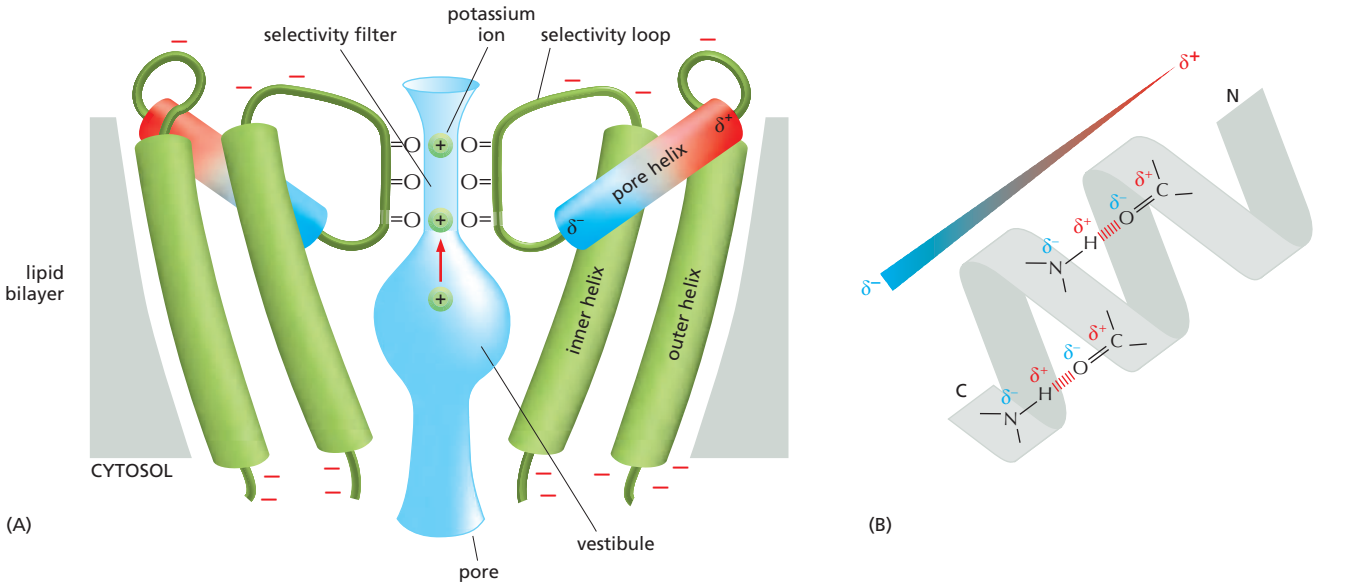


Figure 11–24 The structure of a bacterial K^+ channel. (A) The transmembrane α helices from only two of the four identical subunits are shown. From the cytosolic side, the pore (schematically shaded in *blue)* opens up into a vestibule in the middle of the membrane. The pore vestibule facilitates transport by allowing the K^+ ions to remain hydrated even though they are more than halfway across the membrane. The narrow selectivity filter of the pore links the vestibule to the outside of the cell. Carbonyl oxygens line the walls of the selectivity filter and form transient binding sites for dehydrated K^+ ions. Two K^+ ions occupy different sites in the selectivity filter, while a third K^+ ion is located in the center of the vestibule, where it is stabilized by electrical interactions with the more negatively charged ends of the pore helices. The ends of the four short "pore helices" (only two of which are shown) point precisely toward the center of the vestibule, thereby guiding K^+ ions into the selectivity filter (Movie 11.8). (B) Peptide bonds have an electric dipole, with more negative charge accumulated at the oxygen of the C=O bond and at the nitrogen of the N–H bond. In an α helix, hydrogen bonds (*red*) align the dipoles. As a consequence, every α helix has an electric dipole along its axis, resulting from summation of the dipoles of the individual peptide bonds, with a more negatively charged C-terminal end (δ^-) and a more positively charged N-terminal end (δ^+). (A, adapted from D.A. Doyle et al., *Science* 280:69–77, 1998.)

the cell where K^+ ions exit from the channel (Figure 11–24). The polypeptide chain that connects the two transmembrane helices forms a short α helix (the *pore helix*) and a crucial loop that protrudes into the wide section of the cone to form the **selectivity filter**. The selectivity loops from the four subunits form a short, rigid, narrow pore, which is lined by the carbonyl oxygen atoms of their polypeptide backbones. Because the selectivity loops of all known K^+ channels have similar amino acid sequences, it is likely that they form a closely similar structure.

The structure of the selectivity filter explains the ion selectivity of the channel. A K^+ ion must lose almost all of its bound water molecules to enter the filter, where it interacts instead with the carbonyl oxygens lining the filter; the oxygens are rigidly spaced at the exact distance to accommodate a K^+ ion. A Na^+ ion, in contrast, cannot enter the filter because the carbonyl oxygens are too far away from the smaller Na^+ ion to compensate for the energy expense associated with the loss of water molecules required for entry (Figure 11–25).

Structural studies of K^+ channels and other ion channels have also indicated some general principles of how these channels open and close. The gating involves movement of the helices in the membrane so that they either obstruct or open the path for ion movement. Depending on the particular type of channel, helices tilt, rotate, or bend during gating. The structure of a closed K^+ channel shows that by tilting the inner helices, the pore constricts like a diaphragm at its cytosolic end (Figure 11–26). Bulky hydrophobic amino acid side chains block the small opening that remains, preventing the entry of ions.

Many other ion channels operate on similar principles: the channel's gating helices are allosterically coupled to domains that form the ion-conducting pathway; and a conformational change in the gate—in response, say, to ligand binding or altered membrane potential—brings about conformational change in the conducting pathway, either opening it or blocking it off.

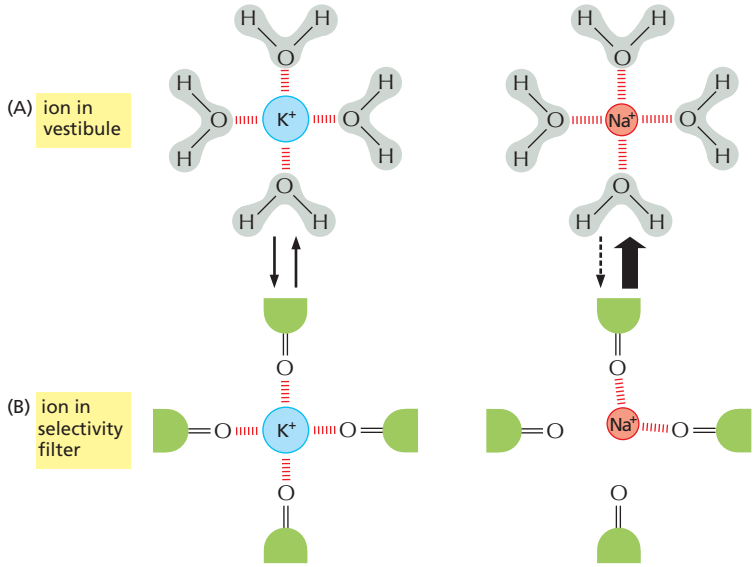


Figure 11–25 K^+ specificity of the selectivity filter in a K^+ channel. The drawings show K^+ and Na^+ ions (A) in the vestibule and (B) in the selectivity filter of the pore, viewed in cross section. In the vestibule, the ions are hydrated. In the selectivity filter, they have lost their water, and the carbonyl oxygens are placed to accommodate a dehydrated K^+ ion. The dehydration of the K^+ ion requires energy, which is precisely balanced by the energy regained by the interaction of the ion with all of the carbonyl oxygens that serve as surrogate water molecules. Because the Na^+ ion is too small to interact with the oxygens, it can enter the selectivity filter only at a great energetic expense. The filter therefore selects K^+ ions with high specificity. (A, adapted from Y. Zhou et al., *Nature* 414:43–48, 2001. With permission from Macmillan Publishers Ltd.)

Mechanosensitive Channels Protect Bacterial Cells Against Extreme Osmotic Pressures

All organisms, from single-cell bacteria to multicellular animals and plants, must sense and respond to mechanical forces in their external environment (such as sound, touch, pressure, shear forces, and gravity) and in their internal environment (such as osmotic pressure and membrane bending). Numerous proteins are known to be capable of responding to such mechanical forces, and a large subset of those proteins has been identified as possible **mechanosensitive channels**, but very few of the candidate proteins have been shown directly to be mechanically activated ion channels. One reason for this dearth in our knowledge is that most such channels are extremely rare. Auditory hair cells in the human cochlea, for example, contain extraordinarily sensitive mechanically gated ion channels, but each of the approximately 15,000 individual hair cells is thought to have a total of only 50–100 of them (**Movie 11.9**). Additional difficulties arise because the gating mechanisms of many mechanosensitive channel types require the channels to be embedded in complex architectures that require attachment to the extracellular matrix or to the cytoskeleton and are difficult to reconstitute in the test tube. The study of mechanosensitive receptors is a field of active investigation.

A well-studied class of mechanosensitive channels is found in the bacterial plasma membrane. These channels open in response to mechanical stretching of the lipid bilayer in which they are embedded. When a bacterium experiences a low-ionic-strength external environment (hypotonic conditions), such as

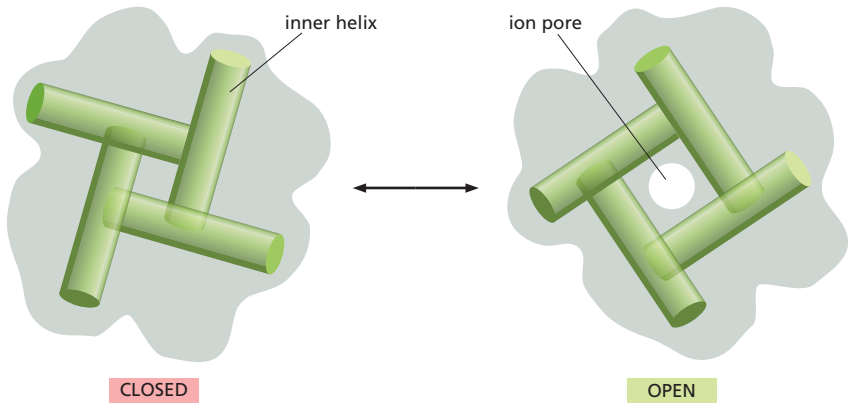


Figure 11–26 A model for the gating of a bacterial K^+ channel. The channel is viewed in cross section. To adopt the closed conformation, the four inner transmembrane helices that line the pore on the cytosolic side of the selectivity filter (see Figure 11–24) rearrange to close the cytosolic entrance to the channel. (Adapted from E. Perozo et al., *Science* 285:73–78, 1999.)

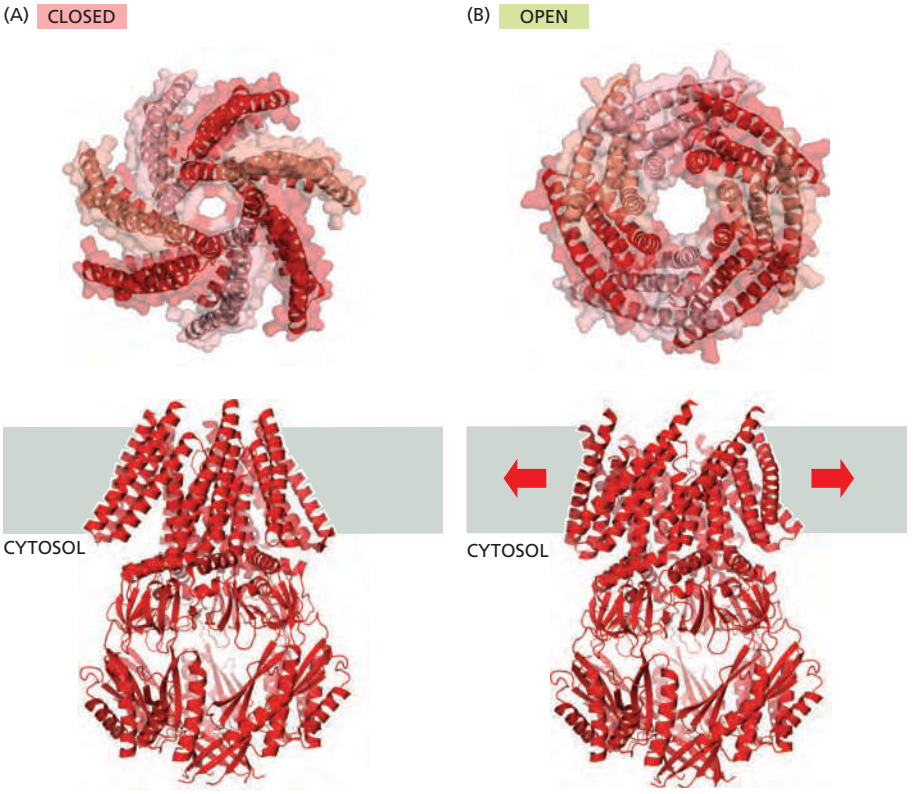


Figure 11–27 The structure of mechanosensitive channels. The crystal structures of MscS in its (A) closed and (B) open conformation are shown. The side views (lower panels) show the entire protein, including the large intracellular domain. The face views (upper panels) show the transmembrane domains only. The open structure occupies more area in the lipid bilayer and is energetically favored when a membrane is stretched. This may explain why the MscS channel opens as pressure builds up inside the cell. (PDB codes: 2OAU, 2VV5.)

rainwater, the cell swells as water seeps in due to an increase in the osmotic pressure. If the pressure rises to dangerous levels, the cell opens mechanosensitive channels that allow small molecules to leak out. Bacteria that are experimentally placed in fresh water can rapidly lose more than 95% of their small molecules in this manner, including amino acids, sugars, and potassium ions. However, they keep their macromolecules safely inside and thus can recover quickly after environmental conditions return to normal.

Mechanical gating has been demonstrated using biophysical techniques in which force is exerted on pure lipid bilayers containing the bacterial mechanosensitive channels; for example, by applying suction with a micropipette. Such measurements demonstrate that the cell has several different channels that open at different levels of pressure. The mechanosensitive channel of small conductance, called the MscS channel, opens at low and moderate pressures (**Figure 11–27**). It is composed of seven identical subunits, which in the open state form a pore about 1.3 nm in diameter—just big enough to pass ions and small molecules. Large cytoplasmic domains limit the size of molecules that can reach the pore. The mechanosensitive channel of large conductance, called the MscL channel, opens to over 3 nm in diameter when the pressure gets so high that the cell might burst.

The Function of a Neuron Depends on Its Elongated Structure

The cells that make most sophisticated use of channels are neurons. Before discussing how they do so, we digress briefly to describe how a typical neuron is organized.

The fundamental task of a **neuron**, or **nerve cell**, is to receive, conduct, and transmit signals. To perform these functions, neurons are often extremely elongated. In humans, for example, a single neuron extending from the spinal cord to a muscle in the foot may be as long as 1 meter. Every neuron consists of a cell body (containing the nucleus) with a number of thin processes radiating outward from it. Usually one long **axon** conducts signals away from the cell body toward distant

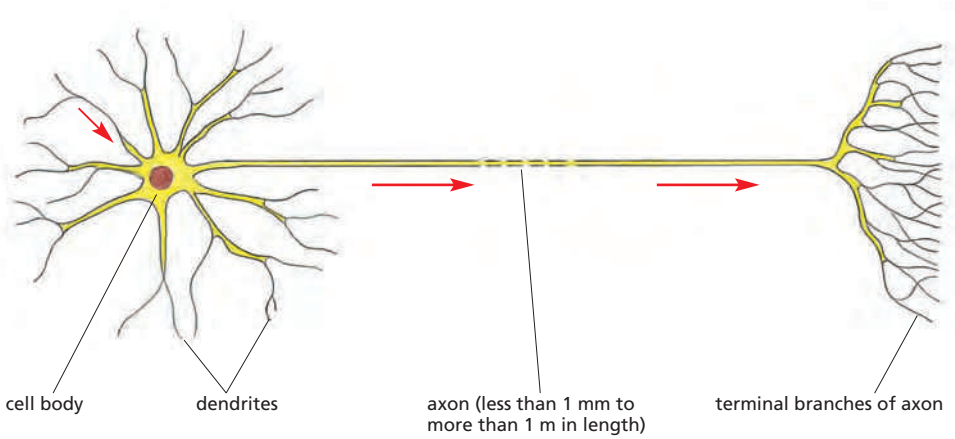


Figure 11–28 A typical vertebrate neuron. The arrows indicate the direction in which signals are conveyed. The single axon conducts signals away from the cell body, while the multiple dendrites (and the cell body) receive signals from the axons of other neurons. The axon terminals end on the dendrites or cell body of other neurons or on other cell types, such as muscle or gland cells.

targets, and several shorter, branching **dendrites** extend from the cell body like antennae, providing an enlarged surface area to receive signals from the axons of other neurons (**Figure 11–28**), although the cell body itself also receives such signals. A typical axon divides at its far end into many branches, passing on its message to many target cells simultaneously. Likewise, the extent of branching of the dendrites can be very great—in some cases sufficient to receive as many as 100,000 inputs on a single neuron.

Despite the varied significance of the signals carried by different classes of neurons, the form of the signal is always the same, consisting of changes in the electrical potential across the neuron's plasma membrane. The signal spreads because an electrical disturbance produced in one part of the membrane spreads to other parts, although the disturbance becomes weaker with increasing distance from its source, unless the neuron expends energy to amplify it as it travels. Over short distances, this attenuation is unimportant; in fact, many small neurons conduct their signals passively, without amplification. For long-distance communication, however, such passive spread is inadequate. Thus, larger neurons employ an active signaling mechanism, which is one of their most striking features. An electrical stimulus that exceeds a certain threshold strength triggers an explosion of electrical activity that propagates rapidly along the neuron's plasma membrane and is sustained by automatic amplification all along the way. This traveling wave of electrical excitation, known as an **action potential**, or *nerve impulse*, can carry a message without attenuation from one end of a neuron to the other at speeds of 100 meters per second or more. Action potentials are the direct consequence of the properties of voltage-gated cation channels, as we now discuss.

Voltage-Gated Cation Channels Generate Action Potentials in Electrically Excitable Cells

The plasma membrane of all electrically excitable cells—not only neurons, but also muscle, endocrine, and egg cells—contains **voltage-gated cation channels**, which are responsible for generating the action potentials. An action potential is triggered by a **depolarization** of the plasma membrane—that is, by a shift in the membrane potential to a less negative value inside. (We shall see later how the action of a neurotransmitter causes depolarization.) In nerve and skeletal muscle cells, a stimulus that causes sufficient depolarization promptly opens the **voltage-gated Na^+ channels**, allowing a small amount of Na^+ to enter the cell down its electrochemical gradient. The influx of positive charge depolarizes the membrane further, thereby opening more Na^+ channels, which admit more Na^+ ions, causing still further depolarization. This self-amplification process (an example of *positive feedback*, discussed in Chapters 8 and 15) continues until, within a fraction of a millisecond, the electrical potential in the local region of membrane has shifted from its resting value of about –70 mV (in squid giant axon; about –40 mV in human) to almost as far as the Na^+ equilibrium potential of about +50 mV (see

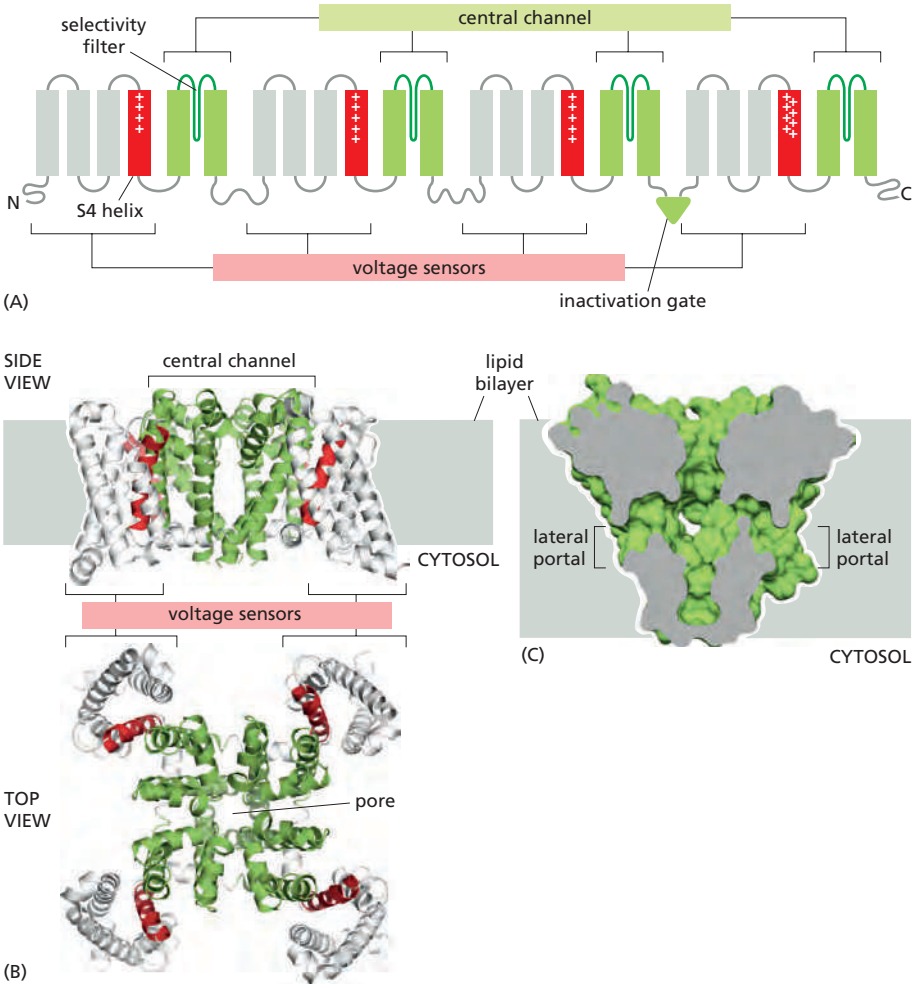


Figure 11–29 Structural models of voltage-gated Na^+ channels. (A) The channel in animal cells is built from a single polypeptide chain that contains four homologous domains. Each domain contains two transmembrane α helices *(green)* that surround the central ion-conducting pore. They are separated by sequences *(blue)* that form the selectivity filter. Four α additional helices *(gray* and *red)* in each domain constitute the voltage sensor. The S4 helices *(red)* are unique in that they contain an abundance of positively charged arginines. An inactivation gate that is part of a flexible loop connecting the third and fourth domains acts as a plug that obstructs the pore in the channel's inactivated state, as shown in Figure 11–30. (B) Side and top views of a homologous bacterial channel protein showing its arrangement within the membrane. (C) A cross section of the pore domain of the channel shown in (B) shows lateral portals, through which the central cavity is accessible from the hydrophobic core of the lipid bilayer. In the crystals, lipid acyl chains were found to intrude into the pore. These lateral portals are large enough to allow entry of small, hydrophobic, pore-blocking drugs that are commonly used as anesthetics and block ion conductance. (PDB code: 3RVZ.)

Panel 11–1, p. 616). At this point, when the net electrochemical driving force for the flow of Na^+ is almost zero, the cell would come to a new resting state, with all of its Na^+ channels permanently open, if the open conformation of the channel were stable. Two mechanisms act in concert to save the cell from such a permanent electrical spasm: the Na^+ channels automatically inactivate and **voltage-gated K^+ channels** open to restore the membrane potential to its initial negative value.

The Na^+ channel is built from a single polypeptide chain that contains four structurally very similar domains. It is thought that these domains evolved by gene duplication followed by fusion into a single large gene (**Figure 11–29**A). In bacteria, in fact, the Na^+ channel is a tetramer of four identical polypeptide chains, supporting this evolutionary idea.

Each domain contributes to the central channel, which is very similar to the K^+ channel. Each domain also contains a *voltage sensor* that is characterized by an unusual transmembrane helix, S4, that contains many positively charged amino acids. As the membrane depolarizes, the S4 helices experience an electrostatic pulling force that attracts them to the now negatively charged extracellular side of the plasma membrane. The resulting conformational change opens the channel. The structure of a bacterial voltage-gated Na^+ channel provides insights how the structural elements are arranged in the membrane (Figure 11–29B and C).

The Na^+ channels also have an automatic inactivating mechanism, which causes the channels to reclose rapidly even though the membrane is still depolarized (see Figure 11–30). The Na^+ channels remain in this *inactivated* state, unable to reopen, until after the membrane potential has returned to its initial negative value. The time necessary for a sufficient number of Na^+ channels to recover from inactivation to support a new action potential, termed the *refractory period*, limits

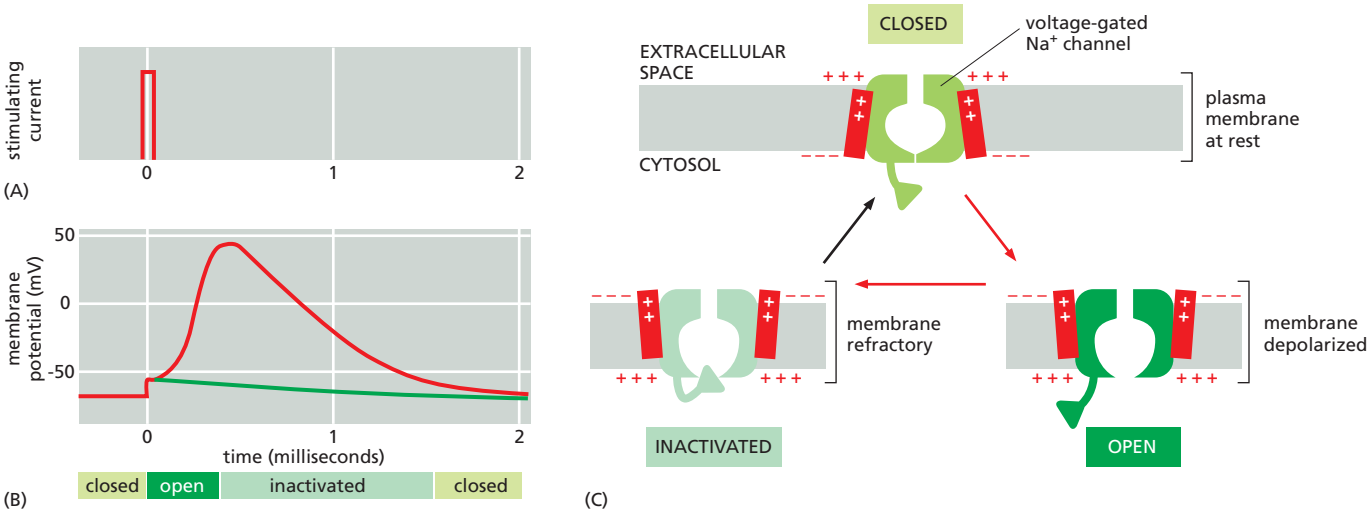


Figure 11–30 Na^+ channels and an action potential. (A) An action potential is triggered by a brief pulse of current, which (B) partially depolarizes the membrane, as shown in the plot of membrane potential versus time. The *green curve* shows how the membrane potential would have simply relaxed back to the resting value after the initial depolarizing stimulus if there had been no voltage-gated Na^+ channels in the membrane. The *red curve* shows the course of the action potential that is caused by the opening and subsequent inactivation of voltage-gated Na^+ channels. The states of the Na^+ channels are indicated in (B). The membrane cannot fire a second action potential until the Na^+ channels have returned from the inactivated to the closed conformation; until then, the membrane is refractory to stimulation. (C) The three states of the Na^+ channel. When the membrane is at rest (highly polarized), the closed conformation of the channel has the lowest free energy and is therefore most stable; when the membrane is depolarized, the energy of the *open* conformation is lower, so the channel has a high probability of opening. But the free energy of the *inactivated* conformation is lower still; therefore, after a randomly variable period spent in the open state, the channel becomes inactivated. Thus, the open conformation corresponds to a metastable state that can exist only transiently when the membrane depolarizes (Movie 11.10).

the repetitive firing rate of a neuron. The cycle from initial stimulus to the return to the original resting state takes a few milliseconds or less. The Na^+ channel can therefore exist in three distinct states—closed, open, and inactivated—which contribute to the rise and fall of the action potential (Figure 11–30).

This description of an action potential applies only to a small patch of plasma membrane. The self-amplifying depolarization of the patch, however, is sufficient to depolarize neighboring regions of membrane, which then go through the same cycle. In this way, the action potential sweeps like a wave from the initial site of depolarization over the entire plasma membrane, as shown in Figure 11–31.

The Use of Channelrhodopsins Has Revolutionized the Study of Neural Circuits

Channelrhodopsins are photosensitive ion channels that open in response to light. They evolved as sensory receptors in photosynthetic green algae to allow the algae to swim toward light. The structure of channelrhodopsin closely resembles that of bacteriorhodopsin (see Figure 10–31). It contains a covalently bound retinal group that absorbs light and undergoes an isomerization reaction, which triggers a conformational change in the protein, opening an ion channel in the plasma membrane. In contrast to bacteriorhodopsin, which is a light-driven proton pump, channelrhodopsin is a light-driven cation channel.

Using genetic engineering techniques, channelrhodopsin can be expressed in virtually any cell type in vertebrates and invertebrates. Researchers first introduced the gene into cultured neurons and showed that flashing light could now activate the channelrhodopsin and induce the neurons to fire action potentials. Because the frequency of the light flashes determined the frequency of the action potentials, one can control the frequency of neuronal firing with millisecond precision.

Next, neurobiologists used the approach to activate specific neurons in the brain of experimental animals. Using a tiny fiber optic cable implanted near the

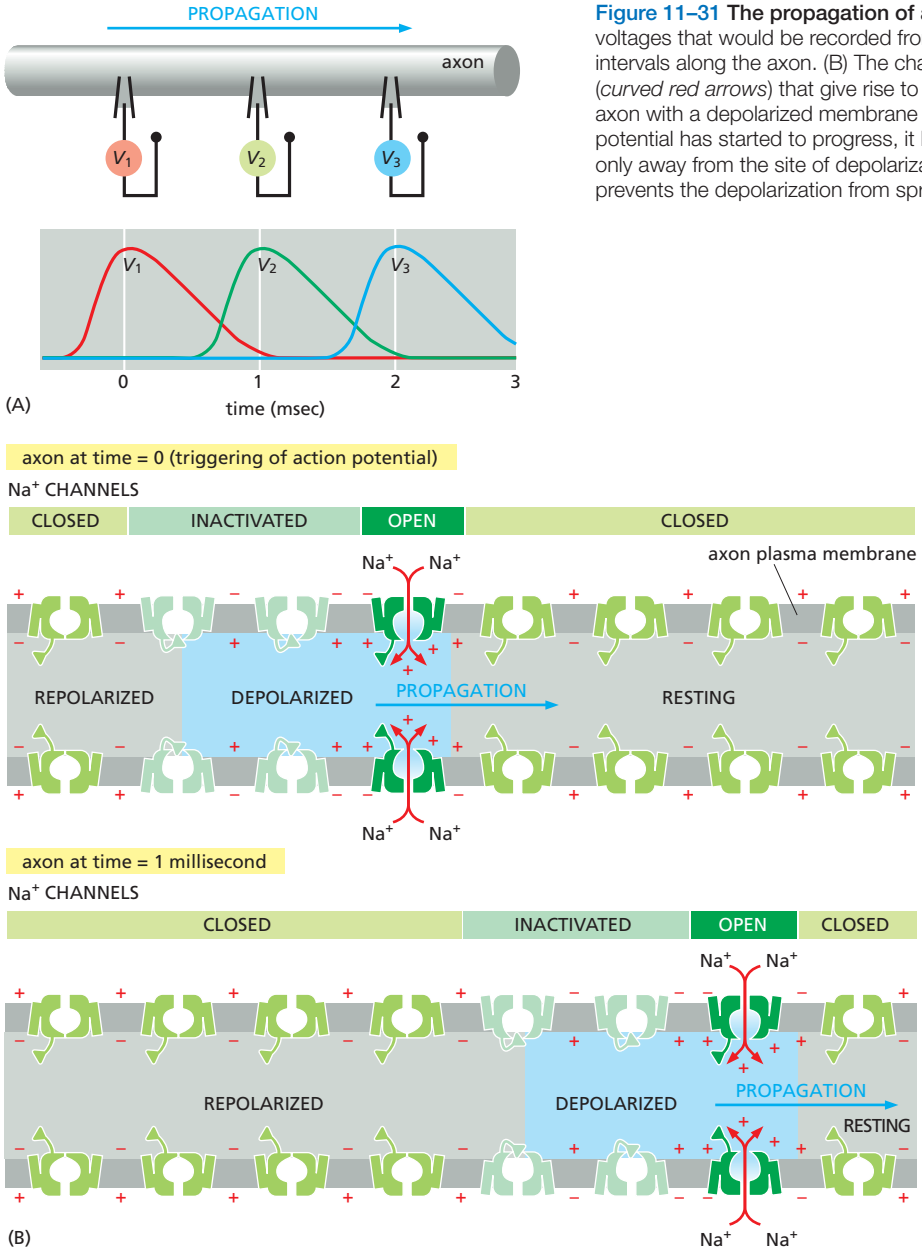


Figure 11–31 The propagation of an action potential along an axon. (A) The voltages that would be recorded from a set of intracellular electrodes placed at intervals along the axon. (B) The changes in the Na^+ channels and the current flows (*curved red arrows*) that give rise to a traveling action potential. The region of the axon with a depolarized membrane is shaded in *blue.* Note that once an action potential has started to progress, it has to continue in the same direction, traveling only away from the site of depolarization, because Na^+-channel inactivation prevents the depolarization from spreading backward.

relevant brain region, they could flash light to specifically activate the channelrhodopsin-containing neurons to fire action potentials. One group of researchers expressed channelrhodopsin in a subset of mouse neurons thought to be involved in aggression: when these cells were activated by light, the mouse immediately attacked anything in its environment—including other mice or even an inflated rubber glove (Figure 11–32); when the light was switched off, the neurons fell silent and the mouse's behavior returned to normal.

Since these pioneering studies, researchers have engineered additional light-responsive ion channels and transporters, including some that can rapidly

Figure 11–32 Optogenetic control of aggression neurons in a living mouse. A gene encoding channelrhodopsin was introduced into a subpopulation of neurons in the hypothalamus of a mouse. When the neurons were exposed to flashing blue light using a tiny, implanted fiber optic cable, the channelrhodopsin channels opened, depolarizing and activating the cells. When the light was switched on, the mouse immediately became aggressive and attacked the inflated rubber glove; when the light was switched off, its behavior immediately returned to normal (Movie 11.11). (From D. Lin et al., *Nature* 470:221–226, 2011. With permission from Macmillan Publishers Ltd.)

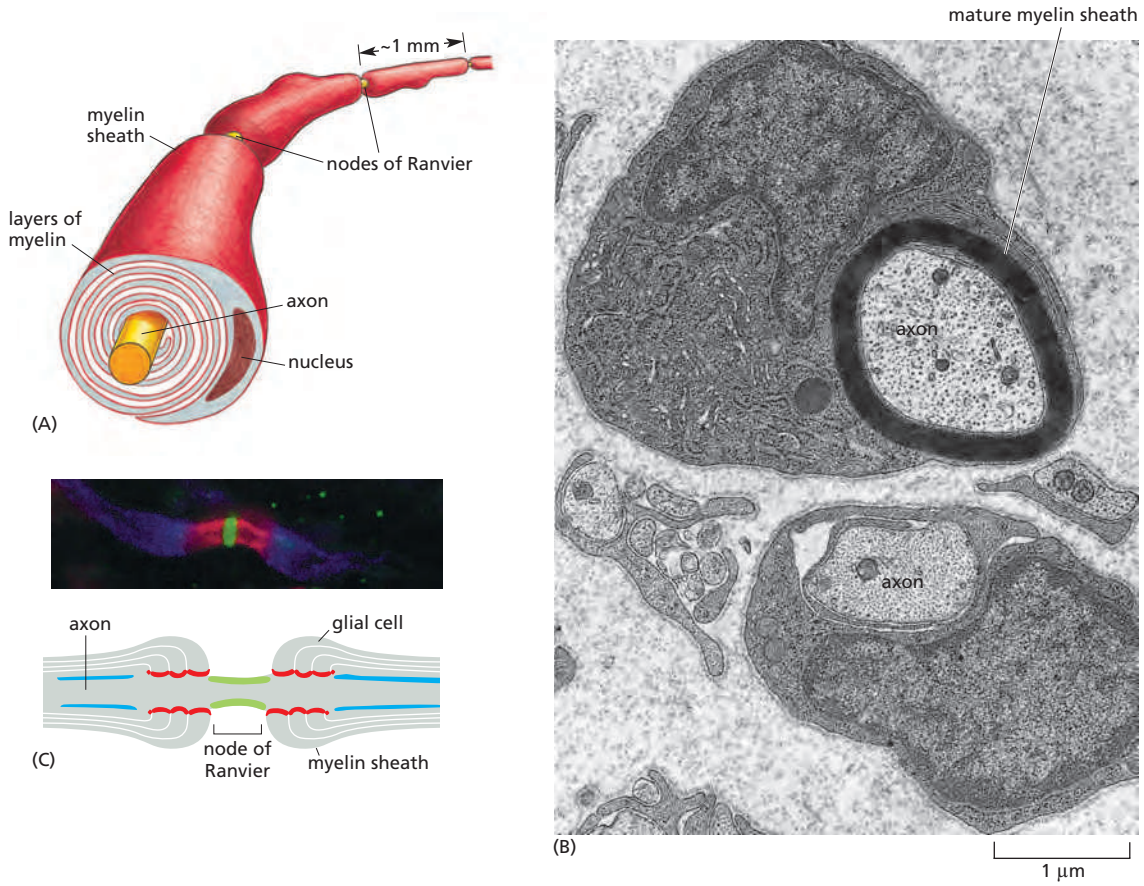


Figure 11–33 Myelination. (A) A myelinated axon from a peripheral nerve. Each Schwann cell wraps its plasma membrane concentrically around the axon to form a segment of myelin sheath about 1 mm long. For clarity, the membrane layers of the myelin are shown less compacted than they are in reality (see part B). (B) An electron micrograph of a nerve in the leg of a young rat. Two Schwann cells can be seen: one near the bottom is just beginning to myelinate its axon; the one above it has formed an almost mature myelin sheath. (C) Fluorescence micrograph and diagram of individual myelinated axons teased apart in a rat optic nerve, showing the confinement of the voltage-gated Na^+ channels (*green*) in the axonal membrane at the node of Ranvier. A protein called Caspr (*red*) marks the junctions where the myelinating glial cell plasma membrane tightly abuts the axon on either side of the node. Voltage-gated K^+ channels (*blue*) localize to regions in the axon plasma membrane well away from the node. (B, from Cedric S. Raine, in Myelin [P. Morell, ed.]. New York: Plenum, 1976; C, from M.N. Rasband and P. Shrager, *J. Physiol.* 525:63–73, 2000. With permission from Blackwell Publishing.)

inactivate specific neurons. It is therefore now possible to transiently activate or inhibit specific neurons in the brains of awake animals with remarkable spatial and temporal precision. In this way, the rapidly expanding new field of **optogenetics** is revolutionizing neurobiology, allowing neuroscientists to analyze the neurons and circuits underlying even the most complex behaviors in experimental animals, including nonhuman primates.

Myelination Increases the Speed and Efficiency of Action Potential Propagation in Nerve Cells

The axons of many vertebrate neurons are insulated by a **myelin sheath**, which greatly increases the rate at which an axon can conduct an action potential. The importance of myelination is dramatically demonstrated by the demyelinating disease *multiple sclerosis*, in which the immune system destroys myelin sheaths in some regions of the central nervous system; in the affected regions, nerve impulse propagation greatly slows or even fails, often with devastating neurological consequences.

Myelin is formed by specialized non-neuronal supporting cells called **glial cells**. **Schwann cells** are the glial cells that myelinate axons in peripheral nerves, and **oligodendrocytes** do so in the central nervous system. These myelinating glial cells wrap layer upon layer of their own plasma membrane in a tight spiral around the axon (**Figure 11–33**A and B), thereby insulating the axonal membrane so that little current can leak across it. The myelin sheath is interrupted at regularly spaced *nodes of Ranvier*, where almost all the Na^+ channels in the axon are concentrated (Figure 11–33C). This arrangement allows an action potential to propagate along a myelinated axon by jumping from node to node, a process called *saltatory conduction*. This type of conduction has two main advantages: action potentials travel very much faster, and metabolic energy is conserved because the active excitation is confined to the small regions of axonal plasma membrane at nodes of Ranvier.

Patch-Clamp Recording Indicates That Individual Ion Channels Open in an All-or-Nothing Fashion

Neuron and skeletal muscle cell plasma membranes contain many thousands of voltage-gated Na^+ channels, and the current crossing the membrane is the sum of the currents flowing through all of these. An intracellular microelectrode can record this aggregate current, as shown in Figure 11–31A. Remarkably, however, it is also possible to record current flowing through individual channels. **Patch-clamp recording**, developed in the 1970s and 1980s, revolutionized the study of ion channels and made it possible to examine transport through a single channel in a small patch of membrane covering the mouth of a micropipette (**Figure 11–34**). With this simple but powerful technique, one can study the detailed properties of ion channels in all sorts of cell types. This work led to the discovery that even cells that are not electrically excitable usually have a variety of ion channels in their plasma membrane. Many of these cells, such as yeasts, are too small to be investigated by the traditional electrophysiologist's method of impalement with an intracellular microelectrode.

Patch-clamp recording indicates that individual ion channels open in an all-or-nothing fashion. For example, a voltage-gated Na^+ channel opens and closes at random, but when open, the channel always has the same large conductance, allowing more than 1000 ions to pass per millisecond (**Figure 11–35**). Therefore, the aggregate current crossing the membrane of an entire cell does not indicate the *degree* to which a typical individual channel is open but rather the *total number* of channels in its membrane that are open at any one time.

Some simple physical principles allow us to refine our understanding of voltage-gating from the perspective of a single Na^+ channel. The interior of the resting neuron or muscle cell is at an electrical potential about 40–100 mV more negative than the external medium. Although this potential difference seems small, it exists across a plasma membrane only about 5 nm thick, so that the resulting voltage gradient is about 100,000 V/cm. Charged proteins in the membrane such as Na^+ channels are thus subjected to a very large electrical field that can profoundly affect their conformation. Each conformation can "flip" to another conformation if given a sufficient jolt by the random thermal movements of the surroundings, and it is the relative stability of the closed, open, and inactivated conformations against flipping that is altered by changes in the membrane potential (see Figure 11–30C).

Voltage-Gated Cation Channels Are Evolutionarily and Structurally Related

Na^+ channels are not the only kind of voltage-gated cation channel that can generate an action potential. The action potentials in some muscle, egg, and endocrine cells, for example, depend on *voltage-gated Ca^{2+} channels* rather than on Na^+ channels.

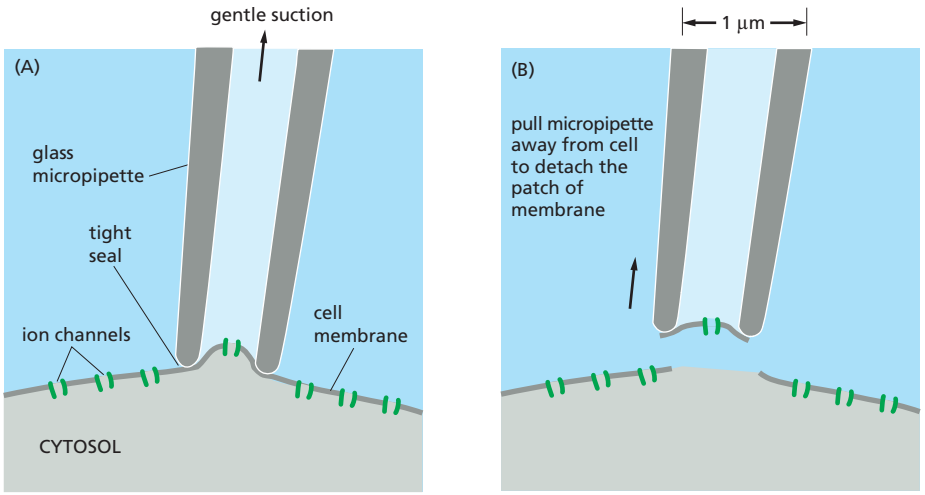


Figure 11–34 The technique of patch-clamp recording. Because of the extremely tight seal between the micropipette and the membrane, current can enter or leave the micropipette only by passing through the ion channels in the *patch* of membrane covering its tip. The term *clamp* is used because an electronic device is employed to maintain, or "clamp," the membrane potential at a set value while recording the ionic current through individual channels. The current through these channels can be recorded with the patch still attached to the rest of the cell, as in (A), or detached, as in (B). 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. A detached patch can also be produced with the opposite orientation, so that the cytoplasmic surface of the membrane faces the inside of the pipette.

There is a surprising amount of structural and functional diversity within each of the different classes of voltage-gated cation channels, generated both by multiple genes and by the alternative splicing of RNA transcripts produced from the same gene. Nonetheless, the amino acid sequences of the known voltage-gated Na^+, K^+, and Ca^{2+} channels show striking similarities, demonstrating that they all belong to a large superfamily of evolutionarily and structurally related proteins and share many of the design principles. Whereas the single-celled yeast *S. cerevisiae* contains a single gene that codes for a voltage-gated K^+ channel, the genome of the worm *C. elegans* contains 68 genes that encode different but related K^+ channels. This complexity indicates that even a simple nervous system made up of only 302 neurons uses a large number of different ion channels to compute its responses.

Humans who inherit mutant genes encoding ion channels can suffer from a variety of nerve, muscle, brain, or heart diseases, depending in which cells the channel encoded by the mutant gene normally functions. Mutations in genes that encode voltage-gated Na^+ channels in skeletal muscle cells, for example, can cause *myotonia*, a condition in which there is a delay in muscle relaxation after voluntary contraction, causing painful muscle spasms. In some cases, this occurs because the abnormal channels fail to inactivate normally; as a result, Na^+ entry persists after an action potential finishes and repeatedly reinitiates membrane depolarization and muscle contraction. Similarly, mutations that affect Na^+ or K^+ channels in the brain can cause *epilepsy*, in which excessive synchronized firing of large groups of neurons causes epileptic seizures (convulsions, or fits).

The particular combination of ion channels conducting Na^+, K^+, and Ca^{2+} that are expressed in a neuron largely determines how the cell fires repetitive sequences of action potentials. Some nerve cells can repeat action potentials up to 300 times per second; other neurons fire short bursts of action potentials separated by periods of silence; while others rarely fire more than one action potential at a time. There is a remarkable diversity of neurons in the brain.

Different Neuron Types Display Characteristic Stable Firing Properties

It is estimated that the human brain contains about 10^{11} neurons and 10^{14} synaptic connections. To make matters more complex, neural circuitry is continuously sculpted in response to experience, modified as we learn and store memories, and irreversibly altered by the gradual loss of neurons and their connections as we age. How can a system so complex be subject to such change and yet continue to function stably? One emerging theory suggests that individual neurons are self-tuning devices, constantly adjusting the expression of ion channels and neurotransmitter receptors in order to maintain a stable function. How might this work?

Neurons can be categorized into functionally different types, based in part on their propensity to fire action potentials and their pattern of firing. For example, some neurons fire action potentials at high frequencies, while others fire rarely. The firing properties of each neuron type are determined to a large extent by the ion channels that the cell expresses. The number of ion channels in a neuron's membrane is not fixed: as conditions change, a neuron can modify the numbers of depolarizing (Na^+ and Ca^{2+}) and hyperpolarizing (K^+) channels and keep their proportions adjusted so as to maintain its characteristic firing behavior—a remarkable example of homeostatic control. The molecular mechanisms involved remain an important mystery.

Transmitter-Gated Ion Channels Convert Chemical Signals into Electrical Ones at Chemical Synapses

Neuronal signals are transmitted from cell to cell at specialized sites of contact known as **synapses**. The usual mechanism of transmission is indirect. The cells are

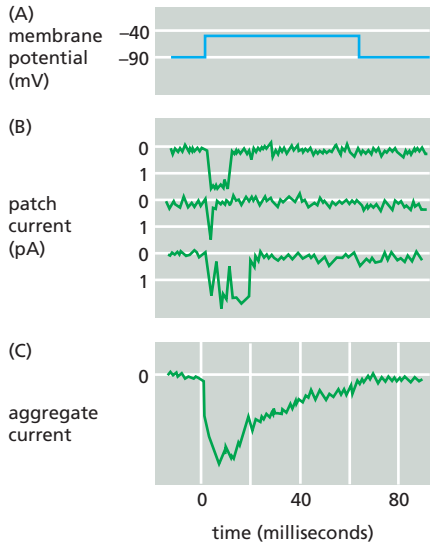
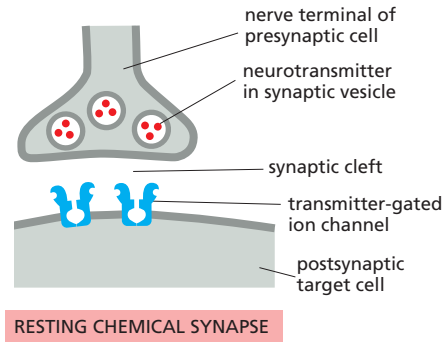
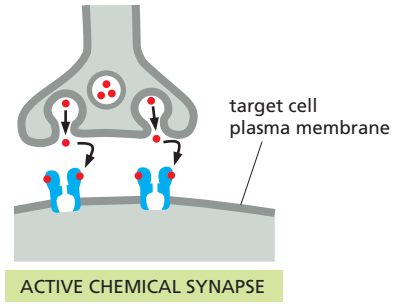
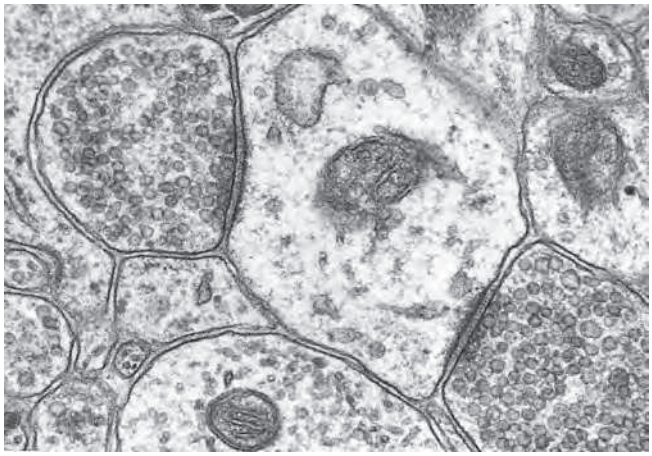


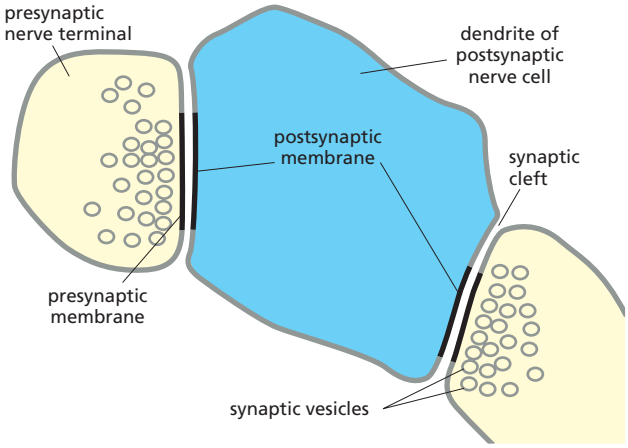
Figure 11–35 Patch-clamp measurements for a single voltage-gated Na^+ channel. A tiny patch of plasma membrane was detached from an embryonic rat muscle cell, as in Figure 11–34. (A) The membrane was depolarized by an abrupt shift of potential from –90 to about –40 mV. (B) Three current records from three experiments performed on the same patch of membrane. Each major current step in (B) represents the opening and closing of a single channel. A comparison of the three records shows that, whereas the durations of channel opening and closing vary greatly, the rate at which current flows through an open channel (its conductance) is practically constant. The minor fluctuations in the current records arise largely from electrical noise in the recording apparatus. Current flowing into the cell, measured in picoamperes (pA), is shown as a downward deflection of the curve. By convention, the electrical potential on the outside of the cell is defined as zero. (C) The sum of the currents measured in 144 repetitions of the same experiment. This aggregate current is equivalent to the usual Na^+ current that would be observed flowing through a relatively large region of membrane containing 144 channels. A comparison of (B) and (C) reveals that the time course of the aggregate current reflects the probability that any individual channel will be in the open state; this probability decreases with time as the channels in the depolarized membrane adopt their inactivated conformation. (Data from J. Patlak and R. Horn, *J. Gen. Physiol.* 79:333–351, 1982. With permission from The Rockefeller University Press.)





(A)





(B)

Figure 11–36 A chemical synapse. (A) When an action potential reaches the nerve terminal in a presynaptic cell, it stimulates the terminal to release its neurotransmitter. The neurotransmitter molecules are contained in synaptic vesicles and are released to the cell exterior when the vesicles fuse with the plasma membrane of the nerve terminal. The released neurotransmitter binds to and opens the transmitter-gated ion channels concentrated in the plasma membrane of the postsynaptic target cell at the synapse. The resulting ion flows alter the membrane potential of the postsynaptic membrane, thereby transmitting a signal from the excited nerve (Movie 11.12). (B) A thin-section electron micrograph of two nerve terminal synapses on a dendrite of a postsynaptic cell. (B, courtesy of Cedric Raine.)

electrically isolated from one another, the *presynaptic cell* being separated from the *postsynaptic cell* by a narrow *synaptic cleft.* When an action potential arrives at the presynaptic site, the depolarization of the membrane opens voltage-gated Ca^{2+} channels that are clustered in the presynaptic membrane. Ca^{2+} influx triggers the release into the cleft of small signal molecules known as **neurotransmitters**, which are stored in membrane-enclosed *synaptic vesicles* and released by exocytosis (discussed in Chapter 13). The neurotransmitter diffuses rapidly across the synaptic cleft and provokes an electrical change in the postsynaptic cell by binding to and opening *transmitter-gated ion channels* (**Figure 11–36**). After the neurotransmitter has been secreted, it is rapidly removed: it is either destroyed by specific enzymes in the synaptic cleft or taken up by the presynaptic nerve terminal or by surrounding glial cells. Reuptake is mediated by a variety of Na^+-dependent neurotransmitter symporters (see Figure 11–8); in this way, neurotransmitters are recycled, allowing cells to keep up with high rates of release. Rapid removal ensures both spatial and temporal precision of signaling at a synapse. It decreases the chances that the neurotransmitter will influence neighboring cells, and it clears the synaptic cleft before the next pulse of neurotransmitter is released, so that the timing of repeated, rapid signaling events can be accurately communicated to the postsynaptic cell. As we shall see, signaling via such *chemical synapses* is far more versatile and adaptable than direct electrical coupling via gap junctions at *electrical synapses* (discussed in Chapter 19), which are also used by neurons but to a much smaller extent.

Transmitter-gated ion channels, also called **ionotropic receptors**, are built for rapidly converting extracellular chemical signals into electrical signals at

chemical synapses. The channels are concentrated in a specialized region of the postsynaptic plasma membrane at the synapse and open transiently in response to the binding of neurotransmitter molecules, thereby producing a brief permeability change in the membrane (see Figure 11–36A). Unlike the voltage-gated channels responsible for action potentials, transmitter-gated channels are relatively insensitive to the membrane potential and therefore cannot by themselves produce a self-amplifying excitation. Instead, they produce local permeability increases, and hence changes of membrane potential, that are graded according to the amount of neurotransmitter released at the synapse and how long it persists there. Only if the summation of small depolarizations at this site opens sufficient numbers of nearby voltage-gated cation channels can an action potential be triggered. This may require the opening of transmitter-gated ion channels at numerous synapses in close proximity on the target nerve cell.

Chemical Synapses Can Be Excitatory or Inhibitory

Transmitter-gated ion channels differ from one another in several important ways. First, as receptors, they have highly selective binding sites for the neurotransmitter that is released from the presynaptic nerve terminal. Second, as channels, they are selective in the type of ions that they let pass across the plasma membrane; this determines the nature of the postsynaptic response. **Excitatory neurotransmitters** open cation channels, causing an influx of Na^+, and in many cases Ca^{2+}, that depolarizes the postsynaptic membrane toward the threshold potential for firing an action potential. **Inhibitory neurotransmitters**, by contrast, open either Cl^- channels or K^+ channels, and this suppresses firing by making it harder for excitatory neurotransmitters to depolarize the postsynaptic membrane. Many transmitters can be either excitatory or inhibitory, depending on where they are released, what receptors they bind to, and the ionic conditions that they encounter. *Acetylcholine*, for example, can either excite or inhibit, depending on the type of acetylcholine receptors it binds to. Usually, however, acetylcholine, *glutamate*, and *serotonin* are used as excitatory transmitters, and γ-*aminobutyric acid* (*GABA*) and *glycine* are used as inhibitory transmitters. Glutamate, for instance, mediates most of the excitatory signaling in the vertebrate brain.

We have already discussed how the opening of Na^+ or Ca^{2+} channels depolarizes a membrane. The opening of K^+ channels has the opposite effect because the K^+ concentration gradient is in the opposite direction—high concentration inside the cell, low outside. Opening K^+ channels tends to keep the cell close to the equilibrium potential for K^+, which, as we discussed earlier, is normally close to the resting membrane potential because at rest K^+ channels are the main type of channel that is open. When additional K^+ channels open, it becomes harder to drive the cell away from the resting state. We can understand the effect of opening Cl^- channels similarly. The concentration of Cl^- is much higher outside the cell than inside (see Table 11–1, p. 598), but the membrane potential opposes its influx. In fact, for many neurons, the equilibrium potential for Cl^- is close to the resting potential—or even more negative. For this reason, opening Cl^- channels tends to buffer the membrane potential; as the membrane starts to depolarize, more negatively charged Cl^- ions enter the cell and counteract the depolarization. Thus, the opening of Cl^- channels makes it more difficult to depolarize the membrane and hence to excite the cell. Some powerful toxins act by blocking the action of inhibitory neurotransmitters: strychnine, for example, binds to glycine receptors and prevents their inhibitory action, causing muscle spasms, convulsions, and death.

However, not all chemical signaling in the nervous system operates through these ionotropic ligand-gated ion channels. In fact, most neurotransmitter molecules that are secreted by nerve terminals, including a large variety of neuropeptides, bind to **metabotropic receptors**, which regulate ion channels only indirectly through the action of small intracellular signal molecules (discussed in Chapter 15). All neurotransmitter receptors fall into one or other of these two

major classes—ionotropic or metabotropic—on the basis of their signaling mechanisms:

1. Ionotropic receptors are ion channels and feature at fast chemical synapses. Acetylcholine, glycine, glutamate, and GABA all act on transmitter-gated ion channels, mediating excitatory or inhibitory signaling that is generally immediate, simple, and brief.
2. Metabotropic receptors are *G-protein-coupled receptors* (discussed in Chapter 15) that bind to all other neurotransmitters (and, confusingly, also acetylcholine, glutamate, and GABA). Signaling mediated by ligand-binding to metabotropic receptors tends to be far slower and more complex than that at ionotropic receptors, and longer-lasting in its consequences.

The Acetylcholine Receptors at the Neuromuscular Junction Are Excitatory Transmitter-Gated Cation Channels

A well-studied example of a transmitter-gated ion channel is the **acetylcholine receptor** of skeletal muscle cells. This channel is opened transiently by acetylcholine released from the nerve terminal at a **neuromuscular junction**—the specialized chemical synapse between a motor neuron and a skeletal muscle cell (**Figure 11–37**). This synapse has been intensively investigated because it is readily accessible to electrophysiological study, unlike most of the synapses in the central nervous system, that is, the brain and spinal cord in vertebrates. Moreover, the acetylcholine receptors are densely packed in the muscle cell plasma membrane at a neuromuscular junction (about 20,000 such receptors per μm^2), with relatively few receptors elsewhere in the same membrane.

The receptors are composed of five transmembrane polypeptides, two of one kind and three others, encoded by four separate genes (**Figure 11–38**A). The four genes are strikingly similar in sequence, implying that they evolved from a single ancestral gene. The two identical polypeptides in the pentamer each contribute one acetylcholine-binding site. When two acetylcholine molecules bind to the pentameric complex, they induce a conformational change that opens the channel. With ligand bound, the channel still flickers between open and closed states, but now it has a 90% probability of being open. This state continues—with acetylcholine binding and unbinding—until hydrolysis of the free acetylcholine by the enzyme *acetylcholinesterase* lowers its concentration at the neuromuscular junction sufficiently. Once freed of its bound neurotransmitter, the acetylcholine receptor reverts to its initial resting state. If the presence of acetylcholine persists for a prolonged time as a result of excessive nerve stimulation, the channel inactivates. Normally, the acetylcholine is rapidly hydrolyzed and the channel closes within about 1 millisecond, well before significant desensitization occurs. Desensitization would occur after about 20 milliseconds in the continued presence of acetylcholine.

The five subunits of the acetylcholine receptor are arranged in a ring, forming a water-filled transmembrane channel that consists of a narrow pore through the lipid bilayer, which widens into vestibules at both ends. Acetylcholine binding opens the channel by causing the helices that line the pore to rotate outward, thus disrupting a ring of hydrophobic amino acids that blocks ion flow in the closed state. Clusters of negatively charged amino acids at either end of the pore help to exclude negative ions and encourage any positive ion of diameter less than 0.65 nm to pass through (Figure 11–38B). The normal through-traffic consists chiefly of Na^+ and K^+, together with some Ca^{2+}. Thus, unlike voltage-gated cation channels, such as the K^+ channel discussed earlier, there is little selectivity among cations, and the relative contributions of the different cations to the current through the channel depend chiefly on their concentrations and on the electrochemical driving forces. When the muscle cell membrane is at its resting potential, the net driving force for K^+ is near zero, since the voltage gradient nearly balances the K^+ concentration gradient across the membrane (see Panel 11–1, p. 616). For Na^+, in contrast, the voltage gradient and the concentration gradient both act in the same direction to drive the ion into the cell. (The same is true for Ca^{2+}, but the

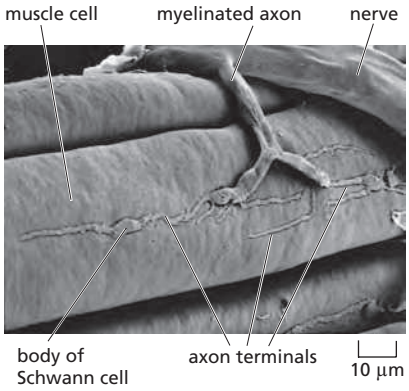


Figure 11–37 A low-magnification scanning electron micrograph of a neuromuscular junction in a frog. The termination of a single axon on a skeletal muscle cell is shown. (From J. Desaki and Y. Uehara, *J. Neurocytol.* 10:101–110, 1981. With permission from Kluwer Academic Publishers.)

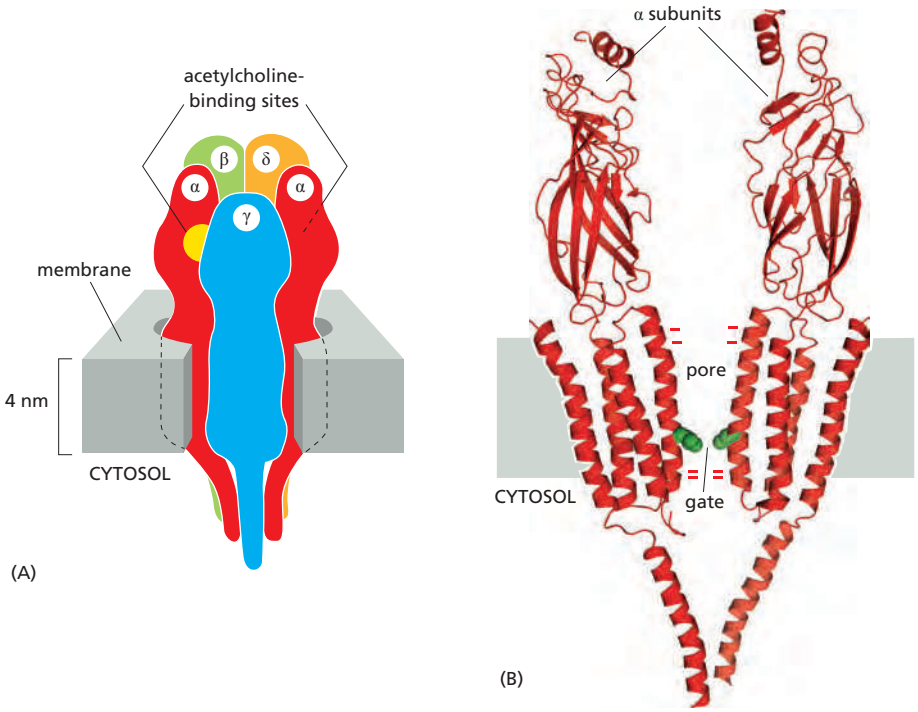


Figure 11–38 A model for the structure of the skeletal muscle acetylcholine receptor. (A) Five homologous subunits (α, α, β, γ, δ) combine to form a transmembrane pore. Both of the α subunits contribute an acetylcholine-binding site nestled between adjoining subunits. (B) The pore is lined by a ring of five transmembrane α helices, one contributed by each subunit (just the two α subunits are shown). In its closed conformation, the pore is occluded by the hydrophobic side chains of five leucines (*green*), one from each α helix, which form a gate near the middle of the lipid bilayer. When acetylcholine binds to both α subunits, the channel undergoes a conformational change that opens the gate by an outward rotation of the helices containing the occluding leucines. Negatively charged side chains (indicated by the "–" signs) at either end of the pore ensure that only positively charged ions pass through the channel. (PDB code: 2BG9.)

extracellular concentration of Ca^{2+} is so much lower than that of Na^+ that Ca^{2+} makes only a small contribution to the total inward current.) Therefore, the opening of the acetylcholine-receptor channels leads to a large net influx of Na^+ (a peak rate of about 30,000 ions per channel each millisecond). This influx causes a membrane depolarization that signals the muscle to contract, as discussed below.

Neurons Contain Many Types of Transmitter-Gated Channels

The ion channels that open directly in response to the neurotransmitters acetylcholine, serotonin, GABA, and glycine contain subunits that are structurally similar and probably form transmembrane pores in the same way as the ionotropic acetylcholine receptor, even though they have distinct neurotransmitter-binding specificities and ion selectivities. These channels are all built from homologous polypeptide subunits, which assemble as a pentamer. Glutamate-gated ion channels are an exception, in that they are constructed from a distinct family of subunits and form tetramers resembling the K^+ channels discussed earlier (see Figure 11–24A).

For each class of transmitter-gated ion channel, there are alternative forms of each type of subunit, which may be encoded by distinct genes or else generated by alternative RNA splicing of a single gene product. The subunits assemble in different combinations to form an extremely diverse set of distinct channel subtypes, with different ligand affinities, different channel conductances, different rates of opening and closing, and different sensitivities to drugs and toxins. Some vertebrate neurons, for example, have acetylcholine-gated ion channels that differ from those of muscle cells in that they are formed from two subunits of one type and three of another; but there are at least nine genes coding for different versions of the first type of subunit and at least three coding for different versions of the second. Subsets of such neurons performing different functions in the brain express different combinations of the genes for these subunits. In principle, and already to some extent in practice, it is possible to design drugs targeted against these narrowly defined subsets, thereby specifically influencing particular brain functions.

Many Psychoactive Drugs Act at Synapses

Transmitter-gated ion channels have for a long time been important drug targets. A surgeon, for example, can relax muscles for the duration of an operation

by blocking the acetylcholine receptors on skeletal muscle cells with *curare*, a plant-derived drug that was originally used by South American Indians to make poison arrows. Most drugs used to treat insomnia, anxiety, depression, and schizophrenia exert their effects at chemical synapses, and many of these act by binding to transmitter-gated channels. Barbiturates, tranquilizers such as Valium, and sleeping pills such as Ambien, for example, bind to GABA receptors, potentiating the inhibitory action of GABA by allowing lower concentrations of this neurotransmitter to open Cl^- channels. Our increasing understanding of the molecular biology of ion channels should allow us to design a new generation of psychoactive drugs that will act still more selectively to alleviate the miseries of mental illness.

In addition to ion channels, many other components of the synaptic signaling machinery are potential targets for psychoactive drugs. As mentioned earlier, after release into the synaptic cleft, many neurotransmitters are cleared by reuptake mechanisms mediated by Na^+-driven symports. Inhibiting such transporters prolongs the effect of the neurotransmitter, thereby strengthening synaptic transmission. Many antidepressant drugs, including Prozac, inhibit the reuptake of serotonin; others inhibit the reuptake of both serotonin and norepinephrine.

Ion channels are the basic molecular units from which neuronal devices for signaling and computation are built. To provide a glimpse of how sophisticated these devices can be, we consider several examples that demonstrate how the coordinated activities of groups of ion channels allow you to move, feel, and remember.

Neuromuscular Transmission Involves the Sequential Activation of Five Different Sets of Ion Channels

The following process, in which a nerve impulse stimulates a muscle cell to contract, illustrates the importance of ion channels to electrically excitable cells. This apparently simple response requires the sequential activation of at least five different sets of ion channels, all within a few milliseconds (**Figure 11–39**).

1. The process is initiated when a nerve impulse reaches the nerve terminal and depolarizes the plasma membrane of the terminal. The depolarization transiently opens voltage-gated Ca^{2+} channels in this presynaptic membrane. As the Ca^{2+} concentration outside cells is more than 1000 times

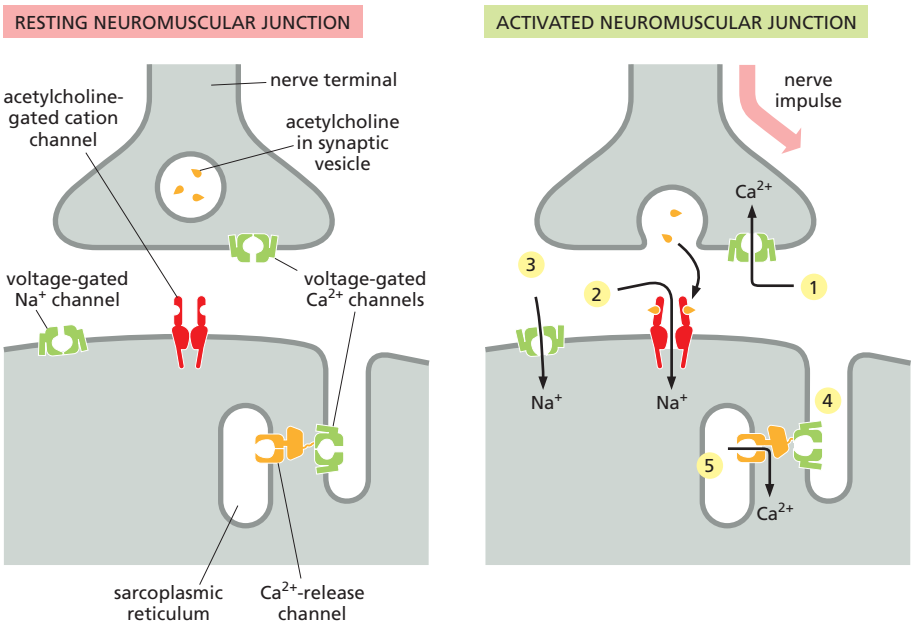


Figure 11–39 The system of ion channels at a neuromuscular junction. These gated ion channels are essential for the stimulation of muscle contraction by a nerve impulse. The various channels are numbered in the sequence in which they are activated, as described in the text.

greater than the free Ca^{2+} concentration inside, Ca^{2+} flows into the nerve terminal. The increase in Ca^{2+} concentration in the cytosol of the nerve terminal triggers the local release of acetylcholine by exocytosis into the synaptic cleft.

2. The released acetylcholine binds to acetylcholine receptors in the muscle cell plasma membrane, transiently opening the cation channels associated with them. The resulting influx of Na^+ causes a local membrane depolarization.
3. The local depolarization opens voltage-gated Na^+ channels in this membrane, allowing more Na^+ to enter, which further depolarizes the membrane. This, in turn, opens neighboring voltage-gated Na^+ channels and results in a self-propagating depolarization (an action potential) that spreads to involve the entire plasma membrane (see Figure 11–31).
4. The generalized depolarization of the muscle cell plasma membrane activates voltage-gated Ca^{2+} channels in the transverse tubules (T tubules—discussed in Chapter 16) of this membrane.
5. This in turn causes *Ca^{2+}-release channels* in an adjacent region of the sarcoplasmic reticulum (SR) membrane to open transiently and release Ca^{2+} stored in the SR into the cytosol. The T-tubule and SR membranes are closely apposed with the two types of channel joined together in a specialized structure, in which activation of the voltage-sensitive Ca^{2+} channel in the T-tubule plasma membrane causes a channel conformational change that is mechanically transmitted to the Ca^{2+}-release channel in the SR membrane, opening it and allowing Ca^{2+} to flow from the SR lumen into the cytoplasm (see Figure 16–35). The sudden increase in the cytosolic Ca^{2+} concentration causes the myofibrils in the muscle cell to contract.

Whereas the initiation of muscle contraction by a motor neuron is complex, an even more sophisticated interplay of ion channels is required for a neuron to integrate a large number of input signals at its synapses and compute an appropriate output, as we now discuss.

Single Neurons Are Complex Computation Devices

In the central nervous system, a single neuron can receive inputs from thousands of other neurons, and it can in turn form synapses with many thousands of other cells. Several thousand nerve terminals, for example, make synapses on an average motor neuron in the spinal cord, almost completely covering its cell body and dendrites (**Figure 11–40**). Some of these synapses transmit signals from the brain or spinal cord; others bring sensory information from muscles or from the skin. The motor neuron must combine the information received from all these sources and react, either by firing action potentials along its axon or by remaining quiet.

Of the many synapses on a neuron, some tend to excite it, while others inhibit it. Neurotransmitter released at an excitatory synapse causes a small depolarization in the postsynaptic membrane called an *excitatory postsynaptic potential* (*excitatory PSP*), whereas neurotransmitter released at an inhibitory synapse generally causes a small hyperpolarization called an *inhibitory PSP.* The plasma membrane of the dendrites and cell body of most neurons contains a relatively low density of voltage-gated Na^+ channels, and so an individual excitatory PSP is generally too small to trigger an action potential. Instead, each incoming signal initiates a local PSP, which decreases with distance from the site of the synapse. If signals arrive simultaneously at several synapses in the same region of the dendritic tree, the total PSP in that neighborhood will be roughly the sum of the individual PSPs, with inhibitory PSPs making a negative contribution to the total. The PSPs from each neighborhood spread passively and converge on the cell body. For long-distance transmission, the combined magnitude of the PSP is then translated, or *encoded*, into the *frequency* of firing of action potentials: the greater the stimulation (depolarization), the higher the frequency of action potentials.

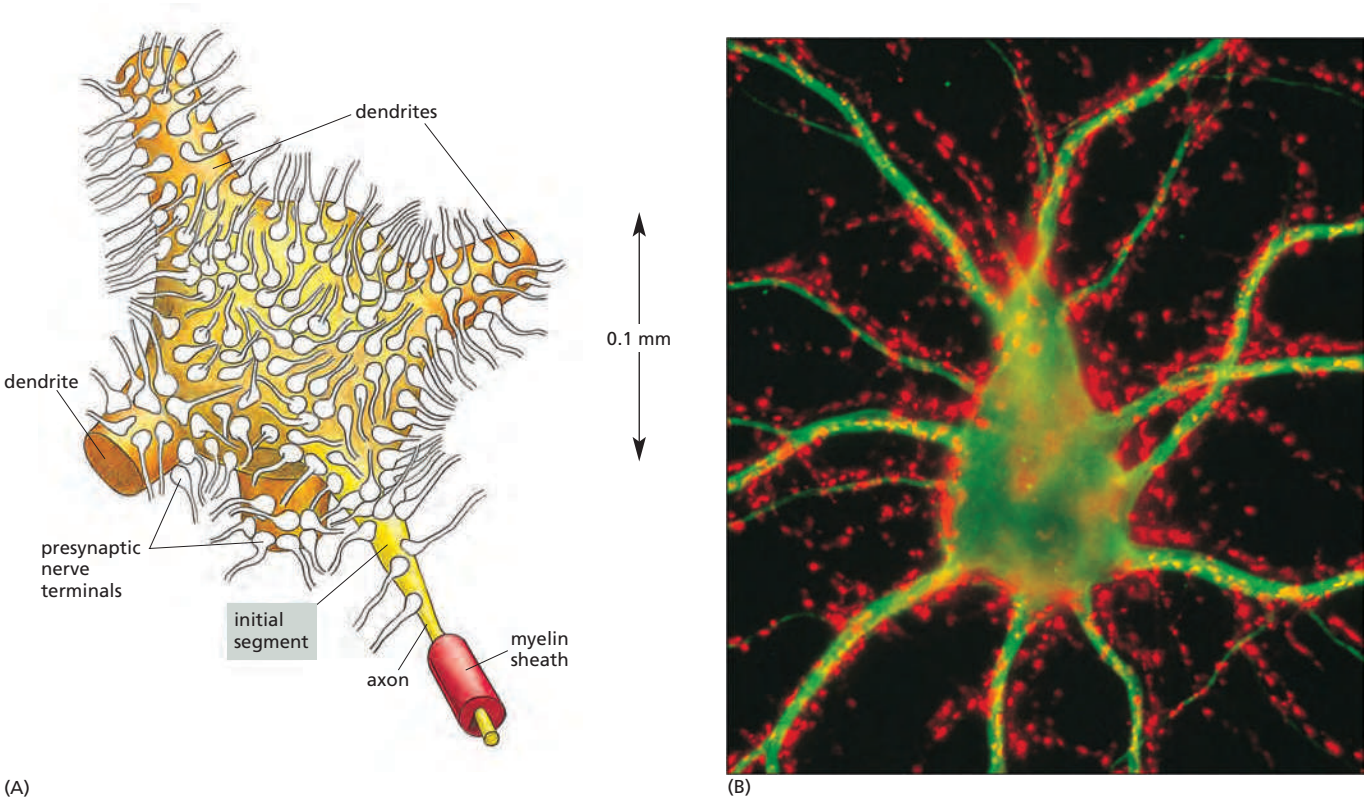


Figure 11–40 A motor neuron in the spinal cord. (A) Many thousands of nerve terminals synapse on the cell body and dendrites. These deliver signals from other parts of the organism to control the firing of action potentials along the single axon of this large cell. (B) Fluorescence micrograph showing a nerve cell body and its dendrites stained with a fluorescent antibody that recognizes a cytoskeletal protein *(green)* that is not present in axons. Thousands of axon terminals *(red)* from other nerve cells (not visible) make synapses on the cell body and dendrites; the terminals are stained with a fluorescent antibody that recognizes a protein in synaptic vesicles. (B, courtesy of Olaf Mundigl and Pietro de Camilli.)

Neuronal Computation Requires a Combination of at Least Three Kinds of K^+ Channels

The intensity of stimulation that a neuron receives is encoded by that neuron into action potential frequency for long-distance transmission. The encoding takes place at a specialized region of the axonal membrane known as the **initial segment**, or *axon hillock*, at the junction of the axon and the cell body (see Figure 11–40). This membrane is rich in voltage-gated Na^+ channels; but it also contains at least four other classes of ion channels—three selective for K^+ and one selective for Ca^{2+}—all of which contribute to the axon hillock's encoding function. The three varieties of K^+ channels have different properties; we shall refer to them as *delayed*, *rapidly inactivating*, and *Ca^{2+}-activated K^+ channels.*

To understand the need for multiple types of channels, consider first what would happen if the only voltage-gated ion channels present in the nerve cell were the Na^+ channels. Below a certain threshold level of synaptic stimulation, the depolarization of the initial-segment membrane would be insufficient to trigger an action potential. With gradually increasing stimulation, the threshold would be crossed, the Na^+ channels would open, and an action potential would fire. The action potential would be terminated by inactivation of the Na^+ channels. Before another action potential could fire, these channels would have to recover from their inactivation. But that would require a return of the membrane voltage to a very negative value, which would not occur as long as the strong depolarizing stimulus (from PSPs) was maintained. An additional channel type is needed, therefore, to repolarize the membrane after each action potential to prepare the cell to fire again.

The **delayed K^+ channels** perform this task, as discussed previously in relation to the propagation of the action potential (see Figure 11–31). They are voltage-gated, but because of their slower kinetics they open only during the falling phase of the action potential, when the Na^+ channels are inactive. Their opening permits an efflux of K^+ that drives the membrane back toward the K^+ equilibrium potential, which is so negative that the Na^+ channels rapidly recover from their inactivated state. Repolarization of the membrane also closes the delayed K^+ channels. The initial segment is now reset so that the depolarizing stimulus from

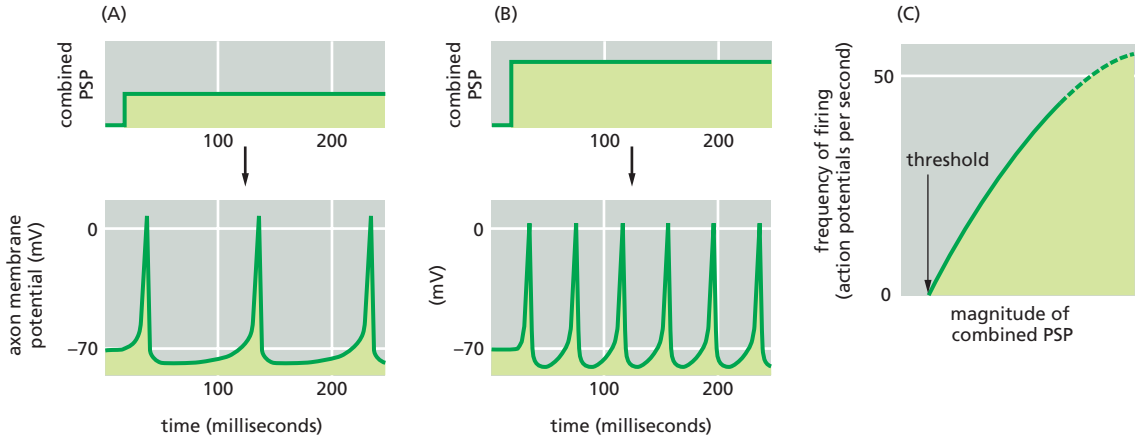


Figure 11–41 The magnitude of the combined postsynaptic potential (PSP) is reflected in the frequency of firing of action potentials. The mix of excitatory and inhibitory PSPs produces a *combined PSP* at the initial segment. A comparison of (A) and (B) shows how the firing frequency of an axon increases with an increase in the combined PSP, while (C) summarizes the general relationship.

synaptic inputs can fire another action potential. In this way, sustained stimulation of the dendrites and cell body leads to repetitive firing of the axon.

Repetitive firing in itself, however, is not enough. The frequency of firing has to reflect the intensity of stimulation, and a simple system of Na^+ channels and delayed K^+ channels is inadequate for this purpose. Below a certain threshold level of steady stimulation, the cell will not fire at all; above that threshold level, it will abruptly begin to fire at a relatively rapid rate. The **rapidly inactivating K^+ channels** solve the problem. These, too, are voltage-gated and open when the membrane is depolarized, but their specific voltage sensitivity and kinetics of inactivation are such that they act to reduce the rate of firing at levels of stimulation that are only just above the threshold required for firing. Thus, they remove the discontinuity in the relationship between the firing rate and the intensity of stimulation. The result is a firing rate that is proportional to the strength of the depolarizing stimulus over a very broad range (**Figure 11–41**).

The process of encoding is usually further modulated by the two other types of ion channels in the initial segment that were mentioned earlier—voltage-gated Ca^{2+} channels and Ca^{2+}-activated K^+ channels. They act together to decrease the response of the cell to an unchanging, prolonged stimulation—a process called **adaptation**. These Ca^{2+} channels are similar to the Ca^{2+} channels that mediate the release of neurotransmitter from presynaptic axon terminals; they open when an action potential fires, transiently allowing Ca^{2+} into the axon cytosol at the initial segment.

The **Ca^{2+}-activated K^+ channel** opens in response to a raised concentration of Ca^{2+} at the channel's cytoplasmic face (**Figure 11–42**). Prolonged, strong depolarizing stimuli will trigger a long train of action potentials, each of which permits a brief influx of Ca^{2+} through the voltage-gated Ca^{2+} channels, so that local cytosolic Ca^{2+} concentration gradually builds up to a level high enough to open the Ca^{2+}-activated K^+ channels. Because the resulting increased permeability of the membrane to K^+ makes the membrane harder to depolarize, the delay between one action potential and the next is increased. In this way, a neuron that is stimulated continuously for a prolonged period becomes gradually less responsive to the constant stimulus.

Such adaptation, which can also occur by other mechanisms, allows a neuron—indeed, the nervous system generally—to react sensitively to *change*, even against a high background level of steady stimulation. It is one of the computational strategies that help us, for example, to feel a light touch on the shoulder and yet ignore the constant pressure of our clothing. We discuss adaptation as a general feature in cell signaling processes in more detail in Chapter 15.

Other neurons do different computations, reacting to their synaptic inputs in myriad ways, reflecting the different assortments of ion channels in their membrane. There are several hundred genes that code for ion channels in the human genome, with over 150 encoding voltage-gated channels alone. Further complexity is introduced by alternative splicing of RNA transcripts and assembling channel subunits in different combinations. Moreover, ion channels are selectively

localized to different sites in the plasma membrane of a neuron. Some K^+ and Ca^{2+} channels are concentrated in the dendrites and participate in processing the input that a neuron receives. As we have seen, other ion channels are located at the axon's initial segment, where they control action potential firing; and some ligand-gated channels are distributed over the cell body and, depending on their ligand occupancy, modulate the cell's general sensitivity to synaptic inputs. The multiplicity of ion channels and their locations evidently allows each of the many types of neurons to tune the electrical behavior to the particular tasks they perform.

One of the crucial properties of the nervous system is its ability to learn and remember. This property depends in part on the ability of individual synapses to strengthen or weaken depending on their use—a process called **synaptic plasticity**. We end this chapter by considering a remarkable type of ion channel that has a special role in some forms of synaptic plasticity. It is located at many excitatory synapses in the central nervous system, where it is gated by both voltage and the excitatory neurotransmitter glutamate. It is also the site of action of the psychoactive drug phencyclidine, or angel dust.

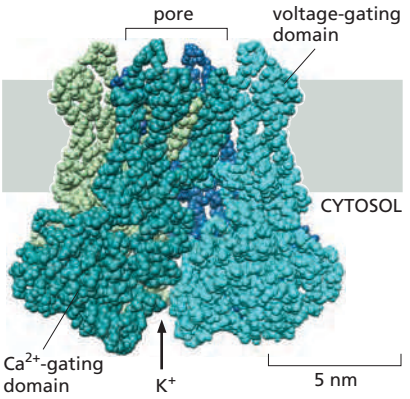


Figure 11–42 Structure of a Ca^{2+}-activated K^+ channel. The channel contains four identical subunits (which are shown in different colors for clarity). It is both voltage- and Ca^{2+}-gated. The structure shown is a composite of the cytosolic and membrane portions of the channel that were separately crystallized. (PDB codes: 2R99, 1LNQ.)

Long-Term Potentiation (LTP) in the Mammalian Hippocampus Depends on Ca^{2+} Entry Through NMDA-Receptor Channels

Practically all animals can learn, but mammals seem to learn exceptionally well (or so we like to think). In a mammal's brain, the region called the *hippocampus* has a special role in learning. When it is destroyed on both sides of the brain, the ability to form new memories is largely lost, although previous long-established memories remain. Some synapses in the hippocampus show a striking form of synaptic plasticity with repeated use: whereas occasional single action potentials in the presynaptic cells leave no lasting trace, a short burst of repetitive firing causes **long-term potentiation** (**LTP**), such that subsequent single action potentials in the presynaptic cells evoke a greatly enhanced response in the postsynaptic cells. The effect lasts hours, days, or weeks, according to the number and intensity of the bursts of repetitive firing. Only the synapses that were activated exhibit LTP; synapses that have remained quiet on the same postsynaptic cell are not affected. However, while the cell is receiving a burst of repetitive stimulation via one set of synapses, if a single action potential is delivered at *another* synapse on its surface, that latter synapse also will undergo LTP, even though a single action potential delivered there at another time would leave no such lasting trace.

The underlying rule in such events seems to be that *LTP occurs on any occasion when a presynaptic cell fires (once or more) at a time when the postsynaptic membrane is strongly depolarized* (either through recent repetitive firing of the same presynaptic cell or by other means). This rule reflects the behavior of a particular class of ion channels in the postsynaptic membrane. Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system, and glutamate-gated ion channels are the most common of all transmitter-gated channels in the brain. In the hippocampus, as elsewhere, most of the depolarizing current responsible for excitatory PSPs is carried by glutamate-gated ion channels called **AMPA receptors**, which operate in the standard way (**Figure 11–43**). But the current has, in addition, a second and more intriguing component, which is mediated by a separate subclass of glutamate-gated ion channels known as **NMDA receptors**, so named because they are selectively activated by the artificial glutamate analog *N*-methyl-D-aspartate. The NMDA-receptor channels are doubly gated, opening only when two conditions are satisfied simultaneously: glutamate must be bound to the receptor, and the membrane must be strongly depolarized. The second condition is required for releasing the Mg^{2+} that normally blocks the resting channel. This means that NMDA receptors are normally activated only when AMPA receptors are activated as well and depolarize the membrane. The NMDA receptors are critical for LTP. When they are selectively blocked with a specific inhibitor or inactivated genetically, LTP does not occur, even though ordinary synaptic transmission continues, indicating the importance of NMDA receptors

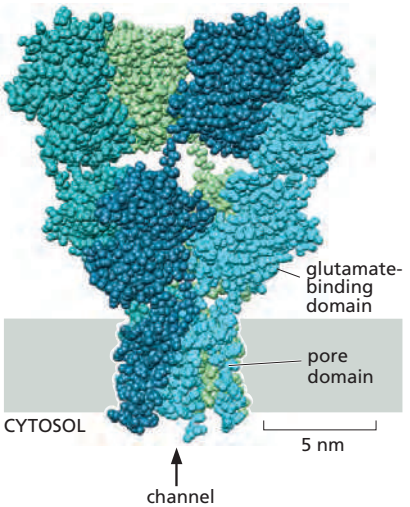


Figure 11–43 The structure of the AMPA receptor. This ionotropic glutamate receptor (named after the glutamate analog α-Amino 3-hydroxy 5-Methyl 4-isoxazole Propionic Acid) is the most common mediator of fast, excitatory synaptic transmission in the central nervous system (CNS). (PDB code: 3KG2.)

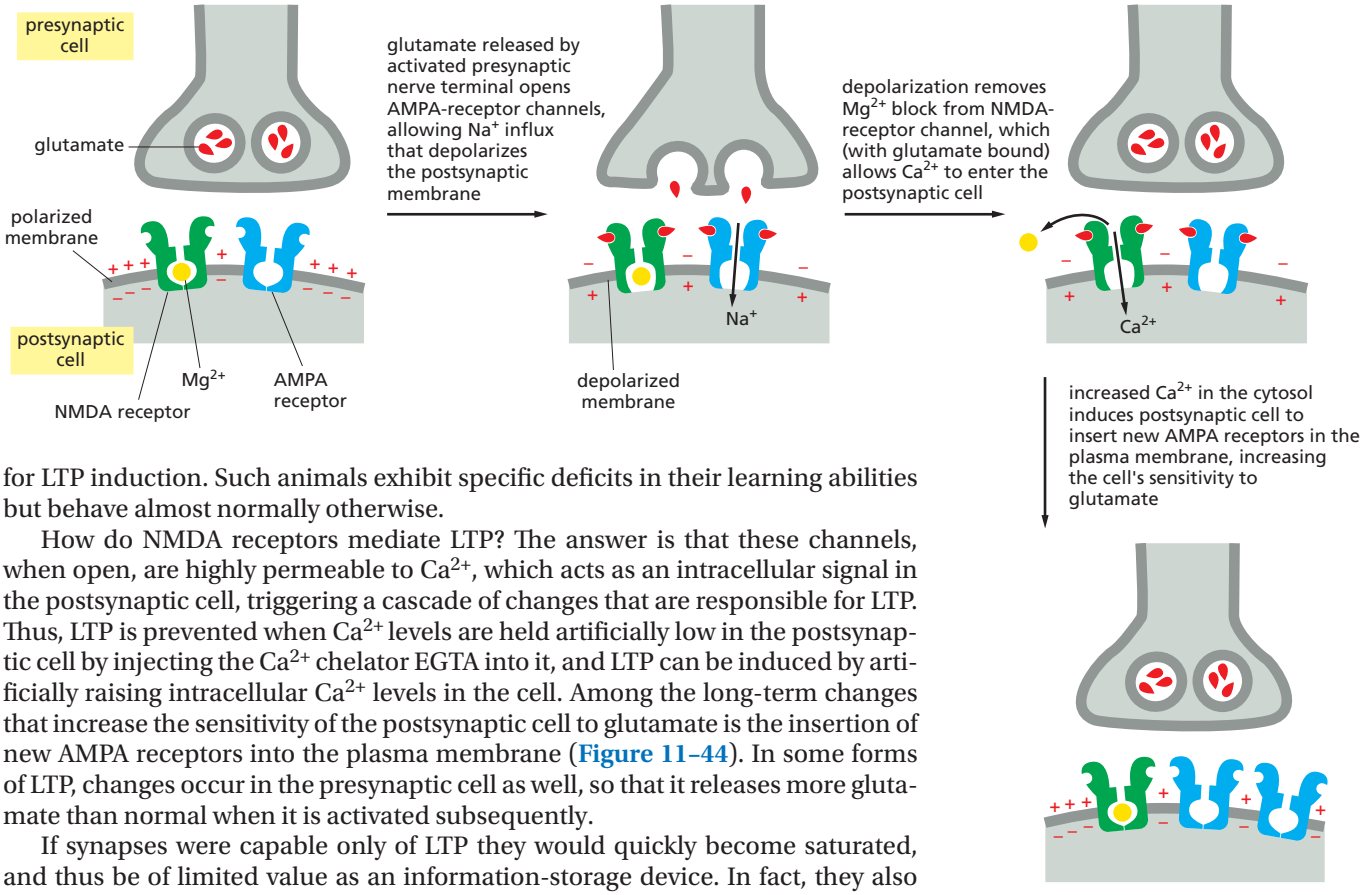


Figure 11–44 The signaling events in long-term potentiation. Although not shown, transmission-enhancing changes can also occur in the presynaptic nerve terminals in LTP, which may be induced by retrograde signals from the postsynaptic cell.

for LTP induction. Such animals exhibit specific deficits in their learning abilities but behave almost normally otherwise.

How do NMDA receptors mediate LTP? The answer is that these channels, when open, are highly permeable to Ca^{2+}, which acts as an intracellular signal in the postsynaptic cell, triggering a cascade of changes that are responsible for LTP. Thus, LTP is prevented when Ca^{2+} levels are held artificially low in the postsynaptic cell by injecting the Ca^{2+} chelator EGTA into it, and LTP can be induced by artificially raising intracellular Ca^{2+} levels in the cell. Among the long-term changes that increase the sensitivity of the postsynaptic cell to glutamate is the insertion of new AMPA receptors into the plasma membrane (**Figure 11–44**). In some forms of LTP, changes occur in the presynaptic cell as well, so that it releases more glutamate than normal when it is activated subsequently.

If synapses were capable only of LTP they would quickly become saturated, and thus be of limited value as an information-storage device. In fact, they also exhibit **long-term depression** (**LTD**), with the long-term effect of reducing the number of AMPA receptors in the post-synaptic membrane. This feat is accomplished by degrading AMPA receptors after their selective endocytosis. Surprisingly, LTD also requires NMDA receptor activation and a rise in Ca^{2+}. How does Ca^{2+} trigger opposite effects at the same synapse? It turns out that this bidirectional control of synaptic strength depends on the magnitude of the rise in Ca^{2+}: high Ca^{2+} levels activate protein kinases and LTP, whereas modest Ca^{2+} levels activate protein phosphatases and LTD.

There is evidence that NMDA receptors have an important role in synaptic plasticity and learning in other parts of the brain, as well as in the hippocampus. Moreover, they have a crucial role in adjusting the anatomical pattern of synaptic connections in the light of experience during the development of the nervous system.

Thus, neurotransmitters released at synapses, besides relaying transient electrical signals, can also alter concentrations of intracellular mediators that bring about lasting changes in the efficacy of synaptic transmission. However, it is still uncertain how these changes endure for weeks, months, or a lifetime in the face of the normal turnover of cell constituents.

Summary

Ion channels form aqueous pores across the lipid bilayer and allow inorganic ions of appropriate size and charge to cross the membrane down their electrochemical gradients at rates about 1000 times greater than those achieved by any known transporter. The channels are "gated" and usually open transiently in response to a specific perturbation in the membrane, such as a change in membrane potential (voltage-gated channels), or the binding of a neurotransmitter to the channel (transmitter-gated channels).

K^+-selective leak channels have an important role in determining the resting membrane potential across the plasma membrane in most animal cells.

Voltage-gated cation channels are responsible for the amplification and propagation of action potentials in electrically excitable cells, such as neurons and skeletal muscle cells. Transmitter-gated ion channels convert chemical signals to electrical signals at chemical synapses. Excitatory neurotransmitters, such as acetylcholine and glutamate, open transmitter-gated cation channels and thereby depolarize the postsynaptic membrane toward the threshold level for firing an action potential. Inhibitory neurotransmitters, such as GABA and glycine, open transmitter-gated Cl^- or K^+ channels and thereby suppress firing by keeping the postsynaptic membrane polarized. A subclass of glutamate-gated ion channels, called NMDA-receptor channels, is highly permeable to Ca^{2+}, which can trigger the long-term changes in synapse efficacy (synaptic plasticity) such as LTP and LTD that are thought to be involved in some forms of learning and memory.

Ion channels work together in complex ways to control the behavior of electrically excitable cells. A typical neuron, for example, receives thousands of excitatory and inhibitory inputs, which combine by spatial and temporal summation to produce a combined postsynaptic potential (PSP) at the initial segment of its axon. The magnitude of the PSP is translated into the rate of firing of action potentials by a mixture of cation channels in the initial segment membrane.

WHAT WE DON'T KNOW

- How do individual neurons establish and maintain their characteristic intrinsic firing properties?
- Even organisms with very simple nervous systems have dozens of different K^+ channels. Why is it important to have so many?
- Why do cells that are not electrically active contain voltage-gated ion channels?
- How are memories stored for so many years in the human brain?

PROBLEMS

Which statements are true? Explain why or why not.

11–1 Transport by transporters can be either active or passive, whereas transport by channels is always passive.

11–2 Transporters saturate at high concentrations of the transported molecule when all their binding sites are occupied; channels, on the other hand, do not bind the ions they transport and thus the flux of ions through a channel does not saturate.

11–3 The membrane potential arises from movements of charge that leave ion concentrations practically unaffected, causing only a very slight discrepancy in the number of positive and negative ions on the two sides of the membrane.

Discuss the following problems.

11–4 Order Ca^{2+}, CO_2, ethanol, glucose, RNA, and H_2O according to their ability to diffuse through a lipid bilayer, beginning with the one that crosses the bilayer most readily. Explain your order.

11–5 How is it possible for some molecules to be at equilibrium across a biological membrane and yet not be at the same concentration on both sides?

11–6 Ion transporters are "linked" together—not physically, but as a consequence of their actions. For example, cells can raise their intracellular pH, when it becomes too acidic, by exchanging external Na^+ for internal H^+, using a Na^+–H^+ antiporter. The change in internal Na^+ is then redressed using the Na^+-K^+ pump.

A. Can these two transporters, operating together, normalize both the H^+ and the Na^+ concentrations inside the cell?

B. Does the linked action of these two pumps cause imbalances in either the K^+ concentration or the membrane potential? Why or why not?

11–7 Microvilli increase the surface area of intestinal cells, providing more efficient absorption of nutrients. Microvilli are shown in profile and cross section in **Figure Q11–1**. From the dimensions given in the figure, estimate the increase in surface area that microvilli provide (for the portion of the plasma membrane in contact with the lumen of the gut) relative to the corresponding surface of a cell with a "flat" plasma membrane.

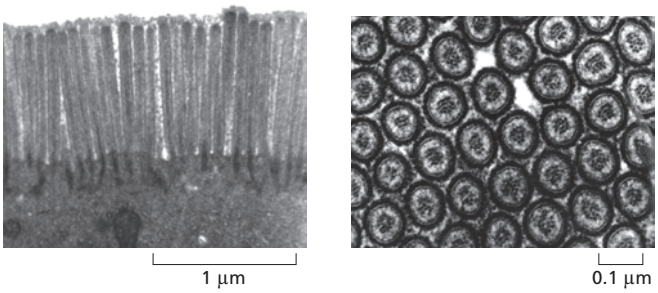


Figure Q11–1 Microvilli of intestinal epithelial cells in profile and cross section (Problem 11–7). (Left panel, from Rippel Electron Microscope Facility, Dartmouth College; right panel, from David Burgess.)

11–8 According to Newton's laws of motion, an ion exposed to an electric field in a vacuum would experience a constant acceleration from the electric driving force, just as a falling body in a vacuum constantly accelerates due to gravity. In water, however, an ion moves at constant velocity in an electric field. Why do you suppose that is?

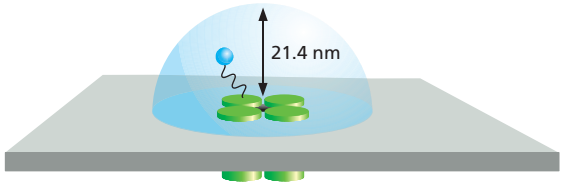


Figure Q11–2 A "ball" tethered by a "chain" to a voltage-gated K^+ channel (Problem 11–9).

TABLE Q11–1 Ionic composition of seawater and of the cytosol in the squid giant axon (Problem 11–10).

Ion	Cytosol	Seawater
Na^+	65 mM	430 mM
K^+	344 mM	9 mM

11–9 In a subset of voltage-gated K^+ channels, the N-terminus of each subunit acts like a tethered ball that occludes the cytoplasmic end of the pore soon after it opens, thereby inactivating the channel. This "ball-and-chain" model for the rapid inactivation of voltage-gated K^+ channels has been elegantly supported for the *shaker* K^+ channel from *Drosophila melanogaster*. (The *shaker* K^+ channel in *Drosophila* is named after a mutant form that causes excitable behavior—even anesthetized flies keep twitching.) Deletion of the N-terminal amino acids from the normal *shaker* channel gives rise to a channel that opens in response to membrane depolarization, but stays open instead of rapidly closing as the normal channel does. A peptide (MAAVAGLYGLGEDRQHRKKQ) that corresponds to the deleted N-terminus can inactivate the open channel at 100 μM.

Is the concentration of free peptide (100 μM) that is required to inactivate the defective K^+ channel anywhere near the local concentration of the tethered ball on a normal channel? Assume that the tethered ball can explore a hemisphere [volume = $(2/3)\pi r^3$] with a radius of 21.4 nm, which is the length of the polypeptide "chain" (**Figure Q11–2**). Calculate the concentration for one ball in this hemisphere. How does that value compare with the concentration of free peptide needed to inactivate the channel?

11–10 The giant axon of the squid (**Figure Q11–3**) occupies a unique position in the history of our understanding of cell membrane potentials and nerve action. When an electrode is stuck into an intact giant axon, the membrane potential registers –70 mV. When the axon, suspended in a bath of seawater, is stimulated to conduct a nerve impulse, the membrane potential changes transiently from –70 mV to +40 mV.

Figure Q11–3 The squid *Loligo* (Problem 11–10). This squid is about 15 cm in length.

For univalent ions and at 20°C (293 K), the Nernst equation reduces to

$$V = 58 \text{ mV} \times \log (C_o/C_i)$$

where C_o and C_i are the concentrations outside and inside, respectively.

Using this equation, calculate the potential across the resting membrane (1) assuming that it is due solely to K^+ and (2) assuming that it is due solely to Na^+. (The Na^+ and K^+ concentrations in the axon cytosol and in seawater are given in **Table Q11–1**.) Which calculation is closer to the measured resting potential? Which calculation is closer to the measured action potential? Explain why these assumptions approximate the measured resting and action potentials.

11–11 Acetylcholine-gated cation channels at the neuromuscular junction open in response to acetylcholine released by the nerve terminal and allow Na^+ ions to enter the muscle cell, which causes membrane depolarization and ultimately leads to muscle contraction.

A. Patch-clamp measurements show that young rat muscles have cation channels that respond to acetylcholine (**Figure Q11–4**). How many kinds of channel are there? How can you tell?

B. For each kind of channel, calculate the number of ions that enter in one millisecond. (One ampere is a current of one coulomb per second; one pA equals 10^{-12} ampere. An ion with a single charge such as Na^+ carries a charge of 1.6×10^{-19} coulomb.)

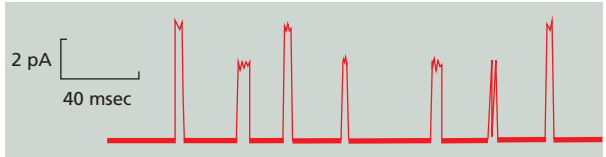


Figure Q11–4 Patch-clamp measurements of acetylcholine-gated cation channels in young rat muscle (Problem 11–11).

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