Membrane Transport of Small Molecules and the Electrical Properties of Membranes

11

Because of its hydrophobic interior, the lipid bilayer of cell membranes prevents 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 transmembrane proteins to transport inorganic ions and small water-soluble organic molecules across the lipid bilayer. 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. The importance of membrane transport is reflected in the large number of genes in all organisms that code for transport proteins, which make up 15–30% of the membrane proteins in all cells. Some specialized mammalian cells devote up to two-thirds of their total metabolic energy consumption to membrane transport processes.

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 traffic of molecules back and forth across lipid bilayers: *transporters*, which have moving parts to transport specific molecules across membranes, and *channels*, which form a narrow hydrophilic pore, allowing passive transmembrane movement, primarily of small inorganic ions. Transporters can be coupled to a source of energy to catalyze active transport, and a combination of selective passive permeability and active transport 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 ionic 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 eucaryotic 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 human brain's astonishing feats.

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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 Ion Concentrations Inside and Outside a Typical Mammalian Cell

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

*The 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-}, proteins, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of Ca^{2+} and Mg^{2+} given are for the free ions. There is a total of about 20 mM Mg^{2+} and 1–2 mM Ca^{2+} in cells, but both are mostly bound to proteins and other substances and, for Ca^{2+}, stored within various organelles.

Protein-Free Lipid Bilayers Are Highly 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 solubility in oil. In general, the smaller the molecule and the more soluble it is in oil (the more hydrophobic, or nonpolar, it is), the more rapidly 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**). By contrast, lipid bilayers are highly 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. Thus, synthetic lipid bilayers are 10^9 times more permeable to water than to even such small ions as Na^+ or K^+ (**Figure 11–2**).

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

Like synthetic lipid bilayers, cell membranes allow water and nonpolar molecules to permeate by simple diffusion. Cell membranes, however, also have to allow the passage of various polar molecules, such as ions, sugars, amino acids, nucleotides, 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 transports a particular class of molecule (such as ions, sugars, or amino acids) and often only certain molecular species of the class. 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 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 blood; the resulting accumulation of cystine in the urine leads to the formation of cystine stones in the kidneys.

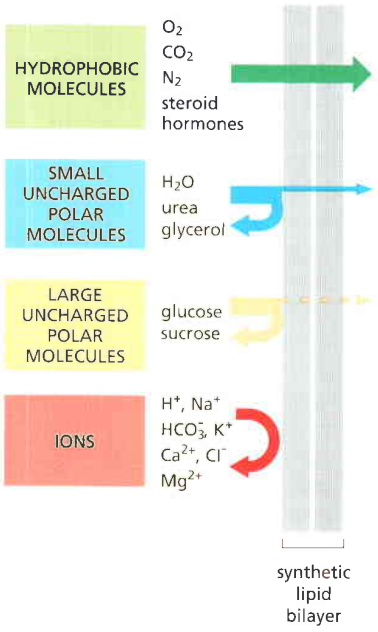


Figure 11–1 The relative permeability of a synthetic lipid bilayer to different classes of molecules. The smaller the molecule and, more importantly, the less strongly it associates with water, the more rapidly the molecule diffuses across the bilayer.

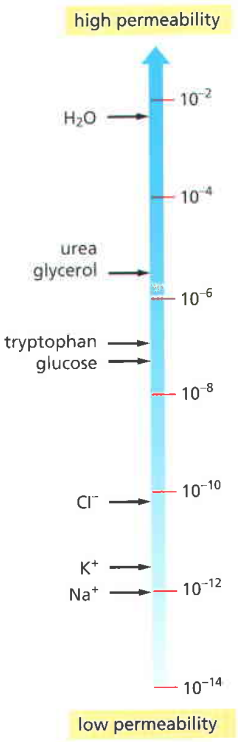


Figure 11–2 Permeability coefficients for the passage of various molecules through synthetic lipid bilayers. The rate of flow of a solute across the bilayer is directly proportional to the difference in its concentration on the two sides of the membrane. Multiplying this concentration difference (in 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 ($10^{-4}/10^{-3}$ L = 0.1 M), for example, would cause a flow of 10^{-4} mol/cm^3 $\times$ 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.

All membrane transport proteins that have been studied in detail have been found to be multipass transmembrane proteins—that is, their polypeptide chains traverse the lipid bilayer multiple times. By forming a continuous protein 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.

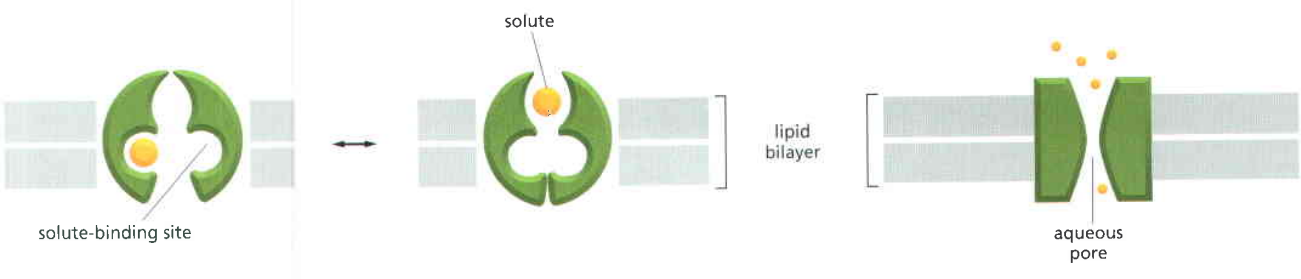
Transporters and channels are the two major classes of membrane transport proteins (**Figure 11–3**). **Transporters** (also called *carriers*, or *permeases)* bind the specific solute to be transported and undergo a series of conformational changes to transfer the bound solute across the membrane. **Channels**, in contrast, interact with the solute to be transported much more weakly. They form aqueous pores that extend across the lipid bilayer; when open, these pores allow specific solutes (usually inorganic ions of appropriate size and charge) 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 diffuse across synthetic lipid bilayers, all cells contain specific channel proteins (called *water channels*, or *aquaporins*) that greatly increase the permeability of these 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**, or **facilitated diffusion**. 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 11–4**A).

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 in Chapter 14. In fact, almost all plasma membranes have an electrical potential difference (voltage gradient) across them, with the inside usually negative with respect to the outside. This potential difference favors the entry of positively charged ions into the cell but opposes the entry of negatively 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 water-filled pore across the bilayer through which specific solutes can diffuse.



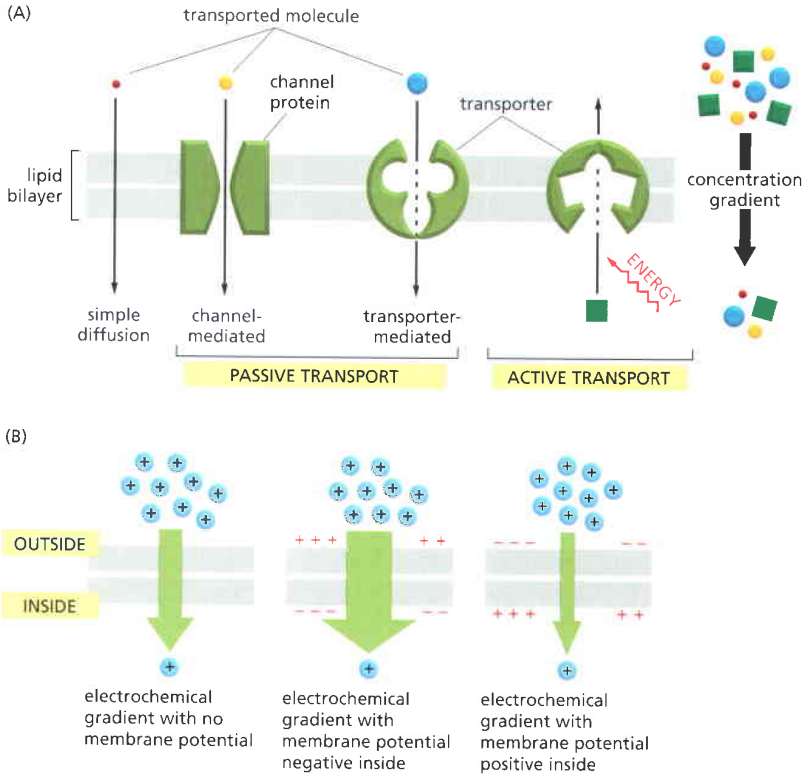


Figure 11–4 Passive and active transport compared. (A) Passive transport down an electrochemical gradient occurs spontaneously, either by simple diffusion through the lipid bilayer or by facilitated diffusion through channels and passive transporters. By contrast, active transport requires an input of metabolic energy and is always mediated by transporters that harvest metabolic energy to pump the solute against its electrochemical gradient. (B) An electrochemical gradient combines the membrane potential and the concentration gradient; they can work additively to increase the driving force on an ion across the membrane *(middle)* or can work against each other *(right)*.

Cells also require transport proteins that will actively pump certain solutes across the membrane against their electrochemical gradients ("uphill"); this process, known as **active transport**, is mediated by transporters, which are also called *pumps*. In active transport, the pumping activity of the transporter is directional because it is tightly coupled to a source of metabolic energy, such as ATP hydrolysis or an ion gradient, as discussed later. Thus, transmembrane movement of small molecules mediated by transporters can be either active or passive, whereas that mediated by channels is always passive.

Summary

Lipid bilayers are highly 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 continuous 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.

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. In 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

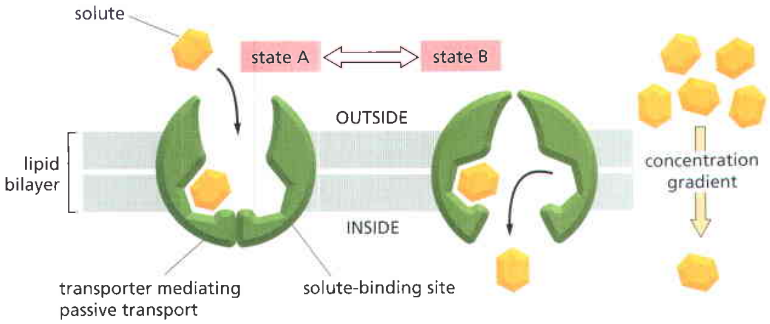


Figure 11–5 A model of how a conformational change in a transporter could mediate the passive movement of a solute. The transporter shown can exist in two conformational states: in state A, the binding sites for solute are exposed on the outside of the lipid bilayer; in state B, the same sites are exposed on the other side of the bilayer. The transition between the two states can occur randomly. It is completely reversible and does not depend on whether the solute binding site is occupied. Therefore, if the solute concentration is higher on the outside of the bilayer, more solute binds to the transporter in the A conformation than in the B conformation, and there is a net transport of solute down its concentration gradient (or, if the solute is an ion, down its electrochemical gradient).

reversible conformational changes that alternately expose the solute-binding site first on one side of the membrane and then on the other. Figure 11–5 shows a schematic model of how a transporter operates. 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 with which the carrier can flip between its two 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 specifically 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 specifically alter the structure of the transporter).

As we discuss below, 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* 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-driven pumps*, which are found mainly in bacteria and archaea, couple uphill transport to an input of energy from light, as with bacteriorhodopsin (discussed in Chapter 10).

Amino acid sequence comparisons suggest that, in many cases, there are strong similarities in molecular design 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 transport by considering transporters that are driven by ion 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^+ pump that is found in the plasma membrane of almost all cells.

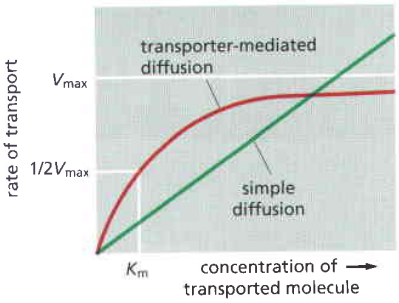


Figure 11–6 The kinetics of simple diffusion and transporter-mediated diffusion. Whereas the rate of simple diffusion is always proportional to the solute concentration, the rate of transporter-mediated diffusion reaches a maximum (V_{max}) when the transporter is saturated. The solute concentration when transport 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.

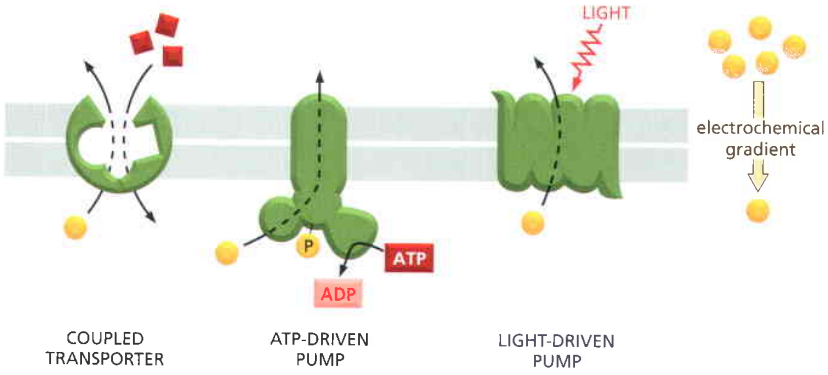


Figure 11–7 Three ways of driving active transport. The actively transported molecule is shown in *yellow*, and the energy source is shown in *red*.

Active Transport Can Be Driven by Ion Gradients

Some transporters simply 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 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 these coupled transporters to harvest the energy stored in the electrochemical gradient of one solute, typically an 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 principle 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, the electrochemical gradient of which provides a large driving force for the active transport of a second molecule. The Na^+ that enters the cell during transport is subsequently pumped out by an ATP-driven Na^+ pump in the plasma membrane (as we discuss later), which, by maintaining the Na^+ gradient, indirectly drives the transport. (For this reason ion-driven carriers are said to mediate *secondary active transport*, whereas ATP-driven carriers are said to mediate *primary active transport*.)

Intestinal and kidney epithelial cells, for example, 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, and the solute and Na^+ bind to different sites on the transporter. 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 greater the rate of solute

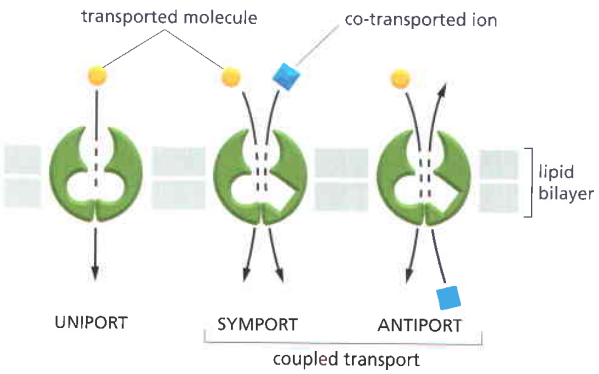


Figure 11–8 Three types of transporter-mediated movement. <ACCC> This schematic diagram shows transporters functioning as uniporters, symporters, and antiporters.

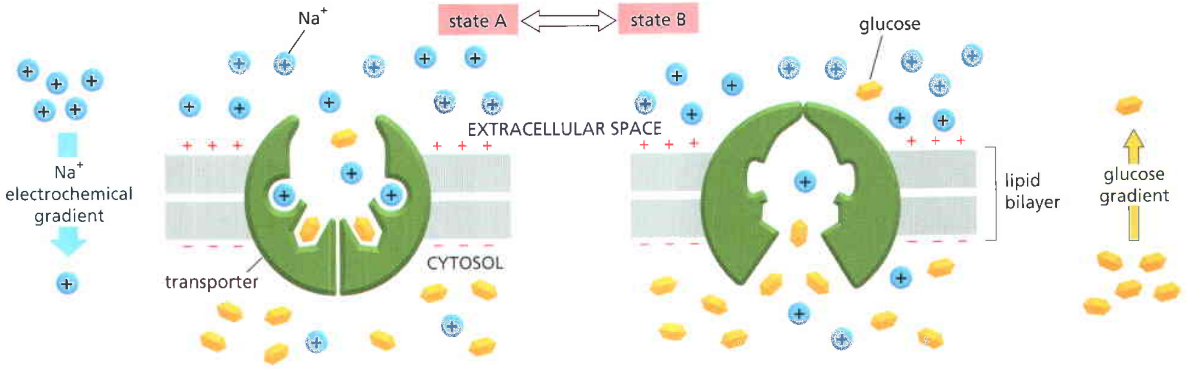


Figure 11–9 One way in which a glucose transporter can be driven by a Na^+ gradient. As in the model shown in Figure 11–5, the transporter oscillates between two alternate states, A and B. In the A state, the protein is open to the extracellular space; in the B state, it is open to the cytosol. Binding of Na^+ and glucose is cooperative—that is, the binding of either ligand induces a conformational change that increases the protein's affinity for the other ligand. 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 A state. Therefore, both Na^+ and glucose enter the cell (via an A → B transition) much more often than they leave it (via a B → A transition). The overall result is the net transport of both Na^+ and glucose into the cell. Note that, because the binding is cooperative, if one of the two solutes is missing, the other fails to bind to the transporter. Thus, the transporter undergoes a conformational switch between the two states only if both solutes or neither are bound.

entry; conversely, if the Na^+ concentration in the extracellular fluid is reduced, solute transport decreases (**Figure 11–9**).

In bacteria and yeasts, as well as in many membrane-enclosed organelles of animal cells, most active transport systems driven by ion gradients depend on H^+ rather than Na^+ gradients, reflecting the predominance of H^+ pumps and the virtual absence of Na^+ pumps in these membranes. The electrochemical H^+ gradient drives the active transport of many sugars and amino acids across the plasma membrane and into bacterial cells. One well-studied H^+-driven symporter is **lactose permease**, which transports lactose across the plasma membrane of *E. coli*. Structural and biophysical studies of the permease, as well as extensive analyses of mutant forms of the protein, have led to a detailed model of how the symporter works. The permease consists of 12 loosely packed transmembrane α helices. During the transport cycle, some of the helices undergo sliding motions that cause them to tilt. These motions alternately open and close a crevice between the helices, exposing the binding sites for lactose and H^+, first on one side of the membrane and then on the other (**Figure 11–10**).

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

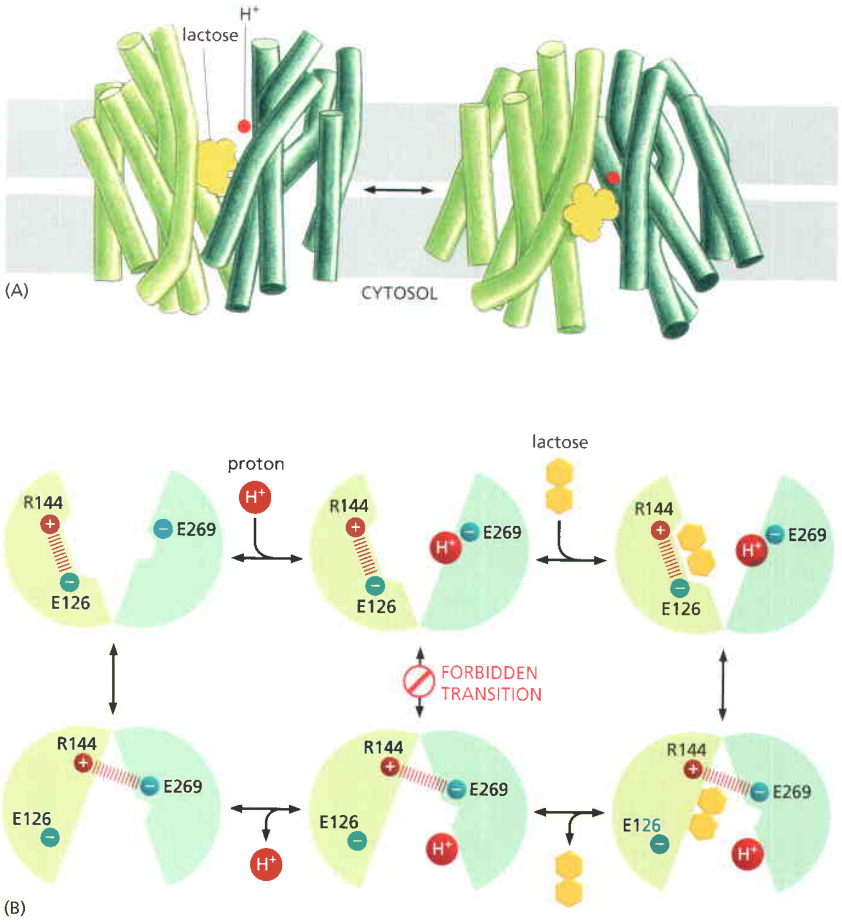


Figure 11–10 The molecular mechanism of the bacterial lactose permease suggested from its crystal structure. (A) The 12 transmembrane helices of the permease are clustered into two lobes, shown in two shades of *green*. The loops that connect the helices on either side of the membrane are omitted for clarity. During transport, the helices slide and tilt in the membrane, exposing binding sites for the disaccharide lactose *(yellow)* and H^+ to either side of the membrane. (B) In one conformational state, the H^+- and lactose-binding sites are accessible to the extracellular space *(top row)*; in the other, they are exposed to the cytosol *(bottom row)*. Loading the solutes on the extracellular side is favored because arginine (R) 144 forms a bond with glutamic acid (E) 126, leaving E269 free to accept H^+. Unloading the solutes on the cytosolic side is favored because R144 forms a bond with E269, which destabilizes the bound H^+. In addition, the lactose-binding site is partially disrupted due to the rearrangement of the helices. Because the transition between the two protonated states *(middle)* is forbidden, H^+ can only be transported when a lactose is also transported. In this way, the electrochemical H^+ gradient drives lactose import. (Adapted from J. Abramson et al., *Science* 301: 610–615, 2003. With permission from AAAS.)

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 10–41) 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 these coupled transporters: ATP-driven H^+ pumps are also 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 action of

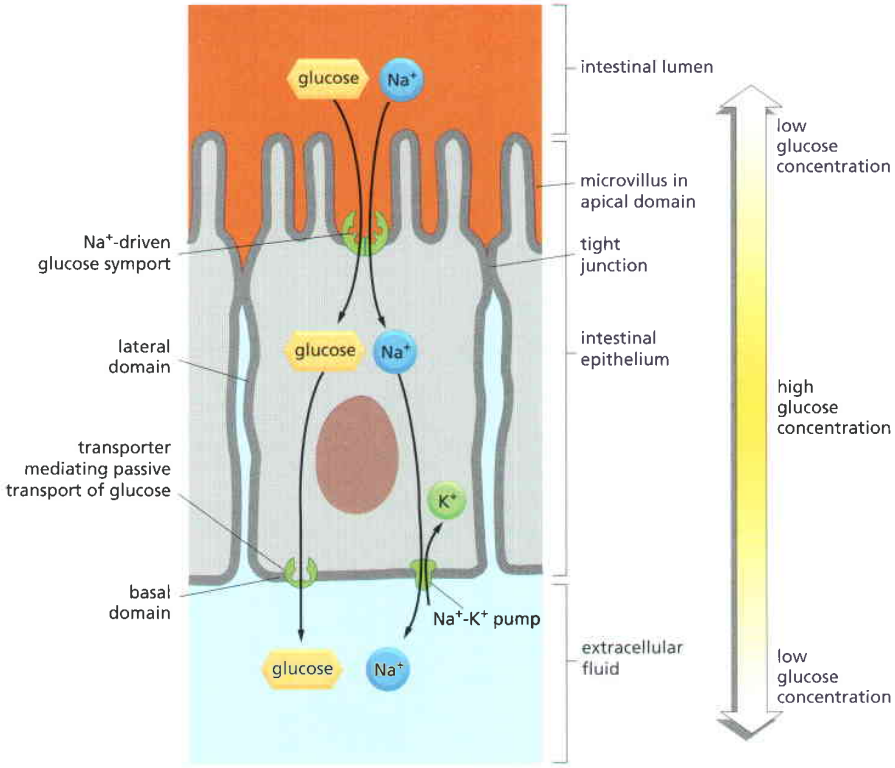


Figure 11–11 Transcellular transport. <GGAT> The transcellular transport of glucose across an intestinal epithelial cell depends on the nonuniform distribution of transporters in the cell's plasma membrane. The process shown here results in the transport of glucose from the intestinal lumen to the extracellular 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 different glucose transporter in the basal and lateral membrane domains. The Na^+ gradient driving the glucose symport is maintained by a Na^+ pump in the basal and lateral plasma membrane domains, which keeps the internal concentration of Na^+ low. 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, and they also serve as diffusion barriers within the plasma membrane, which help confine the various transporters to their respective membrane domains (see Figure 10–37).

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. Na^+-independent transport proteins in the basal and lateral (basolateral) domain 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.

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 procaryotic and eucaryotic 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. **F-type pumps** are turbine-like proteins, constructed from multiple different subunits. They differ structurally from P-type ATPases and are found in the plasma membrane of bacteria, the inner membrane of mitochondria,

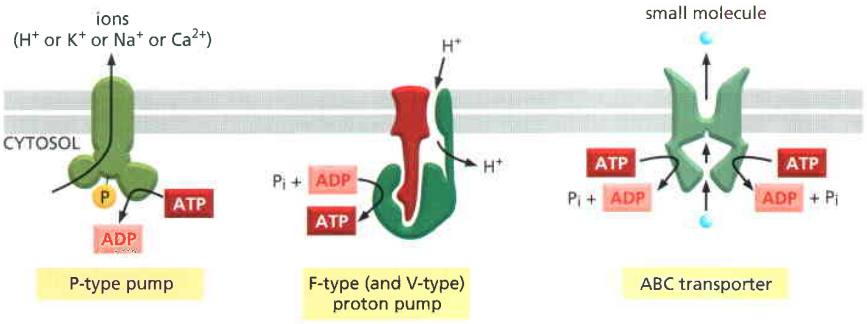


Figure 11–12 Three types of ATP-driven pumps. The different molecular designs of the pumps are cartooned here. Like any enzyme, pumps can work in reverse: when the electrochemical gradients of the solutes are reversed and the ATP/ADP ratio is low, they can synthesize ATP from ADP, as shown for the F-type ATPase, which normally works in this mode.

and the thylakoid membrane of chloroplasts. They are often 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. 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-activated H^+ pump (bacteriorhodopsin) in *Halobacterium*. We discuss these proteins in detail in Chapter 14.

Structurally related to the F-type ATPases is a distinct family of *V-type ATPases* that normally pump H^+ rather than synthesize ATP. They pump H^+ into organelles, such as lysosomes, synaptic vesicles, and plant vacuoles to acidify the interior of these organelles (see Figure 13–36).

3. **ABC transporters** primarily pump small molecules across cell membranes, in contrast to P-type and the F- or V-type ATPases, which exclusively transport ions.

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

The Ca^{2+} Pump Is the Best-Understood P-type ATPase

Eucaryotic cells maintain very low concentrations of free Ca^{2+} in their cytosol ($\sim 10^{-7}$ M) in the face of a very much higher extracellular Ca^{2+} concentration ($\sim 10^{-3}$ M). 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 important, therefore, that the cell maintain 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 across the membrane (see Figure 15–41).

The best-understood P-type transport ATPase is the **Ca^{2+} pump**, or **Ca^{2+} ATPase**, in the *sarcoplasmic reticulum* (SR) membrane of skeletal muscle cells. The SR is a specialized type of endoplasmic reticulum that forms a network of tubular sacs in the muscle cell cytoplasm and 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, as discussed in Chapter 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.

The three-dimensional structure of the SR Ca^{2+} pump has been determined by x-ray crystallography. This structure and the analysis of a related fungal H^+ pump have provided the first views of P-type transport ATPases, which are all thought to have similar structures. They contain 10 transmembrane α helices, three of which line a central channel that spans the lipid bilayer. In the unphosphorylated state, two helices are disrupted and form a cavity that binds two Ca^{2+}

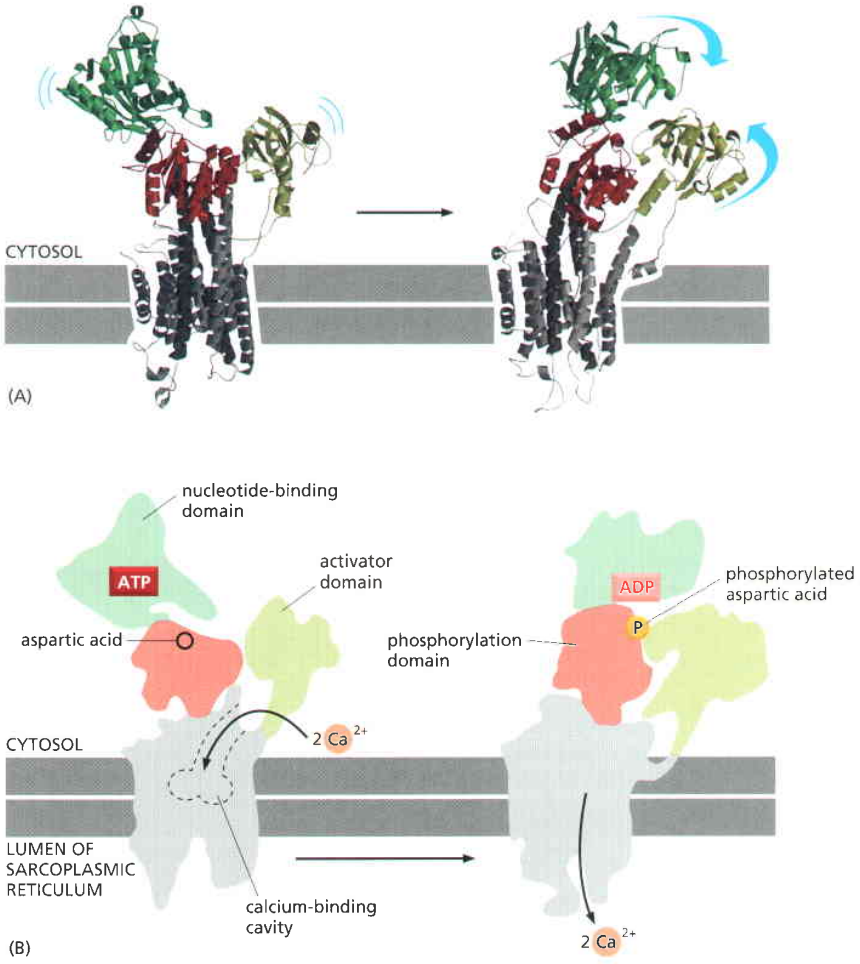


Figure 11–13 A model of how the sarcoplasmic reticulum Ca^{2+} pump moves Ca^{2+}. (A) The structures of the unphosphorylated, Ca^{2+}-bound state *(left)* and the phosphorylated, Ca^{2+}-free state *(right)* were determined by x-ray crystallography. (B) The model shows how ATP binding and hydrolysis cause drastic conformational changes, bringing the nucleotide-binding and phosphorylation domains into close proximity. This change is thought to cause a 90° rotation of the activator domain, which leads to a rearrangement of the transmembrane helices. The rearrangement of the helices disrupts the Ca^{2+}-binding cavity and releases the Ca^{2+} into the lumen of the sarcoplasmic reticulum. (Adapted from C. Toyoshima et al., *Nature* 405:647–655, 2000. With permission from Macmillan Publishers Ltd.)

ions and is accessible from the cytosolic side of the membrane. The binding of ATP to a binding site on the same side of the membrane and the subsequent transfer of the terminal phosphate group of the ATP to an aspartic acid of an adjacent domain lead to a drastic rearrangement of the transmembrane helices. The rearrangement disrupts the Ca^{2+}-binding site and releases the Ca^{2+} ions on the other side of the membrane, into the lumen of the SR (**Figure 11–13**). An essential characteristic of all P-type pumps is that the pump transiently phosphorylates itself during the pumping cycle.

The Plasma Membrane P-type Na^+-K^+ Pump Establishes the Na^+ Gradient 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. 652). A **Na^+-K^+ pump**, or **Na^+ pump**, found in the plasma membrane of virtually all animal cells, maintains these concentration differences. The pump operates as an ATP-driven antiporter, actively pumping Na^+ out of the cell against its steep electrochemical gradient and pumping K^+ in (**Figure 11–14**). Because the pump hydrolyzes ATP to pump Na^+ out and K^+ in, it is also known as a **Na^+-K^+ ATPase**. The pump belongs to the family of P-type ATPases and functions very similarly to the Ca^{2+} pump (**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

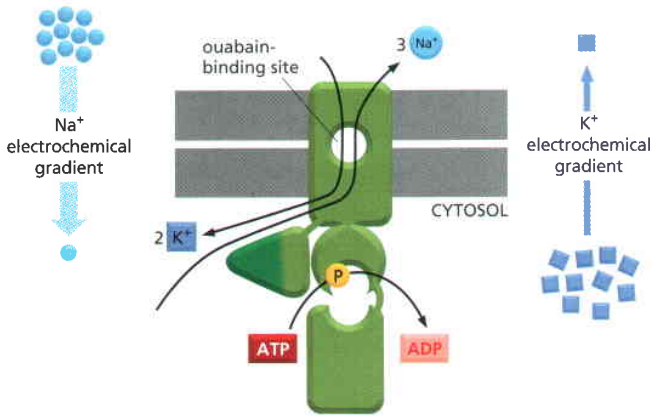


Figure 11–14 The Na^+-K^+ pump. <GAGT> This transporter actively pumps Na^+ out of and K^+ into a cell against their electrochemical gradients. For every molecule of ATP hydrolyzed inside the cell, three Na^+ are pumped out and two K^+ are pumped in. The specific inhibitor ouabain and K^+ compete for the same site on the extracellular side of the pump.

electrically active nerve cells, which, as we shall see, repeatedly gain small amounts of Na^+ and lose small amounts of K^+ during the propagation of nerve impulses.

Like any enzyme, the Na^+-K^+ pump can be driven in reverse, in this case to produce ATP. When the Na^+ and K^+ gradients are experimentally increased to such an extent that the energy stored in their electrochemical gradients is greater than the chemical energy of ATP hydrolysis, these ions move down their electrochemical gradients and ATP is synthesized from ADP and phosphate by the Na^+-K^+ pump. Thus, the phosphorylated form of the pump (step 2 in Figure 11–15) can relax by either donating its phosphate to ADP (step 2 to step 1) or changing its conformation (step 2 to step 3). Whether the overall change in free energy is used to synthesize ATP or to pump Na^+ out of the cell depends on the relative concentrations of ATP, ADP, and phosphate, as well as on the electrochemical gradients for Na^+ and K^+.

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 current across the

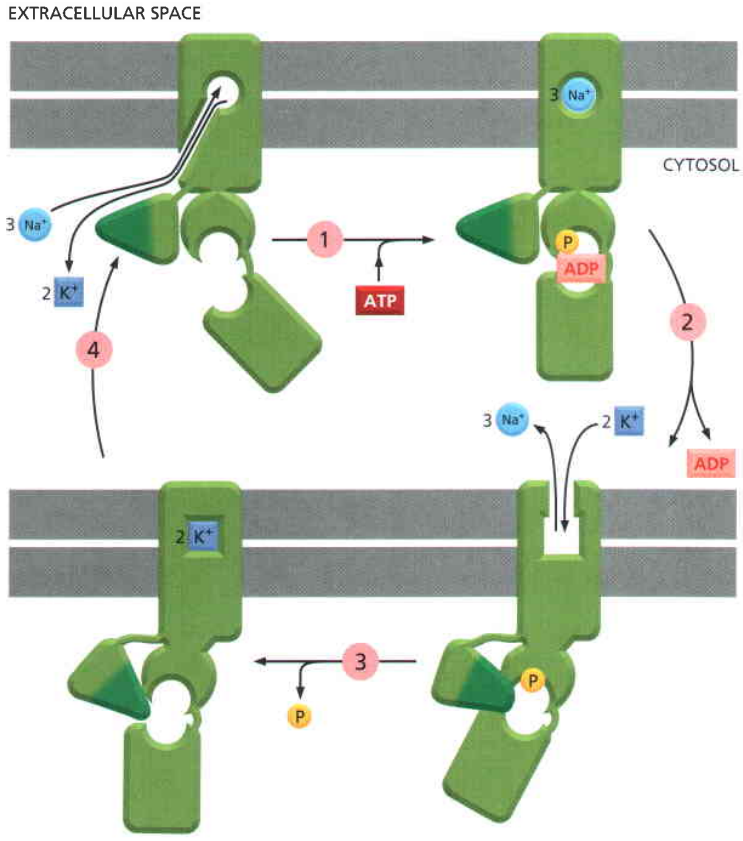


Figure 11–15 A model of the pumping cycle of the Na^+-K^+ pump. (1) The binding of intracellular Na^+ and the subsequent phosphorylation by ATP of the cytoplasmic face of the pump induce a conformational change in the protein that (2) transfers the Na^+ across the membrane and releases it on the outside of the cell. (3) Then, the binding of K^+ on the extracellular surface and the subsequent dephosphorylation of the pump return the protein to its original conformation, which (4) transfers the K^+ across the membrane and releases it into the cytosol. These changes in conformation are analogous to the A ↔ B transitions shown in Figure 11–5, except that here the Na^+-dependent phosphorylation and the K^+-dependent dephosphorylation of the protein cause the conformational transitions to occur in an orderly manner, enabling the protein to do useful work. Although for simplicity the diagram shows only one Na^+- and one K^+-binding site, in the real pump there are three Na^+- and two K^+-binding sites.

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 contributes more than 10% to the membrane potential. The remaining 90%, as we discuss later, depends only indirectly on the Na^+-K^+ pump.

On the other hand, the Na^+-K^+ pump does have a direct role in controlling the solute concentration inside the cell and thereby helps regulate **osmolarity** (or *tonicity*) of the cytosol. All cells contain specialized water channel proteins called *aquaporins* (discussed in detail on p. 673) in their plasma membrane to facilitate water flow across this membrane. Thus, water moves into or out of cells down its concentration gradient, a process called *osmosis*. As explained in **Panel 11–1**, cells 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 tends to create a large osmotic gradient that tends to "pull" water into the cell. Animal cells counteract this effect by an opposite osmotic gradient due to a high concentration of inorganic ions—chiefly Na^+ and Cl^-—in the extracellular fluid. The Na^+-K^+ pump helps maintain osmotic balance by pumping out the Na^+ that leaks in down its steep electrochemical gradient. The Cl^- is kept out by the membrane potential.

In the special case of human red blood cells, which lack a nucleus and other organelles and have a plasma membrane that has an unusually high permeability to water, osmotic water movements can greatly influence cell volume, and the Na^+-K^+ pump plays an important part in maintaining red cell volume. If these cells are placed in a *hypotonic solution* (that is, a solution having a low solute concentration and therefore a high water concentration), there is net movement of water into the cells, causing them to swell and burst (lyse); conversely, if the cells are placed in a *hypertonic solution*, they shrink (**Figure 11–16**). The role of the Na^+-K^+ pump in controlling red cell volume is indicated by the observation that the cells swell, and may eventually burst, if they are treated with *ouabain*, which inhibits the Na^+-K^+ pump. For most animal cells, however, osmosis and the Na^+-K^+ pump have only minor roles 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.

Nonanimal cells cope with their osmotic problems in various ways. Plant cells and many bacteria are prevented from bursting by the semirigid cell wall that surrounds their plasma membrane. In amoebae, the excess water that flows in osmotically is collected in contractile vacuoles, which periodically discharge their contents to the exterior (see Panel 11–1). Bacteria have also evolved strategies that allow them to lose ions, and even macromolecules, quickly when subjected to an osmotic shock.

ABC Transporters Constitute the Largest Family of Membrane Transport Proteins

The last type of carrier protein that we discuss is the family of the **ABC transporters**, so named because each member contains two highly conserved ATPase domains or ATP-binding "cassettes" (**Figure 11–17**). ATP binding leads to dimerization of the two ATP-binding domains, and ATP hydrolysis leads to

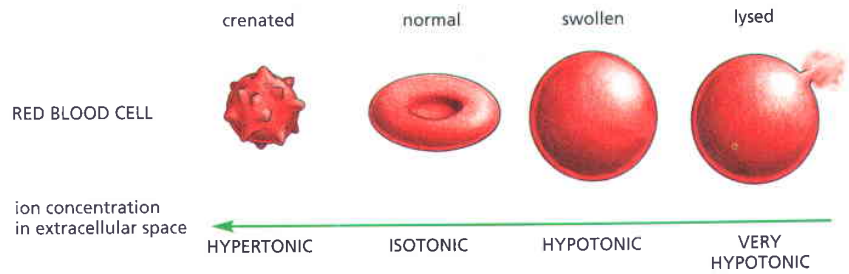
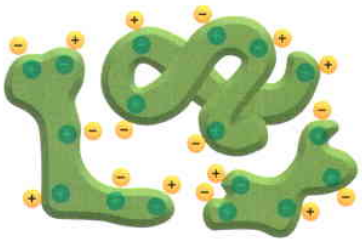
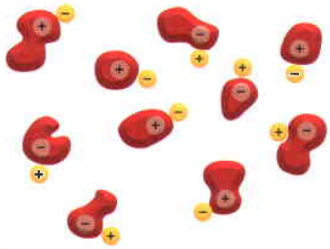


Figure 11–16 Response of a human red blood cell to changes in osmolarity of the extracellular fluid. <GTAC> The cell swells or shrinks as water moves into or out of the cell down its concentration gradient.

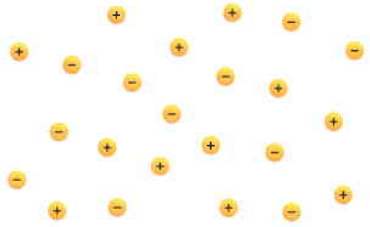
SOURCES OF INTRACELLULAR OSMOLARITY



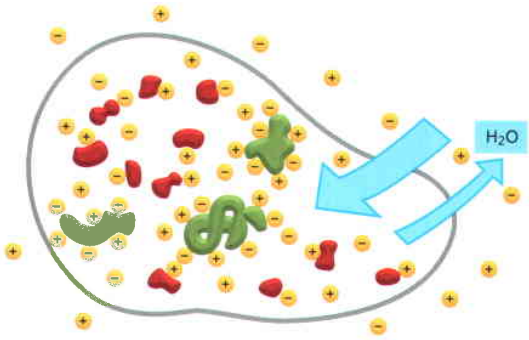
1 Macromolecules themselves contribute very little to the osmolarity of the cell interior since, despite their large size, each one counts only as a single molecule and there are relatively few of them compared to the number of small molecules in the cell.However, most biological macromolecules are highly charged, and they attract many inorganic ions of opposite charge. Because of their large numbers, these counterions make a major contribution to intracellular osmolarity.



2 As the result of active transport and metabolic processes, the cell contains a high concentration of small organic molecules, such as sugars, amino acids, and nucleotides, to which its plasma membrane is impermeable. Because most of these metabolites are charged, they also attract counterions. Both the small metabolites and their counterions make a further major contribution to intracellular osmolarity.



3 The osmolarity of the extracellular fluid is usually due mainly to small inorganic ions. These leak slowly across the plasma membrane into the cell. If they were not pumped out, and if there were no other molecules inside the cell that interacted with them so as to influence their distribution, they would eventually come to equilibrium with equal concentrations inside and outside the cell. However, the presence of charged macromolecules and metabolites in the cell that attract these ions gives rise to the Donnan effect: it causes the total concentration of inorganic ions (and therefore their contribution to the osmolarity) to be greater inside than outside the cell at equilibrium.

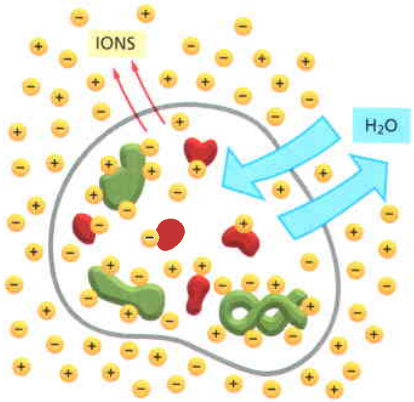


THE PROBLEM

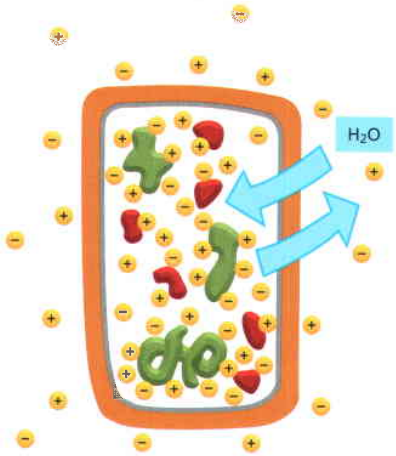
Because of the above factors, a cell that does nothing to control its osmolarity will have a higher concentration of solutes inside than outside. As a result, water will be higher in concentration outside the cell than inside. This difference in water concentration across the plasma membrane will cause water to move continuously into the cell by osmosis.

THE SOLUTION

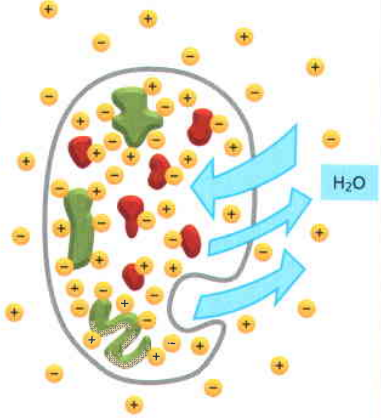
Animal cells and bacteria control their intracellular osmolarity by actively pumping out inorganic ions, such as Na^+, so that their cytoplasm contains a lower total concentration of inorganic ions than the extracellular fluid, thereby compensating for their excess of organic solutes.



Plant cells are prevented from swelling by their rigid walls and so can tolerate an osmotic difference across their plasma membranes: an internal turgor pressure is built up, which at equilibrium forces out as much water as enters.



Many protozoa avoid becoming swollen with water, despite an osmotic difference across the plasma membrane, by periodically extruding water from special contractile vacuoles.



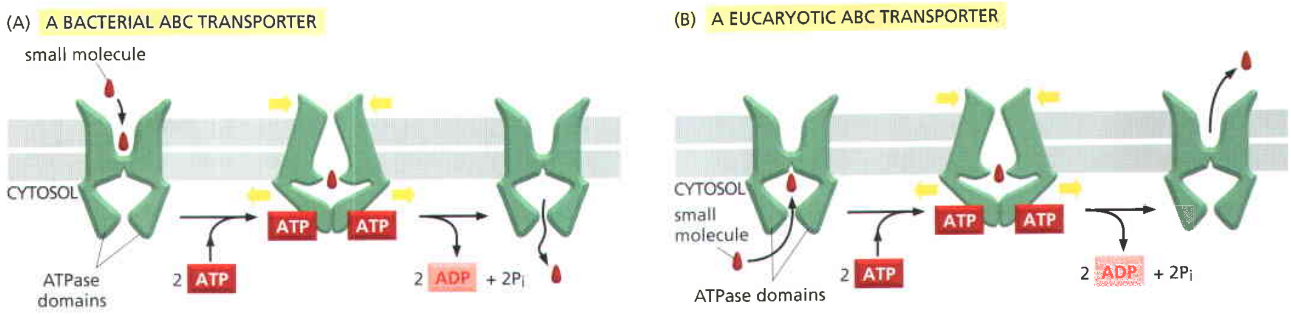


Figure 11–17 Typical ABC transporters in procaryotes (A) and eucaryotes (B). Transporters consist of multiple domains: typically, two hydrophobic domains, each built of six membrane-spanning segments that form the translocation pathway and provide substrate specificity, and two ATPase domains (also called ATP-binding cassettes) protruding 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 (see Figure 10–24). Without ATP bound, the transporter exposes a substrate-binding site to either the extracellular space (in procaryotes) or the intracellular space (in eucaryotes or procaryotes). ATP binding induces a conformational change that exposes the substrate-binding pocket to the opposite face; ATP hydrolysis followed by ADP dissociation returns the transporter to its original conformation. Most individual ABC transporters are unidirectional. Both importing and exporting ABC transporters are found in bacteria, but in eucaryotes almost all ABC transporters export substances from the cytosol—either to the extracellular space or to a membrane-bound intracellular compartment such as the ER or the mitochondria.

their dissociation. These structural changes in the cytosolic domains are thought to be transmitted to the transmembrane segments, driving cycles of conformational changes that alternately expose substrate-binding sites on one side of the membrane and then on the other. In this way, ABC transporters use ATP binding and hydrolysis to transport small molecules across the bilayer.

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 pump a variety of nutrients into the cell. Bacteria also have transport ATPases that use the energy of ATP hydrolysis to import certain small molecules. In bacteria such as *E. coli*, which have double membranes (**Figure 11–18**), the transport ATPases are located in the inner membrane, and an auxiliary mechanism operates to capture the nutrients and deliver them to the transporters (**Figure 11–19**).

In *E. coli*, 78 genes (an amazing 5% of the bacterium's genes) encode ABC transporters, and animal genomes encode more. 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, and even proteins. Whereas bacterial ABC transporters are used for both import and export, those identified in eucaryotes seem mostly specialized for export.

Indeed, the first eucaryotic 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**, the overexpression

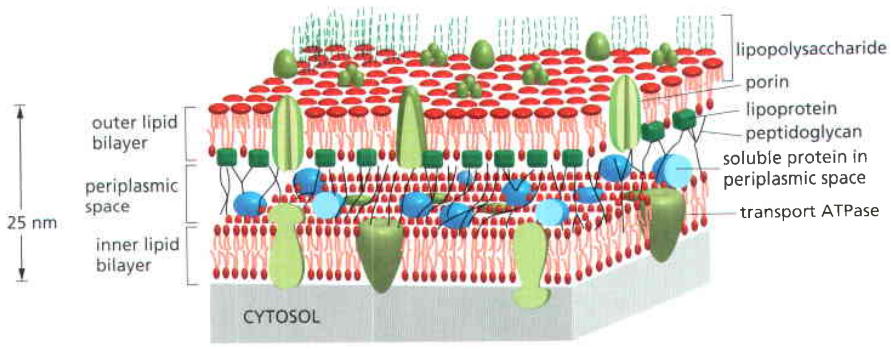


Figure 11–18 A small section of the double membrane of an *E. coli* bacterium. The inner membrane is the cell's plasma membrane. Between the inner and outer lipid bilayer membranes is a highly porous, rigid peptidoglycan layer, composed of protein and polysaccharide, that constitutes the bacterial cell 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 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.

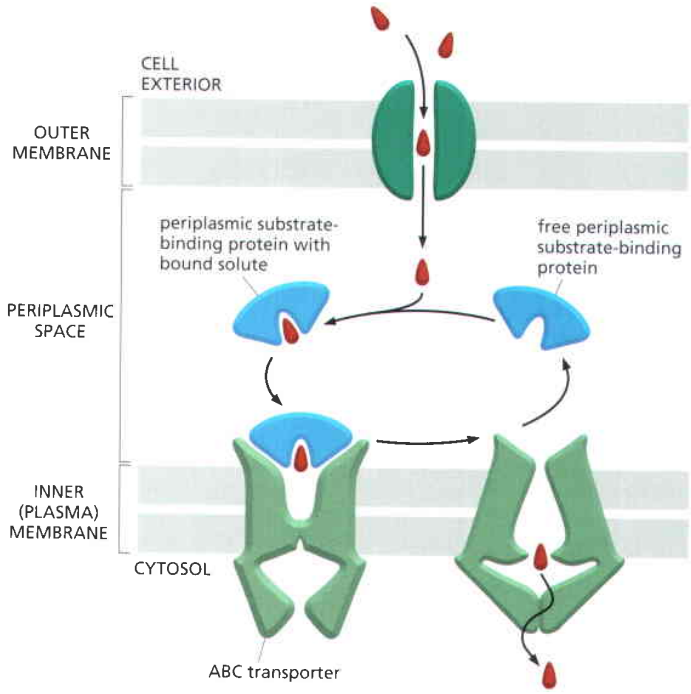


Figure 11–19 The auxiliary transport system associated with transport ATPases in bacteria with double membranes. The solute diffuses through channel-forming proteins (porins) in the outer membrane and binds to a *periplasmic substrate-binding protein,* which undergoes a conformational change that enables it to bind to an ABC transporter in the plasma membrane. The ABC transporter then picks up the solute and actively transfers it across the plasma membrane in a reaction driven by ATP hydrolysis. The peptidoglycan is omitted for simplicity; its porous structure allows the substrate-binding proteins and water-soluble solutes to move through it by simple diffusion.

of which in human cancer cells can make 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 more of the MDR transporter. These cells can pump drugs out of the cell very efficiently and are therefore relatively resistant to the toxic effects of the anticancer drugs. Selection for cancer cells with resistance to one drug can thereby lead to resistance to a wide variety of anti-cancer 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 more than a million people every year. The development of resistance to the antimalarial drug *chloroquine* hampers the control of malaria. The resistant *P. falciparum* have amplified a gene encoding an ABC transporter that pumps out the chloroquine.

In most vertebrate cells, an ABC transporter in the endoplasmic reticulum (ER) membrane actively transports 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 will kill the cell if the peptides are derived from a virus or other microorganisms lurking in the cytosol of an infected cell (discussed in Chapter 25).

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, which functions as a Cl^- channel 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 do not drive the transport process. Instead, they control the opening and closing of the Cl^- channel, which provides a passive conduit for Cl^- to move down its electrochemical gradient. Thus, ABC proteins can apparently function as either transporters or channels.

Summary

Transporters bind specific solutes and transfer them across the lipid bilayer by undergoing conformational changes that expose the solute-binding site sequentially on one side of the membrane and then on the other. Some transporters simply 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 contains proteins of similar amino acid sequences that are thought to have evolved from a common ancestral protein and to 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, as well as for drug resistance in both cancer cells and malaria-causing parasites.

ION CHANNELS AND THE ELECTRICAL PROPERTIES OF MEMBRANES

Unlike carrier proteins, channel proteins form hydrophilic pores across membranes. One class of channel proteins found in virtually all animals forms *gap junctions* between two 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 channel-forming proteins of the outer membranes of bacteria, mitochondria, and chloroplasts (discussed in Chapter 10), have relatively large and permissive pores, which 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 channel proteins 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 carriers in that up to 100 million ions can pass through one open channel each second—a rate 10^5 times greater than the fastest rate of transport mediated by any known carrier protein. However, channels cannot be coupled to an energy source to perform active transport, so the transport that 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. As we shall see, 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 shall consider how they use many different ion channels to receive, conduct, and transmit signals.

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

Two important properties distinguish ion channels from simple 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,

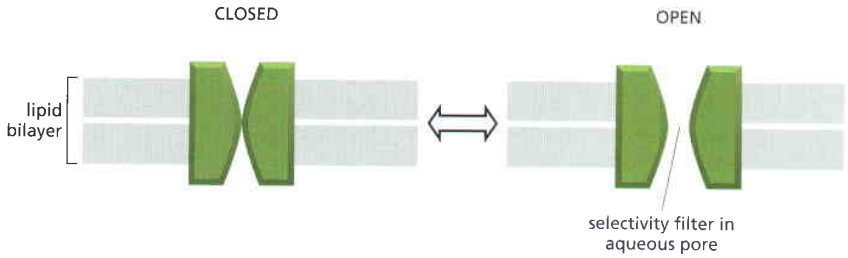


Figure 11–20 A typical ion channel, which fluctuates between closed and open conformations. The channel protein shown here in cross section forms a hydrophilic pore across the lipid bilayer only in the "open" conformational state. Polar groups are thought to line the wall of the pore, while hydrophobic amino acid side chains interact with the lipid bilayer (not shown). The pore narrows to atomic dimensions in one region (the selectivity filter), where the ion selectivity of the channel is largely determined.

through the narrowest part of the channel, which is called the *selectivity filter;* this limits their rate of passage (**Figure 11–20**). 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.

The second important distinction between ion channels and simple aqueous pores is that ion channels are not continuously open. Instead, they are *gated,* which allows them to open briefly and then close again (**Figure 11–21**). Moreover, with prolonged (chemical or electrical) stimulation, most 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. 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 described 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. 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 might typically contain 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, and allow the single-celled *Paramecium* to reverse direction after a collision.

Perhaps the most common ion channels are those that are permeable mainly to K^+. These channels are found in the plasma membrane of almost all

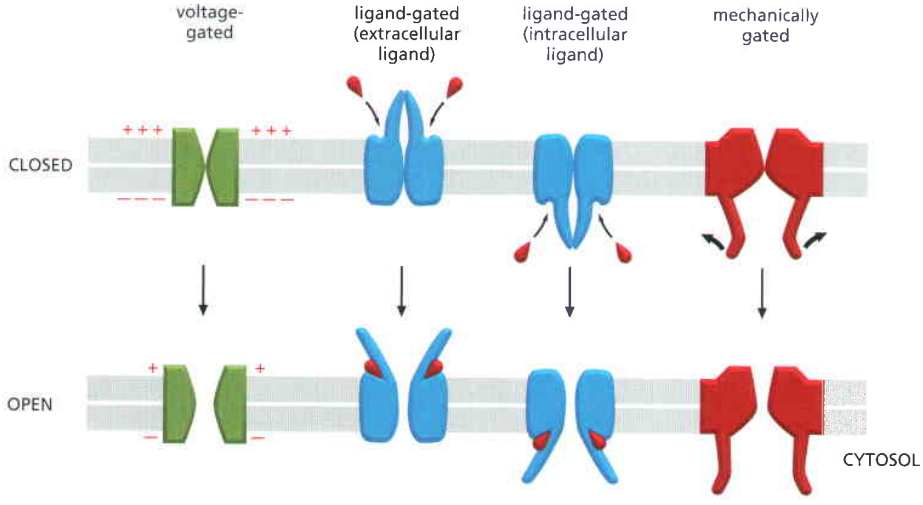


Figure 11–21 The gating of ion channels. This drawing shows different kinds of stimuli that open ion channels. Mechanically gated channels often have cytoplasmic extensions that link the channel to the cytoskeleton (not shown).

animal cells. An important subset of K^+ channels opens even in an unstimulated or "resting" cell, and hence these channels are sometimes 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.

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. 662) 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, the Na^+-K^+ pump helps maintain an osmotic balance across the animal cell membrane by keeping the intracellular concentration of Na^+ low. Because there is little Na^+ inside the cell, other cations have to be plentiful there 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 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 11–2**, 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

Only a minute number of ions must move across the plasma membrane 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

THE NERNST EQUATION AND ION FLOW

The flow of any ion through a membrane channel protein 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 (2 cal mol^{-1} K^{-1})
- T = the absolute temperature (K)
- F = Faraday's constant (2.3×10^4 cal 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. 830.)

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 equlibrium 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 ion,

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

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^+.

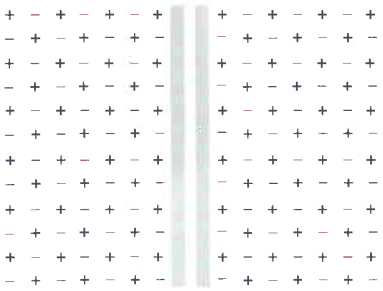
For any particular membrane potential, V_M, the net force tending to drive a particular type of ion out of the cell, is proportional to the difference between V_M and the equilibrium potential for the ion: hence,

for K^+ it is $V_M - V_K$

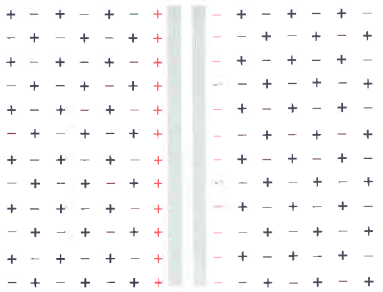
and for Na^+ it is $V_M - V_{Na}$.

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 11–22 The ionic basis of a membrane potential. A small flow of ions 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.

and result in only a very slight discrepancy in the number of positive and negative ions on the two sides of the membrane (**Figure 11–22**). 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 small direct contribution to the membrane potential by pumping out three Na^+ for every two K^+ that it pumps in. However, switching off the pump does not abolish the major component of the resting potential, which is generated by the K^+ equilibrium mechanism described above. 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 osmotic balance is upset, and water seeps into the cell (see Panel 11–1, p. 664), and 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 better 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.

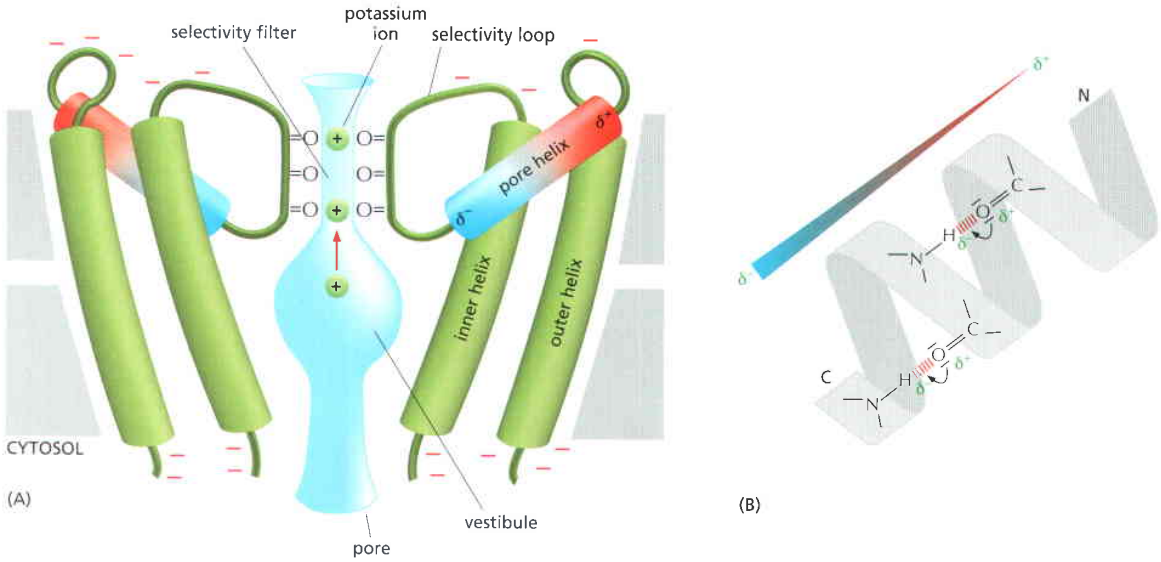


Figure 11–23 The structure of a bacterial K^+ channel. <ATTA> (A) Two transmembrane α helices from only two of the four identical subunits are shown. From the cytosolic side, the pore opens up into a vestibule in the middle of the membrane. The vestibule facilitates transport by allowing the K^+ ions to remain hydrated even though they are halfway across the membrane. The narrow selectivity filter 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. The positions of the K^+ ions in the pore were determined by soaking crystals of the channel protein in a solution containing rubidium ions, which are more electron-dense but only slightly larger than K^+ ions; from the differences in the diffraction patterns obtained with K^+ ions and with rubidium ions in the channel, the positions of the ions could be calculated. Two K^+ ions occupy 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 pore helices (only two of which are shown) point precisely toward the center of the vestibule, thereby guiding K^+ ions into the selectivity filter. Negatively charged amino acids (indicated by *red* minus signs) are concentrated near the channel entrance and exit. (B) Because of the polarity of the hydrogen bonds (*red*) that link adjacent turns of an α helix, every α helix has an electric dipole along its axis, 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. With permission from AAAS.)

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 (**Figure 11–23**). Negatively charged amino acids concentrated at the cytosolic entrance to the pore are thought to attract cations and repel anions, making the channel cation-selective. 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 the cell where K^+ ions exit from the channel. 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 crystal structure shows two K^+ ions in single file within the selectivity filter, separated by about 0.8 nm. Mutual repulsion between the two ions is thought to help move them through the pore into the extracellular fluid.

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 11–24**).

Structural studies of K^+ channels and other channels have also indicated some general principles of how channels may open and close. This gating seems

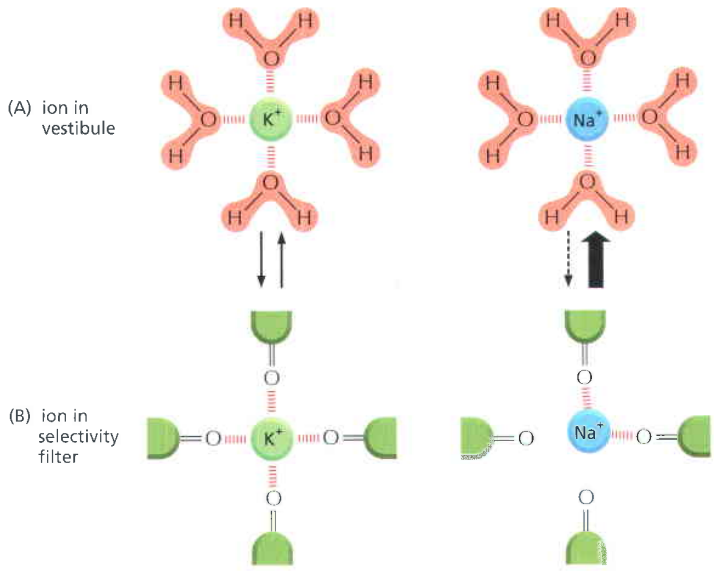


Figure 11–24 K^+ specificity of the selectivity filter in a K^+ channel. The drawing shows K^+ and Na^+ ions (A) in the vestibule and (B) in the selectivity filter of the pore, viewed in cross section. In the vestibule, the ions are hydrated. In the selectivity filter, they have lost their water, and the carbonyl oxygens are placed precisely 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 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. (Adapted from D.A. Doyle et al., *Science* 280:69–77, 1998. With permission from AAAS.)

to involve movement of the helices in the membrane so that they either obstruct (in the closed state) or free (in the open state) the path for ion movement. Depending on the particular type of channel, helices are thought to 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 11–25**). Bulky hydrophobic amino acid side chains block the small opening that remains, preventing the entry of ions.

Most ion channels are constructed from multiple identical subunits, each of which contributes to a common central pore. A recently determined crystal structure of a Cl^- channel, however, has revealed that some ion channels are built very differently. Although the protein is a dimer formed by two identical subunits, each of the subunits contains its own pore through which Cl^- ions move. In the center of the membrane, amino acid side chains form a selectivity filter, which is conceptually similar to that in K^+ channels. But, unlike the filter in K^+ channels, different regions of the protein contribute the side chains, and they are not symmetrically arranged (**Figure 11–26**).

Aquaporins Are Permeable to Water But Impermeable to Ions

We discussed earlier that procaryotic and eucaryotic cells have **water channels**, or **aquaporins**, embedded in their plasma membrane to allow water to move readily across this membrane. Aquaporins are especially abundant in cells that must transport water at particularly high rates, such as the epithelial cells of the kidney.

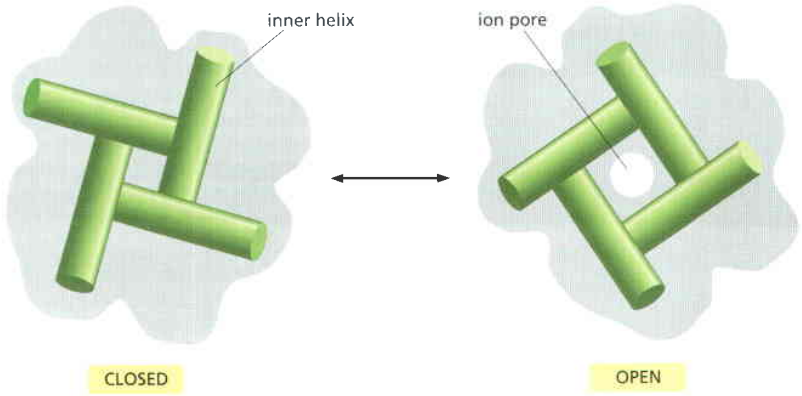


Figure 11–25 A model for the gating of a bacterial K^+ channel. The channel is viewed in cross section. To adopt the closed conformation, the four inner transmembrane helices that line the pore on the cytosolic side of the selectivity filter (see Figure 11–23) rearrange to close the cytosolic entrance to the channel. (Adapted from E. Perozo et al., *Science* 285:73–78, 1999. With permission from AAAS.)

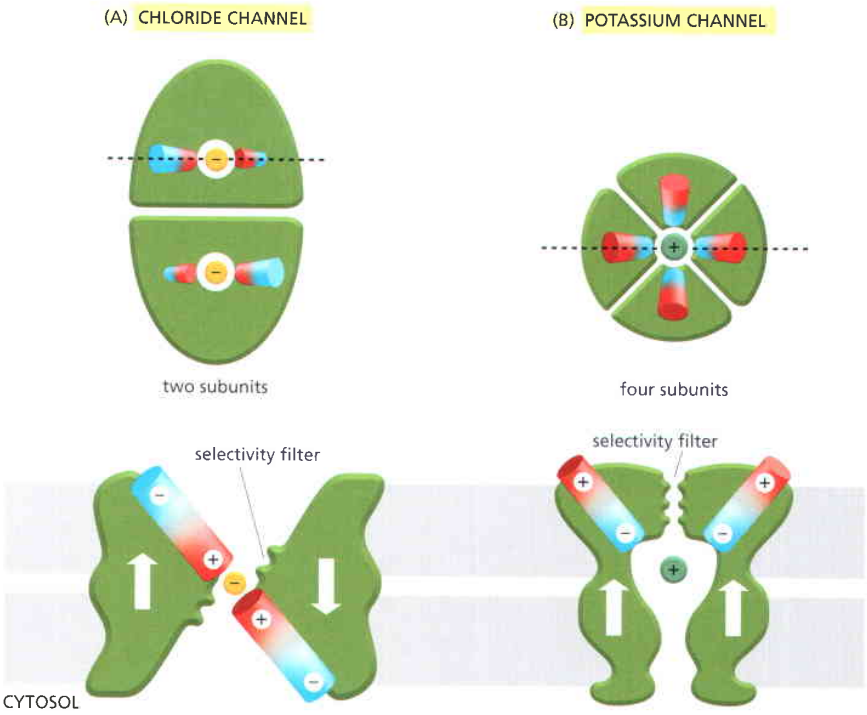


Figure 11–26 Comparison of Cl^- and K^+ channel architectures. (A) The Cl^- channel is a "double-channel" dimer built of two identical subunits, each of which contains its own ion-conducting pore. The upper cartoon is a schematic view of the extracellular face of the channel, showing the two identical ion-conducting pores. The lower cartoon shows a cross section through one subunit, viewed from within the membrane. (*Dotted black line* in top cartoon indicates the plane of section.) The subunit is a single polypeptide chain consisting of two portions, which, though similar, span the membrane with opposite orientations *(white arrows)*: each portion contributes one pore helix oriented such that its positively charged end points towards a centrally positioned selectivity filter. Both elements, the selectivity filter and the helix dipoles, contribute to the selectivity of the channel for negatively charged Cl^- ions. (B) By contrast, the K^+ channel is a tetramer built of four identical subunits, each of which contributes to a centrally located pore. All four subunits have the same orientation in the membrane *(white arrows)*. Four pore helices, one contributed by each subunit, point their negatively charged ends towards a vestibule, stabilizing a positively charged K^+ ion there (also see Figure 11–23). (Lower cartoons in A and B, adapted from R. Dutzler et al., *Nature* 415:287–294, 2002. With permission from Macmillan Publishers Ltd.)

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 crystal 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 11–27**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^+, Na^+, Ca^{2+}, or Cl^- ions. To understand why these channels are also impermeable to H^+, recall that most protons are present in cells as H_3O^+,

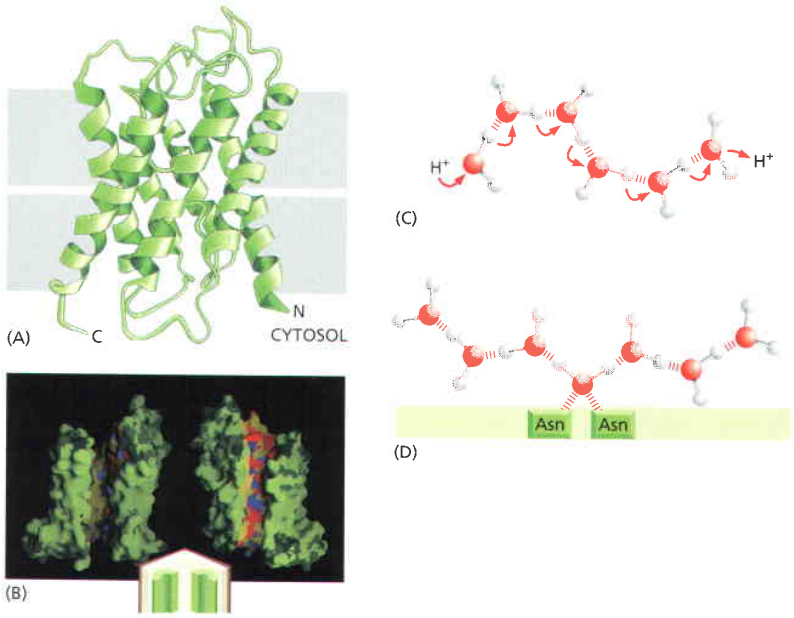


Figure 11–27 The structure of aquaporins. (A) A ribbon diagram of an aquaporin monomer. In the membrane, aquaporins form tetramers, with each monomer containing a pore in its center (not shown). (B) A space-filling model of an aquaporin monomer, which has been cut and opened like a book, so that the inside of the pore is visible. Hydrophilic amino acids lining the pores are colored *red* and *blue,* whereas hydrophobic amino acids lining the pore are colored *yellow.* The amino acids not involved in forming the pore are shown in *green.* Note that 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 membrane. By contrast, the other side of the pore is devoid of such amino acids, providing a *hydrophobic slide* that does not allow hydrogen bonds to form. (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) Two strategically placed asparagines in the center of each aquaporin pore help tether a central water molecule such that both valencies on its oxygen are occupied, thereby preventing an H^+ relay. (A and B, adapted from R.M. Stroud et al., *Curr. Opin. Struct. Biol.* 13:424–431, 2003. With

which diffuses through water extremely rapidly, using a molecular relay mechanism that requires the making and breaking of hydrogen bonds between adjacent water molecules (Figure 11–27C). 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. Because both valences of this oxygen are unavailable for hydrogen bonding, the central water molecule cannot participate in an H^+ relay, and the pore is therefore impermeable to protons (Figure 11–27C and D).

Some bacterial water channels similar to aquaporins also conduct glycerol and small sugars, which interact with similarly positioned carbonyl oxygens lining the pore. Such transient contacts that solutes make with the pore walls ensure that the transport is highly specific, without significantly impeding the speed with which the solute passes. Each individual aquaporin channel passes about 10^9 water molecules per second.

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 Function of a Neuron Depends on Its Elongated Structure

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. A single neuron in a human, extending, for example, 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 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 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 cell 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

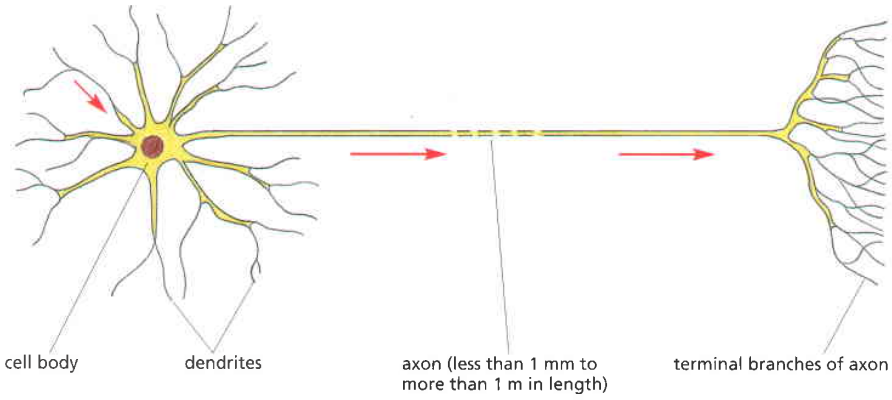


Figure 11–28 A typical vertebrate neuron. The arrows indicate the direction in which signals are conveyed. The single axon conducts signals away from the cell body, while the multiple dendrites (and the cell body) receive signals from the axons of other neurons. The nerve terminals end on the dendrites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

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 Chapter 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 to almost as far as the Na^+ equilibrium potential of about +50 mV (see Panel 11–2, p. 670). 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 that act in concert to save the cell from such a permanent electrical spasm: the Na^+ channels inactivate and voltage-gated K^+ channels open.

The Na^+ channels have an automatic inactivating mechanism, which causes the channels to reclose rapidly even though the membrane is still depolarized. The Na^+ channels remain in this *inactivated* state, unable to reopen, until after the membrane potential has returned to its initial negative value. The whole 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. **Figure 11–29** shows how the changes in state contribute to the rise and fall of the action potential.

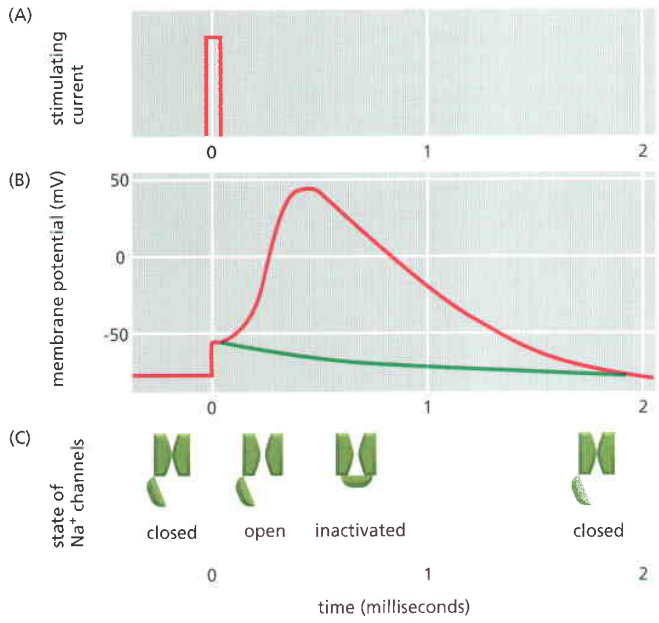


Figure 11–29 An action potential. <CGAG> (A) An action potential is triggered by a brief pulse of current, which (B) partially depolarizes the membrane, as shown in the plot of membrane potential versus time. The *green curve* shows how the membrane potential would have simply relaxed back to the resting value after the initial depolarizing stimulus if there had been no voltage-gated Na^+ channels in the membrane; this relatively slow return of the membrane potential to its initial value of –70 mV in the absence of open Na^+ channels occurs because of the efflux of K^+ through voltage-gated K^+ channels, which open in response to membrane depolarization and drive the membrane back toward the K^+ equilibrium potential. The *red curve* shows the course of the action potential that is caused by the opening and subsequent inactivation of voltage-gated Na^+ channels, whose state is shown in (C). The membrane cannot fire a second action potential until the Na^+ channels have returned to the closed conformation; until then, the membrane is refractory to stimulation.

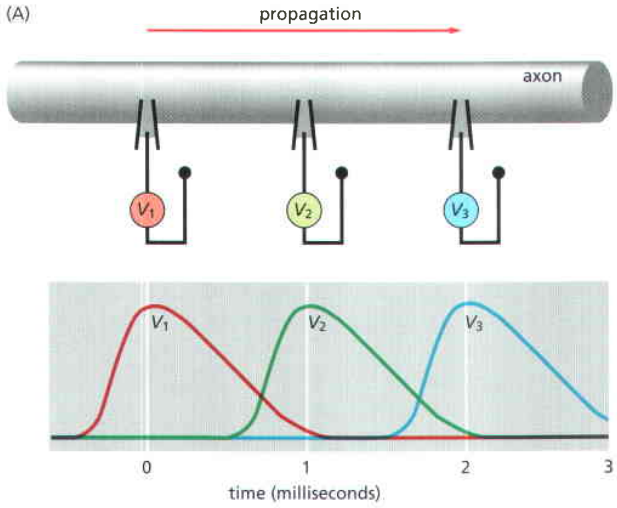
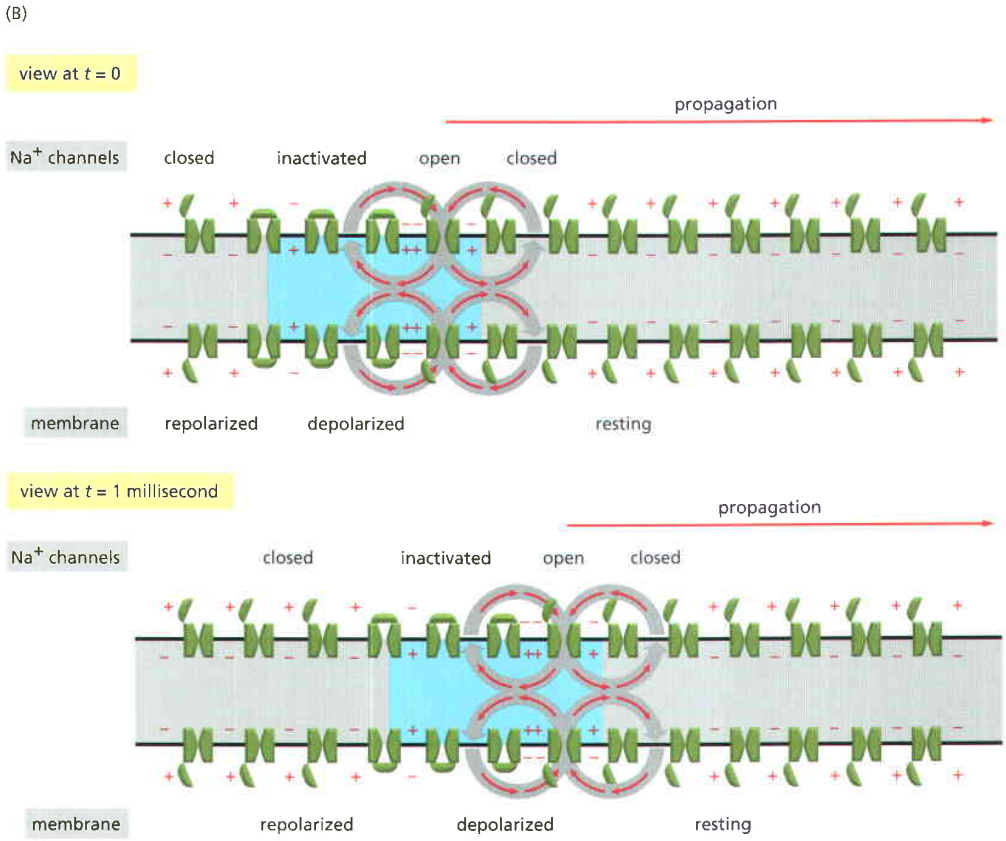


Figure 11–30 The propagation of an action potential along an axon. (A) The voltages that would be recorded from a set of intracellular electrodes placed at intervals along the axon. (B) The changes in the Na^+ channels and the current flows *(orange arrows)* that give rise to the traveling disturbance of the membrane potential. The region of the axon with a depolarized membrane is shaded in *blue.* Note that an action potential can only travel away from the site of depolarization, because Na^+-channel inactivation prevents the depolarization from spreading backward.



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 11–30.

Voltage-gated K^+ channels provide a second mechanism in most nerve cells to help bring the activated plasma membrane more rapidly back toward its original negative potential, ready to transmit a second impulse. These channels open in response to membrane depolarization in much the same way that the Na^+ channels do, but with slightly slower kinetics; for this reason they are some-

times called *delayed K^+ channels*. Once the K^+ channels open, the efflux of K^+ rapidly overwhelms the transient influx of Na^+ and quickly drives the membrane back toward the K^+ equilibrium potential, even before the inactivation of the Na^+ channels is complete.

Like the Na^+ channel, the voltage-gated K^+ channels automatically inactivate. Studies of mutant voltage-gated K^+ channels show that the N-terminal 20 amino acids of the channel protein are required for rapid inactivation: altering this region changes the kinetics of channel inactivation, and removal of the region abolishes inactivation. Amazingly, in the latter case, exposing the cytoplasmic face of the plasma membrane to a small synthetic peptide corresponding to the missing N-terminus restores inactivation. These findings suggest that the N-terminus of each K^+ channel subunit acts like a tethered ball that occludes the cytoplasmic end of the pore soon after it opens, thereby inactivating the channel (**Figure 11–31**). A similar mechanism is thought to operate in the rapid inactivation of voltage-gated Na^+ channels (which we discuss later), although a different segment of the protein seems to be involved.

The electrochemical mechanism of the action potential was first established by a famous series of experiments carried out in the 1940s and 1950s. Because the techniques for studying electrical events in small cells had not yet been developed, the experiments exploited the giant neurons in the squid. Despite the many technical advances made since then, the logic of the original analysis continues to serve as a model for present-day work. **Panel 11–3** summarizes some of the key original experiments.

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, the propagation of nerve impulses is greatly slowed, often with devastating neurological consequences.

Myelin is formed by specialized supporting cells, called **glial cells**. **Schwann cells** myelinate axons in peripheral nerves, and **oligodendrocytes** do so in the central nervous system. These glial cells wrap layer upon layer of their own plasma membrane in a tight spiral around the axon (**Figure 11–32**A and B), thereby insulating the axonal membrane so that little current can leak across it.

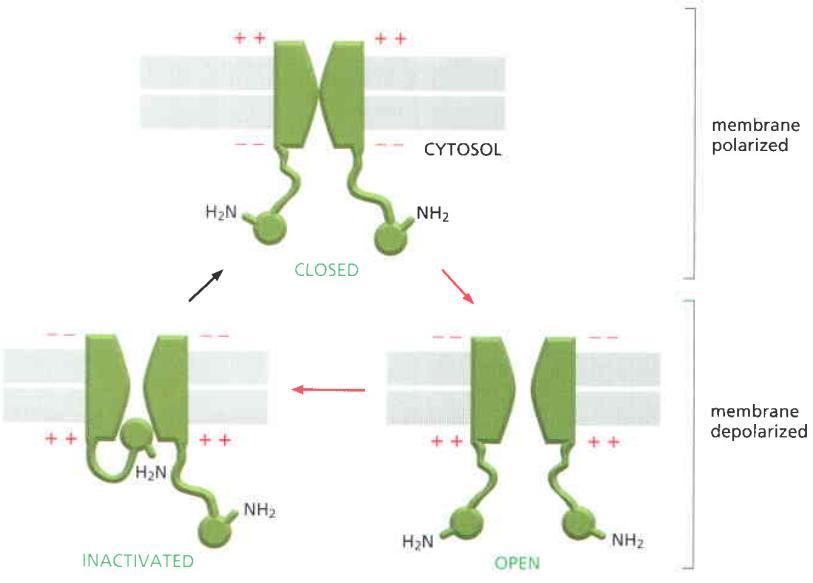
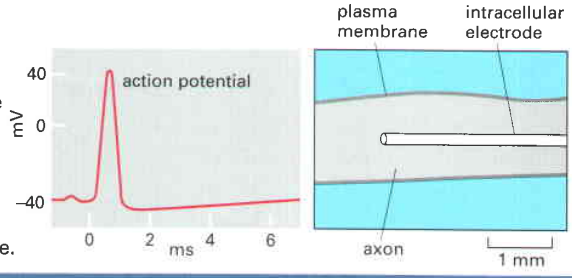


Figure 11–31 The "ball-and-chain" model of rapid inactivation of a voltage-gated K^+ channel. When the membrane is depolarized, the channel opens and begins to conduct ions. If the depolarization is maintained, the open channel adopts an inactive conformation, in which the pore is occluded by the N-terminal 20 amino acid "ball," which is linked to the channel proper by a segment of unfolded polypeptide chain that serves as the "chain." For simplicity, only two balls are shown; in fact, there are four, one from each subunit. A similar mechanism, using a different segment of the polypeptide chain, is thought to operate in Na^+ channel inactivation. Internal forces stabilize each state against small disturbances, but a sufficiently violent collision with other molecules can cause the channel to flip from one of these states to another. The state of lowest energy depends on the membrane potential because the different conformations have different charge distributions. When the membrane is at rest (highly polarized), the closed conformation 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. The *red arrows* indicate the sequence that follows a sudden depolarization; the *black arrow* indicates the return to the original conformation as the lowest energy state after the membrane is repolarized.

1. Action potentials are recorded with an intracellular electrode

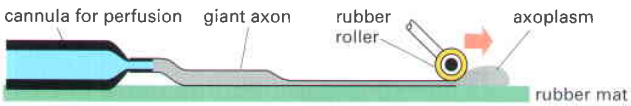
The squid giant axon is about 0.5–1 mm in diameter and several centimeters long. An electrode in the form of a glass capillary tube containing a conducting solution can be thrust down the axis of the axon so that its tip lies deep in the cytoplasm. With its help, one can measure the voltage difference between the inside and the outside of the axon—that is, the membrane potential—as an action potential sweeps past the electrode. The action potential is triggered by a brief electrical stimulus to one end of the axon. It does not matter which end, because the excitation can travel in either direction; and it does not matter how big the stimulus is, as long as it exceeds a certain threshold: the action potential is all or none.

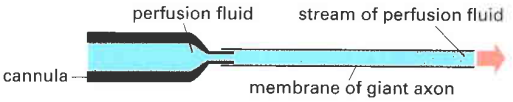


2. Action potentials depend only on the neuronal plasma membrane and on gradients of Na^+ and K^+ across it

The three most plentiful ions, both inside and outside the axon, are Na^+, K^+, and Cl^-. As in other cells, the Na^+-K^+ pump maintains a concentration gradient: the concentration of Na^+ is about 9 times lower inside the axon than outside, while the concentration of K^+ is about 20 times higher inside than outside. Which ions are important for the action potential?

The squid giant axon is so large and robust that it is possible to extrude the gel-like cytoplasm from it, like toothpaste from a tube, and then to perfuse it internally with pure artificial solutions of Na^+, K^+, and Cl^- or SO_4^{2-}. Remarkably, if (and only if) the concentrations of Na^+ and K^+ inside and outside approximate those found naturally, the axon will still propagate action potentials of the normal form. The important part of the cell for electrical signaling, therefore, must be the plasma membrane; the important ions are Na^+ and K^+; and a sufficient source of free energy to power the action potential must be provided by the concentration gradients of these ions across the membrane, because all other sources of metabolic energy have presumably been removed by the perfusion.

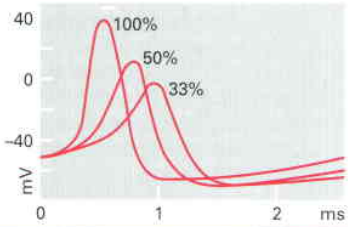




3. At rest, the membrane is chiefly permeable to K^+; during the action potential, it becomes transiently permeable to Na^+

At rest the membrane potential is close to the equilibrium potential for K^+. When the external concentration of K^+ is changed, the resting potential changes roughly in accordance with the Nernst equation for K^+ (see Panel 11–2). At rest, therefore, the membrane is chiefly permeable to K^+: K^+ leak channels provide the main ion pathway through the membrane.

If the external concentration of Na^+ is varied, there is no effect on the resting potential. However, the height of the peak of the action potential varies roughly in accordance with the Nernst equation for Na^+. During the action potential, therefore, the membrane appears to be chiefly permeable to Na^+: Na^+ channels have opened. In the aftermath of the action potential, the membrane potential reverts to a negative value that depends on the external concentration of K^+ and is even closer to the K^+ equilibrium potential than the resting potential is: the membrane has lost most of its permeability to Na^+ and has become even more permeable to K^+ than before—that is, Na^+ channels have closed, and additional K^+ channels have opened.

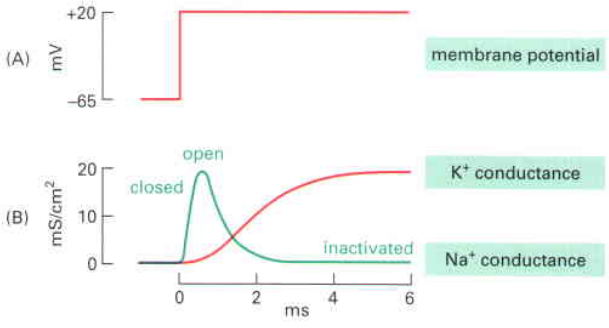


The form of the action potential when the external medium contains 100%, 50%, or 33% of the normal concentration of Na^+.

4. Voltage clamping reveals how the membrane potential controls opening and closing of ion channels

The membrane potential can be held constant ("voltage clamped") throughout the axon by passing a suitable current through a bare metal wire inserted along the axis of the axon while monitoring the membrane potential with another intracellular electrode. When the membrane is abruptly shifted from the resting potential and held in a depolarized state (A), Na^+ channels rapidly open until the Na^+ permeability of the membrane is much greater than the K^+ permeability; they then close again spontaneously, even though the membrane potential is clamped and unchanging. K^+ channels also open but with a delay, so that the K^+ permeability increases as the Na^+ permeability falls (B). If the experiment is now very promptly repeated, by returning the membrane briefly to the resting potential and then quickly depolarizing it again, the response is different: prolonged depolarization has caused the Na^+ channels to enter an inactivated state, so that the second depolarization fails to cause a rise and fall similar to the first. Recovery from this state requires a relatively long time—about 10 milliseconds—spent at the repolarized (resting) membrane potential.

In a normal unclamped axon, an inrush of Na^+ through the opened Na^+ channels produces the spike of the action potential; inactivation of Na^+ channels and opening of K^+ channels bring the membrane rapidly back down to the resting potential.



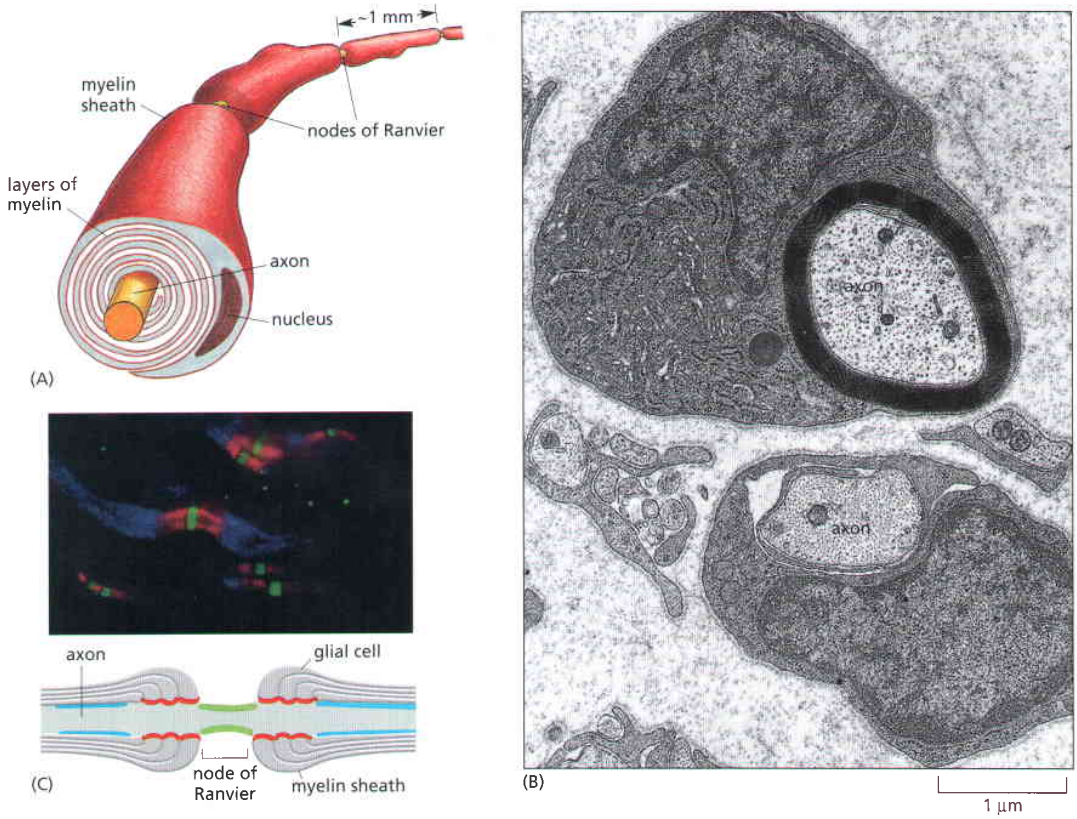


Figure 11–32 Myelination. (A) A myelinated axon from a peripheral nerve. Each Schwann cell wraps its plasma membrane concentrically around the axon to form a segment of myelin sheath about 1 mm long. For clarity, the membrane layers of the myelin in this drawing are shown less compacted than they are in reality (see part B). (B) An electron micrograph of a section from 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 nerve. Three different proteins are detected by staining with antibodies. Voltage-gated Na^+ channels (stained in *green*) are concentrated in the axonal membrane at the nodes of Ranvier. An extracellular protein (called *Caspr*, stained in *red*) marks the end of each myelin sheath. Caspr assembles at the junctions where the glial cell plasma membrane tightly abuts the axon to provide the electrical seal. Voltage-gated K^+ channels (stained in *blue*) localize to regions in the axon plasma membrane that are close to the nodes. (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.)

The myelin sheath is interrupted at regularly spaced *nodes of Ranvier,* where almost all the Na^+ channels in the axon are concentrated (see Figure 11–32C). Because the ensheathed portions of the axonal membrane have excellent cable properties (in other words, they behave electrically much like well-designed underwater telegraph cables), a depolarization of the membrane at one node almost immediately spreads passively to the next node. Thus, an action potential propagates 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 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 Gated 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

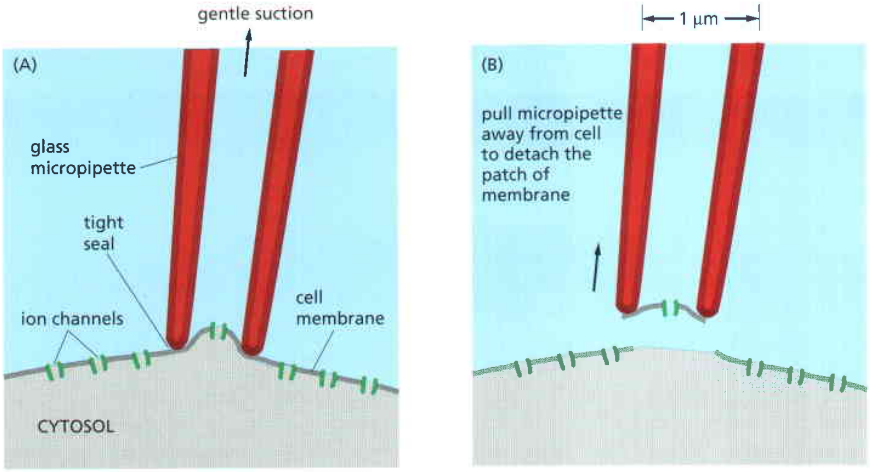


Figure 11–33 The technique of patch-clamp recording. Because of the extremely tight seal between the micropipette and the membrane, current can enter or leave the micropipette only by passing through the channels in the *patch* of membrane covering its tip. The term *clamp* is used because an electronic 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.

record this aggregate current, as shown in Figure 11–30. Remarkably, however, it is also possible to record current flowing through individual channels. **Patch-clamp recording**, developed in the 1970s and 80s has revolutionized the study of ion channels, making it possible to examine transport through a single molecule of channel protein in a small patch of membrane covering the mouth of a micropipette (**Figure 11–33**). With this simple but powerful technique, one can study the detailed properties of ion channels in all sorts of cell types. This work has led to the discovery that even cells that are not electrically excitable usually have a variety of gated 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 voltage-gated Na^+ channels open in an all-or-nothing fashion. A 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. 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 (**Figure 11–34**).

Some simple physical principles allow us to understand voltage-gating. The interior of the resting neuron or muscle cell is at an electrical potential about 50–100 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. Proteins in the

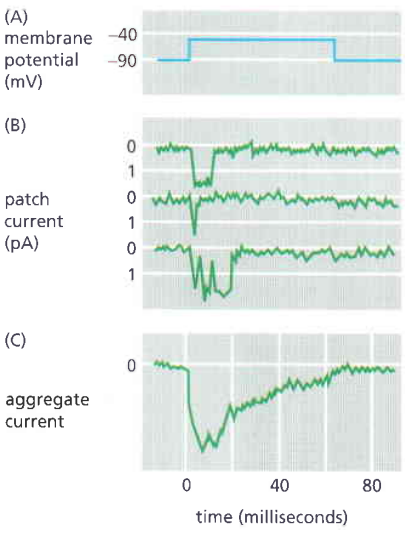


Figure 11–34 Patch-clamp measurements for a single voltage-gated Na^+ channel. A tiny patch of plasma membrane was detached from an embryonic rat muscle cell, as in Figure 11–33. (A) The membrane was depolarized by an abrupt shift of potential. (B) Three current records from three experiments performed 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 is practically constant. The minor fluctuations in the current records arise largely from electrical noise in the recording apparatus. Current is measured in picoamperes (pA). 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.)

membrane are thus subjected to a very large electrical field that can profoundly affect their conformation. These proteins, like all others, have many charged groups, as well as polarized bonds between their various atoms. The electrical field therefore exerts forces on the molecular structure. For many membrane proteins the effects of changes in the membrane electrical field are probably insignificant, but voltage-gated ion channels can adopt alternative conformations whose stabilities depend on the strength of the field. Voltage-gated Na^+, K^+, and Ca^{2+} channels, for example, have characteristic positively charged amino acids in one of their transmembrane segments that respond to depolarization by moving outward, triggering conformational changes that open the channel. 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 legend to Figure 11–31).

Voltage-Gated Cation Channels Are Evolutionarily and Structurally Related

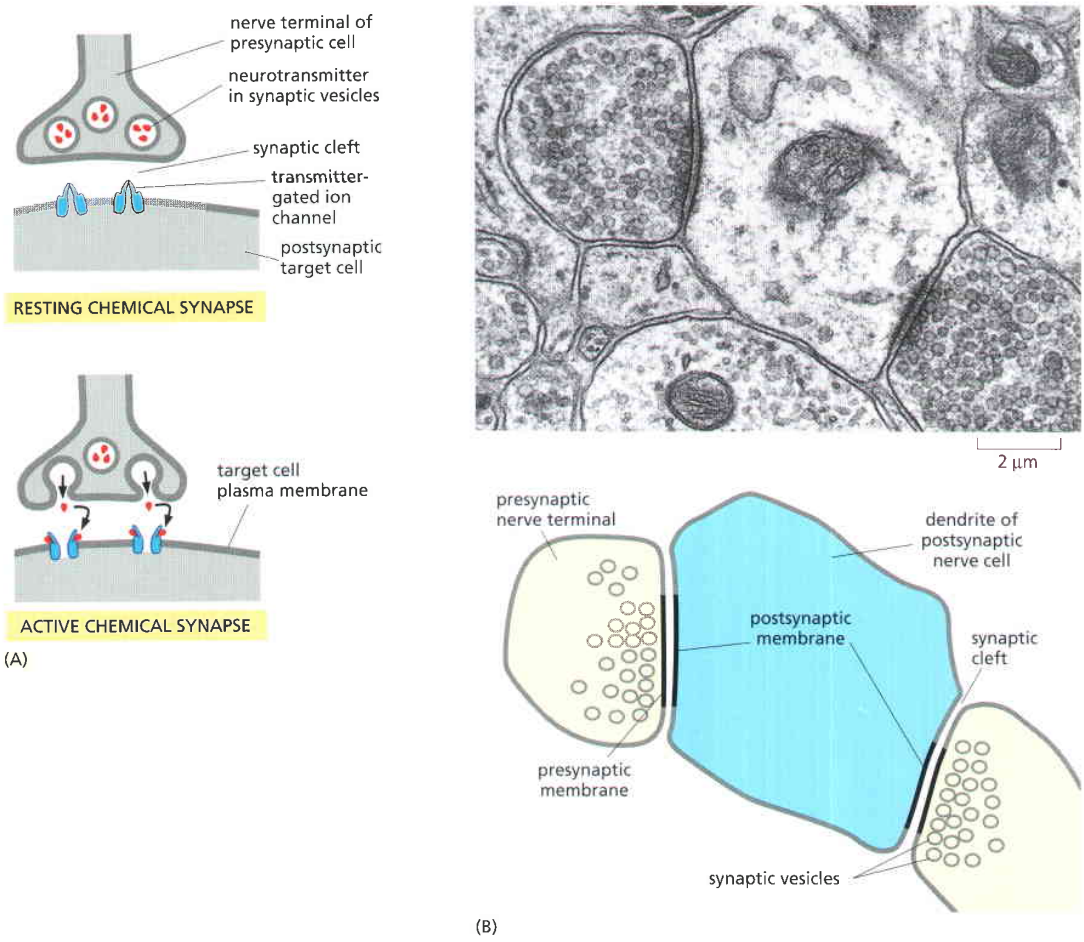
Na^+ channels are not the only kind of voltage-gated cation channel that can generate an action potential. The action potentials in some muscle, egg, and endocrine cells, for example, depend on *voltage-gated Ca^{2+} channels* rather than on Na^+ channels.

There is a surprising amount of structural and functional diversity within each of these three classes, 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 channel proteins 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 cause epileptic seizures (convulsions, or fits).

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 electrically isolated from one another, the *presynaptic cell* being separated from the *postsynaptic cell* by a narrow *synaptic cleft.* A change of electrical potential in the presynaptic cell triggers it to release small signal molecules known as **neurotransmitters**, which are stored in membrane-enclosed synaptic vesicles and released by exocytosis. The neurotransmitter diffuses rapidly across the synaptic cleft and provokes an electrical change in the postsynaptic cell by binding to *transmitter-gated ion channels* (**Figure 11–35**) and opening them. 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 nerve terminal that released it or by surrounding glial cells. Reuptake is mediated by a variety of Na^+-dependent neurotransmitter transporters; 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 are specialized for rapidly converting extracellular chemical signals into electrical signals at chemical synapses. The channels are concentrated in the plasma membrane of the postsynaptic cell in the region of the synapse and open transiently in response to the binding of neurotransmitter molecules, thereby producing a brief permeability change in the membrane (see Figure 11–35A). 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 changes, 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. An action potential can be triggered from this site only if the local membrane potential depolarizes enough to open a sufficient number of nearby voltage-gated cation channels that are present in the same target cell membrane.

Figure 11–35 A chemical synapse. <CTGA> (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 target cell, thereby transmitting a signal from the excited nerve. (B) A thin section electron micrograph of two nerve terminal synapses on a dendrite of a postsynaptic cell. (B, courtesy of Cedric Raine.)

Chemical Synapses Can Be Excitatory or Inhibitory

Transmitter-gated ion channels differ from one another in several important ways. First, as receptors, they have a highly selective binding site 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^+ 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 influences 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 cation channels depolarizes a membrane. We can understand the effect of opening Cl^- channels as follows. The concentration of Cl^- is much higher outside the cell than inside (see Table 11–1, p. 652), 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 depolarizatrion. Thus, the opening of Cl^- channels makes it more difficult to depolarize the membrane and hence to excite the cell. The opening of K^+ channels has a similar effect. The effects of toxins that block their action demonstrate the importance of inhibitory neurotransmitters: strychnine, for example, by binding to glycine receptors and blocking the inhibitory action of glycine, causes muscle spasms, convulsions, and death.

However, not all chemical signaling in the nervous system operates through ligand-gated ion channels. Many of the signaling molecules that are secreted by nerve terminals, including a large variety of neuropeptides, bind to receptors that regulate ion channels only indirectly. We discuss these so-called *G-protein-coupled receptors* and *enzyme-coupled receptors* in detail in Chapter 15. Whereas signaling mediated by excitatory and inhibitory neurotransmitters binding to transmitter-gated ion channels is generally immediate, simple, and brief, signaling mediated by ligands binding to G-protein-coupled receptors and enzyme-coupled receptors tends to be far slower and more complex, and longer lasting in its consequences.

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

The best-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 11–36**). This synapse has been intensively investigated because it is readily accessible to electrophysiological study, unlike most of the synapses in the central nervous system.

The **acetylcholine receptor** has a special place in the history of ion channels. It was the first ion channel to be purified, the first to have its complete amino acid sequence determined, the first to be functionally reconstituted in synthetic lipid bilayers, and the first for which the electrical signal of a single open channel was recorded. Its gene was also the first ion channel gene to be cloned and sequenced, and its three-dimensional structure has been determined, albeit at

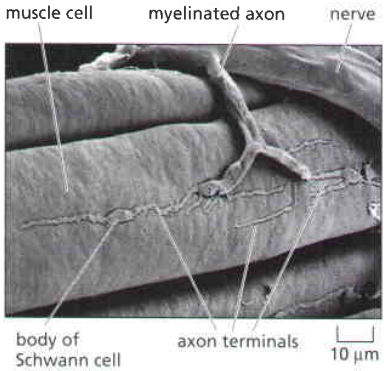


Figure 11–36 A low-magnification scanning electron micrograph of a neuromuscular junction in a frog. The termination of a single axon on a skeletal muscle cell is shown. (From J. Desaki and Y. Uehara, *J. Neurocytol.* 10:101–110, 1981. With permission from Kluwer Academic Publishers.)

only moderate resolution. There were at least two reasons for the rapid progress in purifying and characterizing this receptor. First, an unusually rich source of the acetylcholine receptors exists in the electric organs of electric fish and rays (these organs are modified muscles designed to deliver a large electric shock to prey). Second, certain neurotoxins (such as *α-bungarotoxin*) in the venom of certain snakes bind with high affinity ($K_a = 10^9$ liters/mole) and specificity to the receptor and can therefore be used to purify it by affinity chromatography. Fluorescent or radiolabeled α-bungarotoxin can also be used to localize and count acetylcholine receptors. In this way, researchers have shown that the 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 acetylcholine receptor of skeletal muscle is composed of five transmembrane polypeptides, two of one kind and three others, encoded by four separate genes. 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 to one of two binding sites for acetylcholine that are nestled between adjoining subunits. When two acetylcholine molecules bind to the pentameric complex, they induce a conformational change: the helices that line the pore rotate to disrupt a ring of hydrophobic amino acids that blocks ion flow in the closed state. 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 until hydrolysis by a specific enzyme *(acetylcholinesterase)* located at the neuromuscular junction lowers the concentration of acetylcholine 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 (**Figure 11–37**).

The general shape of the acetylcholine receptor and the likely arrangement of its subunits have been determined by electron microscopy (**Figure 11–38**). The five subunits 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. 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. The normal 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 11–2, p. 670). 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 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.)

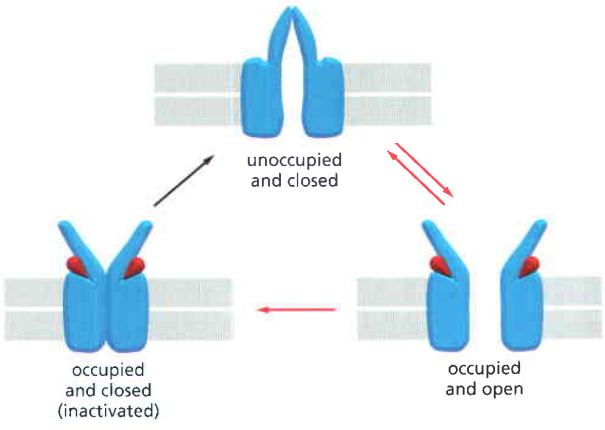


Figure 11–37 Three conformations of the acetylcholine receptor. The binding of two acetylcholine molecules opens this transmitter-gated ion channel. It then maintains a high probability of being open until the acetylcholine has been hydrolyzed. In the persistent presence of acetylcholine, however, the channel inactivates (desensitizes). 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.

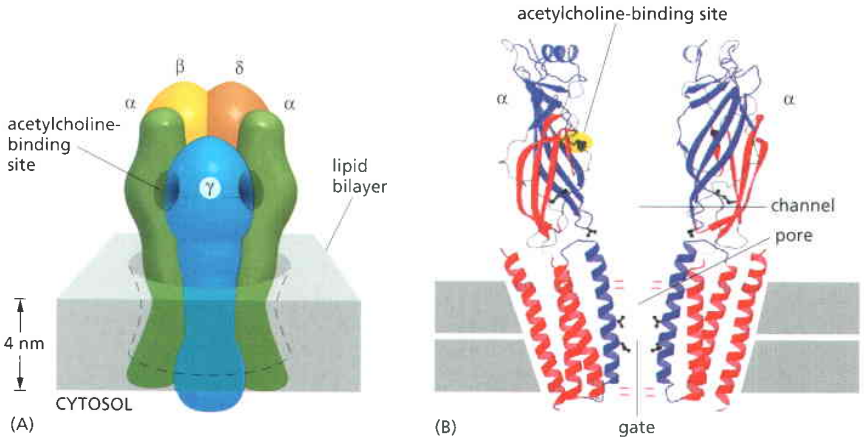


Figure 11–38 A model for the structure of the acetylcholine receptor. (A) Five homologous subunits (α, α, β, γ, δ) combine to form a transmembrane aqueous pore. The pore is lined by a ring of five transmembrane α helices, one contributed by each subunit. In its closed conformation, the pore is thought to be occluded by the hydrophobic side chains of five leucines, one from each α helix, which form a gate near the middle of the lipid bilayer. The negatively charged side chains at either end of the pore ensure that only positively charged ions pass through the channel. (B) Both of the α subunits contribute to an acetylcholine-binding site nestled between adjoining subunits; when acetylcholine binds to both sites, the channel undergoes a conformational change that opens the gate, possibly by rotating the helices containing the occluding leucines to move outward. In the structural drawing *(right)*, the parts of the channel that move in response to AChR binding to open the pore are colored in *blue*. (Adapted from N. Unwin, *Cell* 72[Suppl.]:31–41, 1993. With permission from Elsevier.)

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.

Transmitter-Gated Ion Channels Are Major Targets for Psychoactive Drugs

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, even though they have distinct neurotransmitter-binding specificities and ion selectivities. These channels are all built from homologous polypeptide subunits, which probably assemble as a pentamer resembling the acetylcholine receptor. Glutamate-gated ion channels are constructed from a distinct family of subunits and are thought to form tetramers resembling the K^+ channels discussed earlier.

For each class of transmitter-gated ion channel, there are alternative forms of each type of subunit, either encoded by distinct genes or generated by alternative RNA splicing of the same 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. Vertebrate neurons, for example, have acetylcholine-gated ion channels that differ from those of muscle cells in that they are usually 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, with further diversity due to alternative RNA splicing. Subsets of acetylcholine-sensitive neurons performing different functions in the brain express different combinations of these subunits. This, in principle, and already to some extent in practice, makes it possible to design drugs targeted against narrowly defined groups of neurons or synapses, thereby specifically influencing particular brain functions.

Indeed, transmitter-gated ion channels have for a long time been important targets for drugs. 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 drug from a plant 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. Both barbiturates and tranquilizers, such as Valium and Librium, for example, bind to GABA receptors, potentiating the inhibitory action of GABA by allowing lower concentrations of this neurotransmitter to open Cl^- channels. The new molecular biology of ion

channels, by revealing both their diversity and the details of their structure, holds out the hope of designing 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 transporters. The inhibition of such a transporter prolongs the effect of the transmitter and thereby strengthens synaptic transmission. Many antidepressant drugs, including Prozac, for example, inhibit the uptake of serotonin; others inhibit the uptake of both serotonin and norepinephrine.

Ion channels are the basic molecular components from which neuronal devices for signaling and computation are built. To provide a glimpse of how sophisticated the functions of these devices can be, we consider several examples that demonstrate how groups of ion channels work together in synaptic communication between electrically excitable cells.

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 the 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 membrane. As the Ca^{2+} concentration outside cells is more than 1000 times 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 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 of the muscle cell plasma membrane 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 11–30).

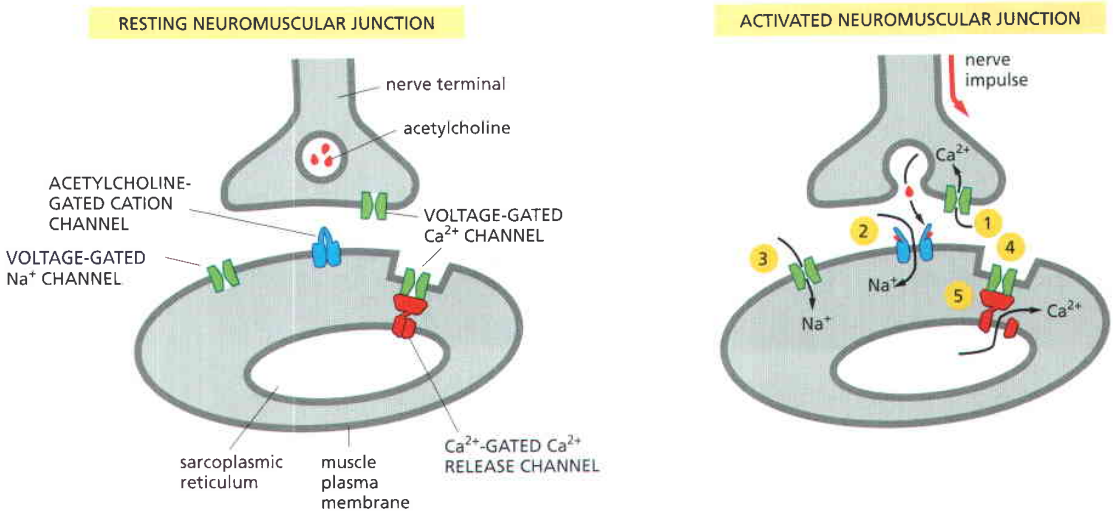


Figure 11–39 The system of ion channels at a neuromuscular junction. These gated ion channels are essential for the stimulation of muscle contraction by a nerve impulse. The various channels are numbered in the sequence in which they are activated, as described in the text.

4. The generalized depolarization of the muscle cell plasma membrane activates voltage-gated Ca^{2+} channels in specialized regions (the transverse [T] tubules—discussed in Chapter 16) of this membrane.
5. This, in turn, causes Ca^{2+}-gated *Ca^{2+} release channels* in an adjacent region of the sarcoplasmic reticulum (SR) membrane to open transiently and release the Ca^{2+} stored in the SR into the cytosol. The T-tubule and SR membranes are closely apposed with the two types of channels joined together in a specialized structure (see Figure 16–77). It is the sudden increase in the cytosolic Ca^{2+} concentration that causes the myofibrils in the muscle cell to contract.

Whereas the activation 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 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 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; its cell body and dendrites are almost completely covered with them (**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, others to inhibit it. Neurotransmitter released at an excitatory synapse causes a small depolarization in the postsynaptic membrane called an *excitatory postsynaptic potential (excitatory PSP)*, while neurotransmitter released at an inhibitory synapse generally causes a small hyperpolarization called an *inhibitory PSP*. The membrane of the dendrites and cell body of most neurons contains a relatively low density of voltage-gated Na^+ channels, and an individual excitatory PSP is generally too small to

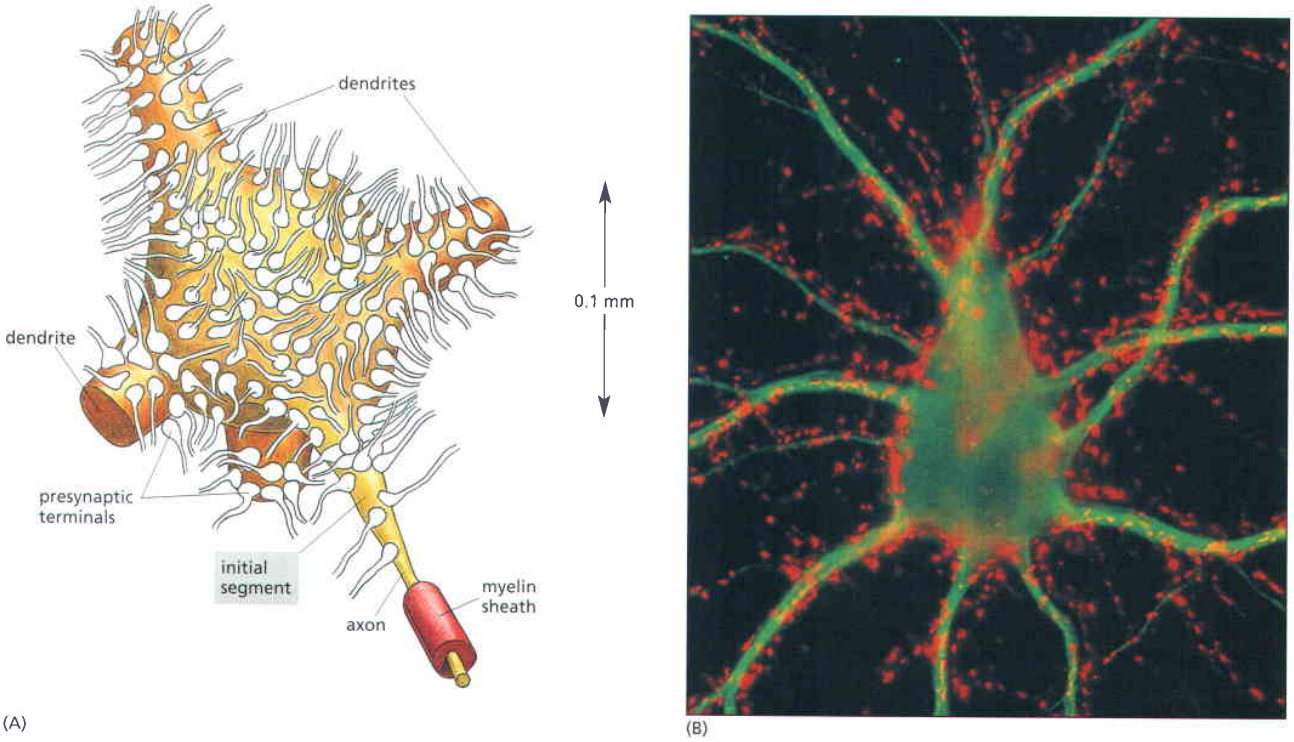


Figure 11–40 A motor neuron cell body in the spinal cord. (A) Many thousands of nerve terminals synapse on the cell body and dendrites. These deliver signals from other parts of the organism to control the firing of action potentials along the single axon of this large cell. (B) Micrograph showing a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

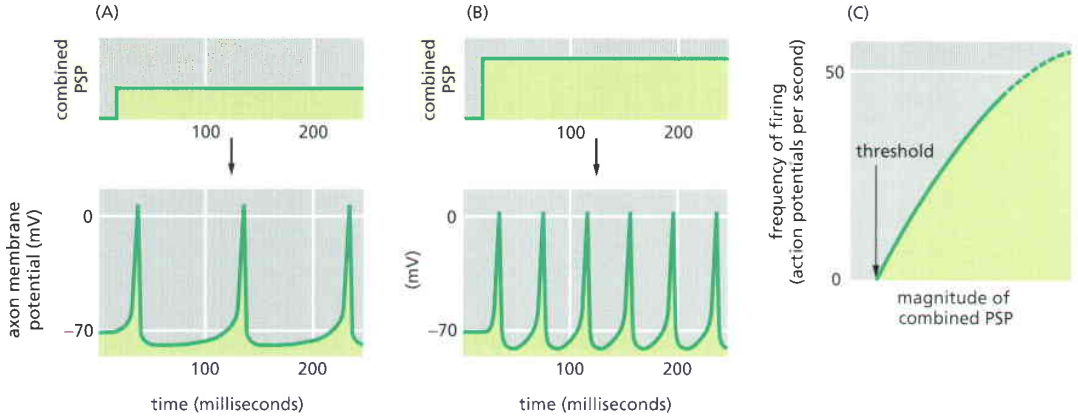


Figure 11–41 The magnitude of the combined postsynaptic potential (PSP) is reflected in the frequency of firing of action potentials. When successive action potentials arrive at the same synapse, each PSP produced adds to the preceding one to produce a larger combined PSP. A comparison of (A) and (B) shows how the firing frequency of an axon increases with an increase in the combined PSP, while (C) summarizes the general relationship.

trigger an action potential. Instead, each incoming signal is reflected in a local PSP of graded magnitude, 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 (**Figure 11–41**). This encoding is achieved by a special set of gated ion channels that are present at high density at the base of the axon, adjacent to the cell body, in a region known as the *initial segment*, or *axon hillock* (see Figure 11–40).

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

We have seen that the intensity of stimulation a neuron receives is encoded for long-distance transmission by the frequency of action potentials that the neuron fires: the stronger the stimulation, the higher the frequency of action potentials. Action potentials are initiated at the **initial segment**, a unique region of each neuron with plentiful voltage-gated Na^+ channels. But to perform its special function of encoding, the membrane of the initial segment also contains at least four other classes of ion channels—three selective for K^+ and one selective for Ca^{2+}. 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 in the usual way 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 p. 677). 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 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 the firing has to reflect the intensity of the 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 (see Figure 11–41C).

The process of encoding is usually further modulated by the two other types of ion channels in the initial segment that were mentioned at the outset, namely 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 initial segment.

The **Ca^{2+}-activated K^+ channel** is both structurally and functionally different from any of the channel types described earlier. It opens in response to a raised concentration of Ca^{2+} at the cytoplasmic face of the nerve cell membrane. Suppose we apply a strong depolarizing stimulus for a long time, triggering a long train of action potentials. Each action potential permits a brief influx of Ca^{2+} through the voltage-gated Ca^{2+} channels, so that the intracellular 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, it increases the delay between one action potential and the next. 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 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 members of the various ion channel families that reside in their membranes. 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 RNAs and assembling channels from different combinations of diverse subunits. The multiplicity of ion channels evidently allows for many different types of neurons, the electrical behavior of which is specifically tuned to the particular tasks that they must perform.

One of the crucial properties of the nervous system is its ability to learn and remember, which seems to depend largely on long-term changes in specific synapses. We end this chapter by considering a remarkable type of ion channel that is thought to have a special role in some forms of learning and memory. It is located at many 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.

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. Correspondingly, some synapses in the hippocampus show marked functional alterations 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 synapses seems to be that *LTP occurs on any occasion when a presynaptic cell fires (once or more) at a time when the post-synaptic 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**, that operate in the standard way. 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 in transgenic animals in which the gene has been knocked out, LTP does not occur, even though ordinary synaptic transmission continues. Such animals exhibit specific deficits in their learning abilities but behave almost normally otherwise.

How do NMDA receptors mediate such a remarkable effect? The answer is that these channels, when open, are highly permeable to Ca^{2+}, which acts as an intracellular mediator 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. 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 11–42**). Evidence also indicates that changes can occur in the presynaptic cell as well, so that it releases more glutamate than normal when it is activated subsequently.

If synapses expressed only LTP they would quickly become saturated and, thus, be of limited value as an information storage device. In fact, synapses also exhibit **long-term depression (LTD)**, which surprisingly 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.

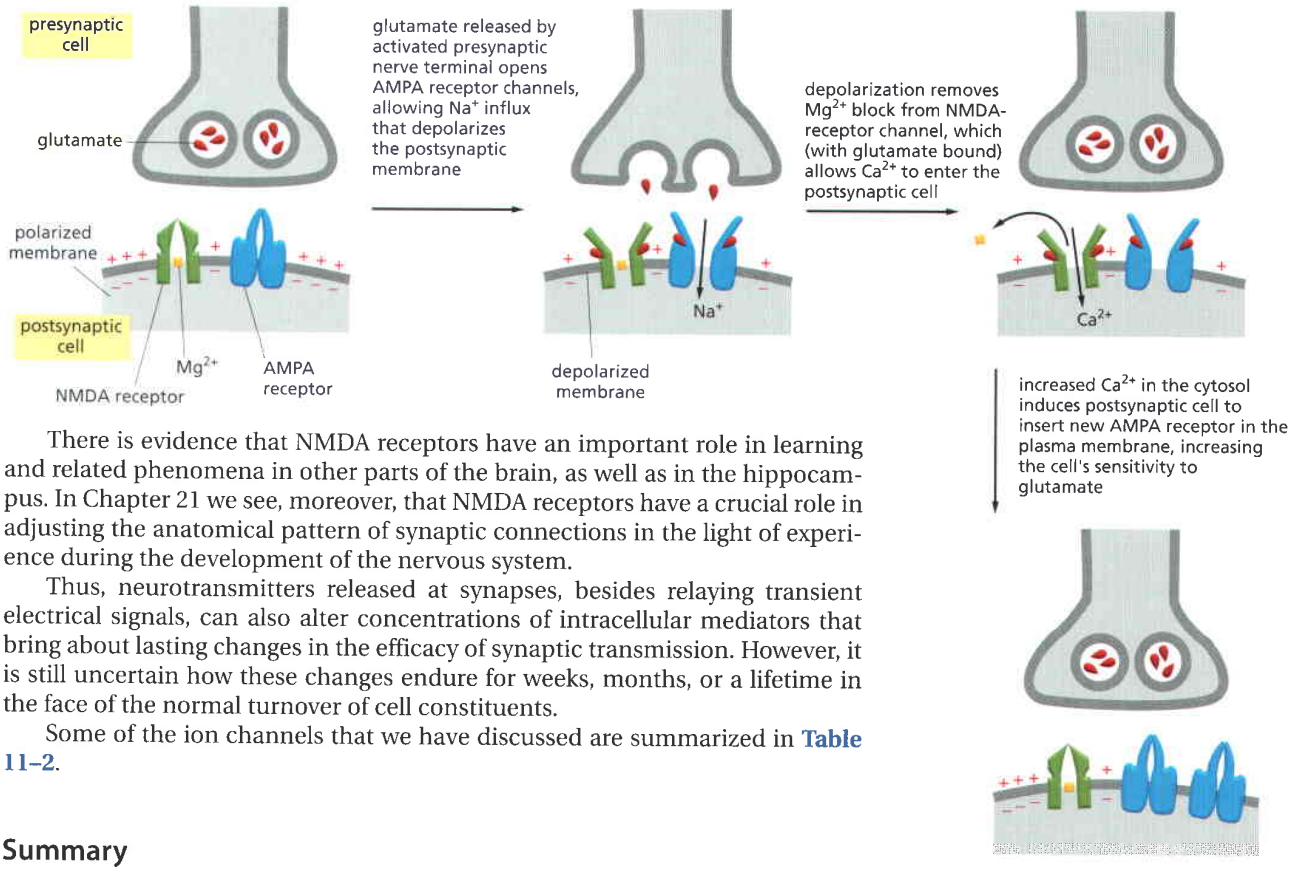


Figure 11–42 The signaling events in long-term potentiation. Although not shown, evidence suggests that changes can also occur in the presynaptic nerve terminals in LTP, which may be stimulated by retrograde signals from the postsynaptic cell.

There is evidence that NMDA receptors have an important role in learning and related phenomena in other parts of the brain, as well as in the hippocampus. In Chapter 21 we see, moreover, that NMDA receptors 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.

Some of the ion channels that we have discussed are summarized in **Table 11–2**.

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 (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 generation of self-amplifying 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 synapses such as LTP and LTD that are thought to be involved in some forms of learning and memory.

Table 11–2 Some Ion Channel Families

CHANNEL TYPE	REPRESENTATIVE EXAMPLE	
Voltage-gated cation channels	voltage-gated Na^+ channels voltage-gated K^+ channels (including delayed and early) voltage-gated Ca^{2+} channels	
Transmitter-gated ion channels	acetylcholine-gated cation channels glutamate-gated Ca^{2+} channels serotonin-gated cation channels	excitatory
	GABA-gated Cl^- channels glycine-gated Cl^- channels	inhibitory

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 postsynaptic potential (PSP) in the cell body. The magnitude of the PSP is translated into the rate of firing of action potentials by a mixture of cation channels in the membrane of the initial segment.

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.

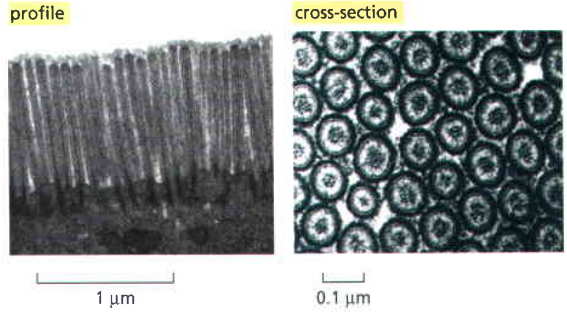


Figure Q11–1 Microvilli of intestinal epithelial cells in profile and cross section (Problem 11–7). (Left panel, from Rippel Electron Microscope Facility, Dartmouth College; Right panel, from David Burgess.)

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?

11–9 The "ball-and-chain" model for the rapid inactivation of voltage-gated K^+ channels has been elegantly confirmed 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 normal 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, 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?

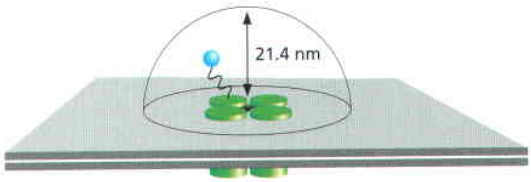


Figure Q11–2 A "ball" tethered by a "chain" to a voltage-gated K^+ channel (Problem 11–9).

11–10 The squid giant axon 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.

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

$$V = 58 \text{ mV} \times \log (C_0/C_i)$$

where C_0 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 axon cytoplasm 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.

Table Q11–1 Ionic composition of seawater and of cytoplasm from the squid giant axon (Problem 11–10).

ION	CYTOPLASM	SEAWATER
Na^+	65 mM	430 mM
K^+	344 mM	9 mM

REFERENCES

General

Martonosi AN (ed) (1985) The enzymes of Biological membranes, vol 3: Membrane transport. 2nd edn. New York: Penum Press.

Stein WD (1990) Channels, carriers and pumps: An introduction to membrane transport. San Diego: Academic Press

Principles of Membrane Transport

Al-Awqati Q (1999) One hundred years of membrane permeability: does Overton still rule? *Nature Cell Biol* 1: E201–E202.

Forrest LR & Sansom MS (2000) Membrane simulations: bigger and better? *Curr Opin Struct Biol* 10:174–181.

Gouaux E and MacKinnon R (2005) Principles of selective ion transport in channels and pumps. *Science* 310:1461–1465.

Mitchell P (1977) Vectorial chemiosmotic processes. *Annu Rev Biochem* 46:996–1005.

Tanford C (1983) Mechanism of free energy coupling in active transport. *Annu Rev Biochem* 52:379–409.

Carrier Proteins and Active Membrane Transport

Almers W & Stirling C (1984) Distribution of transport proteins over animal cell membranes. *J Membr Biol* 77:169–186.

Baldwin SA & Henderson PJ (1989) Homologies between sugar transporters from eukaryotes and prokaryotes. *Annu Rev Physiol* 51:459–471.

Borst P & Elferink RO (2002) Mammalian ABC transporters in health and disease. *Annu Rev Biochem* 71:537–592.

Carafoli E & Brini M (2000) Calcium pumps: structural basis for and mechanism of calcium transmembrane transport. *Curr Opin Chem Biol* 4:152–161.

Dean M, Rzhetsky A et al (2001) The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res* 11:1156–1166.

Doige CA & Ames GF (1993) ATP-dependent transport systems in bacteria and humans: relevance to cystic fibrosis and multidrug resistance. *Annu Rev Microbiol* 47:291–319.

Gadsby DC, Vergani P & Csanady L (2006) The ABC protein turned chloride channel whose failure causes cystic fibrosis. *Nature* 440:477–83.

Higgins CF (2007) Multiple molecular mechanisms for multidrug resistance transporters. *Nature* 446:749–57.

Kaback HR, Sahin-Toth M et al (2001) The kamikaze approach to membrane transport. *Nature Rev Mol Cell Biol* 2:610–620.

Kühlbrandt W (2004) Biology, structure and mechanism of P-type ATPases. *Nature Rev Mol Cell Biol* 5:282–295.

Lodish HF (1986) Anion-exchange and glucose transport proteins: structure, function, and distribution. *Harvey Lect* 82:19–46.

Pedersen PL & Carafoli E (1987) Ion motive ATPases. 1. Ubiquity, properties, and significance to cell function. *Trends Biochem Sci* 12:146–150.

Romero MF & Boron WF (1999) Electrogenic Na^+/HCO_3^- cotransporters: cloning and physiology. *Annu Rev Physiol* 61:699–723.

Saier MH, Jr (2000) Vectorial metabolism and the evolution of transport systems. *J Bacteriol* 182:5029–5035.

Scarborough GA (2003) Rethinking the P-type ATPase problem. *Trends Biochem Sci* 28:581–584.

Stein WD (2002) Cell volume homeostasis: ionic and nonionic mechanisms. The sodium pump in the emergence of animal cells. *Int Rev Cytol* 215:231–258.

Ion Channels and the Electrical Properties of Membranes

Armstrong C (1998) The vision of the pore. *Science* 280:56–57.

Choe S (2002) Potassium channel structures. *Nature Rev Neurosci* 3:115–21.

Choe S, Kreusch A & Pfaffinger PJ (1999) Towards the three-dimensional structure of voltage-gated potassium channels. *Trends Biochem Sci* 24:345–349.

Franks NP & Lieb WR (1994) Molecular and cellular mechanisms of general anaesthesia. *Nature* 367:607–614.

Greengard P (2001) The neurobiology of slow synaptic transmission. *Science* 294:1024–30.

Hille B (2001) Ionic Channels of Excitable Membranes, 3rd ed. Sunderland, MA: Sinauer.

Hucho F, Tsetlin VI & Machold J (1996) The emerging three-dimensional structure of a receptor. The nicotinic acetylcholine receptor. *Eur J Biochem* 239:539–557.

Hodgkin AL & Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* 117:500–544.

Hodgkin AL & Huxley AF (1952) Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J Physiol* 116:449–472.

Jessell TM & Kandel ER (1993) Synaptic transmission: a bidirectional and self-modifiable form of cell–cell communication. *Cell* 72[Suppl]:1–30.

Kandel ER, Schwartz JH & Jessell TM (2000) Principles of Neural Science, 4th ed. New York: McGraw-Hill.

Karlin A (2002) Emerging structure of the nicotinic acetylcholine receptors. *Nature Rev Neurosci* 3:102–114.

Katz B (1966) Nerve, Muscle and Synapse. New York: McGraw-Hill.

King LS, Kozono D & Agre P (2004) From structure to disease: the evolving tale of aquaporin biology. *Nature Rev Mol Cell Biol* 5:687–698.

MacKinnon R (2003) Potassium channels. *FEBS Lett* 555:62–65.

Malenka RC & Nicoll RA (1999) Long-term potentiation—a decade of progress? *Science* 285:1870–1874.

Moss SJ & Smart TG (2001) Constructing inhibitory synapses. *Nature Rev Neurosci* 2:240–250.

Neher E and Sakmann B (1992) The patch clamp technique. *Sci Am* 266:44–51.

Nicholls JG, Fuchs PA, Martin AR & Wallace BG (2000) From Neuron to Brain, 4th ed. Sunderland, MA: Sinauer.

Numa S (1987) A molecular view of neurotransmitter receptors and ionic channels. *Harvey Lect* 83:121–165.

Scannevin RH & Huganir RL (2000) Postsynaptic organization and regulation of excitatory synapses. *Nature Rev Neurosci* 1:133–141.

Seeburg PH (1993) The molecular biology of mammalian glutamate receptor channels. *Trends Neurosci* 16:359–365.

Snyder SH (1996) Drugs and the Brain. New York: WH Freeman/Scientific American Books.

Stevens CF (2004) Presynaptic function. *Curr Opin Neurobiol* 14:341–345.

Tsien RW, Lipscombe D, Madison DV et al (1988) Multiple types of neuronal calcium channels and their selective modulation. *Trends Neurosci* 11:431–438.

Unwin N (2003) Structure and action of the nicotinic acetylcholine receptor explored by electron microscopy. *FEBS Lett* 555:91–95.