

## **11. Membrane Transport of Small Molecules and the Electrical Properties of Membranes**

Because of its hydrophobic interior, the lipid bilayer of cell membranes serves as a barrier to the passage of most polar molecules. This barrier function is crucially important because it allows the cell to maintain concentrations of solutes in its cytosol that are different from those in the extracellular fluid and in each of the intracellular membrane-enclosed compartments. To make use of this barrier, however, cells have had to evolve ways of transferring specific water-soluble molecules across their membranes in order to ingest essential nutrients, excrete metabolic waste products, and regulate intracellular ion concentrations. The transport of inorganic ions and small water-soluble organic molecules across the lipid bilayer is achieved by specialized transmembrane proteins, each of which is responsible for the transfer of a specific ion, molecule, or group of closely related ions or molecules. Cells can also transfer macromolecules and even large particles across their membranes, but the mechanisms involved in most of these cases are different from those used for transferring small molecules, and they are discussed in [Chapters 12](#) and [13](#). The importance of membrane transport is indicated by the large number of genes in all organisms that code for transport proteins, which make up between 15 and 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 considering 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 the transfer: *carrier proteins*, which have moving parts to shift specific molecules across the membrane, and *channel proteins*, which form a narrow hydrophilic pore, allowing the passive movement primarily of small inorganic ions. Carrier proteins 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 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 are used to drive various transport processes, to convey electrical signals in electrically excitable cells, and (in mitochondria, chloroplasts, and bacteria) to 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 astonishing feats of which the human brain is capable

## 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.

### 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 at which it does so, 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<sub>2</sub> and CO<sub>2</sub>, 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 bilayers are 10<sup>9</sup> times more permeable to water than to even such small ions as Na<sup>+</sup> or K<sup>+</sup> (Figure 11-2). [↑ TOP](#)

### There Are Two Main Classes of Membrane Transport Proteins: Carriers 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 are responsible for transferring 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. The specificity of membrane transport proteins was first indicated in the mid-1950s by studies in which single gene mutations were found to abolish the ability of bacteria to transport specific sugars across their plasma membrane. Similar mutations have now been discovered in humans suffering from a variety of inherited diseases that affect the transport of a specific solute in the kidney, intestine, or many other cell types. Individuals with the inherited disease *cystinuria*, for example, are unable to 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.

# Molecular Biology of the Cell 4th ed. Alberts et al., 2002

Table 11-1. A Comparison of Ion Concentrations Inside and Outside a Typical Mammalian Cell

| COMPONENT        | INTRACELLULAR CONCENTRATION (mM)                      | EXTRACELLULAR CONCENTRATION (mM)                      |
|------------------|-------------------------------------------------------|-------------------------------------------------------|
| <b>Cations</b>   |                                                       |                                                       |
| Na <sup>+</sup>  | 5-15                                                  | 145                                                   |
| K <sup>+</sup>   | 140                                                   | 5                                                     |
| Mg <sup>2+</sup> | 0.5                                                   | 1-2                                                   |
| Ca <sup>2+</sup> | 10 <sup>-4</sup>                                      | 1-2                                                   |
| H <sup>+</sup>   | 7 × 10 <sup>-5</sup> (10 <sup>-7.2</sup> M or pH 7.2) | 4 × 10 <sup>-5</sup> (10 <sup>-7.4</sup> M or pH 7.4) |
| <b>Anions*</b>   |                                                       |                                                       |
| Cl <sup>-</sup>  | 5-15                                                  | 110                                                   |

\* The cell must contain equal quantities of positive and negative charges (that is, be electrically neutral). Thus, in addition to Cl<sup>-</sup>, the cell contains many other anions not listed in this table; in fact, most cellular constituents are negatively charged (HCO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, proteins, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of Ca<sup>2+</sup> and Mg<sup>2+</sup> given are for the free ions. There is a total of about 20 mM Mg<sup>2+</sup> and 1-2 mM Ca<sup>2+</sup> in cells, but this is mostly bound to proteins and other substances and, for Ca<sup>2+</sup>, stored within various organelles.

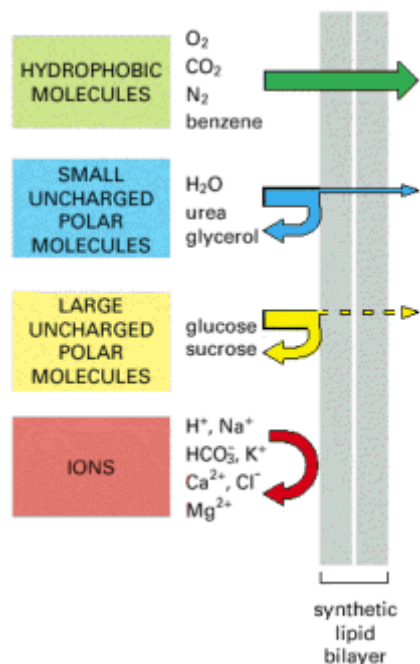


Figure 11-1. The relative permeability of a synthetic lipid bilayer to different classes of molecules. The smaller the molecule and, more importantly, the less strongly it associates with water, the more rapidly the molecule diffuses across the bilayer.

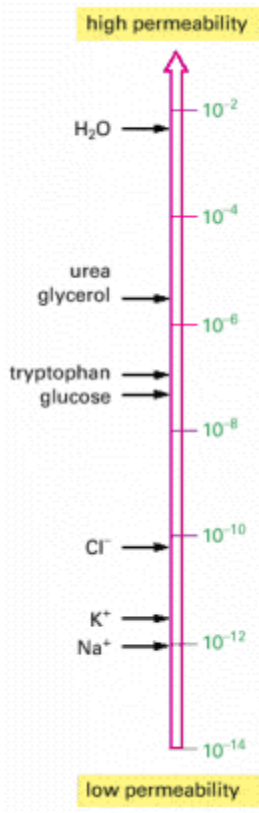


Figure 11-2. Permeability coefficients for the passage of various molecules through synthetic lipid bilayers. The rate of flow of a solute across the bilayer is directly proportional to the difference in its concentration on the two sides of the membrane. Multiplying this concentration difference (in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

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.

Carrier proteins and channel proteins are the two major classes of membrane transport proteins. Carrier proteins (also called *carriers*, *permeases*, or *transporters*) bind the specific solute to be transported and undergo a series of conformational changes to transfer the bound solute across the membrane (Figure 11-3). Channel proteins, in contrast, interact with the solute to be transported much more weakly. They form aqueous pores that extend across the lipid bilayer; when these pores are open, they allow specific solutes (usually inorganic ions of appropriate size and charge) to pass through them and thereby cross the membrane (see Figure 11-3). Not surprisingly, transport through channel proteins occurs at a much faster rate than transport mediated by carrier proteins. [↑ TOP](#)

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

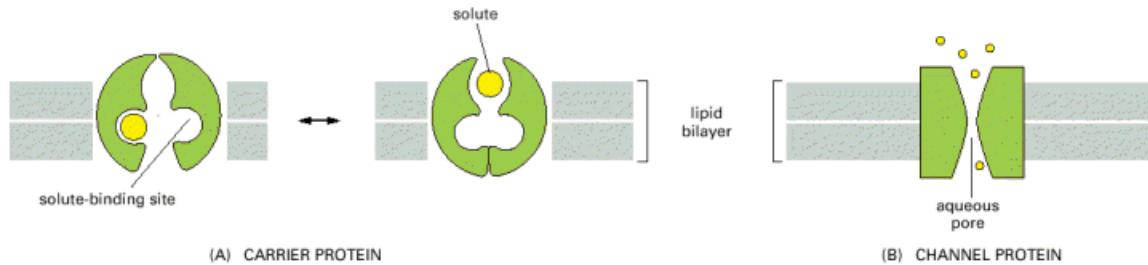


Figure 11-3. Carrier proteins and channel proteins. (A) A carrier protein alternates between two conformations, so that the solute-binding site is sequentially accessible on one side of the bilayer and then on the other. (B) In contrast, a channel protein forms a water-filled pore across the bilayer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

### Active Transport Is Mediated by Carrier Proteins Coupled to an Energy Source

All channel proteins and many carrier proteins 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, it is simply the difference in its concentration on the two sides of the membrane—its *concentration gradient*—that drives passive transport and determines its direction (Figure 11-4A).

If the solute carries a net charge, however, both its concentration gradient and the electrical potential difference across the membrane, the *membrane potential*, influence its transport. The concentration gradient and the electrical gradient can be combined to calculate a net driving force, the electrochemical gradient, for each charged solute (Figure 11-4B). We discuss this 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.

Cells also require transport proteins that will actively pump certain solutes across the membrane against their electrochemical gradient (“uphill”); this process, known as active transport, is mediated by carriers, which are also called *pumps*. In active transport, the pumping activity of the carrier protein 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, transport by carriers can be either active or passive, whereas transport by channel proteins is always passive. [↑ TOP](#)

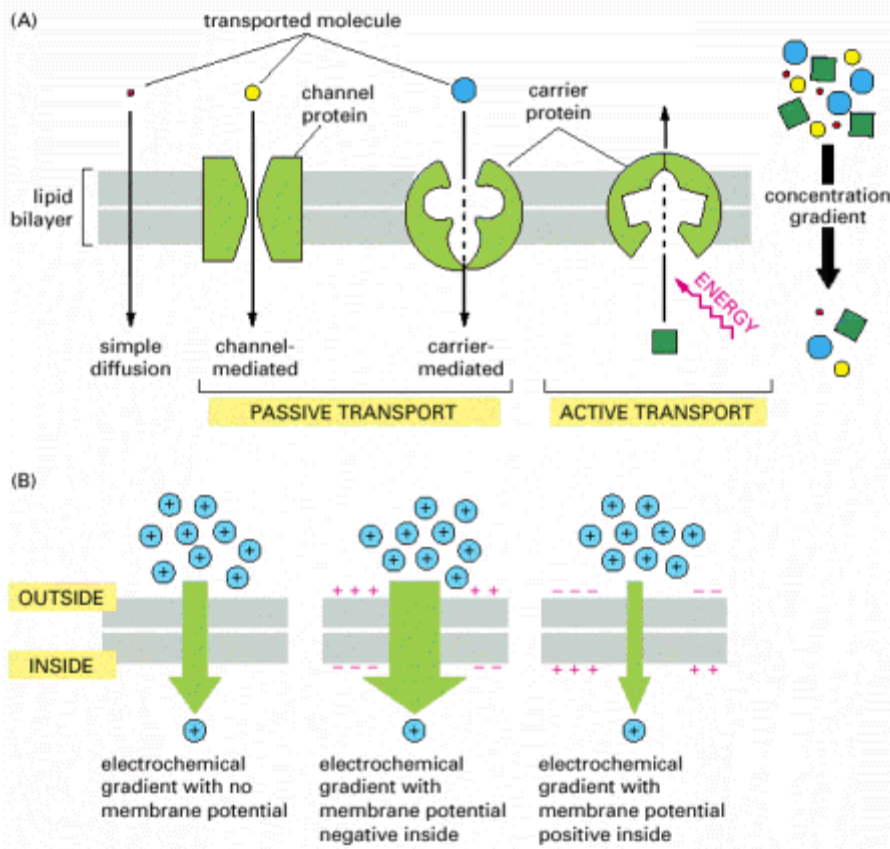


Figure 11-4. Passive and active transport compared. (A) Passive transport down an electrochemical gradient occurs spontaneously, either by simple diffusion through the lipid bilayer or by facilitated diffusion through channels and passive carriers. By contrast, active transport requires an input of metabolic energy and is always mediated by carriers that harvest metabolic energy to pump the solute against its

electrochemical gradient. (B) An electrochemical gradient combines the membrane potential and the concentration gradient, which can work additively to increase the driving force on an ion across the membrane (*middle*) or can work against each other (*right*).

## Ionophores Can Be Used as Tools to Increase the Permeability of Membranes to Specific Ions

Ionophores are small hydrophobic molecules that dissolve in lipid bilayers and increase their permeability to specific inorganic ions. Most are synthesized by microorganisms (presumably as biological weapons against competitors or prey). They are widely used by cell biologists as tools to increase the ion permeability of membranes in studies on synthetic bilayers, cells, or cell organelles. There are two classes of ionophores—**mobile ion carriers** and **channel formers** (Figure 11-5). Both types operate by shielding the charge of the transported ion so that it can penetrate the hydrophobic interior of the lipid bilayer. Since ionophores are not coupled to energy sources, they permit the net movement of ions only down their electrochemical gradients.

*Valinomycin* is an example of a mobile ion carrier. It is a ring-shaped polymer that transports  $K^+$  down its electrochemical gradient by picking up  $K^+$  on one side of the

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

membrane, diffusing across the bilayer, and releasing  $K^+$  on the other side. Similarly, *FCCP*, a mobile ion carrier that makes membranes selectively leaky to  $H^+$ , is often used to dissipate the  $H^+$  electrochemical gradient across the mitochondrial inner membrane, thereby blocking mitochondrial ATP production. *A23187* is yet another example of a mobile ion carrier, only it transports divalent cations such as  $Ca^{2+}$  and  $Mg^{2+}$ . When cells are exposed to *A23187*,  $Ca^{2+}$  enters the cytosol from the extracellular fluid down a steep electrochemical gradient. Accordingly, this ionophore is widely used to increase the concentration of free  $Ca^{2+}$  in the cytosol, thereby mimicking certain cell-signaling mechanisms (discussed in [Chapter 15](#)).

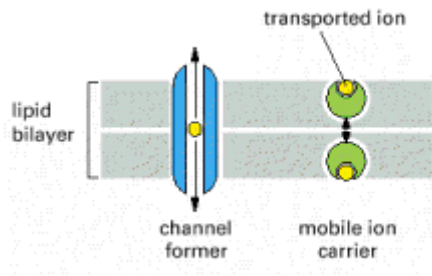


Figure 11-5. Ionophores: a channel-former and a mobile ion carrier. In both cases, net ion flow occurs only down an electrochemical gradient.

*Gramicidin A* is an example of a channel-forming ionophore. It is a dimeric compound of two linear peptides (of 15 hydrophobic amino acids each), which wind around each other to form a double helix. Two gramicidin dimers are thought to come together end to end across the lipid bilayer to form what is probably the simplest of all transmembrane channels, which selectively allows monovalent cations to flow down their electrochemical gradients. Gramicidin is made by certain bacteria, perhaps to kill other microorganisms by collapsing the  $H^+$ ,  $Na^+$ , and  $K^+$  gradients that are essential for their survival, and it has been useful as an antibiotic. [↑ TOP](#)

### 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—carriers and channels. Both form continuous protein pathways across the lipid bilayer. Whereas transport by carriers can be either active or passive, solute flow through channel proteins is always passive. Ionophores, which are small hydrophobic molecules made by microorganisms, can be used as tools to increase the permeability of cell membranes to specific inorganic ions.



## Carrier Proteins and Active Membrane Transport

The process by which a carrier protein transfers a solute molecule across the lipid bilayer resembles an enzyme-substrate reaction, and in many ways carriers behave like enzymes. In contrast to ordinary enzyme-substrate reactions, however, the transported solute is not covalently modified by the carrier protein, but instead is delivered unchanged to the other side of the membrane.

Each type of carrier protein has one or more specific binding sites for its solute (substrate). It transfers the solute across the lipid bilayer by undergoing reversible conformational changes that alternately expose the solute-binding site first on one side of the membrane and then on the other. A schematic model of how such a carrier protein is thought to operate is shown in [Figure 11-6](#). When the carrier is saturated (that is, when all solute-binding sites are occupied), the rate of transport is maximal. This rate, referred to as  $V_{\max}$ , is characteristic of the specific carrier and reflects the rate with which the carrier can flip between its two conformational states. In addition, each transporter protein has a characteristic binding constant for its solute,  $K_m$ , equal to the concentration of solute when the transport rate is half its maximum value ([Figure 11-7](#)). 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 by the carrier) or noncompetitive inhibitors (which bind elsewhere and specifically alter the structure of the carrier).

As we discuss below, it requires only a relatively minor modification of the model shown in [Figure 11-6](#) to link the carrier protein 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 11-8](#)):

1. *Coupled carriers* 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 bacterial cells, couple uphill transport to an input of energy from light, as with bacterio-rhodopsin (discussed in [Chapter 10](#)).

Amino acid sequence comparisons suggest that, in many cases, there are strong similarities in molecular design between carrier proteins that mediate active transport and those that mediate passive transport. Some bacterial carriers, for example, which 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 carriers that mediate passive glucose transport into most animal cells. This suggests an evolutionary relationship between various carrier proteins; and, given the importance of small metabolites and sugars as an energy source, it is not surprising that the superfamily of carriers is an ancient one.



# Molecular Biology of the Cell 4th ed. Alberts et al., 2002

We begin our discussion of active transport by considering carrier proteins 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  $\text{Na}^+$  pump that is found in the plasma membrane of almost all cells.

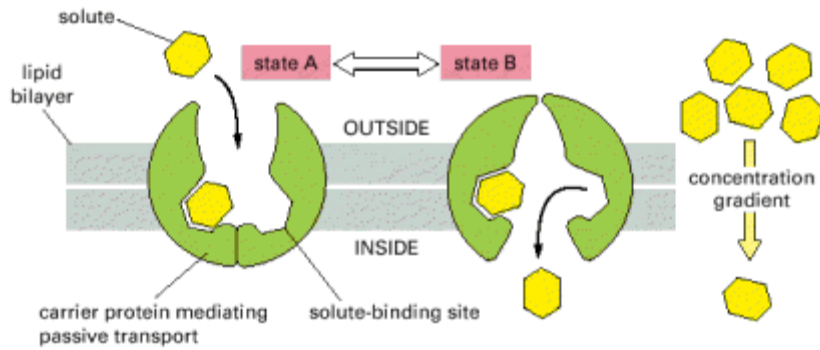


Figure 11-6. A model of how a conformational change in a carrier protein could mediate the passive transport of a solute. The carrier protein shown can exist in two conformational states: in state A, the binding

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 carrier protein 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).

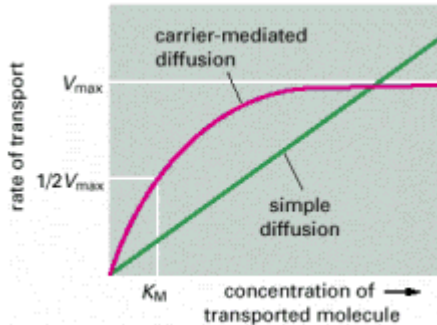


Figure 11-7. The kinetics of simple diffusion and carrier-mediated diffusion. Whereas the rate of the former is always proportional to the solute concentration, the rate of the latter reaches a maximum ( $V_{\max}$ ) when the carrier protein is saturated. The solute concentration when transport is at half its maximal value approximates the binding constant ( $K_m$ ) of the carrier for the solute and is analogous to the  $K_m$  of an enzyme for its substrate. The graph applies to a carrier transporting a single solute; the kinetics of coupled

transport of two or more solutes (see text) is more complex but shows basically similar phenomena.

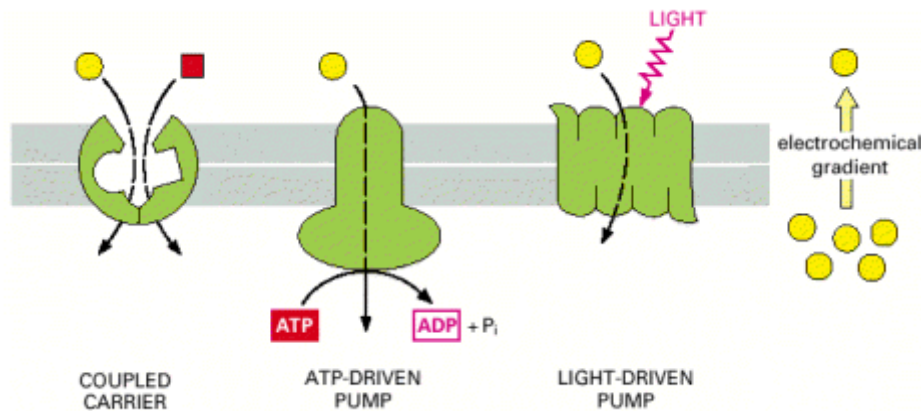


Figure 11-8. Three ways of driving active transport. The actively transported molecule is shown in yellow, and the energy source is shown in red.

## Active Transport Can Be Driven by Ion Gradients

Some carrier proteins simply transport a single solute from one side of the membrane to the other at a rate determined as above by  $V_{\max}$  and  $K_m$ ; they are called uniporters. Others, with more complex kinetics, function as *coupled carriers*, 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, or the transfer of a second solute in the opposite direction, performed by antiporters (Figure 11-9).

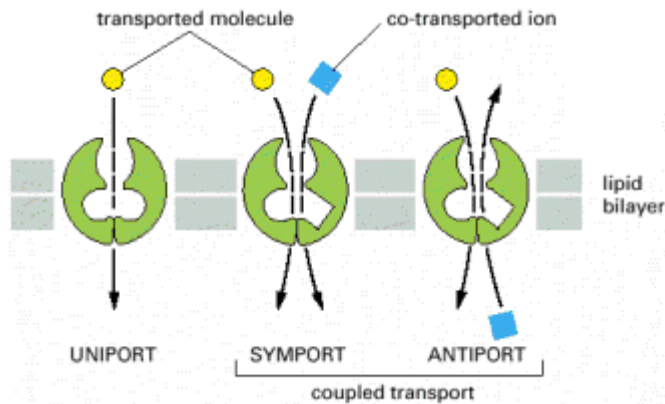


Figure 11-9. Three types of carrier-mediated transport. This schematic diagram shows carrier proteins functioning as uniporters, symporters, and antiporters.

The tight coupling between the transport of two solutes allows these carriers 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 carriers function as symporters, others as antiporters. In the plasma membrane of animal cells,  $\text{Na}^+$  is the usual co-transported ion whose electrochemical gradient provides a large driving force for the active transport of a second molecule. The  $\text{Na}^+$  that enters the cell during transport is subsequently pumped out by an ATP-driven  $\text{Na}^+$  pump in the plasma membrane (as we discuss later), which, by maintaining the  $\text{Na}^+$  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 symport systems that are driven by the  $\text{Na}^+$  gradient across the plasma membrane; each system is specific for importing a small group of related sugars or amino acids into the cell. In these systems, the solute and  $\text{Na}^+$  bind to different sites on a carrier protein. Because the  $\text{Na}^+$  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  $\text{Na}^+$ , the greater the rate of solute entry; conversely, if the  $\text{Na}^+$  concentration in the extracellular fluid is reduced, solute transport decreases (Figure 11-10).

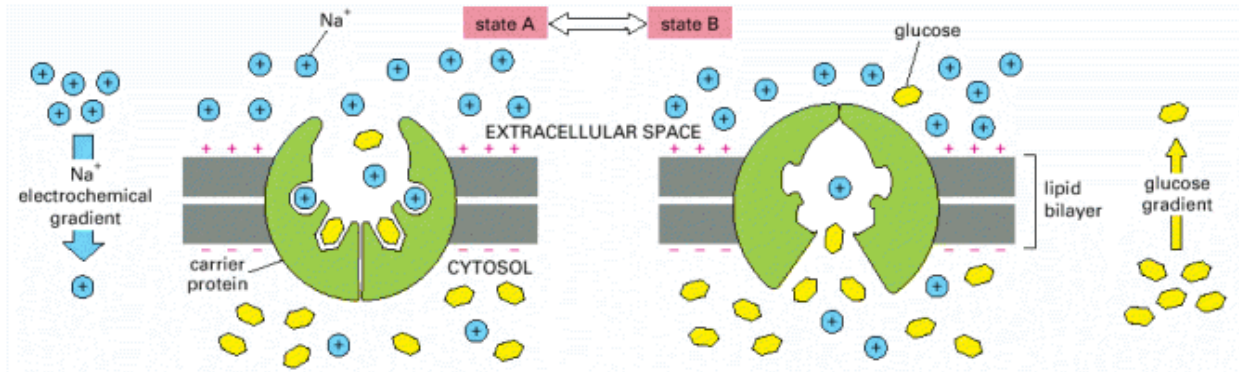


Figure 11-10. One way in which a glucose carrier can be driven by a Na<sup>+</sup> gradient. As in the model shown in [Figure 11-6](#), the carrier oscillates between two alternate states, A and B. In the A state, the protein is open to the extracellular space; in the B state, it is open to the cytosol. Binding of Na<sup>+</sup> and glucose is cooperative—that is, the binding of either ligand induces a conformational change that greatly increases the protein's affinity for the other ligand. Since the Na<sup>+</sup> concentration is much higher in the extracellular space than in the cytosol, glucose is more likely to bind to the carrier in the A state. Therefore, both Na<sup>+</sup> 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<sup>+</sup> 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 carrier. Thus, the carrier undergoes a conformational switch between the two states only if both solutes or neither are bound.

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<sup>+</sup> rather than Na<sup>+</sup> gradients, reflecting the predominance of H<sup>+</sup> pumps and the virtual absence of Na<sup>+</sup> pumps in these membranes. The active transport of many sugars and amino acids into bacterial cells, for example, is driven by the electrochemical H<sup>+</sup> gradient across the plasma membrane. One well-studied H<sup>+</sup>-driven symport is **lactose permease**, which transports lactose across the plasma membrane of *E. coli*. Although the folded structure of the permease is unknown, biophysical studies and extensive analyses of mutant proteins have led to a detailed model of how the symport works. The permease consists of 12 loosely packed transmembrane  $\alpha$  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 the solutes lactose and H<sup>+</sup>, first on one side of the membrane and then on the other ([Figure 11-11](#)). [↑ TOP](#)

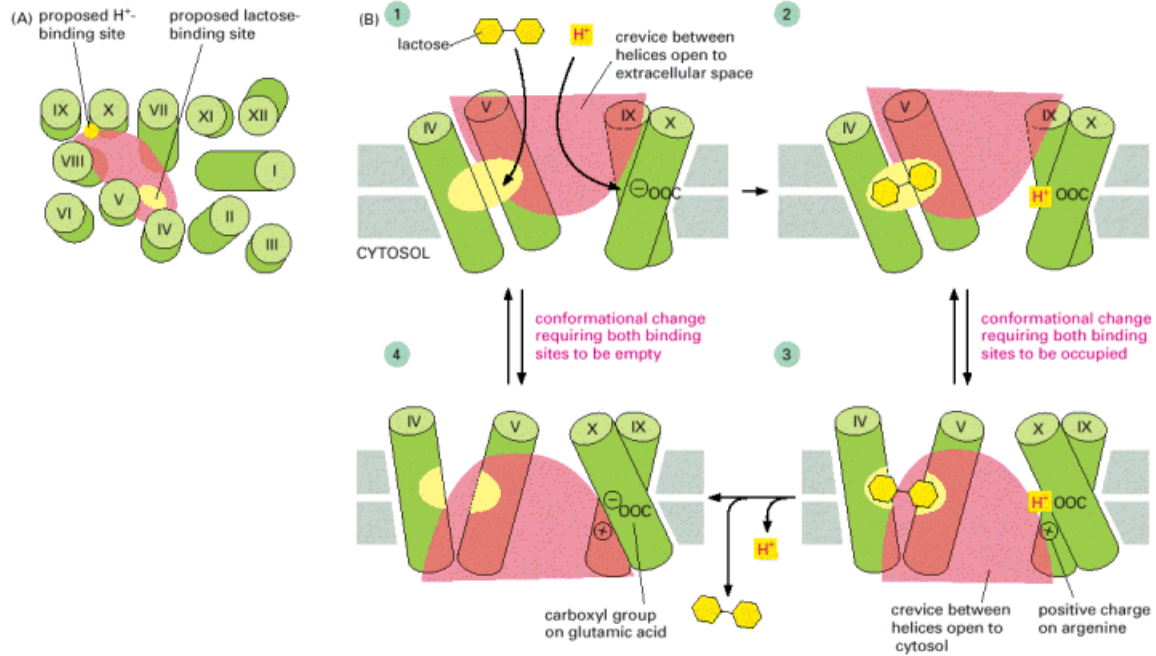


Figure 11-11. A model for the molecular mechanism of action of the bacterial lactose permease. (A) A view from the cytosol of the proposed arrangement of the 12 predicted transmembrane helices in the membrane. The loops that connect the helices on either side of the membrane are omitted for clarity. A glutamic acid on helix X binds H<sup>+</sup>, and amino acids contributed by helices IV and V bind lactose. (B) During a transport cycle, the carrier flips between two conformational states: in one, the H<sup>+</sup>- and lactose-binding sites are accessible to the extracellular space (1 and 2); in the other, they are exposed to the cytosol (3 and 4). Unloading of the solutes on the cytosolic face (3 → 4) is favored because the lactose-binding site is partly disrupted and a positive charge contributed by an arginine on helix IX displaces the H<sup>+</sup> from the glutamic acid on helix X. (A, adapted from H.R. Kaback and J. Wu, *Accts. Chem. Res.* 32:805-813, 1999.)

## Na<sup>+</sup>-driven Carrier Proteins in the Plasma Membrane Regulate Cytosolic pH

The structure and function of most macromolecules are greatly influenced by pH, and 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 be able to control the pH of their intracellular compartments.

Most cells have one or more types of Na<sup>+</sup>-driven antiporters in their plasma membrane that help to maintain the cytosolic pH (pH<sub>i</sub>), at about 7.2. These proteins use the energy stored in the Na<sup>+</sup> gradient to pump out excess H<sup>+</sup>, which either leaks in or is produced in the cell by acid-forming reactions. Two mechanisms are used: either H<sup>+</sup> is directly transported out of the cell or HCO<sub>3</sub><sup>-</sup> is brought into the cell to neutralize H<sup>+</sup> in the cytosol

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

(according to the reaction  $\text{HCO}_3^- + \text{H}^+ \rightarrow \text{H}_2\text{O} + \text{CO}_2$ ). One of the antiporters that uses the first mechanism is a *Na<sup>+</sup>-H<sup>+</sup> exchanger*, which couples an influx of  $\text{Na}^+$  to an efflux of  $\text{H}^+$ . Another, which uses a combination of the two mechanisms, is a *Na<sup>+</sup>-driven Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchanger* that couples an influx of  $\text{Na}^+$  and  $\text{HCO}_3^-$  to an efflux of  $\text{Cl}^-$  and  $\text{H}^+$  (so that  $\text{NaHCO}_3$  comes in and  $\text{HCl}$  goes out). The  $\text{Na}^+$ -driven  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchanger is twice as effective as the  $\text{Na}^+$ - $\text{H}^+$  exchanger, in the sense that it pumps out one  $\text{H}^+$  and neutralizes another for each  $\text{Na}^+$  that enters the cell. If  $\text{HCO}_3^-$  is available, as is usually the case, this antiporter is the most important carrier protein regulating  $\text{pH}_i$ . Both exchangers are regulated by  $\text{pH}_i$  and increase their activity as the pH in the cytosol falls.

An *Na<sup>+</sup>-independent Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchanger* also has an important role in  $\text{pH}_i$  regulation. Like the  $\text{Na}^+$ -dependent transporters, the  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchanger is regulated by  $\text{pH}_i$ , but the movement of  $\text{HCO}_3^-$ , in this case, is normally out of the cell, down its electrochemical gradient. The rate of  $\text{HCO}_3^-$  efflux and  $\text{Cl}^-$  influx increases as  $\text{pH}_i$  rises, thereby decreasing  $\text{pH}_i$  whenever the cytosol becomes too alkaline. The  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchanger is similar to the band 3 protein in the membrane of red blood cells discussed in [Chapter 10](#). In red blood cells, band 3 protein facilitates the quick discharge of  $\text{CO}_2$  as the cells pass through capillaries in the lung.

ATP-driven  $\text{H}^+$  pumps are also used to control the pH of many intracellular compartments. As discussed in [Chapter 13](#), the low pH in lysosomes, as well as in endosomes and secretory vesicles, is maintained by such  $\text{H}^+$  pumps, which use the energy of ATP hydrolysis to pump  $\text{H}^+$  into these organelles from the cytosol. [↑ TOP](#)

### An Asymmetric Distribution of Carrier Proteins in Epithelial Cells Underlies the Transcellular Transport of Solutes

In epithelial cells, such as those involved in absorbing nutrients from the gut, carrier proteins are distributed nonuniformly in the plasma membrane and thereby contribute to the transcellular transport of absorbed solutes. As shown in [Figure 11-12](#),  $\text{Na}^+$ -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.  $\text{Na}^+$ -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 (see [Figure 11-12](#)). Such microvilli can increase the total absorptive area of a cell by as much as 25-fold, thereby enhancing its transport capabilities.

Although, as we have seen, ion gradients have a crucial role in driving many essential transport processes in cells, the ion pumps that use the energy of ATP hydrolysis are responsible mainly for establishing and maintaining these gradients, as we next discuss. [↑ TOP](#)



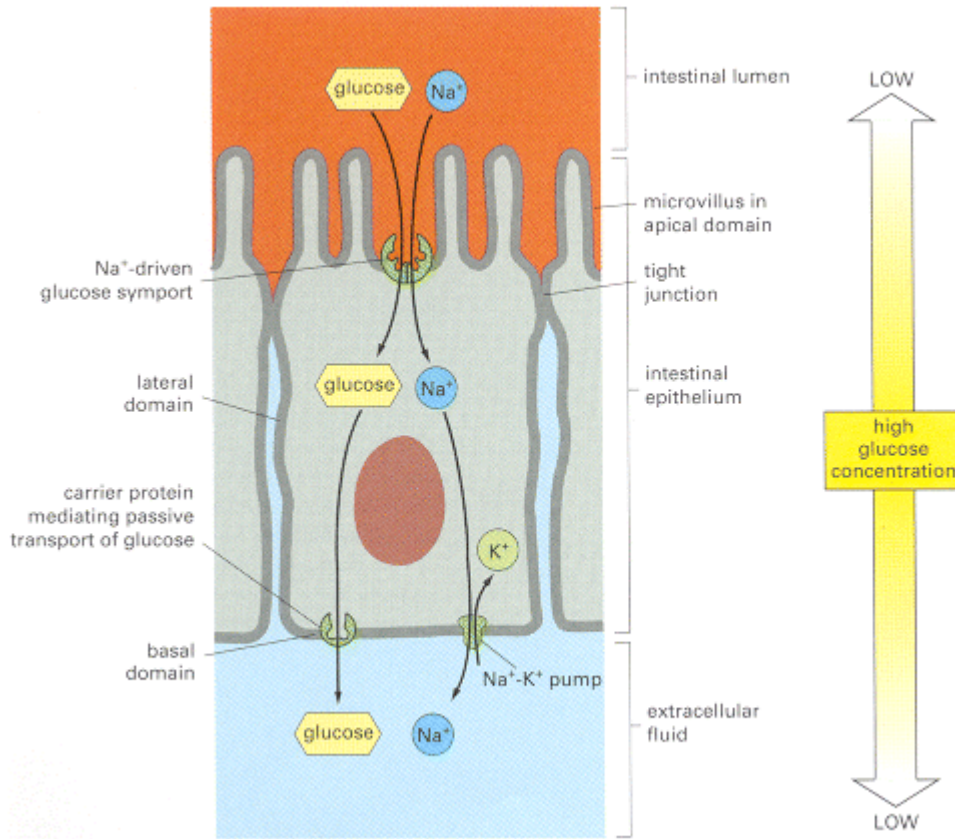


Figure 11-12. Transcellular transport. The transcellular transport of glucose across an intestinal epithelial cell depends on the nonuniform distribution of transport proteins in the cell's plasma membrane. The process shown here results in the transport of glucose from the intestinal lumen to the extracellular fluid (from where it passes into the blood). Glucose is pumped into the cell through the apical domain of the membrane by a Na<sup>+</sup>-powered glucose symport. Glucose passes out of the cell (down its concentration gradient) by passive transport mediated by a different glucose carrier protein in the basal and lateral membrane domains. The Na<sup>+</sup> gradient driving the glucose symport is maintained by a Na<sup>+</sup> pump in the basal and lateral plasma membrane domains, which keeps the internal concentration of Na<sup>+</sup> 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 carrier proteins to their respective membrane domains (see [Figure 10-41](#)).

### The Plasma Membrane Na<sup>+</sup>-K<sup>+</sup> Pump Is an ATPase

The concentration of K<sup>+</sup> is typically 10 to 20 times higher inside cells than outside, whereas the reverse is true of Na<sup>+</sup> (see [Table 11-1](#)). These concentration differences are maintained by a Na<sup>+</sup>-K<sup>+</sup> pump, or Na<sup>+</sup> pump, found in the plasma membrane of virtually all animal cells. The pump operates as an antiporter, actively pumping Na<sup>+</sup> out of the cell against its steep electrochemical gradient and pumping K<sup>+</sup> in. Because the

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

pump hydrolyzes ATP to pump  $\text{Na}^+$  out and  $\text{K}^+$  in, it is also known as a  $\text{Na}^+ - \text{K}^+$  ATPase (Figure 11-13).

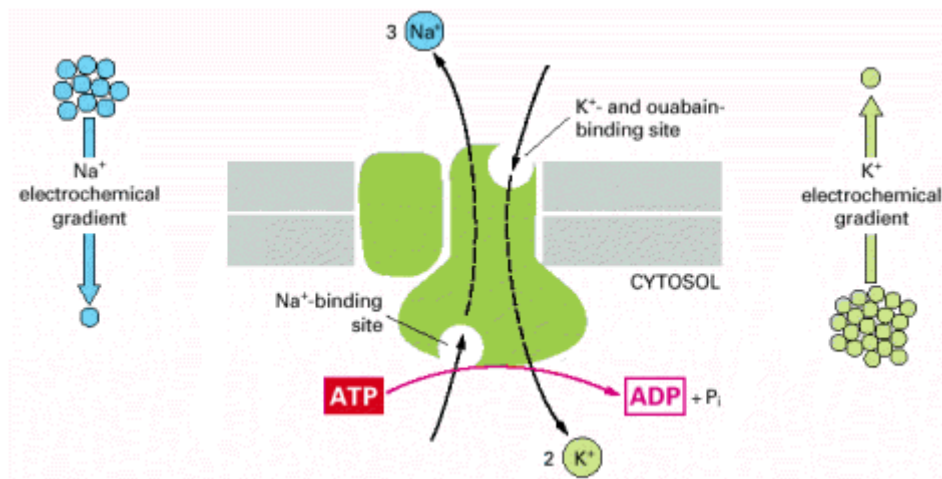


Figure 11-13. The  $\text{Na}^+ - \text{K}^+$  pump. This carrier protein actively pumps  $\text{Na}^+$  out of and  $\text{K}^+$  into a cell against their electrochemical gradients. For every molecule of ATP hydrolyzed inside the cell, three  $\text{Na}^+$  are pumped out and two  $\text{K}^+$  are pumped in. The specific inhibitor ouabain and  $\text{K}^+$  compete for the same site on the extracellular side of the pump.

We have seen that the  $\text{Na}^+$  gradient produced by the pump drives the transport of most nutrients into animal cells and also has a crucial role in regulating cytosolic pH. As we discuss below, the pump also regulates cell volume through its osmotic effects; indeed, it keeps many animal cells from bursting. Almost one-third of the energy requirement of a typical animal cell is consumed in fueling this pump. In electrically active nerve cells, which, as we shall see, repeatedly gain small amounts of  $\text{Na}^+$  and lose small amounts of  $\text{K}^+$  during the propagation of nerve impulses, this number approaches two-thirds of the cell's energy requirement.

An essential characteristic of the  $\text{Na}^+ - \text{K}^+$  pump is that the transport cycle depends on autophosphorylation of the protein. The terminal phosphate group of ATP is transferred to an aspartic acid residue of the pump and is subsequently removed, as explained in [Figure 11-14](#). Different states of the pump are thus distinguished by the presence or absence of the phosphate group. Ion pumps that phosphorylate themselves in this way are called *P-type transport ATPases*. They constitute a family of structurally and functionally related proteins, which includes a variety of  $\text{Ca}^{2+}$  pumps and  $\text{H}^+$  pumps, as we discuss below.

Like any enzyme, the  $\text{Na}^+ - \text{K}^+$  pump can be driven in reverse, in this case to produce ATP. When the  $\text{Na}^+$  and  $\text{K}^+$  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  $\text{Na}^+ - \text{K}^+$  pump. Thus, the phosphorylated form of the pump (step 2 in [Figure 11-14](#)) 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



## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

overall change in free-energy is used to synthesize ATP or to pump  $\text{Na}^+$  out of the cell depends on the relative concentrations of ATP, ADP, and phosphate, as well as on the electrochemical gradients for  $\text{Na}^+$  and  $\text{K}^+$ . [↑ TOP](#)

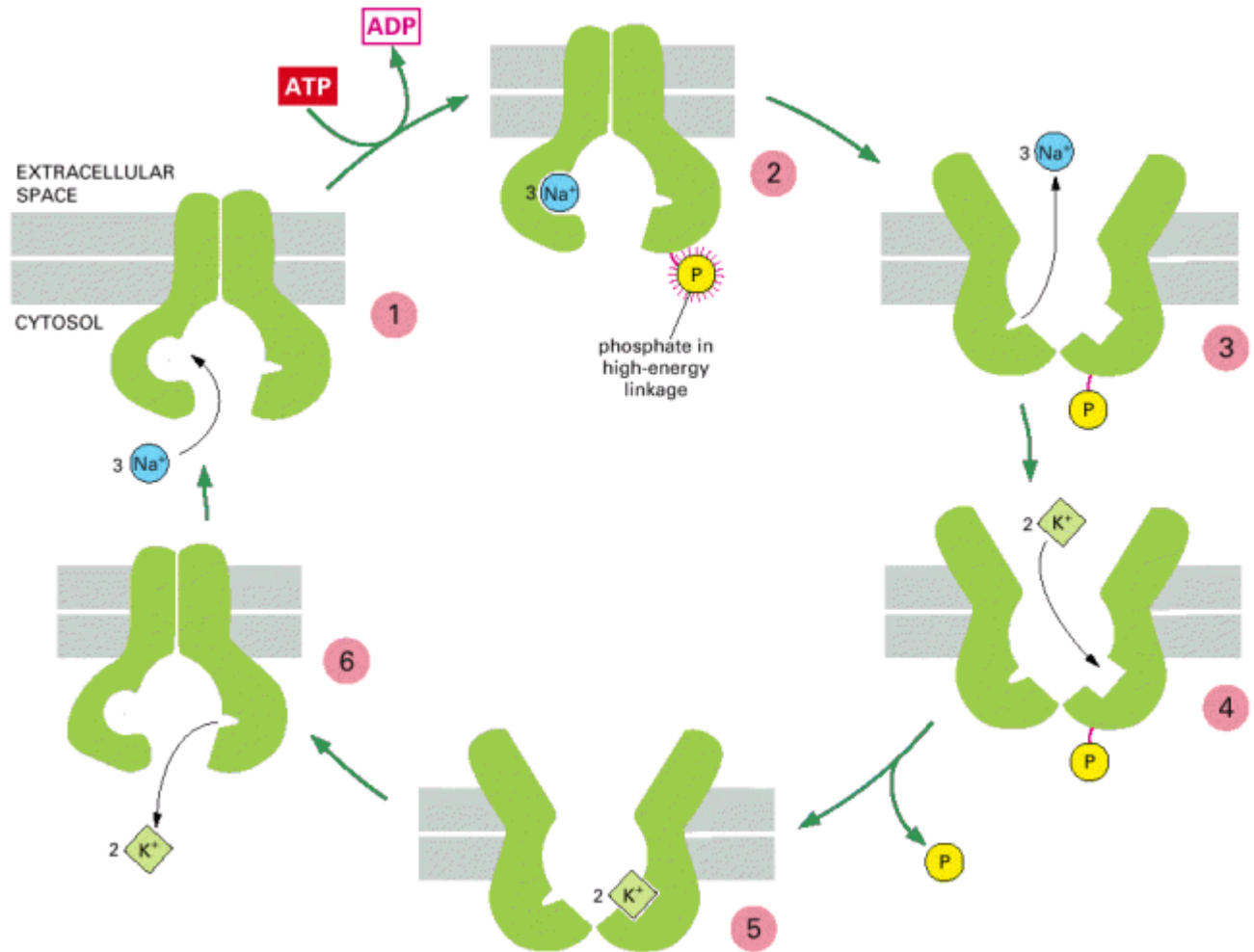


Figure 11-14. A model of the pumping cycle of the  $\text{Na}^+$ - $\text{K}^+$  pump. (1) The binding of  $\text{Na}^+$  and (2) the subsequent phosphorylation by ATP of the cytoplasmic face of the pump induce the protein to undergo a conformational change that (3) transfers the  $\text{Na}^+$  across the membrane and releases it on the outside. (4) Then, the binding of  $\text{K}^+$  on the extracellular surface and (5) the subsequent dephosphorylation return the protein to its original conformation, which (6) transfers the  $\text{K}^+$  across the membrane and releases it into the cytosol. These changes in conformation are analogous to the  $A \leftrightarrow B$  transitions shown in [Figure 11-6](#), except that here the  $\text{Na}^+$ -dependent phosphorylation and the  $\text{K}^+$ -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 only one  $\text{Na}^+$ - and one  $\text{K}^+$ -binding site are shown, in the real pump there are thought to be three  $\text{Na}^+$ - and two  $\text{K}^+$ -binding sites. Moreover, although the pump is shown as alternating between two conformational states only, there is evidence that it goes through a more complex series of conformational changes during the pumping cycle.

### Some $\text{Ca}^{2+}$ and $\text{H}^+$ Pumps Are Also P-type Transport ATPases

In addition to the  $\text{Na}^+$ - $\text{K}^+$  pump, the P-type transport ATPase family includes  $\text{Ca}^{2+}$  pumps that remove  $\text{Ca}^{2+}$  from the cytosol after signaling events and the  $\text{H}^+$ - $\text{K}^+$  pumps that secrete acid from specialized epithelial cells in the lining of the stomach. The  $\text{Ca}^{2+}$  pumps are especially important. Eucaryotic cells maintain very low concentrations of free  $\text{Ca}^{2+}$  in their cytosol ( $\sim 10^{-7}$  M) in the face of very much higher extracellular  $\text{Ca}^{2+}$  concentrations ( $\sim 10^{-3}$  M). Even a small influx of  $\text{Ca}^{2+}$  significantly increases the concentration of free  $\text{Ca}^{2+}$  in the cytosol, and the flow of  $\text{Ca}^{2+}$  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](#)). The maintenance of a steep  $\text{Ca}^{2+}$  gradient is therefore important to the cell. The  $\text{Ca}^{2+}$  gradient is maintained by  $\text{Ca}^{2+}$  transporters in the plasma membrane that actively move  $\text{Ca}^{2+}$  out of the cell. One of these is a P-type  $\text{Ca}^{2+}$  ATPase; the other is an antiporter (called a  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchanger) that is driven by the  $\text{Na}^+$  electrochemical gradient (see [Figure 15-38A](#)).

The best-understood P-type transport ATPase is the  $\text{Ca}^{2+}$  pump, or  $\text{Ca}^{2+}$  ATPase, in the sarcoplasmic reticulum membrane of skeletal muscle cells. The sarcoplasmic reticulum is a specialized type of endoplasmic reticulum that forms a network of tubular sacs in the muscle cell cytosol and serves as an intracellular store of  $\text{Ca}^{2+}$ . (When an action potential depolarizes the muscle cell membrane,  $\text{Ca}^{2+}$  is released from the sarcoplasmic reticulum through the  $\text{Ca}^{2+}$ -release channels into the cytosol, stimulating the muscle to contract, as discussed in [Chapter 16](#).) The  $\text{Ca}^{2+}$  pump, which accounts for about 90% of the membrane protein of the organelle, is responsible for moving  $\text{Ca}^{2+}$  from the cytosol back into the sarcoplasmic reticulum. The endoplasmic reticulum of nonmuscle cells contains a similar  $\text{Ca}^{2+}$  pump, but in smaller quantities.

The three-dimensional structure of the sarcoplasmic reticulum  $\text{Ca}^{2+}$  pump has been determined at high resolution. This structure and the analysis of a related fungal  $\text{H}^+$  pump have provided the first views of P-type transport ATPases, which are all thought to have similar structures. They contain 10 transmembrane  $\alpha$  helices, three of which line a central channel that spans the lipid bilayer. In the unphosphorylated state of the  $\text{Ca}^{2+}$  pump, two of these helices are disrupted and form a cavity that is accessible from the cytosolic side of the membrane and binds two  $\text{Ca}^{2+}$  ions. The binding of ATP to a binding site on the same side of the membrane and the subsequent phosphorylation of an adjacent domain lead to a drastic rearrangement of the transmembrane helices. The rearrangement disrupts the  $\text{Ca}^{2+}$ -binding site and releases the  $\text{Ca}^{2+}$  ions on the other side of the membrane, into the lumen of the sarcoplasmic reticulum ([Figure 11-15](#)). [↑ TOP](#)

### The $\text{Na}^+$ - $\text{K}^+$ Pump Is Required to Maintain Osmotic Balance and Stabilize Cell Volume

Since the  $\text{Na}^+$ - $\text{K}^+$  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 membrane, tending to create an electrical potential, with the cell's inside negative relative to the outside. This electrogenic effect of the pump, however, seldom contributes more than 10% to the

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

membrane potential. The remaining 90%, as we discuss later, depends on the  $\text{Na}^+ - \text{K}^+$  pump only indirectly.

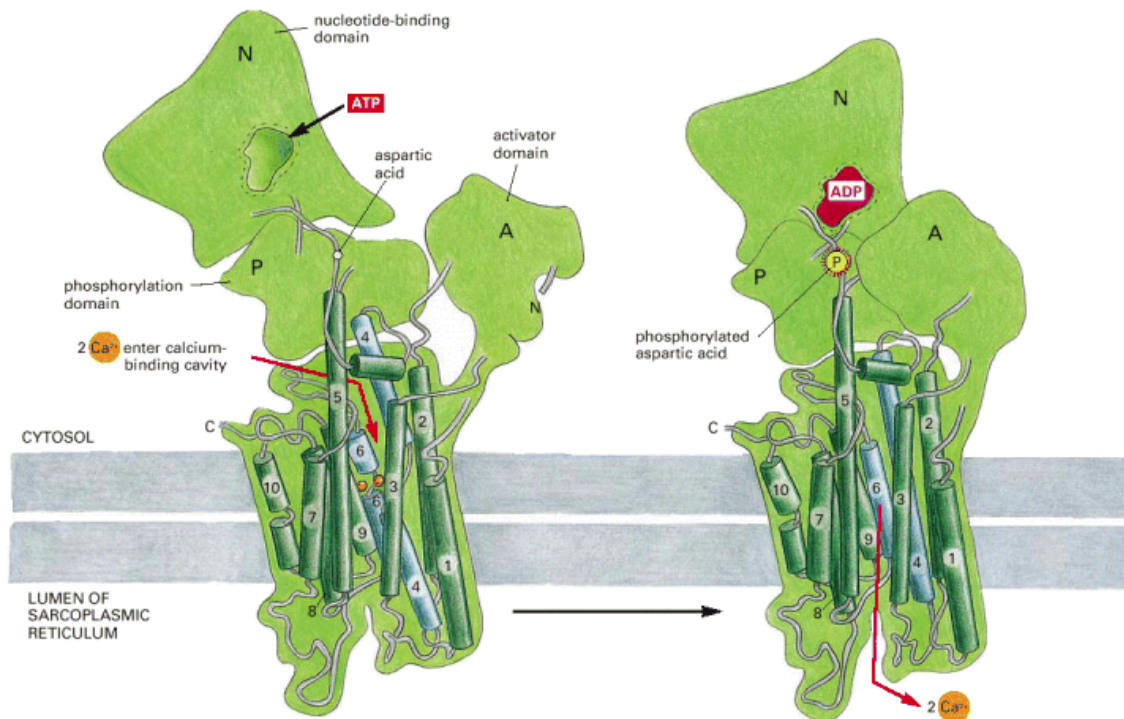


Figure 11-15. A model of how the sarcoplasmic reticulum  $\text{Ca}^{2+}$  pump moves  $\text{Ca}^{2+}$ . The structure of the unphosphorylated  $\text{Ca}^{2+}$ -bound state (*left*) is based on the X-ray crystallographic structure of the pump. The structure of the phosphorylated,  $\text{Ca}^{2+}$ -free state (*right*) is based on lower-resolution structures determined by electron microscopy, and so the arrangement of the transmembrane helices is speculative. Helices 4 and 6 are disrupted in the unphosphorylated state and form the  $\text{Ca}^{2+}$ -binding site on the cytosolic side of the membrane. ATP binding and hydrolysis cause drastic conformational changes, bringing the nucleotide-binding (N) and phosphorylation (P) domains into close proximity. This change is thought to cause a  $90^\circ$  rotation of the actuator domain (A), which leads to a rearrangement of the transmembrane helices. The rearrangement eliminates the breaks in helices 4 and 6 abolishing the  $\text{Ca}^{2+}$ -binding sites and releasing the  $\text{Ca}^{2+}$  ions on the other side of the membrane into the lumen of the sarcoplasmic reticulum. (Adapted from C. Toyoshima et al., *Nature* 405:647–655, 2000.)

On the other hand, the  $\text{Na}^+ - \text{K}^+$  pump does have a direct and crucial role in regulating cell volume. It controls the solute concentration inside the cell, thereby regulating the osmolarity (or *tonicity*) that can make a cell swell or shrink (Figure 11-16). Because the plasma membrane is weakly permeable to water, water moves slowly into or out of cells down its concentration gradient, a process called *osmosis*. If cells are placed in a *hypotonic solution* (that is, a solution having a low solute concentration and therefore a high water concentration), there is a net movement of water into the cells, causing them to swell and burst (lyse). Conversely, if cells are placed in a *hypertonic solution*, they shrink (see Figure 11-16). Many animal cells also contain specialized *water channels* in their plasma membrane to facilitate osmotic water flow called *aquaporins*.

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

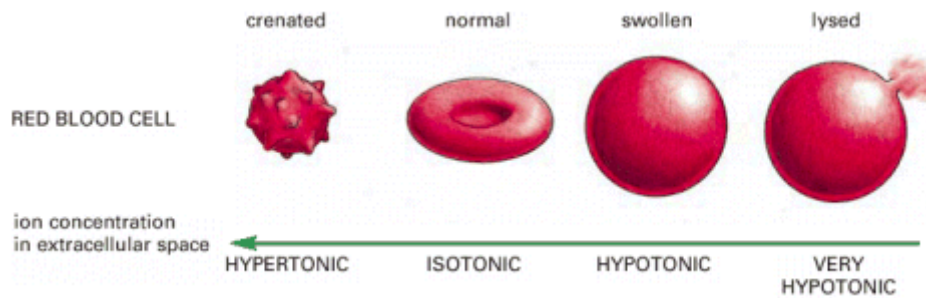


Figure 11-16. Response of a human red blood cell to changes in osmolarity of the extracellular fluid. The cell swells or shrinks as water moves into or out of the cell down its concentration gradient.

The importance of the  $\text{Na}^+$ - $\text{K}^+$  pump in controlling cell volume is indicated by the observation that many animal cells swell, and often burst, if they are treated with *ouabain*, which inhibits the  $\text{Na}^+$ - $\text{K}^+$  pump. 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, unless balanced, would tend to “pull” water into the cell. For animal cells this effect is counteracted by an opposite osmotic gradient due to a high concentration of inorganic ions—chiefly  $\text{Na}^+$  and  $\text{Cl}^-$ —in the extracellular fluid. The  $\text{Na}^+$ - $\text{K}^+$  pump maintains osmotic balance by pumping out the  $\text{Na}^+$  that leaks in down its steep electrochemical gradient. The  $\text{Cl}^-$  is kept out by the membrane potential.

There are, of course, other ways for a cell to cope with its osmotic problems. 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. But for most animal cells, the  $\text{Na}^+$ - $\text{K}^+$  pump is crucial. [↑ TOP](#)

### Membrane-bound Enzymes That Synthesize ATP Are Transport ATPases Working in Reverse

The plasma membrane of bacteria, the inner membrane of mitochondria, and the thylakoid membrane of chloroplasts all contain transport ATPases. These, however, belong to the family of **F-type ATPases** and are structurally very different from P-type ATPases. F-type ATPases are turbine-like structures, constructed from multiple different protein subunits. We shall discuss them in detail in [Chapter 14](#).

The F-type ATPases are known as *ATP synthases* because they normally work in reverse: instead of ATP hydrolysis driving ion transport,  $\text{H}^+$  gradients across their membranes drive the synthesis of ATP from ADP and phosphate. The  $\text{H}^+$  gradients are generated

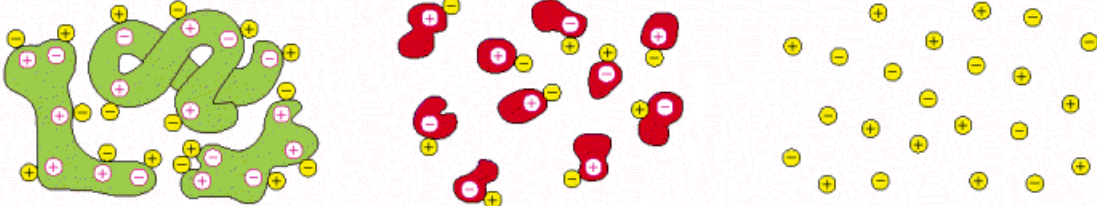


# Molecular Biology of the Cell 4th ed. Alberts et al., 2002

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*.

## 11-1. Intracellular Water Balance: the Problem and Its Solution

**SOURCES OF INTRACELLULAR OSMOLARITY**



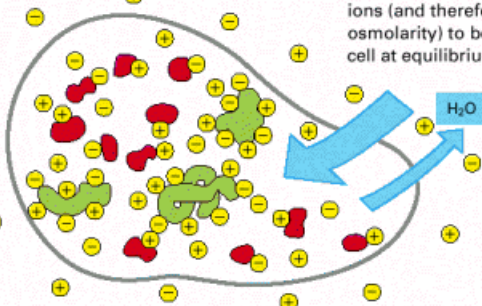
**Macromolecules themselves contribute very little to the osmolarity of the cell interior since, despite their large size, each one counts only as a single molecule and there are relatively few of them compared to the number of small molecules in the cell. However, most biological macromolecules are 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.**

**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.**

**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**, causing it to rupture.

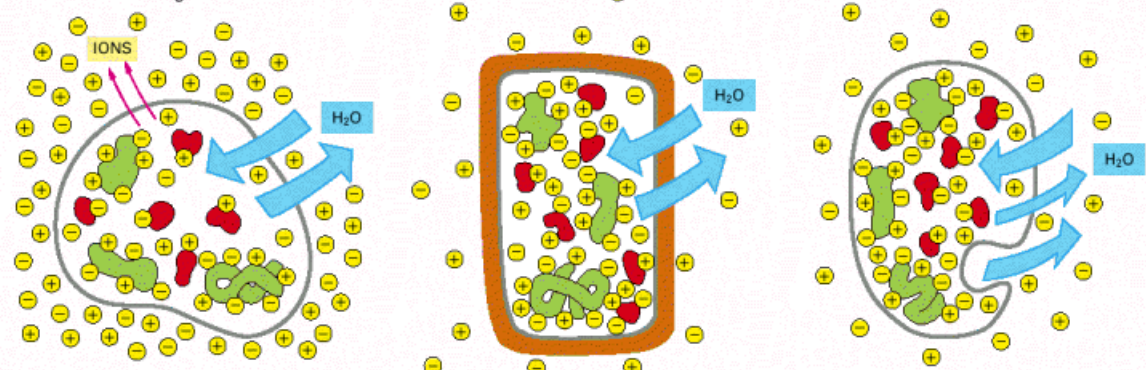


**THE SOLUTION**

**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.**



## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

The ATP synthases can, like the P-type transport ATPases, work in either direction: when the electrochemical gradient across their membrane drops below a threshold value, they will hydrolyze ATP and pump  $H^+$  across the membrane. Structurally related to the F-type ATPases is a distinct family of *V-type ATPases*. Certain organelles, such as lysosomes, synaptic vesicles, and plant vacuoles, contain V-type ATPases that pump  $H^+$  into the organelles and hence are responsible for acidification of their interiors. Thus, V-type ATPases normally pump protons rather than synthesize ATP. [↑ TOP](#)

### ABC Transporters Constitute the Largest Family of Membrane Transport Proteins

The last type of carrier protein that we discuss is a family of transport ATPases that are of great clinical importance, even though their normal functions in eucaryotic cells are only just beginning to be discovered. The first of these proteins to be characterized was found in bacteria. We have already mentioned that the plasma membranes of all bacteria contain carrier proteins that use the  $H^+$  gradient across the membrane to pump a variety of nutrients into the cell. Many 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 11-17), the transport ATPases are located in the inner membrane, and an auxiliary mechanism exists to capture the nutrients and deliver them to the transporters (Figure 11-18).

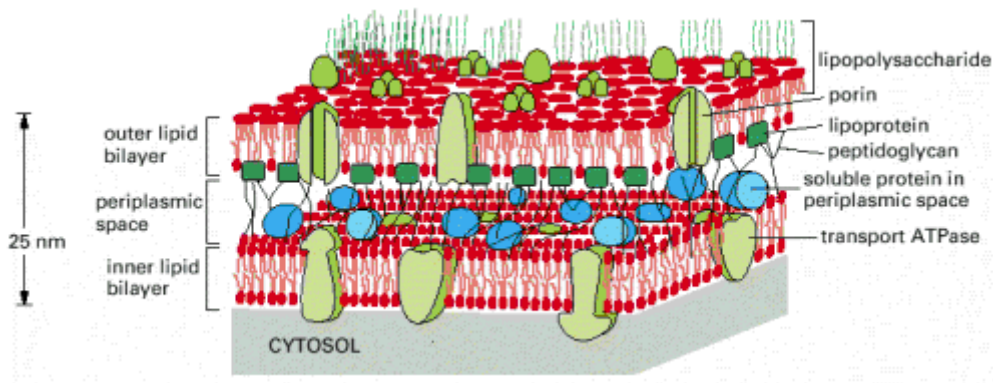


Figure 11-17. A small section of the double membrane of an *E. coli* bacterium. The inner membrane is the cell's plasma membrane. Between the inner and outer lipid bilayer membranes is a highly porous, rigid peptidoglycan, composed of protein and polysaccharide, that constitutes the bacterial cell wall. It is attached to lipoprotein molecules in the outer membrane and fills the *periplasmic space* (only a little of the peptidoglycan 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 therefore are called *Gram-positive*; their single membrane is analogous to the inner (plasma) membrane of Gram-negative bacteria.

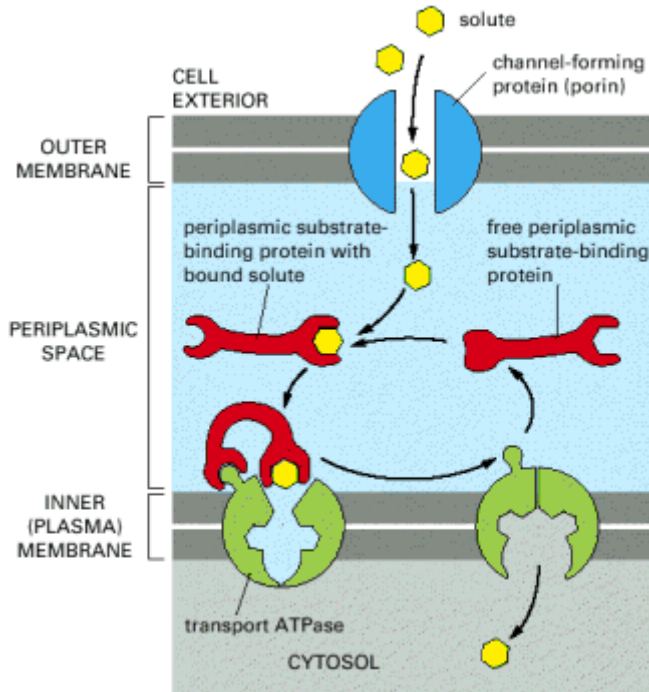


Figure 11-18. The auxiliary transport system associated with transport ATPases in bacteria with double membranes. The solute diffuses through channel-forming proteins (porins) in the outer membrane and binds to a *periplasmic substrate-binding protein*. As a result, the substrate-binding protein undergoes a conformational change that enables it to bind to an ABC transporter in the plasma membrane, which then picks up the solute and actively transfers it across the bilayer 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.

The transport ATPases in the bacterial plasma membrane belong to the largest and most diverse family of transport proteins known. It is called the **ABC transporter superfamily** because each member contains two highly conserved ATP-binding cassettes (Figure 11-19). ATP binding leads to dimerization of the two ATP-binding domains, and ATP hydrolysis leads to 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 to one or the other side of the membrane. In this way, ABC transporters use ATP binding and hydrolysis to transport molecules across the bilayer.

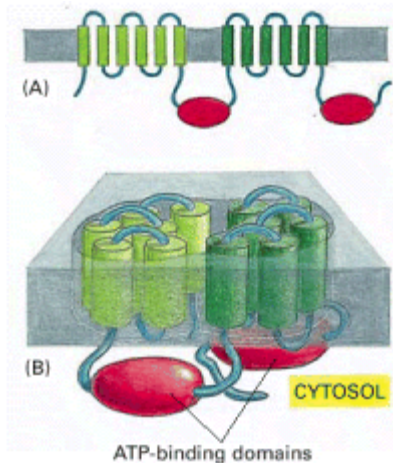


Figure 11-19. A typical ABC transporter. (A) A topology diagram. (B) A hypothetical arrangement of the polypeptide chain in the membrane. The transporter consists of four domains: two highly hydrophobic domains, each with six putative membrane-spanning segments that somehow form the translocation pathway, and two ATP-binding catalytic domains (or cassettes). In some cases the two halves of the transporter are formed by a single polypeptide (as shown), whereas in other cases they are formed by two or more separate polypeptides that assemble into a similar structure (see Figure 10-32).



## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

In *E. coli*, 78 genes (an amazing 5% of the bacterium's genes) encode ABC transporters, and animal cells contain many more. Although each is thought to be specific for a particular substrate or class of substrates, the variety of substrates transported by this superfamily is great and includes amino acids, sugars, inorganic ions, polysaccharides, peptides, and even proteins. Whereas bacterial ABC transporters are used for both import and export, those described in eucaryotes mostly seem specialized for export. ABC transporters also catalyze the flipping of lipids from one face of the lipid bilayer to the other, and thus have an important role in membrane biogenesis and maintenance, as discussed in [Chapter 12](#). When the substrates are lipids or overall hydrophobic molecules, the binding sites for them must be exposed on the surface of the transporter that is in contact with the hydrophobic interior of the lipid bilayer.

Indeed, the first eucaryotic ABC transporters identified were discovered because of their ability to pump hydrophobic drugs out of the cytosol. One of these is the **multidrug resistance (MDR) protein**, whose overexpression 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 selection of cells that overexpress the MDR transport protein. The transporter pumps the drugs out of the cell, thereby reducing their toxicity and conferring resistance to a wide variety of therapeutic agents. 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 with this parasite, which remains a major cause of human death, killing more than a million people every year. The control of malaria is hampered by the development of resistance to the antimalarial drug *chloroquine*, and resistant *P. falciparum* have been shown to have amplified a gene encoding an ABC transporter that pumps out the chloroquine.

In yeasts, an ABC transporter is responsible for exporting a mating pheromone (which is a peptide 12 amino acids long) across the yeast cell plasma membrane. In most vertebrate cells, an ABC transporter in the endoplasmic reticulum (ER) membrane actively transports a wide variety of peptides, produced by protein degradation, from the cytosol into the ER. This is the first step in a pathway of great importance in the surveillance of cells by the immune system (discussed in [Chapter 24](#)). The transported protein fragments, having entered the ER, are eventually carried to the cell surface, where they are displayed for scrutiny by cytotoxic T lymphocytes, which kill the cell if the fragments seem foreign (as they do if they derive from a virus or other microorganisms lurking in the cytosol).

Yet another member of the ABC family has been discovered through studies of the common genetic disease *cystic fibrosis*. This disease is caused by a mutation in a gene encoding an ABC transporter that functions as a regulator of a Cl<sup>-</sup> channel in the plasma membrane of epithelial cells. One in 27 white persons carries a mutant gene encoding this protein; and, in 1 in 2500, both copies of the gene are mutant, causing the disease. It is

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

still uncertain how the ABC transporter acts to regulate Cl<sup>-</sup> conductance across the membrane. [↑ TOP](#)

### Summary

Carrier proteins 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 carrier proteins simply transport a single solute “downhill,” whereas others can act as pumps to transport a solute “uphill” against its electrochemical gradient, using energy provided by ATP hydrolysis, by a downhill flow of another solute (such as Na<sup>+</sup> or H<sup>+</sup>), or by light to drive the requisite series of conformational changes in an orderly manner. Carrier proteins belong to a small number of families. Each family comprises proteins of similar amino acid sequence 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 the ubiquitous Na<sup>+</sup> - K<sup>+</sup> pump, 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 cancer cells and in 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 25](#)).

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. Because these proteins are concerned specifically with inorganic ion transport, they are referred to as **ion channels**. For transport efficiency, 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  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , or  $\text{Cl}^-$ —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 a diversity of such channels for receiving, conducting, and transmitting 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, through the narrowest part of the channel, which is called the *selectivity filter*; this limits their rate of passage. Thus, as ion concentrations are increased, the flux of ions 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 11-20](#)). In most cases, the gate opens in response to a specific stimulus. The main types of stimuli that are known to cause ion

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

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*) (Figure 11-21). The activity of many ion channels is regulated, in addition, by protein phosphorylation and dephosphorylation; this type of channel regulation is discussed, together with nucleotide-gated ion channels, in Chapter 15. 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.

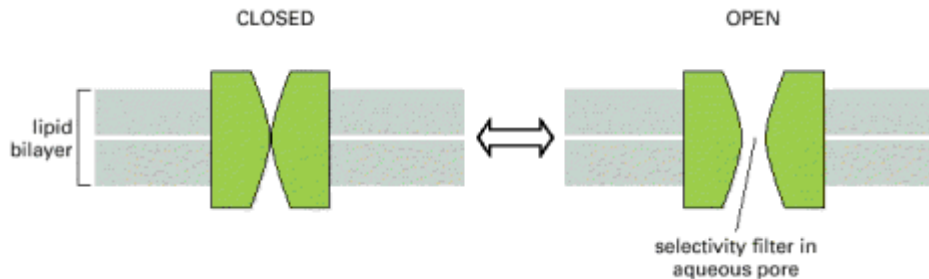


Figure 11-20. A typical ion channel, which fluctuates between closed and open conformations. The channel protein shown here in cross section forms a hydrophilic pore across the lipid bilayer only in the “open” conformational state. Polar groups are thought to line the wall of the pore, while hydrophobic 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.

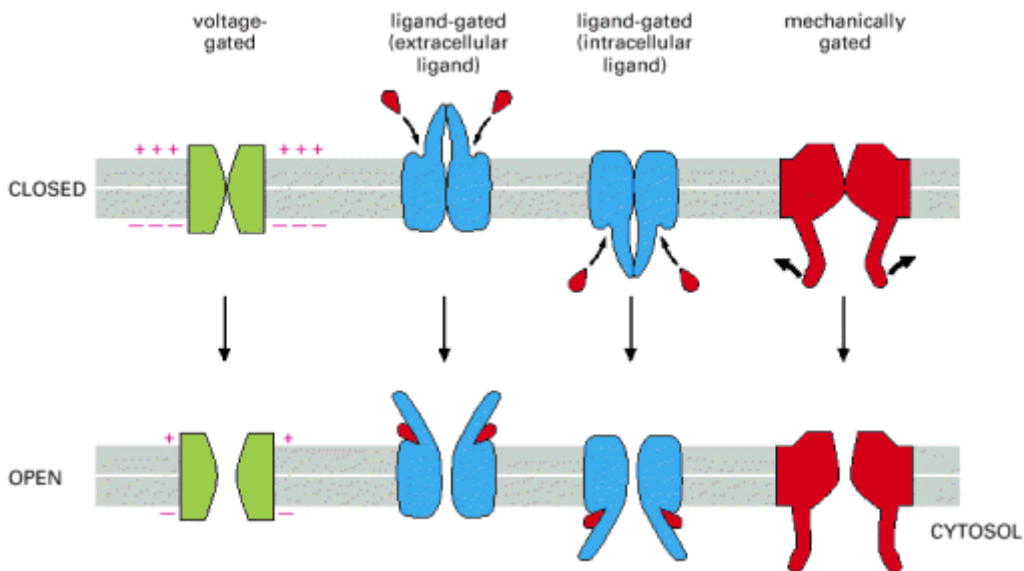


Figure 11-21. The gating of ion channels. This drawing shows different kinds of stimuli that open ion channels. Mechanically gated channels often have cytoplasmic extensions that link the channel to the cytoskeleton (not shown).

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

More than 100 types of ion channels have been described thus far, and new ones are still being added to the list. They 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 kinds of ion channels or more, 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 animal cells. An important subset of  $K^+$  channels are open even in an unstimulated or “resting” cell and are hence sometimes called  **$K^+$  leak channels**. Although this term covers a variety of 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. [↑ TOP](#)

### 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 Figure 11-22) and from passive ion diffusion. As we discuss in [Chapter 14](#), most of the membrane potential of the mitochondrion is generated by electrogenic  $H^+$  pumps in the mitochondrial inner 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 its equilibrium value can be calculated 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

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

concentration gradient. As  $K^+$  moves out, it 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, expresses the equilibrium condition quantitatively and, as explained in Panel 11-2, makes it possible to calculate the theoretical resting membrane potential if the ratio of internal and external ion concentrations is known. 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

The number of ions that must move across the plasma membrane to set up the membrane potential is minute. Thus, one can think of the membrane potential as arising from movements of charge that leave ion *concentrations* practically unaffected and result in only a very slight discrepancy in the number of positive and negative ions on the two sides of the membrane (Figure 11-22). Moreover, these movements of charge are generally rapid, taking only a few milliseconds or less.

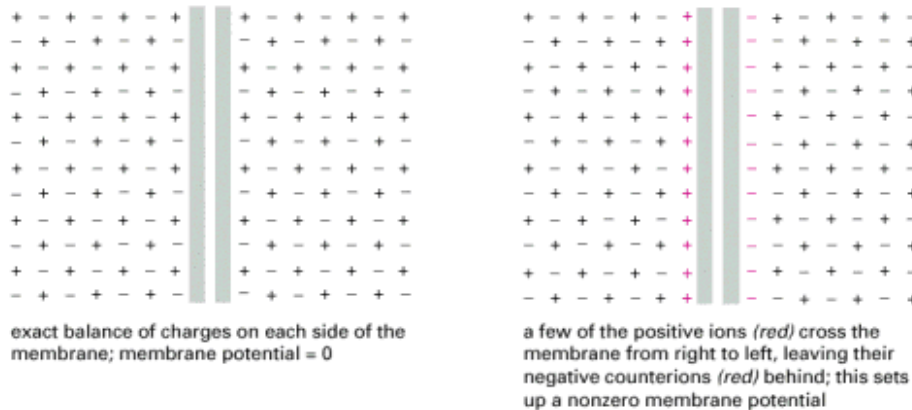


Figure 11-22. The ionic basis of a membrane potential. A small flow of ions carries sufficient charge to cause a large change in the membrane potential. The ions that give rise to the membrane potential lie in a thin ( $< 1$  nm) surface layer close to the membrane, held there by their electrical attraction to their oppositely charged counterparts (counterions) on the other side of the membrane. For a typical cell, 1 microcoulomb of charge ( $6 \times 10^{12}$  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  $10 \mu\text{m}$ , 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.



## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

It is illuminating to consider what happens to the membrane potential in a real cell if the  $\text{Na}^+$ - $\text{K}^+$  pump is suddenly inactivated. A slight drop in the membrane potential immediately occurs. This is because the pump is electrogenic and, when active, makes a small direct contribution to the membrane potential by pumping out three  $\text{Na}^+$  for every two  $\text{K}^+$  that it pumps in. However, switching off the pump does not abolish the major component of the resting potential, which is generated by the  $\text{K}^+$  equilibrium mechanism outlined above. This component persists as long as the  $\text{Na}^+$  concentration inside the cell stays low and the  $\text{K}^+$  ion concentration high—typically for many minutes. But the plasma membrane is somewhat permeable to all small ions, including  $\text{Na}^+$ . Therefore, without the  $\text{Na}^+$ - $\text{K}^+$  pump, the ion gradients set up by pumping will eventually run down, and the membrane potential established by diffusion through the  $\text{K}^+$  leak channels will fall as well. As  $\text{Na}^+$  enters, the osmotic balance is upset, and water seeps into the cell (see [Panel 11-1](#)). If the cell does not burst, it eventually comes to a new resting state where  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  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  $\text{Na}^+$ - $\text{K}^+$  pump.

The potential difference across the plasma membrane of an animal cell at rest varies between -20 mV and -200 mV, depending on the organism and cell type. Although the  $\text{K}^+$  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, one needs to know their atomic structure. The first ion channel to be crystallized and studied by x-ray diffraction was a bacterial  $\text{K}^+$  channel. The details of its structure revolutionized our understanding of ion channels. [↑ TOP](#)

### The Three-dimensional Structure of a Bacterial $\text{K}^+$ Channel Shows How an Ion Channel Can Work

The remarkable ability of ion channels to combine exquisite ion selectivity with a high conductance has long puzzled scientists.  $\text{K}^+$  leak channels, for example, conduct  $\text{K}^+$  10,000-fold better than  $\text{Na}^+$ , yet the two ions are featureless spheres with similar diameters (0.133 nm and 0.095 nm, respectively). A single amino acid substitution in the pore of a  $\text{K}^+$  channel can result in a loss of ion selectivity and cell death. The normal selectivity cannot be explained by pore size, because  $\text{Na}^+$  is smaller than  $\text{K}^+$ . Moreover, the high conductance rate is incompatible with the channel's having selective, high-affinity  $\text{K}^+$ -binding sites, as the binding of  $\text{K}^+$  ions to such sites would greatly slow their passage.



## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

The puzzle was solved when the structure of a *bacterial K<sup>+</sup> 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 are concentrated at the cytosolic entrance to the pore and are thought to attract cations and repel anions, making the channel cation-selective. Each subunit contributes two transmembrane 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<sup>+</sup> ions exit the channel. The polypeptide chain that connects the two transmembrane helices forms a short  $\alpha$  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<sup>+</sup> channels have similar amino acid sequences, it is likely that they form a closely similar structure. The crystal structure shows two K<sup>+</sup> ions in single file within the selectivity filter, separated by about 8 Å. Mutual repulsion between the two ions is thought to help move them through the pore into the extracellular fluid.

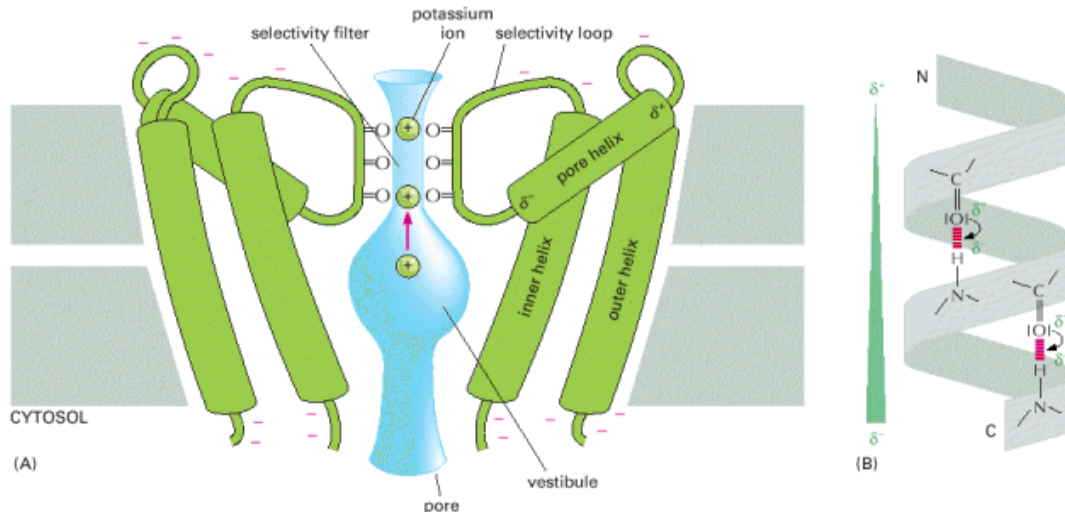


Figure 11-23. The structure of a bacterial K<sup>+</sup> channel. (A) Only two of the four identical subunits are shown. From the cytosolic side, the pore opens up into a vestibule in the middle of the membrane. The vestibule facilitates transport by allowing the K<sup>+</sup> ions to remain hydrated 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<sup>+</sup> ions. The positions of the K<sup>+</sup> 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<sup>+</sup> ions; from the differences in the diffraction patterns with K<sup>+</sup> ions and with rubidium ions in the channel, the positions of the ions could be calculated. Two K<sup>+</sup> ions occupy sites in the selectivity filter, while a third K<sup>+</sup> 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 point precisely toward the center of the vestibule, thereby guiding K<sup>+</sup> ions into the selectivity filter. (B) Because of the polarity of the hydrogen bonds (in red) that link adjacent turns of an  $\alpha$  helix, every  $\alpha$  helix has an electric dipole along its axis, with a more negatively charged C-terminal end ( $\delta^-$ ) and a more positively charged N-terminal end ( $\delta^+$ ). (A, Adapted from D.A. Doyle et al., *Science* 280:69–77, 1998.)

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

The structure of the selectivity filter explains the exquisite ion selectivity of the channel. For a  $K^+$  ion to enter the filter, it must lose almost all of its bound water molecules and interact instead with the carbonyl oxygens lining the selectivity filter, which 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).

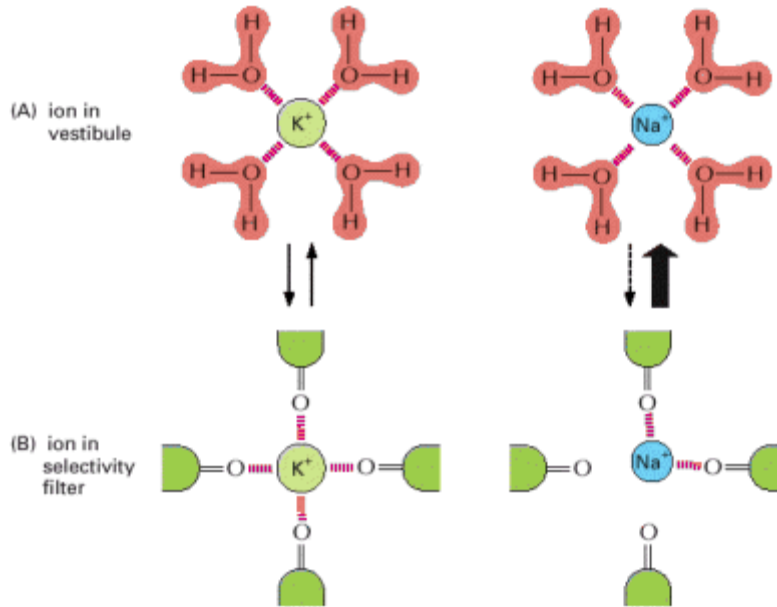


Figure 11-24.  $K^+$  specificity of the selectivity filter in a  $K^+$  channel. The drawing shows  $K^+$  and  $Na^+$  ions (A) in the vestibule and (B) in the selectivity filter of the pore, viewed in cross section. In the vestibule, the ions are hydrated. In the selectivity filter, the carbonyl oxygens 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 could 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.) Structural studies of the bacterial  $K^+$  channel have indicated how these channels may open and close. The loops that form the selectivity filter are rigid and do not change conformation when the channel opens or closes. In contrast, the inner and outer transmembrane helices that line the rest of the pore rearrange when the channel closes, causing the pore to constrict like a diaphragm at its cytosolic end (Figure 11-25). Although the pore does not close completely, the small opening that remains is lined by hydrophobic amino acid side chains, which block the entry of ions.

The cells that make most use of ion channels are neurons. Before discussing how they do so, we must digress to review briefly how a typical neuron is organized. [↑ TOP](#)

## The Function of a Nerve Cell 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 nerve cell in a human being, 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 nerve cells (Figure 11-26). Signals are also received on the cell body itself. The 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.

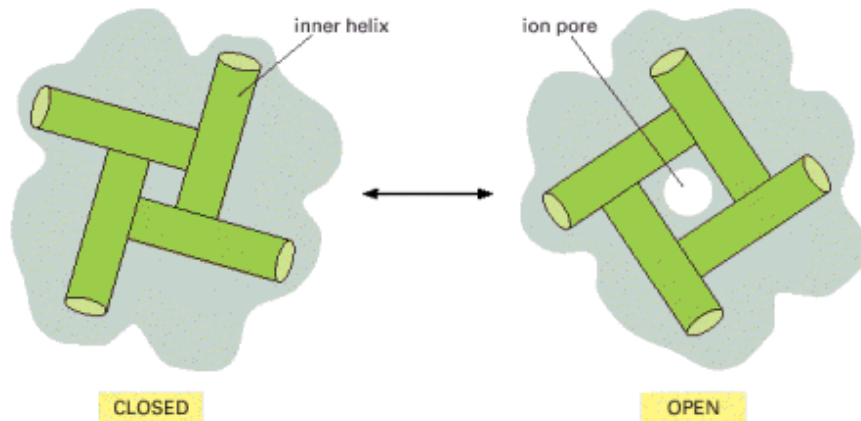


Figure 11-25. A model for the gating of a bacterial  $K^+$  channel. The channel is viewed in cross section. To adopt the closed conformation, the four inner transmembrane helices that line the pore on the cytosolic side of the selectivity filter (see Figure 11-22) rearrange to close the cytosolic entrance to the channel. (Adapted from E. Perozo et al., *Science* 285:73–78, 1999.)

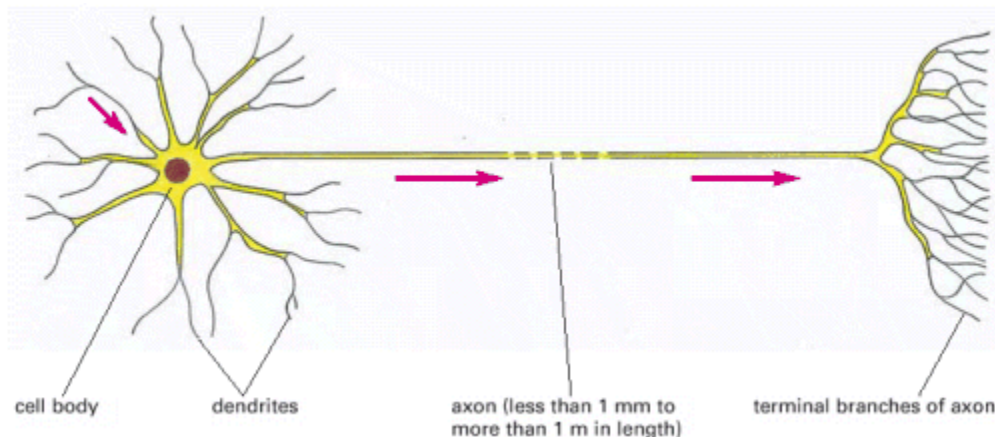


Figure 11-26. A typical vertebrate neuron. The arrows indicate the direction in which signals are conveyed. The single axon conducts signals away from the cell body, while the multiple dendrites receive signals from the axons of other neurons. The nerve terminals end on the dendrites or cell body of other neurons or on other cell types, such as muscle or gland cells.

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

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. Communication occurs because an electrical disturbance produced in one part of the cell spreads to other parts. Such a disturbance becomes weaker with increasing distance from its source, unless energy is expended 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, 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 is propagated rapidly along the neuron's plasma membrane and is sustained by automatic amplification all along the way. This traveling wave of electrical excitation, known as an action potential, or *nerve impulse*, can carry a message without attenuation from one end of a neuron to the other at speeds as great as 100 meters per second or more. Action potentials are the direct consequence of the properties of voltage-gated cation channels, as we shall now see. [↑ TOP](#)

### 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. (We shall see later how this can be caused by the action of a neurotransmitter.) In nerve and skeletal muscle cells, a stimulus that causes sufficient depolarization promptly causes **voltage-gated Na<sup>+</sup> channels** to open, allowing a small amount of Na<sup>+</sup> to enter the cell down its electrochemical gradient. The influx of positive charge depolarizes the membrane further, thereby opening more Na<sup>+</sup> channels, which admit more Na<sup>+</sup> ions, causing still further depolarization. This process continues in a self-amplifying fashion 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<sup>+</sup> equilibrium potential of about +50 mV (see Panel 11-2). At this point, when the net electrochemical driving force for the flow of Na<sup>+</sup> is almost zero, the cell would come to a new resting state, with all of its Na<sup>+</sup> channels permanently open, if the open conformation of the channel were stable. The cell is saved from such a permanent electrical spasm by two mechanisms that act in concert: inactivation of the Na<sup>+</sup> channels, and opening of voltage-gated K<sup>+</sup> channels.

The Na<sup>+</sup> channels have an automatic inactivating mechanism, which causes the channels to reclose rapidly even though the membrane is still depolarized. The Na<sup>+</sup> channels remain in this *inactivated* state, unable to reopen, until a few milliseconds after the membrane potential has returned to its initial negative value. The Na<sup>+</sup> channel can therefore exist in three distinct states—closed, open, and inactivated. How they contribute to the rise and fall of the action potential is shown in Figure 11-27.



# Molecular Biology of the Cell 4th ed. Alberts et al., 2002

## 11-2. The Derivation of the Nernst Equation

### 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 ( $2 \text{ cal mol}^{-1} \text{ K}^{-1}$ )

$T$  = the absolute temperature (K)

$F$  = Faraday's constant ( $2.3 \times 10^4 \text{ cal V}^{-1} \text{ mol}^{-1}$ )

$z$  = 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 and discussed in Panel 14-1, p. 784.) The free-energy change per mole of solute moved across the plasma membrane ( $\Delta G_{\text{conc}}$ ) 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  $\Delta G_{\text{volt}} = zFV$ . At the point where the concentration and voltage gradients just balance,  $\Delta G_{\text{conc}} + \Delta G_{\text{volt}} = 0$  and the ion distribution is at equilibrium across the membrane. Thus,

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

and, therefore,

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

For a univalent ion,

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

Thus, for such an ion at  $37^\circ\text{C}$ ,  $V = +61.5 \text{ mV}$  for  $C_o / C_i = 10$ , whereas  $V = 0$  for  $C_o / C_i = 1$ .

The  $\text{K}^+$  equilibrium potential ( $V_K$ ), for example, is  $61.5 \log_{10}([K^+]_o / [K^+]_i)$  millivolts ( $-89 \text{ mV}$  for a typical cell where  $[K^+]_o = 5 \text{ mM}$  and  $[K^+]_i = 140 \text{ mM}$ ). At  $V_K$ , there is no net flow of  $\text{K}^+$  across the membrane. Similarly, when the membrane potential has a value of  $61.5 \log_{10}([Na^+]_o / [Na^+]_i)$ , the  $\text{Na}^+$  equilibrium potential ( $V_{Na}$ ), there is no net flow of  $\text{Na}^+$ .

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  $\text{K}^+$  it is  $V_M - V_K$  and for  $\text{Na}^+$  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  $\text{Na}^+$  ions across  $1 \mu\text{m}^2$  of membrane will carry sufficient charge to shift the membrane potential by about  $100 \text{ mV}$ . Because there are about  $3 \times 10^7$   $\text{Na}^+$  ions in a typical cell ( $1 \mu\text{m}^3$  of bulk cytoplasm), such a movement of charge will generally have a negligible effect on the ion concentration gradients across the membrane.

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

The description just given of an action potential concerns only 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 spreads as a traveling wave from the initial site of depolarization to involve the entire plasma membrane, as shown in [Figure 11-28](#).

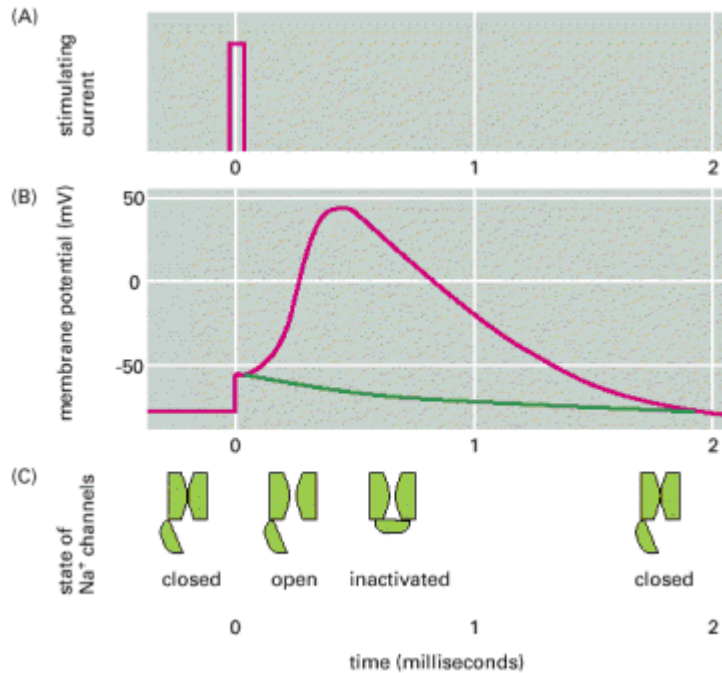


Figure 11-27. An action potential. (A) An action potential is triggered by a brief pulse of current, which (B) partially depolarizes the membrane, as shown in the plot of membrane potential versus time. The *green curve* shows how the membrane potential would have simply relaxed back to the resting value after the initial depolarizing stimulus if there had been no voltage-gated ion 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<sup>+</sup> channels occurs because of the efflux of K<sup>+</sup> through K<sup>+</sup> channels, which open in response to membrane depolarization and drive the membrane back toward the K<sup>+</sup> 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<sup>+</sup> channels, whose state is shown in (C). The membrane cannot fire a second action potential until the Na<sup>+</sup> channels have returned to the closed conformation; until then, the membrane is refractory to stimulation.

**Voltage-gated K<sup>+</sup> 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, so that the transient influx of Na<sup>+</sup> is rapidly overwhelmed by an efflux of K<sup>+</sup>, which quickly drives the membrane back toward the K<sup>+</sup> equilibrium potential, even before the inactivation of the Na<sup>+</sup> channels is complete. These K<sup>+</sup> channels respond to changes in membrane potential in much the same way as the Na<sup>+</sup> channels do, but with slightly slower kinetics; for this reason; they are sometimes called *delayed K<sup>+</sup> channels*.

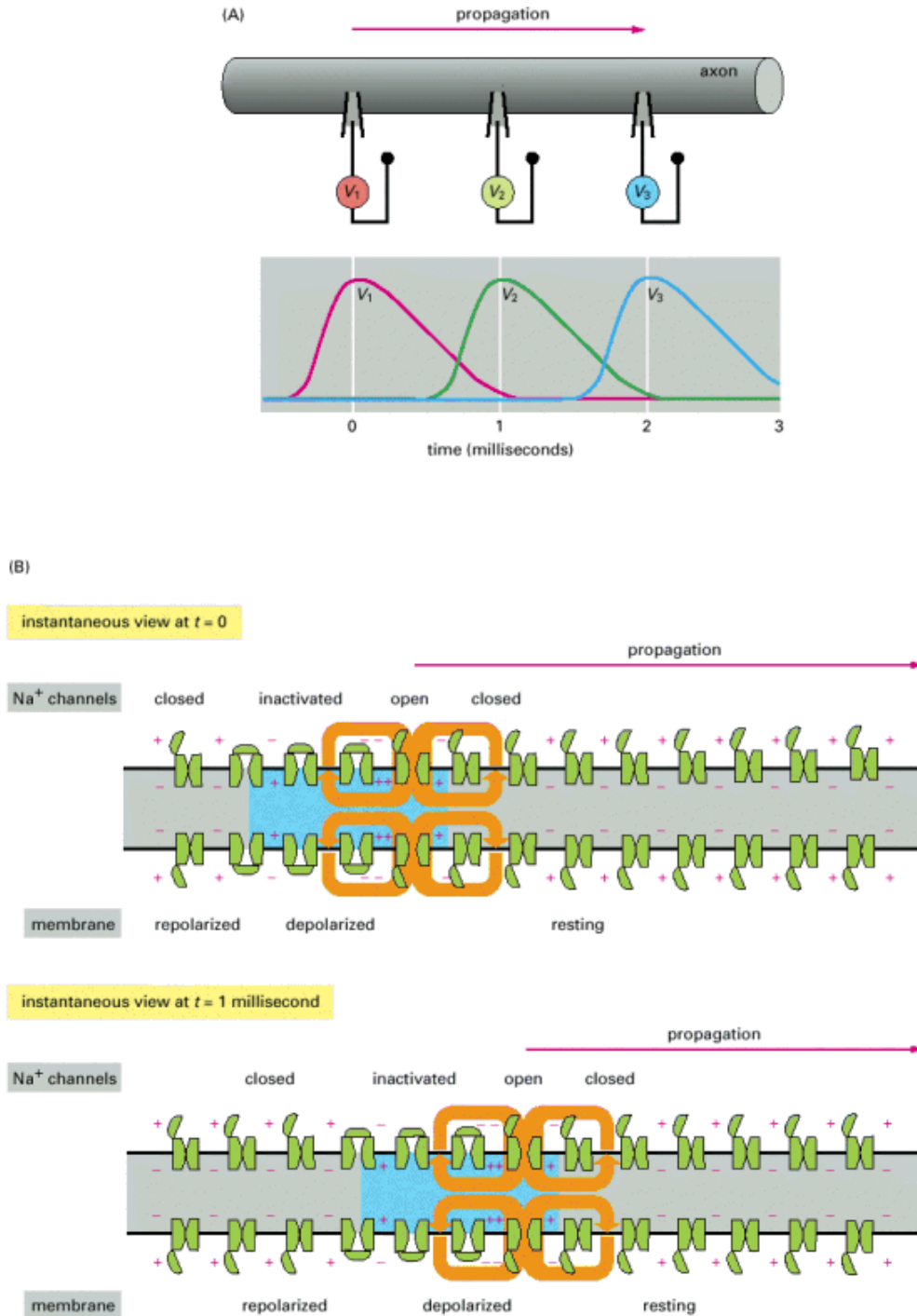


Figure 11-28. The propagation of an action potential along an axon. (A) The voltages that would be recorded from a set of intracellular electrodes placed at intervals along the axon. (B) The changes in the  $\text{Na}^+$  channels and the current flows (*orange arrows*) that give rise to the traveling 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  $\text{Na}^+$ -channel inactivation prevents the depolarization from spreading backward (see also [Figure 11-30](#)). On myelinated axons, clusters of  $\text{Na}^+$  channels can be millimeters apart from each other.



## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

Like the  $\text{Na}^+$  channel, voltage-gated  $\text{K}^+$  channels can inactivate. Studies of mutant voltage-gated  $\text{K}^+$  channels show that the N-terminal 20 amino acids of the channel protein are required for rapid inactivation of the channel. If this region is altered, the kinetics of channel inactivation are changed, and if the region is entirely removed, inactivation is abolished. Amazingly, in the latter case, inactivation can be restored by exposing the cytoplasmic face of the plasma membrane to a small synthetic peptide corresponding to the missing amino terminus. These findings suggest that the amino terminus of each  $\text{K}^+$  channel subunit acts like a tethered ball that occludes the cytoplasmic end of the pore soon after it opens, thereby inactivating the channel (Figure 11-29). A similar mechanism is thought to operate in the rapid inactivation of voltage-gated  $\text{Na}^+$  channels (which we discuss later), although a different segment of the protein seems to be involved.

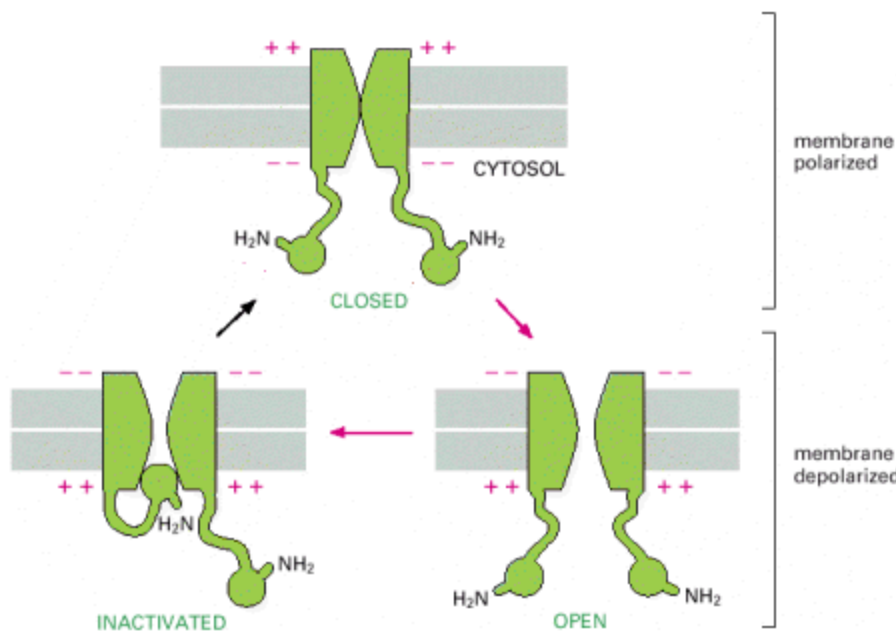


Figure 11-29. The “ball-and-chain” model of rapid inactivation for a voltage-gated  $\text{K}^+$  channel. When the membrane potential is depolarized, the channel opens and begins to conduct ions. If the depolarization is maintained, the open channel adopts an inactive conformation, where the pore is 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  $\text{Na}^+$  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.

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

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](#) outlines 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 myelin sheaths in some regions of the central nervous system are destroyed; where this happens, 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 11-30](#)), thereby insulating the axonal membrane so that little current can leak across it. The myelin sheath is interrupted at regularly spaced *nodes of Ranvier*, where almost all the  $\text{Na}^+$  channels in the axon are concentrated. 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. [↑ TOP](#)

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

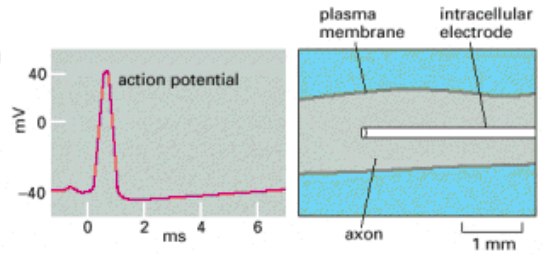
Neuron and skeletal muscle cell plasma membranes contain many thousands of voltage-gated  $\text{Na}^+$  channels, and the current crossing the membrane is the sum of the currents flowing through all of these. This aggregate current can be recorded with an intracellular microelectrode, as shown in [Figure 11-28](#). Remarkably, however, it is also possible to record current flowing through individual channels. This is achieved by means of patch-clamp recording, a method that has revolutionized the study of ion channels by allowing researchers to examine transport through a single molecule of channel protein in a small patch of membrane covering the mouth of a micropipette ([Figure 11-31](#)). With this simple but powerful technique, the detailed properties of ion channels can be studied in

# Molecular Biology of the Cell 4th ed. Alberts et al., 2002

## 11-3. Some Classical Experiments on the Squid Giant Axon

### 1. Action potentials are recorded with an intracellular electrode

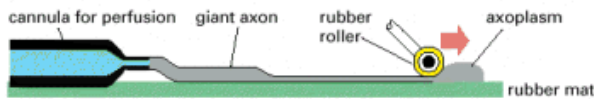
The squid giant axon is about 0.5–1 mm in diameter and several centimeters long. An electrode in the form of a glass capillary tube containing a conducting solution can be thrust down the axis of the axon so that its tip lies deep in the cytoplasm. With its help, one can measure the voltage difference 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.



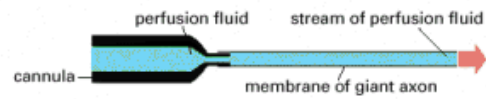
### 2. Action potentials depend only on the neuronal plasma membrane and on gradients of Na<sup>+</sup> and K<sup>+</sup> across it

The three most plentiful ions, both inside and outside the axon, are Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup>. As in other cells, the Na<sup>+</sup>-K<sup>+</sup> pump maintains a concentration gradient: the concentration of Na<sup>+</sup> is about 9 times lower inside the axon than outside, while the concentration of K<sup>+</sup> 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 cytoplasm from it, like toothpaste from a tube, and then



to perfuse it internally with pure artificial solutions of Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup> or SO<sub>4</sub><sup>2-</sup>. Remarkably, if (and only if) the concentrations of Na<sup>+</sup> and K<sup>+</sup> 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<sup>+</sup> and K<sup>+</sup>; and a sufficient source of free energy to power the action potential must be provided by their concentration gradients across the membrane, because all other sources of metabolic energy have presumably been removed by the perfusion.

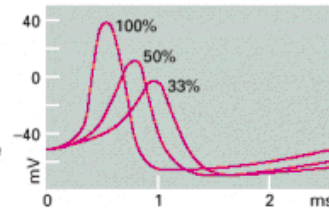


### 3. At rest, the membrane is chiefly permeable to K<sup>+</sup>; during the action potential, it becomes transiently permeable to Na<sup>+</sup>

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

If the external concentration of Na<sup>+</sup> 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<sup>+</sup>. During the action potential, therefore, the membrane appears to be chiefly permeable to Na<sup>+</sup>: Na<sup>+</sup> 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<sup>+</sup> and is even closer to the K<sup>+</sup> equilibrium potential than the resting potential is: the membrane has lost most of its permeability to Na<sup>+</sup> and has become even more permeable to K<sup>+</sup> than before—that is, Na<sup>+</sup> channels have closed, and additional K<sup>+</sup> channels have opened.



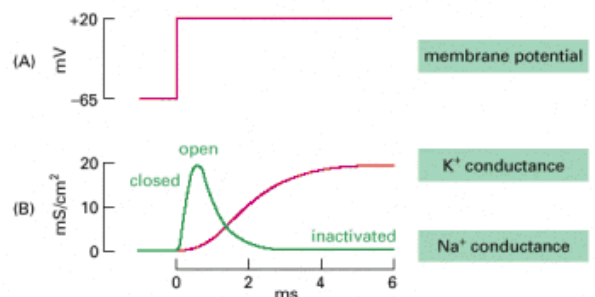
The form of the action potential when the external medium contains 100%, 50%, or 33% of the normal concentration of Na<sup>+</sup>.

### 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<sup>+</sup> channels rapidly open until the Na<sup>+</sup> permeability of the membrane is much greater than the K<sup>+</sup> permeability; they then close again spontaneously, even though the membrane potential is clamped and unchanging. K<sup>+</sup> channels also open but with a delay, so that the K<sup>+</sup> permeability increases as the Na<sup>+</sup> 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<sup>+</sup> 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 influx of Na<sup>+</sup> through the opened Na<sup>+</sup> channels produces the spike of the action potential; inactivation of Na<sup>+</sup> channels and opening of K<sup>+</sup> channels bring the membrane rapidly back down to the resting potential.





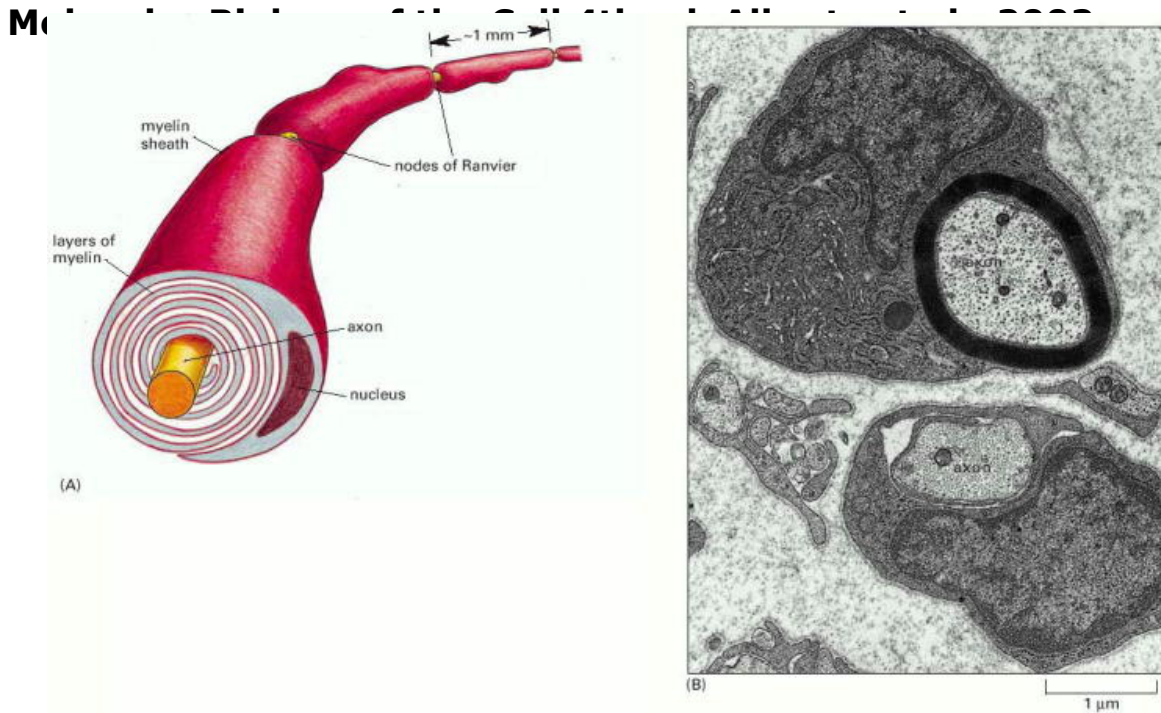


Figure 11-30. Myelination. (A) A myelinated axon from a peripheral nerve. Each Schwann cell wraps its plasma membrane concentrically around the axon to form a segment of myelin sheath about 1 mm long. For clarity, the layers of myelin in this drawing are not shown compacted together as tightly as they 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. (B, from Cedric S. Raine, in *Myelin* [P. Morell, ed.]. New York: Plenum, 1976.)

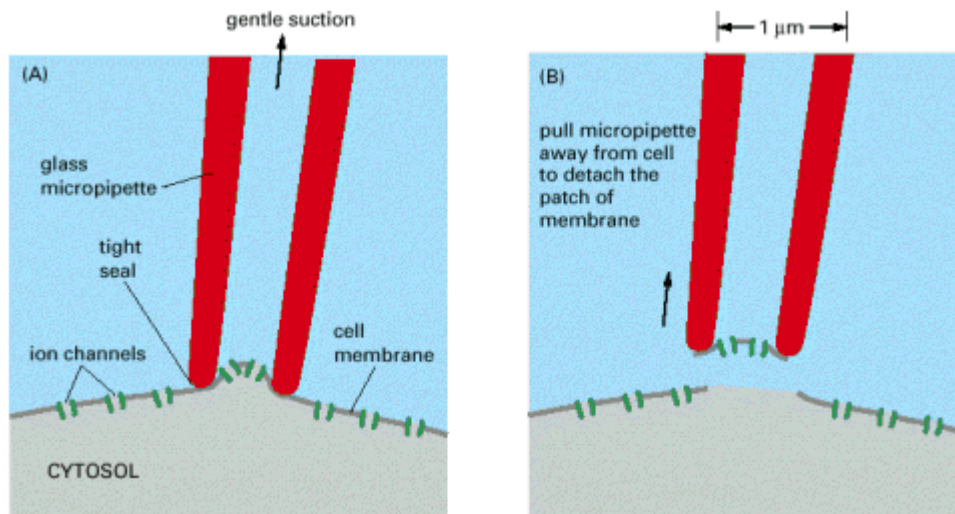


Figure 11-31. The technique of patch-clamp recording. Because of the extremely tight seal between the micropipette and the membrane, current can

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. Recordings of the current through these channels can be made 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.

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

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  $\text{Na}^+$  channels open in an all-or-nothing fashion. The times of a channel's opening and closing are 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 11-32).

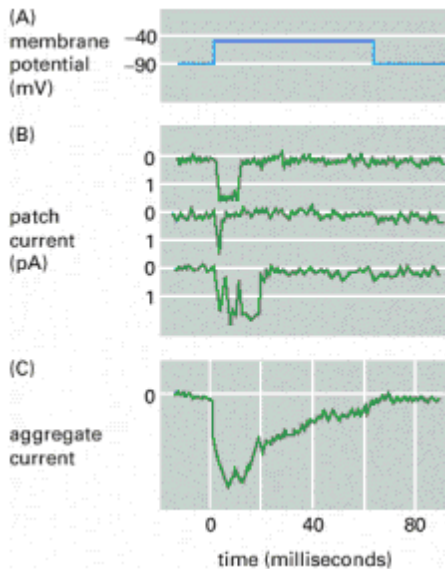


Figure 11-32. Patch-clamp measurements for a single voltage-gated  $\text{Na}^+$  channel. A tiny patch of plasma membrane was detached from an embryonic rat muscle cell, as in [Figure 11-31](#). (A) The membrane was depolarized by an abrupt shift of potential. (B) Three current records from three experiments 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). (C) The sum of the currents measured in 144 repetitions of the same experiment.

This aggregate current is equivalent to the usual  $\text{Na}^+$  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. © The Rockefeller University Press.)

The phenomenon of voltage gating can be understood in terms of simple physical principles. 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 membrane are thus



## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

subjected to a very large electrical field. These proteins, like all others, have a number of 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 a number of alternative conformations whose stabilities depend on the strength of the field. Voltage-gated  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  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 11-29](#)). [↑ TOP](#)

### Voltage-gated Cation Channels Are Evolutionarily and Structurally Related

$\text{Na}^+$  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  $\text{Ca}^{2+}$  channels* rather than on  $\text{Na}^+$  channels. Moreover, voltage-gated  $\text{Na}^+$ ,  $\text{K}^+$ , or  $\text{Ca}^{2+}$  channels of unknown function are found in some cell types that are not normally electrically active.

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  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  channels show striking similarities, suggesting 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  $\text{K}^+$  channel, the genome of the worm *C. elegans* contains 68 genes that encode different but related  $\text{K}^+$  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 on where the gene is expressed. Mutations in genes that encode voltage-gated  $\text{Na}^+$  channels in skeletal muscle cells, for example, can cause *myotonia*, a condition in which muscle relaxation after voluntary contraction is greatly delayed, causing painful muscle spasms. In some cases this is because the abnormal channels fail to inactivate normally; as a result,  $\text{Na}^+$  entry persists after an action potential finishes and repeatedly reinitiates membrane depolarization and muscle contraction. Similarly, mutations that affect  $\text{Na}^+$  or  $\text{K}^+$  channels in the brain can cause *epilepsy*, where excessive synchronized firing of large groups of nerve cells cause epileptic seizures (convulsions, or fits). [↑ TOP](#)

## 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 a 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 11-33). 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  $\text{Na}^+$ -dependent neurotransmitter carrier proteins; 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.

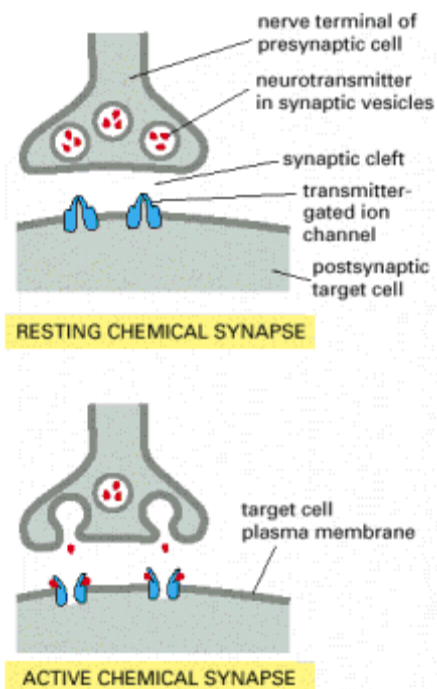


Figure 11-33. A chemical synapse. When an action potential reaches the nerve terminal in a presynaptic cell, it stimulates the terminal to release its neurotransmitter. The neurotransmitter molecules are contained in synaptic vesicles and are released to the cell exterior when the vesicles fuse with 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.

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

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 11-33](#)). 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 how much neurotransmitter is 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 increases enough to open a sufficient number of nearby voltage-gated cation channels that are present in the same target cell membrane. [↑ TOP](#)

### 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 as to 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  $\text{Na}^+$  that depolarizes the postsynaptic membrane toward the threshold potential for firing an action potential. Inhibitory neurotransmitters, by contrast, open either  $\text{Cl}^-$  channels or  $\text{K}^+$  channels, and this suppresses firing by making it harder for excitatory influences to depolarize the postsynaptic membrane. Many transmitters can be either excitatory and 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  *$\gamma$ -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. The effect of opening  $\text{Cl}^-$  channels can be understood as follows. The concentration of  $\text{Cl}^-$  is much higher outside the cell than inside (see [Table 11-1](#)), but its influx is opposed by the membrane potential. In fact, for many neurons, the equilibrium potential for  $\text{Cl}^-$  is close to the resting potential—or even more negative. For this reason, opening  $\text{Cl}^-$  channels tend to buffer the membrane potential; as the membrane starts to depolarize, more negatively charged  $\text{Cl}^-$  ions enter the cell and counteract the effect. Thus, the opening of  $\text{Cl}^-$  channels makes it more difficult to depolarize the membrane and hence to excite the cell. The opening of  $\text{K}^+$  channels has a similar effect. The importance of inhibitory neurotransmitters is demonstrated by the effects of toxins that block their

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

action: strychnine, for example, by binding to glycine receptors and blocking the 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. These so-called *G-protein-linked receptors* and *enzyme-linked receptors* are discussed 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-linked receptors and enzyme-linked receptors tends to be far slower and more complex, and longer lasting in its consequences. [↑ TOP](#)

### 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 11-34](#)). This synapse has been intensively investigated because it is readily accessible to electrophysiological study, unlike most of the synapses in the central nervous system.

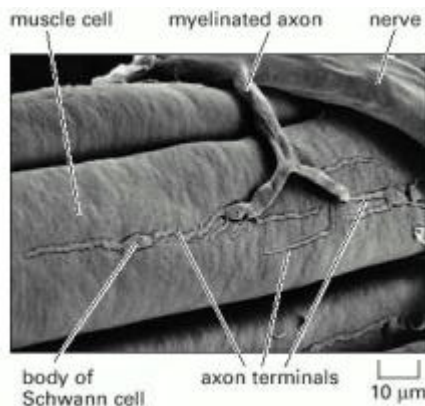


Figure 11-34. A low-magnification scanning electron micrograph of a neuromuscular junction in a frog. The

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 it is the only ligand-gated channel whose three-dimensional structure has been determined, albeit at 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 electrical organs of electric fish and rays (these organs are modified muscles designed to deliver a large electrical shock to prey). Second, certain neurotoxins (such as

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

*$\alpha$ -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  *$\alpha$ -bungarotoxin* can also be used to localize and count acetylcholine receptors. In this way, it has been shown that the receptors are densely packed in the muscle cell plasma membrane at a neuromuscular junction (about 20,000 such receptors per  $\mu\text{m}^2$ ), with relatively few receptors elsewhere in the same membrane. termination of a single axon on a skeletal muscle cell is shown. (From J. Desaki and Y. Uehara, *J. Neurocytol.* 10:101–110, 1981. © Kluwer Academic Publishers.)

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 have binding sites for acetylcholine. When two acetylcholine molecules bind to the pentameric complex, they induce a conformational change that opens the channel. With ligand bound, the channel still flickers between open and closed states, but now has a 90% probability of being in the open state. This state continues until the concentration of acetylcholine is lowered sufficiently by hydrolysis by a specific enzyme (acetylcholinesterase) located in the neuromuscular junction. 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 11-35](#)).

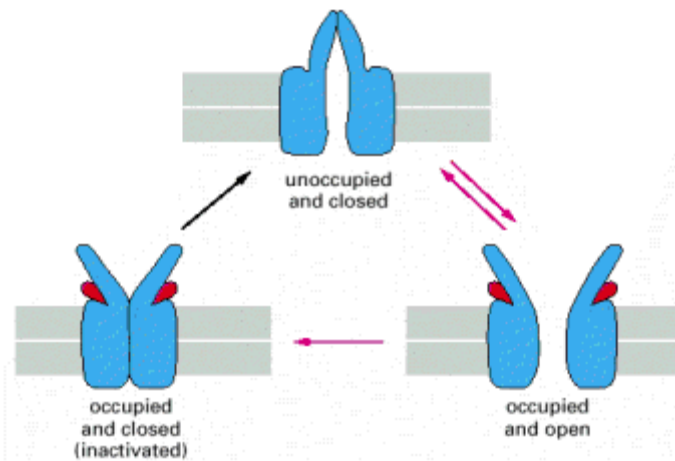


Figure 11-35. Three conformations of the acetylcholine receptor. The binding of two acetylcholine molecules opens this transmitter-gated ion channel. It then maintains a high probability of being open until the acetylcholine has been hydrolyzed. In the persistent presence of acetylcholine, however, the 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.



## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

The general shape of the acetylcholine receptor and the likely arrangement of its subunits have been determined by electron microscopy (Figure 11-36). 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  $\text{Na}^+$  and  $\text{K}^+$ , together with some  $\text{Ca}^{2+}$ . Thus, unlike voltage-gated cation channels, such as the  $\text{K}^+$  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  $\text{K}^+$  is near zero, since the voltage gradient nearly balances the  $\text{K}^+$  concentration gradient across the membrane (see Panel 11-2). For  $\text{Na}^+$ , 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  $\text{Ca}^{2+}$ , but the extracellular concentration of  $\text{Ca}^{2+}$  is so much lower than that of  $\text{Na}^+$  that  $\text{Ca}^{2+}$  makes only a small contribution to the total inward current.) Therefore, the opening of the acetylcholine receptor channels leads to a large net influx of  $\text{Na}^+$  (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. [↑ TOP](#)

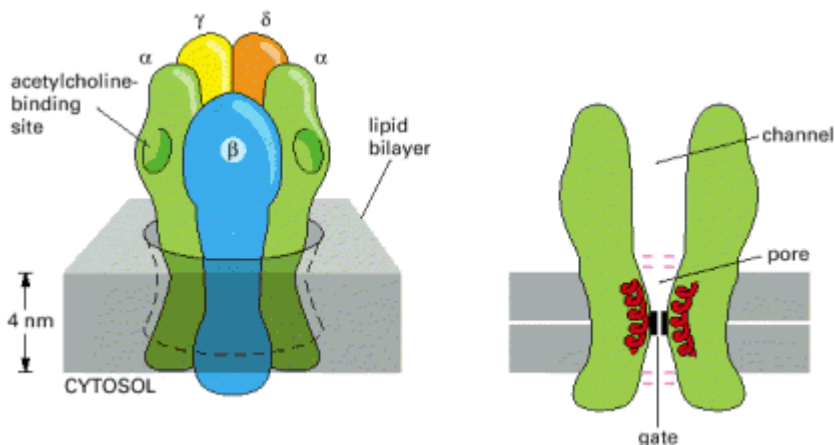


Figure 11-36. A model for the structure of the acetylcholine receptor. Five homologous subunits ( $\alpha$ ,  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) combine to form a transmembrane aqueous pore. The pore is lined by a ring of five transmembrane  $\alpha$  helices, one contributed by each subunit. In its closed conformation, the pore is thought to be occluded by the hydrophobic side chains of five leucines, one from each  $\alpha$  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. Both of the  $\alpha$  subunits contain an acetylcholine-binding site; when acetylcholine binds to both sites, the channel undergoes a conformational change that opens the gate, possibly by causing the leucines to move outward. (Adapted from N. Unwin, *Cell/Neuron* 72/10 [Suppl.]:31-41, 1993.)

## 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, suggesting that they are evolutionarily related and probably form transmembrane pores in the same way, even though their neurotransmitter-binding specificities and ion selectivities are distinct. These channels seem to have a similar overall structure, in each case formed by 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, like the  $K^+$  channels discussed earlier.

For each class of transmitter-gated ion channels, alternative forms of each type of subunit exist, either encoded by distinct genes or generated by alternative RNA splicing of the same gene product. These combine in different variations 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 are characterized by 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 influencing particular brain functions specifically.

Indeed, transmitter-gated ion channels have for a long time been important targets for drugs. A surgeon, for example, can make muscles relax 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 poison arrows. Most drugs used in the treatment of 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 discussed earlier, many neurotransmitters are cleared from the synaptic cleft after release by  $Na^+$ -driven carriers. The inhibition of such a carrier prolongs the effect of the transmitter and thereby strengthens synaptic

# Molecular Biology of the Cell 4th ed. Alberts et al., 2002

transmission. Many antidepressant drugs, including Prozac, for example, act by inhibiting 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. [↑ TOP](#)

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

The importance of ion channels to electrically excitable cells can be illustrated by following the process whereby a nerve impulse stimulates a muscle cell to contract. This apparently simple response requires the sequential activation of at least five different sets of ion channels, all within a few milliseconds ([Figure 11-37](#)).

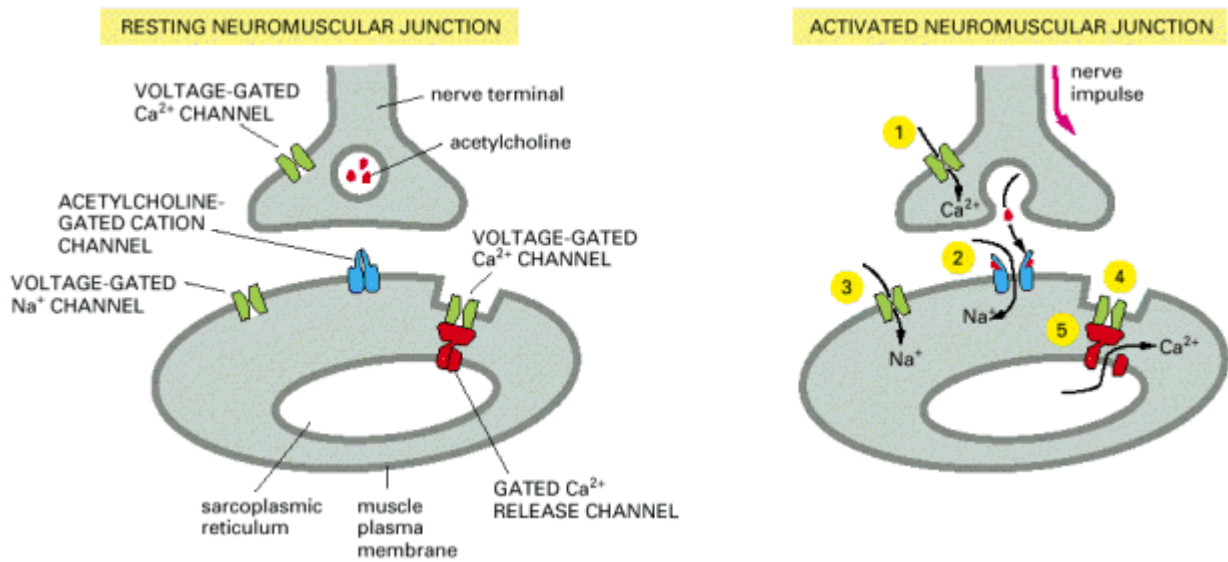


Figure 11-37. The system of ion channels at a neuromuscular junction. These gated ion channels are essential for the stimulation of muscle contraction by a nerve impulse. The various channels are numbered in the sequence in which they are activated, as described in the text.

**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<sup>2+</sup> channels in this membrane. As the Ca<sup>2+</sup> concentration outside cells is more than 1000 times greater than the free Ca<sup>2+</sup> concentration inside, Ca<sup>2+</sup> flows into the nerve terminal. The increase in Ca<sup>2+</sup> concentration in the cytosol of the nerve terminal triggers the localized release of acetylcholine into the synaptic cleft.

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

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  $\text{Na}^+$  causes a localized membrane depolarization.
3. The local depolarization of the muscle cell plasma membrane opens voltage-gated  $\text{Na}^+$  channels in this membrane, allowing more  $\text{Na}^+$  to enter, which further depolarizes the membrane. This, in turn, opens neighboring voltage-gated  $\text{Na}^+$  channels and results in a self-propagating depolarization (an action potential) that spreads to involve the entire plasma membrane (see [Figure 11-28](#)).
4. The generalized depolarization of the muscle cell plasma membrane activates voltage-gated  $\text{Ca}^{2+}$  channels in specialized regions (the transverse [T] tubules—discussed in [Chapter 16](#)) of this membrane.
5. This, in turn, causes  *$\text{Ca}^{2+}$  release channels* in an adjacent region of the sarcoplasmic reticulum membrane to open transiently and release the  $\text{Ca}^{2+}$  stored in the sarcoplasmic reticulum into the cytosol. It is the sudden increase in the cytosolic  $\text{Ca}^{2+}$  concentration that causes the myofibrils in the muscle cell to contract. It is not certain how the activation of the voltage-gated  $\text{Ca}^{2+}$  channels in the T-tubule membrane leads to the opening of the  $\text{Ca}^{2+}$  release channels in the sarcoplasmic reticulum membrane. The two membranes are closely apposed, however, with the two types of channels joined together in a specialized structure (see [Figure 16-74](#)). It is possible, therefore, that a voltage-induced change in the conformation of the plasma membrane  $\text{Ca}^{2+}$  channel directly opens the  $\text{Ca}^{2+}$  release channel in the sarcoplasmic reticulum through a mechanical coupling.

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. [↑ TOP](#)

### 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 synapse on 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 11-38](#)). 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

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

hyperpolarization called an *inhibitory PSP*. The membrane of the dendrites and cell body of most neurons contains a relatively low density of voltage-gated  $\text{Na}^+$  channels, and an individual excitatory PSP is generally too small to 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. Because the cell body is small compared with the dendritic tree, its membrane potential is roughly uniform and is a composite of the effects of all the signals impinging on the cell, weighted according to the distances of the synapses from the cell body. The *combined PSP* of the cell body thus represents a **spatial summation** of all the stimuli being received. If excitatory inputs predominate, the combined PSP is a depolarization; if inhibitory inputs predominate, it is usually a hyperpolarization.

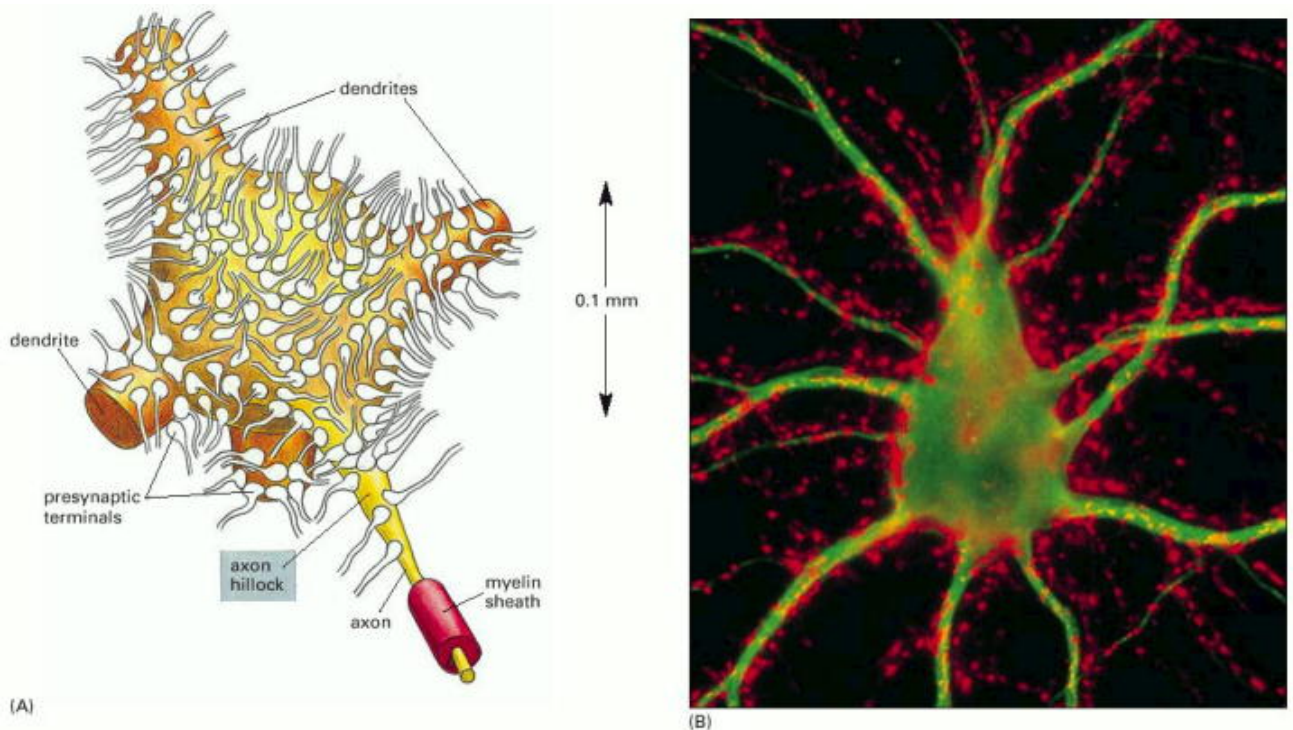


Figure 11-38. A motor neuron cell body in the spinal cord. (A) Many thousands of nerve terminals synapse on the cell body and dendrites. These deliver signals from other parts of the organism to control the firing of action potentials along the single axon of this large cell. (B) Micrograph showing a 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).



## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

Whereas spatial summation combines the effects of signals received at different sites on the membrane, **temporal summation** combines the effects of signals received at different times. If an action potential arrives at a synapse and triggers neurotransmitter release before a previous PSP at the synapse has decayed completely, the second PSP adds to the remaining tail of the first. If many action potentials arrive in quick succession, each PSP adds to the tail of the preceding PSP, building up to a large sustained average PSP whose magnitude reflects the rate of firing of the presynaptic neuron (Figure 11-39). This is the essence of temporal summation: it translates the *frequency* of incoming signals into the *magnitude* of a net PSP.

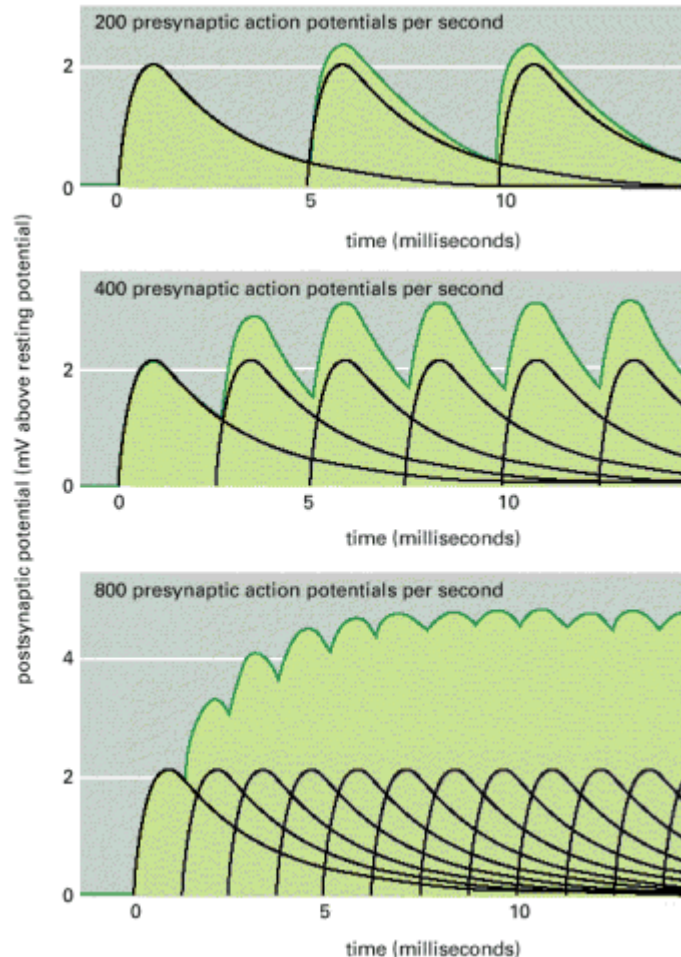


Figure 11-39. The principle of temporal summation. Each presynaptic action potential arriving at a synapse produces a small postsynaptic potential, or PSP (*black lines*). When successive action potentials arrive at the same synapse, each PSP produced adds to the tail of the preceding one to produce a larger combined PSP (*green lines*). The greater the frequency of incoming action potentials, the greater the size of the combined PSP.

Temporal and spatial summation together provide the means by which the rates of firing of many presynaptic neurons jointly control the membrane potential in the body of a single postsynaptic cell. The final step in the neuronal computation made by the postsynaptic cell is the generation of an output, usually in the form of action potentials, to relay a signal to other cells. The output signal reflects the magnitude of the PSP in the cell body. While the PSP is a continuously graded variable, however, action potentials are always all-or-nothing and uniform in size. The only variable in signaling by action potentials is the time interval between one action potential and the next. For long-distance

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

transmission, the magnitude of the PSP is therefore translated, or *encoded*, into the *frequency* of firing of action potentials (Figure 11-40). 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 axon hillock (see Figure 11-38). [↑ TOP](#)

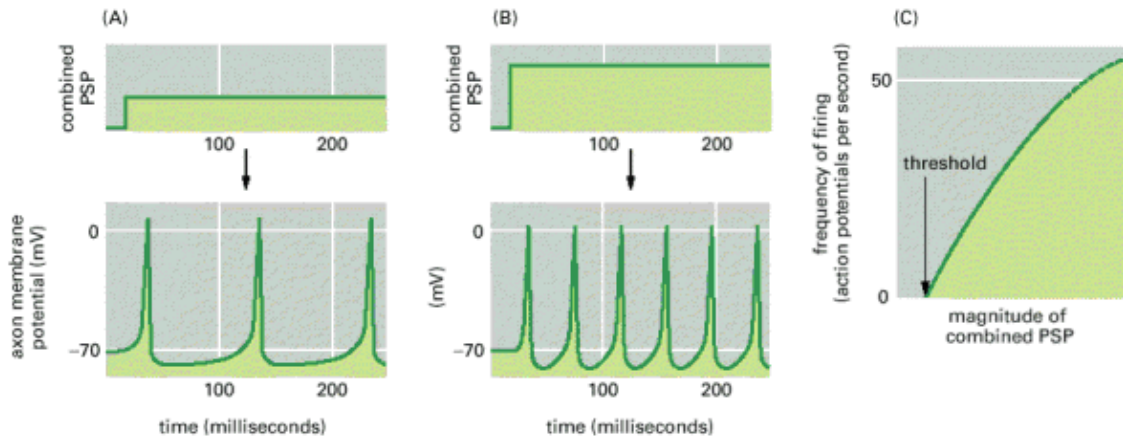


Figure 11-40. The encoding of the combined PSP in the form of the frequency of firing of action potentials by an axon. A comparison of (A) and (B) shows how the firing frequency of an axon increases with an increase in the combined PSP, while (C) summarizes the general relationship.

### Neuronal Computation Requires a Combination of at Least Three K<sup>+</sup> Channels

We have seen that the intensity of stimulation received by a neuron is determined 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 axon hillock, a unique region of each neuron where voltage-gated Na<sup>+</sup> channels are plentiful. But to perform its special function of encoding, the membrane of the **axon hillock** also contains at least four other classes of ion channels—three selective for K<sup>+</sup> and one selective for Ca<sup>2+</sup>. The three varieties of K<sup>+</sup> channels have different properties; we shall refer to them as the *delayed*, the *early*, and the *Ca<sup>2+</sup>-activated K<sup>+</sup> 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<sup>+</sup> channels. Below a certain threshold level of synaptic stimulation, the depolarization of the axon hillock membrane would be insufficient to trigger an action potential. With gradually increasing stimulation, the threshold would be crossed, the Na<sup>+</sup> channels would open, and an action potential would fire. The action potential would be terminated in the usual way by inactivation of the Na<sup>+</sup> channels. Before another action potential could fire, these channels would have to recover from their inactivation. But that would require a return of

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

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.

This task is performed by the **delayed K<sup>+</sup> channels** discussed previously in relation to the propagation of the action potential. They are voltage-gated, but because of their slower kinetics they open only during the falling phase of the action potential, when the Na<sup>+</sup> channels are inactive. Their opening permits an efflux of K<sup>+</sup> that drives the membrane back toward the K<sup>+</sup> equilibrium potential, which is so negative that the Na<sup>+</sup> channels rapidly recover from their inactivated state. Repolarization of the membrane also causes the delayed K<sup>+</sup> channels to close. The axon hillock 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<sup>+</sup> channels and delayed K<sup>+</sup> 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 **early K<sup>+</sup> 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 11-40](#)).

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

The **Ca<sup>2+</sup>-activated K<sup>+</sup> 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<sup>2+</sup> at the cytoplasmic face of the nerve cell membrane. Suppose that a strong depolarizing stimulus is applied for a long time, triggering a long train of action potentials. Each action potential permits a brief influx of Ca<sup>2+</sup> through the voltage-gated Ca<sup>2+</sup> channels, so that the intracellular Ca<sup>2+</sup> concentration gradually builds up to a level high enough to open the Ca<sup>2+</sup>-activated K<sup>+</sup> channels. Because the resulting increased permeability of the membrane to K<sup>+</sup> 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.

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

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, for example, at least five known types of voltage-gated  $\text{Ca}^{2+}$  channels in the vertebrate nervous system and at least four known types of voltage-gated  $\text{K}^{+}$  channels. The multiplicity of genes evidently allows for a host of different types of neurons, whose electrical behavior 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. [↑ TOP](#)

### Long-term Potentiation (LTP) in the Mammalian Hippocampus Depends on $\text{Ca}^{2+}$ 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 postsynaptic membrane is strongly depolarized* (either through recent repetitive firing of the same presynaptic cell or by other means). This rule reflects the behavior of a particular class of ion channels in

## Molecular Biology of the Cell 4th ed. Alberts et al., 2002

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 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 only activated when conventional glutamate-gated ion channels are activated as well and depolarize the membrane. The NMDA receptors are critical for LTP. When they are selectively blocked with a specific inhibitor, LTP does not occur, even though ordinary synaptic transmission continues. An animal treated with this inhibitor exhibits specific deficits in its learning abilities but behaves 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 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 conventional glutamate receptors into the plasma membrane ([Figure 11-41](#)). 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.

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 channel families that we have discussed are summarized in [Table 11-2](#).



**Molecular Biology of the Cell 4th ed. Alberts et al., 2002**

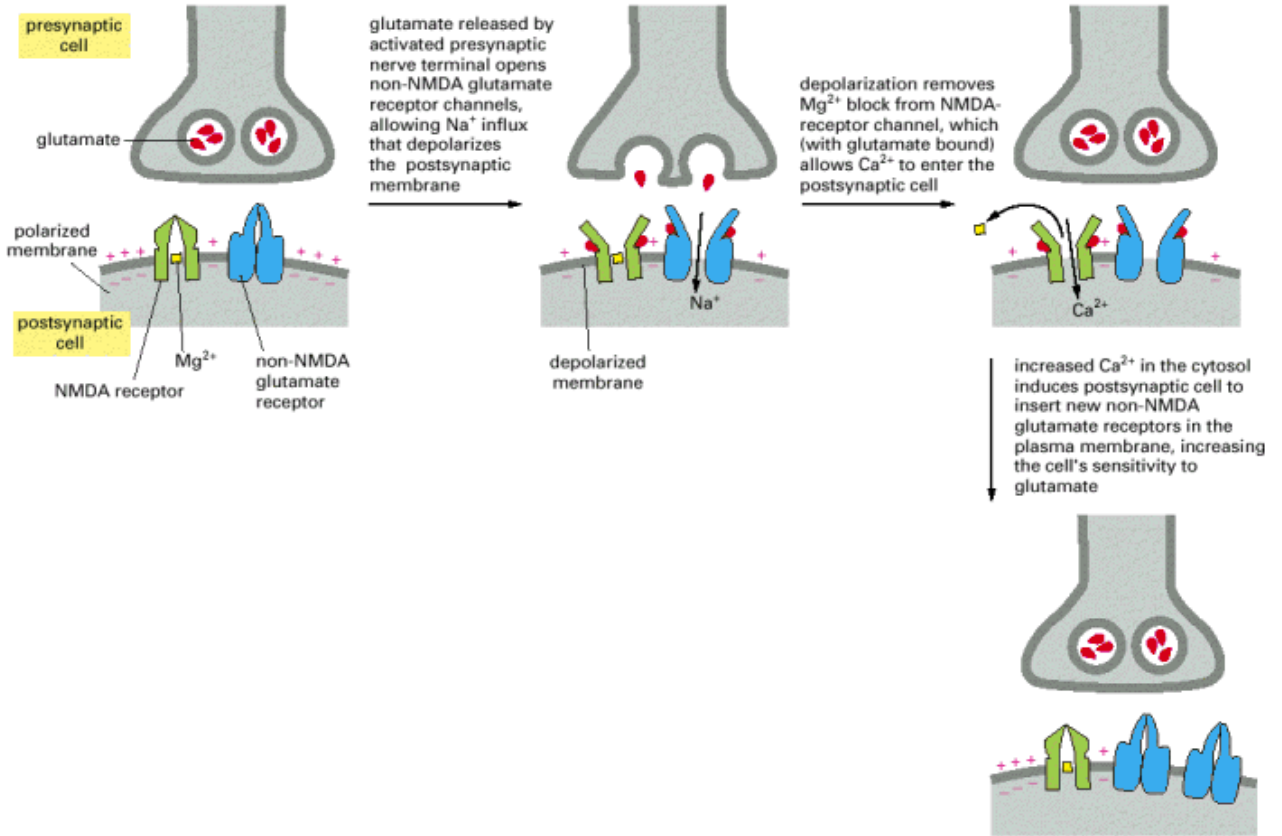


Figure 11-41. The signaling events in long-term potentiation. Although not shown, evidence suggests that changes can also occur in the presynaptic nerve terminals in LTP, which may be stimulated by retrograde signals from the postsynaptic cell.

Table 11-2. Some Ion Channel Families

| FAMILY*                        | REPRESENTATIVE SUBFAMILIES                                          |              |
|--------------------------------|---------------------------------------------------------------------|--------------|
| Voltage-gated cation channels  | voltage-gated Na <sup>+</sup> channels                              |              |
|                                | voltage-gated K <sup>+</sup> channels (including delayed and early) |              |
|                                | voltage-gated Ca <sup>2+</sup> channels                             |              |
| Transmitter-gated ion channels | acetylcholine-gated cation channels                                 | } excitatory |
|                                | glutamate-gated Ca <sup>2+</sup> channels                           |              |
|                                | serotonin-gated cation channels                                     |              |
|                                | GABA-gated Cl <sup>-</sup> channels                                 | } inhibitory |
|                                | glycine-gated Cl <sup>-</sup> channels                              |              |

\*The members of a family are similar in amino acid sequence and are therefore thought to have derived from a common ancestor; within subfamilies, the resemblances are usually even closer.

### 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 carrier. 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, are highly permeable to  $Ca^{2+}$ , which can trigger the long-term changes in synapses that are thought to be involved in some forms of learning and memory.

Ion channels work together in complex ways to control the behavior of electrically excitable cells. A typical neuron, for example, receives thousands of excitatory and inhibitory inputs, which combine by spatial and temporal summation to produce a 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 axon hillock.