Some ion channels can be blocked by certain free ions or molecules in the cytoplasm or extracellular fluid that bind either to the mouth of the aqueous pore or somewhere within the pore. If the blocker is an ion that binds to a site within the pore, it will be influenced by the membrane electric field as it enters the channel. For example, if a positively charged blocker enters the channel from outside the membrane, then making the cytoplasmic side of the membrane more negative will drive the blocker into the channel by electrostatic attraction, increasing the block. Although some blockers are toxins or drugs that originate outside the body, others are common ions normally present in the cell or its environment. Physiological blockers of certain classes of channels include Mg^{2+}, Ca^{2+}, Na^{+}, and polyamines such as spermine.

The Opening and Closing of a Channel Involve Conformational Changes

In ion channels that mediate electrical signaling, the channel protein has two or more conformational states that are relatively stable. Each conformation represents a different functional state. For example, each ion channel has at least one open state and one or two closed states. The transition of a channel between these different states is called *gating*.

The molecular mechanisms of gating are only partially understood. In some cases, such as the voltage-gated Cl^{-} channel described later in the chapter, a local conformational change along the channel lumen gates the channel (Figure 8–4A). In most cases, channel gating involves widespread changes in the channel's conformation (Figure 8–4B). For example, concerted movements, such as twisting, bending, or tilting, of the subunits that line the channel pore mediate the opening and closing of some ion channels (see Figure 8–14 and Chapters 11 and 12). The molecular rearrangements that occur during the transition from closed to open states appear to enhance ion conduction through the channel not only by creating a wider lumen, but also by positioning relatively more polar amino acid constituents at the surface that lines the aqueous pore. In other cases (eg, inactivation of K^{+} channels described in Chapter 10), part of the channel protein acts as a particle that can close the channel by blocking the pore (Figure 8–4C).

Three major transduction mechanisms have evolved to control channel opening in neurons. Certain channels are opened by the binding of chemical ligands, known as agonists (Figure 8–5A). Some ligands bind directly to the channel either at an extracellular or intracellular site; transmitters bind at extracellular sites, whereas certain cytoplasmic constituents, such as Ca^{2+}, cyclic nucleotides, and GTP-binding proteins, bind at intracellular sites, as do certain dynamically regulated mobile lipid components of the membrane (Chapter 14). Other ligands activate intracellular second messenger signaling cascades that can covalently modify channel gating through protein phosphorylation (Figure 8–5B). Many ion channels are regulated by changes in membrane potential (Figure 8–5C). Some voltage-gated channels act as temperature sensors; changes in temperature shift their voltage gating to higher or lower membrane potentials, giving rise to heat- or cold-sensitive channels. Finally, some channels are regulated by mechanical force (Figure 8–5D).

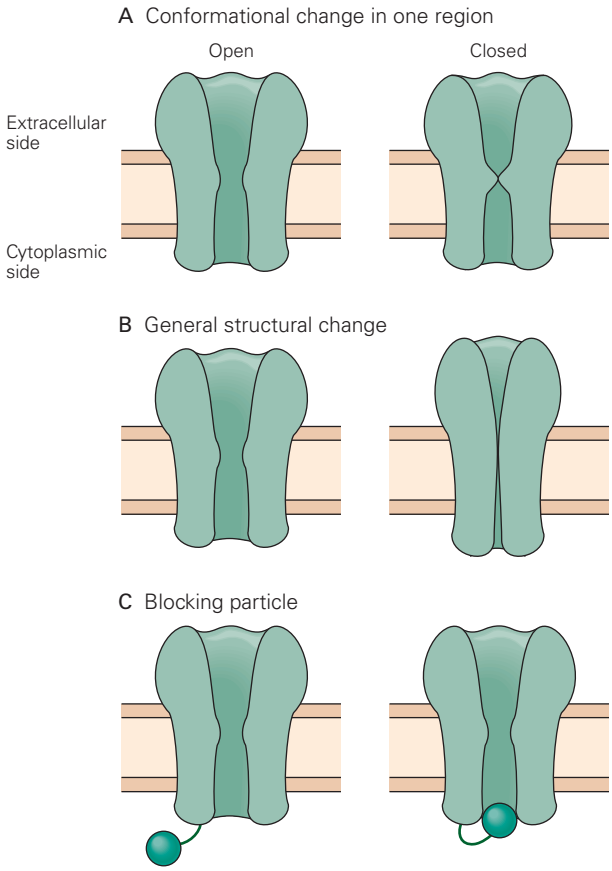

Figure 8–4 Three physical models for the opening and closing of ion channels.

A. A localized conformational change occurs in one region of the channel.

B. A generalized structural change occurs along the length of the channel.

C. A blocking particle swings into and out of the channel mouth.

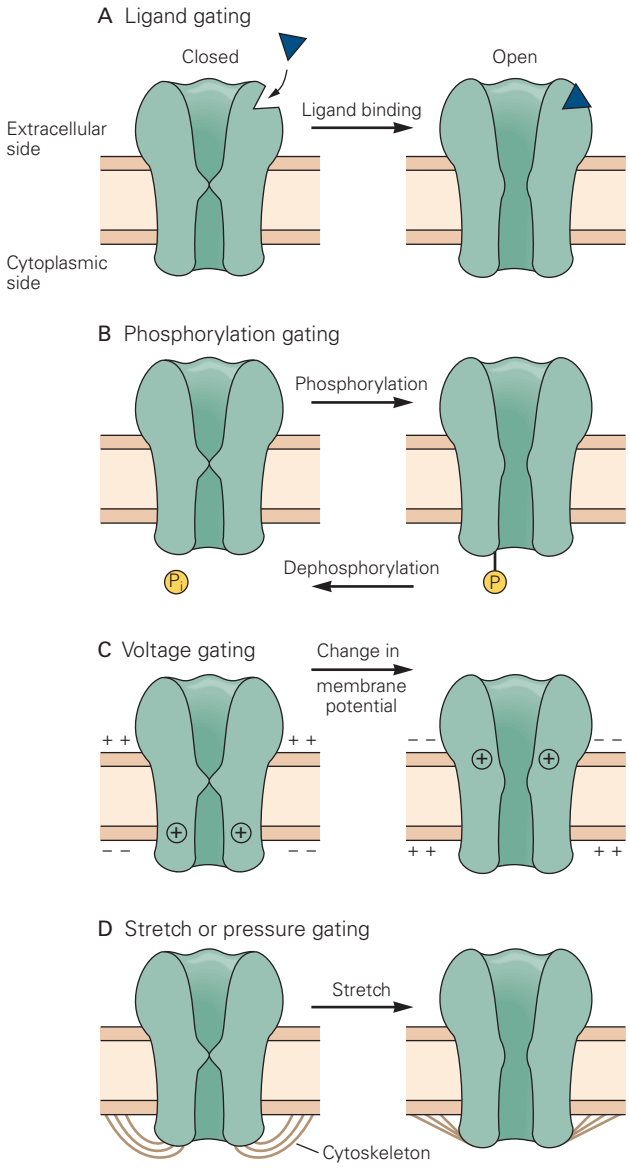

Figure 8–5 Several types of stimuli control the opening and closing of ion channels.

A. A ligand-gated channel opens when a ligand binds a receptor site on the external surface of the channel protein. The energy from ligand binding drives the channel toward an open state.

B. Some channels are regulated by protein phosphorylation and dephosphorylation. The energy for channel opening comes from the transfer of the high-energy phosphate, P_i.

C. Voltage-gated channels open and close with changes in the electrical potential difference across the membrane. The change in membrane potential causes a local conformational change by acting on a region of the channel that has a net charge.

D. Some channels open and close in response to membrane stretch or pressure. The energy for gating may come from mechanical forces that are passed to the channel either directly by distortion of the membrane lipid bilayer or by protein filaments attached to the cytoskeleton or surrounding tissues.

The rapid gating actions necessary for moment-to-moment signaling may also be influenced by certain long-term changes in the metabolic state of the cell. For example, the gating of some K^+ channels is sensitive to intracellular levels of ATP. Some channel proteins contain a subunit with an integral oxidoreductase catalytic domain that is thought to alter channel gating in response to the redox state of the cell.

These regulators control the entry of a channel into one of three functional states: closed and activatable (resting), open (active), or closed and nonactivatable (*inactivated* or *refractory*). A change in the functional state of a channel requires energy. In voltage-gated channels, the energy is provided by the movement of a charged region of the channel protein through the membrane's electric field. This region, the *voltage sensor*, contains a net electric charge, called a *gating charge*, resulting from the presence of basic (positively charged) or acidic (negatively charged) amino acids. The movement of the charged voltage sensor through the electric field in response to a change in membrane potential imparts a change in free energy to the channel that alters the equilibrium between the closed and open states of the channel. For most voltage-gated channels, channel opening is favored by making the inside of the membrane more positive (depolarization).

In transmitter-gated channels, gating is driven by the change in chemical free energy that results when the transmitter binds to a receptor site on the channel protein. Finally, mechanosensitive channels are gated by force transmitted by the distortion of the surrounding lipid bilayer or by protein tethers.

The stimuli that gate the channel also control the rates of transition between the open and closed states of a channel. For voltage-gated channels, the rates are steeply dependent on membrane potential. Although the time scale can vary from several microseconds to a minute, the transition tends to require a few milliseconds on average. Thus, once a channel opens, it stays open for a few milliseconds, and after closing, it stays closed for a few milliseconds before reopening. Once the transition between open and closed states begins, it proceeds virtually instantaneously (in less than 10 µs, the present limit of experimental measurements), thus giving rise to abrupt, all-or-none, step-like changes in current through the channel (Figure 8–2 in Box 8–1).

Ligand-gated and voltage-gated channels enter refractory states through different processes. Ligand-gated channels can enter the refractory state when their exposure to the agonist is prolonged, a process called *desensitization*—an intrinsic property of the interaction between ligand and channel.

Many, but not all, voltage-gated channels enter a refractory state after opening, a process termed *inactivation*. In the inactivated state, the channel is closed and can no longer be opened by positive voltages. Rather, the membrane potential must be returned to its initial negative resting level before the channel can recover from inactivation so that it can again open in response to depolarization. Inactivation of voltage-gated Na^+ and K^+ channels is thought to result from a conformational change, controlled by a subunit or region of the channel separate from that which controls activation. In contrast, the inactivation of certain voltage-gated Ca^{2+} channels is thought to require Ca^{2+} influx. An increase in cytoplasmic Ca^{2+} concentration inactivates the Ca^{2+} channel by binding to the regulatory molecule calmodulin, which is permanently associated with the Ca^{2+} channel protein (Figure 8–6).

Some mechanically gated ion channels that mediate touch sensation inactivate in response to a prolonged stimulus or to a train of brief stimuli. Although the molecular mechanism of this inactivation is not known, it is thought to be an intrinsic property of the channel.

Exogenous factors, such as drugs and toxins, can also affect the gating control sites of an ion channel. Most of these agents tend to inhibit channel opening, but a few facilitate opening. *Competitive antagonists* interfere with normal gating by binding to the same site at which the endogenous agonist normally binds. Antagonist binding, which does not open the channel, blocks access of agonist to the binding site, thereby preventing channel opening. The antagonist binding can be weak and reversible, as in the blockade of the nicotinic ACh-gated channel in skeletal muscle by the plant alkaloid curare, a South American arrow poison (Chapters 11 and 12), or it can be strong and virtually irreversible, as in the blockade of the same channel by the snake venom α-bungarotoxin.

Some exogenous substances modulate gating in a noncompetitive manner, without directly interacting with the transmitter-binding site. For example, binding of the drug diazepam (Valium) to a regulatory site on Cl^- channels that are gated by γ-aminobutyric acid (GABA), an inhibitory neurotransmitter, enhances the frequency with which the channels open in response to GABA binding (Figure 8–7B). This type of indirect, allosteric modulatory effect is found in some voltage- and mechanically gated channels as well.

The Structure of Ion Channels Is Inferred From Biophysical, Biochemical, and Molecular Biological Studies

What do ion channels look like? How does the channel protein span the membrane? What happens to the structure of the channel when it opens and closes? Where along the length of the channel protein do drugs and transmitters bind?

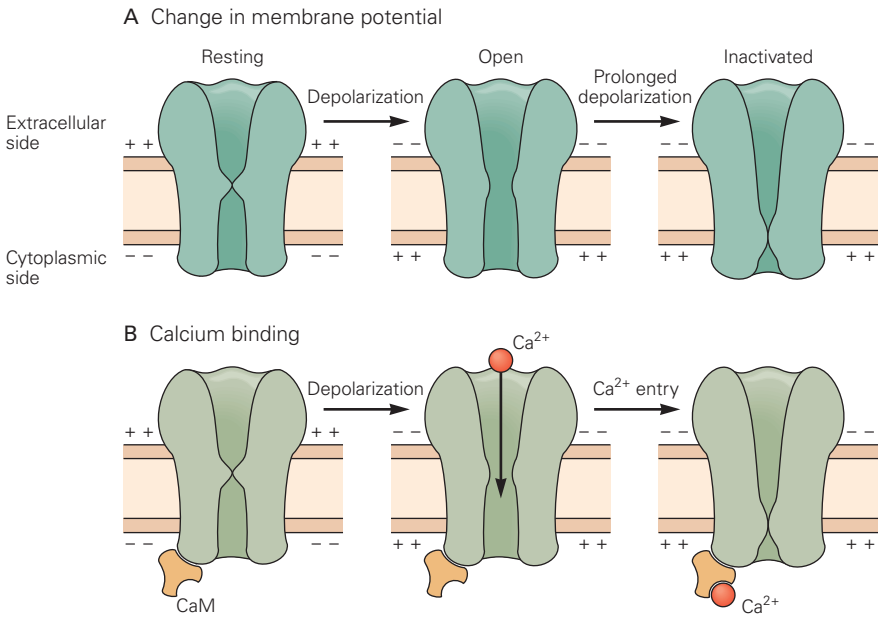

Figure 8–6 Voltage-gated channels are inactivated by two mechanisms.

A. Many voltage-gated channels enter a refractory (inactivated) state after briefly opening in response to depolarization of the membrane. They recover from the refractory state and return to the resting state only after the membrane potential is restored to its resting value.

B. Some voltage-dependent Ca^{2+} channels become inactivated when the internal Ca^{2+} level increases following channel opening. The internal Ca^{2+} binds to calmodulin (**CaM**), a specific regulatory protein associated with the channel.

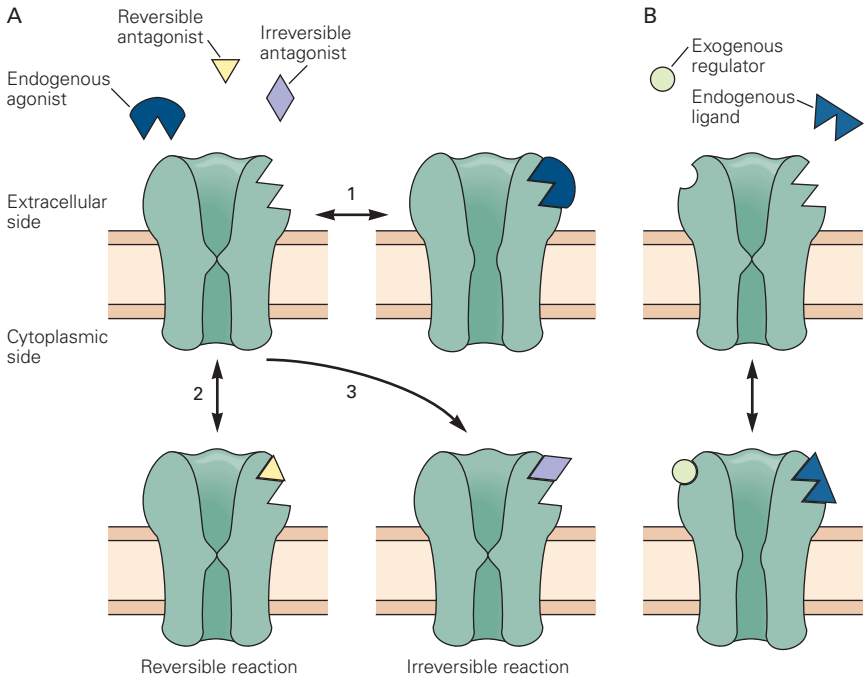

Figure 8–7 Exogenous ligands, such as drugs, can bias an ion channel toward an open or closed state.

A. In channels that are normally opened by the binding of an endogenous ligand (1), a drug or toxin may block the binding of the agonist by means of a reversible (2) or irreversible (3) reaction.

B. Some exogenous agents can bias a channel toward the open state by binding to a regulatory site, distinct from the ligand-binding site that normally opens the channel.

Biophysical, biochemical, and molecular biological studies have provided a basic understanding of channel structure and function. More recent studies using X-ray crystallography and cryo-electron microscopy have provided information about the structure of an increasing number of channels at the atomic level. All ion channels are large integral-membrane proteins with a core transmembrane domain that contains a central aqueous pore spanning the entire width of the membrane. The channel protein often contains carbohydrate groups attached to its external surface. The pore-forming region of many channels is made up of two or more subunits, which may be identical or different. In addition, some channels have auxiliary subunits that may have a variety of effects, including facilitating cell surface expression of the channel, targeting the channel to its appropriate location on the cell surface, and modifying gating properties of the channel. These subunits may be attached to the cytoplasmic end or embedded in the membrane (Figure 8–8).

The genes for all the major classes of ion channels have been cloned and sequenced. The amino acid

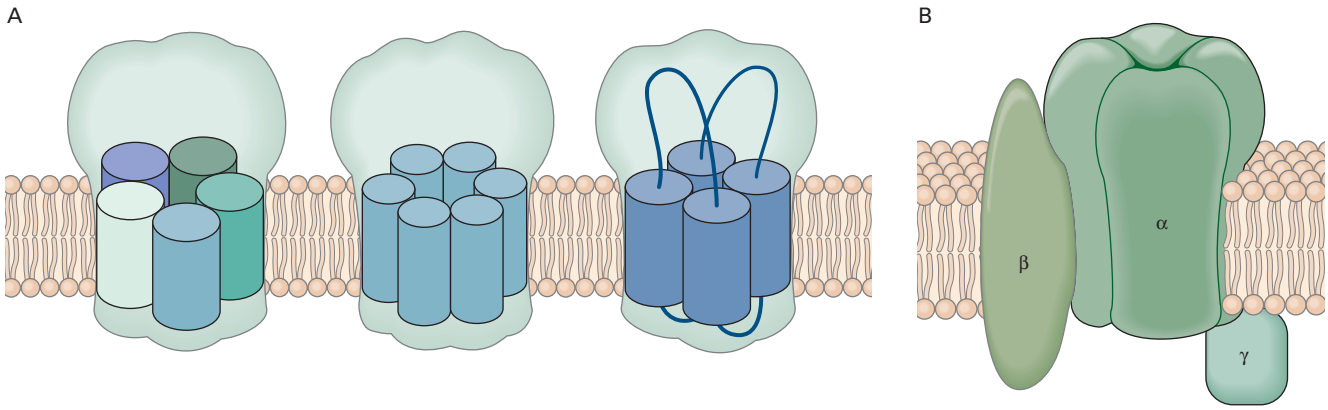

Figure 8–8 Ion channels are integral membrane proteins composed of several subunits.

A. Ion channels can be constructed as hetero-oligomers from distinct subunits (*left*), as homo-oligomers from a single type of subunit (*middle*), or from a single polypeptide chain organized into repeating motifs, where each motif functions as the equivalent of one subunit (*right*).

B. In addition to one or more α-subunits that form a central pore, some channels contain auxiliary subunits (β or γ) that modulate pore gating, channel expression, and membrane localization.

sequence of a channel, inferred from its DNA sequence, can be used to create a structural model of the channel protein. Regions of secondary structure—the arrangement of the amino acid residues into α-helixes and β-sheets—as well as regions that are likely to correspond to membrane-spanning domains of the channel are then predicted based on the structures of related proteins that have been experimentally determined using electron and X-ray diffraction analysis. This type of analysis has identified, for example, the presence of four hydrophobic regions, each around 20 amino acids in length, in the amino acid sequence of a subunit of the ACh receptor channel. Each of these regions is thought to form an α-helix that spans the membrane (Figure 8–9).

Comparing the amino acid sequences of the same type of channel from different species provides additional insights into channel structure and function. Regions that show a high degree of sequence similarity (that is, have been highly conserved throughout evolution) are likely to be important in determining the structure and function of the channel. Likewise, conserved regions in different but related channels are likely to serve a common biophysical function.

The functional consequences of changes in a channel's primary amino acid sequence can be explored through a variety of techniques. One particularly versatile approach is to use genetic engineering to construct channels with parts that are derived from the genes of different species—so-called *chimeric channels*. This technique takes advantage of the fact that the same type of channel can have somewhat different properties in different species. For example, the bovine ACh receptor channel has a slightly greater single-channel conductance than the ACh receptor channel in electric fish. By comparing the properties of a chimeric channel

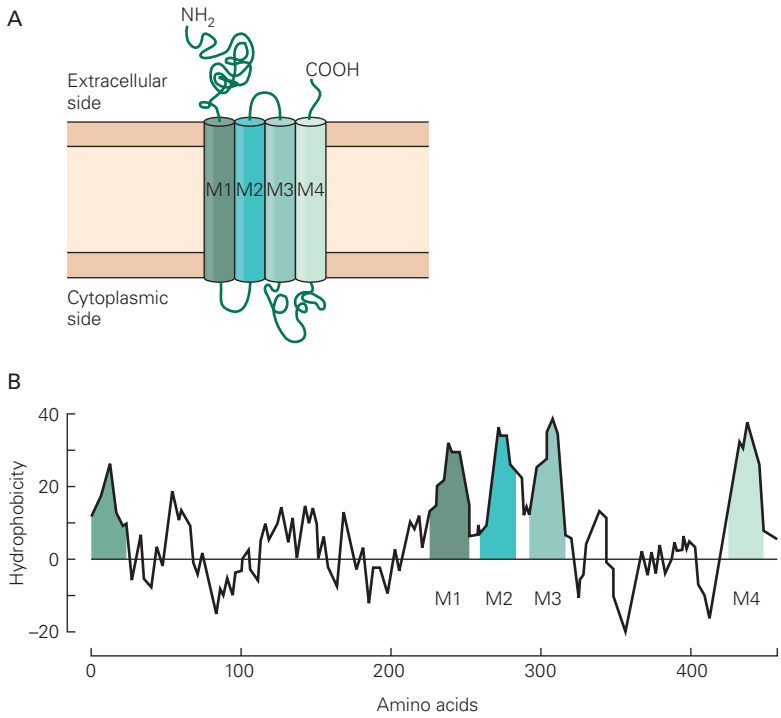

Figure 8–9 The secondary structure of membrane-spanning proteins.

A. A proposed secondary structure for a subunit of the nicotinic acetylcholine (ACh) receptor channel present in skeletal muscle. Each cylinder (**M1–M4**) represents a putative membrane-spanning α-helix comprised of approximately 20 hydrophobic amino acid residues. The membrane segments are connected by cytoplasmic or extracellular segments (loops) of hydrophilic residues. The amino terminus (NH_2) and carboxyl terminus (**COOH**) of the protein lie on the extracellular side of the membrane.

B. The membrane-spanning regions of an ion channel protein can be identified using a hydrophobicity plot. The amino acid sequence of the α-subunit of the nicotinic ACh receptor was inferred from the nucleotide sequence of the cloned receptor subunit gene. Then a running average of hydrophobicity was plotted for the entire amino acid sequence of the subunit. Each point in the plot represents an average hydrophobic index of a sequence of 19 amino acids and corresponds to the midpoint of the sequence. Four of the hydrophobic regions (M1–M4) correspond to the membrane-spanning segments. The hydrophobic region at the far left in the plot is the signal sequence, which positions the hydrophilic amino terminus of the protein on the extracellular surface of the cell during protein synthesis. The signal sequence is cleaved from the mature protein. (Reproduced, with permission, from Schofield et al. 1987.)

to those of the two original channels, one can assess the functions of specific regions of the channel. This technique has been used to identify the membrane-spanning segment that forms the lining of the pore of the ACh receptor channel (Chapter 12).

The roles of different amino acid residues or stretches of residues can be tested using *site-directed mutagenesis*, a type of genetic engineering in which specific amino acid residues are substituted or deleted. Finally, one can exploit the naturally occurring mutations in channel genes. A number of inherited and spontaneous mutations in the genes that encode ion channels in nerve or muscle produce changes in channel function that can underlie certain neurological diseases. Many of these mutations are caused by localized changes in single amino acids within channel proteins, demonstrating the importance of that region for channel function. The detailed functional changes in such channels can then be examined in an artificial expression system.

Ion Channels Can Be Grouped Into Gene Families

The great diversity of ion channels in a multicellular organism is illustrated by the human genome. Our genome contains nine genes encoding variants of voltage-gated Na^+ channels, 10 genes for different Ca^{2+} channels, 80 genes for K^+ channels, 70 genes for ligand-gated channels, and more than a dozen genes for Cl^- channels. Fortunately, the evolutionary relationships between the genes that encode ion channels provide a relatively simple framework with which to categorize them.

Most of the ion channels that have been described in nerve and muscle cells fall into a few gene superfamilies. Members of each gene superfamily have similar amino acid sequences and transmembrane topology and, importantly, related functions. Each superfamily is thought to have evolved from a common ancestral gene by gene duplication and divergence. Several superfamilies can be further subdivided into families of genes encoding channels with more closely related structure and function.

One superfamily encodes ligand-gated ion channels that are receptors for the neurotransmitters ACh, GABA, glycine, or serotonin (Chapter 12). All of these receptors are composed of five subunits, each of which has four transmembrane α-helixes (Figure 8–10A). In addition, the N-terminal extracellular domain that forms the receptor for the ligand contains a conserved loop of 13 amino acids flanked by a pair of cysteine residues that form a disulfide bond. As a result, this receptor superfamily is referred to as the *cys-loop receptors*. Ligand-gated channels can be classified by their ion selectivity in addition to their agonist. The genes that encode glutamate receptor channels belong to a separate gene family.

Gap-junction channels, which bridge the cytoplasm of two cells at electrical synapses (Chapter 11), are encoded by a separate gene superfamily. A gap-junction channel is composed of two hemi-channels, one from each connected cell. A hemi-channel has six identical subunits, each of which has four membrane-spanning segments (Figure 8–10B).

The genes that encode the voltage-gated ion channels responsible for generating the action potential belong to another superfamily (Chapter 10). These channels are selective for Ca^{2+}, Na^+, or K^+. Comparative DNA sequence data suggest that most voltage-sensitive cation channels stem from a common ancestral channel—perhaps a K^+ channel—that can be traced to a single-cell organism living more than 1.4 billion years ago, before the evolution of separate plant and animal kingdoms.

All voltage-gated cation channels have a similar four-fold symmetric architecture, with a core motif composed of six transmembrane α-helical segments termed S1–S6. A seventh hydrophobic region, the *P-region*, connects the S5 and S6 segments by dipping into and out of the extracellular side of the membrane (Figures 8–10C and 8–11A); it forms the channel's selectivity filter. Voltage-gated Na^+ and Ca^{2+} channels are composed of a large subunit that contains four repeats of this basic motif (Figure 8–10C). Voltage-gated K^+ channels are tetramers, with each separate subunit containing one copy of the basic motif (Figure 8–11A). Each subunit contributes one P-region to the pore of the fully assembled channel. This structural configuration is also shared by other, more distantly related channel families described later and in Chapter 10.

The S4 segment is thought to play a particularly important role in voltage gating. It contains an unusual pattern of amino acids in which every third position contains a positively charged arginine or lysine residue. This region was originally proposed to be the voltage sensor because, according to fundamental biophysical principles, voltage-gating must involve the movement of intramembrane gating charges within the membrane electric field. Additional evidence implicating S4 as the voltage sensor comes from the finding that this pattern of positive charges is highly conserved in all voltage-gated cation-selective channels but is absent in channels that are not voltage-gated. Further support comes from site-directed mutagenesis experiments showing that neutralization of these positive charges in S4 decreases the voltage sensitivity of channel activation.

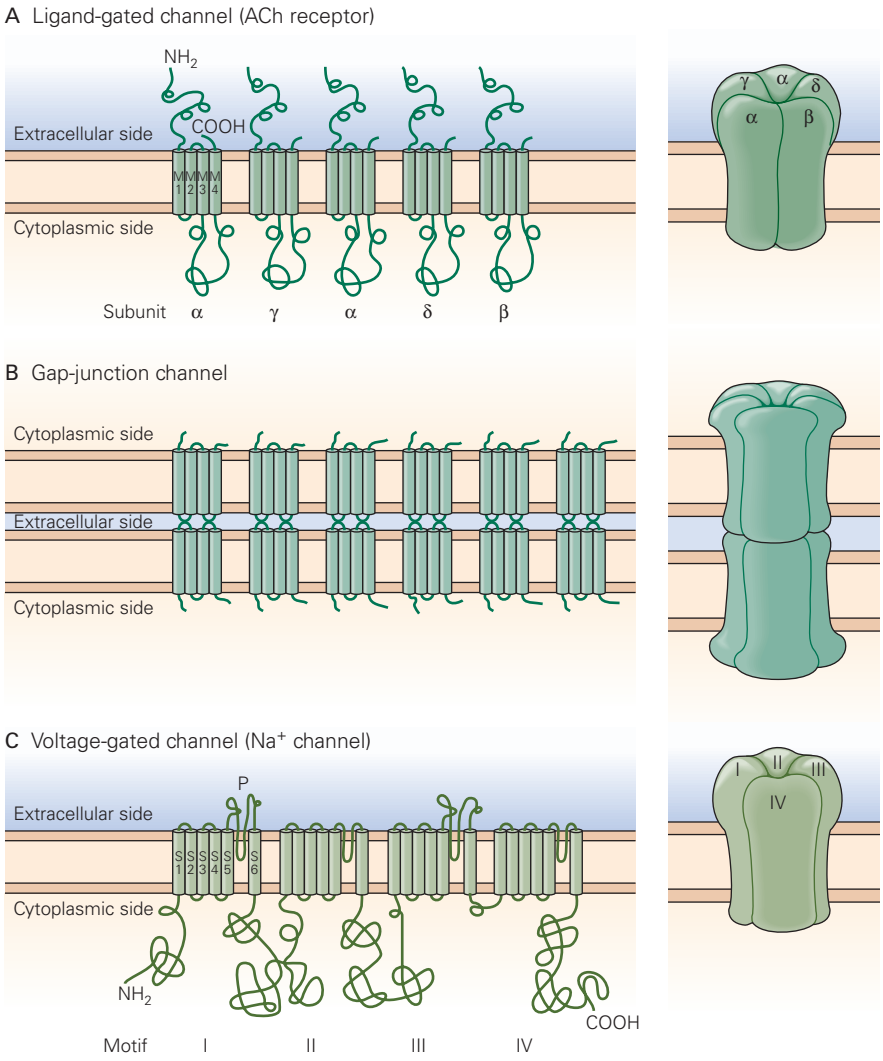

Figure 8–10 Three superfamilies of ion channels.

A. Members of a large family of ligand-gated channels, such as the acetylcholine receptor channel, are composed of five identical or closely related subunits, each of which contains four transmembrane α-helixes (M1–M4). Each cylinder in the figure represents a single transmembrane α-helix.

B. The gap-junction channel is formed from a pair of hemichannels, one each in the pre- and postsynaptic cell membranes, that join in the space between two cells. Each hemichannel is made of six identical subunits, each containing four transmembrane α-helixes. Gap-junction channels serve as conduits between the cytoplasm of the pre- and postsynaptic cells at electrical synapses (Chapter 11).

C. The voltage-gated Na^+ channel is formed from a single polypeptide chain that contains four homologous domains or repeats (motifs I–IV), each with six membrane-spanning α-helixes (**S1–S6**). The S5 and S6 segments are connected by an extended strand of amino acids, the **P**-region, which dips into and out of the external surface of the membrane to form the selectivity filter of the pore. Voltage-gated Ca^{2+} channels share the same general structural pattern, although the amino sequences are different.

The major gene family encoding the voltage-gated K^+ channels is related to three additional families of K^+ channels, each with distinctive properties and structure. One family includes genes encoding three types of channels activated by either intracellular Na^+ or Ca^{2+} or by intracellular Ca^{2+} plus depolarization. A second family consists of the genes encoding inward-rectifying K^+ channels. Because they are open at the resting potential and rapidly occluded by cytosolic cations during depolarization, they conduct ions more readily in the inward than in the outward direction. Each channel subunit has only two transmembrane segments connected by a pore-forming P-region. A third family of genes encodes K2P channels composed of subunits with two repeated pore-forming segments (Figure 8–11). Various members are regulated by temperature, mechanical force, and intracellular ligands. These channels may also contribute to the K^+ permeability of the resting membrane.

The sequencing of the genomes of a variety of species, from bacteria to humans, has led to the identification of additional ion channel gene families. These include channels with related P-regions but that are only very distantly related to the family of voltage-gated channels. An example is the excitatory postsynaptic glutamate-gated channel, in which the P-region is inverted, entering and leaving the internal surface of the membrane (Figure 8–11D).

Finally, the transient receptor potential (TRP) family of nonselective cation channels (named after a mutant *Drosophila* strain in which light evokes a brief receptor potential in photoreceptors) comprises a very large and diverse group of tetrameric channels that contain P-regions. Like the voltage-gated K^+ channels,

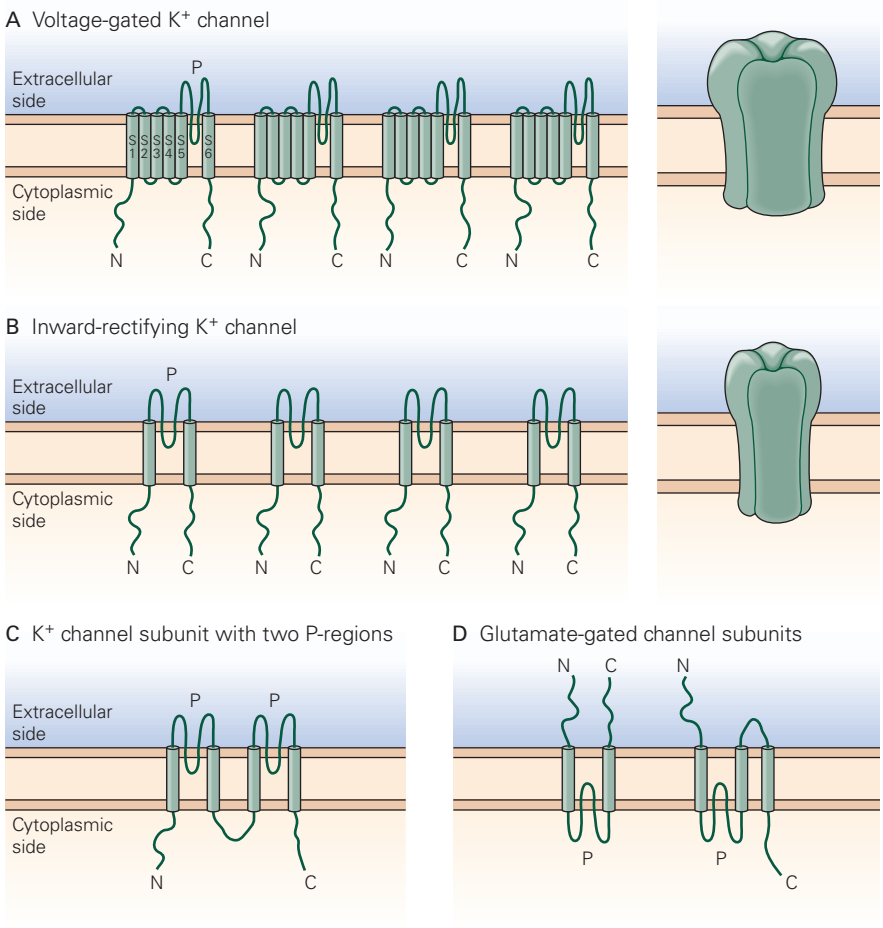

Figure 8–11 Four related families of ion channels with P-regions.

A. Voltage-gated K^+ channels are composed of four subunits, each of which corresponds to one repeated domain of voltage-gated Na^+ or Ca^{2+} channels, with six transmembrane segments and a pore-forming P-region (see Figure 8–10C).

B. Inward-rectifying K^+ channels are composed of four subunits, each of which has only two transmembrane segments connected by a P-region.

C. The K2P K^+ channels are composed of subunits that contain two repeats similar to the inward-rectifying K^+ channel subunit, with each repeat containing a P-region. Two of these subunits combine to form a channel with four P-regions.

D. Glutamate receptors constitute a distinct family of tetrameric channels with P-regions. Their pore regions are nonselectively permeable to cations. In these receptors, the amino terminus is extracellular and the P-region enters and exits the cytoplasmic side of the membrane. The distantly related bacterial GluR0 K^+-permeable glutamate receptor has four subunits, which contain two transmembrane segments (*left*); in higher organisms, the subunits contain three (*right*).

TRP channels also contain six transmembrane segments, but are in most cases gated by intracellular or intramembrane ligands. TRP channels are important for Ca^{2+} metabolism in all cells, visual signaling in insects, and pain, heat, and cold sensation in the nervous system of higher animals (Chapter 18). TRP channels have been implicated in osmoreception and certain taste sensations in mammals.

A number of other families of channels have been identified, structurally unrelated to those considered earlier. These include CLC Cl^- channels that help set the resting potential of skeletal muscle cells and certain neurons, nonspecific cation-permeable Piezo channels that are activated by mechanical stimuli (Chapter 18), Na^+ channels that are activated by H^+ ions released during inflammation, and ligand-gated cation channels that are activated by ATP, which functions as a neurotransmitter at certain excitatory synapses. With the completion of the human genome project, it is likely that nearly all of the major classes of ion channel genes have now been identified.

The diversity of ion channels is even greater than the large number of ion channel genes. Because most channels in a subfamily are composed of multiple subunits, each type of which may be encoded by a family of closely related genes, combinatorial permutations of these subunits can generate a diverse array of heteromultimeric channels with different functional properties. Additional diversity can be produced by posttranscriptional and posttranslational modifications. These subtle variations in structure and function presumably allow channels to perform highly specific functions. As with enzyme isoforms, variants of a channel with slightly different properties may be expressed at distinct stages of development, in different cell types throughout the brain, and even in different regions within a cell. Changes in neuronal activity can also lead to changes in ion channel expression patterns (Chapter 10).

Biochemical, biophysical, and molecular biological approaches have been important in defining structure–function relationships among the large variety of ion

channels. The use of X-ray crystallography and cryo-electron microscopy to define the structure of channels at atomic resolution provides a framework for achieving greater understanding of the mechanisms of ion channel function and malfunction due to disease-causing mutations. Combining a wide array of data from these various approaches makes possible the construction of detailed molecular models, which can be tested by further experiments, as well as by theoretical approaches such as molecular dynamics simulation.

X-Ray Crystallographic Analysis of Potassium Channel Structure Provides Insight Into Mechanisms of Channel Permeability and Selectivity

The first high-resolution X-ray crystallographic analysis of the molecular architecture of the pore region of an ion-selective channel was provided by Rod MacKinnon and his colleagues. To overcome the difficulties inherent in obtaining crystals of large integral membrane proteins, they initially focused on a non–voltage-gated K^+ channel, termed KcsA, from a bacterium. This channel is advantageous for crystallography as it can be expressed at high levels for purification, is relatively small, and has a simple transmembrane topology similar to that of the inward-rectifying K^+ channel in higher organisms, including mammals (Figure 8–11B).

The crystal structure of the KcsA protein provides several important insights into the mechanisms by which the channel facilitates the movement of K^+ ions across the hydrophobic lipid bilayer. The channel is made up of four identical subunits arranged symmetrically around a central pore (Figure 8–12A). Each subunit has two membrane-spanning α-helixes, an inner and outer helix. They are connected by the P-loop, which forms the selectivity filter of the channel. The amino acid sequence of these subunits is homologous to that of the S5-P-S6 region of vertebrate voltage-gated K^+ channels. The two α-helixes of each subunit tilt away from the central axis of the pore such that the structure resembles an inverted tepee (Figure 8–12B,C).

The four inner α-helixes from each of the subunits line the cytoplasmic end of the pore. At the intracellular mouth of the channel, these four helixes cross, forming a very narrow opening—the "smoke hole" of the tepee. Because this hole is too small to allow passage of K^+ ions, the crystal structure is presumed to represent the channel in the closed state. The inner helixes are homologous to the S6 membrane-spanning segment of voltage-gated K^+ channels (Figure 8–11A). At the extracellular end of the channel, the transmembrane helixes in each subunit are connected by a region consisting of three elements: (1) a chain of amino acids that surrounds the mouth of the channel (the turret region), (2) an abbreviated α-helix (the pore helix) approximately 10 amino acids in length that projects toward the central axis of the pore, and (3) a stretch of 5 amino acids near the C-terminal end of the P-region that forms the selectivity filter.

The shape and structure of the pore determine its ion-conducting properties. Both the inner and outer mouths of the pore are lined with acidic amino acids whose negative charges help attract cations from the bulk solution. Going from inside to outside, the pore consists of a medium-wide tunnel, 18 Å in length, which leads into a wider (10 Å diameter) spherical inner chamber. This chamber is lined predominantly by the side chains of hydrophobic amino acids. These relatively wide regions are followed by the very narrow selectivity filter, only 12 Å in length, which is rate-limiting for the passage of K^+ ions. A high K^+ ion throughput rate is ensured by the fact that the inner 28 Å of the pore, from the cytoplasmic entrance to the selectivity filter, lacks polar groups that could delay ion passage by binding and unbinding the ion (Figure 8–12C,D).

An ion passing from the polar solution through the nonpolar lipid bilayer encounters the least energetically favorable region in the middle of the bilayer. The large energy difference between these two regions for a K^+ ion is minimized by two details of channel structure. The inner chamber is filled with water, which provides a highly polar environment, and the pore helixes provide dipoles whose electronegative carboxyl ends point toward this inner chamber (Figure 8–12C).

The high energetic cost incurred as a K^+ ion sheds its waters of hydration is partially compensated by the presence of 20 electronegative oxygen atoms that line the walls of the selectivity filter and form favorable electrostatic interactions with the permeant ions. Each of the four subunits contributes four main-chain carbonyl oxygen atoms from the protein backbone and one side-chain hydroxyl oxygen atom to form a total of four binding sites for K^+ ions. Each bound K^+ ion is thus stabilized by interactions with a total of eight oxygen atoms, which lie in two planes above and below the bound cation. In this way, the channel is able to compensate for the loss of the K^+ ion's waters of hydration. The selectivity filter is stabilized at a critical width, such that it provides optimal electrostatic interactions with K^+ ions as they pass but is too wide for the smaller Na^+ ions to interact effectively with all eight oxygen atoms at any point along the length of the filter (Figure 8–12C).

In light of the extensive interactions between a K^+ ion and the channel, how does the KcsA channel