

Molecular Properties of Ion Channels

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Ion channels are present in most, if not all, cells. The cell membrane, being a hydrophobic bilayer of lipids lined with negative phosphate charges on both sides, is normally impermeable to all but small, uncharged molecules. In order to allow important physiological ions to cross this barrier, channels serve as highly selective passageways through which ions can pass into and out of the cell. In the nervous system, the activity of ion channels sets the resting membrane potential of neurons and muscles and controls the firing pattern and waveform of action potentials. To fulfill these functions, each type of ion channel is selectively permeable to only certain ions. In addition, they open and close in response to the actions of transmitters and the metabolic state of the cell so as to modulate neuronal excitability (Hille, 2001).

How does an ion channel determine when to open and when to close? How does it allow certain ions to pass while blocking other ions? These key questions of ion channel function have been the focus of molecular studies during the past several decades. Following a brief overview of how ion channels are grouped into different families based on their molecular properties, we will discuss how channel gating and ion selectivity are achieved. Our discussion will focus mainly on the pore-loop family of ion channels as this group of channels has served as the model for many studies of ion channel function and mechanism.

The first questions to be examined in this chapter concern channel gating—the opening and closing of channels:

- How does voltage open channels?
- Why don't ion channels stay open indefinitely?
- How does calcium affect channel opening?
- How are ion channels influenced by metabotropic signaling pathways?
- How could the metabolic state of a cell influence channel opening?

The second set of questions concern ion selectivity:

- Why do potassium channels let the larger potassium ions rather than the smaller sodium ions go through? How does the channel accomplish this fast enough to allow millions of ions per second to stream single-file through a channel?
- The narrowest part of a sodium channel pore is actually larger than that of a potassium channel pore. How does a sodium channel allow sodium rather than potassium ions to pass through?
- How does a calcium channel allow only calcium ions to pass through under physiological conditions when calcium ions are far outnumbered by sodium ions of nearly the same size?

FAMILIES OF ION CHANNELS

The most fundamental feature of an ion channel is the pore, a tunnel that allows ions to stream across the membrane (see Fig. 11.1). The pore is made from transmembrane segments organized to form a hole through the lipid bilayer, and, in most channels, is selective for certain types of ions over others. The ion conductance properties of the pore are determined by the unique arrangement of amino acids and transmembrane segments in the protein. In addition, most channels can regulate the movement of ions through the pore with a gate that can open or close depending on stimuli. The stimulus can be in the form of voltage depolarization, extracellular ligands, or intracellular second messengers. Once the pore has been opened, many channels also have unique mechanisms to either deactivate or inactivate and stop the flow of current.

Ion channels are grouped into several families based on similarities in their structure and mechanism of action. Channels in the same family typically share the

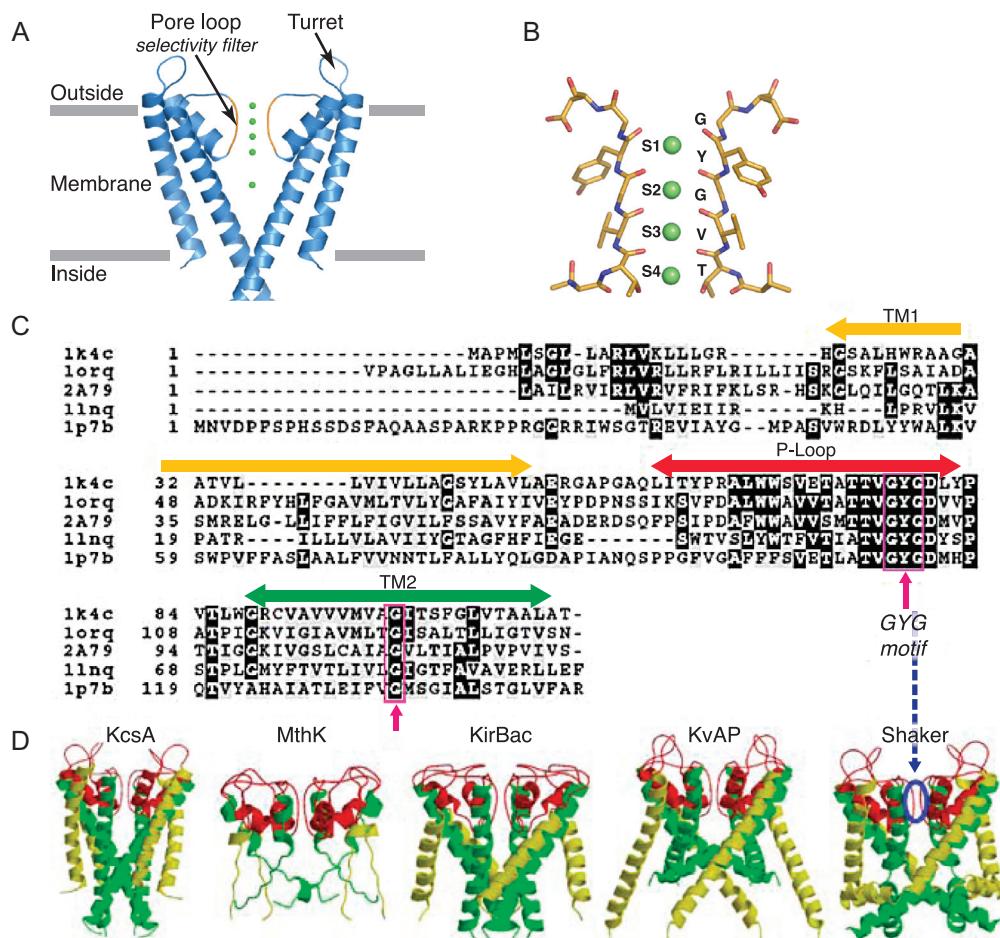


FIGURE 11.1 Examples of ion channel pores from various potassium channels. (A) A water-filled cavity is formed from four protein subunits, two of which are shown in the bacterial potassium channel, KcsA. The cavity creates a passageway through which ions can flow across the membrane, into or out of the cell. (B) The unique amino acid sequence of each family of channels allows it to selectively filter out particular ions. In the case of KcsA, K^+ but not Na^+ ions are allowed to pass through the selectivity filter, even though K^+ ions are bigger than Na^+ ions are. S1–4 refer to the four K^+ ion-binding sites in the selectivity filter, each composed of eight oxygen atoms from the TVGYG signature motif. (C) Pore-region sequence alignments of five structurally known potassium channels are shown with the GYG signature motif boxed in magenta and other highly conserved regions labeled in black. (D) Structural comparison of the pore regions from the same five potassium channels. (A) and (B) adapted from Lockless et al. (PloS 2007, p. e121); (C) and (D) from Srivastava et al. (2006).

same membrane topology for their pore-lining α subunits and display significant sequence similarity. In addition to forming the ion permeation pathway, α subunits often also contain other domains to support the pore and influence its dynamics. Auxiliary or β subunits can confer regulatory or modulatory effects on channel function. Interestingly, some families of ion channels bear weak though recognizable resemblance with one another, indicating that different families of ion channels may be evolutionarily related.

In this section, we introduce several well-studied families of ion channels and describe their physiological function (Fig. 11.2). The main focus is on channels gated by voltage and calcium; ligand-gated ion channels are covered in Chapter 10.

Pore-Loop Channels

The group of pore-loop (“p-loop”) channels is a large superfamily of evolutionarily related proteins that share a similar architecture characterized by four homologous subunits each contributing two transmembrane helices joined by a p-loop toward the pore-lining region of the channel. This protein family contains over a hundred different gene members, including channels in the voltage-gated sodium (Na_v), voltage-gated calcium (Ca_v), voltage-gated potassium (K_v), calcium-activated potassium (K_{Ca}), cyclic nucleotide gated (CNG), transient receptor potential (TRP), inwardly-rectifying potassium (K_{ir}) and two-pore potassium (K_{2P}) subfamilies (Yu and Catterall, 2004).

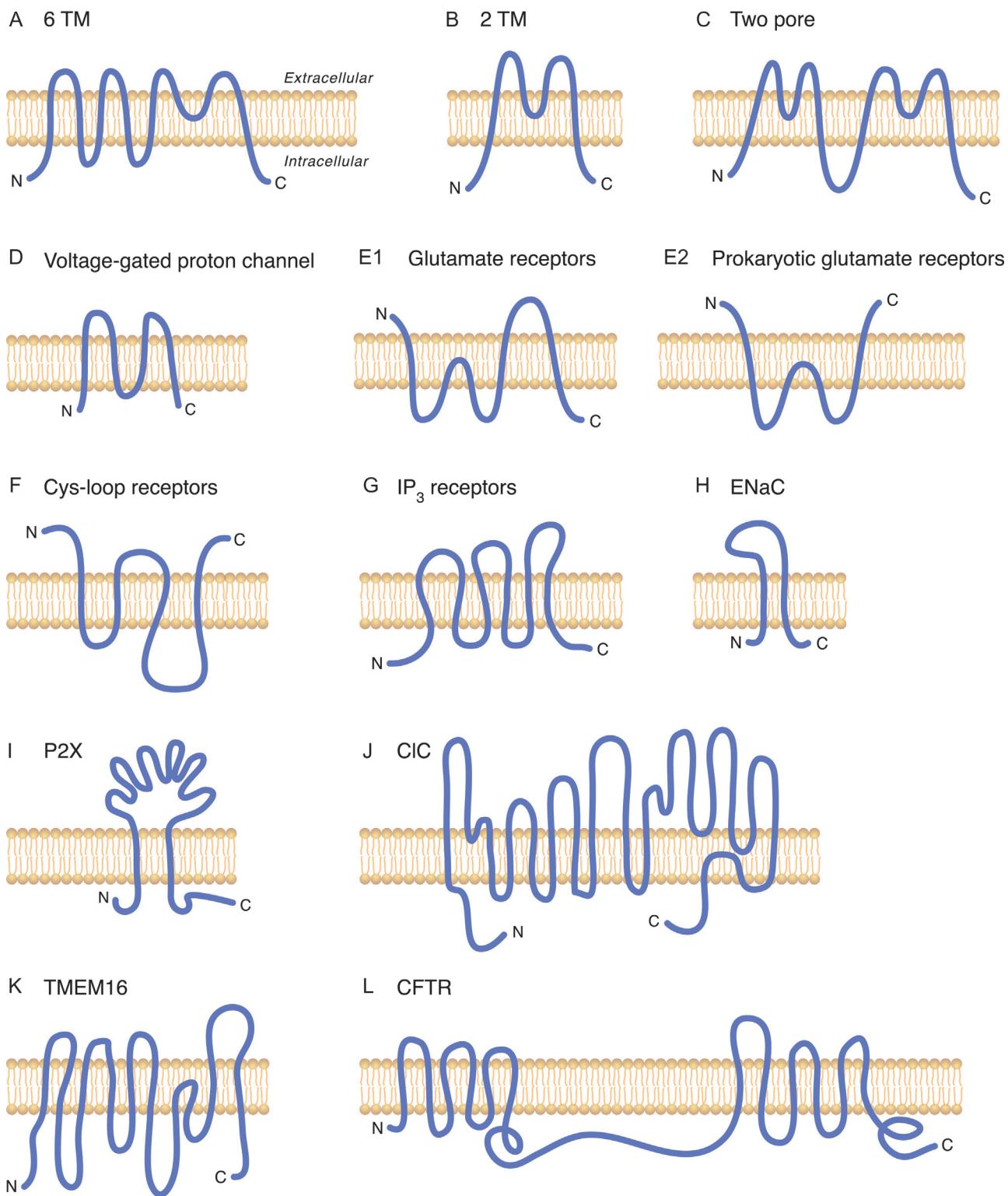


FIGURE 11.2 (Continued)

Crystallographic studies of these proteins have revealed an “inverted teepee” topology (Fig. 11.1) with the p-loop located at the wider end of the teepee facing the extracellular solution and the narrower portion of the teepee pointing toward the intracellular compartment. The p-loop and the transmembrane helices flanking it form the narrowest portion of the ion permeation pathway (Doyle et al., 1998), and the order and identity of the amino acid residues in the p-loop determine the precise ion selectivity properties of the channel (Heinemann et al., 1992; Lu et al., 2001).

Voltage-gated sodium, calcium, and potassium channels. The founding members of this family of proteins are the voltage-gated channels, including Nav, Cav, and Kv (Figs. 11.2A and 11.3A–B) (Catterall, 1998; Catterall, 2000; Jan and Jan, 1997). Voltage-gated channels are opened by changes in membrane potential detected by a voltage sensor connected to the pore domain. In the voltage-gated potassium channel, four homologous subunits assemble post-translationally into a pore-forming protein complex; in sodium and calcium channels, four homologous pseudosubunits are linked together in a long polypeptide to form a large α subunit. Each subunit or pseudosubunit contains six transmembrane segments organized with a four-transmembrane voltage sensor that is N-terminal to the two-transmembrane pore domain. By responding to changes in membrane potential, these channels play critical roles in shaping action potentials and facilitating neuronal responses to depolarization. For example, activation of voltage-gated sodium channels initiate and propagate action potentials, voltage-gated potassium channels hyperpolarize the cell and modulate action potential duration, and voltage-gated calcium channels regulate the activity of second messengers important for downstream signaling events

(see Chapter 4). Although most voltage-gated channels are activated by depolarization, other family members include hyperpolarization-activated cation channels that are involved in rhythmic activities (Lüthi and McCormick, 1998) and plant potassium channels that are activated by hyperpolarization (Schachtman et al., 1992; Gaymard et al., 1996; Marten et al., 1999).

Calcium-activated potassium channels. As their name suggests, calcium-activated potassium channels have potassium-selective pores that are gated by calcium (Berkefeld et al., 2010). These channels can be divided into two groups: large-conductance BK channels (Atkinson et al., 1991; Lee and Cui, 2010) and small-conductance SK channels (Köhler et al., 1996; Weatherall et al., 2010). BK channels are maximally activated by simultaneous depolarization and micro-molar intracellular calcium to facilitate the termination of action potentials and regulate neuronal excitability. Similar to the voltage-gated potassium channel, BK channels are complexes of four subunits that each contain six transmembrane segments forming a voltage sensor and a pore domain. In addition, BK channels have an extra S0 domain that places the N-terminus on the extracellular side and a large cytosolic calcium-sensing domain that uses the free energy of calcium interaction with acidic residues arranged in a “calcium gating ring” to drive changes in gate conformation (Fig. 11.3C) (Yuan et al., 2010). SK channel topology is also similar to that of the voltage-gated potassium channel topology, with six transmembrane segments per subunit and four subunits per channel protein. Unlike the BK channel, however, the SK channel is not gated by voltage, and its calcium-sensing domain is an auxiliary calmodulin (CaM) subunit. The CaM molecule is constitutively associated with the cytosolic CaM-binding domain of the SK channel, and the

FIGURE 11.2 Several major families of ion channels use similar designs. (A) The 6-TM family includes voltage-gated sodium, calcium, and potassium channels, the calcium-gated potassium channels, the cyclic nucleotide-gated cation channels, and the transient receptor potential channels. (B) The 2-TM family includes inwardly rectifying potassium channels and the bacterial potassium channel KcsA. (C) Two-pore potassium channels contain four transmembrane segments. (D) The voltage-gated proton channel contains just the voltage sensor domain from the 6-TM channels. (E) Ionotropic glutamate receptors contain pore-lining domains that appear to be upside down relative to other channels in this figure. Glutamate receptors in the animal kingdom (left) are cation channels whereas a prokaryotic glutamate receptor (right) is selective for potassium ions. (F) The cys-loop receptors are composed of five subunits, each with four transmembrane segments. The M2 segment lines the pore and rotates to open the channel upon acetylcholine binding. (G) IP₃ is a major second messenger that leads to Ca²⁺ ion influx from internal stores by binding intracellular calcium channels, such as InsP₃R Ca²⁺ release channels. The NH₂-terminal region includes an IP₃ binding domain. (H) The epithelia sodium channels have two transmembrane segments and a large extracellular domain between them. The extracellular domain respond to environmental stimuli to regulate channel function. (I) The P2X ionotropic purinergic cation channels also have two membrane spanning segments with a large extracellular domain that bind ligands. (J) Each subunit of the CIC channel has eighteen membrane segments, not all of which completely cross the membrane. Each subunit is arranged with approximate two-fold symmetry around a central pore. (K) The topology of TMEM16 family of ion channels has not been fully characterized. There are approximately eight transmembrane domains per subunit and two subunits assemble to form a pore. (L) The pivotal role of the chloride Cystic Fibrosis Transmembrane Regulator channel becomes clear when mutations in the channel lead to a deficiency in movement of salt and water out of cells, blocking passageways and ultimately leading to increased morbidity and mortality in human patients. It is a member of the ABC family and contains both transmembrane domains and nucleotide binding domains on the same polypeptide.

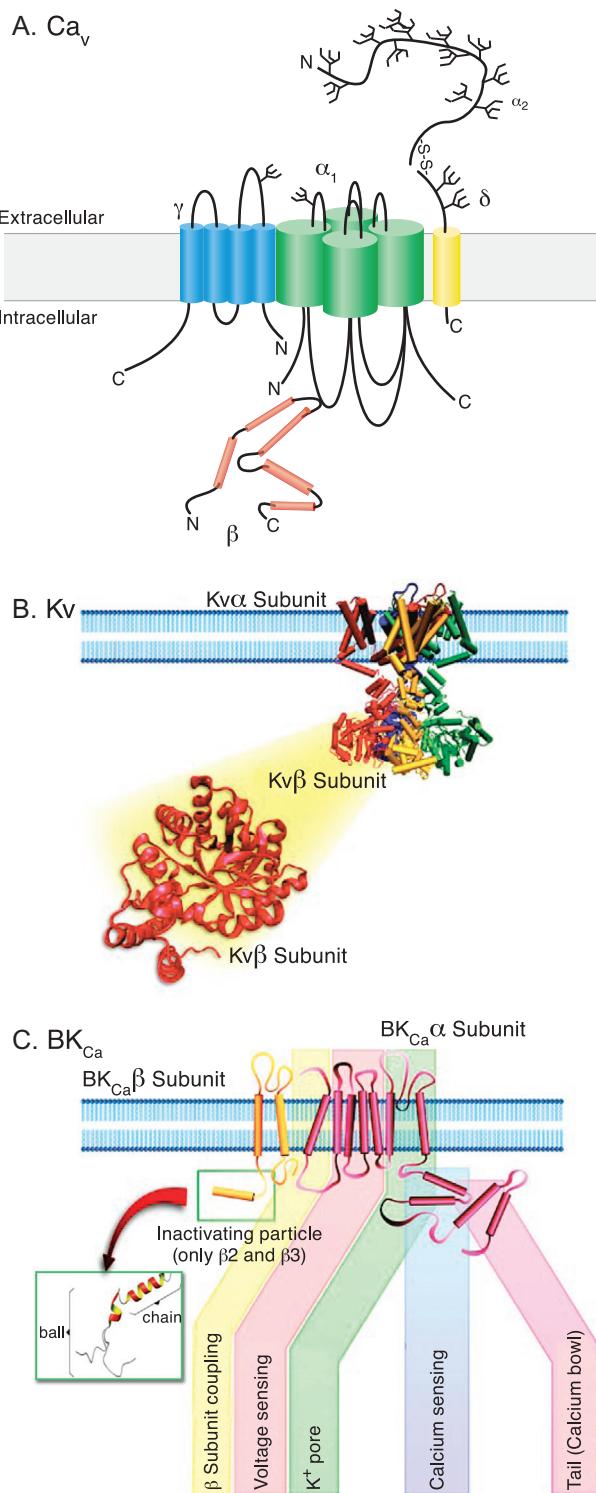


FIGURE 11.3 Voltage-gated Ca^{2+} and K^+ channels share a 6-TM architecture. (A) As with many other channels, Ca_V channels consist of many protein domains that allow the channel to be regulated by a variety of extra- and intracellular signals in addition to voltage sensitivity. The pore is formed by the α_1 subunit (green) while the β (red), γ (blue), and δ (yellow) subunits modulate channel function. Pitchforks on extracellular side indicate glycosylation sites and S-S indicate a disulfide bridge. N and C indicate N- and C-

binding of calcium to CaM causes conformational changes that are transmitted to the SK protein gate (Schumacher et al., 2001). By hyperpolarizing the neuron at the synapse during calcium influx, SK channels reduce AMPA-dependent EPSPs, thereby modulating synaptic plasticity and learning and memory (Adelman et al., 2012) (see Chapter 18).

Cyclic nucleotide gated channels. CNG channels are cation-selective and play a critical role in signal transduction pathways that use cGMP or cAMP as second messengers (Biel and Michalakis, 2009; Mazzolini et al., 2010). Although they share the six-transmembrane topology of Kv channels and have a functional voltage sensor (Tang and Papazian, 1997), CNG channels are largely insensitive to changes in membrane potential and are gated by cyclic nucleotides. Ligand binding is facilitated by a cytosolic cyclic nucleotide binding domain located at the C-terminus of each subunit. Cyclic nucleotide binding to each subunit triggers centripetal movement of the gating ring to open the channel (Taraska and Zagotta, 2007).

Other channels with architecture similar to CNG channels include the hyperpolarization-activated and cyclic nucleotide gated (HCN) channels (Wahl-Schott and Biel, 2009) and the *ether-a-go-go* (EAG) potassium channels (Bauer and Schwarz, 2001). HCN channels are sodium- and potassium-permeable membrane proteins that are activated by hyperpolarization. HCN channels are widely expressed in the brain and in the heart, where they are important for the regulation of the pacemaker current. Ligand interaction with its cyclic nucleotide domains modulates HCN channel activity by shifting its activation curves toward depolarizing potentials. EAG, along with eag-related (ERG) and eag-like (ELK) channels, are potassium-selective channels activated by depolarizing membrane potentials. Although these channels have a cyclic nucleotide binding domain, ligand binding does not seem to have a pronounced functional effect on channel gating.

termini of the polypeptide respectively. (B) Voltage-gated K^+ channels consist of four α subunits that together form a pore for the passage of ions, as well as a cytoplasmic β subunit that modulates channel function. (C) BK Ca is an example of a K^+ channel that has an additional domain sensitive to Ca^{2+} . The α subunit contains seven transmembrane domains. Four of these form a voltage-sensing domain and two of these form the potassium ion pore. The calcium-sensing domain is located in the cytoplasmic C-terminus. The β subunits assemble 1:1 with α subunits and modulate channel function. Certain β subunits contain an N-terminal inactivating particle that confers N-type inactivation onto the assembled channel. (A) adapted from Arkkath and Campbell (2003); (B) and (C) adapted from Torres et al. (2007).

Transient receptor potential channels. The transient receptor potential (TRP) channels are a diverse class of nonselective cation channels that are important for sensory transduction. They share a six transmembrane architecture with the K_V channels, but are generally not voltage sensitive and may be permeable to divalent cations like calcium (Montell, 2005). TRP channels may be activated by a variety of stimuli and thus serve key signal transduction roles in several sensory modalities. Different subtypes of TRP channels have been shown to be necessary for thermo-, mechano-, chemo-, and photosensation. In some cases, such as in the TRPV family responsible for thermosensation and mechanosensation, the ion channels are the physical sensors responsible for transducing environmental stimuli to electrochemical reactions. In other cases, such as in the TRPC family, which responds to second messengers of the phospholipase C (PLC) pathway and to calcium efflux from internal stores, the channels act as intermediaries in the signal transduction pathway. This is the case in the pheromone chemosensing pathway in the mammalian vomeronasal organ and the photon detection pathway in the *Drosophila* photoreceptor cells, where environmental stimuli are converted to neuronal depolarization by the activation of G protein-coupled receptors that signal through second messengers to activate TRP channels (Venkatachalam and Montell, 2007). Notwithstanding the prominence of TRP channels in sensory transduction, the mechanism by which these channels gate their pores remains poorly understood. Channel gating may involve several structural elements, including the N-terminal ankyrin repeats (Cordero-Morales et al., 2011), the pore turret (Yang et al., 2010), the C terminal cytoplasmic domain (Brauchi et al., 2006), and the transmembrane pore domain (Salazar et al., 2009).

Inwardly rectifying potassium channels. The inwardly rectifying potassium (K_{ir}) channels are also members

of the p-loop superfamily. Although the concentration of potassium ions is usually much higher inside the cell than outside the cell, K_{ir} channels are named for their ability to allow much larger potassium influx than efflux. Like K_V channels, K_{ir} channels have four α subunits lining the pore, but they only contain two transmembrane domains, which correspond to the pore domain of the K_V channel alpha subunit without the voltage sensor (Fig. 11.2B) (Jan and Jan, 1997). The observed inward rectification arises from a voltage-dependent block of the pore by the strong interaction of divalent cations with several acidic residues in the second transmembrane domain (Fig. 11.4). At hyperpolarizing potentials, extracellular cations—most of which are monovalent ions—tend to flow into the cell. Because the p-loop selectivity filter is selective for potassium over sodium, the inward flow of current consists mostly of potassium ions. However, since the cytoplasm contains high concentrations of magnesium and other di- and polyvalent cations, the efflux of these multivalent cations at depolarizing potentials tends to clog the pore. As a result, current is blocked in the outward direction (Nichols and Lopatin, 1997).

Channels in the classical $K_{ir}2$ subfamily are constitutively open while channels in the $K_{ir}3$ G-protein gated potassium channel (GIRK) and $K_{ir}6$ ATP-sensitive potassium channel (K_{ATP}) subfamilies open in response to ligand interaction. GIRK channels are activated by interaction of their cytoplasmic C-terminal domain directly with $G_{\beta\gamma}$ subunits. Their activation allows inhibitory transmitters such as GABA to generate slow inhibitory postsynaptic potentials (IPSPs) in the brain, and the parasympathetic transmitter acetylcholine to slow the heart rate (Lüscher et al., 1997; Wickman et al., 1998). K_{ATP} are inactivated by ATP interaction with $K_{ir}6$'s cytoplasmic domains and are activated by ADP interaction with the nucleotide-binding domain of an auxiliary sulphonylurea receptor (SUR) subunit that

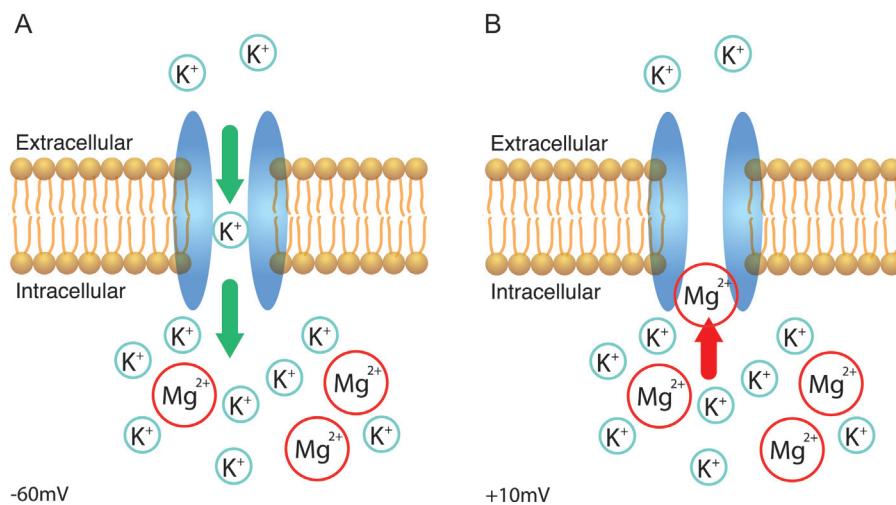


FIGURE 11.4 Voltage dependent block of K_{ir} channels. (A) At hyperpolarized voltages (e.g., -60 mV), potassium ions flow freely into the cell. (B) At depolarized voltages (e.g., $+10$ mV), movement of large divalent cations (Mg^{2+}) into the channel block potassium ion exit.

associates 1:1 with Kir6 subunits (Hibino et al., 2010). Besides regulating insulin release from the pancreas in response to changes in blood glucose levels, ATP-sensitive potassium channels are present the brain, the heart, and skeletal and smooth muscles (Quayle et al., 1997; Ashcroft and Gribble, 1998; Babenko et al., 1998; Ashcroft, 2000). In the central nervous system, these channels may be responsible for regulating food intake and metabolism (Yang et al., 2012b). In addition, metabolic stress may activate these channels to protect neurons from death (Yamada et al., 2001).

Two-pore potassium channels. Two-pore potassium (K_{2P}) channels differ from other p-loop channels in that they contain two pore-loop domains per subunit. Each pore-lining α subunit of these channels appears to be a tandem dimer of two K_{ir} -like, or one K_{ir} -like and one K_V -like, α subunits (Fig. 11.2C) (Lesage and Lazdunski, 2000). Because of their unique two-pore architecture, each subunit actually contributes two p-loops to the channel pore and channels assemble as dimers instead of tetramers. These channels are responsible for a “leak” potassium current that is active at rest, determining the resting membrane potential with their activities. Although many two-pore potassium channels are constitutively active, their activity can be modulated by various environmental stimuli, which makes them ideal candidates for sensory receptors. Among these candidates are the TREK channel subtype that can be modulated by mechanical perturbation of the membrane and by changes in temperature and pH, the TASK channel subtype that is sensitive to acid, and the TALK channel subtype that is sensitive to alkaline. Additional channels in the K_{2P} family include the TWIK, THIK, and TRESK groups (Enyedi and Czirák, 2010). Channel gating in the K_{2P} family may be mediated by alpha helices near the inner opening of the pore domain or in the plasma membrane bilayer (Miller and Long, 2012; Brohawn et al., 2012). In addition, many of these channels may have novel pharmacological profiles due to the presence of a “cap” that blocks access to their pore domain from the extracellular side (Patel et al., 1999).

Voltage-Gated Proton Channel

Members of the voltage-gated proton channel family have a voltage-sensor domain homologous to that of the K_V channels (Fig. 11.2D). Instead of having a separate pore domain, however, movement of the voltage sensor in response to depolarization opens a proton permeation pathway in the voltage-sensor domain itself (Berger and Isacoff, 2011). This pathway is filled with a continuous “wire” of water molecules that extend from the intracellular to the extracellular side, and protons are conducted along this wire by jumping from one

water molecule to the next (Ramsey et al., 2010). Each proton channel is a dimer composed of two separate voltage-sensor domains, each with its own independent pore that is allosterically coupled to the other so that channel gating exhibits positive cooperativity (Tombola et al., 2010). In excitable cells, these channels are responsible for extruding acid during action potentials, presumably in response to excess protons produced as a result of metabolic activity (Decoursey, 2003).

Intracellular Calcium Channels

Cation channels composed of four subunits with large cytoplasmic domains and six transmembrane segments each are responsible for releasing calcium from internal stores such as the endoplasmic reticulum (ER) (Mikoshiba, 1997; George et al., 2004). Members of this family include the inositol 1,4,5-trisphosphate (IP_3) receptors, which are activated by interaction with IP_3 second messengers, and the ryanodine receptors (RyR), which can be activated by direct interaction with voltage-gated calcium channels on the plasma membrane (Fig. 11.2G). No crystallographic structure of these channels has yet been resolved, but high-resolution electron microscopy has revealed a “square-mushroom” shape with a pore extending down the central axis. Similar to pore-loop potassium channels, the selectivity filter is thought to be a narrow constriction in the pore formed by TM5 and TM6 that is gated by the bending and rotation of a bundle of transmembrane helices located at the cytoplasmic surface (Foskett et al., 2007). In IP_3 receptors, the free energy used to drive channel activation comes from the interaction between phosphate groups on the IP_3 ligand and basic residues in the clam-shaped IP_3 binding core domain (Taylor and Tovey, 2010). Although the allosteric activation mechanism for ryanodine receptors is not well understood, gating for RyR channels is likely to be complex and involve several modulators such as CaV channels, calmodulin, calsequestrin, CaMKII, and FK506 binding proteins that dock onto its large cytoplasmic domain (Lanner et al., 2010).

Epithelial Sodium Channels

Epithelial sodium channels (ENaC) are voltage-independent, amiloride-sensitive, trimeric protein complexes that contain two membrane-spanning segments per subunit (Fig. 11.2H) and are responsible for the detection of a variety of sensory stimuli (Ben-Shahar, 2011). Members of the ENaC family include FMRFamide-activated sodium channels that are gated by ligands (Coscoy et al., 1998), acid-sensing ion channels (ASIC) that are activated by protons (Waldmann et al., 1997),

chemosensory channels that respond to salt (Chandrashekhar et al., 2010), and mechanosensory channels responsible for touch sensation (O'Hagan et al., 2005). The pore of these channels is lined by the second transmembrane helix of each subunit and is gated by a kink in the helix that rotates away from the central axis to open the ion permeation pathway. Ion selectivity is most likely achieved by the negative electrostatic potential of the pore vestibule and a narrow selectivity filter on the cytoplasmic side of the gate. A large extracellular domain is poorly conserved between different ENaC channels and acts as the sensor for the various stimuli detected by different ENaC channels (Jasti et al., 2007; Kashlan and Kleyman, 2011).

ClC Chloride Channels

ClC chloride channels are widely expressed throughout the body. In the nervous system, they regulate the excitability of neuronal membranes as well as the acidification of synaptic vesicles (Jentsch et al., 2005). These channels evolved from prokaryotic chloride/proton exchangers, and many of them remain voltage and pH sensitive. Structurally, ClC channels are dimeric proteins containing one pore per subunit (Fig. 11.2J). Each subunit is a two-fold symmetrical polypeptide with 18 transmembrane segments. The C-terminal half of the polypeptide is homologous to the N-terminal half, but oriented in the opposite, anti-parallel direction. The two halves of each subunit fold around a central pore running down the axis of symmetry, each orienting the N termini of two alpha-helices toward the ion permeation pathway and creating two positive electrostatic sites in the pore that selectively interact with chloride (Dutzler et al., 2002). The pore is gated by the side chain of a negatively charged glutamate residue that occludes the pore in the closed state and swings out of the way when it is protonated by low pH to reveal a third chloride binding site (Dutzler et al., 2003). The movement of chloride into this site is voltage dependent, and the competition between the ion and the glutamate side chain for this site is responsible for the voltage-dependent gating of ClC channels (Pusch et al., 1995; Pusch, 2004).

Cystic Fibrosis Transmembrane Regulator

The Cystic Fibrosis Transmembrane Regulator (CFTR) protein is a member of the ATP-binding cassette (ABC) superfamily that forms chloride channels in the heart, airway epithelium, and exocrine tissue (Sheppard and Welsh, 1999). Encoded by a single polypeptide, the CFTR protein contains a regulatory R domain in addition to the two transmembrane domains and two nucleotide binding domains found

in other ABC proteins (Gadsby et al., 2006). Full channel activation requires the phosphorylation of the R domain by protein kinase A (Cheng et al., 1991) followed by the interaction of the nucleotide binding domains with ATP (Vergani et al., 2005). This interaction drives the dimerization of the nucleotide binding domains, sandwiching two ATP molecules between them to open the gate. Simultaneously, a Walker motif in the second nucleotide binding domain begins to hydrolyze its ATP, which returns the channel to the closed state (Gunderson and Kopito, 1995; Berger et al., 2005). Each transmembrane domain contains six membrane-spanning segments (Fig. 11.2K), and homology models based on nonchannel ABC transporters as well as site-directed mutagenesis studies suggest that lysine and arginine residues in transmembrane segments 1, 5, and 6 form a positively charged environment in the pore and are important for ion selectivity (El Hiani and Linsdell, 2012).

TMEM16 Calcium-Activated Channels

There are ten genes in the mammalian genome that code for integral membrane proteins of the TMEM16 family (Fig. 11.2L) (Duran and Hartzell, 2011). Although not all of these genes have been characterized yet, at least three of them are calcium-activated ion channels. TMEM16A and TMEM16B are chloride channels that are responsible for regulating mucus secretion in the airway epithelium and membrane excitability in central and peripheral neurons (Huang et al., 2012b; Huang et al., 2012a), while TMEM16F is a nonselective cation channel that is involved in plasma membrane lipid scrambling in platelets (Yang et al., 2012a). As of this writing, biochemical studies have described these channels as dimeric proteins (Fallah et al., 2011; Sheridan et al., 2011; Tien et al., 2013), but other structural mechanisms responsible for calcium sensitivity and ion selectivity remain largely unknown.

Different Families of Ion Channels May Share Common Functional Modules

Some resemblance is found between the structures of different families of ion channels. For example, p-loop channels share a similar pore lining architecture with ionotropic glutamate receptors and a similar voltage sensor domain with voltage-gated proton channels. Likewise, even though P2X and ENaC channels share no homology on the sequence level, P2X and ASIC channels appear to have similar pore structures (Gonzales et al., 2009). In addition, there is a weak but discernible similarity between the pore-lining domain of potassium channels and the potassium transporters

of bacteria and yeast, which appear to contain K_{ir} -like pseudosubunits linked in tandem (Durell et al., 1999; Jan and Jan, 1997). Apparently, different ion channels and transporters may adopt the same fundamental structural motif for transporting ions across the membrane although the specific designs vary due to divergence of the physiological requirements.

Different Members of the Same Family May Have Divergent Functions

Whereas ion channels from the same family often have similar functional modules for ion permeation and/or channel gating, their functions can diverge to a remarkable degree. For example, pore-loop channels can be permeable to either sodium, potassium, or calcium, or be nonselective for cations (Yellen, 2002). Also, the same voltage module in pore-loop channels can be adapted to sense depolarization or hyperpolarization (Männikö et al., 2002), and other modules can be added to open channels in response to calcium, ATP, or metabotropic second messengers. These differences in function can be further varied by expressing channels at different times or in different cellular compartments. Such diversity of function may have arisen following expression amplification by channel gene duplication.

CHANNEL GATING

In general, the energy required to change the conformation of a channel gate from a closed to an open state is provided by the interaction of the sensor domain with a stimulus. These interactions cause movements throughout the channel that bend, twist, or otherwise distort the gate to allosterically open the pore. In ligand-gated channels, these deformations begin when an extracellular ligand-binding domain flexes to accommodate electrostatic and steric contacts with a ligand. In voltage-sensitive channels, the stimulus is detected by the movement of a voltage sensor pulled by an electrostatic force. In this section, we explore the mechanisms by which stimuli can activate channels in more detail. We focus on the pore-loop family of ion channels, a well-characterized group of proteins that, as described above, respond to many different types of stimuli and thus illustrate several different strategies used by channel proteins to link pore gating to the external environment.

The pore of many channels in the p-loop family share a common gating mechanism located at the intracellular end of the inner pore lining helix (called S6 in some channels and M2 in others). Much of our understanding of this gate comes from structural studies of crystallized

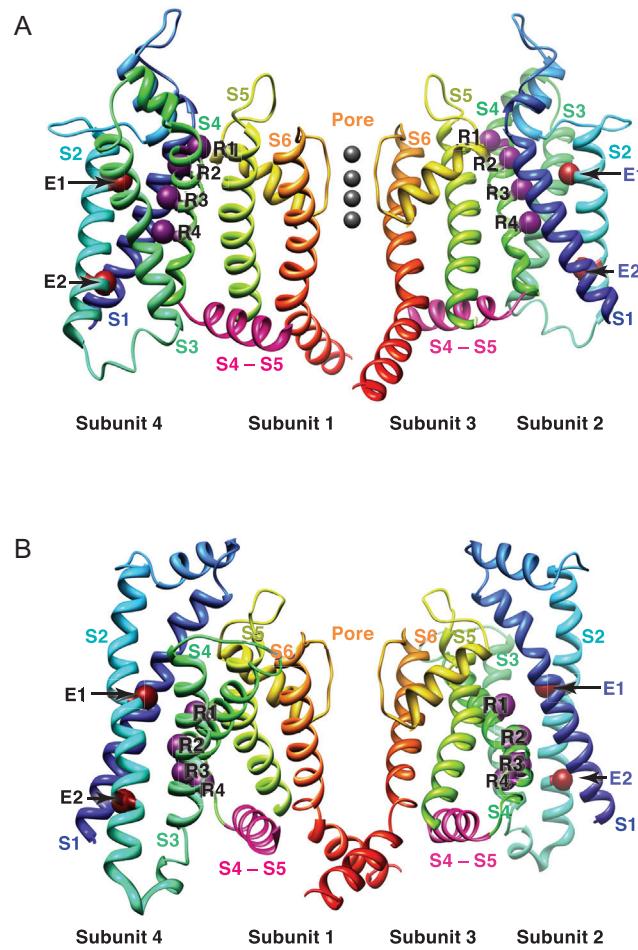


FIGURE 11.5 Movement of the S6 channel gate in the Kv1.2 channel. S6 helices are colored orange. The magenta S4–S5 linker helix is covalently linked to the voltage sensor and transmits sensor movement to the gate by pushing on the S6 pore helix. (A) In the open state, S6 helices are kinked outward, allowing ions (green circles) to flow freely. (B) In the closed state, the S4–S5 linker pushes down on the S6 pore helix, straightening out the structure. The straightened S6 helices cross each other at the intracellular end and occlude the pore. From Catterall (2010).

ion channels. The first potassium channel to be examined at atomic resolution was the two-transmembrane, proton-activated potassium channel KcsA from bacteria (Fig. 11.1). In the closed state, the inner pore segment is straight and forms a crossed helical bundle at the cytoplasmic end of the pore to occlude ion permeation (Doyle et al., 1998). Comparison of this channel with the calcium-gated bacterial MthK channel in the open conformation (Jiang et al., 2002a; Jiang et al., 2002b; Webster et al., 2004; Elinder et al., 2007) indicates that these inner pore helices bend at a hinge point to splay away from the pore and open the channel (Fig. 11.5). Such a model is in agreement with structures of eukaryotic potassium channels where the inner pore helix also bends at the

cytoplasmic end to constrict and dilate the pore (Long et al., 2005b; Long et al., 2007).

Instead of (and sometimes in addition to) a bend in the inner pore helix, K_{ir} channels use the rigid body movement of an alpha helix to constrict the pore. As seen in the structure of the closed bacterial KirBac1.1 channel, a large hydrophobic residue blocks water and ion access to the pore from the cytoplasmic side (Kuo et al., 2003). A direct comparison between closed and open conformations provided by the crystal structure of a eukaryotic Kir3 chimeric channel suggests that this residue is rotated away from the axis of the pore by rigid-body rotations and displacements of channel subunits relative to each other during gate

opening and closing (Fig. 11.6B–C) (Nishida et al., 2007; Whorton and MacKinnon, 2011). This model is in agreement with functional studies of this region based on Kir2.1 (Minor et al., 1999) and Kir3.2 (Yi et al., 2001) proteins that map this residue's position in the membrane and indicate its importance in channel gating.

How Does Voltage Open Channels?

Voltage-gated ion channels contain intrinsic voltage sensors. Voltage-gated ion channels typically are closed at the resting membrane potential but open upon membrane depolarization. These channels detect changes in

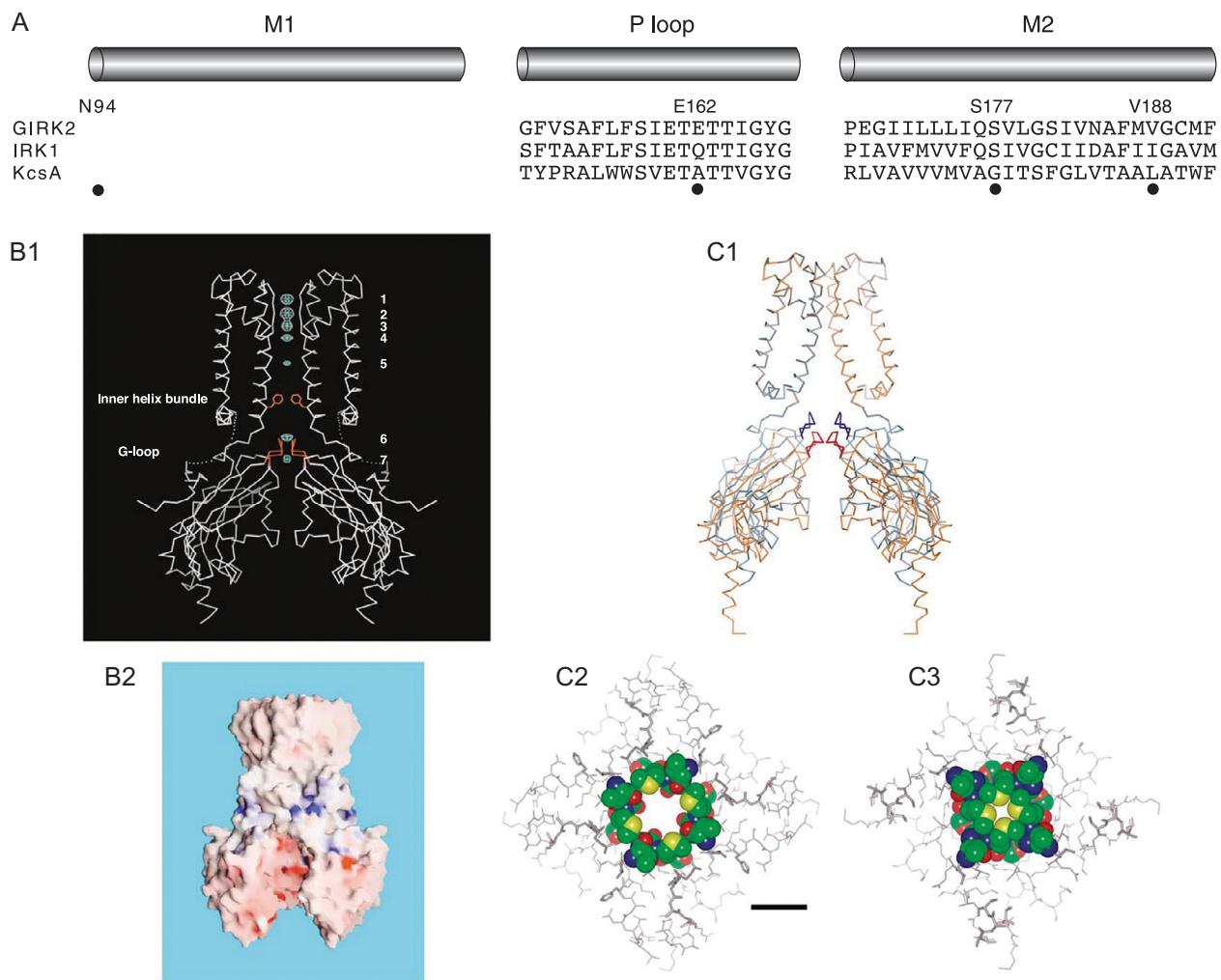


FIGURE 11.6 Gating model for G protein-activated inwardly rectifying potassium channel Kir3.2 (GIRK2). (A) Sequence alignment of the first transmembrane segment M1, the P loop and the second transmembrane segment M2 of GIRK2, IRK1, and KcsA. Yeast screens of randomly mutagenized GIRK2 channels reveal that mutations of the four residues marked above the GIRK2 sequence cause the channel to be constitutively open. (B1) Side view of a chimeric Kir3.1-prokaryotic Kir potassium channel showing the conduction pore in its open conformation. (B2) Space-filling model of the channel shown in (B1). (C) Models of the transmembrane helical arrangement in the open and closed forms. The pore region of the dilated (blue) and constricted (red) conformations have been superimposed in (C1) to show the movements of the G loop from the open (C2) to closed (C3) positions of the channel. (A) Adapted from Yi et al. (2001); (B) and (C) from Nishida et al. (2007).

electric potential across the membrane through a domain responsible for sensing voltage. The voltage sensor spans the membrane and is thus exposed to the electric field across the phospholipid bilayer. Charged residues in this sensor move in response to changes in membrane potential and trigger conformational changes of the channel. The movement of these gating charges results in a gating current that has been measured in biophysical experiments (Keynes, 1994; Sigworth, 1994).

The S4 segment corresponds to the voltage sensor. In the voltage-gated ion channels, these gating charges correspond to several basic, positively charged residues at every third position in the S4 transmembrane alpha helix. Each of the four subunits or pseudosubunits of these channels contain one voltage sensor, and depolarization of the cell membrane exerts an electrostatic force on the gating charges that causes the S4 segment to move outward (Fig. 11.7). The concerted movement of the four S4 segments cooperatively opens the channel gate.

S4 moves in the voltage-gated sodium channel. Movement of the S4 segment in the fourth pseudosubunit of the voltage-gated sodium channel has been detected experimentally (Horn, 2000). By replacing an arginine of this S4 segment with a cysteine, which can react with thiol reagents such as MTSET, Horn and

colleagues tested whether this cysteine is exposed to water. If the cysteine can access MTSET dissolved in water, the cysteine-thiol reaction sometimes results in an alteration in the kinetic properties of the channel, thereby providing an electrophysiological readout of the covalent modification. Remarkably, a cysteine at the position of the third arginine of this S4 segment is accessible from the cytoplasmic side of the membrane when the channel is closed, but becomes accessible from the extracellular side when the channel is activated by depolarization, suggesting movement of the S4 segment (Yang et al., 1996).

S4 moves in the voltage-gated potassium channel. Similarly, this movement has been confirmed by observations made in the homotetrameric Shaker potassium channel. As shown by Isacoff and colleagues, only the second arginine of the S4 segment is buried in the closed potassium channel; a cysteine at that position cannot react with thiol reagents from either side of the membrane. As the channel opens upon membrane depolarization, the second arginine becomes exposed to the extracellular side of the membrane, whereas the third, fourth and fifth basic residues move from the cytoplasmic side to sites buried in the membrane (Larsson et al., 1996). This S4 movement can also be monitored by a rhodamine fluorophore that is covalently attached to the S4 segment and reports changes in its environment by changing its fluorescence. The time course of the fluorescence change parallels the time course of the gating current (Mannuzzu et al., 1996), allowing verification that the S4 movement reflects channel gating.

Gating charges are stabilized in the membrane by interaction with acidic residues. The existence of basic residues in a hydrophobic membrane environment is usually energetically unfavorable. To stabilize these positively charged side chains, acidic residues are positioned along the movement path of the S4 gating charges as they move from one side of the membrane to the other. The existence of these neutralizing groups are detected in experiments where charge reversal mutations of S4 basic residues can be rescued with compensatory mutations of acidic residues in S2 (Papazian et al., 1995). In addition, cysteine mutations of basic residues in S4 and acidic residues in S2 can form disulfide bonds and prevent voltage sensor movement. Depending on whether the specific basic-acidic residue pair interacts when the voltage sensor is in its activated or deactivated positions, the channel will lock into either an open or closed state. Sequential mutations along S2 have shown that multiple S2 acidic residues will interact with a single gating charge as it traverses the membrane to shield it from the hydrophobic lipid environment (DeCaen et al., 2008; DeCaen et al., 2009).

S4 likely moves in a helical screw motion. Because voltage-gated ion channels typically are activated at

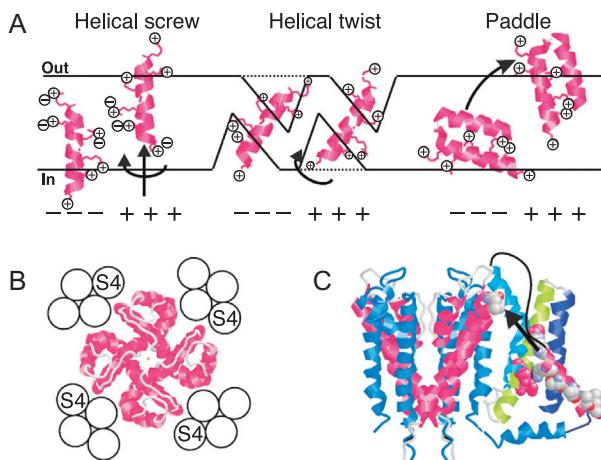


FIGURE 11.7 Various voltage-sensor movement models. (A) Three major movement models have been proposed for how the S4 voltage sensor moves outward during depolarization to open the channel. First proposed was the helical screw model, which involves both rotation and translational movement of the S4 segment. The helical twist model proposed rotation but not translation. A novel paddle model that suggests S4 movement as a hydrophobic cation through the lipid bilayer has stirred considerable controversy. (B) The position of the S4 segment has been another subject of considerable debate. The proposed position of S4 shown here with KcsA in the center places the S4 segment in the channel periphery, facing both lipids and proteins. (C) This position of S4 would allow large-scale translocation and is consistent with the helical screw model, shown here by the direction of the arrow. From Elinder et al. (2007).

0 mV, none of the atomic resolution structures of these channels have captured the voltage sensor in a resting state. As a result, the location of S4 during hyperpolarization and its movement during depolarization have remained hotly debated subjects (Tombola et al., 2006). Most modern models of voltage sensor movement describe it as an outward spiral motion of the S4 helix along its central axis (Fig. 11.7A) (Zhang et al., 2012). The vertical movement of the helix is quite large as disulfide locking experiments have shown that the same acidic residue on S2 contacts the first gating charge in the resting state and the last gating charge in the activated state (Broomand and Elinder, 2008), suggesting that at least four charges are moved across the membrane during activation. At the same time, a rotation of the S4 segment probably takes place during this motion since atomic distances between residues on S4 and S2 change during sensor movement in a way that cannot be explained by simple vertical translation (Cha et al., 1999; Glauner et al., 1999).

The four S4 segments of four identical subunits undergo first independent then concerted movements. The presence of four S4 segments that function as intrinsic voltage sensors of a voltage-gated ion channel accounts for the steep voltage dependence of channel activation. Upon depolarization, the S4 segments of the four identical subunits in a Shaker potassium channel move outward in two discernible steps, initially independently of one another then cooperatively in a concerted step, leading to channel opening (Mannuzzu and Isacoff, 2000). In crystal structures of the voltage gated potassium channel (Long et al., 2005a), the voltage sensor domain of one subunit interacts with the pore domain of an adjacent subunit, providing a mechanism by which inter-subunit cooperativity can be achieved.

Movement of S4 is coupled to channel gate. The movement of the voltage sensor is coupled to pore opening by the S4-S5 linker, which packs against one end of the S6 pore helix. In the open state, a glycine residue in S6 creates a kink in the structure, causing the helix to bend away from the pore and allowing ions to pass. In the closed state, the S4 voltage sensor moves inward, pulling the S4-S5 linker down to push on S6. This movement straightens out the S6 pore helix, causing the four rods to cross each other at the pore and occluding the movement of ions (Fig. 11.5) (Doyle et al., 1998; Jiang et al., 2002a; Jiang et al., 2002b; Long et al., 2005b).

Lipid environment support voltage-sensor function. Although research into the voltage sensor has traditionally centered on the protein itself, recent research has clarified the importance of the lipid environment in which the sensor is embedded. Crystal structures of voltage-gated potassium and sodium channels reveal an intimate contact between the ion channel protein and the surrounding lipids (Payandeh et al., 2011;

Long et al., 2007). The negatively charged head groups of phospholipids help stabilize the positive gating charges as they move across the membrane (Schmidt et al., 2006); enzymatic removal of these negative charges immobilizes the voltage sensor (Xu et al., 2008). The hydrophobic fatty acids of the lipids, meanwhile, are important for regulating the conformational flexibility of the voltage sensor. Changing the lipid environment of voltage-gated channels can change the energetically favored conformation at depolarizing voltages from an activated to a deactivated state (Zheng et al., 2011) or lower their maximal open probabilities (Li et al., 2005). In addition, the lipid environment of the voltage sensor has been proposed to be important for positioning the S4 gating charges in the membrane (Hessa et al., 2005, Cuello et al., 2004), and the use of nonphysiological detergents may be responsible for distortions of the voltage sensor orientation in the first crystal structures of the voltage-gated potassium channels (Lee et al., 2005).

Why Don't Ion Channels Stay Open Indefinitely?

Channels inactivate after they activate and open. Whereas some voltage-gated ion channels such as the M-type voltage-gated potassium channel stay open as long as the membrane potential is above the threshold for channel activation, most voltage-gated ion channels inactivate. In other words, the channel stops conducting ions even though the membrane potential is maintained at a depolarized level. Such a mechanism is important for the unidirectional movement of the action potential. As the action potential propagates, it activates voltage-gated sodium channels along the axon, which further depolarize the membrane. Because sodium channels inactivate, the channels located along the section of the axon that has recently been depolarized cannot be reactivated, preventing the action potential from spreading backwards.

Channels can inactivate in different ways. N-type (“fast”) inactivation takes place via interaction of an “inactivation ball” at the N-terminus of the α or β subunit with the cytoplasmic end of the pore, whereas C-type (“slow”) inactivation involves the extracellular end of the pore.

N-type inactivation takes place near the cytoplasmic end of the pore. N-type inactivation is also known as the “ball and chain” mechanism for inactivation (Armstrong and Bezanilla, 1977; Stotz and Zamponi, 2001). A cytoplasmic ball peptide at the N-terminus of the α or β subunit (Rettig et al., 1994; Wallner et al., 1999; Zagotta et al., 1990) appears to bind to a “receptor” within the channel pore that becomes accessible

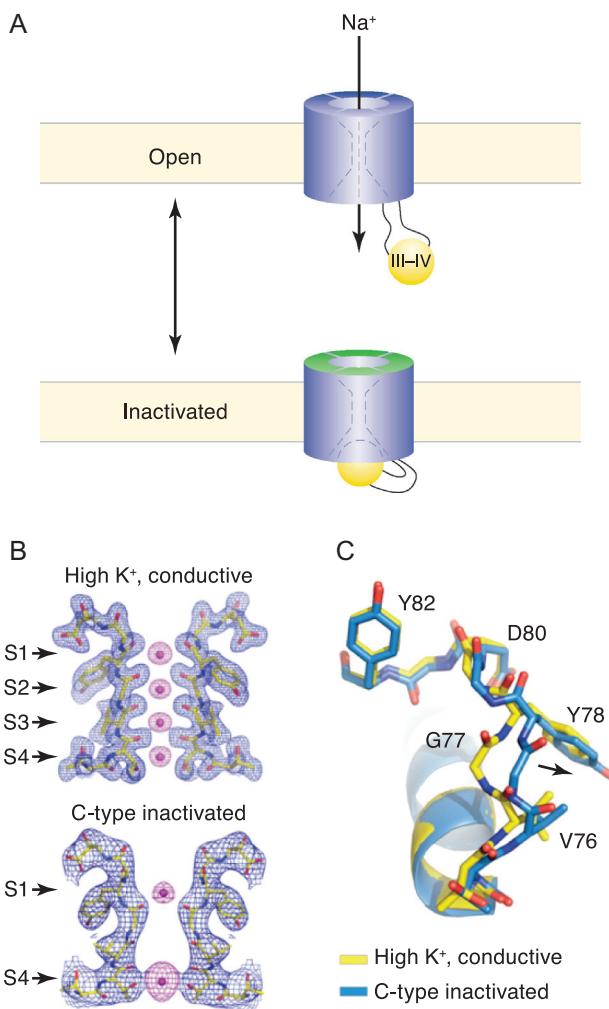


FIGURE 11.8 Inactivation in pore-loop channels. (A) In N-type inactivation, a cytoplasmic ball peptide binds to the intracellular side of the channel pore in the inactivated state. (B) In C-type inactivation, movement of ion-binding residues in the selectivity filter prevents ion flow. In the open state (top), four ion binding sites are available and electrostatic repulsion between bound ions at those sites pushes ions out of the pore. In the closed state (bottom), two ion binding sites are lost and ion flux is stopped. (C) Comparison of the filter in the open (blue) state and the closed (yellow) state. Oxygen atoms are colored in red and their movement prevents coordination of ions in the filter. (A) from Stotz and Zamponi (2001); (B) from Cuello et al. (2010b).

when the channel opens (Fig. 11.8A). The interaction of the ball peptide with the pore blocks ion permeation and inactivates the channel. Residues implicated in either electrostatic or physical interaction with the inactivation ball have been found in the sequences just preceding the first transmembrane segment S1 (Gulbis et al., 2000), the cytoplasmic loop connecting the S4 and the S5 segments, and the S4-S5 loop itself (Isacoff et al., 1991; Yellen, 1998). Although the inactivation ball is thought to physically plug the pore at the

cytoplasmic side (Zhou et al., 2001a), no atomic resolution structure of an inactivated channel exists, and there is little room in known channel structures to allow access of the inactivation ball to the pore. Therefore, it is also conceivable that inactivation could result from conformational changes triggered by allosteric channel interaction with the ball, which is functional even in isolation from the channel.

N-type inactivation may couple to voltage gating in different ways, and the details of these interactions may vary for different channels. Some channels can inactivate only after some or all of their voltage sensors have moved in response to membrane depolarization. In addition, the voltage sensors may be “immobilized” in their activated configuration once the channel has entered the N-type inactivated state. In this case, bringing the membrane potential from hyperpolarized to depolarized would cause a channel to shift from the deactivated to the activated and then to the N-type inactivated state. Upon reversing the membrane potential to a hyperpolarized level, the channel would have to reverse course, going from the N-type inactivated to the activated state before returning to the deactivated state. Thus, the channel would open briefly after the membrane potential is brought back to a hyperpolarized level. Such reopening of voltage-gated calcium channels could cause the “delayed release” of transmitters observed following an action potential (Slesinger and Lansman, 1991).

C-type inactivation is slower than N-type inactivation. Even in the absence of an N-terminal ball peptide, channels can exhibit so-called C-type inactivation arising from structural changes along the pore region of the channel that affect the outer pore, the selectivity filter, and the voltage sensor (Kurata and Fedida, 2006). The first step in this process involves movements of pore-lining structures near the extracellular end of the pore and the attached p-loop (Loots and Isacoff, 1998; Gandhi et al., 2000; Larsson and Elinder, 2000). Because the p-loop forms the narrowest portion of the ion permeation pathway, these movements collapse the pore and prevent further ion conduction (Fig. 11.8B–C) (Kiss and Korn, 1998). The presence of ions in the selectivity filter can modulate this process by propping open the pore and prevent its collapse (Cuello et al., 2010b). Increasing the concentration of permeable ions will slow C-type inactivation, while blocking permeable ions from entering the pore will accelerate C-type inactivation (Baukowitz and Yellen, 1995).

Despite the importance of the selectivity filter, C-type inactivation is not just a local phenomenon and involves global movements of the other parts of the channel, including the voltage-sensing domain. First, movement of the voltage sensor during activation is responsible for tilting the S2 helix toward the pore domain and deforming the selectivity filter. This

mechanical distortion allosterically couples channel activation to C-type inactivation (Cuello et al., 2010a). Second, subsequent to pore collapse, gating charges in the voltage sensor are immobilized. During depolarization, fewer gating charges move across the membrane (Olcese et al., 1997) and there are smaller S4 sensor movements (Loots and Isacoff, 1998) when the channel is in an inactive rather than a deactivated state. This immobilization of the voltage sensor is dependent on the state of the selectivity filter, because occupancy of the pore by permeant ions is able to prevent this phenomenon (Chen et al., 1997). Once the channel has entered the C-type inactivated state, it must be hyperpolarized in order to “open” the inactivation gate and return the channel to a deactivated state, from where it can be once again be opened by depolarization.

Channel inactivation may be modulated by permeant ions and the lipid environment. In addition to N- and C-type inactivation, other mechanisms of inactivation have been described. For example, channels can be inactivated by the ions to which they are permeable, as is the case with calcium-induced calcium channel inactivation (Lee et al., 1999, Zühlke et al., 1999). Also, the pore of the channel is affected by the membrane environment in which it is embedded, and the ability of a channel to inactivate can depend on the identity of its surrounding lipids (Oliver et al., 2004).

How Does Calcium Affect Channel Activity?

Neuronal activities may regulate channel activities via calcium. Besides modulating membrane potential, neuronal activities may also involve changes in cytosolic calcium concentration. These changes occur by either the entry of calcium through voltage- or transmitter-gated calcium channels or the release of calcium from internal stores by second messengers. In response, some ion channels use the concentration of intracellular calcium as cues to open or close their pores. There are at least two ways by which channel function can be modulated by calcium. First, the channel may have a calcium sensor domain that interacts with calcium directly and transmits these motions to the gate. Second, the channel may instead rely on other proteins like calmodulin, which bind calcium and indirectly modulate channel activities.

BK channels are activated by a calcium gating ring. Direct calcium action underlies the calcium activation of the large conductance calcium-activated potassium (BK) channels (Lee and Cui, 2010). BK channels are sensitive to both voltage and calcium. Increasing intracellular calcium concentrations over six orders of magnitude causes the current-voltage dependence curve of channel activation to shift progressively to the left,

meaning that increasing internal Ca^{2+} enables lower voltages to generate the same current through these channels (Cui et al., 1997; Meera et al., 1997; Barrett et al., 1982). How is this amazing feat accomplished? In addition to a voltage-sensing domain similar to the voltage-gated channels, BK channels have a C-terminal cytoplasmic region that contains two calcium-binding RCK (Regulator of K^+ Conductance) domains. In the assembled protein, the four channel subunits form a large intracellular calcium-gating ring that binds eight calcium ions and facilitates calcium sensing (Fig. 11.9A) (Yuan et al., 2010). In the presence of calcium, negatively charged aspartate residues in each RCK domain coordinate one calcium ion, and this interaction pries open the interface between two RCK domains like a clamshell and stretches the diameter of the gating ring (Fig. 11.9B). Because the gating ring is covalently linked to the S6 pore helix, this motion pulls on the gate to tug it open (Jiang et al., 2002a; Ye et al., 2006). This mechanism by which calcium opens the pore independently from the voltage sensor is consistent with observations that the two activation gates are at most weakly cooperative (Cui et al., 1997; Cui et al., 2009).

Additional motifs responsible for calcium sensing may also lie outside the C-terminal domain. BK channels truncated right after S6 are still gated by calcium (Piskorowski and Aldrich, 2002), and coexpression of the $\beta 1$ subunit can partially rescue calcium-dependent gating in BK channels whose RCK calcium-binding sites have been mutated (Qian and Magleby, 2003). However, as of this writing, no additional calcium-binding sites have yet been identified.

Other channels, such as SK, are activated by interaction with calmodulin. Ion channels may also be gated by calcium indirectly through soluble calcium sensors not covalently linked to the pore domain, such as free calmodulin (CaM) molecules. CaM is a soluble cytosolic protein with four calcium-binding sites and interaction with calcium causes CaM to undergo conformational changes (Meador et al., 1993; Hoeflich and Ikura, 2002). An ion channel’s activity may be modulated by CaM by either interacting with CaM in a calcium-dependent manner or by sensing the conformational changes of a constitutively bound CaM molecule. The latter mechanism is responsible for the calcium-sensitivity of small conductance calcium-activated potassium channels (SK) (Xia et al., 1998), the inactivation of calcium-induced calcium channels (Budde et al., 2002), and the calcium modulation of cyclic nucleotide-gated cation channels (Chen and Yau, 1994).

In the SK channel, this CaM molecule is bound by a cytosolic CaM binding domain C-terminal to the sixth transmembrane helix. In the absence of calcium, CaM and its binding domain are in an extended, monomeric conformation. When the N-lobe of the CaM molecule

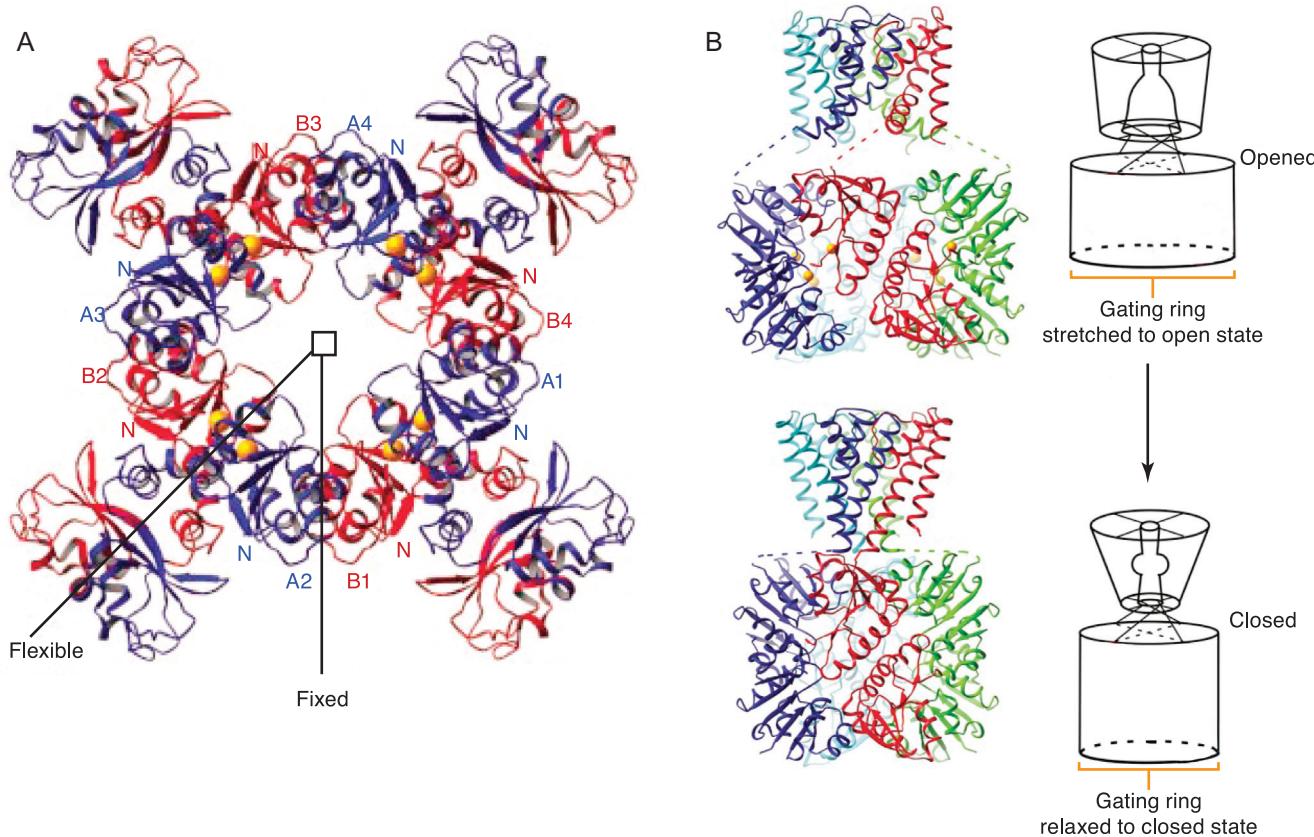


FIGURE 11.9 Calcium-dependent gating in BK channels. (A) Structural model of the calcium gating ring in BK channels. A1–A4 and B1–B4 are calcium-binding RCK domains. (B) In the closed conformation (bottom), the diameter of the closing ring is hypothesized to be smaller and the RCK domains are not bound to calcium. In the open conformation (top), interaction with RCK domains stretches the gating ring through a rotational motion of rigid bodies in the RCK domains, and this pulls on the S6 helices to open the pore. Adapted from Jiang *et al.* (2002a).

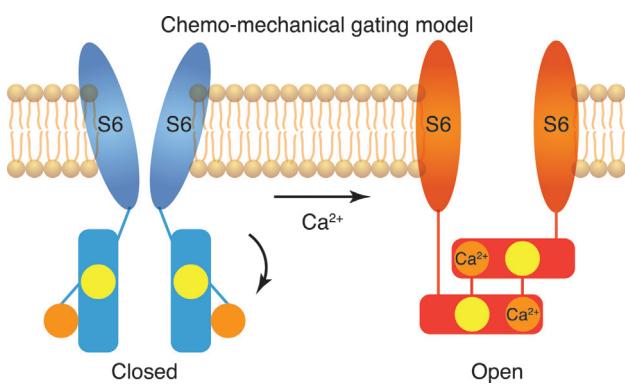


FIGURE 11.10 Calcium-dependent gating in SK channels. Cartoon model of the S6 helices in closed (blue) and open (red) states. Calmodulin binds the calmodulin-binding domain (cytoplasmic rectangles) with its C-terminal lobes (yellow) and binds calcium with its N-terminal lobes (orange). Upon calcium interaction, the N-terminal lobes bind to the adjacent SK channel subunit, causing dimerization. This movement pulls on the S6 helices to open the gate. From Schumacher *et al.* (2001).

binds calcium, however, it wraps itself around the CaM binding domain of an adjacent subunit, reorienting the CaM binding helix and forming a dimer between the two molecules. Because the CaM binding domain is covalently linked to the S6 inner helix, this movement pulls on the pore domain and opens the gate (Fig. 11.10) (Schumacher *et al.*, 2001; Schumacher *et al.*, 2004).

How are Ion Channels Influenced by Metabotropic Receptor Signaling Pathways?

Metabotropic transmitter receptors are coupled to the trimeric G protein. Some ion channels are indirectly activated by intercellular transmitter molecules through enzymatic signaling pathways. Many of these transmitters act through a family of transmembrane G protein-coupled receptor (GPCR) proteins (see also Chapters 10 and 16). In the resting state, the α subunit of the trimeric G protein is bound to a molecule of GDP. Transmitter activation of GPCRs facilitates the

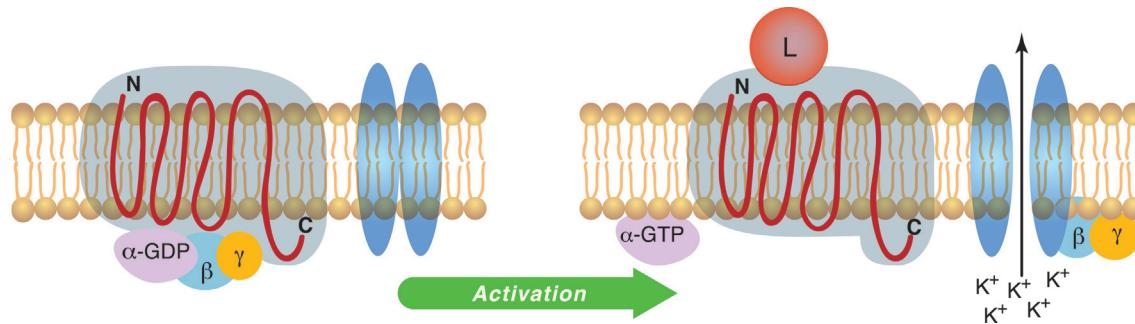


FIGURE 11.11 G protein-activated inwardly rectifying potassium channels (K_{ir}3) are activated by direct interaction with the $\beta\gamma$ subunits of G proteins. L represents the ligand for the G protein-coupled receptor with seven transmembrane segments, e.g., the parasympathetic transmitter acetylcholine for slowing the heart rate or the inhibitory transmitter GABA for generating the slow inhibitory postsynaptic potential in the central nervous system. Ligand interaction with the receptor causes exchange of GTP for GDP in the α subunit and the dissociation of the $\beta\gamma$ subunit to interact with K_{ir}3.

exchange of GTP for GDP, leading to the dissociation or rearrangement of the α -GTP subunit from the $\beta\gamma$ subunit (Fig. 11.11 and see also Chapter 4) (Bünemann et al., 2003; Papin et al., 2005). Depending on the identity of the GPCR and the G protein, the α and $\beta\gamma$ subunits may then activate adenylyl cyclase (G_s), inhibit adenylyl cyclase (G_i), or activate phospholipase C (PLC) (G_q). These G protein subunits may also modulate the activities of other effectors and second messengers, such as calcium, cyclic nucleotides, phosphoinositides, and kinases. Each of these downstream targets may then transduce the signal to alter ion channel activities.

GIRK channels are modulated directly by interaction with G protein $\beta\gamma$ subunits. For example, the gate to G protein-activated inwardly rectifying potassium channels are opened by a direct interaction between GIRK channels and both the $\beta\gamma$ subunit of G proteins and the second messenger phosphatidylinositol-4,5-biphosphate (PIP₂) (Sadja et al., 2003). PIP₂ association with GIRK channels is a prerequisite for channel activation. When PIP₂ lipid molecules are bound between the two GIRK transmembrane segments, the negatively charged PIP₂ phosphate residues are coordinated by positively charges of the transmembrane helices, causing the pore helices to rotate and splay apart. This, however, does not fully open the channel. GIRK channels, unlike other members of the pore-loop family, have an additional pore constriction in their cytoplasmic domain immediately underneath the pore in addition to the gate formed by the crossing of the pore helices. This second gate, called the G-loop, is opened when the $\beta\gamma$ subunit interacts with the cytoplasmic N and C terminal domains of the GIRK protein, rearranging the protein folds of the cytoplasmic domains and widening the G-loop gate (Fig. 11.6C) (Whorton and MacKinnon, 2011).

Other channels can be differentially modulated by metabotropic pathways. In the case of some Ca_V ion channels, interaction with $\beta\gamma$ subunits causes channel

inhibition instead of activation. Although voltage remains the primary stimulus that regulates the gate, its effects can be modified by the presence of other signals (Herlitze et al., 1996; Ikeda, 1996).

Multiple second messengers may converge on the same channel, resulting in integration of signaling processes. Integrative processes are particularly useful because they allow the cell to combine signals from many different pathways to regulate output at a single ion channel. These second messenger systems are described in more detail in Chapter 6, but here we briefly illustrate how they can exert major effects on ion channels. We have already discussed how GIRK channel gating is controlled by both the $\beta\gamma$ subunit of G proteins and the PIP₂ second messenger (from the G_q pathway) (Huang et al., 1998; Gamper and Shapiro, 2007). In the heart, acetylcholine first activates GIRK channels via m₂ muscarinic acetylcholine receptors by direct action of the G protein $\beta\gamma$ subunits on the channel. At the same time, acetylcholine causes GIRK channel desensitization by activating m₃ muscarinic acetylcholine receptors, which in turn activate PLC and reduce PIP₂ levels (Kobrinsky et al., 2000). Thus, by activating two separate signaling pathways, the activity of GIRK channels can be precisely controlled.

Similarly, the metabotropic modulation of Ca_V channels lies at the intersection of several signaling pathways. Protein kinase C (PKC) phosphorylation not only increases calcium channel activities but also prevents channel inhibition by the $\beta\gamma$ subunits of the G protein (Zamponi et al., 1997). In addition, PIP₂ also inhibits voltage-gated calcium channels by altering their voltage-dependence for channel activation; this inhibition is alleviated by phosphorylation by protein kinase A (PKA) (Wu et al., 2002). In these two examples multiple second messengers converge on the α subunit of the channel. In many other cases, second messengers impinge on both α and regulatory β subunits to modulate channel activities.

How Could the Metabolic State of a Cell Influence Channel Activity?

The K_{ATP} channel is inactivated by ATP. Intracellular ATP, an indicator of a cell's energy status, may also alter the activity of channels. As discussed above, ATP-sensitive potassium channels are inhibited by ATP but stimulated by ADP and contain four pore-lining Kir6 subunits as well as four regulatory ATP-binding cassette family SUR subunits (Fig. 11.12). Unlike other members of the pore-loop family discussed so far, activation of the K_{ATP} channel gate is facilitated by a binding site on the auxiliary subunit while deactivation is regulated by a binding site on the alpha subunit itself (Nichols, 2006). While no atomic resolution structure of the K_{ATP} channel is yet available, it is thought that PIP_2 and ATP share the same cytoplasmic binding sites located at the interface between adjacent channel subunits. In the absence of ATP, PIP_2 is bound and, depending on ligand interaction with the SUR subunit, the channel can enter its open state. Cytosolic ATP displaces the PIP_2 molecule and closes the gate by crossing the helix bundles in the pore domain (Antcliff et al., 2005).

The K_{ATP} channel is activated by ADP. At the same time, SUR1 contains a nucleotide binding site that is able to bind both ATP and ADP. When bound to ADP, SUR1 subunits interact with Kir6 subunits to open the gate and allow ion permeation. Although the exact

mechanism is not yet known, SUR interacts with the first transmembrane segment of Kir6 via its transmembrane domain and the allosteric modulation of the gate may occur through this interface (Tucker et al., 1997; Aittoniemi et al., 2009). When bound to ATP, the nucleotide-binding domains of SUR1 hydrolyze ATP molecules into ADP molecules, thereby opening the channel (Zingman et al., 2001). In this way, the K_{ATP} channel is sensitive to the ratio of cytoplasmic ATP and ADP, which provides an intricate mechanism by which to regulate channel activity.

ION PERMEATION

We now turn our attention to the selectivity filter of ion channels. How does a potassium channel select for the larger potassium ions over the smaller sodium ions, while permitting permeation rates close to the ion's diffusion limit? Given that the narrowest part of a sodium channel pore is actually larger than that of a potassium channel pore, how does a sodium channel allow sodium but not potassium ions to pass through? How does a calcium channel allow only calcium ions to pass through even though sodium ions are of nearly the same size and are much more abundant under physiological conditions? Many biophysical and molecular studies have been performed to try to

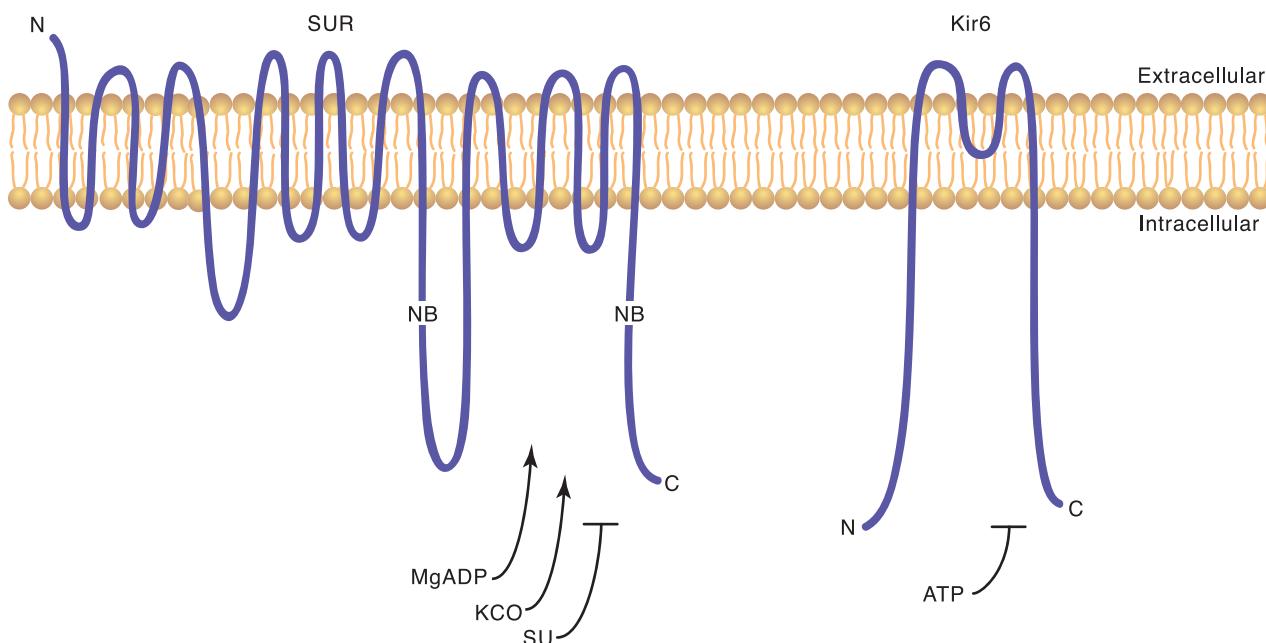


FIGURE 11.12 The ATP-sensitive potassium channels contain four pore-lining α subunits (Kir6) and four regulatory β subunits (SUR). SUR is a member of the ATP-binding cassette (ABC) family and contains two nucleotide binding (NB) domains. ATP acts on Kir6 to inhibit the channel whereas Mg-ADP acts on SUR to activate the channel. Sulfonylurea (SU) drugs that inhibit the channel and KCO compounds that activate the channel also act on SUR.

answer these questions over the past several decades (Hille, 2001). In this section, we will use the pore-loop channels as an example to describe current models of ion selectivity.

Potassium Selectivity

To achieve high selectivity while maintaining a high flux of ions through the pore, the voltage-gated potassium channel has multiple binding sites in its selectivity filter for the permeant ion. The high-affinity binding sites allow the channel to selectively interact with potassium, thereby making it energetically favorable for potassium ions to move through the pore while excluding other ions. But if the channel binds to potassium, why then does not the ion get stuck in the pore? Because the pore contains multiple potassium binding sites, several ions occupy the selectivity filter simultaneously. Electrostatic or other long-range interactions between these ions in the pore facilitate their dissociation from their binding sites, thereby allowing rapid flow of ions across the membrane.

Evidence for potassium binding sites in the K_V selectivity filter. Early indications for potassium binding sites in the selectivity filter arose from examining the amount of current flowing through a channel as a function of ion concentration: the fact that current through the pore saturates at high ion concentrations suggests that there is a rate-limiting interaction between the ion and the channel. Another indication for ion binding sites came from the “test of independence.” If one assumes that permeant ions move through the channel without interacting with the pore, their flux should be independent of the concentration of other permeant ions in solution. This prediction does not fit the experimental data, indicating that there are ion interaction sites in the channel pore, resulting in inter-dependence between ions as they permeate the channel (Hille, 2001).

Evidence that there is more than one potassium binding site in the pore. The presence of multiple ion binding sites in the K_V channel pore has been known since the 1950s, when Hodgkin and Keynes (1955) showed that the ratio of potassium efflux and influx has a slope of 2.5 when plotted against driving force. If there is only one ion binding site in the pore, one would expect that the ratio of potassium ions leaving the cell to those entering the cell to be equal to the ratio of potassium ion concentration inside the cell to that outside the cell because ions can move freely in both directions. However, if the pore has multiple ion binding sites, the flow of ions cannot easily change directions. Like a narrow bridge, if an ion is in the middle of the pore and a second ion comes in behind it, the first ion can only move forward because the reverse route is

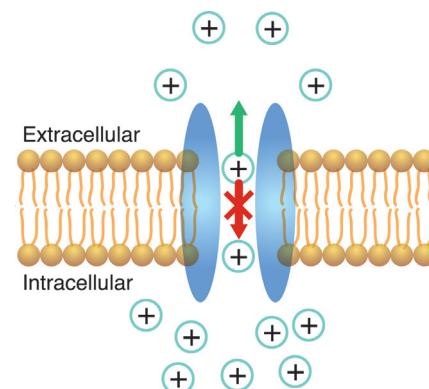


FIGURE 11.13 Movement of multiple ions in a channel pore in concert. If the ion near the intracellular end of the pore is moving outward due to the imbalance of ions across the membrane, the ion in the next binding site is blocked from moving inward. Thus, the ratio of potassium efflux to influx is greater than one even though each potassium ion carries one unit charge.

blocked (Fig. 11.13). As a result, if the concentration of potassium ions is higher inside the cell than out, the ratio of ions that move out to those that move in would be higher than the driving force would suggest (Hagiwara et al., 1976; Hille, 2001).

Another indication that there are multiple ion binding sites comes from the so-called anomalous mole-fraction effect. Let us suppose that a channel is permeable to two types of ions (Fig. 11.14). Compared to permeant ion B, permeant ion A binds to the channel's external binding site more tightly and also moves through the channel at a slower rate. If the extracellular solution contains predominantly the B-type permeant ions mixed with a small amount of the A-type permeant ions, occupation of the channel's external binding site by permeant ion A would block the binding and passage of permeant B through the channel, and the flux of ion B would be smaller than what would be expected if ion B were the only species present. However, because of the difference in affinity, it is also more difficult for permeant ion B than for another permeant ion A to displace the A ion at the external binding site. Since the extracellular solution contains mostly ion B, most of the ions trying to enter the pore are the B ions that cannot displace the bound A ion and there is no mass action force pushing A forward. As a result, the flux of ion A is also smaller than what one would expect if ion A were the only species present. This apparently paradoxical, or anomalous, phenomenon arises when a channel contains more than one ion binding site and has been observed in potassium channels (Hagiwara et al., 1977; Hille, 2001).

Crystallographic evidence for multiple ions in the pore. Direct evidence for the presence of multiple permeant ions in the channel pore is provided by visualization

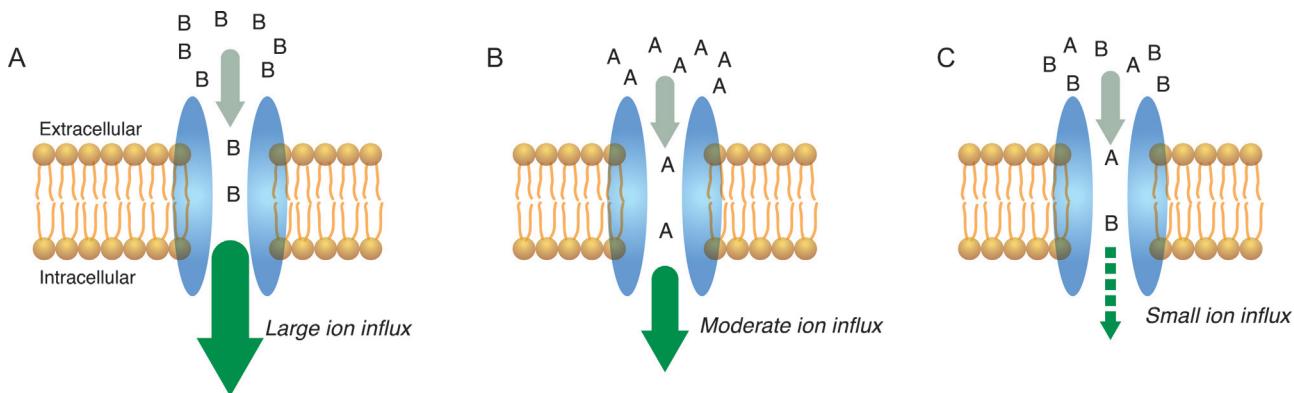


FIGURE 11.14 Anomalous mole-fraction effect. (A) In the presence of only permeant ion B the ion flux is large because permeant ion B moves through the channel at a fast rate. (B) In the presence of only permeant ion A the ion flux is moderate because permeant ion A binds more tightly to the channel's external binding site and moves through the channel more slowly. (C) In the presence of predominantly permeant ion B mixed with a small fraction of permeant ion A, the ion flux is smaller than in either of the above cases, because permeant ion A binds tightly to the external binding site in the channel and blocks passage of permeant ion B.

of the bacterial KcsA potassium channel in the crystal form (Fig. 11.1) (Doyle et al., 1998; Morais-Cabral et al., 2001). The selectivity filter is located within the upper half of the pore adjacent to the extracellular surface, and it contains four potassium binding sites in a row. Each site is coordinated by the backbone carbonyl groups of eight residues of the p-loop region (Fig. 11.15).

Potassium ions in solution are typically surrounded by a shell of water molecules. Because the selectivity filter is so narrow, though, ions have to lose most of these water molecules in order to enter the filter. To balance out the energetically unfavorable reaction of separating the charged potassium ion from the dipole moment of water molecules, potassium ions interact with the dipole moments of the carbonyl oxygens in the selectivity filter. Sodium ions are excluded from the filter because their atomic radius is smaller than that of potassium and thus cannot fully interact with all of the coordinating carbonyl groups (Zhou et al., 2001b). In this way, potassium channels are selective for the larger potassium ions over the smaller sodium ions.

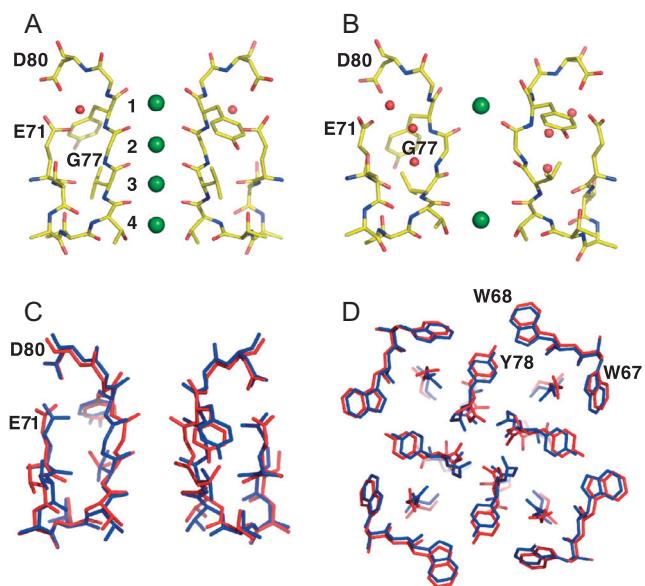
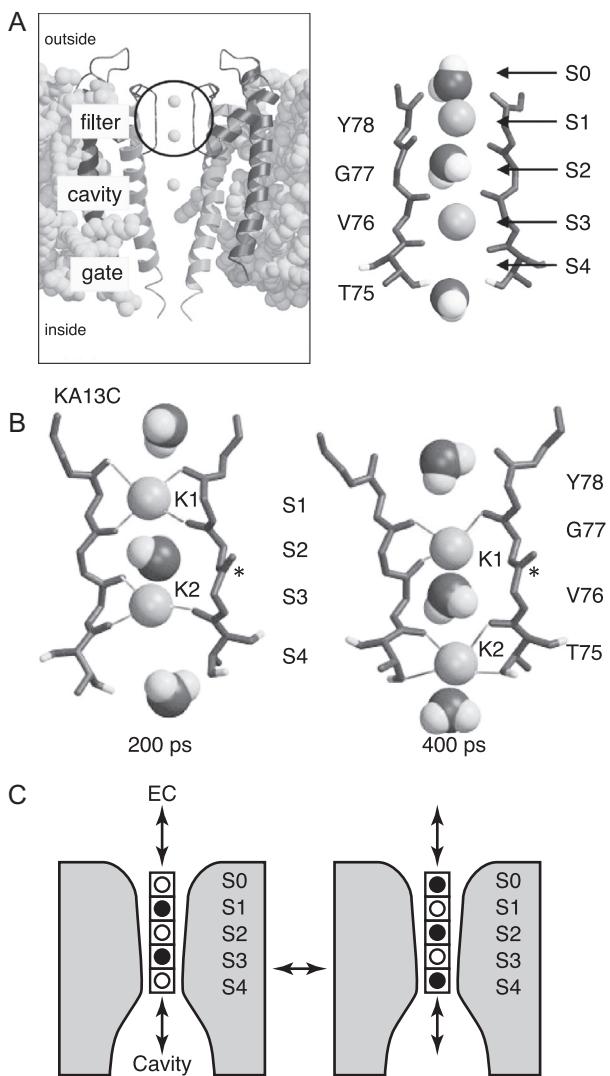
The channel then utilizes repulsion between bound potassium ions to free the ions from the selectivity filter and push them out of the pore. Even though the KcsA channel has four potassium binding sites in its selectivity filter, there can only be two simultaneously bound ions because the sites are so close together that electrostatic interactions prevent potassium ions from occupying adjacent sites. Suppose that there are two ions already in this narrow constriction and a third ion wants to enter. Only two possible energetically equivalent states exist for this scenario: either sites 1 and 3 are currently occupied and an ion wants to enter at site 4; or sites 2 and 4 are currently occupied and an ion wants to enter at site 1 (Fig. 11.15C). In the first

case, the addition of an ion at site 4 will push the ion at site 3 into site 2, and that will push the ion in site 1 out of the selectivity filter. Similarly, in the second case, the addition of an ion at site 1 will push the ion at site 2 into site 3, and that will push the ion in site 4 out of the selectivity filter. If there is an imbalance of ions across the membrane such that there are more potassium ions in the intracellular compartment (which is closer to site 4) compared to the extracellular compartment, ions are more likely to enter the channel at site 4 than at site 1 and drive the net movement of ions out of the cell (Morais-Cabral et al., 2001). In this way, the potassium channel is able to achieve high ion flux even while selectively binding to potassium ions.

These crystallographic studies have also indicated that the selectivity filter of the KcsA channel is not rigid, and its flexibility is important for its role in ion permeation. As we have already discussed, C-type inactivation arises from the collapse of the selectivity filter from the absence of permeant ions in the pore to prop the channel open. If the selectivity filter is mutated to prevent this collapse, however, the mutant channel loses its selectivity for potassium ions and is able to conduct sodium ions even in the absence of potassium ions. Thus, the channel's ability to adapt structurally to the presence of potassium or sodium ions in solution is fundamental to ion selectivity (Fig. 11.16) (Valiyaveetil et al., 2006).

Sodium Selectivity

Sodium channels conduct partially hydrated ions. Although the sodium p-loop channel is structurally very similar to the potassium channel, early analysis of its pore dimension with organic ions of different sizes showed that the narrowest part of sodium



low-field-strength carbonyl oxygens from the amide backbone and are wide enough to accommodate a sodium ion simultaneously with four water molecules. The outermost ion binding site, though, is surrounded by four negatively charged, high-field-strength acidic side chains (in vertebrates, instead of acidic side chains, these four residues are Asp-Glu-Lys-Ala or the DEKA motif) and can only fit a sodium ion hydrated with two water molecules. Thus, as an ion moves through the sodium channel, it is dehydrated by the acidic side chains and is rehydrated as it interacts with the backbone carbonyl groups. Because the outermost ion binding site can strip water molecules from smaller sodium ions more efficiently than from larger potassium ions, the pore is selective for sodium (Payandeh et al., 2011).

channels is actually wider than that of potassium channels (Hille, 1971) and thus cannot fully strip the hydration shell from permeant ions. Rather, sodium ions move through the channel with part of its hydration shell intact (Fig. 11.17) (Catterall, 2012). Crystal structures of a bacterial sodium channel indicate that there are three ion binding sites in the selectivity filter. The two inner ion binding sites are coordinated by

Calcium Permeability

Calcium channels of the p-loop family are very similar to sodium channels in structure and function. Yet, subtle differences in its amino acid sequence allow calcium channels to be selectively permeable to calcium even though the extracellular sodium concentration is much higher.

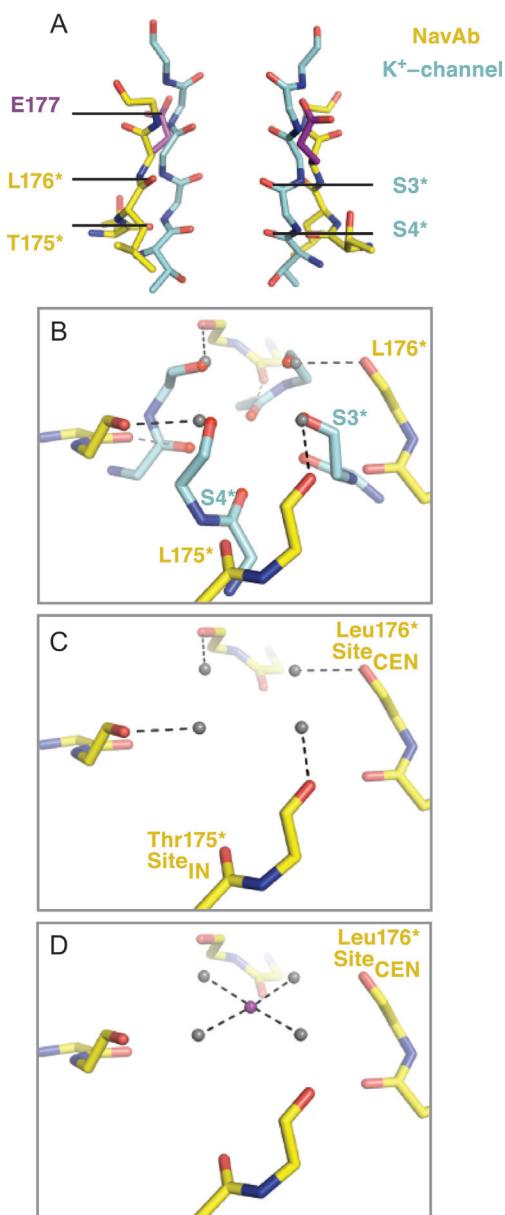


FIGURE 11.17 Sodium selectivity of a voltage-gated sodium channel pore. (A) Comparison of the sodium channel (yellow) versus potassium channel (blue) selectivity filters. The sodium channel filter is wider even though sodium ions are smaller than potassium ions. The purple residue at E177 forms ring of acidic residues responsible for calcium selectivity. L176 and T175 are pore-lining residues in the sodium channel at positions homologous to S3 and S4 in the potassium channel pore. (B) Close-up view of selectivity filter of the sodium channel (yellow) and potassium channel (blue). The grey spheres are water molecules bound to the sodium channel filter. Their hypothesized positions match where the carbonyl backbones of the potassium channel are. (C) Same as (B) with the potassium channel removed. (D) Same as (B) with a central sodium ion coordinated by the four water molecules. Modified from Payandeh et al. (2011).

Calcium channels bind to calcium ions with high affinity. One clue to the mechanism by which calcium selectivity is achieved comes from the dependence of its ion selectivity on extracellular ion concentration. Under physiological conditions with millimolar concentrations of calcium, these channels selectively conduct calcium ions. If the extracellular calcium concentration is reduced to submicromolar levels, though, calcium channels become permeable to sodium ions. At intermediate calcium concentrations, permeability of both ions is substantially reduced (Fig. 11.18A) (Almers and McCleskey, 1984; Hess and Tsien, 1984).

This behavior of the calcium channel could be explained if the channel has more than one binding site with higher affinity for calcium than for sodium. In the absence of calcium ions, sodium ions occupy the binding sites and go through the channel. As the calcium concentration is raised, one of the binding sites becomes occupied by calcium. Because the channel has higher affinity for calcium than for sodium, it is difficult for a sodium ion to displace the calcium ion. Thus, the bound calcium ion in effect blocks current flux through the channel. As the concentration of calcium is raised even further, multiple binding sites in the pore become occupied by calcium. Like in the potassium channel, electrostatic repulsion between the multiple bound calcium ions would provide a driving force to move calcium through the pore despite the tight binding (Hille, 2001).

Single amino acid residue changes are responsible for calcium versus sodium selectivity. Although no crystal structure of a p-loop calcium channel is yet available, these channels are highly homologous to sodium channels and probably use a similar mechanism for ion selectivity. The difference in ion permeability between the two species is conferred by the DEKA residues forming the outermost cation binding site in the selectivity filter of the sodium channel discussed above. In calcium channels, this motif is replaced by four glutamates, EEEE, and these negatively charged residues are crucial for the channel's affinity for calcium (Fig. 11.18B–E) (Yang et al., 1993; Payandeh et al., 2011). Glutamate substitutions of DEKA in sodium channels cause the mutant sodium channel to become calcium selective (Heinemann et al., 1992). Taken together these studies suggest that calcium channels utilize a similar permeation mechanism as sodium channels, and the greater affinity of the calcium channel pore for calcium ions confers calcium selectivity under physiological conditions.

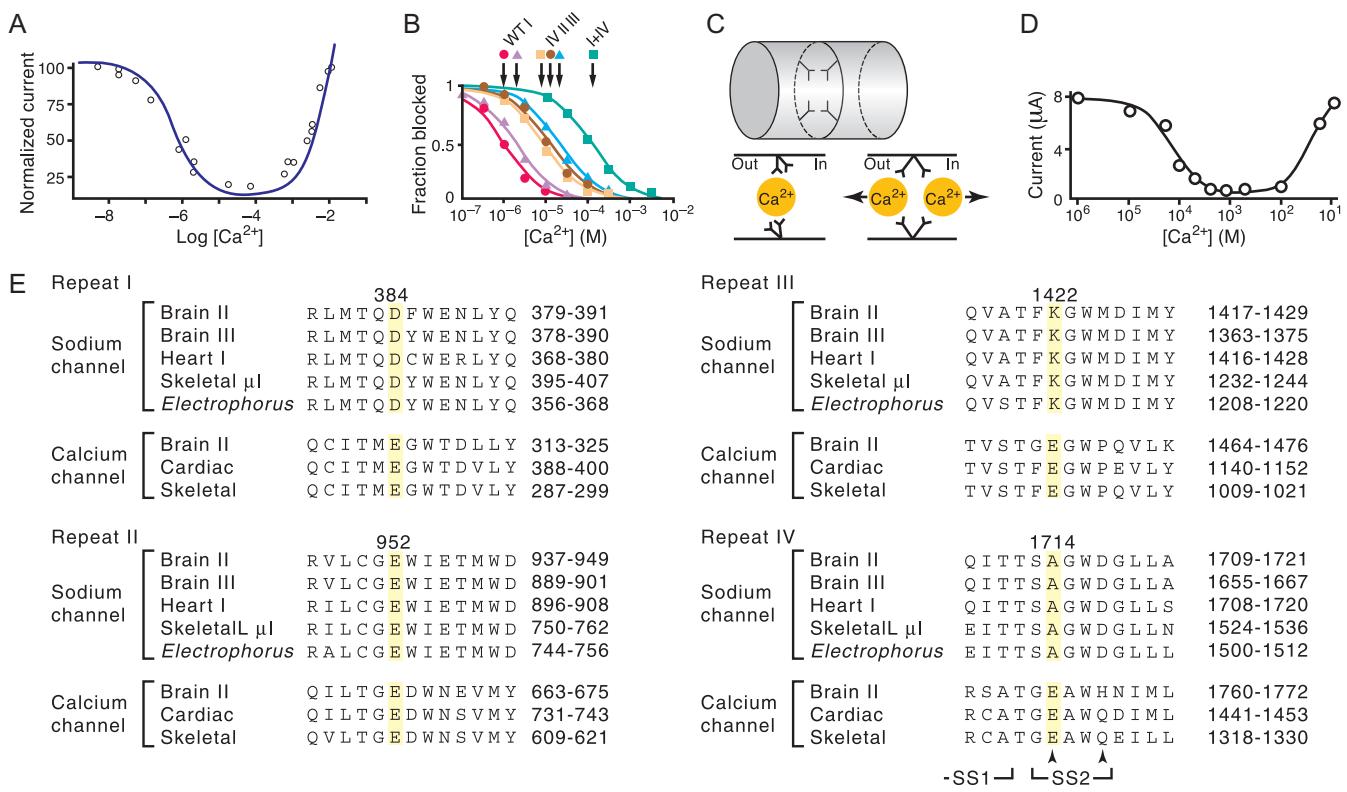


FIGURE 11.18 Dependence of voltage-gated calcium channel ion selectivity on calcium concentration. (A) In the presence of sodium ions and varying concentration of calcium ions, calcium channels are permeable to sodium ions at submicromolar calcium concentration. At submillimolar calcium concentration, a calcium ion occupies one binding site in the channel and blocks sodium permeation. At still higher calcium concentration, calcium may occupy multiple binding sites; the presence of multiple calcium ions in the same channel pore allows them to dissociate from the binding site more readily and pass through the channel. (B) The affinity of the calcium binding site as indicated by the blocking action of calcium on lithium permeation is reduced by substituting a glutamate in the p-loop with glutamine. WT, wild-type calcium channel. I, II, III, and IV indicate glutamine substitution in the first, second, third, and fourth repeats of the channel. I + IV indicates double mutations in the first and fourth repeats. (C) How the ring of four glutamate residues in the calcium channel pore might bind one or two calcium ions. (D) Glutamate substitution of lysine 1422 of the p-loop in the third repeat of voltage-gated sodium channels causes the mutant channel to behave like a calcium channel. (E) Alignment of the p-loop sequences for each of the four repeats of the voltage-gated sodium channels and calcium channels. (A) adapted from Almers and McCleskey (1984) (B) and (C) adapted from Yang et al. (1993) (D) and (E) are adapted from Heinemann et al. (1992).

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