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Taking Apart the Gating of Voltage-Gated K^+ Channels

Minireview

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Voltage-gated ion channels constitute a subset of ion channels that are intrinsically sensitive to changes in the membrane potential. Like clockwork, these ion channels open and close to generate complex electrical signals, such as action potentials, in neurons and other excitable cells. How voltage-gated ion channels "sense" changes in the membrane potential and the mechanism by which those changes are translated into channel gating, the conformational changes that allow channels to open and close, are questions that have fascinated researchers for decades.

Avid readers of classic papers will recognize that Hodgkin and Huxley, realizing that a molecular description for the ionic currents they observed was out of their reach, posed these questions in 1952. Today, a direct approach to solving this problem might seem transparent, if not venturesome. A crystal structure of a voltage-gated ion channel in the closed state and the open state would give a detailed picture of how the channel works. Structures of the voltage-gated ion channel in various intermediate states would also be helpful for dissecting individual steps in the gating process. Through a combination of well-crafted experiments and bold deductive reasoning, however, a great deal has already been learned about how these fantastic machines work (for review, see Yellen, 1998). In this article, we will aim to summarize what is known about how various structural components in voltage-gated K^+ channels control gating.

Voltage-gated K^+ channels are assembled from four subunits that each contains six helical transmembrane segments, numbered S1–S6 (see Figure 1). From what we know thus far, voltage-gated ion channels are built from two parts: a domain that senses voltage and a domain that forms the pore (Kubo et al., 1993). S5 and S6 make up the pore-forming domain; S6 is believed to be the inner helix, which lines much of the pore. S1–S4 surround the pore domain and function as the voltage-sensing domain.

"Evolving" Gates

Voltage-gated ion channels open and close by multiple mechanisms. What do the gates look like? For decades, the activation gates have been described figuratively as a "trap door" or a "hinged lid" that sits over the inner mouth of the pore. Though simplistic, these descriptions modeled well many of the early observations of gating such as the "foot-in-the-door" effect and "blocker trapping." A structural description of the activation gates of voltage-gated ion channels remains elusive. Spin-labeling studies of KcsA suggest that K^+ channels may gate by rotating the inner helices that line the pore (Perozo et al., 1999). In this model, the degree of rotation of the helices regulates the aperture of the inner mouth of the pore that opens and closes like the shutters of a camera. For voltage-gated K^+ channels, this model is broadly consistent with measurements of the state-dependent reactivity of cysteines in S6 and the identification of mutations that affect gating in this region (for review, see Yellen, 1998).

A second gate uses the N-terminal tip of the voltage-gated K^+ channel protein and is hence termed N-type inactivation. N-type inactivation closes the channel during long, sustained depolarizations and works by a "ball-and-chain" mechanism. The opening of the activation gates is believed to create a binding site for the extreme N terminus to enter and occlude the inner mouth of the pore. A third gate, responsible for C-type inactivation, is located at the outer mouth of the pore and works by collapsing the pore selectivity filter. In Shaker K^+ channels, N-type inactivation is generally faster than C-type inactivation; therefore, they are also referred to as fast and slow inactivation, respectively. In some K^+ channels, however, C-type inactivation can be fast. HERG channels, for example, display a fast form of C-type inactivation that plays a part in determining its physiological role in the repolarization of the cardiac action potential and the suppression of arrhythmias.

One of the revelations from the first voltage-gated ion channel sequences was the prediction of a transmembrane segment S4 that contains basic residues. This fueled the notion that changes in the membrane potential moved a charged region of the ion channel across the electric field, an idea endorsed by Hodgkin and Huxley in 1952: "Details of the mechanism will probably not be settled for some time, but it seems difficult to escape the conclusion that the changes in ionic permeability depend on the movement of some component of the membrane which behaves as though it had a large charge or dipole moment" (Hodgkin and Huxley, 1952). A great deal of evidence suggests that, when the membrane is depolarized, the positive charges in S4 are moved across the membrane, thus making S4 the "voltage sensor" (for review, see Horn, 2000). By attaching fluorescent probes onto Shaker K^+ channels, it has been possible to derive a picture of how S4 moves during channel activation (Cha et al., 1999; Glauner et al., 1999). The four individual S4 segments in a voltage-gated K^+ channel are situated in canals that surround the pore domain. The exact motion of S4, during channel activation, is still uncertain, but there is agreement that the S4 helix rotates considerably along its long axis, possibly up to 180°. What is unclear is whether the S4 segment slides up through the gating canal. One group argued that there is little translation of S4 (Cha et al., 1999). The second group suggested that, while rotation of S4 without translation was a possibility, an alternative and equally plausible model was that S4 rachets in a "helical screw" motion as it rotates (Glauner et al., 1999).

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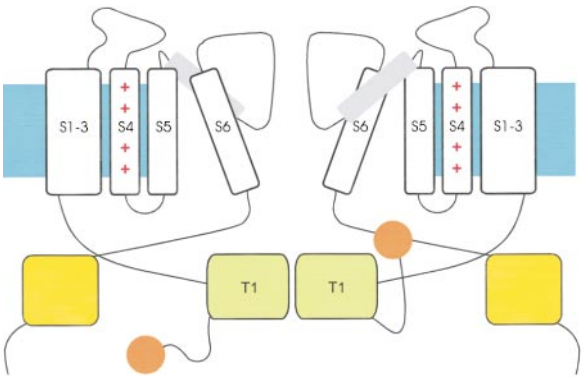

Figure 1. A Side View Representation of a Voltage-Gated K^+ Channel

This model shows two subunits. The pore is lined by residues in the P region and S6. The T1 domain (green) is suspended below the transmembrane domain connected by a linker from S1. The inactivation ball (orange) accesses the cytoplasmic mouth of the pore. The C-terminal cytoplasmic domain is drawn off to the side of the T1 domain, but its relative location is unknown.

Coupling the Voltage Sensor to the Gate

How does the movement of S4 trigger the channel gates to open and close? The answer is unknown. The linkage and proximity between S4 and the pore-forming domain suggest that any movement in the S4 segment may be transmitted directly to the pore domain. This is consistent with the identification of mutations in the inner half of S4 and the S4–S5 linker that affect the voltage dependence of gating. In addition, Li-Smerin et al. (2000) systematically mutagenized positions in S5 and S6 that correspond to lipid-facing residues in KcsA in order to identify positions that may disrupt an interface between the voltage-sensing domain and the pore domain. As they point out, their analysis cannot rule out that those mutations directly affect gating; however, their mutations, in fact, cluster in a region where adjacent pore-forming subunits interact. This region may serve to communicate structural changes in the voltage-sensing domain to the pore.

Mechanically, one can imagine a number of mechanisms that might serve to couple the movement in S4 to the activation gates. One possibility is that the 180° rotation of S4 is transmitted through the S4–S5 linker as a torque or a twist in the cord. An attractive aspect of this model is the similarity in the voltage-sensing mechanism—a twisting in S4—and the activation gating mechanism—a twisting in S6. Alternatively, could they be coupled like cogwheels in a machine? It is also possible that outward extrusion of S4 allows the activation gates to open.

Gating and the Cytoplasmic T1 Domain

Does gating concern only the transmembrane domains? There has recently been considerable discussion in the field as to a possible role of the N-terminal T1 domain in gating. The T1 tetramerization domain resides between the N-terminal inactivation "ball" and S1 and was identified as an ~120 amino acid stretch that is important in specifying the assembly of voltage-gated K^+ channels that belong to the same subfamily; voltage-gated K^+ channels can assemble with other channels from the same subfamily but not with members of different subfamilies. The T1 domain crystallizes as a tetramer with a narrow hole that runs through the center leading to the possibility that the T1 pore is a continuation of the ion channel pore and thus participates in ion permeation and channel gating (Kreusch et al., 1998). If the T1 domain, however, juxtaposes the cytoplasmic face of the transmembrane domain, there would leave little room for the sizable C-terminal cytoplasmic tail that continues after S6. In addition, that location would place the T1 domain in the direct path of the N-terminal inactivation ball from reaching the inner mouth of the pore.

Nonetheless, a role in channel gating is suggested by mutations in T1 that alter the gating properties of voltage-gated K^+ channels. Mutations along the T1 subunit interface (Minor et al., 2000) and the C-terminal aspect of the T1 pore (Cushman et al., 2000) affect both the biochemical stability of the T1 tetramer complex and the apparent stability of the closed state relative to the open state as assessed by the voltage dependence of activation.

Without the T1 domain, voltage-gated K^+ channels form fully functional channels (Kobertz and Miller, 1999). This implies that gating per se may involve principally the transmembrane region. The Miller group has also been able to design disulfide linkages across adjacent T1 subunits with paired cysteine mutagenesis (Kobertz et al., 2000). These T1-cross-linked channels appear to form disulfide bonds irrespective of the closed or open state of the channel, suggesting that the T1 domain does not have to undergo radical conformational changes during gating. An alternative model places the T1 domain suspended beneath the membrane by the S1–T1 linker like a "hanging gondola" (Kobertz et al., 2000). Windows created by the ~30 amino acid long tethers would provide enough space for the N-terminal "ball" and permeant ions to reach the pore. This is consistent with the identification of mutations in the S1–T1 linker that couple to mutations in the N-terminal "ball" and affect the rate of N-type inactivation (Gulbis et al., 2000). What about the shifts in voltage dependence observed (Cushman et al., 2000; Minor et al., 2000)? One possibility is that the T1 domain still contacts the transmembrane domain in a way that leaves enough room for ions and the inactivation ball to enter through the window. This would be consistent with evidence that the T1 domain is a target for second messengers that modulate potassium channels (Cachero et al., 1998).

Slow Inactivation in and around the Pore

Though the precise mechanism of C-type inactivation is unknown, several observations suggest that the mechanism involves a partial collapse of the selectivity filter in the pore (for review, see Yellen, 1998). More recent evidence suggests that C-type inactivation may occur in two steps. In the first step, termed P-type inactivation, the outer mouth of the pore closes. In the second step, the closure of the outer gates is stabilized by an interaction with the extruded S4 segment to reach what generally has been described before as the C-type inactivated state (Olcese et al., 1997; Loots and Isacoff, 1998).

In this issue of *Neuron*, Gandhi et al. (2000) explored the potential interaction between S4 and the pore do-

main further by tracking gating motions in Shaker K^+ channels under voltage clamp. This was accomplished by systematically measuring the fluorescence changes of covalently linked fluorophores at numerous sites in S4 and the pore domain and looking for emerging patterns. A couple observations reinforce the notion that the S4 segment interacts with the pore during slow inactivation. First, based on the kinetics of ΔF_{on}, positions along S4 can be divided into three zones that run vertically in stripes along S4. This pattern is consistent with an outward movement of S4 during channel activation. Second, at many positions in S4, the ΔF_{on}s and the ΔF_{off}s are asymmetric, a phenomenon they refer to as fluorescence hysteresis. They convincingly demonstrate that this originates from an interaction between S4 and the inactivating pore. Overall, their results are consistent with S4 segment undergoing a "helical screw" motion during channel activation. Following P-type inactivation, they propose that the S4 segment tilts inward and stabilizes the inactivated pore.

What type of rearrangements occurs within the pore domain itself? Larsson and Elinder (2000), also in this issue of *Neuron*, describe the use of cysteine mutagenesis to demonstrate the role of a conserved glutamate at the end of S5. First, they demonstrate that this side chain, E418, is likely to hydrogen bond with another residue in the pore domain. Using the KcsA structure to identify candidate interacting partners, they introduced cysteines into paired positions and induced the formation of disulfide bonds. This worked for two sets of mutations: E418C/G452C and E418C/V451C. Both of these interacting partners are located near the end of the P–S6 linker. Surprisingly, a disulfide bridge between E418C and G452C stabilizes the open state, while a disulfide bridge between E418C and V451C stabilizes the inactivated state. The opposing effects of disulfide bridges in two neighboring positions suggest that during slow inactivation the P–S6 loop rotates. This leads to a model of how the pore constricts during P-type inactivation. In KcsA, the selectivity filter is held open by a network of tryptophans that interact between subunits to form an aromatic cuff that encircles the pore (Doyle et al., 1998). The side chain of a conserved proline (P450 in Shaker) is perched just above where two tryptophans meet. A rotation of the P–S6 loop would cause this proline to rotate outward and allow the tryptophans to move closer together. This could tighten the aromatic cuff and cause the selectivity filter to narrow. As the authors point out, this model agrees well with experiments showing that a cysteine in P450 in Shaker becomes more exposed to the extracellular solution during slow inactivation (for review, see Yellen, 1998).

We are becoming increasingly familiar with the parts that comprise a voltage-gated K^+ channel. The next major objective will be to understand how these different parts—the selectivity filter, the gates, the voltage sensor, the T1 domain, and others—operate together. Once these long-standing questions are "settled," we will have a completed picture of how a voltage-gated ion channel works.

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