Reviews

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Voltage-Gated K Channels

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Abstract: Ion channels and the electrical properties they confer on cells are involved in every human characteristic that distinguishes us from the stones in a field. Every perception, thought, movement, and heartbeat depends on electrical signals generated by the activity of ion channels. Early views of the relationship between channel structure and function have undergone substantial modification following the cloning of various ion channels and the determination of the structure of a simple bacterial K channel, the KcsA channel. This review focuses on the relationship between the structure and function of voltage-dependent K channels, covering the molecular bases of channel selectivity, conduction, and gating. The evolution of ion channels in bacteria is discussed, as well as the basis of channel selectivity and conduction in the KcsA channel. More complex channels have evolved molecular "gatekeepers," allowing them to respond to appropriate stimuli by opening, closing, and inactivating.

Introduction

Ion channels and the electrical properties they confer on cells are involved in every human characteristic that distinguishes us from the stones in a field. Every perception, thought, movement, and heartbeat depends on electrical signals generated by the activity of ion channels. Ultimately, the magical properties that animate us arise from the ability of channels to facilitate the movement of selected ions across membranes, which inherently are strong barriers to ion passage. Just as important as the abilities to select and conduct ions are the abilities to open and close (gate) in a timely manner and to respond to gating signals thanks to a "gatekeeper." There are a variety of gatekeepers, some of which respond to mechanical stimuli, as in touch receptors and cochlear hair cells; others to chemicals, as in olfaction and vision; and others to voltage, as in axonal

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transmission and the timing of the heartbeat. The focus here is the voltage-gated K channel, which has been intensely studied since the great work of Hodgkin and Huxley (1). Excellent progress has been made in the last two decades, beginning with the cloning of channels (2, 3, 4, 5), the description of the inactivation mechanism in K channels (6), the discovery of the pore region (7, 8), and determination of the structure of a simple K channel (9).

Early Views

An early view of a voltage-gated potassium channel is shown in Fig. 1. This view was based largely on evidence from experiments with tetraethylammonium (TEA) and its derivatives (10, 11) and influenced by the intriguing experiments of Hodgkin and Keynes (12), who suggested that several K^+ ions move simultaneously through a long K pore. At the inner side of the membrane, there is a gate, which operates in an all-or-none manner; in other words, it is entirely open or entirely closed. The gate controls access to a vestibule that is large enough to accept a hydrated K^+ ion, or a TEA^+ ion, which is about the same size. The pore then narrows to the diameter of a dehydrated K^+ ion in the filter region. A K^+ ion can enter the filter by shedding its hydration waters, but this is not possible for a TEA^+ ion with covalently attached arms: It remains in the vestibule and blocks conduction. The filter is lined with dipolar carbonyl groups, which attract the K^+ ion and substitute for the hydration waters. On leaving the filter at the outer end, a K^+ ion rehydrates. TEA^+ can be driven out of the vestibule by making the internal voltage negative, in the presence of external K^+ ions, which drive TEA^+ inward. Experiments showed that Na^+ ions in the internal medium can be forced into the vestibule (13), but, as does TEA^+, they block ion movement because they cannot enter the filter, even though in dehydrated form they are smaller than K^+ (Pauling radii Na^+ 0.95 Å, K^+ 1.33 Å). This paradox is explained under *Selectivity* below.

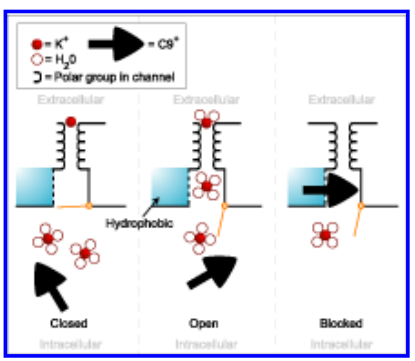

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Fig. 1.

An early view of K channel architecture. A K channel is shown at left with closed gate, and with a dehydrated K^+ in its filter. The filter is lined with polar carbonyl groups capable of replacing the water molecules that surround a K^+ ion in solution. When the gate opens (center) hydrated K^+ ions enter the vestibule, dehydrate, pass through the filter, and rehydrate. When the gate is open, $C9^+$ (nonyltriethylammonium ion) present in the cytoplasm can enter the vestibule because its triethylammonium portion is about the size of a hydrated K^+ ion. Once in, its position is stabilized by the binding of its nonyl arm to a hydrophobic region in the vestibule

wall. Because of its covalently linked arms, $C9^+$ is too large to enter the filter, and it remains in the vestibule blocking K^+ movement until driven out. Experiment shows that the gate can be forced with difficulty to close behind the $C9^+$. [Reprinted from (23), p. 332, courtesy of Marcel Dekker, Inc.]

The vestibule has a hydrophobic moiety that binds the hydrophobic arm of $C9^+$, a TEA derivative formed by extending one ethyl arm with seven methylenes (nonyltriethylammonium ion). $C9^+$ can be trapped in the channel by closing of the gate, showing that the vestibule remains at least partially intact in the closed state. This was summarized by drawing the gate as a flap covering the inner end of an invariant pore, a view that now must be modified, as described under *The Gate* below.

As pointed out by Hodgkin and Huxley (1), the presence of mobile charge in the membrane that moves in response to voltage changes provides the only possible means of conferring voltage dependence on the opening and closing of the gate. Movement of these charges proved to generate a detectable gating current, first found in Na channels and later in K channels. These charges serve both to sense the membrane voltage and to drive the conformational changes that open and close the channel (14).

Our first direct chemical knowledge of voltage-gated channels came in a blinding flash from Numa and his colleagues, who sequenced the sodium channel (2). The Na channel is composed of four similar domains, each containing six transmembrane segments. It is striking that the fourth segment (S4) of each domain contains four to eight positively charged residues, mostly arginines. This segment was the clear choice for the voltage sensor. The K channel, sequenced a few years later (3, 4, 5), proved to be very similar, but is composed of four identical subunits rather than four domains. Similar to the Na channel, the fourth segment of each domain contains many positively charged residues. The functions of conductivity and selectivity were assigned to a connecting segment called the P region between the fifth (S5) and sixth (S6) transmembrane crossings (7, 8).

In the Beginning: Bacterial K Channels

The voltage-gated channel is a specialized and sophisticated structure with an interesting (although speculative) evolutionary history. Ion channels (15) and energy-driven ion pumps (16) got their start in bacteria. A central fact of bacterial life is a negative internal voltage of about –150 mV, necessary for membrane transport and the production of ATP (17). A side effect of the negative interior is the tendency to concentrate cations inside: monovalent cations by a factor of about 300, and divalent cations by a factor of 10^5. To prevent saturation or crystallization in its interior, a bacterium must limit the permeability of cations that are at relatively high concentration in the environment. In most environments (soil,

brackish water or sea water, blood), sodium is more plentiful than potassium, and magnesium and calcium are present at low concentrations. If our blood is used as an example of a growth medium, a bacterium freely permeable to all cations would have equilibrium internal concentrations of Na^+, Ca^{2+}, and Mg^{2+} that are impossibly high, 40 to 100 M, leading to crystallization inside the bacterium. K^+ would be high but below saturation, at about 1.3 M. Thus, to regulate its internal environment, the bacterium needs a highly selective K^+ channel, allowing entry of K^+ but severely limiting entry of Na^+ and divalent cations. Furthermore, it needs energy-consuming pumps to drive out Na^+ and divalent cations, which would slowly accumulate despite low permeability. A pump for Cl^-, which is repelled from the inside by the negative internal voltage, would be unnecessary, and few examples have been suggested in the animal kingdom. From these early housekeeping origins evolved the wide variety of channels and pumps that are found in our cells.

Structure of a Bacterial Channel

An example of a bacterial K channel is the KcsA channel, whose structure is known from the groundbreaking work of MacKinnon and his associates (9). This relatively simple channel is composed of four identical subunits clustered around a central conducting pore (Fig. 2A). Each subunit has two transmembrane crossings, TM1 and 2 (Fig. 2B), which are analogous to the S5 and S6 segments of the more complex, voltage-gated, Shaker channel (Fig. 2C). TM1 crosses from inside to out, where it connects to a "turret" that is important in toxin binding, a short pore helix that extends inward through about one-third of the membrane, and the selectivity filter with its characteristic glycine-tyrosine-glycine (GYG) motif that turns toward the outer surface. TM2 crosses from outside to in, and lines the "cavity," a large space just internal to the filter, and the gating region, which is at the inner end of the pore. As crystallized, this simple K^+ channel lacks a gatekeeper, but has the three other key ingredients of a gated ion channel: conduction, selectivity, and a gate. We begin with the selectivity filter.

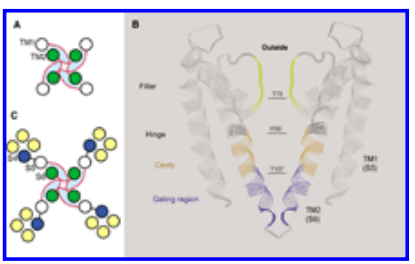

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Fig. 2.

K channel structure. (**A** and **B**) The KcsA channel. This KcsA channel is composed of four identical subunits in a square array around a central pore (A). From the x-ray crystallography analysis of Doyle *et al.* (9), two subunits are shown in (B) with the pore axis in the plane of the page. Each subunit has two transmembrane segments, TM1 (white circle) and TM2 (green circle). Beginning at the outer end, the labeled regions are the selectivity filter (yellow), a glycine that serves as a hinge for gating movements (black), a large water-filled cavity (orange), and a gating region where the TM2 helices

converge (blue). (The labeled lines in the pore give the level of residues referred to in later figures.) In the more complex voltage-gated channel (**C**), there are six transmembrane segments, with the fifth (S5) and sixth (S6) segments (analogous to TM1 and TM2) forming the core channel. Four additional transmembrane segments, S1 to S4, provide voltage sensing.

Selectivity

The essential structural feature of the selectivity filter is the close spacing of carbonyl groups around the pore axis, shown in Fig. 3. A dehydrated K^+ ion fits well between the carbonyl groups in the filter. In the absence of a pore, a cation cannot jump into the bilayer from the adjacent water, because it is bound electrostatically to water molecules, which are dipolar and turn their negative ends toward the K^+ ion, as shown in Fig. 3C, a cross section through the cavity. The cumulative energy of binding to water is about equal to a covalent bond. Because the ion cannot bond electrostatically to the nonpolar lipid molecules, leaving water and entering the bilipid layer would require breaking all bonds to water without replacing them, at an enormous energetic cost of about 75 kCal/mol. The carbonyls of the selectivity filter solve this problem by replacing the hydration waters, making it energetically possible for the ion to leave water and enter the filter. The carbonyls bind tightly to a potassium ion (Fig. 3, A and B), in part because the carbonyls have a higher dipole moment (~3.5 D) than water molecules (1.8 D). This is helpful in overcoming the electrostatic forces that work against the entry of ions into the membrane.

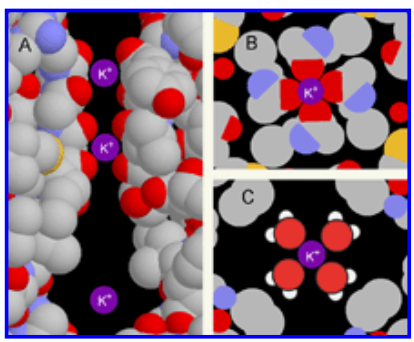

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Fig. 3.

Ions in the pore. (**A**) Longitudinal view of the selectivity filter, with carbonyl oxygens shown in red. Two dehydrated K^+ ions are shown in the filter and a hydrated K^+ ion in the cavity (waters not shown). (**B**) Cross section through the filter at the level of KcsA residue Thr^{75} (see Fig. 2), with a K^+ ion snugly fitted in the carbonyls. (**C**) Cross section of the large aqueous cavity at the level of KcsA residue Ile^{100}. A K^+ ion easily enters this large aqueous space, surrounded by its hydration waters.

The main task of the filter is to discriminate against anions, sodium ion, and divalent cations. Anion exclusion is inherent in the design of the filter, because the

negative ends of the carbonyl dipoles lining the filter repel anions. Why can't Na^+ pass through the filter? Paradoxically, because it is too small! In Fig. 4 [taken from (13)], K^+ and Na^+ ions are shown in water and in the filter. The K^+ ion is bound tightly by oxygens both in water and in the filter, whereas the Na^+ ion is bound well only in water: the filter, for structural reasons, cannot constrict sufficiently to bring more than two of the carbonyls within good bonding distance of the Na^+. As a result, the energy of the Na^+ in the pore is very high compared with its energy in water. Overall, there is a low energy barrier for K^+ entry into the filter, as required for fast throughput, and a high energy barrier for Na^+ entry, as required for selectivity [see the appendix of (13)].

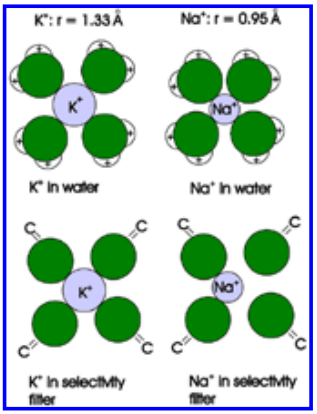

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Fig. 4.

A model for K^+-Na^+ discrimination by the filter (13). A K^+ ion (1.33 Å crystal radius) in the selectivity filter has about the same energy as in water and is bound to either oxygens of water molecules or carbonyl oxygens in the pore. The carbonyl groups have higher dipole moments than the water molecules, which helps to overcome the image forces that arise from the low dielectric constant of the membrane (54). An Na^+ ion (0.95 Å crystal radius) in the filter has higher energy than one in water, because it binds effectively to only two of the four carbonyl oxygens shown. The other two carbonyls are prevented from collapsing around the Na^+ ion by structural constraints in the filter wall. [Reproduced from The Journal of General Physiology, **60**, 588 (1972); by copyright permission of The Rockefeller University Press.]

The filter does not automatically reject divalent cations. Ba^{2+}, which is the same size as K^+, binds tightly in the pore and will remain there indefinitely under appropriate conditions. It enters the filter very slowly, presumably because of a barrier imposed by the need to dehydrate. Fortunately, Ba^{2+} is present at very low concentration in most environments. Ca^{2+} and Mg^{2+} apparently do not enter the filter, presumably for the same reason that Na^+ does not--they are too small and, consequently, bind more tightly to water than to the carbonyls in the filter.

Conduction

As just noted, high ion flux through the pore depends on low energy barriers for transfer of an ion from water to the pore and back to water. Because a K^+ ion is bound quite tightly in water, it follows that its binding in the filter must be tight as well. This seems indeed to be the case. Complete removal of K^+ irreversibly destroys K^+ conductance in the squid giant axon (18, 19), presumably because in the absence of cations the repulsion among carbonyl dipoles is strong enough to

destroy the integrity of the filter. Experiments by Gomez-Lagunas (20) in Shaker show a similar result, with the interesting addition that the gate must be opened after removal of K^+ in order to abolish K^+ conductance. K^+ conductance remains intact indefinitely if the gate stays shut, but decreases by about 40% with each opening. This intriguing experiment suggests that, although K^+ ions are bound in the filter quite strongly, they can be displaced if bombarded in an open channel by a suitable replacement ion such as Na^+. In this case, the rare entry of a Na^+ ion into the filter displaces the bound K^+ ion, which drifts away in the K^+-free solution. The Na^+ ion is not bound tightly enough to maintain the integrity of the filter.

If binding to the filter is tight enough to immobilize the K^+ ions in the experiment just cited, how is fast ion throughput achieved when the gate is open? A plausible answer is that a K^+ ion cannot move unless its replacement is at hand. In this view, a sufficiently energetic ion approaching the filter repels the nearest ion in the filter, which in turn repels and displaces the next, and so forth. The startling results of MacKinnon and colleagues (21), in fact, show a K^+ ion at the inner and outer ends of the filter, poised to displace ions occupying the filter. (Under normal ionic conditions, it seems likely that only the ion at the inner end, in the cavity, would usually be present.) The waters surrounding the ions at either end of the filter show an interesting order (21): They are in two layers, and in each layer, four waters are disposed with fourfold symmetry around the K^+ ion. The symmetry is probably imposed by the fourfold nature of the channel (22). At the outer end of the selectivity filter, the backbone carbonyl of a glycine residue (G^{79} probably displaces the water layers as the K^+ enters the filter) (21). The cavity and the inner portion of an open channel (see below) are large and easily negotiated by hydrated K^+ (Fig. 3). Interestingly, the inner pore shows significant discrimination against Na^+ ion (22), presumably because the hydrated radius of Na^+ is larger than that of K^+.

One of the original pieces of evidence suggesting that ions crossed membranes through channels was the "long-pore effect" (12). This phenomenon can be understood by saying that transfer of a single radioactively labeled K^+ ion through the pore requires that the labeled ion enter the pore, say from the outside, followed by enough successive ion entries from outside to drive the labeled ion from binding site to binding site all the way through the pore. If there are two binding sites, the first entry (the labeled ion entering site 1) must be followed by a second entry from the same side that drives it to site 2, followed by a third collision that drives it from site 2 to the cytoplasm (23). The tracer flux in this example would be proportional to the likelihood of three successive collisions from outside, and therefore to the 3rd power of the external K^+ concentration. Measured simultaneously, inward charge flux (K^+ current) would be proportional to the first power of external K^+ concentration, because each collision transfers one charge across the membrane (unless saturation is reached). The flux data (12, 24) and the KcsA structure are in good agreement.

The Gate

It came as a surprise that a simple K channel like KcsA would have a gate, but a likely candidate was identified at the convergence of the TM2 helices near the inner end of the pore (9). In this region, it can be argued that there are three

"hydrophobic seals," at locations Thr^{107}, Ala^{111}, and Val^{115}. At each of these points, hydrophobic methylenes from the cited residues crowd together so closely that even a dehydrated K^+ ion cannot fit between them (Fig. 5). They thus form a succession of high-energy barriers that prohibit passage of K^+ ions. Means of crystallizing the KcsA channel in the open state have not been found, but MacKinnon and colleagues (25) have recently reported a similar two-TM channel that serves as a model for the open state. This channel (Mthk) has a large ring structure at its cytoplasmic end that binds calcium and, apparently, pulls the TM2 helices apart. The open channel thus has a large tube connecting the cytoplasm and the inner end of the filter. The closed state of the Mthk channel has not yet been described. An overlay of the two channels (Fig. 6) leads to the conclusion that the inner part of S6 rotates about a glycine hinge (arrow) during the open-to-closed transition. These snapshots of the closed and open channel must be essentially correct. Although there is still much to learn, they are fully compatible with biophysical data. An important finding by del Camino and Yellen (26) is that the gate near the inner end of the channel closes tightly enough to exclude Ag^+ ions, which are slightly smaller than K^+ ions.

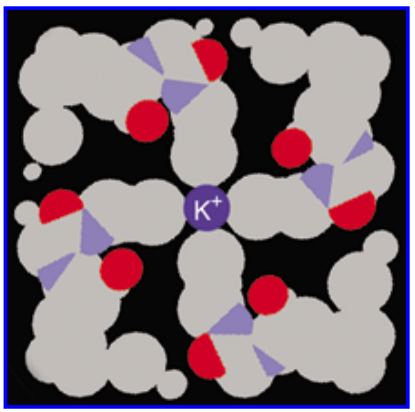

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Fig. 5.

One of three hydrophobic seals in the KcsA structure, this one at the level of Thr^{107} (see Fig. 2). At each seal, methylene groups from the four subunits converge too closely to admit passage of even a dehydrated K^+ ion. The energy barrier at each of the seals would thus approximate the hydration energy of K^+, estimated to be about 75 kCal/mol, close to the strength of a covalent bond.

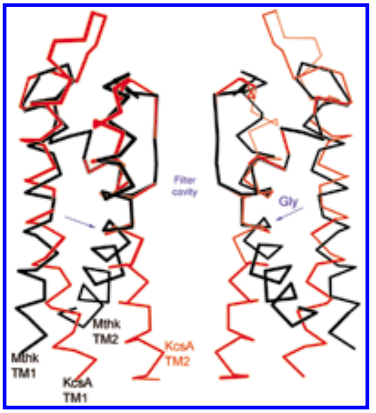

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Fig. 6.

Likely configurations for the gate, open and closed. Superimposed are the KcsA channel (red), which serves as a model for the closed state, and the Mthk channel (black), a model for the open state. The backbone structure of the TM2 helices converge in KcsA to form seals like the one shown in Fig. 5. The TM2 helices of Mthk diverge to form a large entryway for ions.

Gatekeepers

Over time, gatekeepers of various types evolved to open and close the gate in response to chemical or electrical signals. The gatekeepers are separate parts added to the KcsA structure. The main focus here is the voltage-gated K channel, which was constructed by adding four additional membrane crossing segments (S1 to S4) to the core channel formed by S5 and S6 (TM1 and 2 in KcsA are very similar to S5 and 6 in Shaker). Of the added segments, the highly charged S4 segment, with a lysine or an arginine in every third position, is the obvious candidate for the voltage sensor. It has been shown by methanethiosulfonate (MTS) labeling (27) that S4 is a transmembrane segment and that it moves when the voltage changes. The precise nature of the movement is not yet clear (28, 29, 30, 31), but it seems certain that the S4 serves both as a voltage sensor and as the engine whose movement opens the channel. The modular nature of the pore is emphasized by experiments showing that a hybrid channel made from the pore module of KcsA, and the voltage-sensing region of Shaker shows voltage-dependent gating (32).

Given the KcsA structure, it is fun to speculate on the events as the gate opens. Opening is a single, all-or-none event, and there is reason, as noted above, to associate this with an outward pull on the S6 helices that breaks hydrophobic seals similar to the ones in KcsA. [Some caution is in order because the gate region in Shaker K channels seems unlikely to be shaped exactly like the one in KcsA. In Shaker, there is a proline-valine-proline (PVP) motif, with the V at the same location as the outermost of the hydrophobic seals noted above in KcsA (33).] The pull originates in all probability from movement of the S4 helices. A diagram showing what might happen to the gate subjected to opening-pulls is given in Fig. 7. In the closed state, four methylene groups like the ones in Fig. 5 are held together by hydrophobic bonds. Each methylene is connected to a "gating spring,"

whose other end is connected, indirectly, to S4, and is stretched by S4 movement. Pulling on one (B), two (C, D), or three (E) of the methylenes (arrows in Fig. 7) simply leads to rearrangement of the methylenes but no opening: In all cases each methylene is well bound to others, as in the closed state (with the possible exception of D). Only when the springs of all four methylenes are pulling outward simultaneously are the bonds broken effectively. Contributing to the all-or-none nature of the event, conduction does not begin until the separation is large enough to allow passage of a hydrated K^+ ion. [Caution: The methylenes in the KcsA crystal structure (9) are too close together to admit a water molecule between them, but may be too far apart to form effective van der Waal's bonds.]

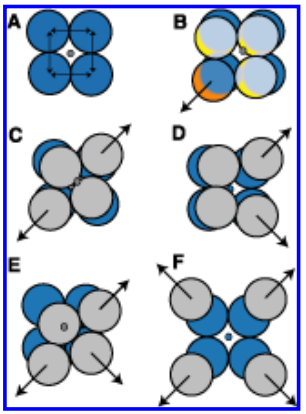

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Fig. 7.

Opening the gate. The four methylenes of the hydrophobic seal shown in Fig. 5 are shown bound together in the resting state (**A**) and as they are pulled outward from the pore axis by activation of one to four (**B** to **F**) of the S4 helices. Effective opening requires activation of all four S4s (F).

There is another phenomenon observed in the interaction between the S4 helices and the gate that is not captured by the picture of the S4s pulling on a spring. When the gate is open, the S4s are almost completely locked in activated position, as is evident from the low amplitude of the gating current when the voltage is returned to the resting level (34). If the gate is prevented from opening by the application of 4-aminopyridine (4-AP) (35), the S4s can be driven into the activated position without becoming locked. A mechanical analog of the locking phenomenon and its prevention by 4-AP is shown in Fig. 8. A flange attached to each S6 helix moves outward from the pore axis as the gate opens after upward movement of all four S4 segments. The flange prevents (or, if slanted downward, hinders) inward movement of the S4 as long as the gate remains open. When closed, pressure of the S4 helices on the flanges (see Fig. 8A) locks the gate closed, which helps to explain the very low open probability reached at negative voltages (36). The presence of 4-AP in the cavity makes gate opening improbable, as described below, but hinders movement of the S4s only slightly [the Q-V curve is shifted by about 12 mV (37)]. The near-normal movement of gating charge when 4-AP prevents channel opening implies that the work involved in stretching the gating spring is not very large. If the spring were very stiff, failure of the gate to open would strongly impede movement of the gating charge. The impediment is small, suggesting that

the work required to stretch the spring is much less than the work done by the field on the S4 helix as it moves. The spring in this view has only a modest effect on open probability, increasing it by a factor of about ten, from perhaps 0.08 to 0.8, the maximum observed. The interaction of the flange with S4 provides the strong coupling between S4 movement and the gate noted as necessary by Islas and Sigworth (36).

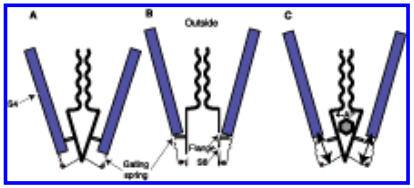

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Fig. 8.

Two modes of controlling the gate. (**A**) The closed state. The S4 segment and the gate region of S6 are connected by a gating spring that, purely for simplicity, is shown as a direct connection. The spring is not stretched in the closed state. The deactivated S4s prevent opening of the gate by preventing lateral motion of the hypothetical flanges connected to the S6 segments. (**B**) When all of the S4 segments move outward to activated position, the gate opens because (i) the S4s pull the gate region outward by means of the gating springs, and (ii) the S4s have moved out of the way, allowing the flanges to move laterally. The flanges prevent inward movement of the S4s until the gate closes. (**C**) If the gate is glued shut by 4-AP in the cavity (see text), the gating springs are under maximum tension when the S4s are activated. The relatively easy outward movement of the S4s in this condition, found in gating current experiments, shows that the stretched spring does not have enough tension to seriously disturb S4 motion. Similarly, inward movement of the S4s, judged from gating current, is completely free, because the flanges do not interfere. S4 activation-deactivation is shown here as a simple sliding motion, though many types of S4 motion would work just as well with the flange idea.

Two means of governing the open-closed state of a channel have just been noted: pulling the gate open or forcing it closed [compare (38)]. K channels may vary in their use of these two approaches. The KcsA channel in studies so far reported is usually closed, which suggests strong adhesion between the gating region of the S6 helices. However, the data above suggest that Shaker's gate has only a mild preference for the closed state, and is locked closed by pressure of the S4 helices

on a flange. Interestingly, introducing electrostatic repulsion between the S6s by the proline to aspartate mutation P476D in the gating region renders the gate of Shaker incapable of closing (39).

Cavity Occupancy and Gating

As described above, blocking cations that act internally have long been thought to lodge in the cavity, near the inner end of the selectivity filter (10). MacKinnon and colleagues have in fact seen a tetrabutylammonium analogue at this position (40). When the gate is open, the cavity is also accessible to MTS reagents (27), which have been used by Yellen and colleagues to determine precisely which positions are protected by the closed gate (41). Mutation of isoleucine 470 (in the cavity wall) shows that occupancy of the cavity is closely related to the ability of the gate to open and close (42). TEA^+ can enter a Shaker K channel with open gate and block it; and, once in blocking position, it prevents the gate from closing. TEA^+ thus cannot be trapped in the cavity by the gate, because its presence there makes gate-closing impossible. Replacement of isoleucine 470 by cysteine makes it possible for the gate to close and trap TEA^+, presumably because the cavity is enlarged slightly (43). This suggests that the cavity constricts somewhat as the gate closes, making the cavity of unmutated Shaker too small to accommodate TEA^+. This is quite consistent with the open and closed K-channel structures presented by Jiang *et al.* (25).

Does the presence of other cation species in the cavity of Shaker have a similar effect on gating? Suspicion immediately falls on K^+, which with one hydration shell is about the same size as TEA^+. In fact, other cations in the cavity, including K^+, appear to prevent gate-closing in Shaker but not in the I470C mutant (43). These experiments, like the ones with TEA^+, suggest that the cavity changes shape or constricts as the gate closes. Of interest is the suggestion that 4-AP, a clinically used K-channel blocker, has the opposite effect when it occupies the cavity: It induces gate-closing, which biases the closed-to-open transition toward closed by about the energy of a hydrogen bond (33). 4-AP apparently enters the channel when the gate is open, then pulls the gate closed behind it.

Inactivation

Some Shaker K channels inactivate by a ball-and-chain mechanism, in which one of the "balls" found at the N terminus of each K channel subunit enters and occludes the channel (6). When it does so, it prevents the gate from closing, and freezes the gating charge in activated position. When the ball comes out of a channel during recovery from inactivation, the channel conducts transiently before its gate closes (44, 45). If the extracellular K^+ concentration is high, recovery is faster, because K^+ entry displaces the inactivating particle from the channel.

The inner end of the channel is partially covered by a "hanging gondola" that presumably protects the entrance and plays a structural role. Large pores between the shrouds of the gondola allow free access by ions (46, 47). Precisely how the inactivation balls of Shaker interact with the gondola is not clear, but one possibility is that the gondola serves to keep them near the channel mouth. It should be noted in passing that inactivation of the Na channel is similar but more

complicated: Inactivation can occur to some extent from the closed state (48, 49, 50), and the channel does not conduct during recovery from inactivation (51, 52, 53).

Conclusions and Questions

Crystallization and x-ray analysis have transformed the K channel from an idea to a concrete structure. We now have a good understanding of selectivity and conduction, the location of the gate, and the broad strokes of the rearrangements as the gate opens and closes. Intriguing questions remain, and many of them concern precisely the portions of the K channel that have not been crystallized: the S4 and the links connecting it to the gate. Some of these questions are treated speculatively in the text above. How does the S4 move in response to voltage changes? Does it rotate (31), translate (28, 29, 30), or both? What and where are the counterions for the arginines and lysines of the S4? The counterions are not a part of the polypeptide itself, and must come from the internal and/or external solution, but which? and are there preferred anions? Do they move during gating? What are the nature of the aqueous clefts that give the anions access to the positive residues in the S4 (29, 55)? What more is there to learn about the influence on the gate of cavity occupancy by ions are drugs? Answering these questions will certainly be aided by crystallization of a complete voltage-gated K channel, but there is much to be learned in the meantime.

Note added in proof. Since preparation of this review, MacKinnon and colleagues have accomplished the crystallization and structural analysis of a bacterial voltage-gated channel (56). Crystallization required the use of an antibody scaffold to stabilize the protein. The observed structure is quite unexpected, with helices S1 to S4 lying within the plain of the membrane rather than crossing it, as suggested by MTS labeling experiments in the literature (cited in the text). One must ask how much of this unexpected structure was imposed by the antibody scaffold. The structure leads to a model for activation of each S4 in which four charges move together through a lipid environment at the periphery of the channel complex. Energetically, this seems highly unlikely.

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