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Potassium channel structures: do they conform?

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Potassium channels are signalling elements vital to vertebrate neurotransmission, and cardiac and renal function. Two inherent qualities equip them for their role in the interconversion of chemical and electrical messages: high selectivity for potassium ions and the ability to open (gate) on cue. The crystal structure of KcsA, published in 1998, explained much about potassium selectivity and high ion flux. The enormous diversity of potassium channels (some hundreds of genes in humans) may have hampered similar progress in understanding gating processes. The recent determination of several representative structures has provided us with a valuable reference for discriminating between features that are utilized in gating across the potassium channel genre and features that determine responsiveness to family-specific gating cues.

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Introduction

In the five and a half years since the first three-dimensional structure of a K^+ channel pore (KcsA) [1] was elucidated by X-ray crystallography, structures of representative K^+ channels from three major families (voltage gated, calcium gated and inwardly rectifying) have been reported. Although the pore architecture is shared, the structures exhibit considerable conformational variability (Figure 1). This review will explore K^+ channel structure/function relationships in the light of the recently determined multidomain structures of MthK [2], KvAP [3••] and KirBac [4••]. We first clarify what can reasonably be inferred from each structure within the bounds of the experiment, highlighting salient features, and then evaluate this in a broader physiological context. The latest structures provide a fresh perspective on a key issue in K^+ channel biophysics: whether central aspects of architecture and gating are likely to be conserved throughout the entire genre, or whether structural and functional homology extends only as far as permeation characteristics, allowing the various families to evolve distinct means of gating.

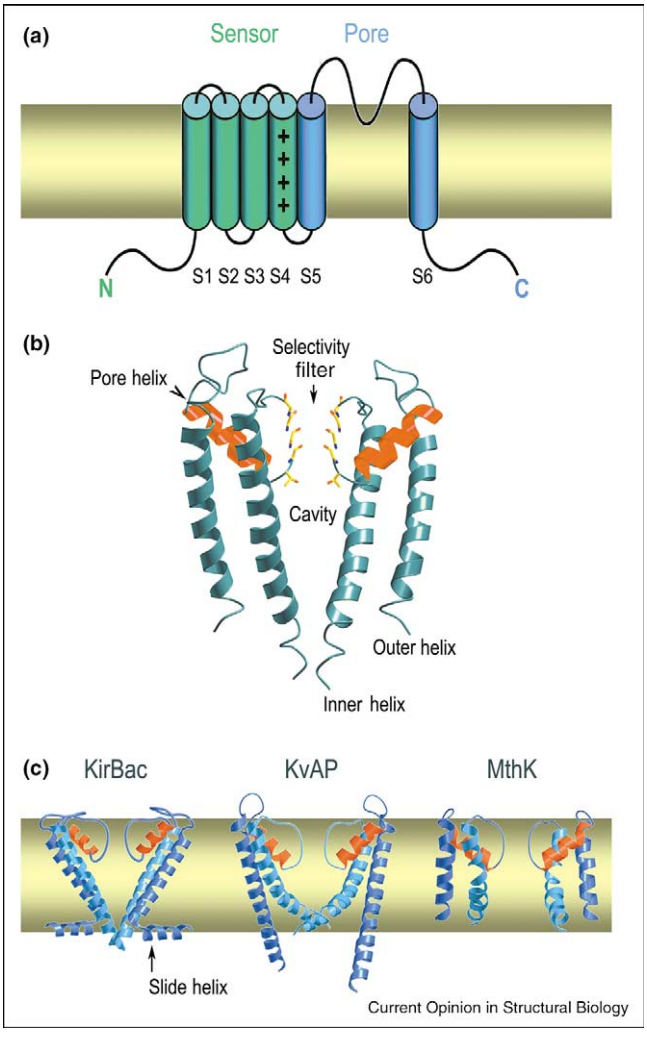
K^+ channel structures

Native conformations of K^+ channels are attained in a planar lipid bilayer. The extraction of multidomain channels from membranes without introducing structural artifacts represents a major technical hurdle of which we are increasingly aware. All K^+ channel structures reported to date represent integral membrane proteins crystallised in an aqueous environment. Parts of the protein exposed to lipid *in vivo* are sequestered in detergent or protein within the crystals. The plasticity of detergent-mediated crystal contacts presents another challenge, typically yielding weak, anisotropic diffraction.

A voltage-gated K^+ channel: KvAP

The voltage-dependent channel KvAP [3••] has six transmembrane helical segments, organised into two structural domains (pore and voltage sensor), in each monomer. The structure of the integral membrane assembly was crystallographically determined to moderate resolution and was accompanied by a high-resolution structure of the isolated voltage sensor. To improve diffraction, each protein was crystallised in complex with an antibody to the S3–S4 loop. It is enlightening to compare the sequence-identical regions of the two KvAP structures. Despite similar secondary structures, the two proteins do not superimpose (Figure 2a,b). To understand why, it may help to look at K^+ channels as being formed by a series of lipophilic α-helices separated by more polar sequences amenable to forming sharp turns or structured loops. Some of these separating sequences have the propensity to form helices as well as turns. In the lipid bilayer, many of these segments are functionally important and reside at the membrane–solution interface. Once extracted into micelles, however, any functional role is unimportant, and whether and where helix disruption occurs is dependent on the local environment of the protein and will be strongly influenced by the need to shield exposed hydrophobic residues. In the two KvAP structures, the helical periodicity is disrupted such that turns occur at different relative points in the linear sequence (Figure 2a,b). The isolated voltage sensor is relatively compact, but its counterpart in the channel assemblage is convoluted and makes extensive interactions with the antibody (Figure 2c). The KvAP structure, although providing a remarkable first view of a voltage-gated channel, has

Figure 1



K^+ channel architecture. **(a)** Most K^+ channels have six membrane-spanning elements in each subunit. All have at least two, corresponding to the S5–S6 region. The ion conduction pathway forms at the centre of a symmetric tetramer of these subunits. **(b)** Two diametrically opposed subunits of KcsA are depicted to show the cavity in the membrane. **(c)** Conformational plasticity of the pore. Two subunits of each of three K^+ channel pores are shown. PDB codes: KirBac, 1P7B; KvAP, 1ORQ; MthK, 1LNQ.

proved controversial, conflicting with a swathe of biophysical data. Although antibody binding clearly compromises the structure, simple removal of the channel from the membrane also contributes to the confusion. Correct juxtaposition of the pore and sensor domains requires a planar lipid environment. The S4–S5 hinge that connects them is susceptible to angular distortion, particularly if the two domains are incorporated into separate micelles. Because of these complicating factors, it is not possible to draw any firm conclusions about the relationship between the pore and the voltage sensor from the KvAP structure.

Ligand-gated K^+ channels

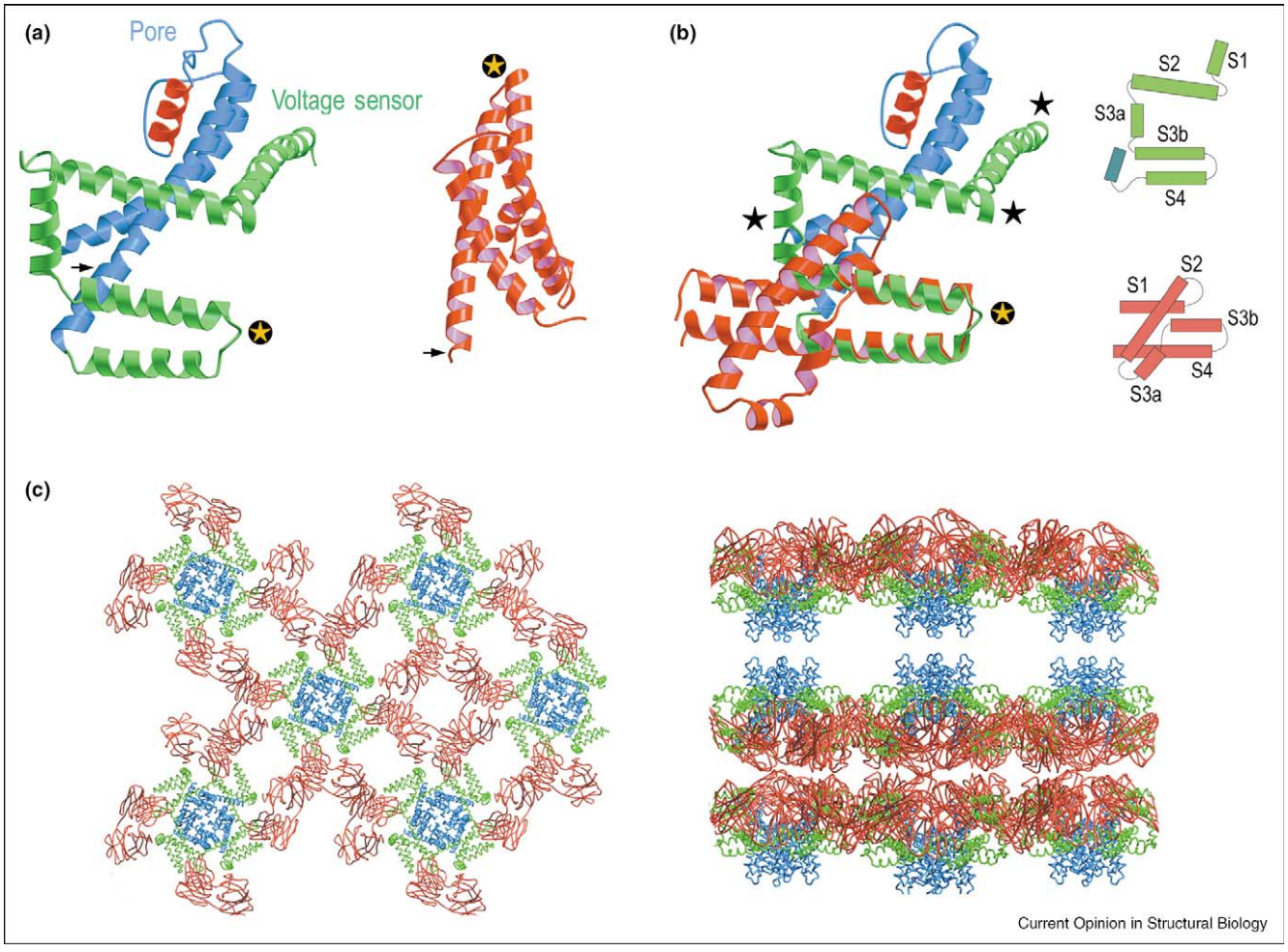
MthK

MthK is a Ca^{2+}-gated K^+ channel assembly from *Methanococcus* [2] with sequence homology to mammalian BK voltage-Ca^{2+}-gated channels. The crystal lattice is formed exclusively by contacts between intracellular *Rck* domains, whereas the pore domains 'float' in conduits along the sixfold crystallographic axis (Figure 3a). Electron density for the membrane-spanning pore region of MthK is ill defined and indeed can only be seen in averaged maps (Figure 3b). It is not possible to confirm the register of the amino acid sequence from these maps. Notwithstanding, the channel itself is in a realistic configuration, undistorted by crystal packing forces. The structure of an isolated *Rck* dimer from an *Escherichia coli* channel does not form a tetramer [5], suggesting that, despite their faint presence, the pore domains in the MthK crystal mediate tetramer formation and thereby determine the lattice symmetry. Although a single gene encodes the pore and intracellular domains, in the wide-open state that crystallised, the polypeptide segments linking the two regions are so poorly ordered as to be invisible. This leaves a clear passage for ions to reach the pore entrance. We note that the open MthK channel structure is inconsistent with a high-energy state.

To prepare recombinant MthK for crystallisation, a secondary translation initiation point just upstream of the *Rck* coding region was removed by mutagenesis [2]. This site is responsible for providing wild-type channels with duplicate intracellular domains by co-translating a second copy of *Rck*. The crystallised channels have compensated for the loss of this second domain by tail-to-tail dimerisation. The asymmetric unit of the crystal thus contains a complex of two K^+ channels, each with a single 'ring' of four *Rck* domains (Figure 3c). The entire assembly has 422 point symmetry. The isolated *Rck* structure [5] is again helpful, as it validates subunit interfaces between the two rings, despite their origin on separate channels. It is difficult to tell whether the presence of a second pore beneath the biological unit (Figure 3b,c) affects the relative disposition of the two *Rck* rings to the pore.

Each *Rck* domain has two parts, a larger N terminus and a smaller peripheral C-terminal segment, connected by a flexible helix-turn-helix motif. Superposition of the Cα backbones of *Rck* domain dimers from MthK and *E. coli* shows that the two structures markedly diverge at this hinge-like connection. Thus, in the MthK structure, each upper and lower *Rck* pair interleaves (Figure 3c, inset), whereas in *E. coli*, they do not. In MthK, two Ca^{2+} are each coordinated by three glutamates. Acidic residues are not conserved at these positions in eukaryotic BK channels. A high residual peak in the fourfold averaged difference density is evident adjacent to the carboxylate moiety of E133 (equivalent to D362 in mammalian *mSlo1*

Figure 2



KvAP structures. **(a)** Ribbon diagram showing a single subunit of KvAP (left) and the isolated voltage sensor (right), oriented with respect to the membrane. Arrows indicate approximately where the slide helices would be expected to begin in each structure. Bound antibody chains are not shown, but the epitope-binding site is denoted with an encircled yellow star. **(b)** Voltage sensor domains from the two structures are superimposed. Black stars show additional regions that make significant contacts with the antibody in the channel assembly. Schematics of the voltage sensors are included to aid interpretation. **(c)** Perpendicular views of the crystal packing of the KvAP assembly. Antibodies to the S3–S4 loop form composite layers in the crystal (antibody in dark pink and voltage sensor in green). These support the pores (blue). The sensors make interprotein contacts with the antibody, whereas the pore domains are paired in a head-to-head fashion between layers, with hydrophobic surfaces stabilized in detergent micelles.

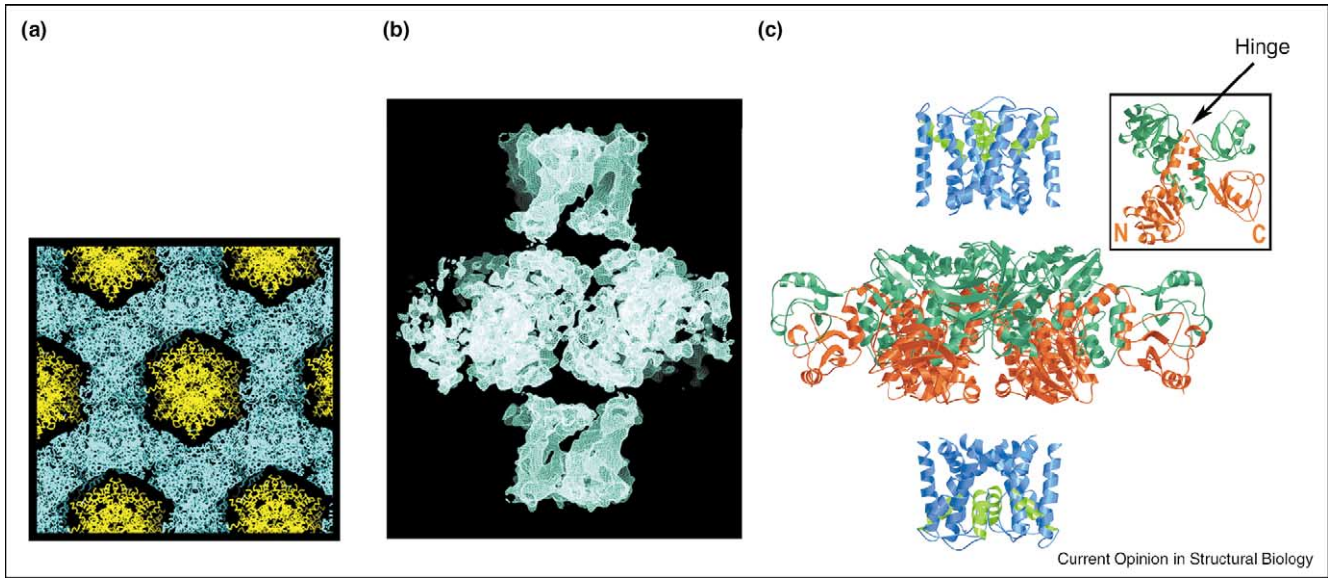
BK channels). This could be assigned as an additional Ca^{2+}. Interestingly, D362 is one of three residues in the intracellular domains implicated in moderate-range Ca^{2+} sensitivity in *mSlo1* [6•]. The other two (D367 and E399) are homologous to E138 and N158, which are positioned at the membrane surface facing the pore.

A gating mechanism presented on the basis of the MthK structure [2] indicates that Ca^{2+} binding reshapes a cleft between the two 'gating rings', altering their relative positions and causing the transmembrane helices of the channel to be dragged apart. It should be noted that the model largely disregards any contribution from the C-terminal *Rck* subdomains, which, in mammalian *mSlo1* channels, confer responsiveness to micromolar concentrations of Ca^{2+}.

KirBac

KirBac is an inward rectifier K^+ channel and, like MthK, its structure was determined as an assembly with its intracellular domains (Figure 4) [4••]. By contrast, the pore is intrinsically well ordered. KirBac is the only K^+ channel structure unambiguously closed to ion conduction and provides a much needed reference point to which we shall return later. This most recent structure enabled the identification of specific intramolecular contacts that are responsible for complete occlusion of the pore at the intracellular face of the membrane. We suggest these

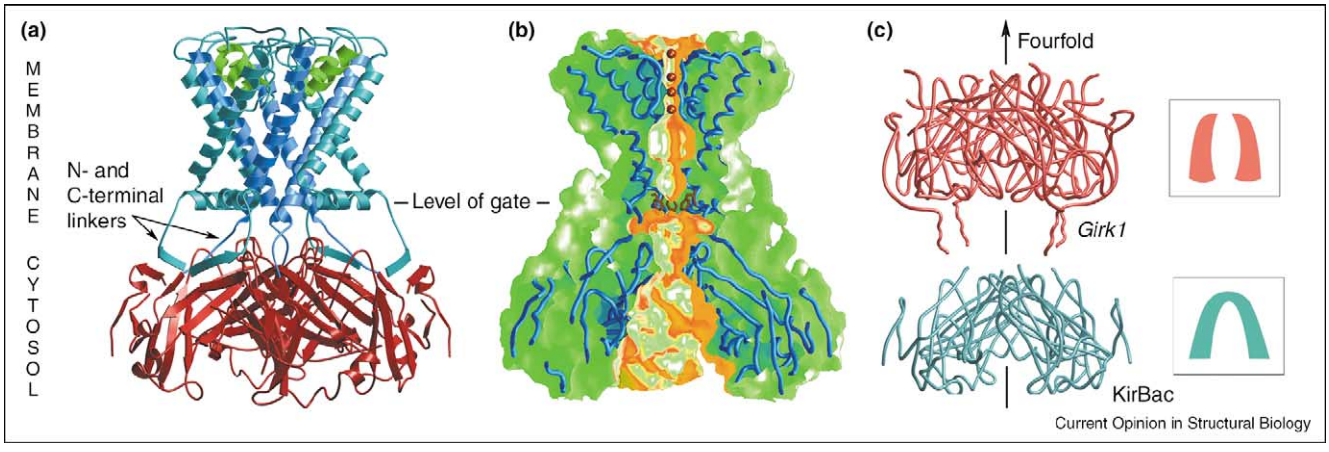
Figure 3



MthK: a Ca^{2+}-gated channel assembly. **(a)** Projection of the MthK lattice along the crystallographic sixfold, showing tightly packed cytosolic *Rck* domains (blue). The pore domains (yellow) are relatively unconstrained. **(b)** Marked regional differences can be seen in averaged $2F_o$-F_c density covering an asymmetric unit of the MthK crystal. The centrally located *Rck* domains are consistent with the 3.3 Å resolution, but electron density for the pore is exceedingly diffuse. **(c)** Ribbon diagram of the published structure corresponding to the map. The *Rck* domains of each channel are discriminated by colour: green for the upper ring and orange for the lower. Although the channel and *Rck* domains are on a single polypeptide chain, the connections between them cannot be seen in the diffuse density. Inset: interleaving of distal and proximal *Rck* dimers in the channel assembly. N- and C-terminal subdomains and the central hinge portion of the orange *Rck* domain are indicated.

contacts represent the physical 'activation gate' in all K^+ channels. The KirBac channel adopts an hourglass shape, due to severe constriction of the pore on the intracellular face. This constriction is the consequence of an extensive hydrogen-bonding network involving intracellular domains and structural lipids. A reinforced turn in the outer helix at the same junction creates an additional structural element — an amphipathic 'slide helix' that lies

Figure 4



An inward rectifier K^+ channel assembly. **(a)** The hourglass form of KirBac is quite distinct from MthK. The narrowest region is at the intracellular face of the membrane. Taut N- and C-terminal linkers connect the pore and intracellular domains. **(b)** Cutaway surface showing the effect of steric obstruction by Phe146 on the ion conduction pathway. Four phenylalanine residues (brown), one from each subunit, effectively divide the ion path (shown in orange) in two at the level of the gate. Note the absence of any side entrances to the ion conduction pathway. **(c)** Isolated intracellular tetramers of *Girk1* (pink) and KirBac (blue) shown as coloured tubes. Although a single subunit superimposes closely, the tetramers look quite different. KirBac is pinched in at the top and widened at the base relative to *Girk1*. Insets show the effect of pinching at the gate on the shape of the internal cavity. In *Girk1*, a channel runs right through the centre, whereas in KirBac the intracellular domains resemble an upturned bowl. We suggest that these correspond to the configurations adopted in alternate physiological gating states.

parallel to the bilayer (Figure 1c). A short slide helix also appears to be present in diffuse density calculated for the open MthK channel (Figure 3b), although it was not built into the model. The KirBac channel employs additional devices that result in a total shutdown of activity. The central cavity in the KirBac conduction pathway is markedly smaller than in open channels, such that it cannot harbour a large ion cloud, and there are no side entrances providing access to the pore. Unlike open channels, in which four pore helix dipoles focus in unison at a central point in the membrane, the pore helices in KirBac are slightly twisted away. This causes a tiny distortion in the precisely suspended ion filter, which we believe disfavours conduction. In crystal structures, one sees a time-averaged snapshot. KirBac has three sites in the filter occupied by ions. This can be attributed to contributions from two arrangements: a configuration with one ion at a central site and a second configuration with two ions about 7 Å apart (as in conducting K^+ channel structures) [1,7]. The temperature factor, B_{iso}, for the central single K^+ is almost an order of magnitude lower than that for the other two ions in the filter. Our data do not discriminate between two possible interpretations of this low thermal parameter, that is, whether the central site is more highly occupied than the other two or whether the central ion is simply immobile. Either way, it suggests an impediment to conduction. The high ion flux that typifies K^+ channels has been ascribed to a mechanism based on electrostatic repulsion, such that when a new ion enters the selectivity filter, the distal K^+ is knocked out like a billiard ball. By this measure, crystallised KirBac exists predominantly in a non-conducting configuration.

With respect to the intracellular domains of KirBac, an interesting comparison can be made to the structure of the fused N- and C-terminal intracellular regions of the mammalian homologue *Girk1* [8]. With the exclusion of a few loops, two of which are disordered in KirBac, the topology is identical and a single subunit of each superimposes closely. The tetrameric assemblies do not superimpose, however, as they differ in the tilt of each monomer with respect to the molecular fourfold. Thus, relative to *Girk1*, the cytosolic domains of KirBac are constricted at the face abutting the membrane and widened at the furthermost edge (Figure 4c). This configuration is constrained by multiple tethers to the pore. Intriguingly, this tilt with respect to a central tetrad is reminiscent of a similar effect on the cytosolic domain (T1) of voltage-gated K^+ (Kv) channels [9] when an ancillary subunit (Kvβ) is attached [10]. It has also been demonstrated that activation of Kv channels is sensitive to the tilt of intracellular T1 domain subunits relative to a molecular fourfold [11,12]. Indeed, a small destabilization conferred on Kv1 channels by co-expressing them with Kvβ [13] could plausibly be ascribed to this effect. We suggest that the structures of the isolated cytosolic domains of the Kv and KirBac channels have crystallised in a low-energy or relaxed state, as one might expect, and that transmembrane components, auxiliary subunits and/or binding partners may enforce the adoption of a more strained configuration when incorporated into a biological assembly.

K^+ channel gating

We now tackle the central issue of this review. What do the current crop of K^+ channel structures reveal about gating? The paradigm is that closed K^+ channels are in a low-energy resting state in the membrane and that opening is driven by the conversion of input energy (in the form of a depolarising pulse, ligand binding, etc.) into mechanical work. In voltage-dependent Kv channels, three alternative models hinge on the type and degree of movement of conserved charged residues in S4 (see Figure 1a) relative to the pore. The traditional 'moving helix' and recent 'paddle' [3••] models suggest that opening occurs via outward motion of S4, which, by implication, draws the transmembrane helices of the pore apart. A newer 'transporter' model [14•] proposes that minimal movement of S4 is required and that the electric field of the membrane moves relative to these same charged residues. It is conceivable that a small S4 movement may be sufficient to promote Kv opening, after which charged residues can, but do not have to, move further across the membrane.

What can structure contribute to this debate, for so long the sole realm of channel biophysicists? A major stumbling block is that, to date, only one physiological conformer of any K^+ channel has been solved, making it difficult to infer any hard and fast rules. The pore domains do share a common architecture, suggesting parallel function, and it is therefore likely that similar processes perturb the closed state of all K^+ channels. These recent structures bring to light some anomalies in the currently accepted views on gating. Key issues that need addressing, in our opinion, are: reconciliation of the strongly constrained structure of KirBac with a 'resting' state; the part played by the electric field of the membrane and its constituent lipids in promoting and maintaining a closed state; and whether the transmembrane helices of the pore really need to be physically pulled or pushed apart to open a pathway for conduction.

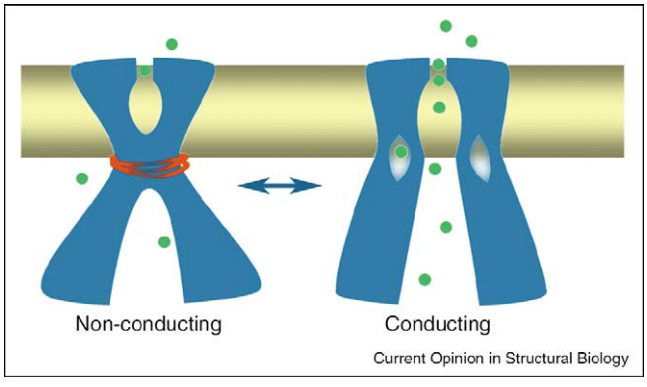
Contrasting the collective open K^+ channel structures, in particular MthK, with KirBac is revealing and raises new possibilities that we shall briefly outline below. We stated earlier that an intricate web of intramolecular contacts weaves the outer, inner and slide helices together, constricting the base of the pore and the proximal face of the cytosolic domains in the closed KirBac channel. This is evident in the striking hourglass form of KirBac. The transmembrane and intracellular regions of the channel are coupled together by taut, structured N- and C-terminal linkers, which preclude the independent movement

of either domain. What would happen if the positional restraints on the linkers were broken? That few such contacts are evident in MthK and KvAP implies that restraints are not as important in open state channels. The conformational differences between isolated and complexed forms of *Girk1* (and T1) cytosolic domains suggest that disrupting these constraints would result in decentralisation of the structural elements, freeing the coupled transmembrane helices to spontaneously move away from one another. This would almost certainly unblock the ion conduction pathway and obviates the need for any mechanical dragging apart of helices during opening. Such a process must be entropy driven, facilitating rapid channel opening but prolonging closure because an extensive network of interactions must be re-established to reach a fully closed and inactivated state. It does not preclude rapid attainment of a lesser non-conductive state, such as that exhibited by the KcsA structure. An analogy that may be helpful is to imagine that closed K^+ channels are constricted, as if by an elastic band, at the edge that meets the intracellular solution (Figure 5). Snipping the elastic band triggers immediate release and a refastening process to restore the original form may require external input. Restated, imposition of numerous molecular constraints on the closed channel may maintain it in a state of suppressed action. The driving force for gating would be relaxation of pore and cytosolic domains into a lower energy configuration.

So how might this relate to K^+ channels in general? All K^+ channels have intracellular regions, which vary from peptides to large multisubunit structures. These may function, in part, to stabilize the closed state. Gating cues, such as a voltage pulse or ligand binding, act on receptive elements in the intracellular or transmembrane domains. One can imagine that a tiny change on a molecular level, such as the disruption of a specific salt bridge or hydrogen bond, could instigate a chain of events that destabilizes a web of molecular tethers holding the gate. For example, in Kv channels, the initial movement of S4 occurring independently in each channel subunit [15,16] may disrupt an interaction between the pore and voltage sensor in the closed state. In Ca^{2+}-gated channels, Ca^{2+} binding facilitates opening. Perhaps Ca^{2+} ions compete for the carboxylates of conserved aspartate residues, disrupting ionic interactions that bridge domain interfaces to maintain the closed state. Thus, the possibility arises that transmembrane (e.g. voltage sensor) and intracellular gating domains may simply confer alternative means of adopting a general process, and add nuance to the response. This presents a new and interesting alternative, based on structural comparisons, to the prevailing viewpoint.

Is the hypothesis presented here in keeping with biophysical data? In our opinion, it is not inconsistent, but clearly this requires detailed investigation. The advent of further structures of complete K^+ channel assemblies will allow these ideas, and others, to evolve.

Figure 5



Hypothetical conformers of a K^+ channel: tense (left) and relaxed (right). Red bands denote the intramolecular constraints in the closed state. When these yield, the ion conduction pathway widens. Intracellular entrances to the pore may form as the transmembrane helices move from a closely packed formation to a loosely arranged one.

Conclusions

Structural characterisation of K^+ channel assemblies has not yet yielded any specific gating mechanisms in the way that the first KcsA structure illuminated the basis of ion selectivity and flux. It is, however, flinging new possibilities into the ring, bringing us ever closer to a thorough understanding of K^+ channel function. Some of these ideas will inevitably confront existing views, as here. Productive collaborations between structural biologists and channel biophysicists should be fostered, and will be invaluable in bringing K^+ channel gating events into even sharper focus.

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