Potassium channels: life in the post-structural world

Daniel L Minor Jr

More than three years have passed since the first structure of a potassium channel protein revealed fundamental molecular details of a platform for ion-selective conduction. Recent efforts have turned to understanding what this structure tells us about potassium channel structure and function in general and, most importantly, which questions remain unanswered. Successes in solving membrane protein structures are still hard won and slow. High-resolution studies of cytoplasmic channel domains and channel-associated proteins, the most tractable entry points for dissecting large, complex eukaryotic channels, are revealing a modularity of function commonly seen in many other biological systems. Studies of these domains bring into sharp focus issues of channel regulation, how these domains and associated proteins are coupled to the transmembrane domains to influence channel function, and how ion channels are integrated into cellular signaling pathways.

Addresses
Department of Biochemistry and Biophysics, Cardiovascular Research Institute, 513 Parnassus Avenue, HSE 1308, Box 0130, University of California, San Francisco, CA 94143-0130, USA;
e-mail: minor@itsa.ucsf.edu

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Introduction

Electrical signals are essential for life in higher organisms and are governed by the action of specialized membrane proteins known as ion channels [1]. These proteins affect electrical excitability by controlling the passage of ions across a cell's lipid bilayer in a highly regulated manner. Of these, potassium channels are the largest, most diverse group [2]. The opening of potassium channels allows potassium to flow out, driving the membrane potential in the negative direction and quieting cell activity. Thus, these channels are essential for the activity of excitable tissues, such as the brain, heart and nervous system. Dysfunctions in these proteins are linked to an ever-increasing number of human diseases [3–5]. The potassium channel field is vast. This review covers only recent insights into a few central structural issues regarding gating and the role of intracellular domains in controlling channel activity.

KcsA as a model for potassium channel pores

The structure of KcsA (Figure 1) [6], a bacterial potassium channel with four subunits, each of which has two transmembrane helices bridged by the potassium selectivity filter region (known as the P-region), has been used as a simulacrum of the inner core of channels from the larger, six-transmembrane voltage-gated potassium channel family. Indeed, features of the highly conserved potassium channel selectivity filter amino acid sequence GYG and the extracellular channel vestibule of KcsA match the known properties of voltage-gated potassium channels remarkably well. The homology between the external vestibules of KcsA and these channels has proven robust enough to make a high-affinity binding site on KcsA for a small peptide toxin (charybdotoxin) known to bind voltage-gated (Kv or Shaker-type) channels [7], and for the design of charybdotoxin variants with altered specificity for different six-transmembrane channels [8].

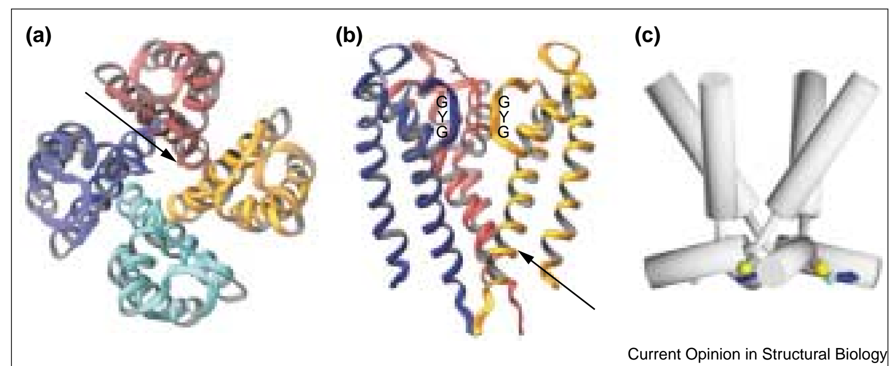
Many potassium channels are sensitive to block by barium ions [1]. Recent crystallographic work with barium-soaked KcsA crystals confirms conclusions from electrophysiological measurements of the interaction of barium ions with the selectivity filter of calcium-activated potassium channels [9•]. These data further support the good correspondence between the selectivity filter structure in KcsA and that likely to be found in more complex channels.

Kinked helices

Substantial divergence is found among potassium channel sequences beyond the selectivity filter. For instance, all Kv channels have a conserved PVP sequence in the middle of the pore-lining, last transmembrane segment (S6). These amino acids are predicted to reside in an area analogous to the narrow portion of the bundle crossing formed by the KcsA inner transmembrane helices, which lack prolines. Multiple (but not single) prolines in transmembrane helices frequently cause breaks or irregularities in the helix structure [10]. Open channel blocker protection experiments on cysteine mutants of the Shaker S6 domain indicate that much more of this segment is accessible than might be expected from strict comparison with the KcsA structure [11••]. These observations, together with a constraint provided by a pair of residues that form an intersubunit cadmium-binding site that locks the channel open [12], suggest that, at least for the Kv family, the structure of the pore-lining segment may be significantly different from that represented by the KcsA structure — bearing a sharp bend or break in the presumed S6 helix at the position of the conserved prolines [11••].

Even in membrane proteins that are 'simple' helical bundles, significant structural differences may exist in the details of helix packing. The recent story of the seven-transmembrane proteins bacteriorhodopsin and rhodopsin beautifully illustrates this subtlety [13]. Although both have similar topologies, the high-resolution model of the former was never able to provide an accurate template for understanding the latter. The other major potassium channel families, Two-P and inward rectifier (Kir) potassium channels, do not have the conserved PXP sequence in their transmembrane segments, so it remains an open question whether the narrow bundle crossing seen in KcsA is common to these

Figure 1



Architecture of the bacterial potassium channel KcsA [6] and the proposed differences in the inner helix of Kv (Shaker-type) potassium channels. **(a)** Extracellular view of KcsA. The arrow indicates the position of the structure formed by the conserved potassium channel selectivity filter sequence GYG for the red subunit. **(b)** Side view of KcsA. The front subunit has been removed so that the position of the narrow bundle crossing can be seen (indicated by the arrow). The positions of the GYG sequences are also indicated. **(c)** Proposed model for the bent helices in voltage-gated (Kv) potassium channels (after [11••]). The yellow and blue balls represent the positions of the residues found to coordinate cadmium and lock the channel in the open state.

types of channels [14–17] and how similar their helical-bundle arrangements are to that of KcsA [18•].

Moving helices

There is a growing consensus that the KcsA crystal structure represents a closed state of the channel. KcsA is opened by low pH (~4.5) [19], whereas the crystals were grown under conditions (pH 7.5) in which the predominant conformation of the channel is closed [20]. The narrowest part of the inner helix bundle (3–4 Å diameter) is lined by hydrophobic residues and appears to be too small to allow the passage of hydrated ions [21••]. Quaternary amine compounds with diameters larger than this opening (~5–8 Å) gain access to the ion conduction pathway and block the open state of KcsA ([22]; L Heginbotham, personal communication). Moreover, electron paramagnetic spin resonance (EPR) experiments suggest that significant conformational changes happen near this narrow portion of the channel when the channel opens [21••]. Together, these data suggest that the open state of the channel must be different from the form seen in the crystal structure and that the narrow region of KcsA must widen to allow ions to pass. EPR experiments suggest that the narrow portion widens through a rotation and tilt of the transmembrane helices [21••]. If and how much the selectivity filter or pore helices move as a consequence of these changes are unknown.

A common point for channel activation?

Genetic selections can be extremely powerful tools for probing protein function. Two studies of the inward rectifying channels Kir3.1 and Kir3.2 that used random mutagenesis and functional complementation of potassium-transport-deficient yeast have identified key positions that affect gating [23•,24•]. Both studies found channel-opening mutations in the transmembrane segments, even though Kir3 channels are normally opened by the binding of G protein βγ subunits to their cytoplasmic domains. These mutations seem to correspond to regions that are involved in the opening of both KcsA [21••] and Kv channels [11••], suggesting that changes in the pore-lining segments of potassium channels may be generally involved in gating transitions.

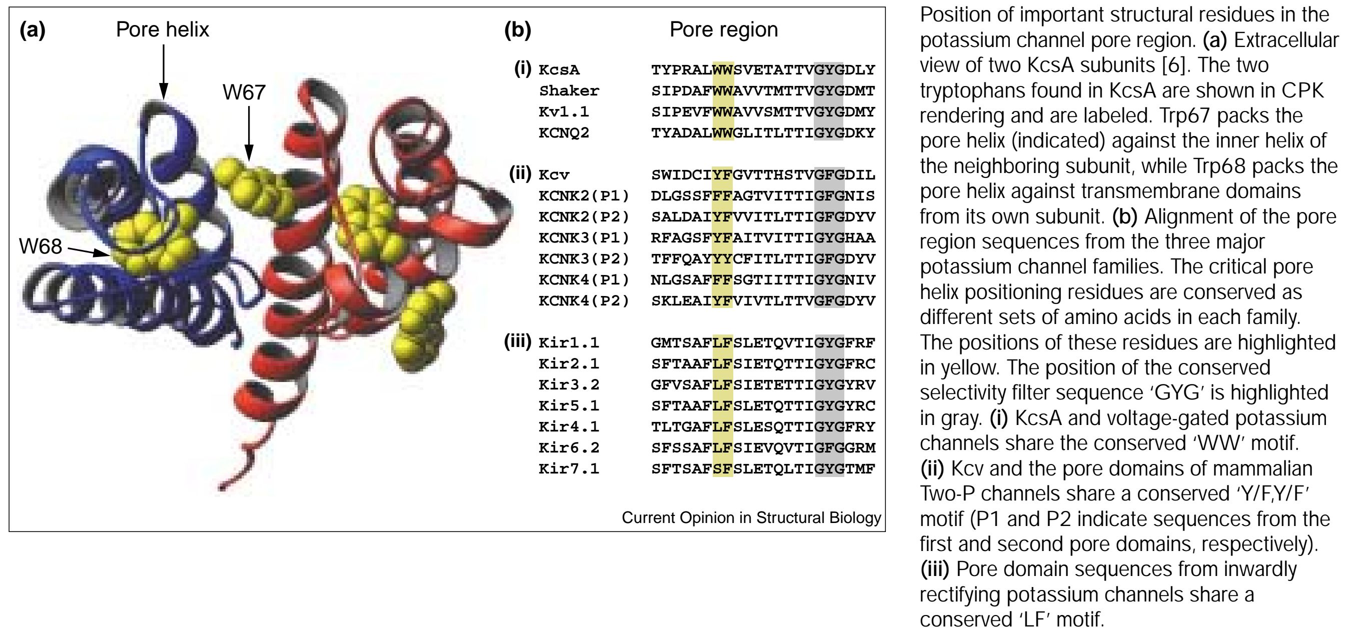
Questions about the pore helix and the selectivity filter

One of the most remarkable features of KcsA is its size (160 residues). An even smaller potassium channel (94 residues) from a form of life lower than a bacterium, a virus, has now been discovered. Kcv is the first known viral potassium channel [25••] and has two transmembrane segments separated by a canonical potassium channel P-region. Unlike KcsA, Kcv forms functional channels in *Xenopus* oocytes, a feature that could make it extremely useful for future structure/function studies. The region of the channel corresponding to the expected pore helix contains features that resemble the family of Two-P potassium channels (for a recent review of Two-P channels, see [26•]) and highlights an important structural issue regarding pore region differences among potassium channel families.

Voltage-gated Kv and KCNQ potassium channels share a conserved pore region feature with KcsA — a pair of tandem tryptophans that support the position of the pore helix. The first of these in KcsA, W67, makes subunit–subunit contacts between the pore helix and the inner transmembrane helix of the neighboring subunit (Figure 2a). The second, W68, packs the pore helix against its own subunit. These same positions are strongly conserved within other families of potassium channels as different sets of residues (Figure 2b). In Two-P channels and Kcv, these positions are a combination of the two smaller aromatics — Y–Y, F–F, Y–F and F–Y. In Kir channels, these positions are strongly conserved as L–F pairs.

Should these small differences in pore helix packing matter? The answer would appear to be ‘yes’. Mutation of the intersubunit tryptophan (W434F) in Shaker Kv channels (converting the pair from W–W to F–W) completely eliminates potassium conductance while permitting channel assembly and proper function of the voltage sensors [27,28].

Figure 2



Position of important structural residues in the potassium channel pore region. **(a)** Extracellular view of two KcsA subunits [6]. The two tryptophans found in KcsA are shown in CPK rendering and are labeled. Trp67 packs the pore helix (indicated) against the inner helix of the neighboring subunit, while Trp68 packs the pore helix against transmembrane domains from its own subunit. **(b)** Alignment of the pore region sequences from the three major potassium channel families. The critical pore helix positioning residues are conserved as different sets of amino acids in each family. The positions of these residues are highlighted in yellow. The position of the conserved selectivity filter sequence 'GYG' is highlighted in gray. **(i)** KcsA and voltage-gated potassium channels share the conserved 'WW' motif. **(ii)** Kcv and the pore domains of mammalian Two-P channels share a conserved 'Y/F,Y/F' motif (P1 and P2 indicate sequences from the first and second pore domains, respectively). **(iii)** Pore domain sequences from inwardly rectifying potassium channels share a conserved 'LF' motif.

This mutation appears to distort the selectivity filter in a way that prevents potassium flow [27], but that allows these 'inactivated' channels to pass smaller ions (Li^+ and Na^+) that are usually excluded [29,30]. As both Two-P and Kir channels are selective for potassium and bear residues that have smaller volumes than tryptophan, there must be compensatory packing differences in this region, a feature that has been suggested for Kir channels from studies of the transmembrane domains [18•]. What these changes are and how such changes impact channel conduction properties remains to be understood.

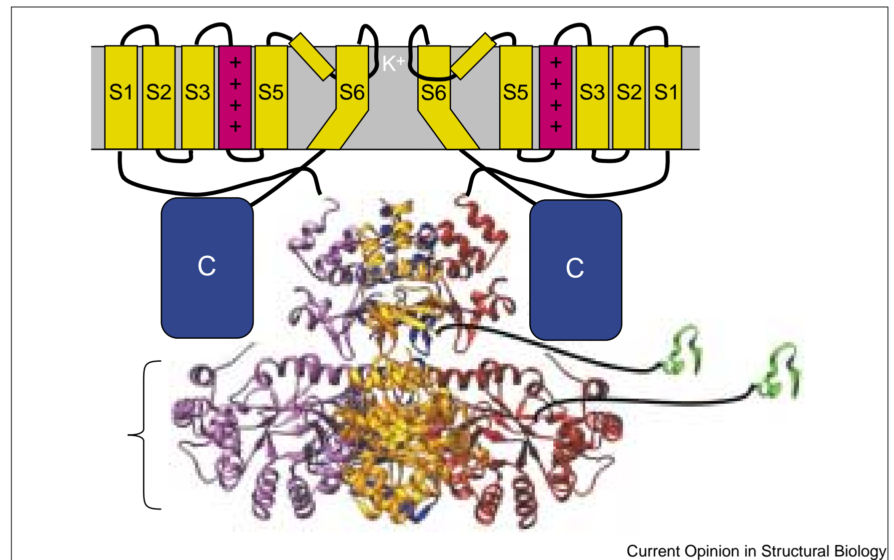
The suggestion that the selectivity filter may act as a gate permitting or denying the passage of ions through the channel is further emphasized by recent studies of a set of Kir channel selectivity filter mutants [31••,32]. Reducing the electronegativity of the carbonyl oxygens of the conserved selectivity filter glycines by making an ester bond (rather than an amide) through unnatural amino acid suppression methods changes the open times and subconductance levels of the channel [31••]. Mutations of the 'Y' of the GYG motif were found only to affect the dwell time of the open state, not the selectivity [32]. One of the biggest structural puzzles regarding ion conduction is the physical explanation for the subconductance levels observed in many types of channels. Although these experiments suggest that some sort of conformational distortions in the filter are responsible, one is left wanting to understand a real atomic-level description of these events.

T1 – more than just an assembly domain

One of the most perplexing pieces of Kv channel architecture is the N-terminal cytoplasmic assembly domain, known as the T1 domain. The first T1 structure revealed a tetramer with two rather peculiar features [33], a water-filled hole along the fourfold symmetry axis and subunit–subunit contacts largely involving polar residues. There has been much debate about the position of T1 relative to the rest of the channel [34,35] and even about the relevance of the T1 structure itself [36]. Recent studies have provided some answers to these questions and uncovered new functions for this domain that raise a new set of questions awaiting a definitive answer.

Mutational and structural analyses of T1 domains reveal a previously unrecognized function for this domain in affecting voltage-dependent gating [37••,38••]. Isosteric replacement of T46V in the Kv1.2 T1 domain increased both the stability of the closed state of the channel and the stability of the isolated T1 domain, without causing significant structural changes [37••]. Together with studies of other mutants of the subunit interface [37••] and the C-terminal face of T1 [37••,38••] that affect channel gating, this suggests that there is a conformational change in T1 as the transmembrane portions of the channel move between open and closed states. Large, buried polar surfaces are rare in protein complexes and it has been suggested that they exist to facilitate domain-scale rearrangements [37••,39]. It is notable that both the T1 domain and the cytoplasmic Kvβ subunit structures [40] have large buried polar surfaces between the subunits. The scale of the changes and how T1 couples to transmembrane segments are unknown. Two likely means of coupling are through the approximately 30 amino acids that connect the C-terminal end of T1 to the first transmembrane segment (the T1–S1 linker) and through interactions between the C-terminal face of T1 and the cytoplasmic loops that link the transmembrane segments.

Figure 3



Cartoon diagram of a voltage-gated (Kv) potassium channel. Only two transmembrane domains are shown and are colored yellow and labeled. The primary voltage sensor, S4, is indicated in magenta and is labeled with plus signs to indicate its positive charge. The positions of the potassium-binding sites in the selectivity filter are indicated by the 'K+'. The S6 segment is bent, as suggested in [11••]. The structures and putative positions of the T1 domain and Kvβ subunits (indicated by the bracket) are shown. The T1 domain is from Kv1.2 [37••] and contains an extra C-terminal helix not found in the T1–Kvβ complex structure [41••]. The four subunits in both T1 and Kvβ are colored yellow, purple, blue and red. The expected positions of the C-terminal cytoplasmic domains (currently of unknown structure, 100–400 amino acids in Kv channels) are shown as blue boxes. The N-terminal inactivation peptide from Kv3.4 is shown in green [61]. Inactivation peptides are found on some T1 domains and some Kvβ subunits. The expected positions of their connections to the known structures are shown. For the sake of clarity, only one inactivation peptide is shown for each.

Any doubts about the orientation of T1 with respect to the membrane are firmly quelled by the structure of the complex between a T1 domain and the cytoplasmic Kvβ subunit [41••]. This structure shows that 'stirrups' on the N-terminal face of T1 are used to interface with a relatively flat surface on the large Kvβ tetramer, leaving the C-terminal face of T1 free. Evidence for the role of Kvβ subunits as functional oxidoreductase enzymes [40] remains elusive. The direct contacts between Kvβ and T1, together with the role of T1 in channel gating, may offer a means for redox-sensitive channel regulation should the Kvβ subunits prove to be active oxidoreductases.

The most pressing issue now is understanding how these cytoplasmic domains dock onto and communicate with the transmembrane complex. The T1 mutational studies and the T1–Kvβ complex suggest that the C-terminal side of T1 faces the transmembrane part of the channel below the ion conduction pathway [37••,38••,41••]. This placement of T1 raises an interesting topological issue (Figure 3). The T1–S1 linker is likely to reach from close to the central axis of the channel to the periphery of the transmembrane helical bundle, where the polypeptide enters the membrane [37••]. The last transmembrane segment, S6, exits the membrane near the central axis of the channel and is followed by large (100–400 residue) C-terminal cytoplasmic domains. Thus, it would seem that the polypeptide chain must thread outward past T1 into an area in which there would be space for the C-terminal domains. This would create a coaxial arrangement in which the exterior of T1 contacts the C-terminal cytoplasmic domains, an interaction that is supported by cross-linking experiments [42]. The proposed bend in S6 [11••] could be an important feature for allowing the polypeptide chain to exit the membrane farther from the central axis of the channel, near the periphery of T1.

All of this protein mass below the channel pore raises questions regarding how the ions themselves and modulators, such as the N-terminal inactivation peptide (or 'ball'), find their way to the pore. To give the ions and the inactivation peptide room to access the channel pore, it has been proposed that T1 is suspended below the transmembrane segments in a manner that creates a gap or 'windows' through which ions and the inactivation peptide enter [41••,43,44]. Mutant cycle analysis supports the idea that the inactivation peptide interacts strongly with the T1–S1 linker [41••]. However, the ball does interact with channels lacking T1, although less well [36,41••,45•], suggesting that the T1–S1 linker is not absolutely necessary. Mutations in the loops between transmembrane segments affect the activity of the inactivation peptide [46,47] and may represent other portions of the binding target. It is striking that the ball peptide does not offer much more protection in the lower portion of S6 relative to quaternary ammonium ions despite its larger size [11••]. Not enough is known yet about the conformation of the channel cytoplasmic domains in the open state to understand these issues clearly.

Regulatory roles of cytoplasmic domains in gating

It is evident from the work on T1 that, just like the cytoplasmic domains in calcium-sensitive potassium channels and cyclic-nucleotide-gated and hyperpolarization-activated cation channels, the cytoplasmic domains of Kv channels have important effects on the opening and closing of the channel pore. Substitution of T1 with a four-stranded

coiled coil results in Kv channels that assemble, but are substantially more willing to open than wild-type channels [37••,45•], supporting the idea that T1 plays a role in the channel gating process.

Regulation of gating may be a general property of potassium channel cytoplasmic domains. Deletions or mutations in the cytoplasmic domains of Two-P channels [26•,48], KcsA [21••,49] and the RCK domain of BK channels [50] have important effects on channel gating. Defining the roles of such domains, how they are coupled to the channel pore and how they are coupled to channel auxiliary subunits and cellular signaling pathways [41••,51–55] will be critical for understanding both the mechanics of channel operation and how channels work and are regulated within excitable cells.

Channel properties switched by transmembrane subunits

Besides cytoplasmic factors, it has recently been discovered that the single-pass transmembrane channel subunits of the MinK family (for a review, see [56]) have profound effects on the gating of Kv3.4 channels [57] and convert KCNQ family voltage-gated channels into constitutively open channels [58••,59••]. How interactions between these membrane subunits and voltage-gated channels cause these radical changes is not known, although the interactions within the membrane seem to be important [60].

Perspectives

Proteins are complicated. One thing that is becoming ever clearer is that channels act like big, allosteric machines, with many coupled moving parts that change conformation as the channel moves between open and closed states [35]. As more high-resolution information about ion channels comes to light, we will be confronted with the need to move from simple models of channel structure and action to models that integrate the details of protein structure, dynamics and energetics. The field now possesses two extremely sharp tools, high-resolution structure determination and the ability to make exquisitely precise functional measurements on single molecules. The combination of these techniques promises a very rich time ahead indeed for life in the post-structural world.

Update

Recent work by Schumacher *et al.* [62] shows the structure of calmodulin bound to a cytoplasmic domain of small-conductance calcium-activated potassium channels. The authors suggest a gating model that explains how the action of calcium on channel-bound calmodulin opens these channels.

Further work from the MacKinnon laboratory [63] using co-crystals of KcsA with tetrabutylantimony (TBSb) and tetrabutylammonium (TBA) locates the position of TBSb in the central cavity of KcsA. The similarity in structure and function of TBSb to TBA suggests that this location is the binding site for quaternary ammonium ions when they block the channel. This paper also includes mutant cycle analysis of the effects of mutations on the inner helix of Kv1.4 and its interactions with TBA and the inactivation peptide. This analysis suggests that TBA and the inactivation peptide act by a common mechanism.

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- •• of outstanding interest

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