Architecture of a membrane protein: The voltage-gated K^+ channel

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The vast array of neuronal action potential waveforms can be ascribed, in large part, to the sculpting of their falling phases by potassium channels. These membrane proteins play several other roles in cell physiology such as the regulation of heartbeat and of insulin release from pancreatic cells as well as auditory signal processing in the cochlea. The functional channel is a tetramer with either six or two transmembrane segments per monomer. Selectivity filters, voltage sensors and gating elements have been mapped to residues within the transmembrane region. Cytoplasmic residues, which are accessible targets for signal transduction cascades and provide attractive means of regulation of channel activity, are now seen to be capable of modulating various aspects of channel function. In this review, we focus on basic channel properties: activation, inactivation and ionic selectivity. We discuss these physiological parameters in the light of recent X-ray crystal structures of bacterial Kv channels, and a body of work combining mutagenesis with electrophysiology and spectroscopy.

THE generation, maintenance and propagation of electrical signals are vital for excitable cells like neurons and cardiac muscle. The unequal distribution of inorganic cations across cell membranes, together with selective permeability of the membranes, generates a transmembrane potential. Electrically excitable cells use gradients of inorganic cations like Ca^{++}, Na^+ and K^+ to generate electrical signals including the firing of action potentials and for the regulation of heartbeat. Electrical signaling in these systems involves pulses of ions flowing down their electrochemical gradients, generating transient alterations in transmembrane potentials. Voltage-gated ion channels mediate these fluxes at rates (~100 million ions per second) which are close to diffusion limits but are paradoxically almost ideally ion-selective (over 3000 : 1 selectivity for Ca^{++} over Na^+ in the Ca^{++} channel). Functionally they may be thought of as water-filled pores traversing the membrane, with elements of ion selectivity and the ability to open and close in response to environmental stimuli. The stimuli which gate ion channels are quite diverse. Some channels are opened by ligands like neurotransmitters or cytoplasmic messenger molecules. Others are opened by changing voltage across the membrane and yet others by sensory stimuli of various kinds. These two aspects of channel functioning – gating and selectivity are conceptually distinct from one another.

Ion channels are among the most efficient biomolecules known. Opening Na^+ channels across a lipid bilayer lowers the energy barrier for Na^+ transport from over 25 kcal/mole to less than 1 kcal/mole, amplifying a small change in input potential into a very large change in output current. Amplification is even higher for calcium transport through calcium channels, critical for its role as a signaling molecule in cell physiology. Ion channels have been found in organisms ranging from viruses to mammals through bacteria and plants.

Voltage-gated potassium channels (Kv channels), originally postulated by Hodgkin and Huxley (see ref. 1 for a detailed discussion on HH models), have been shown to be tetrameric assemblies, each subunit consisting of six transmembrane segments and contributing a re-entrant Pore-lining loop (P-loop) to the ion conduction pathway. Other K^+ channels, such as Kir, IRK and KcsA channels have a different architecture, with only two transmembrane segments and included P-loop per channel subunit; while TWAK and TREK channels have two P-loops per monomer and presumably dimerize to form a functional channel pore.

How do these molecular devices work? How do they allow ion flux at rates close to the diffusion limit and yet maintain high selectivity? What is the molecular basis for voltage sensing? Understanding the molecular basis of ion channel function is a field of intense research today. This review will present our current understanding of these important questions.

Ion channels: Historical perspective

In Peter Medawar's evocative phrase, we are merely bags of thinking water; a bag with 'holes' punched in it for communication with the world outside the 'bag'. In the late eighteenth century, Galvani observed that frog muscles contracted when a metal hook piercing the spinal medulla made contact with an iron railing, suggesting an electrical source for the contraction. Spikes of electrical activity called Action Potentials were shown to underlie nerve conduction.

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In 1952, Hodgkin and Huxley showed that the action potential consisted of an initial transient phase due to sodium influx followed by a slower maintained outward current due to potassium efflux. Further, they developed empirical kinetic descriptions that would be simple enough to make practical calculations of electrical responses, yet good enough to predict correctly the major features of excitability such as the action potential shape and conduction velocity (see ref. 1 for detailed discussion). These required the postulate that the membrane permeability to Na^+ and K^+ depended on the transmembrane potential. In 1964, Narahashi and coworkers selectively blocked sodium current with tetrodotoxin, leaving potassium currents untouched to demonstrate, for the first time, that there were distinct molecules responsible for sodium and potassium transport[2]. The purification of the nicotinic AcetylCholine Receptor (nAChR)[3–5] and sodium channels[6] later proved that these ion transporters were proteins. Finally, the development of the patch clamp technique to study single channel physiology confirmed the molecular identity of these ion transporters[7–9].

The next major breakthrough was the cloning of voltage-gated ion channels – first the Na^+ channel[10], then K^+ channels[11–13], and finally Ca^{++} channels[14]. The functional channel consists of four subunits. In the case of Na^+ and Ca^{++} channels, the four subunits are strung together on one polypeptide chain, while in the case of the K^+ channels, each subunit is a separate polypeptide. The small size and functional variety of K^+ channels has made them the system of choice to investigate structure–function relationships in voltage-gated ion channels. This review describes voltage-gated neuronal potassium channels and discusses various structural aspects of these membrane proteins with a functional perspective.

A channel's a hole
With a physiological role
A peptide chain
In a lipid domain
With its helical coil
Twisted through oil
And its aqueous pore
An open pore
A path for glow
Where ions may go
If not too large
With a electrostatic charge
Examined kinetically
Determined frenetically
With equipment deluxe
Measuring currents and flux
Just a spike on the screen
In fluorescent green
Does it really exist?
Does the channel persist?
Or is it invention
To bemuse the convention?

—Rothstein, 1980

K^+ channel: Relating form and function

After five decades of electrophysiology and biophysics, combined with a little over a decade of extensive molecular biological work and a few important crystal structures of some bacterial relatives of Kv channels over the past 5 years, we now have a relatively clear mapping of channel function to distinct regions of Kv channel proteins. Figure 1 schematically depicts the putative transmembrane topology of a voltage-dependent potassium (Kv) channel. Kv channels are tetrameric assemblies of subunits each containing six transmembrane helices and a re-entrant pore-lining P-loop. The fourth transmembrane segment (S4) is positively charged with arginine or lysine amino acid residues at every third position; and has been shown to move in response to changes in transmembrane potential (see ref. 15 for a review on voltage sensing). The movements of this 'sensor' are transduced by an effector mechanism culminating in the rotation of S5 and S6 helices which opens the channel pore (see refs 16 and 17 for reviews). The P-loop lines the aqueous pore and carries

A channel is not a mere hole
With a physiological role
It is the brain's soul
That keeps the ionic dole
Opens by variety of means
Voltage, pH, ATP and all other
Cell's kith and kin
It's the channel's molecular interior
That makes them far superior
A selectivity filter
That passes the ions in a glitter
Followed by an aqueous cavity
That holds ions in captivity
Than there is bundle cris-cross
That opens a channel in gross
And lets the ions go across
There's the ball and chain
For the ionic train
And shuts the main
And then there are beta subunits
That makes a duplex
Of a channel complex
So you see...
The data panels insist
That channels exist!
It is the invention
That justifies the convention!

—AV

the elements of ionic selectivity[18]. The N-terminal cytoplasmic tail contains the T1 domain that tetramerises in solution[19] and regulates the promiscuity of subunit assembly[20]. Rapidly inactivating channels like shaker, Kv1.4 and Kv3.4 have an inactivation domain at their extreme N-terminus, commonly known as the 'ball and chain' apparatus which is responsible for rapid inactivation.

Depending on the cellular localization of the channel and its physiological function, K^+ channels may be gated by a host of factors such as intracellular Ca^{++} for Kca channels; pH for KcsA channels; changes in transmembrane potentials for Kv channels; ATP for K_{ATP} channels; mechanical pressure for TREK channels; and intracellular polyamines or Mg^{++} for inward rectifiers like Kir channels. In this review we will focus on voltage-gated K^+ channels.

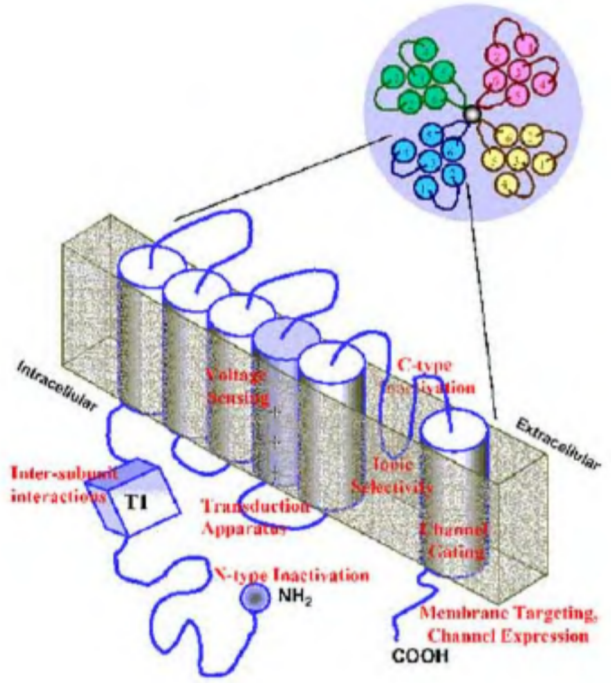

Figure 1. The putative transmembrane topology of potassium channel. A subunit of six transmembrane segments and a re-entrant P-loop tetramerizes to constitute the functional channel pore, as shown in inset (top view). The assigned functional roles of each structural segment are indicated. The fourth transmembrane segment is positively charged and functions as the voltage sensor. The cytoplasmic part of the channel consists of a T1 domain, involved in the specificity of channel assembly, and an inactivation 'ball' domain responsible for rapid inactivation. The activation gate lies near the cytoplasmic mouth of the channel. Activation gating involves the rotational transitions in S6 helices that are arranged as a bundle crossing (see ref. 16). As a result of S6 rotations, the minimal internal diameter increases sufficiently to allow K^+ cation to pass through. An open channel can go back to the closed state by reversing the movement of S6 helices. Alternatively, the ionic conduction can also be stopped by two mechanisms of channel inactivation. C-type inactivation is the slower mechanism and involves the 'pinch shutting' of the external channel mouth just above the selectivity filter. The better known inactivation mechanism of K^+ channels is the 'ball and chain' type which is rapid and involves the auto-inhibitory channel blockade by an inactivation domain of Kv channels like shaker, Kv1.4 and Kv3.4.

Architecture of K^+ channels

The prototype for the pore-forming domain of the channel is the bacterial K^+ channel, KcsA, whose crystal structure has been solved[18,21]. Each subunit of KcsA has two transmembrane helices; the outer helix (M1) is homologous to S5 and the inner helix (M2) to S6 of Kv channels. Figure 2 shows the interior of the KcsA protein (Figure 2 *a* top view; 2 *b* bottom view), with secondary structure shown as ribbons for three of the four subunits (Figure 2 *c*). The narrow selectivity filter is seen at the top (lining residues shown as orange sticks), with the extended selectivity filter loop supported by the pore helices (Figure 2 *c*). The four yellow spheres mark the four K^+ ion binding sites; these are typically occupied by alternating K^+ ions and water molecules[22]. The four inner helices cross near the base of the channel and constitute the narrowest point of the pore (the 'bundle crossing') (Figure 2 *c*). Between the

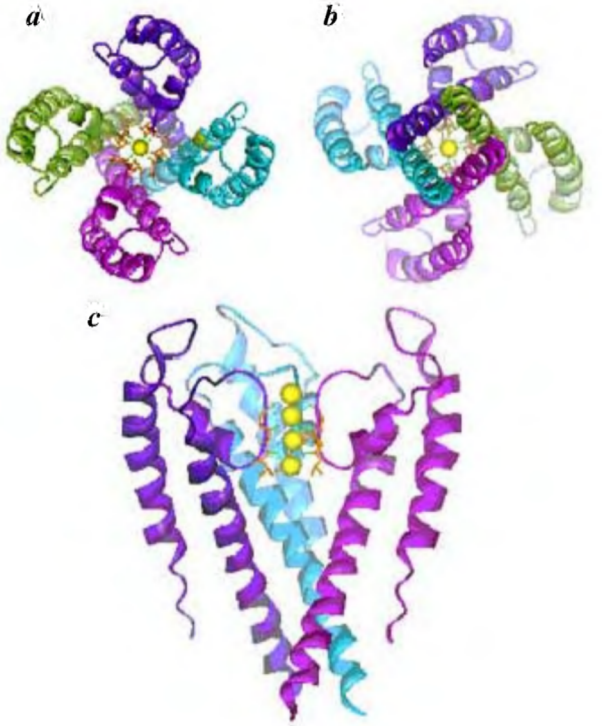

Figure 2. The structure of the K^+ channel pore: KcsA. The crystal structure of KcsA channel that is equivalent to S5, S6 helices and the P-loop of Kv channels. Images are reconstructed using the PDB coordinates (1J95). Axial views from the extracellular side (***a***) and from the intracellular side (***b***) of the channel tetramer. The K^+ ion is shown in yellow and the selectivity filter in orange. The backbone carbonyls that surrounds the K^+ ion are shown in green. (*c*), The transmembrane structure of KcsA. The front subunit has been removed in order to expose the interiors of the selectivity filter and the single file trooping of K^+ ions within the filter.

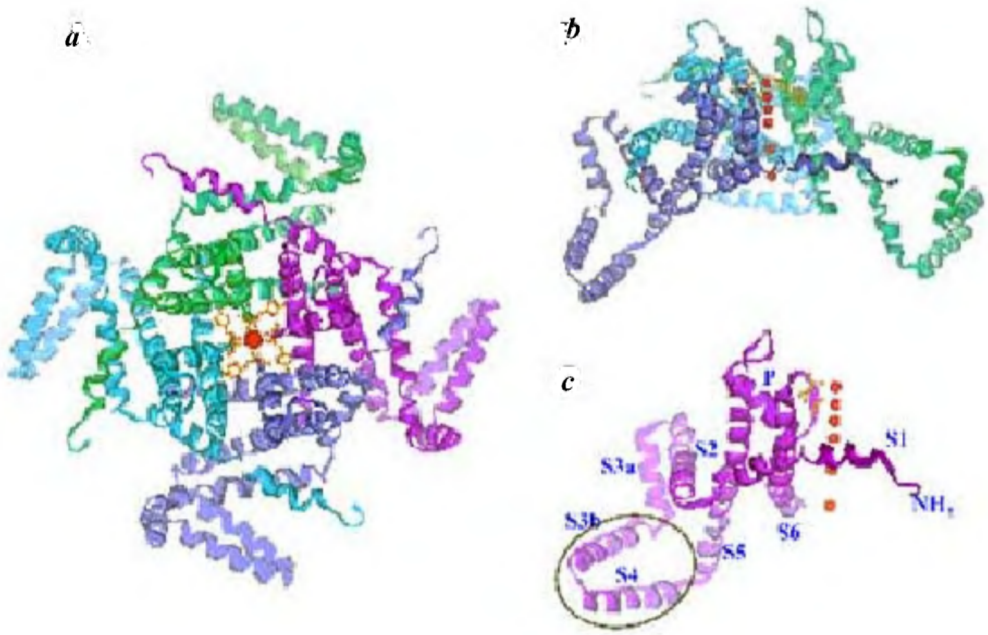

Figure 3. The transmembrane structure of the Kv channel: KvAP. The molecular architecture of an archaebacterial Kv channel, KvAP. Images are reconstructed using the PDB co-ordinates (1ORQ) from protein data bank. ***a***, Axial view of the channel tetramer from extracellular side; ***b***, Molecular interiors of the channel (lateral view). The front subunit has been removed in order to expose the interiors of selectivity filter and the linear arrangement of K^+ ions within the filter. The K^+ ions are shown in red and the selectivity filter in orange. ***c***, The transmembrane packing of a single monomer of KvAP protein. The segment assignments in Figure 1 are depicted here next to their corresponding structure. Note the 'voltage sensing paddles' towards the cytoplasmic face of the channel (gray circle).

selectivity filter and bundle crossing is the water-filled 'cavity'. This spectacular structure is now being mined for insights into the manner in which ions traverse the membrane[23] and the process of selectivity[22], as discussed in a later section on ionic selectivity.

The much-awaited high-resolution structure of a six-TM Kv channel is now available. The crystal structure of a voltage-dependent K^+ channel, KvAP, from the thermophilic archaebacteria *Aeropyrum pernix* has been solved (Figure 3) by MacKinnon and his coworkers[24]. This channel exhibits high amino-acid sequence homology to known eukaryotic Kv channels and exhibits strongly voltage-dependent K^+ currents[25]. The polypeptide backbones of the KvAP channel and that of KcsA are almost superimposable over the pore region, as expected for K^+-selective channels. The structure of the remaining segments, comprising the voltage sensing apparatus, departs significantly from expectations, as will be discussed later.

Low-resolution (> 1.8 nm) cryo-electron micrographs (cryo-EM) of shaker[26] and Kv1[27] channel have been reported. These EM structures suggest a 'Hanging Gondola' type of architectural design of Kv channels with the T1 tetramer coaxial to transmembrane channel pore. The cytoplasmic β-subunits of Kv channels have also been crystallized in solution[28] and have been shown to form central symmetric tetramers. In fact, the Kv1 EM structure shows a tetramerized β-subunit docked to the T1-tetramer as suggested earlier by co-crystallization of a β-subunit with T1 domain in solution[28,29].

Some additional crystal structures of bacterial K^+ channel have been reported, all of which belong to the two-TM class of the channels. MthK is an intracellular Ca^{++} gated channel[30] while MscS is a mechano-sensitive channel[31]. The MthK structure, the first open pore channel structure, shows a broadening of the intracellular channel entryway (up to 12 Å) on channel activation[32]. KirBac is yet another bacterial channel whose structure has been recently solved[33]. KirBac is in the closed state in the crystal structure and suggests that channel gating involves coupling between the intracellular and membrane domains.

The cytoplasmic termini of K^+ channel proteins are responsible for a variety of channel functions. The extreme N-terminal 'ball' is responsible for rapid 'ball and chain' inactivation[34,35]; the more distal T1 domain limits the promiscuity of subunit–subunit interactions and tetramerizes in solution[19,20]; and residues on the C-terminal chain are involved in regulating channel assembly and targeting the plasma membrane. Their cytoplasmic accessibility makes the cytoplasmic tails potential targets of cell signaling cascades involving K^+ channels. (For a review of the structures and physiological roles of these cytoplasmic tails, see ref. 36.)

K^+ channel gating mechanisms

In cellular events of electrical signaling, ionic permeability can be modulated by ion channels. At the molecular level, three different gates have been recognized in Kv channels. These are: (1) Activation gate or S6 gate in the case of six-TM channels, that opens the channel by rotation of S6 helices[16]. (2) Selectivity filter or the pore gate, that involves pinching shut the selectivity filter itself[16,37], also called as 'C-type inactivation' gate. (3) Inactivation gate, that plugs the open channel with the auto-inhibitory 'ball' domain[35,38,39], often referred to as 'N-type inactivation' gate.

The first two gating mechanisms involve conformational changes that are often associated with sensor mechanics. The inactivation 'ball' domain, constituting the third gate, is located at the extreme N-terminus of rapidly inactivating channels like shaker[34,40], Kv1.4 (refs 41 and 42) and Kv3.4 (ref. 43) and will be discussed later.

Opening of the channel pore: Activation gating

The opening of the channel pore requires the rotation of S6 helices, the activation gate, which essentially are the 'effectors' of the signal transduced from the stimulus 'sensors'. Depending upon the stimulus and its physiological roles, various sensing mechanisms have evolved in potassium channels. All sensing pathways are presumed to converge to a similar 'effector' mechanism that finally opens the channel pore. Perturbations in either sensor or gating apparatus would affect activation gating.

Gating coupled with TM potentials: S4, the voltage sensor

Activation gating in voltage-gated K^+ channels is a highly cooperative phenomenon. The fourth transmembrane segment (S4) carries several positive charges and functions as the voltage sensor, moving in response to changes in transmembrane potential. This movement is transduced into channel opening by mechanisms that are yet to be elucidated. Movement of the charges constituting the voltage sensor results in a small, but measurable, current. Detailed analysis of measurements of this 'gating' current in shaker channels and mutants has been interpreted in terms of S4 segments moving independently of each other[15,44], followed by a cooperative transition of the entire protein leading to channel opening[45,46]. Residues at the N-terminus of S4 and in the S3–S4 loop are accessible to chemical labelling from the extracellular medium in the closed state. Several S4 residues show differential accessibility in the closed vs. open states, suggesting substantial rearrangement of the structure[15]. Movement of residues at the N-terminus of S4, as well as other residues in S2, S3 and S5, has been directly observed using Fluorescence Resonance Energy Transfer (FRET).

The structure of an archaebacterial voltage-gated K^+ channel, KvAP, has recently been reported[24]. The channel, crystallized with the help of Fab fragments to restrict the motion of its voltage sensor, exhibits a structure at odds with established preconceptions in that the voltage sensor appears to consist of residues previously designated as forming the end of S3, the S3–S4 loop and much of S4 forming what the authors refer to as the 'voltage-sensing paddle' (see Figure 3 *c*)[47]. The paddle is oriented parallel to the membrane surface close to the cytoplasmic face. Another structure of the S1 to S4 segments, again stabilized by a Fab fragment binding to the S3–S4 loop, has the paddle located close to the extracellular surface. These structures would suggest that the voltage sensor is located at an extremity of the channel, rather than its interior; that it moves through a lipidic environment rather than the interior of the protein; and that its movement is directly coupled to channel opening inasmuch as movement of S4 tugs on S5 which appears to move in concert with S6 (refs 24 and 47) (also see ref. 48 for comments). Interestingly, there is a leucine zipper holding the paddle together. The presence of such heptad repeats of leucine has been noted in the S4–S5 linker of animal channels and has been suggested as a mediator of channel opening[49,50]. However, the crystal structures may need to be reconciled with existing labelling and electrophysiological data.

The voltage-sensing S4 helix is located at the membrane interface with the cytoplasm in the closed state structure, which would position it at the periphery of the membrane electric field. Intriguingly, the KvAP structure shows intra-membrane locations of the N-terminus of the channel and of the S1–S2 linker, neither of which had been predicted to lie within the lipid bilayer. Indeed, the S1–S2 linker has been labelled from the extracellular medium and the N-terminus is linked to the cytosolic T1 domain in animal K^+ channels. The location of the S3–S4 linker at the cytoplasmic face of the membrane needs to be reconciled with extensive labelling studies which indicate that these residues are accessible from the extracellular surface in the resting state of the cell. Furthermore, deletions made in the S3–S4 linker affect voltage dependence in a manner consistent with an extension of the S4 helix beyond its traditionally designated N-terminus[51]. Distances estimated between residues near the N-termini of the S4 segments in a tetramer using FRET[15,52,53] are much shorter than those estimated from the structure (~ 45 Å by FRET vs ~ 90 Å in the structure). On the other hand, the EM reconstruction of shaker indicates a structure that extends over 100 Å at its widest point, which is near the base of the transmembrane domain. The FRET labels, it may be noted, were attached from the extracellular surface. Figure 4 overlays the KcsA and KvAP structures onto the EM reconstruction of shaker, demonstrating the excellent overlap in the pore region and also the fact that shaker appears to have a lot more material in the transmembrane domain than KvAP does.

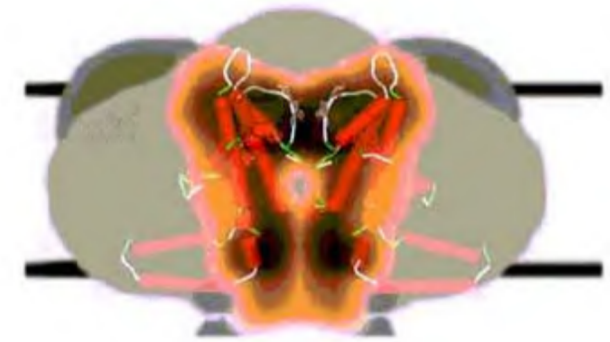

Figure 4. Structures in conflict: KvAP and Kv1 architectures. The crystal structure of bacterial KvAP channel[24] superimposed on the CryoEM structure of mammalian Kv channel[27] at same scale. The outline of the KcsA structure[18] has also been included to delineate the ion conduction region. The overlay demonstrates the high degree of homology at the acqueous pore while there is significantly more membrane embedded area in the Kv1 structure than in KvAP.

Movement of gating charges can be dissociated from channel opening in mutant channels such as the V2[44] and ILT[46] mutants of Shaker. The ILT channel opens only on depolarizing to +50 mV, while much of the gating charge moves over the voltage range −100 mV to −50 mV. These data would indicate that there must be intermediate steps between movement of the voltage sensor and channel opening, at odds with the suggestions made on the basis of the KvAP structure[24]. Moreover, with the voltage sensor located on the cytoplasmic face, there would not appear to be any scope for movement of the sensor in an inward direction from the position reported for the channel at resting membrane potentials. Alternatively, the paddle could be in the 'up' position at resting potentials in inward rectifiers and move down in response to hyperpolarization. It is not immediately obvious how such a movement would result in channel opening. Inward gating currents have been reported for a plant inward rectifier[54–56] and in a methanococcal inward rectifier[57].

The conflict between the reported structure and earlier experimental studies will require a re-examination of the structure and of the interpretation of earlier experimental results. It should be kept in mind that membrane proteins are purified using detergents and crystallized in media that do not resemble the anisotropic lipid bilayer. Thus, crystallization into non-physiological states due to lack of structural constraints imposed by the bilayer may be worth considering. In this context the possibility of Fab fragments inducing structural rearrangements that may not have been feasible within the membrane should not be lightly ruled out.

Channel terminals: The innate gating modifiers

Kv channels exhibit a wide array of voltage-dependent gating profiles and are the major determinants of variation in action potential waveforms. The voltage-sensing domain in Kv channels is well conserved and channels utilize their highly variant cytoplasmic termini to generate the range of physiologies. Kv1.4 channels from a variety of sources exhibit a much shallower voltage dependence of activation than other members of the family[58–60], for reasons that have not been well understood. A chimaeric channel, 1N/4, consisting of the N-terminal chain of hKv1.1 transplanted onto the transmembrane portion of hKv1.4, exhibits a significantly increased cooperativity of the conductance–voltage (G–V) curve with little, if any, shift in the potential at which currents are first observed[61,62]. This could be brought about by the chain segment of hKv1.1 perturbing the machinery that transduces S4 movements into channel opening, probably during the last, cooperative phase of the gating transition[45,46].

The modulatory potential of the N-terminal cytoplasmic tail is more dramatically illustrated by a family of chimaeric channels where the 'ball and chain' of hKv1.4 are covalently attached to the transmembrane body of hKv1.1. The 4N/1 chimaera, stitched together from the N-terminal 'ball and chain' of hKv1.4 and transmembrane body of hKv1.1, opens on membrane hyperpolarization and behaves much like six transmembrane inward rectifiers[63]. This reversal of rectification by a simple swap of cytoplasmic N-termini suggests a modular role of residues concerned. Indeed, the co-expression of the components of the chimaera as two separate entities reconstituted the inward rectifier[64] while both parental components were 'garden variety' outward rectifying channels. The chimaera junction in 4N/1 chimaera was within the T1 domain, in a manner that is expected to disrupt the tetrameric assembly of this cytoplasmic structure[19,63,64]. The related construct, the 4N/1T1 chimaera, has the chimaera junction shifted so as to retain the entire T1 domain from hKv1.1. This chimaeric channel was closed at resting membrane potentials, but exhibited voltage-dependent inactivating potassium currents on depolarization and non-inactivating currents on hyperpolarization[65]. This switching of the operating range of the channel on chain binding could provide a handle to probe the machinery involved in either limiting the movement of the voltage sensing apparatus or in transducing such movements into channel opening and closing.

Channel opening is accompanied by changes in the inter-helix distances of the S6 helix, interpreted as a rotation of S6 leading to a widening of the 'smoke hole' in the teepee structure[16]. Limits are probably imposed on the movement of S4 and, possibly, S6 in order to achieve channel opening over a restricted voltage range. A chimaera consisting of the N-terminal cytoplasmic tail of hKv1.1 attached to the transmembrane body of hKv1.2, exhibits a distinct maximum in channel conductance at 0 mV[66,67]. The decline in conductance on further depolarization is indicative of a failure to lock the channel into its open state. The perturbation could thus help identify means by which movements of either S4 or S6 is limited.

Shutting the channel pore: Inactivation gating

K^+ currents through Kv channels reduce on prolonged opening. Channels employ two distinct mechanisms for this current decay (inactivation gating). The first mechanism of Kv channel inactivation is N-type inactivation which involves an auto-inhibitory ball domain at the extreme N-terminus of channels like shaker and Kv1.4 (Figure 1). The second mechanism is C-type inactivation and, strangely enough, it has nothing to do with the C-terminus of the channels. Instead, residues in S5, S6 and the P-region have been shown to influence C-type inactivation (Figure 1).

The first few N-terminal residues are critical for 'ball and chain' type inactivation[68] in rapidly inactivating Kv channels (Figure 1). Truncations in the N-terminus eliminate inactivation in shaker[34] and in Kv1.4 (refs 41, 42) channels. Inactivation can be reconstituted by floating in a peptide with N-terminal ball sequences[39,40], in a concentration-dependent manner. A secondary inactivation domain (ID2) has been located more distally on the N-terminus of Kv1.4, which can cause inactivation on removal of the primary inactivation domain (ID1)[69]. As described earlier, the high resolution NMR structure of these tandemly linked inactivation particles of Kv1.4 has been solved[70]. This structural information coupled with functional data suggests that the first inactivation domain (ID1, residues 1–38) may be the predominant pore-occluding ball domain; and the second inactivation domain (ID2, residues 40–50), most likely works as a 'docking domain' that attaches ID1 to the cytoplasmic face of the channel.

K^+ channel permeation and ionic selectivity

Potassium channels are transmembrane proteins with a central aqueous pore through which ions can flow selectively down their electrochemical gradients. The fluxes through these channels are up to 100 million ions per second. Voltage-gated ion channels show selectivity in the ions to which they are permeable. K^+ channels are extremely selective in which ions they allow to pass and yet have transport rates close to the aqueous diffusion limit. One way to generate this high selectivity is to construct a filter consisting of carbonyl and/or carboxyl oxygens that optimally coordinate K^+. The risk in doing so is that the K^+ ion might be strongly bound, and would not exit the pore rapidly, as required for a high throughput.

K^+ channel proteins employ four architectural features to counteract this apparent paradox. First, the channels use plenty of water to make the ion stable. This is accomplished by having a broad water-filled cavity in the middle of the ion permeation pathway containing a large amount of water (see Figures 2 and 3). This explanation was proposed many years ago, for the very large conductance of certain calcium-activated K^+ channels[71], and indeed, the crystal structures of the pores of different bacterial K^+ channels confirm this hypothesis[18,24,30,32]. Second, K^+ channels stabilize the ions and achieve cation selectivity by using the electrostatic influence of the dipole moments of the pore helices. The intracellular vestibule of K^+ channels has the negative ends of four helix dipoles pointed towards its centre[18]. Roux and MacKinnon[23] have proposed that these helix dipoles produce a preferential stabilization of cations near the entrance to the narrow selectivity filter. This principle is reaffirmed in the CIC chloride channel structure that is designed to conduct the negatively charged chloride ions – these channels have the positive ends of multiple helices pointed towards the central ion site[72].

A third feature of the K^+ channel for permeation and ion selectivity is the presence of a series of rings of carbonyl oxygen atoms. As a K^+ ion diffuses through water, it is more or less constantly surrounded by a cage of polar oxygen atoms from the water molecules. As originally proposed by Armstrong in 1974, the selectivity filter of the potassium channel is designed to mimic the water structure around a K^+ ion but is also designed not to adopt the presumably more compact structure around a Na^+ ion. Each K^+ ion in the selectivity filter is surrounded by two groups of four oxygen atoms, just as in water: these oxygen atoms (shown in fluorescent green in Figure 2) are in fact the backbone carbonyl oxygens of the selectivity filter residues from the four subunits[18,24,30,32]. The counterintuitive finding from the structures of KcsA is the hydrophobic lining of the central aqueous cavity effectively 'greasing' the permeation pathway and facilitating the quick exit of ions from the aqueous cavity[73]. Finally, a long-known feature of potassium channels is that K^+ ions pass through in single file, with simultaneous occupancy by multiple (>3) ions (Figures 2 *c* and 3 *c*). The mutual electrostatic repulsion between adjacent K^+ ions destabilizes ions in the pore, permitting the favourable interactions to produce ion selectivity without the overly tight binding that would impair rapid permeation[74,75].

The structural and architectural features of potassium channels are thus perfectly adapted to fit their function. They solve the electrostatic problem of stabilizing ions – without making them as stable as they are in water – by using plenty of water and helix dipoles to counteract the unfavourable dielectric environment within the membrane. Furthermore, they solve the problem of stabilizing potassium in preference to sodium by precisely matching the geometry of oxygen atoms around a partially desolvated potassium ion.

K^+ channels: The present standpoint

The emerging picture of ion channels is that they are modular with critical functions regulated by various intra-

cellular factors. The elements of voltage-sensing, ion permeation and selectivity reside within the transmembrane region of the protein, while rapid inactivation is brought about by residues at the N-terminus. In addition, the specificity of subunit–subunit interactions is brought about by the cytoplasmic T1 domain, and residues responsible for the regulation of assembly and targeting are on the cytoplasmic termini.

While the S4 helix along with S3 helix has been shown to constitute the voltage-sensing module, the precise motions executed by the sensor in response to changes in transmembrane potentials are now being investigated. The coupling of sensor movement to rotation of the effector helices (the S5 and S6 helices) leading to channel opening now moves to the centre stage. The Holy Grail of channel biophysicists has long been the understanding of what these molecules looked like and how that architecture resulted in their remarkable functions. With the determination of the remarkable series of K^+ channel crystal structures by Rod MacKinnon's group, the focus now shifts to an elucidation of who does what and when in response to changes in transmembrane potential, leading to channel opening and closing.

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