POTASSIUM CHANNEL STRUCTURES

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The molecular basis of K^+ channel function is universally conserved. K^+ channels allow K^+ flux and are essential for the generation of electric current across excitable membranes. K^+ channels are also the targets of various intracellular control mechanisms, such that the suboptimal regulation of channel function might be related to pathological conditions. Because of the fundamental role of K^+ channels in controlling membrane excitability, a structural understanding of their function and regulation will provide a useful framework for understanding neuronal physiology. Many recent physiological and crystallographic studies have led to new insights into the workings of K^+ channels.

ION CHANNEL STRUCTURE

Ion channels are membrane proteins that mediate ion flux between the outside and inside of the cell through a small, water-filled hole in the membrane — a pore. Ion-selective pores were originally proposed to explain separate components of Na^+, K^+ and leak currents in the classic experiments of Hodgkin and Huxley[1]. As channels have equipped cells during evolution with the means to control ion flux, they have assumed fundamental roles in the functioning of organisms, from bacteria to humans. In prokaryotes, ion-selective channels are crucial for cell survival and growth. In higher organisms, these channels have become a primary means of intercellular signalling, particularly between neurons. Accurate physical measurements of the sizes of the pores, and the capability to record the activity of individual channels, one at a time, have allowed the correlation of early physiological data[1] with specific physical entities[2]. With the subsequent genetic identification of these ion-selective pores as macromolecular proteins that span the membrane, our understanding of the structure and function of K^+ channels has progressed swiftly in recent years, culminating in the landmark determination of the crystal structure of a K^+ channel — the bacterial channel KcsA (see Protein Data Bank entry 1BL8)[3]. For a review of voltage-gated K^+ channels before the KcsA era, I refer the reader to the insightful writing of Armstrong and Hille[4].

Ion channels generate electrical signals across membranes. As channels can be thought of as catalysts of the passage of ions through the membrane, it has been argued that ion channels bear the same relation to electrical signalling that enzymes bear to metabolism[2]. But unlike enzymes, which have evolved to deal with countless substrates, the substrates of ion channels are limited to a few types of ion. This fact emphasizes the simplicity of the permeation process that all channels catalyse. What are the structural bases of permeation and ion selectivity? This is the first question that I address in this article.

Ion channels exercise a very tight temporal control over the permeation reaction, as opening a pore across the membrane can be energetically costly. Let us consider, for example, a neuron, the volume of which might be ~10^{-12} ml and its intracellular K^+ concentration 100 mM (about 10^8 ions). If ions permeate through a K^+ channel at the rate of 10^7 per second, ~10 channel molecules could drain the cell of K^+ in a second. So, to be effective in altering the membrane potential — the currency of neuronal signalling — channel opening must be brief and fast. To accomplish this brief opening and closing, channels have developed various ways of turning on (gating) and off (inactivation) in response to external signals or according to in-built mechanisms. Indeed, the self-inactivating property of ion channels is unprecedented among biological structures. To achieve these gating and inactivation phenomena, channels are believed to undergo conformational cycles. So, the second question that I will ask

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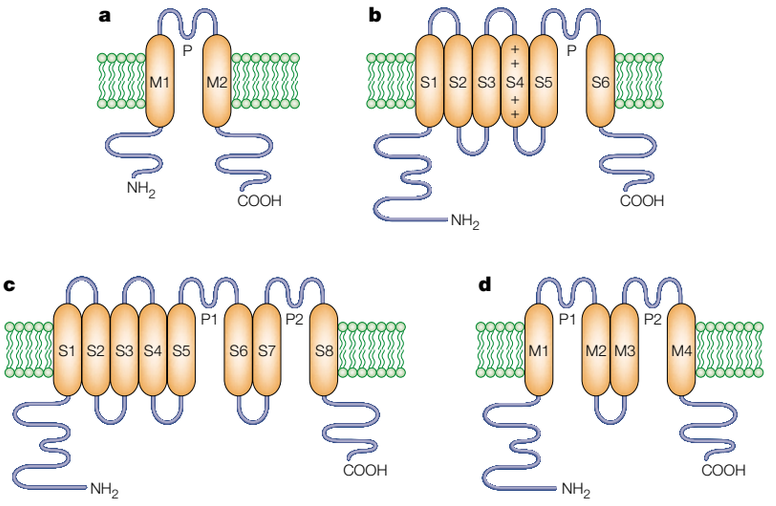

Figure 1 | **The four main classes of potassium channel. a** | 2TM/P channels (which consist of two transmembrane (TM) helices with a P loop between them), exemplified by inwardly rectifying K^+ channels and by bacterial K^+ channels such as KcsA. **b** | 6TM/P channels, which are the predominant class among ligand-gated and voltage-gated K^+ channels. **c** | 8TM/2P channels, which are hybrids of 6TM/P and 2TM/P, and were first found in yeast. **d** | 4TM/2P channels, which consist of two repeats of 2TM/P channels. 8TM/2P and 4TM/2P probably assemble as dimers to form a channel. 4TM/2P channels are far more common than was originally thought. These so-called 'leakage' channels are targets of numerous anaesthetics[39]. S4 is marked with plus signs to indicate its role in voltage sensing in the voltage-gated K^+ channels.

concerns the structural basis of channel gating and inactivation.

Last, the interaction of channels with cytoplasmic proteins can also trigger conformational changes that modulate K^+ channel activity. Although the biological consequences of such interactions are diverse, different interacting proteins share common structural features, which allow conformational changes that regulate ion flux. The nature of these interacting proteins will be the third subject that I will discuss in this article.

K^+ channel architecture

A prototypical K^+ channel consists of four subunits that are clustered to form the ion-permeation pathway across the membrane. Two transmembrane helices and a short loop between them (referred to as the P LOOP) are the trademark of K^+ channels. This canonical architecture of two inner helices and a loop (referred to as 2TM/P) is essentially a universal feature of K^+ channels, but each subfamily of channels is characterized by further, distinct features[5] (FIG. 1). For example, four transmembrane helices (S1–S4) precede the 2TM/P in voltage-gated K^+ channels to endow the channel with the capability to sense and respond to the change in membrane potential. Other variations on the basic K^+ channel architecture include 4TM/2P channels and 8TM/2P channels, two K^+ channel subfamilies that are characterized by the presence of two 2TM/P motifs[6].

The pore-forming 2TM/P domain is flanked by cytoplasmic, protein-interacting domains. They include an amino-terminal tetramerization domain, which is conserved among voltage-gated K^+ channels, and a carboxy-terminal, protein-interacting domain that is characteristic of INWARDLY RECTIFYING K^+ CHANNELS. Another cytoplasmic domain that seems to be crucial for the regulation of K^+ flux is a carboxy-terminal, nucleotide-binding domain referred to as the KTN NAD-binding domain (BOX 1). This domain is highly conserved across all prokaryotic K^+-transport systems and in the K^+ channels of plants, and is related to the RCK domain (so-called because it regulates the conductance of K^+) of the eukaryotic BK K^+ CHANNEL subfamily[7].

The selectivity filter

The opening of K^+ channels allow cations to flow passively across the membrane. Although ion flow is fast, channels are highly selective. Ion selectivity takes place at the narrowest part of the ion-permeation pathway, known as the selectivity filter[8]. The crystal structure of KcsA[3] at a resolution of 3.2 Å showed that the filter is ~12 Å long and ~2.5 Å in diameter (FIG. 2). This structure showed that the main constituents of the filter are MAIN-CHAIN carbonyl oxygens from amino-acid residues Thr–Val–Gly–Tyr–Gly, which are characteristic of the P loop[3]. On the basis of this structure, the selectivity process can be thought of as a series of stereochemical checkpoints. Each checkpoint consists of four oxygen atoms that occupy the corners of a square. This oxygen-lined checkpoint is repeated five times every ~3.0 Å along the filter (FIG. 2).

As ions in solution interact strongly with a layer of water molecules (the hydration shell) in the range of 80 kcal mol^{-1} collectively, the narrow size of the pore has long been thought to strip K^+ ions of their hydration shell as they enter the channel. The oxygen atoms of the filter are indeed well positioned to act as surrogate waters for the dehydrated K^+ ions (1.3 Å radius) within the filter. On the basis of this structure, it has been inferred that the oxygen-lined squares must be conformationally rigid, to prevent the filter from shrinking to a smaller radius. In this way, the coordination of dehydrated Na^+ ions, which have a radius of 0.9 Å, would be precluded. So, the actual energy barrier through the pore would be larger for a physically smaller ion.

How exactly are K^+ ions coordinated by the filter oxygen squares? The structure of KcsA at a resolution of 2.0 Å (REF. 9) provides striking insights into the selectivity and permeation processes, at an atomic level of detail. The basic architecture of the filter, and the presence of oxygen squares with fourfold symmetry, have been confirmed. Furthermore, six K^+-binding sites have been accurately visualized along the selectivity filter — four internal (P1–P4) and two external (P0 and P5). The four internal binding sites are located halfway between two successive oxygen squares, such that eight oxygen atoms can coordinate a K^+ ion at the centre of the antiprism between two rings (FIG. 2). P1–P4 refer to four internal cation-binding sites that are located midway between five successive oxygen squares. The three outermost sites (P1–P3) consist entirely of main-chain carbonyl oxygens, and the innermost site (P4) consists of a carbonyl-oxygen-lined square and another square of oxygens contributed by threonine SIDE CHAINS. Despite minor differences, all sites have a similar antiprism coordination

P LOOP
In an ion channel, the P loop is a short amino-acid segment between two transmembrane helices that dips into the membrane without fully crossing it. The primary sequence of the P loop of K^+ channels has the signature sequence Thr–Val–Gly–Tyr–Gly.

INWARDLY RECTIFYING K^+ CHANNELS
Potassium channels that allow long depolarizing responses, as they close during depolarizing pulses and open with steep voltage dependence on hyperpolarization. They are called inward rectifiers because current flows through them more easily into than out of the cell.

BK K^+ CHANNELS
Potassium channels that are regulated by calcium. As their unitary conductance is big, they are called BK to distinguish them from SK channels, a different population of calcium-regulated channels with smaller conductance. In neurons, BK channels colocalize with calcium channels, shape action potential waveforms and regulate transmitter release.

Box 1 | KTN is a universal regulator of transmembrane K^+ flux

The regulation of intracellular K^+ levels is critical in sustaining life, because K^+ ions serve to control cellular osmolarity against the surrounding environment. All prokaryotic genomes studied so far contain a KTN domain that is associated with K^+-transport systems. The KTN NAD-binding domain is found in a variety of proteins, including K^+ channels, phosphoesterases and various other transporters (see Protein Families Database (Pfam) entry PF02254)[36]. It is similar to the RCK (regulating the conductance of K^+) domain of eukaryotic Ca^{2+}-regulated BK channels[7]. The isolated KTN domain forms a stable tetramer. The canonical scaffold of the KTN domain is a dimeric molecule, and a flexible hinge between dimers might control the assembly of the KTN tetramer. This hinge motion can be physically coupled to the transmembrane loops that face the cytoplasm to control K^+ flux. This mode of gating control is similar to that of glutamate receptor ion channels.

geometry. The non-bonding distance between K^+ and oxygen ranges from 2.7 Å to 3.1 Å. This distance is close to the theoretical distance between oxygen and K^+, and also between two water molecules (2.8 Å, on average).

This coordination geometry is preserved even outside the filter, at its junction with the bulk medium. One external K^+ site (P0) at the external entrance to the filter consists of four carbonyl oxygens from the filter and four water oxygens from the bulk solvent. The last external K^+-binding site (P5) is located at the internal entrance to the filter. It has the same antiprism coordination as the other sites, but the oxygens are entirely provided by immobilized water molecules (FIG. 2). So, the conserved geometry of the oxygens that surround the K^+ ions before they enter the filter, within the filter, and after they have exited, provides strong support for a model in which the hydration shell of the cation is replaced with pore oxygens.

Ion permeation

What is the energetic cost of exchanging hydration waters with pore oxygens? It is difficult to derive an estimate on the basis of a single snapshot of the conformation, such as that provided by a single crystal structure. But by varying the concentration of K^+ in which crystals were obtained, and therefore changing the degree of cation occupancy in the filter, Morais-Cabral *et al.*[10] have recently addressed this question. They showed that, at low K^+ concentration, P1 and P4 are the first sites to fill up. At saturating concentrations of K^+, the filter can harbour two K^+ ions simultaneously, either at P1 and P3 (1,3-configuration), or at P2 and P4 (2,4-configuration). A transition between these two configurations can be initiated by a third ion entering from one side. On the basis of experimental observations of comparable levels of occupancy at all four sites, it has been suggested that the energetic barrier between the two configurations is very low. Therefore, the entering cation can easily exert a repulsive force that is sufficient to push off the cation at the other end of the channel. It is interesting to note that Rb^+, a cation with properties that are similar to those of K^+, occupies the P sites less uniformly at higher Rb^+ concentrations. This fact explains why the overall throughput of Rb^+ through the pore is lower than that of K^+ at high ionic concentrations.

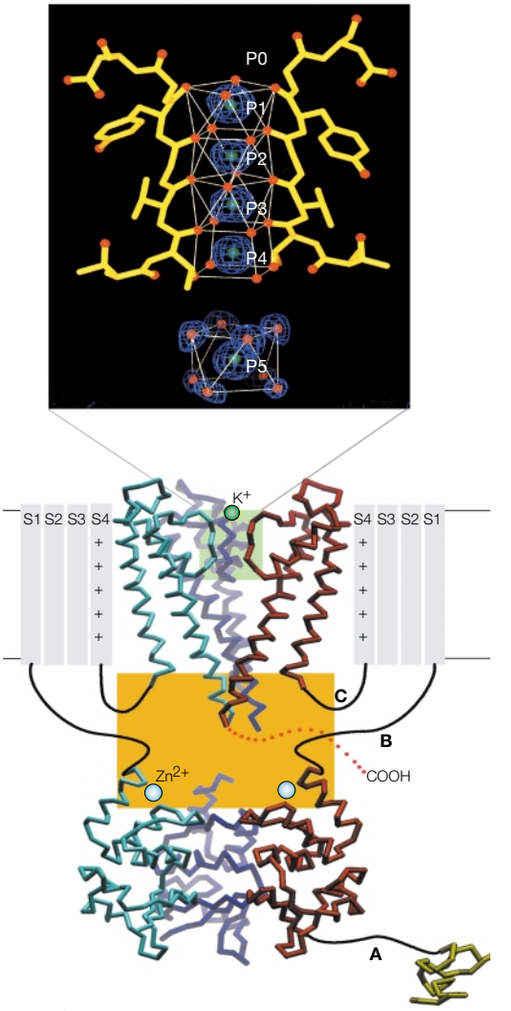

Figure 2 | **Composite structure of voltage-gated K^+ channels.** Top panel: A backbone diagram of the ion-selectivity filter of KcsA. P1–P5 correspond to five K^+-binding sites that are numbered from the outside (top). The P0 site mentioned in the main text is not shown. Each site is formed by eight oxygen atoms (red) that surround each K^+ ion (green) as it passes through the channel. The P1–P4 sites are formed by oxygens contributed by the channel protein. The P5 site is formed by eight immobilized water molecules. Bottom panel: Composite structure of a voltage-gated K^+ channel. The top half shows the transmembrane (TM) domains, including the 2TM/P core, which, in this case, corresponds to the structure of KcsA[3]. The TM helix S4 — the voltage-sensor — is highly charged with basic residues. The cytoplasmic domains are shown in the bottom half. The amino-terminal tetramerization domain (T1) corresponds to the Kv3.1 T1 domain[24]. Zn^{2+} ions, located between four subunits near the carboxy-terminal ends of the chain, are shown in blue. The structure of the inactivation ball (yellow), connected to the T1 tetramer, corresponds to that derived by NMR[40]. The loops denoted by A–C connect these isolated structures and domains, but their relative dispositions are unknown. The transducer box (orange) corresponds to the region between the inner leaflet of the membrane and carboxy-terminal side of the T1 tetramer, which constitutes a putative cytoplasmic vestibule. Other components of the 'transducer box' include linkers A–C, and the carboxy-terminal end of S6 (dotted line in red). All of these components are probably involved in transducing conformational changes that underlie voltage-dependent channel gating, inactivation, and protein–protein interactions.

MAIN CHAIN
The part of an amino acid (NH_3^+ and COO^-) that forms a covalent link with the next amino acid on a polypeptide chain.

SIDE CHAIN
The part of an amino acid that extends from the α-carbon atom that is unique for that amino acid.

NMR
Nuclear magnetic resonance. A technique used to determine the content, purity and molecular structure of a sample. This method is based on the fact that some atomic nuclei have a magnetic moment. When these nuclei are placed in a magnetic field and are simultaneously exposed to electromagnetic radiation, they change their energy state and absorb energy.

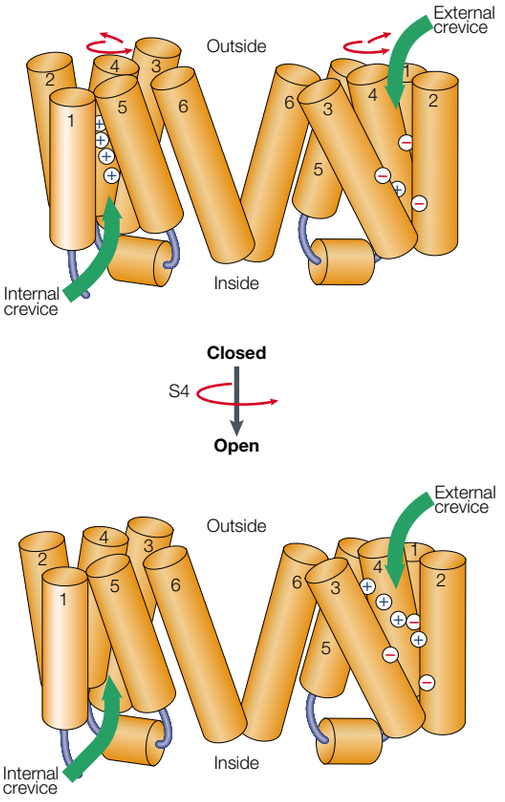

Figure 3 | **Model for the molecular motion associated with voltage-mediated gating.** The top panel shows the closed state, in which charged residues (labelled +) lie within a narrow internal crevice that opens into the intracellular solution. The bottom panel shows the open state. After the transmembrane domain S4 undergoes an anticlockwise rotation and tilt (red arrows above S4), the charged residues become accessible to the extracellular side through the external crevice. Note that the channel subunits are viewed from the front (left) and back (right). Adapted from REF. 13

Is there a significant energy barrier between the 1,3- and 2,4-configurations of K^+ in the course of permeation? If so, is the energy barrier higher for Na^+ than for K^+ ions? Berbeche & Roux[11] have carefully modelled the energy landscape of the selectivity filter. By placing cations every 0.5 Å along the permeation path, and allowing all atoms in the system to adjust dynamically to their new surroundings, the transition between the 1,3- and 2,4-configurations seems to have a very low energy barrier. These data represent a computational confirmation of the results of Morais-Cabral and colleagues[10]. Furthermore, Berbeche & Roux[11] show that the energy barrier is higher for Na^+ than for K^+ by ~3 kcal mol^{-1} at the P1 site and by ~7 kcal mol^{-1} at the P2 site. This difference in energy barrier alone would partly account for the preferred permeation of K^+ over Na^+ ions, which is of the order of ~1,000-fold. Interestingly, the dynamic simulation also indicates that the fluctuation of atomic positions at the filter is too large to account for the delicate positional balance required to distinguish Na^+ (0.9 Å radius) from K^+ (1.3 Å radius)[11]. This result emphasizes the importance of dynamic properties to explain ion selectivity in an accurate way.

The crystal structure of KcsA actually represents the conformation of the channel in a complex with K^+ ions. Does the presence of K^+ stabilize the conformation of the selectivity filter? When K^+ ions are removed from the crystals, their absence causes the selectivity filter to deform significantly[9]. In the absence of K^+, the five oxygen squares do not maintain the same coordinating orientation as before. The main-chain carbonyl oxygens and the amide nitrogens of the filter alter their conformation by intercalating at least three immobilized water molecules between each channel subunit. The immediate interpretation of this observation is that the filter adopts a non-conducting conformation in the absence of K^+ ions[9]. The transition to this presumed non-conducting conformation means that the selectivity filter might not be as rigid as was originally thought. The transition to the ion-permeating conformation might in fact be associated with a more global conformational change in the innermost transmembrane helices. In the case of the KcsA channel, this class of global changes has been observed to occur in response to low pH[12]. As the protein conformation is intrinsically flexible, allowing it to adopt multiple conformational states, dynamic changes of the 'open and rigid' selectivity filter of the channel might operate on a different timescale for different purposes. Global conformational changes of the pore can take place in the order of milliseconds, whereas local atomic movement can occur in the order of nanoseconds. If different conformations cannot be trapped individually in a crystal, purely crystallographic studies will probably be insufficient to resolve the issues that are related to the dynamic properties of the channel. Crystallographic analysis will not give information about the rate of conversion between different conformations, because the electron density that is determined experimentally is the time-averaged ensemble of the entire set of ordered molecules in the crystal. Conformational alteration of the K^+-free selectivity filter is indeed an important indication that the dynamic properties of the pore need to be analysed.

Gating and inactivation

Flow rate through K^+ channels is close to the diffusion limit of the cation, and it is driven solely by its electrochemical potential. Given the uniform K^+ coordination, which is designed to achieve minimal energy barriers in the pore to ensure a fast flow rate[9], other parts of the channel must participate in determining whether the channels are open or closed, and they might do so by propagating conformational changes to the ion-permeation pathway. A large body of research has aimed to unravel the mechanisms by which a channel changes from a non-conducting to a conducting state (gating) and vice versa (inactivation and deactivation). As these studies have already been reviewed elsewhere[4], I will focus on some recent developments that highlight the modular structure of K^+ channels and the possible structural relationship between the domains that are involved in gating and the permeation pathway.

Gating has been studied in most detail in voltage-gated channels. In the case of this subfamily, channel opening is elicited by depolarizing the membrane, and is intimately coupled to the conformation of the S4 transmembrane helix (FIG. 1). Its mechanism of action is built into the channel, and presumably operates through well-coordinated conformational cycles that depend on voltage changes. Many lines of evidence support the idea that S4 is the voltage sensor. For example, spectroscopic probes attached to different parts of the S4 helix experience environmental changes in response to changes in membrane potential, and these changes occur on the same timescale that accompanies voltage activation of the channel[13,14] (FIG. 3). But how are changes in S4 translated at the permeation pathway such that ion flow can proceed? The answer to this question is still incomplete.

When the 2TM/P core of a voltage-gated K^+ channel was replaced by the equivalent part of KcsA, the chimeric channel continued to show voltage-sensitive behaviour[5]. This chimeric channel, built by joining the voltage-sensing module (S1–S4) to the ion-permeation module (S5–S6), provides clear evidence of the modular architecture of voltage-gated K^+ channels. But more importantly, to accomplish the conformational coupling between the two modules, the voltage-sensitive module needed to include relatively short segments of the amino-terminal end of S5 and the carboxy-terminal end of S6, which are exposed to the cytoplasm. These segments are presumably exposed to and interact with other parts of the channel in the cytoplasm. In contrast to this remarkably functional chimaera, simply replacing the entire S5–S6 of a voltage-gated K^+ channel with that of the KcsA core transmembrane module does not equip KcsA with voltage-sensing capability[15].

These unexpected discoveries highlight the fact that there is relatively little direct interaction between membrane-embedded parts of the S1–S4 and S5–S6 helices, and emphasize the importance of concerted conformational changes that are transmitted at the junction between the transmembrane and cytoplasmic domains of the channel. So, the structural coupling at the membrane–cytoplasm junction might be far more complex than was previously thought[16]. The junction at which the transmembrane and cytoplasmic domains meet (orange box in FIG. 2) is clearly at the heart of ion-flux control. To discuss this poorly understood but functionally important part of the channel, I will refer to it as the 'transducer box'.

The main role of the transducer box is to couple conformational changes in different parts of the channel, which ultimately lead to the opening and closing of the permeation pathway. Indeed, all potential moving parts, such as the putative inactivation-ball-binding loop between S4 and S5 (REF. 17), the putative gate near the carboxyl terminus of S6 (REF. 18), the charged loop between the amino-terminal tetramerization domain and S1 (REF. 19), and probably part of the 'inactivation ball' itself, reside in this region. But before discussing the transducer box in the context of these moving parts, it is necessary to consider in more detail the amino-terminal cytoplasmic domain of voltage-gated K^+ channels, of which the inactivating peptide ball is a part.

SITE-DIRECTED MUTAGENESIS
The generation of a mutation at a predetermined position in a DNA sequence. The most common method involves the use of a chemically synthesized mutant DNA strand that can hybridize with the target molecule.

The tetramerization domain

In the case of voltage-gated K^+ channels, the primary sequences of their amino-terminal domains are highly conserved across species, and can be grouped into four main subfamilies (Shaker or Kv1, Shab or Kv2, Shaw or Kv3, and Shal or Kv4). If subunits from the same subfamily are co-expressed, they can co-assemble and form functional heterotetrameric channels across a species barrier. However, co-assembly between different subfamily members does not take place. The molecular determinants of this specificity — the so-called T1 tetramerization domain — are encoded in the amino-terminal domains of the polypeptide chain[20,21]. The core T1 domain consists of ~130 amino acids that immediately precede the first transmembrane helix S1, and its structural role is to stabilize a functional tetrameric channel. Indeed, an engineered peptide that is known to form a stable tetramer by itself can restore functional channel activity when fused to the channel subunits that lack the tetramerization domain[22].

The isolated T1 domain forms a stable tetramer in solution. Detailed structural analyses and comparison between the atomic structures of the isolated T1 tetramers from the Kv4 (REF. 23) and Kv3 (REF. 24) subfamilies of *Aplysia californica* have provided us with valuable insights into the structural basis of channel assembly and modulation. First, the T1 domains of all voltage-gated K^+ channels share a common structural scaffold with fourfold rotational symmetry[23]. Second, the subunit interface is highly polar and Zn^{2+} ions are present at the assembly interface as the main structural determinant of all non-Shaker-type voltage-gated K^+ channels[24]. Third, a water-filled cavity exists at the fourfold-symmetry centre of the T1 tetramer, and the central tip of the narrowest part of the cavity is structurally flexible. However, the T1 cavity is not part of the ion-permeation pathway[25], and it seems that the cytoplasmic vestibule of the channel should lie between the membrane-facing side of T1 and the inner leaflet of the membrane[16,19,25] (FIG. 2). When the narrowest part of the T1 cavity was altered by SITE-DIRECTED MUTAGENESIS, a distinct conformational alteration in the membrane-facing side of the T1 tetramer was systematically accompanied by alterations of the voltage sensitivity of the channel[25,26]. Interestingly, the Kβ subunit, the main cytoplasmic protein known to interact with voltage-gated K^+ channels, coaxially associates with Kv1 subunits by interfacing with the cytoplasm-facing side of T1, modulating excitability and inactivation properties[19]. Remarkably, a conformational change similar to that caused by mutations that alter voltage sensitivity[25] occurs in the membrane-facing side of T1 as a result of binding Kβ, despite the fact that Kβ binds to the cytoplasmic side of T1. These observations reinforce the idea that the membrane-facing side of T1 constitutes part of the transducer box that mediates conformational coupling (FIG. 2).

Box 2 | **KchIP has diverse biological roles**

KchIP is a particularly interesting protein, not least because of its seemingly unrelated roles. It is not known whether KchIP is free in the cytoplasm, but it is most probably bound to the membrane when activated. In any case, the intracellular location of KchIP remains highly elusive.

KchIP has been identified independently on several occasions; consequently, it is known by a number of names. So, KchIP has been identified as calsenilin, a protein that enhances the surface expression of presenilin by interacting with the processing of this protein in the endoplasmic reticulum[37]. KchIP has also been identified as DREAM, the first Ca^{2+}-responsive transcriptional regulator[38], which binds to the upstream region of the gene that encodes the opioid peptide dynorphin.

The identification of KchIP as a protein with such distinct roles points to potentially diverse roles of KchIP in the cell.

The inactivation domain and channel vestibule

In some voltage-gated K^+ channels, the so-called inactivation ball precedes the T1 domain. The inactivation ball is a domain of ~30 amino acids that resides at the amino-terminal end of the protein and is supposedly capable of physically plugging the pore during channel inactivation. Indeed, classic experiments have shown that inactivation can be restored to channels that lack the amino terminus by adding a soluble ball peptide.

The inactivation ball is connected to T1 through a flexible linker of ~30 or more amino acids, which is known as the chain (marked by A in FIG. 2). It has been known for a long time that one inactivation peptide is sufficient to inactivate the channel, despite the fact that there are four identical balls. Similarly, there are four identical entryways in the transducer box that lead to the central pore. These entryways are relatively narrow, lateral openings that are located near the B and C linkers (labelled in FIG. 2). So, how does a single ball reach the channel vestibule and inactivate the channel? As the inactivation ball is structurally flexible, one possible mechanism by which the ball could reach the inside of the pore and physically plug the permeation pathway is through one of the four lateral openings, as an unfolded polypeptide chain[27]. In fact, mutations in the B linker also modulate the inactivation rate, presumably by affecting the rate of access through the lateral openings[19].

It is noteworthy that the inactivation peptide that forms part of the channel might not operate in exactly the same way as the isolated peptide added to the ball-truncated channel. The ball peptide might be bound elsewhere in the channel before it disengages to reach its binding site. The NIP (N-type inactivation prevention) domain was discovered as a short stretch of amino acids that prevents the inactivation process and is located within the amino-terminal segment that precedes the T1 domain of Kv1.5 (REF. 28). Strikingly, the NIP domain is transferable between voltage-gated K^+ channels. Although the mechanism by which the NIP domain prevents inactivation is unclear, it is tempting to speculate that the presence of a similar domain in other K^+ channels might be a general phenomenon that is involved in controlling the disengagement process of the inactivation peptide. In this model, channel activation might affect the structure of the NIP domain, and the inactivation peptide might sense this structural change to trigger the conformational cycle and the inactivation process. To explain the known stoichiometry of one ball being enough to shut off one channel, the conformational change might propagate across the subunits by ALLOSTERIC mechanisms, such that a change in one subunit causes conformational changes in other subunits. Because this idea requires that significant conformational coupling exists between different parts of the channel, it is important to emphasize that there is no experimental evidence to suggest that the chain connecting the ball to T1 has a distinct conformation. Indeed, the opposite is probably true, as the chain can be shortened so that the ball has faster access to its binding site. It will be very interesting to determine to what extent the folding or unfolding of the ball peptide and a flexible chain contributes energetically to the inactivation process. Several unanswered questions about the structural and functional features of K^+ channels await the determination of the structure of the transducer box.

Regulation by protein–protein interactions

In addition to the built-in mechanisms that regulate channel opening and closing, channel function can also be regulated through protein–protein interactions. These interactions allow channels to alter their behaviour in response to various signal-transduction pathways. There are many known examples of channel regulation by signalling pathways. Perhaps the best-known case is the regulation of the inwardly rectifying K^+ channel Kir3 by G-protein-coupled receptors[29]. In this case, regulation depends on the direct interaction of the channel with the $G\beta\gamma$ complex. But there are further examples of protein–protein interactions that control K^+ channels, which highlight the versatility of this type of mechanism in the regulation of channel function. The amino-terminal domain of Kv1.5 interacts with SH3-domain-containing proteins, such as SRC, and many tyrosine kinase receptors[30]. In this case, phosphorylation of the channel renders it inactive. Similarly, the carboxy-terminal domain of Kv4 channels interacts with and is phosphorylated by MITOGEN-ACTIVATED PROTEIN KINASE. In this case, phosphorylation suppresses cellular excitability[31,32]. But perhaps the most striking example is the interaction between the amino-terminal domain of Kv4 channels and a protein known as KchIP (BOX 2). In this case, binding of KchIP enhances the inactivation and recovery rates of the Kv4 channel[33].

These and other cytoplasmic proteins that interact with K^+ channels can often act as monomers or dimers, in contrast to the rotationally symmetrical tetrameric architecture of the K^+ channels. Such a stoichiometric imbalance, in principle, might disrupt a symmetrical relationship that is generally expected between the channel subunit and interacting proteins. The structure of the complex that is formed between the Ca^{2+}-binding protein calmodulin and the calmodulin-binding, carboxy-terminal domain of SK channels[34] was the first example to provide an insight into this type of interaction. The calmodulin-binding

ALLOSTERIC
A term originally used to describe enzymes that have two or more receptor sites, one of which (the active site) binds the principal substrate, whereas the other(s) bind(s) effector molecules that can influence its biological activity. More generally, it is used to describe the indirect coupling of distinct sites within a protein, mediated by conformational changes.

SRC
The first proto-oncogene to be identified. It codes for a non-receptor protein tyrosine kinase.

MITOGEN-ACTIVATED PROTEIN KINASE
Any of a family of protein kinases that are important for relaying signals from the cell membrane to the nucleus.

domain of the channel forms a dimeric complex with calmodulin. The binding of Ca^{2+} to calmodulin triggers dimerization of this dimeric complex, presumably leading to channel activation. However, two rotationally symmetrical dimers cannot convert to a rotationally symmetrical tetramer by simple association of the dimers. This example indicates that allosteric mechanisms probably mediate concerted conformational change between the four subunits of the SK channel; such mechanisms might serve as a general means to achieve concerted conformational changes in K^+ channels, and presumably in cyclic-nucleotide-gated channels[35]. New examples are emerging of a similar non-symmetrical conformational coupling between the transmembrane and cytoplasmic domains of prokaryotic K^+ channels and small molecules (BOX 1). The structural complexity of this type of interaction will no doubt keep us intrigued for some time before we can make any firm generalizations about the mechanisms of K^+ channel regulation by protein–protein interactions.

Perspectives

The resolution of the atomic structure of the ion-conduction pathway of KcsA has served as a springboard from which we have gained a clear understanding of the underlying principles of ion selectivity and permeation. A huge challenge for the field will now be to understand the structural mechanisms of voltage gating and inactivation, and their interaction with the ion-permeation pathway. In this regard, examples of conformational coupling between the pore and the cytoplasmic domains of K^+ channels are already helping us to gain new insights into the fundamental mechanisms of channel regulation. A molecular understanding of these processes will be instrumental in elucidating the mechanisms that underlie the higher-order activity of neuronal networks.

1. Hodgkin, A. L. & Huxley, A. F. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.)* **117**, 500–544 (1952).
2. Hille, B. *Ionic Channels of Excitable Membranes* 3rd edn (Sinauer Associates, Sunderland, Massachusetts, 2001).
3. Doyle, D. *et al.* The structure of a potassium channel: molecular basis of K^+ conduction and selectivity. *Science* **280**, 69–77 (1998).
This article reports the first crystal structure of an ion-selective filter, using the bacterial K^+ channel KcsA as a model system. This study laid the foundation for understanding mechanisms of ion selection and permeation.
4. Armstrong, C. M. & Hille, B. Voltage-gated ion channels and electrical excitability. *Neuron* **20**, 371–380 (1998).
5. Lu, Z., Klem, A. M. & Ramu, Y. Ion conduction pore is conserved among potassium channels. *Nature* **413**, 809–813 (2001).
6. Biggin, P. C., Roosild, T. & Choe, S. Potassium channel structure: domain by domain. *Curr. Opin. Struct. Biol.* **10**, 456–461 (2000).
7. Jiang, Y., Pico, A., Cadene, M., Chait, B. T. & MacKinnon, R. Structure of the RCK domain from the *E. coli* K^+ channel and demonstration of its presence in the human BK channel. *Neuron* **29**, 593–601 (2001).
8. Bezanilla, F. & Armstrong, C. M. Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. *J. Gen. Physiol.* **60**, 588–608 (1972).
9. Zhou, Y., Morais-Cabral, J. H., Kaufman, A. & MacKinnon, R. Chemistry of ion coordination and hydration revealed by a K^+ channel–Fab complex at 2.0 Å resolution. *Nature* **414**, 43–48 (2001).
10. Morais-Cabral, J. H., Zhou, Y. & MacKinnon, R. Energetic optimization of ion conduction rate by the K^+ selectivity filter. *Nature* **414**, 37–42 (2001).
11. Berneche, S. & Roux, B. Energetics of ion conduction through the K^+ channel. *Nature* **414**, 73–77 (2001).
12. Perozo, E., Cortes, D. M. & Cuello, L. G. Structural rearrangements underlying K^+-channel activation gating. *Science* **285**, 73–78 (1999).
This article reports the structural framework for the dynamics of channel activation, using KcsA as a model system.
13. Cha, A., Snyder, G. E., Selvin, P. R. & Bezanilla, F. Atomic scale movement of the voltage-sensing region in a potassium channel measured via spectroscopy. *Nature* **402**, 809–813 (1999).
14. Glauner, K. S., Mannuzzu, L. M., Gandhi, C. S. & Isacoff, E. Y. Spectroscopic mapping of voltage sensor movement in the Shaker potassium channel. *Nature* **402**, 813–817 (1999).
15. Caprini, M. *et al.* Structural compatibility between the putative voltage sensor of voltage-gated K^+ channels and the prokaryotic KcsA channel. *J. Biol. Chem.* **276**, 21070–21076 (2001).
16. Choe, S., Kreusch, A. & Pfaffinger, P. J. Towards the three-dimensional structure of voltage-gated potassium channels. *Trends Biochem. Sci.* **24**, 345–349 (1999).
17. Isacoff, E. Y., Jan, Y. N. & Jan, L. Y. Putative receptor for the cytoplasmic inactivation gate in the Shaker K^+ channel. *Nature* **353**, 86–90 (1991).
18. Liu, Y., Holmgren, M., Jurman, M. E. & Yellen, G. Gated access to the pore of a voltage-dependent K^+ channel. *Neuron* **19**, 175–184 (1997).
19. Gulbis, J. M., Mann, S. & MacKinnon, R. Structure of a voltage-dependent K^+ channel β subunit. *Cell* **97**, 943–952 (1999).
20. Li, M., Jan, Y. N. & Jan, L. Y. Specification of subunit assembly by the hydrophilic amino-terminal domain of the Shaker potassium channel. *Science* **257**, 1225–1230 (1992).
21. Shen, N. V., Chen, X., Boyer, M. M. & Pfaffinger, P. J. Deletion analysis of K^+ channel assembly. *Neuron* **11**, 67–76 (1993).
22. Zerangue, N., Jan, Y. N. & Jan, L. Y. An artificial tetramerization domain restores efficient assembly of functional Shaker channels lacking T1. *Proc. Natl Acad. Sci. USA* **97**, 3591–3595 (2000).
23. Kreusch, A., Pfaffinger, P., Stevens, C. F. & Choe, S. Crystal structure of the tetramerization domain of the Shaker potassium channel. *Nature* **392**, 945–948 (1998).
Shows that the cytoplasmic T1 domain forms a stable tetramer, raising questions about how ions access the transmembrane part of voltage-gated K^+ channels.
24. Bixby, K. *et al.* Zn^{2+}-mediated and molecular determinants of tetramerization in voltage-gated K^+ channels. *Nature Struct. Biol.* **6**, 38–43 (1999).
25. Cushman, S. J. *et al.* Voltage-dependent activation of potassium channels is coupled to T1 domain structure. *Nature Struct. Biol.* **7**, 403–407 (2000).
26. Minor, D. L. *et al.* The polar T1 interface is linked to conformational changes that open the voltage-gated potassium channel. *Cell* **102**, 657–670 (2000).
27. Zhou, M., Morais-Cabral, J., Mann, S. & MacKinnon, R. Potassium channel receptor site for the inactivation gate and quaternary amine inhibitors. *Nature* **411**, 657–661 (2001).
28. Roeper, J. *et al.* NIP domain prevents N-type inactivation in voltage-gated potassium channels. *Nature* **391**, 390–393 (1998).
29. Kubo, Y., Reuveny, E., Slesinger, P. A., Jan, Y. N. & Jan, L. Y. Primary structure and functional expression of a rat G-protein-coupled muscarinic potassium channel. *Nature* **364**, 802–806 (1993).
30. Holmes, T. C., Fadool, D. A., Ren, R. & Levitan, I. B. Association of Src tyrosine kinase with a human potassium channel mediated by SH3 domain. *Science* **274**, 2089–2091 (1996).
31. Adams, J. P. *et al.* The A-type potassium channel Kv4.2 is a substrate for the mitogen-activated protein kinase ERK. *J. Neurochem.* **75**, 2277–2287 (2000).
32. Impey, S., Obrietan, K. & Storm, D. R. Making new connections: role of ERK/MAP kinase signaling in neuronal plasticity. *Neuron* **23**, 11–14 (1999).
33. An, W. F. *et al.* Modulation of A-type potassium channels by a family of calcium sensors. *Nature* **403**, 553–556 (2000).
34. Schumacher, M. A., Rivard, A. F., Bachinger, H. P. & Adelman, J. P. Structure of the gating domain of a Ca^{2+}-activated K^+ channel complexed with Ca^{2+}/calmodulin. *Nature* **410**, 1120–1124 (2001).
35. Flynn, G. E., Johnson, J. P. Jr & Zagotta, W. N. Cyclic nucleotide-gated channels: shedding light on the opening of a channel pore. *Nature Rev. Neurosci.* **2**, 643–651 (2001).
36. Bateman, A. *et al.* The Pfam protein families database. *Nucleic Acids Res.* **28**, 263–266 (2000).
37. Buxbaum, J. D. *et al.* Calsenilin: a calcium-binding protein that interacts with the presenilins and regulates the levels of a presenilin fragment. *Nature Med.* **4**, 1177–1181 (1998).
38. Carrion, A. M., Link, W. A., Ledo, F., Mellstrom, B. & Naranjo, J. R. DREAM is a Ca^{2+}-regulated transcriptional repressor. *Nature* **398**, 80–84 (1999).
39. Goldstein, S. A., Bockenhauer, D., O'Kelly, I. & Zilberberg, N. Potassium leak channels and the KCNK family of two-P-domain subunits. *Nature Rev. Neurosci.* **2**, 175–184 (2001).
40. Antz, C. *et al.* NMR structure of inactivation gates from mammalian voltage-dependent potassium channels. *Nature* **385**, 272–274 (1997).

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