Review

An overview of the potassium channel family

Christopher Miller

Address: HHMI and Department of Biochemistry, Brandeis University, Waltham, MA 02454, USA. E-mail: cmiller@brandeis.edu

Published: 13 October 2000

Genome ***Biology*** 2000, **1(4)**:reviews0004.1–0004.5

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2000/1/4/reviews/0004

Abstract

Potassium channels, tetrameric integral membrane proteins that form aqueous pores through which K^+ can flow, are found in virtually all organisms; the genomes of humans, *Drosophila,* and *Caenorhabditis elegans* contain 30-100 K^+ channel genes each. The structure of a bacterial K^+ channel, sequence comparisons with other channels and electrophysiological measurements have enabled conclusions about the mechanism of gating and ion flow to be drawn for many other channels.

Summary

Potassium channels, originally identified as the molecular entities mediating flows of potassium ions across nerve membranes in action potential generation, are now known in virtually all types of cells in all organisms, where they are involved in a multitude of physiological functions. They are tetrameric integral membrane proteins forming transmembrane aqueous pores through which K^+ specifically permeates. Many molecular subfamilies of K^+ channels are known, and these roughly correspond to the physiological signals by which pore opening is controlled, for example, voltage, Ca^{2+}, G proteins, and polyamines. The high-resolution structure of a bacterial K^+ channel is known, and this reveals the chemical basis of K^+-selective permeation. The genomes of humans, *Drosophila,* and *Caenorhabditis elegans* contain 30-100 K^+ channel genes each, and some of these are subject to elaborate alternative splicing. Several human genetic diseases, such as pathologies involving cardiac arrhythmias, deafness, epilepsy, diabetes, and misregulation of blood pressure, are caused by disruption of K^+ channel genes. In this review I shall summarize what is currently known about the structure of these proteins and the genes encoding them, their evolution, the mechanism by which they transport ions, and the various physiological functions they fulfill.

The organization and evolution of K^+ channel genes

The evolution of K^+ channels is a subject of much speculation, but the appearance of this family throughout the biological universe suggests that they are ancient proteins, not the highly specialized neuro-machines that were envisioned when they were originally described [1]. All fully sequenced genomes - eukaryotic, eubacterial, and archaeal - contain at least one K^+ channel [2]. No other ion channel type displays such ubiquity. K^+ channels are the founding members of the 'S4-superfamily' of ion channels dedicated to electrical signaling, which, apart from the K^+ channels, are found exclusively in eukaryotes. The cyclic nucleotide-gated channels evolved from K^+ channels via acquisition of a cyclic nucleotide-binding domain near the carboxyl terminus; the Ca^{2+} and Na^+ channels, each of which is a monomer containing four internal repeats, evolved from K^+ channels via two gene duplications, with the Ca^{2+} channels appearing in unicellular organisms such as protists, and Na^+ channels arising with the appearance of neurons in multicellular organisms. The pore-forming sequences of these neuronally specialized channels evolved to achieve alternative ion specificities.

In humans, many but not all K^+ channel genes are multiply spliced. Not enough is yet known to make generalizations

about gene structure; in some cases, the entire coding region lies within a single exon, while in others it is segmented into multiple exons, which in some but not all instances delineate clear functional domains. K^+ channel genes are in general not chromosomally clustered.

Characteristic structural features of K^+ channels

There are two broad classes of K^+ channels defined by transmembrane topology, as reflected in primary sequence: the six-transmembrane-helix voltage-gated (K_v) and the two-transmembrane-helix inward-rectifier (K_{ir}) subtypes [3-5] (Figure 1). All K^+ channels display a 'signature sequence' between the two most carboxy-terminal transmembrane helices [6], which reads, with minor variations, TMxTVGYG (using the single-letter amino acid code). In addition, all K_v-type channels have a highly unusual 'S4 sequence' in which lysine or arginine appears in every third or fourth position in an otherwise hydrophobic stretch making up the fourth transmembrane segment [3,4]. Several variations on these gross sequence features are known; the 'slowpoke' subfamily of Ca^{2+}-activated K^+ channels, which are architecturally similar to the K_v subtype, has an extra transmembrane segment near the amino terminus, and the '2P' channels have subunits that are tandem pairs of two K^+ channel sequences. (Most 2P channels are formed from pairs of K_{ir}-like sequences, but a few are known in which a K_v-type and a K_{ir}-type are linked in tandem.)

Currently, direct structural information of K^+ channels is limited to a single bacterial protein, KcsA, of K_{ir} topology [7] (Figure 2). But the structural inferences drawn from electrophysiological experiments on eukaryotic K^+ channels are in such striking harmony with the bacterial channel structure that it is safe to extrapolate the following structural features from KcsA to the entire family. K^+ channels are formed as tetramers [8] of identical or similar subunits arranged in fourfold symmetry around the water-filled ion-conduction pathway (except for the 2P tandem channels, which are presumably dimers.) Common to all K^+ channel subunits is a structural core consisting of two transmembrane helices, readily identified by hydrophobicity algorithms, separated by a re-entrant pore-loop carrying the signature sequence (Figure 1). The most carboxy-terminal transmembrane helix and the signature sequence form most of the lining of the aqueous pore and thus carry the structural determinants of the high K^+ selectivity exhibited by most K^+ channels [6,9,10]. The transmembrane span of the aqueous pore of KcsA, and by inference of other K^+ channels, is highly asymmetric. The narrowest part of the pore, the 'selectivity filter', is a 3 Å diameter tube originating abruptly on the extracellular side of the protein and extending normal to the membrane plane for about 10-15 Å. The wall of this structure is uncharged but highly hydrophilic, lined mainly by twelve backbone carbonyl groups, three from each subunit. The pore then widens to a 10 Å spherical water-filled cavity about halfway through the membrane. This cavity's wall, constructed mostly from aliphatic sidechains, is counterintuitively hydrophobic in nature, as is the intracellular end of the pore. In the KcsA structure (Figure 2), the pore is occluded on the intracellular side by the crossing of four of the transmembrane helices in a 'teepee'-like structure; this occlusion region almost certainly represents the closed state analogous to the 'gate' of the K_v channels, the conformational switch known to open and close the pore. In both K_v

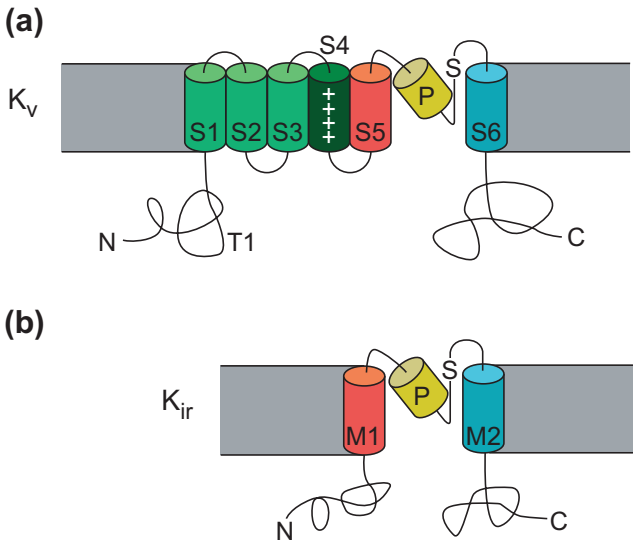

Figure 1
Membrane topologies and main features of the K_v and K_{ir} potassium channel subtypes. **(a,b)** Schematic representation of the membrane topology of (a) K_v and (b) K_{ir} channels. Note that one subunit of the tetrameric structure is shown (see Figures 3b,4). Transmembrane helices are numbered S1-S6 in K_v channels and M1 and M2 in K_{ir} channels; P, pore helix; S, signature sequence; N, amino terminus; C, carboxyl terminus; T1, conserved T1 domain (see Figure 4). The extracellular side is towards the top. Adapted with permission from [24].

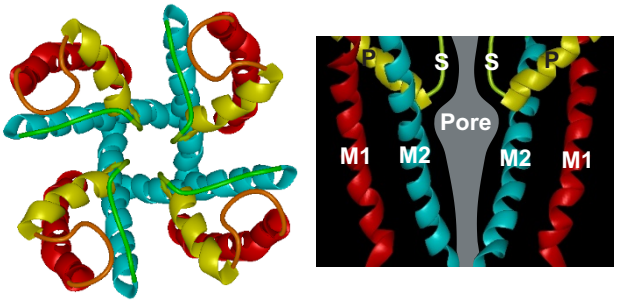

Figure 2
Structure of the *Streptomyces lividans* K^+ channel (KcsA). Labels are as in Figure 1. Reproduced with permission from [24].

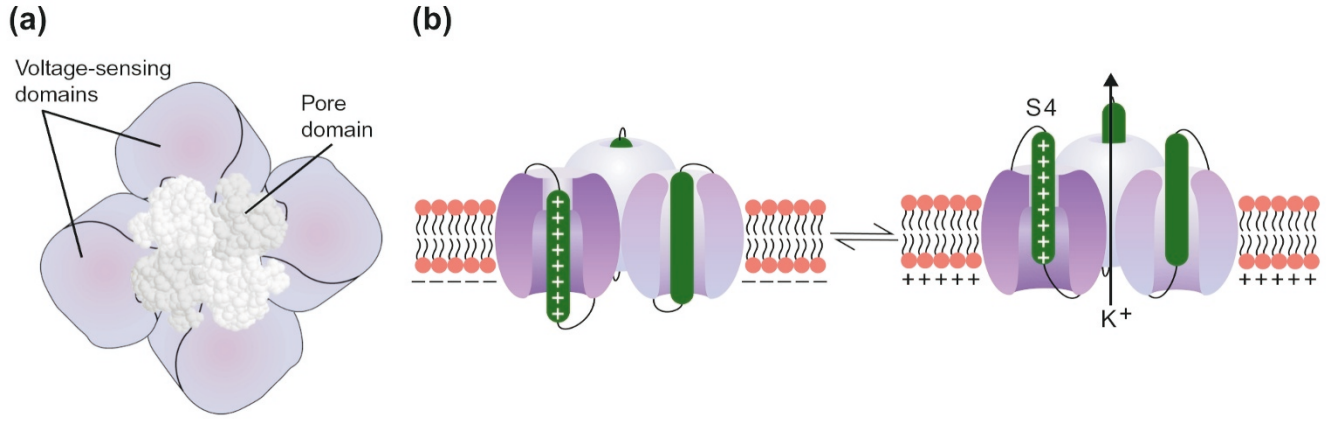

Figure 3
(a) View of a hypothetical K_v-type K^+ channel from the extracellular side of the membrane, showing the central pore-forming region, S5-P-S6, modeled by the structure of KcsA, surrounded by a voltage-gating domain of unknown structure, composed of S1-S4. Reproduced with permission from [25]. **(b)** Diagram of a typical K^+ channel, showing the outward movement of the voltage-sensing transmembrane segment S4 concomitant with channel opening. Reproduced with permission from [26].

and K_{ir} topology, both the amino and carboxyl termini are located on the cytoplasmic side of the membrane.

In the six-transmembrane K_v channels, the first four transmembrane segments, S1-S4, form a module that somehow controls the opening and closing of the pore (Figure 3). The first three of these segments are inferred to be helical along most of their lengths and located on the lipid-exposed periphery of the membrane-embedded complex. The fourth transmembrane segment, S4, which is not lipid-exposed, is thought to form the voltage-sensing element of these channels. This S4 sequence also is found in several classes of channels that have little or no voltage-dependent gating, such as Ca^{2+}-activated K^+ channels and cyclic nucleotide-gated channels.

The K_v channels all have a conserved domain, denoted T1, in the region just to the amino-terminal side of the first transmembrane helix. The structure of the soluble, isolated T1 domain of channel $K_v1.1$ from *Aplysia californica* is known [11] (Figure 4). This structure is preserved in the intact channel in a distinctive 'hanging gondola' formation, in which the tetrameric T1, co-axial with the pore, is separated by about 20 Å from the membrane-embedded part of the channel, connected by strands of protein that define 'windows' of about 20 Å width through which ions gain access to the intracellular side of the pore [12]. Many if not all K_v channels are associated with intracellular, globular 'β subunits' of unknown functions [13], which bind to the lower part of the T1 domain [14].

Localization and function

K^+ channels are found in so many different types of cells carrying out so many different biological tasks that it is impossible to summarize all of them here. All K^+ channels, however, carry out a single basic function: the formation of a transmembrane 'leak' extremely specific for K^+ ions. Since cells almost universally maintain cytoplasmic K^+ concentrations much higher than those extracellularly, the opening of

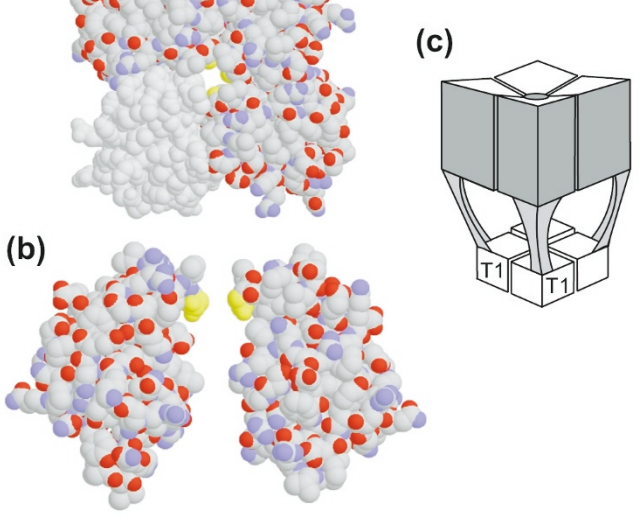

Figure 4
The amino-terminal T1 domain of K_v channels. **(a,b)** Structure of the soluble, isolated T1 domain of $K_v1.1$ from *Aplysia californica*, with views along (a) the fourfold axis and (b) perpendicular to it. **(c)** Schematic diagram of the T1 domain location in the full-length K^+ channel. Reproduced with permission from [18].

a K^+ channel automatically implies a negative-going change in electrical voltage across the cell membrane. This 'membrane hyperpolarization', as it is known in the technical jargon, occurs in different physiological contexts for varied purposes. Obvious examples involve termination of the action potential in electrically excitable cells such as nerve, muscle, hormone-secreting adrenal chromaffin, and pancreatic β cells. But more subtle functions are known in non-excitable cells. K^+ channels play crucial roles in the cellular K^+ recycling required for electrolyte balance effected by the renal epithelium; hyperpolarization of T and B cells is a prerequisite for mitogenesis and proliferation in the immune response; the electrical tuning of mechanosensory cells in auditory transduction relies centrally on the gating kinetics of K^+ channels; guard cells from green plants rely upon K^+ channels to regulate osmotic flow required for regulation of gas and water exchange in leaves; even erythrocytes, often viewed as passive bags of hemoglobin, use a K^+ channel, probably for volume regulation and maintenance of cell shape. The biological functions of prokaryotic K^+ channels are unknown.

In general, K^+ channel activities are elaborately and tightly regulated, both by tissue-specific control of transcription and by biochemical actions on the channel proteins. Some K^+ channels are constitutively active, but most act transiently, being 'gated' by physiological signals. The K_v channels are activated by depolarizing voltage changes; some Ca^{2+}-activated K^+ channels are sensitive to both voltage and cytoplasmic Ca^{2+} levels, whereas others respond only to Ca^{2+}; different classes of K_{ir} channels are directly gated by intracellular factors such as G proteins, nucleotides, or polyamines. In addition, protein phosphorylation is often found to modulate the sensitivity of K^+ channels to their primary physiological signals, or is itself the activating signal [15].

K^+ channels are mainly found on plasma membranes, but they are not in general randomly distributed over the cell surface. For instance, some K^+ channels carry sequences that cause them to associate with scaffolding proteins sporting protein-interaction sequences, such as PDZ domains. This feature provides a possible mechanism for clustering of channels in specific regions of the neuron, as, for example in the co-localization at the presynaptic terminal of Ca^{2+}-activated K^+ channels and Ca^{2+} channels.

Mechanism of permeation and gating

The most fundamental task carried out by all K^+ channels is to catalyze the rapid permeation of K^+ ions while rejecting biologically abundant potential competitors such as Na^+, Ca^{2+}, and Mg^{2+}. The general mechanistic outlines of how these proteins manage to select with high efficiency the larger potassium ion over the smaller sodium ion have been known for decades [16,17], and the recent structure of KcsA provides the molecular details of this catalytic mechanism [18]. There are two features essential to achieving the combination of exquisite ion selectivity and high throughput rate of K^+ channels: first, precise co-ordination of dehydrated K^+ by the protein; second, multiple ion occupancy within the permeation pathway. The basic idea is that K^+ channel pores present electronegative oxygen moieties - now known to be mainly backbone carbonyl groups of the signature sequence - arranged as a cage into which a dehydrated K^+ ion exactly fits, but in which a Na^+ ion would fit so loosely that it energetically prefers to remain in aqueous solution. In other words, K^+ binds tightly to the pore. This feature alone would produce high selectivity for K^+, but it would not allow high throughput because the tightly bound ion would dissociate from its site very slowly. To achieve a fast dissociation rate, three ion-binding sites lie in single file within the pore, close enough that the ions electrostatically repel each other.

Although no high-resolution structures of K_v channels are known, some basic characteristics of the gating of these voltage-dependent channels are understood. The central event initiating channel opening is an outward movement of the S4 transmembrane segment, as depicted in Figure 3b; since this segment carries numerous positively charged residues, this conformational movement is energetically favored by depolarizing voltage [19,20]. How this movement is actually coupled to the opening of the pore, however, is still unknown. It is known, however, that opening of K_v channels is the direct result of movements of S6 near the intracellular end of the pore [21].

Another type of gating seen in some K_v channels is termed 'N-type inactivation', a process leading to the spontaneous closing of the channel upon maintained depolarization. The conformational change producing this inactivation involves the physical plugging of the intracellular end of the open pore by the channel's amino-terminal 20 or so residues, which act as a tethered pore-blocker in a 'ball-and-chain' mechanism [22,23].

Two broad areas of K^+ channel research are beginning to yield to modern experimental assault: molecular structures of the proteins, and the mapping of specific K^+ channel proteins to cellular and physiological contexts. Each of these areas has presented vexing, difficult barriers, which are now being overcome. Structural efforts are directed towards overexpression of prokaryotic K^+ channels, which when successful can lead quickly to crystallographic and spectroscopic investigations of the purified protein. In addition, several isolated water-soluble domains and associated subunits of eukaryotic K^+ channels have been crystallized, and full-length channels are being modeled by piecing together these separate structural elements. The overexpression and crystallization of full-length eukaryotic K^+ channels is still beyond the reach of current techniques, but cryo-electron microscopy and single-particle image reconstruction algorithms are starting to provide low-resolution (about 20 Å) structures of ion channels.

The cell biology and physiology of K^+ channels presents great challenges for the future. Since several K^+ channel subunits are typically expressed in an individual cell, and since subunits within the same subfamily can co-assemble into the same tetramer, the collection of K^+ channels in a given tissue is almost certainly highly heterogeneous. What is the composition of K^+ channels in a given cell? Does the cell regulate K^+ channel function by controlling the mix of subunits available for tetramer assembly? Are K^+ channel subunits segregated within a cell by scaffolding proteins? As molecular probes for K^+ channels become more sophisticated, it will be possible to answer difficult questions like these.

References

1. Hille B. *Ionic Channels of Excitable Membranes.* Sunderland, Massachusetts: Sinauer Associates, 1992.
2. Littleton JT, Ganetzky B: **Ion channels and synaptic organization: analysis of the *Drosophila* genome.** *Neuron* 2000, **26**:35-43.
3. Papazian DM, Schwarz TL, Tempel BL, Jan YN, Jan LY: **Cloning of genomic and complementary DNA from Shaker, a putative potassium channel gene from Drosophila.** *Science* 1987, **237**:749-753.
4. Pongs O, Kecskemethy N, Müller R, Krah-Jentgens I, Baumann A, Kiltz HH, Canal I, Llamazares S, Ferrus A: **Shaker encodes a family of putative potassium channel proteins in the nervous system of Drosophila.** *EMBO J* 1988, **7**:1087-1096.
5. Ho K, Nichols CG, Lederer WJ, Lytton J, Vassilev PM, Kanazirska MV, Hebert SC: **Cloning and expression of an inwardly rectifying ATP-regulated potassium channel.** *Nature* 1993, **362:**31-38.
6. Heginbotham L, Lu Z, Abramson T, MacKinnon R: **Mutations in the K^+ channel signature sequence.** *Biophys J* 1994, **66**:1061-1067.
7. Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R: **The structure of the potassium channel: molecular basis of K^+ conduction and selectivity.** *Science* 1998, **280**:69-76.
8. MacKinnon R, Aldrich RW, Lee AW: **Functional stoichiometry of Shaker potassium channel inactivation.** *Science* 1993, **262**:757-759.
9. Hartmann HA, Kirsch GE, Drewe JA, Taglialatela M, Joho RH, Brown AM: **Exchange of conduction pathways between two related K^+ channels.** *Science* 1991, **251**:942-944.
10. Yellen G, Jurman ME, Abramson T, MacKinnon R: **Mutations affecting internal TEA blockade identify the probable pore-forming region of a K^+ channel.** *Science* 1991, **251**:939-942.
11. Kreusch A, Pfaffinger PJ, Stevens CF, Choe S: **Crystal structure of the tetramerization domain of the *Shaker* potassium channel.** *Nature* 1998, **392**:945-948.
12. Kobertz WR, Williams C, Miller C: **Hanging gondola structure of the T1 domain in a voltage-gated K(+) Channel.** *Biochemistry* 2000, **39**:10347-10352.
13. Scott VE, Rettig J, Parcej DN, Keen JN, Findlay JB, Pongs O, Dolly JO: **Primary structure of a beta subunit of alpha-dendrotoxin-sensitive K^+ channels from bovine brain.** *Proc Natl Acad Sci USA* 1994, **91**:1637-1641.
14. Gulbis JM, Zhou M, Mann S, MacKinnon R: **Structure of the cytoplasmic beta subunit-T1 assembly of voltage-dependent K^+ channels.** *Science* 2000, **289:**123-127.
15. Levitan IB: **Modulation of ion channels by protein phosphorylation. How the brain works.** *Adv Second Messenger Phosphoprotein Res* 1999, **33**:3-22.
16. Hille B, Schwarz W: **Potassium channels as multi-ion single-file pores.** *J Gen Physiol* 1978, **72**:409-442.
17. Neyton J, Miller C: **Discrete Ba^{2+} block as a probe of ion occupancy and pore structure in the high-conductance Ca^{2+}-activated K^+ channel.** *J Gen Physiol* 1988, **92**:569-586.
18. Miller C: **Ion channels: doing hard chemistry with hard ions.** *Curr Opin Chem Biol* 2000, **4**:148-151.
19. Larsson HP, Baker OS, Dhillon DS, Isacoff EY: **Transmembrane movement of the Shaker K^+ channel S4.** *Neuron* 1996, **16**:387-397.
20. Starace DM, Stefani E, Bezanilla F: **Voltage-dependent proton transport by the voltage sensor of the Shaker K^+ channel.** *Neuron* 1997, **19**:1319-1327.
21. Yellen G: **The moving parts of voltage-gated ion channels.** *Quart Rev Biophys* 1998, **31**:239-295.
22. Zagotta WN, Hoshi T, Aldrich RW: **Restoration of inactivation in mutants of *Shaker* potassium channels by a peptide derived from ShB.** *Science* 1990, **250**:568-571.
23. Hoshi T, Zagotta WN, Aldrich RW: **Biophysical and molecular mechanisms of *Shaker* potassium channel inactivation.** *Science* 1990, **250**:533-538.
24. Moczydlowski E: **Chemical basis for alkali cation selectivity in potassium-channel proteins.** *Chem Biol* 1998 **5:**R291-R301.
25. Li-Smerin Y, Hackos DH, Swartz KJ: **A localized interaction surface for voltage-sensing domains on the pore domain of a K+ channel.** *Neuron* 2000, **25:**411-423.
26. Yang N, George AL Jr, Horn R: **Molecular basis of charge movement in voltage-gated sodium channels.** *Neuron* 1996, **16:**113-122.