COMMENTARY
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Two-pore domain potassium channels

Variation on a structural theme

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and
Brohawn SG, del Mármol J, MacKinnon R. Crystal structure of the human K2P TRAAK, a lipid- and mechano-sensitive K⁺ ion channel. Science 2012; 335:436-41; PMID:22282805; http://dx.doi.org/10.1126/science.1213808.

The ability of cells to reliably fire action potentials is critically dependent upon the maintenance of a hyperpolarized resting potential, which allows voltage-gated Na^+ and $Ca2^+$ channels to recover from inactivation and open in response to a subsequent stimulus. Hodgkin and Huxley first recognized the functional importance a small, steady outward leak of K^+ ions to the resting potential, action potential generation and cellular excitability, and we now appreciate the contribution of inward rectifier-type K^+ channels (Kir or KCNJ channels) to this process. More recently, however, it has become evident that two-pore domain K^+ (K2P) channels also contribute to the steady outward leak of K^+ ions, and thus, maintenance of the resting potential. Molecular cloning efforts have demonstrated that K2P channel exist in yeast to humans, and represent a major branch in the K^+ channel superfamily. Humans express 15 types of K2P channels, which are grouped into six subfamilies, based on similarities in amino acid sequence and functional properties. Although K2P channels are not voltage-gated, due to the absence of a canonical voltage sensor domain, their activity can be regulated by a variety of stimuli, including mechanical force, polyunsaturated fatty acids (PUFAs) (e.g., arachidonic acid), volatile anesthetics, acidity/pH, pharmacologic agents, heat and signaling events, such as phosphorylation and protein-protein interactions. K2P channels thus represent important regulators of cellular excitability by virtue of their impact on the resting potential, and as such, have garnered considerable attention in recent years.

Following refinement of experimental strategies to allow investigators to explore the biophysical and functional properties K2P channels with more confidence, structural analysis became an obvious priority. Toward this end, two complementary studies now provide high resolution (i.e., 3.4–3.8 Å) X-ray crystallographic structures of the human K2P channels TWIK-1 and TRAAK, respectively, which have revealed a number of remarkable features. To begin, K2P channel subunits contain four distinct transmembrane helices (TM1–4), and two bona fide pore sequences (P), arranged in the following order: TM1-P-TM2-TM3-P-TM4 with both the N- and C-termini positioned in the cytosol. K2P subunits thus loosely resemble a tandem assembly of two Kir channel subunits. Equally interestingly is that holo-TRAAK and TWIK-1 channels are assembled as homo-dimers, as suggested by earlier biochemical studies, but in sharp contrast to the tetrameric assembly of K^+ channel subunits observed in earlier X-ray crystal structures of mammalian and bacterial K^+ channels. In these latter channels, each subunit contains a single pore sequence (e.g., T-X-G-Y-G-D), which coalesce in the holo-channel to form a 4-fold, symmetric selectivity filter positioned in the center of channel through which dehydrated K^+ ions pass. In K2P channels, the presence of two pore sequences in each subunit, with slightly different amino acid composition, suggested that only two subunits are theoretically required for assembly of a functional selectivity filter, but was this really the case? The two reported crystal structures clearly demonstrate that both TWIK-1

and TRAAK channels contain a central selectivity filter with 4-fold symmetry capable of binding K^+ ions; these images indicate that the four pore sequences in each homodimer K2P channels truly assemble in much the same fashion as the canonical pore sequences present in tetrameric K^+ channels. Closer inspection of the structures reveals that the atomic positions of the carbonyl oxygens responsible for coordinating individual K^+ ions in the selectivity filter of the K2P channel almost superimpose with the equivalent oxygen atoms in the selectivity filter of the bacterial KcsA channel, despite modest differences in amino acid sequence. These observations indicate that the chemical process underlying high K^+ selectivity in the filter can tolerate minor changes in the amino acid composition of individual pore sequences, providing that the 3-dimensional integrity of the ion coordination sites is preserved.

Another noticeable feature of K2P channels is the presence of a large extracellular "cap" structure that arises from an extended portion of the TM1 helix. In the holo-channel, this cap sits directly above the K^+ selectivity filter and conduction pathway and effectively blocks ion efflux in the vertical direction. Ions leaving the selectivity filter are thus forced to exit via two lateral portals positioned above the filter, which are also large enough to allow partial rehydration of K^+ ions as they exit. The presence of the cap also prevents blockade of K2P channels by naturally occurring peptide toxins and other small molecules (e.g., TEA) that typically bind within the external mouth of the conduction pathway and physically plug the channel pore. In Kir2 channels, enlarged pore turret structures extending above the external mouth of the channel also prevent many pore blockers from acting. Such structural modifications thus make these channels insensitive to many peptide toxins that bind to sites within the external conduction pathway.

Somewhat unexpectedly, the K2P structures revealed two lateral openings or fenestrations in the transmembrane section of the holo-channel that create passageways between the lipid bilayer and the ion conduction pathway. As some K2P channels are modulated by hydrophobic compounds and various lipids that readily enter the membrane bilayer, these openings likely represent access points for these agents to enter the channel and influence ion conduction. In the TWIK-1 structure, electron dense material was detected in these fenestrations, and may represent the alkyl chains of membrane phospholipids. Interestingly, the Catterall group has recently reported similar fenestrations in the X-ray crystal structure of a bacterial, voltage-gated sodium channel (Nature 2011;475: 353). As Nav channels are also modulated by hydrophobic compounds that often act through the membrane bilayer (e.g., local anesthetics, such as procaine and lidocaine), the presence of openings in the mid-membrane region of a channel may be a general structural feature that facilitates the regulatory actions of such compounds.

As noted above, K2P channels are responsive to chemical and mechanical stimuli that may influence the channel via impacts on the membrane bilayer, but how this occurs at a molecular level is unclear. Functional studies have implicated a region at the C-terminal end of each TM4 segment, which forms a helical structure that extends along the cytosolic surface of the channel and contacts the TM2 segment in the adjacent protomer. For several types of K2P channels, this C-terminal helix may thus represent a molecular sensor involved in gating, however, this mechanism may not apply to all K2P family members (i.e., TRAAK channels) that are sensitive to regulatory stimuli, such as mechanical forces, PUFAs, phosphorylation events and protein-protein interactions.

In summary, these two studies have provided important new insights into the structural features of K2P channels that underlie their functional attributes. In particular, K2P channels are built with a highly discriminating ion selectivity filter similar to those present in classic K^+ channels, but have been given a novel and somewhat radical, structural framework to support this filter domain in the membrane bilayer. The presence of extensive contact surfaces between subunits at the homodimer interface, together with intersubunit contacts present in the external cap structure, are likely important to stabilize this framework. As we have already seen with other cation-selective channels, K2P channels are modular in design. The highly conserved selectivity filter has been paired with a novel transmembrane supporting framework that displays considerable structural diversity compared with other members of the K^+ channel superfamily. The crystallization of additional cation-selective channels will undoubtedly provide further insights into the extent of this diversity or variation.

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