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PERSPECTIVES

A slick, slack pathway through the pore

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Slo2.1 and Slo2.2 K^+ channels (also called 'Slick' and 'Slack', respectively) are members of an extended gene family of K^+ channels defined by the presence of 'regulator of K^+ conduction' (RCK) domains at their carboxy termini (Yuan *et al.* 2000, 2003; Santi *et al.* 2006). RCK domains serve as cytosolic ligand sensors, and are activated by Na^+ ions in the case of Slo2 channels. Other members of this gene family include BK (Slo1) channels, which are activated by cytosolic Ca^{2+} instead of Na^+, as well as a host of orthologues found in prokaryotes, such as MthK (Rothberg & Magleby, 2000; Jiang *et al.* 2002; Latorre & Brauchi, 2006; Smith *et al.* 2013). These channels have been models for understanding biophysical mechanisms of channel gating, and as recent crystallographic and cryo-EM experiments provide information on channel structure, it becomes even more interesting to incorporate these structural details into our thinking.

For example, as the RCK domains are tethered to the cytosolic face of the channel, it might seem quite reasonable to think that gating of Slo2 channels involves movement of the RCK domains to relieve steric hindrance of K^+ ions by a molecular 'gate' at the cytosolic end of the pore. A combination of structural and functional evidence has supported the idea that the K^+ channel gate is located near the cytosolic threshold of the pore and is formed by a 'bundle-crossing' of the four pore-lining helices of the channel. Support for this model can trace its origins to classical 'blocker-trapping' experiments in the squid axon K^+ channel, which illustrated that a quaternary ammonium blocker molecule could access its binding site from the cytosolic side of the pore only after the K^+ channel gate has been opened, and it can be subsequently 'trapped' following entry if the K^+ channel's activation gate is closed behind it (Armstrong, 1971; Armstrong & Hille, 1972). The molecular basis of the gate as a bundle-crossing was supported by elegant cysteine-modification experiments in the *Shaker* Kv channel and related channels, and finally by the crystal structure of the apparently closed KcsA channel (Holmgren *et al.* 1997; Liu *et al.* 1997; Doyle *et al.* 1998; del Camino & Yellen, 2001; Shin *et al.* 2001; Rothberg *et al.* 2002). However, more recent evidence has suggested that not all K^+ channels may conform to this mechanism, and that the gate may instead lie deeper within the pore (Wilkens & Aldrich, 2006).

In this issue of *The Journal of Physiology*, Giese *et al.* (2017) challenge whether the bundle-crossing hypothesis applies to Slo2 channels with a series of electrophysiological experiments. The authors first target two leucine residues, L267 and L270, using mutagenesis guided by recent structural data, to test their role in gating. These residues line the central axis of the pore not near the bundle-crossing, but instead near the channel's selectivity filter (Hite *et al.* 2015). The authors find, interestingly, that substitution of these residues individually or together can render Slo2.1 channels constitutively open, consistent with the idea that these side chains impact movement of the gate. Such a key role for these deep-pore residues in modulating gating energetics is at odds with a model in which gating consists only of localized movements of the bundle-crossing at the cytosolic end of the pore.

The authors then interrogate residues positioned near a potential bundle-crossing, focusing on M282 in Slo2.1 and its analogue M354 in Slo2.2. These methionine side chains appear to face the central axis of the pore, and would be the most likely to come together to form a hydrophobic gate in the closed conformation. If this idea were true, then one might predict that substituting these methionines with glutamate side chains might result in electrostatic repulsion at the bundle-crossing, to render the channel constitutively active. Interestingly, M282E and M354E channels did not show enhanced activation, again arguing against the idea that bundle-crossing gates the flow of K^+ in these channels.

The work of Giese *et al.* adds to an emerging picture of diversity among K^+ channels in terms of their structural mechanisms to gate K^+ conduction. In voltage-gated K^+ channels (like *Shaker*), there is robust evidence that side chains near the bundle-crossing form a gate that is strongly controlled by the channel's voltage sensor, and can impede the flow of large blocking molecules as well as small ions, such as Cd^{2+} or Ag^+ (del Camino & Yellen, 2001). On the other hand, it now seems the K^+ channels in the RCK family exhibit relatively weak (or 'slack'?) steric hindrance at the bundle-crossing, which may impede the access of large blocking molecules, but not smaller ions (Li & Aldrich, 2004, 2006). These new results provide a rationale to probe deeper into the pore, in search of the elusive gate.

References

Armstrong CM (1971). Interaction of tetraethylammonium ion derivatives with the potassium channels of giant axons. *J Gen Physiol* **58**, 413–437.

Armstrong CM & Hille B (1972). The inner quaternary ammonium ion receptor in potassium channels of the node of Ranvier. *J Gen Physiol* **59**, 388–400.

del Camino D & Yellen G (2001). Tight steric closure at the intracellular activation gate of a voltage-gated K^+ channel. *Neuron* **32**, 649–656.

Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT & MacKinnon R (1998). The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science* **280**, 69–77.

Hite RK, Yuan P, Li Z, Hsuing Y, Walz T & MacKinnon R (2015). Cryo-electron microscopy structure of the Slo2.2 Na^+-activated K^+ channel. *Nature* **527**, 198–203.

Holmgren M, Smith PL & Yellen G (1997). Trapping of organic blockers by closing of voltage-dependent K^+ channels: evidence for a trap door mechanism of activation gating. *J Gen Physiol* **109**, 527–535.

Giese MH, Gardner A, Hansen A & Sanguinetti MC (2017). Molecular mechanisms of Slo2 K^+ channel closure. *J Physiol* **595**, 2321–2336.

Jiang Y, Lee A, Chen J, Cadene M, Chait BT & MacKinnon R (2002). Crystal structure and mechanism of a calcium-gated potassium channel. *Nature* **417**, 515–522.

Latorre R & Brauchi S (2006). Large conductance Ca^{2+}-activated K^+ (BK) channel: activation by Ca^{2+} and voltage. *Biol Res* **39**, 385–401.

Li W & Aldrich RW (2004). Unique inner pore properties of BK channels revealed by quaternary ammonium block. *J Gen Physiol* **124**, 43–57.

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Li W & Aldrich RW (2006). State-dependent block of BK channels by synthesized Shaker ball peptides. *J Gen Physiol* **128**, 423–441.

Liu Y, Holmgren M, Jurman ME & Yellen G (1997). Gated access to the pore of a voltage-dependent K^+ channel. *Neuron* **19**, 175–184.

Rothberg BS & Magleby KL (2000). Voltage and Ca^{2+} activation of single large-conductance Ca^{2+}-activated K^+ channels described by a two-tiered allosteric gating mechanism. *J Gen Physiol* **116**, 75–99.

Rothberg BS, Shin KS, Phale PS & Yellen G (2002). Voltage-controlled gating at the intracellular entrance to a hyperpolarization-activated cation channel. *J Gen Physiol* **119**, 83–91.

Santi CM, Ferreira G, Yang B, Gazula VR, Butler A, Wei A, Kaczmarek LK & Salkoff L (2006). Opposite regulation of Slick and Slack K^+ channels by neuromodulators. *J Neurosci* **26**, 5059–5068.

Shin KS, Rothberg BS & Yellen G (2001). Blocker state dependence and trapping in hyperpolarization-activated cation channels: evidence for an intracellular activation gate. *J Gen Physiol* **117**, 91–101.

Smith FJ, Pau VP, Cingolani G & Rothberg BS (2013). Structural basis of allosteric interactions among Ca^{2+}-binding sites in a K^+ channel RCK domain. *Nat Commun* **4**, 2621.

Wilkens CM & Aldrich RW (2006). State-independent block of BK channels by an intracellular quaternary ammonium. *J Gen Physiol* **128**, 347–364.

Yuan A, Dourado M, Butler A, Walton N, Wei A & Salkoff L (2000). SLO-2, a K^+ channel with an unusual Cl^- dependence. *Nat Neurosci* **3**, 771–779.

Yuan A, Santi CM, Wei A, Wang ZW, Pollak K, Nonet M, Kaczmarek L, Crowder CM & Salkoff L (2003). The sodium-activated potassium channel is encoded by a member of the *Slo* gene family. *Neuron* **37**, 765–773.

Additional information

Competing interests

None declared.

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