

Roberts, E.B., Meredith, M.A., and Ramoa, A.S. (1998). *J. Neurophysiol.* *80*, 1021–1032.

Roberts, E.B., and Ramoa, A.S. (1999). *J. Neurophysiol.* *81*, 2587–2591.

Scheetz, A.J., and Constantine-Paton, M. (1994). *Faseb J.* *8*, 745–752.

Seeburg, P.H. (1993). *Trends Neurosci.* *16*, 359–365.

## Potassium Channel Mechanics

**What is the moving part that switches an ion channel's current on and off? In this issue of *Neuron*, del Camino and Yellen (2001) exploit scanning cysteine mutagenesis and sulfhydryl reagents to show that the intracellular end of the S6 helices forms a mechanical gate for the Shaker potassium channel.**

Ion channels are famous for doing two things very well. They can be very discriminating about the ion species they rapidly conduct across the membrane; they can also open and close according to very specific stimuli. Now that we've seen the beautiful pictures of ions in the multiple binding sites of the KcsA channel (Zhou et al., 2001a), we have the impression that the ion selectivity in potassium channels is basically understood and it will be only a matter of time until the ion transport process is worked out in full thermodynamic detail (see, for example, Bernèche and Roux, 2001). The story is not as far along for the gating process, although the paper by del Camino et al. (2001) in this issue of *Neuron* lays to rest some old, nagging questions about the nature of the gate itself.

The problem with understanding the gating process is that the KcsA crystal structure is a picture of a channel frozen in one state, and we aren't entirely sure which state it's in: is it closed, open, or somewhere in between? To really know what a gate is, we need to see it move; until we have structures of a channel in the open and shut states, we can't be sure which are the moving parts that make it work.

The first hint of the nature of the gate in a voltage-dependent channel was Clay Armstrong's 1966 (Armstrong, 1966) study of the squid axon potassium current. He microinjected tetraethylammonium ions (TEA) into the axoplasm, and saw two intriguing features of the resulting block of the channels. First, the TEA block develops on a millisecond time scale but only after the channels open, as if the closed gate hinders access of TEA to its binding site. Second, once bound, the TEA interferes with the closing of the gate. Specifically, the closing of the channels appeared to be delayed until TEA left its binding site. These results gave rise to the idea that the activation gate is on the intracellular face of the channel, while the TEA site is deeper inside, near the center of the membrane. In fact, later experiments showed that TEA can be trapped inside the channel when the gate is forced closed by a strong hyperpolarization, suggesting that it is like a trap door. This picture finally has been given rigorous confirmation.

More than thirty years later, people are again applying small molecules to the intracellular side of membranes and measuring ion channel currents in an experimental program to deduce the nature of the channel gate. Instead of perfused squid axons, inside-out patches from transfected cells are perfused with solution-switching systems with microsecond response times. The ligands are typically MTS reagents, sulfhydryl-reactive molecules that were developed by Arthur Karlin (Akabas et al., 1992) to map the solvent accessibility of cysteine residues. The strategy has been to perform a scanning mutagenesis, introducing individual cysteines up and down the  $\alpha$  helices lining the channel pore, and to see whether the modification rate is dependent on whether the channel is open or closed.

Recent cysteine accessibility work of this sort has been inspired by the KcsA channel structure. In that channel, the extracellularly disposed 1/3 of the permeation pathway consists of the selectivity filter, a very narrow region with four binding sites for potassium ions. Below this, near the center of the membrane, is a water-filled cavity that is now known to be the site for binding quaternary ammonium ions like TEA (Zhou et al., 2001). The intracellular end of the KcsA ion pathway becomes very narrow (remember, we might be looking at a closed channel structure!) as the four M2 helices come together like the posts of an inverted teepee, producing a narrow "smoke hole" at the bundle crossing. While spin-label studies began to suggest a rotation and spreading of the helices when the KcsA channel is opened (Perozo et al., 1999), investigators of cyclic-nucleotide-gated (CNG) channels and voltage-gated potassium channels have looked for changes in accessibility of residues in the S6 helices (the analogs of KcsA's M2 helices) above the bundle crossing.

For CNG channels, the surprising result has been that, in both the open and closed states, cysteines are accessible to internally applied MTS reagents all the way up the S6 helices to the selectivity filter region (Sun et al., 1996; Flynn and Zagotta, 2001). Although there is a movement of the helices, it appears that the channel gate is in the selectivity filter itself. The situation is quite different in the voltage-gated Shaker channel, where Gary Yellen's group has shown huge decreases in accessibility of MTS and other reagents to residues above the bundle crossing when the channel is closed (Liu et al., 1997). This result would seem to prove that the S6 bundle forms the Shaker gate, except for a few details.

It turns out that Shaker has not just one way of gating the flow of ions, but at least three. Shaker is a voltage-gated channel that opens an "activation gate" on depolarization—this is the sort of gate that Armstrong was studying. It also has a "fast" inactivation process, which results from a plugging of the pore from the intracellular end by the amino-terminal end of an extended peptide chain (Zhou et al., 2001b). It also has one or more "slow" inactivation processes (called "C-type" and "P-type" in the literature) which seem to involve a collapse of the selectivity filter region. If the selectivity filter is an inactivation gate, might it not participate in activation gating too?

There actually is evidence for a strong involvement of the selectivity filter in Shaker's activation gating process. First, several of the mutations that affect selectivity

of the channel were found also to have large effects on the open-closed equilibrium, stabilizing the open state of the channel and slowing the tail current time course (see, for example, Heginbotham et al., 1994). Second, a close examination of a single Shaker channel's behavior as it closes revealed that the ion selectivity changes as the channel passes briefly through subconductance levels on its way to being fully closed (Zheng and Sigworth, 1998). The easiest explanation for this result is that activation gating involves a closing down of the selectivity filter.

If part of the activation gate involves the selectivity filter, could it be that all of the important gating of potassium ions occurs there? Maybe the S6 bundle crossing is indeed a "gate," but a physiologically irrelevant one that is effective only for large ions like TEA or MTSET. To put to rest nagging questions like this, one needs to test the accessibility of a small probe the size and valence of a potassium ion. Fortunately, there is an appropriate cysteine-reactive probe, which is the silver ion. Surmounting a daunting array of technical difficulties, del Camino and Yellen now show that the accessibility of a cysteine at residue 474 of Shaker, lying just above the bundle crossing, is decreased 700-fold when the channel closes. It is difficult to imagine more definitive proof for the existence of an intracellular gate in this location that is responsible for controlling potassium flux.

Del Camino and Yellen pose, and answer, an additional question about the nature of the S6 gate. Might it be that the channel gate works like a field-effect transistor, using electrostatic potentials rather than steric hindrance to switch the cation flux on and off? The answer is no because negatively charged and neutral MTS reagents show the same sort of gated access to residue 474 as the positively charged MTSET.

The conclusion is that Armstrong's picture is correct: there is a physical gate at the intracellular end of the channel. What its exact structure is unclear, but it probably works more like an iris diaphragm than like a trap door. The motions of the S6 helices that open and close this gate are likely also to influence the selectivity filter, where an additional restriction on ion permeation perhaps occurs.

It is a good thing, too, that voltage-gated channels have a gate that closes tightly. Paralysis and myotonias arise from subtle defects in the voltage-gated sodium channels of muscle, where small amounts of leakage are seen to cause big problems (Cannon, 2000). So far, these defects seem all to arise from the "chattering" of gates rather than from their incomplete closure, but incompletely sealed gates would certainly produce similar disorders. The S6 helices of sodium channels are quite different from those of Shaker (they don't have the proline motif that, in Shaker, is suspected to produce a kink near the gate region), but this difference probably just makes them interesting targets for future cysteine accessibility studies.

#### Fred J. Sigworth

Department of Cellular and Molecular Physiology  
Yale University School of Medicine  
New Haven, Connecticut 06520

#### Selected Reading

- Akabas, M.H., Stauffer, D.A., Xu, M., and Karlin, A. (1992). *Science* 258, 307–310.
- Armstrong, C.M. (1966). *J. Gen. Physiol.* 50, 491–503.
- Bernèche, S., and Roux, B. (2001). *Nature* 414, 73–77.
- Cannon, S.C. (2000). *Kidney Int.* 57, 772–779.
- del Camino, D., and Yellen, G. (2001). *Neuron* 32, this issue, 649–656.
- Flynn, G.E., and Zagotta, W.N. (2001). *Neuron* 30, 689–698.
- Heginbotham, L., Lu, Z., Abramson, T., and MacKinnon, R. (1994). *Biophys. J.* 66, 1061–1067.
- Liu, Y., Holmgren, M., Jurman, M.E., and Yellen, G. (1997). *Neuron* 19, 175–184.
- Perozo, E., Cortes, D.M., and Cuello, L.G. (1999). *Science* 285, 73–78.
- Sun, Z.P., Akabas, M.H., Goulding, E.H., Karlin, A., and Siegelbaum, S.A. (1996). *Neuron* 16, 141–149.
- Zheng, J., and Sigworth, F.J. (1998). *J. Gen. Physiol.* 112, 457–474.
- Zhou, Y., Morais-Cabral, J.H., Kaufman, A., and MacKinnon, R. (2001a). *Nature* 414, 43–48.
- Zhou, M., Morais-Cabral, J.H., Mann, S., and MacKinnon, R. (2001b). *Nature* 411, 657–661.