**Bend to Open?**

In this issue of *Structure*, Ludtke et al. (2005) report the 9.6 Å structure of the ryanodine receptor (RyR1) closed state. The structure shows a bent inner helix and raises the question of whether inner helix deformation is really a conserved channel gating mechanism.

How do channels open? This simple question has propelled experimental investigation into ion channel function for decades. Ion channel proteins span plasma membranes and intracellular membranes in all forms of life (Hille, 2001). Their principal function is to provide a gated passageway for charged atoms to cross the membrane. This regulated ionic flow underlies diverse processes that include sensation, muscle contraction, and cognition. Ion channel proteins are diverse. Some, such as potassium channels, have exquisite ion selectivity while others, such as the nicotinic acetylcholine receptor family, are more promiscuous in the types of ions they pass. The stimuli that move ion channels between the open and closed states also vary. Some channels are gated by a single stimulus such as ligand binding or a membrane voltage change. Others respond to polymodal inputs. In the face of this variety, the basic human urge to find common themes has led investigators to look for unifying principles that serve as a framework for understanding the conformational transitions that trigger the simple event of opening a hole for the ions to pass through.

Because they are membrane proteins and offer severe challenges to sample preparation, ion channels remain on the structural biology frontier. Landmark electron microscopy studies of ion channels isolated from native preparations gave the first pictures of gating (Unwin, 1995; Unwin and Ennis, 1984). Investigation of identical channels under closed and open conditions indicated that channel pores might work in an iris-like mechanism where conformational changes between subunits affected pore diameter changes.

Higher resolution pictures have come from studies of different heterologously expressed prokaryotic potassium channels (Jiang et al., 2002a, 2003; Kuo et al., 2003; Zhou et al., 2001). Comparison of the pore structures of a closed channel, KcsA (Zhou et al., 2001), and an open channel, MthK (Jiang et al., 2002a), together with the observation of a highly conserved pore-lining helix glycine originated the hypothesis that inner helix deformation at the conserved glycine might be a common mechanism for opening the constriction that blocks ions from passing through voltage-gated ion channel superfamily member pores (Jiang et al., 2002b). The current paper by Ludtke et al. (2005) provides an important reminder that we still have much to learn regarding this simple question of how channels open.

Ludtke et al. (2005) report the 9.6 Å resolution structure of the type 1 ryanodine receptor (RyR1) determined by single-particle electron cryomicroscopy (EM). RyRs are huge protein complexes (~2.3 MDa) that regulate calcium ion release from internal stores in response to gating cues from plasma membrane calcium channels. RyRs are the largest ion channel proteins known and are found in many cell types. Despite the size difference (~5000 amino acids for RyR subunits versus ~500 for typical voltage-gated potassium channel subunits), sequence similarities in the transmembrane region suggest that RyR pore-forming parts are related to the voltage-gated ion channel superfamily (Wang et al., 2005).

The resolution of the RyR1 structure is far from that required for side chain identification but is sufficient to permit the identification of some helical structures. This new study represents the first time single-particle analysis has achieved this level of detail for a membrane protein. Using an algorithm that locates helical secondary structures, the authors identify five rod-like sequences in the transmembrane region suggesting that RyR pore-forming parts are related to the voltage-gated ion channel superfamily. Helix 1 is bent and lines a large funnel-shaped cavity along the channel's central axis. Comparison of the arrangement of helices 1 and 2 with known channel structures shows a striking similarity to MthK. In contrast to MthK, an open channel with a bent inner helix, the RyR1 structure is that of a closed channel and raises the question of whether inner helix bending is essential for channel gating.

Interpreter bias can be problematic in intermediate resolution structures. The unbiased search methods Ludtke and colleagues implement are important new tools for discerning authentic structures in low-resolution electron density maps. Another recent RyR1 EM study at lower resolution suggested the diametrical conclusion that the closed RyR1 pore resembled the closed channel KcsA, where the inner helices are straight and form a constriction around the ion conduction path (Samso et al., 2005). However, in that analysis the authors searched with full channel models. Determination of the resolution of EM data is a thorny issue that can confuse the nonspecialist (for example, Samso et al. [2005] and Ludtke et al. [2005] report 10.3 and 9.6 Å resolutions, respectively, but estimate the limits by different methods). Applying the same criterion to both datasets shows that the new study is at a higher resolution (13.8 versus 9.6 Å, calculated using the method of Ludtke et al., [2005]; D.L.M.). The better data, unbiased identification of helical structures, and subsequent comparison to known structures lends confidence to the conclusions of the present report.
Does the inner helix really have to bend at a glycine for the channel to open, or are the bends under a different constraint? We have yet to see the high-resolution structure of the same channel in the open and closed states. Glycines in membrane proteins have another role distinct from the facilitation of a conformational change that kinks a formerly straight helix. Conserved glycines often permit close transmembrane helix packing (Curran and Engelman, 2003). In assessing glycine hinge models it might be important to consider that channel inner helices are not stand-alone structures but form close-packed interactions with surrounding channel parts. Thus, the consequences of gating-induced kinks would propagate beyond the inner helix internal structure and directly affect protein-protein contacts with neighboring channel structure. While mutagenesis can offer support for the importance of pore-lining helix glycines (Magidovich and Yifrach, 2004; Zhao et al., 2004), without direct structural data of mutant channels it cannot separate effects that enable bending from those that impact side chain packing.

Does one really have to bend the inner helix to open channel pores? We do yet not have a definitive answer. Ludtke and colleagues provide a new stimulus for pondering this question. In our quest to understand molecular mechanisms, we should keep in mind that Nature holds many secrets that await the light of experimental science and reason, and to draw a lesson from a classic piece of advice:

There are more things in heaven and earth, Horatio, Than are dreamt of in your philosophy.
—Hamlet 1:5

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