Commentary

Capturing Ion Channel Gating: A Little Salt on the Tail Does the Trick

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This is a golden age for ion channels: a time when chemistry, molecular biology, and electrophysiology have come together with structural biology to provide glimpses into some truly amazing membrane proteins. And yet, as usual, the answers yield more questions. Is a new structure the structure of an open channel or of a closed one? How does the voltage sensor actually move? Though providing amazing insights, each crystal structure is just a still life portrait of the ion channel–it doesn't breathe or flicker; it has lost the "soul" that was deduced by electrophysiology. In a real sense, therefore, new structures serve as a starting point for more functional studies, rather than as an end point. Indeed, in this issue, Craven and Zagotta (2004) use functional studies to interpret structural information and enhance our understanding of the gating mechanisms of cyclic nucleotide-regulated channels.

Craven and Zagotta chose to study the bovine retinal CNGA1 channel and the mouse HCN2 channel. CNGA1 is a cyclic nucleotide-gated channel that mediates the light response in retinal rod cells and has very little voltage dependence (for review see Kaupp and Seifert, 2002; Matulef and Zagotta, 2003). The channel is open in the dark, when cyclic GMP is high, and closes in the light, following hydrolysis of cyclic GMP via a G-protein cascade (for review see Pugh and Lamb, 2000; Roof and Makino, 2000; Burns and Baylor, 2001; Zimmerman, 2001). HCN2 is a pacemaker channel found in high abundance in the brain and heart (for review see Santoro and Tibbs, 1999; Accili et al., 2002; Biel et al., 2002; Robinson and Siegelbaum, 2003). This channel is mainly activated by hyperpolarizing voltages, giving rise to the "h-current" (variously called I_h, I_f, or I_q); the binding of cyclic nucleotides (usually cyclic AMP) shifts its voltage activation curve to less hyperpolarized potentials. Both channels exist as tetramers, and each channel has a cyclic nucleotide binding domain (CNBD) on the cytoplasmic COOH-terminal tail of each of its subunits.

The COOH-terminal tails are thought to be involved in intersubunit interactions within cyclic nucleotide-regulated channels. For example, the C-helix portion of the rod channel CNBD appears to form intersubunit disulfide bonds mainly in closed states of the channel, and the C-helices are thought to disconnect and separate during channel opening (Matulef and Zagotta, 2002; Mazzolini et al., 2002). The C-linker regions of neighboring subunits also have been found to form disulfide bonds (Rosenbaum and Gordon, 2002). Furthermore, modulation of the channel by transition metal divalent cations appears to involve coordination of the metal ions by histidines on adjacent subunits (for review see Matulef and Zagotta, 2003). Finally, inhibition of cyclic nucleotide-gated channels by Ca^{2+}/calmodulin may involve the disruption of intersubunit interactions between NH_2- and COOH-terminal domains (for review see Matulef and Zagotta, 2003).

In the last decade, it has become clear that within the COOH-terminal tails, the cytoplasmic C-linker regions (Fig. 1) are very important in transmitting the binding of cyclic nucleotides to the opening of the pore. This is not surprising, because the C-linker physically connects the CNBD to the last transmembrane segment (S6), which is thought to constitute part of the pore lining, and may even represent an intracellular gate in HCN channels (Shin et al., 2001; Rothberg et al., 2002). In rod and olfactory cyclic nucleotide-gated (CNG) channels, transition metal divalent cations can modulate channel gating by binding to histidines in the C-linker region near S6 (Ildefonse and Bennett, 1991; Karpen et al., 1993; Gordon and Zagotta, 1995). In particular, results with Ni^{2+} suggest specific translational and rotational movements of the C-linker in gating; residues closer to the CNBD also have been implicated in the allosteric transition (for review see Matulef and Zagotta, 2003).

Last year Zagotta et al. (2003) presented a crystal structure of the COOH-terminal tail of HCN2. Structural and biochemical studies revealed that the HCN2 fragments formed tetramers, connected mainly at their C-linker regions. This association between C-linkers involved hydrophobic interactions, hydrogen bonding, and salt bridges, and was described as "like an elbow resting on the shoulder of its neighbor." The results with COOH-terminal fragments were extrapolated to

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Abbreviations used in this paper: CNBD, cyclic nucleotide binding domain; CNG, cyclic nucleotide-gated.

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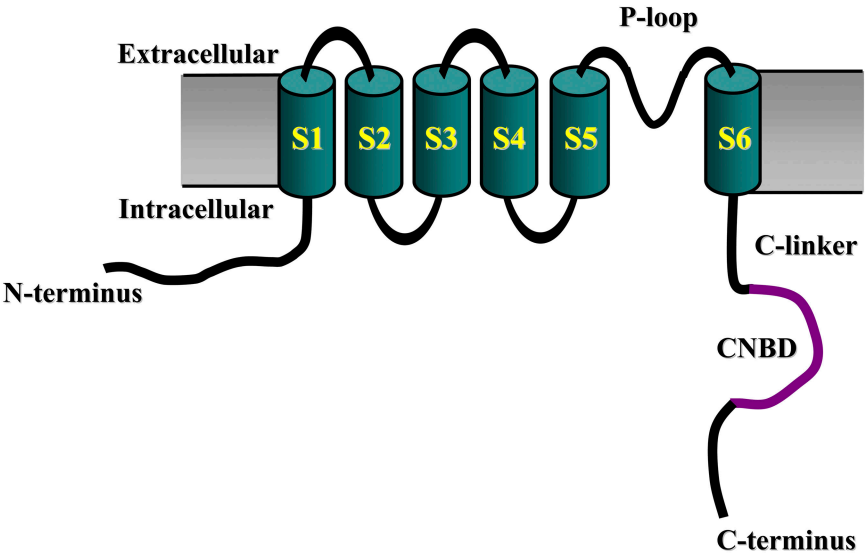

FIGURE 1. Expected subunit topology for a cyclic nucleotide-regulated channel. Cyclic nucleotide binding at CNBD promotes channel opening by disrupting salt bridges formed between the C-linker and CNBD, and between C-linkers of neighboring subunits.

predict similar intersubunit interactions in the intact tetrameric channel.

In the current paper, Craven and Zagotta (2004) use mutagenesis and patch clamp methods to investigate the role of the observed C-linker salt bridges in channel gating. They show that in functioning channels, salt bridges indeed form between C-linker regions of neighboring subunits, as well as between C-linker and CNBD regions within subunits. These salt bridge formations stabilize the closed conformation of the channel, and the binding of cyclic nucleotides disrupts the salt bridges, allowing channel opening. Moreover, the associations that connect subunits also control channel opening; perhaps this coupling can help explain the cooperativity of activation first observed in experiments with the native rod CNG channel (Zimmerman and Baylor, 1986).

A particularly interesting result of the functional studies is the realization that within the crystal structure, the state of activation of the C-linker did not match that of the CNBD. Within the crystallized fragment of HCN2, each CNBD contained bound cAMP and therefore was in the agonist-activated form. However, the C-linkers in the structure were connected by salt bridges, which, based on the functional studies, are characteristic of the closed channel. Thus, it would appear that the CNBD can be in the activated state at the same time the C-linker is in a resting, nonactivated state. This surprising arrangement may have occurred in the structure because the absence of the pore in the crystallized fragments may have stabilized the resting conformation of the C-linker. The ability of the two regions to be activated independently led Craven and Zagotta to suggest a gating model containing three modules, the CNBD, the C-linker, and the pore, and that each of these would have an activated and a nonactivated state. This model simulates the main features of their functional data. The underlying concept also makes sense if we consider what is always seen in single-channel recordings from agonist-activated channels: channels clearly open and close while the agonist remains bound. Indeed, modular models of channel gating have been used before (Horrigan and Aldrich, 2002). In this case, we have some structural data as further support.

Considering the Craven and Zagotta results in the context of previous work on CNG channel gating (for review see Kaupp and Seifert, 2002; Matulef and Zagotta, 2003), one obtains a sense of the structural movements that may occur during channel opening. The binding of cyclic nucleotide in the CNBD involves association of the ligand with the β roll of the CNBD, followed by a pivoting movement of the C-helix portion of the CNBD toward the β roll, enveloping the cyclic nucleotide. This process destabilizes the salt bridges (and probably other interactions) between the C-linker and the CNBD, as well as between the C-linkers of adjacent subunits. These movements then trigger twisting and/or bending of S6, and ultimately pore opening.

The Craven and Zagotta article is an exemplary illustration of how it is possible to derive a cohesive picture of an ion channel based on comparisons of structural and functional data, as has been the case for many years in more conventional enzymology. Similar efforts are underway for other channels. But to what extent are these studies affected by the use of accessory molecules (e.g., antibody fragments) in the crystallizations (Jiang et al., 2003a,b), and by the fact that the channels crystallized to date have been from bacteria, while the functional studies are on eukaryotic channels? Moreover, we are not even close to considering the effects of differences in molecular (e.g., lipid) environment on

the channel structures. Nevertheless, rapid progress is being made, and this is a tremendously exciting time. The crystal structure of a bacterial ClC chloride "channel" (Dutzler et al., 2002) inspired a functional study that determined that the bacterial protein was actually an H^+/Cl^- exchange transporter, rather than a Cl^- channel like the eukaryotic versions of previous electrophysiological studies (Accardi and Miller, 2004). A large body of functional work has been inspired by a proposed structural model for movement of the voltage sensor (S4) in K^+ channels (Jiang et al., 2003a,b; for review see Blaustein and Miller, 2004). Also, although there is widespread acceptance of the KcsA and MthK channels as models of closed and open K^+ channels, respectively (Jiang et al., 2002; for review see Schumacher and Adelman, 2002), these assignments are just assumptions based on two different channels. Clearly, it will be important to have undisputable closed and open structures of the same channel type. In this context, however, it will be important to heed the lessons drawn by the Craven and Zagotta work, that just forming crystals under conditions that are supposed to promote closure or opening of a channel may not necessarily yield the expected structures. Thus, the best path is likely to continue to be the current one, jogging back and forth between structure and function.

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