Commentary

Ion Channel Structure and the Promise of Bacteria: Cyclic Nucleotide-Gated Channels in the Queue

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To date it has proved very difficult to express eukaryotic channels in Escherichia coli or otherwise obtain enough of these proteins for crystallization. So, unbeknownst to bacteria, they have been making a major contribution to ion channel research since 1998. So much so that everyone in the ion channel community eagerly anticipates the next promising bacterial channel and the big payoff that comes from the elucidation of its three-dimensional structure. This golden age of bacterial channels started when Rod MacKinnon and his colleagues crystallized and determined the structure of the KcsA K+$ channel in 1998, giving us the first high-resolution picture of an ion-selective channel (Doyle et al., 1998; Morais-Cabral et al., 2001; Zhou et al., 2001). KcsA is a member of a superfamily of tetrameric cation channels that includes K$+, Na$+, and Ca$+2 channels, as well as cyclic nucleotide-gated (CNG) channels, hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels, and glutamate receptors. All of these channels contain four subunits or domains that surround a central ion-conducting pore (Karpen and Ruiz, 2002). Each subunit of KcsA contains two transmembrane segments and a reentrant loop in between that comprises the outer pore region, including the K$+ selectivity filter. The other members of the superfamily appear to have this basic pore architecture, but most of these channels are more complex, containing one or more sensor regions that control the opening and closing of the pore. There are two general types of sensors: integral membrane regions that respond to changes in membrane potential, and regions near the membrane surface that bind ligands. Both types of sensors are linked to the pore and undergo conformational changes in response to their specific stimuli. These conformational changes move a gate that opens or closes the pore.

In the last couple of years the structures of two bacterial K$+ channels with sensor regions have been solved, providing high-resolution glimpses into the structural bases of channel gating. MthK is opened by the binding of intracellular Ca$+2 (Jiang et al., 2002), and KvAP is opened by membrane depolarization (Jiang et al., 2003). MthK resembles KcsA with two transmembrane segments and a pore loop in each subunit, but MthK contains a much longer cytoplasmic COOH-terminal region that confers Ca$+2 sensitivity. Ca$+2 binds to each COOH-terminal region in a cleft between two RCK (regulator of K$+ conductance) domains. The eight RCK domains in the four subunits form a gating ring at the intracellular membrane surface. Although much remains to be learned about the details of the gating motions, the available evidence suggests that binding of Ca$+2 increases the diameter of the gating ring, which in turn pulls apart the inner helices of the pore to cause opening. In contrast, each KvAP subunit contains six transmembrane segments, S1–S6. The structure of the S5-P-S6 module is nearly identical to the entire KcsA subunit, while the first four transmembrane segments contain the voltage-gating apparatus. The most conspicuous segment is S4, which contains a series of positively charged residues, and which moves in response to changes in membrane potential. Again, the picture is far from complete in this case, but there is general agreement that the displacement of S4 also exerts force on the inner pore helices to open the channel.

In the absence of a complete three-dimensional structure, less is known about the mechanisms of cyclic nucleotide gating in CNG and HCN channels. Both types of channels rapidly link changes in cyclic nucleotide levels to changes in membrane potential. CNG channels are nonselective cation channels that open in response to the direct binding of cGMP or cAMP (Kaupp and Seifert, 2002; Matulef and Zagotta, 2003). They play a central role in the transduction of sensory stimuli. In retinal rods and cones, a light-induced reduction in cGMP concentration causes the closure of channels and a membrane hyperpolarization. In olfactory receptor neurons, an odorant-induced increase in cAMP...
concentration causes the opening of channels and a membrane depolarization. CNG channels have been detected in many other cell types, where their specific roles remain to be determined. HCN channels are cation channels that are selective for K⁺ over Na⁺ by about a factor of four. Unlike CNG channels, they are primarily gated by membrane potential, opening in response to hyperpolarization. The voltage dependence can be modulated by cyclic nucleotide binding (Robinson and Siegelbaum, 2003; Baruscotti and DiFrancesco, 2004). HCN channels have several different functions in the brain and heart: regulating pacemaker activity or rhythmic firing, controlling the resting membrane potential, and altering membrane resistance. Neurotransmitters are known to regulate HCN channels through changes in cyclic nucleotide levels; the best known case is the acceleration of heart rate by β-adrenergic agonists, which cause a rise in cAMP. The molecular functions of CNG and HCN channels have received considerable scrutiny, and some of the basic structural units have been identified in site-directed mutagenesis experiments (Kaupp and Seifert, 2002; Matulef and Zagotta, 2003; Robinson and Siegelbaum, 2003; Baruscotti and DiFrancesco, 2004; Rosenbaum and Gordon, 2004). Last year, Zagotta et al. (2003) solved the structure of a fragment of HCN2, the COOH-terminal cytoplasmic domain bound to cAMP or cGMP. While this was a promising beginning, investigators in this field have still felt somewhat left out of the parade of new bacterial channels, considering the invaluable information that comes from a single complete structure within a channel family.

In this issue, Nimigean et al. (2004) give aficionados of these channels something to look forward to. They report the intriguing discovery of a cyclic nucleotide-modulated K⁺ channel, MloK1, from the bacterium *Mesorhizobium loti*, a plant symbiont. Based on sequence comparisons, MloK1 appears to have most of the modules characteristic of eukaryotic CNG and HCN channels: six putative transmembrane segments (S1–S6), a pore segment between S5 and S6 that contains a K⁺ selectivity sequence, and a COOH-terminal cyclic nucleotide-binding domain. The channels exhibit several functional properties that are consistent with these sequence assignments. The MloK1 protein expresses well in *E. coli*, and it was purified in large quantity. On a gel filtration column the purified protein displays a range of molecular sizes, including aggregates in the void volume. However, if the entire purification is done with cyclic nucleotide present, the protein elutes as a homogeneous peak at the expected size for a tetrameric complex. Cyclic nucleotides appear to ensure proper folding and favor assembly of the channel tetramer. A similar observation was made by Zagotta et al. (2003) in sedimentation equilibrium experiments on the HCN2 COOH-terminal fragment, which runs as an apparent monomer in the absence of cAMP, and as a mixture of monomers and tetramers with cAMP bound. After purification, MloK1 was reconstituted into liposomes. Using ⁸⁶Rb⁺ as a tracer and ionic gradients to electrically polarize the liposomes, Nimigean et al. (2004) make a strong case that the protein behaves as an ion channel rather than a transporter, and that the channel is selective for K⁺ and Rb⁺ over Na⁺ and Li⁺. They show that ⁸⁶Rb⁺ uptake is stimulated by both cAMP and cGMP in a concentration-dependent manner. Both nucleotides exhibit a high apparent affinity, with cAMP (K_{1/2} = 60 nM) more potent than cGMP (K_{1/2} = 600 nM). The concentration dependence of activation for each nucleotide is consistent with a single binding event causing significant opening (Hill coefficient of 1). It is noteworthy that there is substantial basal influx in the absence of cyclic nucleotides, dependent on the presence of the MloK1 protein. This could reflect a voltage dependence of activation, which is difficult to study in a systematic way in liposomes. Unfortunately, attempts to measure currents in planar lipid bilayers so far have been unsuccessful. Trying to predict the voltage dependence of channel gating from an inspection of the S4 sequence is simply not possible given our current level of understanding. MloK1 contains less net positive charge in this region (Fig. 1 of Nimigean et al., 2004) than voltage-gated channels, HCN channels, or even CNG channels, which exhibit little or no voltage dependence. However, HCN channels have S4 segments that are very similar to those of voltage-gated K⁺ channels, and yet the two channels open at completely opposite membrane potentials (Mannikko et al., 2002).

Without a doubt the most striking observation about MloK1 in the paper by Nimigean et al. (2004) is that it contains an extremely short linker between the cyclic nucleotide-binding domain and the transmembrane core. In CNG and HCN channels, the so-called C-linker sequence is ~80 amino acid residues, while in MloK1 it is only 13 residues. The C-linker has received quite a bit of attention as the region that translates the energy from cyclic nucleotide binding into mechanical gate movements in S6 (Kaupp and Seifert, 2002; Matulef and Zagotta, 2003; Rosenbaum and Gordon, 2004). Residues in this region that have been shown to affect the allosteric opening transition span just about the entire 80–amino acid length in CNG channels (Gordon and Zagotta, 1995; Brown et al., 1998; Zong et al., 1998; Paololetti et al., 1999; Johnson and Zagotta, 2001). In the tetrameric structure that forms from the HCN2 COOH-terminal fragment, the C-linker region consists of six α-helices (Zagotta et al., 2003). Thus, it is surprising to find that a 13–amino acid sequence can suffice for the important operation of opening the channel. Although the structures may not be comparable, it is interesting
to note that the linkers connecting the cytoplasmic RCK domain to S6 in both MthK channels (Jiang et al., 2002) and eukaryotic Ca\(^{2+}\)-activated K\(^+\) (BK) channels (Niu et al., 2004) are also short (~17 residues). Furthermore, small changes in the length of this segment have rather large effects on the probability of opening; an e-fold decrease in \(P_o\) was observed for every 1.5-amino acid increase in linker length (Niu et al., 2004). In this case, the short linker segment exhibits the behavior of a passive spring. Clues to the function of the longer C-linker in HCN channels come from the structure of the HCN2 COOH-terminal fragment (Zagotta et al., 2003). The C-linker region from each subunit interacts strongly with its neighbors in the tetrameric complex. In fact, the vast majority of intersubunit contacts in the COOH-terminal fragment occur between the C-linkers regions and not the cyclic nucleotide-binding domains. Evidence from another study indicates that all of the C-linkers in a tetrameric channel are required for cAMP modulation (Ulens and Siegelbaum, 2003). Thus, these regions in the eukaryotic channels may play important roles in subunit assembly, cooperativity of activation, and perhaps regulation by other proteins.

At this point, we hope that high-resolution structural information and more functional information will follow from this important discovery of a bacterial cyclic nucleotide-modulated channel. Several attributes suggest that MloK1 is a homologue of HCN channels and not CNG channels: sensitivity to nucleotides in the submicromolar range, 10-fold higher apparent affinity for cAMP over cGMP, and K\(^+\)-selective ion conduction with the characteristic GYG sequence in the selectivity filter. If this channel does turn out to be HCN-like in most respects, high-resolution structures should ultimately shed light on why HCN channels are much less selective for K\(^+\) than are other K\(^+\) channels, how hyperpolarization activates the channel (a polarity opposite from other voltage-gated K\(^+\) channels), and how voltage activation and cyclic nucleotide modulation interact at the level of the pore gate.

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REFERENCES
