Cyclic Nucleotide-Regulated Ion Channels: Spotlight on Symmetry

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DOI 10.1016/j.str.2007.08.004

In this issue of *Structure*, Chiu et al. (2007) report the 16 Å EM structure of the prokaryotic cyclic nucleotide-regulated K^+ channel MloK1. This structure reveals that the channel is arranged as a four-fold symmetric tetramer.

The energy of ligand binding can be transmitted tens of angstroms through the structure of a protein. Perhaps nowhere is this better illustrated than in ligand-gated ion channels—proteins that facilitate the flow of charged atoms across biological membranes. The fact that many channels are oligomeric and therefore contain multiple ligand-binding sites adds to the complexity of their regulation. The ease of recording the submillisecond function of ion channels by measuring their ionic currents has provided an unmatched view of the behavior of these enzymes. A complete understanding of channel mechanics, however, also requires knowledge of the channel's atomic structure and how this structure rearranges upon ligand binding. This gap in understanding has pushed ion channel research into the arena of structure. High resolution structures of channels (and membrane proteins in general) have come mainly from prokaryotic sources, owing to the relative ease of their preparation in comparison to their eukaryotic counterparts. The paper by Chiu et al. (2007) in this issue shows that, once again, structural studies of prokaryotic channels afford a rare look into the atomic underpinnings of ion channel function, moving us ever closer to understanding how these molecular machines work.

Chiu et al. (2007) present the 16 Å resolution electron microscopy (EM) structure of the full-length prokaryotic cyclic nucleotide-regulated K^+ channel MloK1—a relative of eukaryotic cyclic nucleotide-regulated channels such as CNG and HCN channels and a member of the six transmembrane segment K^+ channel family (Clayton et al., 2004; Nimigean et al., 2004). In cyclic nucleotide-regulated channels, cyclic nucleotides (cAMP or cGMP) bind to a carboxyl-terminal ligand-binding domain producing an increase in the open probability of the ion-conducting pore, a process referred to as gating (Craven and Zagotta, 2006). While the overall resolution of the structure was not high, the authors were able to fit into their EM densities the 1.7 Å resolution structure of the isolated ligand-binding domain from MloK1, as well as the 2.9 Å structure of the transmembrane domains from the K^+ channel Kv1.2, previously solved by X-ray crystallography (Clayton et al., 2004; Long et al., 2005). The authors demonstrate for the first time that in full-length cyclic nucleotide-regulated channels, the cytoplasmic ligand-binding domains are organized as a four-fold symmetric tetramer (Figure 1). The ligand-binding domains are positioned below the transmembrane domains as independent noninteracting units, like four hanging lanterns. Surprisingly, these data diverge from previous models of MloK1, where the ligand-binding domains were proposed to be organized as a dimer-of-dimers (Clayton et al., 2004). In light of this new structure, those models must now be re-examined.

While the tetrameric organization of Mlok1 was a surprise, a similar symmetry has been seen for the related channel HCN2. The crystal structure of a carboxyl-terminal fragment of HCN2 also displays a four-fold symmetry (Zagotta et al., 2003). As in MloK1, the ligand-binding domains in HCN2 are independent noninteracting units, hanging below the membrane (Figure 1). In HCN2, however, the six helix C-linker, a region of the channel not present in Mlok1, intervenes between the ligand-binding domains and the transmembrane domains and forms virtually all of the intersubunit contacts in the carboxyl-terminal region (Figure 1). It seems that even without a C-linker, MloK1 can assemble into a tetramer and exhibit cyclic nucleotide-regulated channel gating.

The independent arrangement of the ligand-binding domains in MloK1 is nicely consistent with two recent reports showing that the binding of cyclic nucleotides in MloK1 is independent, not cooperative (Cukkemane et al., 2007; Nimigean and Pagel, 2007). However, since the binding of ligand to each subunit likely promotes a concerted conformational change in the pore, some binding cooperativity could have occurred even without direct interactions between ligand-binding domains if the coupling was strong and the opening transition was favorable. CNG channels exhibit such cooperativity probably, in part, due to a concerted transition in their C-linkers (Biskup et al., 2007).

How can the four-fold symmetry revealed by the EM structure of intact channels be reconciled with the dimeric arrangement seen in the X-ray structure of the isolated ligand-binding domains? Because both the EM and X-ray structures were solved in the presence of ligand (cAMP), it is unlikely that the different symmetries merely reflect two different functional states of the channel—a model proposed to

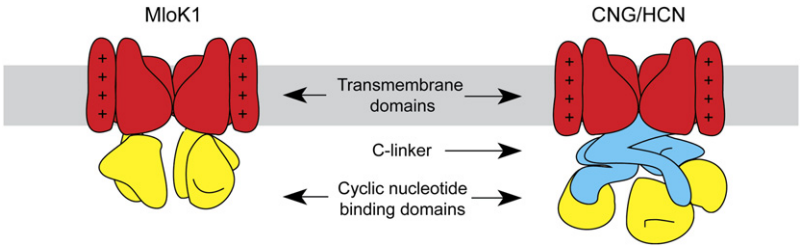

Figure 1. Domain Architectures of Prokaryotic (Left) and Eukaryotic (Right) Cyclic Nucleotide-Regulated Ion Channels

fit the activation of eukaryotic cyclic nucleotide-regulated channels (Ulens and Siegelbaum, 2003). Instead, the difference likely stems from the fact that the X-ray structure was solved from an isolated fragment of the channel, a fragment that likely formed a nonphysiological arrangement in the crystal.

Determining the biological unit—the physiological arrangement of subunits—in a crystal is not a trivial matter. There is no a priori way of knowing which intermolecular contacts are physiological and which are a product of crystal formation. This is particularly problematic for protein fragments. In MloK1, it seems that, without a C-linker, the transmembrane domains are necessary to ensure the correct physiological arrangement of the subunits.

Over and above the results, the work by Chiu et al. (2007) demonstrates the power of EM to solve intractable structural problems. EM studies can help elucidate the structures of full-length proteins and protein complexes that have been difficult to solve with X-ray crystallography. EM also can capture proteins in different conformational states, which opens the window for studies on dynamic structural rearrangements—arguably the future of biochemistry. Improvements in EM will lead only to more beautiful structures. And joined with X-ray crystallography, we should expect new and tantalizing insights into the structures of notoriously difficult proteins. A combination of approaches always leads to a more robust understanding of the microscopic world.

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How to Get All "A"s in Polyadenylation

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DOI 10.1016/j.str.2007.08.002

In an elegant study in this issue of *Structure*, Balbo and Bohm (2007) report the crystal structure of yeast poly(A) polymerase in a ternary complex with its substrate MgATP and the elongating poly(A) tail, providing molecular insights into the mechanism of polyadenylation.

Most eukaryotic mRNA precursors (pre-mRNAs) must undergo extensive processing before they can be exported from the nucleus to the cytoplasm and translated into proteins. At the 3′ end, the pre-mRNA is cleaved at a specific location and a polyadenylate tail (poly(A) tail) of about 200–300 nucleotides is added. A large complex of more than 15 proteins is required for this 3′-end processing. It has been known for over thirty years that poly(A) polymerase (PAP, Pap1p in yeast) catalyzes the addition of the poly(A) tail (Edmonds, 2002). PAP belongs to the DNA polymerase β superfamily of enzymes, but does not require a template.

Earlier structural studies of yeast and mammalian PAP free enzyme and complex with MgATP and dATP show that the enzyme contains three domains: N-terminal domain, middle domain, and C-terminal domain