COMMENTARY

Dynamic measurements for funny channels

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Proteins possess the uncanny ability to telegraph signals over long distances. The presence of a ligand, snugly bound to a site on one end of a molecule, can be "felt" by an effector domain several nanometers away. Determining precisely how this task is accomplished is a major challenge for structural biologists. Hypotheses range from the purely dynamic—for example, a binding event affects the conformational possibilities of nearby domains—to the mechanical view whereby a series of structural changes propagates like dominoes from one domain to the next. In PNAS, Saponaro et al. use NMR spectroscopy to examine the first steps in the process that transduces ligand binding into opening of a hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channel and present a structural basis for the inhibition of HCN by the accessory protein TRIP8b (tetratricopeptide repeat-containing Rab8b-interacting protein) (1).

Funny Channels

HCN channels are responsible for the cationic "funny current" (I_f) that controls pacemaking in the heart and are involved in numerous neuronal properties related to repetitive firing, resting membrane potential, input resistance, and dendritic integration (2). The channels belong to the voltage-gated K^+ channel superfamily, and like other members of this family are tetramers, with each transmembrane subunit containing a voltage sensor domain coupled to a pore domain (Fig. 1A). However, in HCN the voltage sensors are hooked up backwards, such that the channels are activated by hyperpolarization rather than depolarization

(2). At their carboxyl termini, HCN channels have adopted a regulatory cyclic nucleotide-binding domain (CNBD) that is found in enzymes and transcription factors from bacteria to humans. Binding of cAMP to this domain promotes channel opening by shifting the voltage dependence of activation to more positive potentials and increasing the maximal current (2).

The crystal structure of the carboxyl terminal region of HCN2 was solved in the presence of cAMP (Fig. 1B) (3). The CNBD is composed of an eight-stranded β-roll, flanked on the N-terminal side by the A-helix and followed by two helices (the B- and C-helices). cAMP binds to a pocket in the β-roll, making contact with a short helix (the P-helix) between strands β6 and β7 and with the C-helix. A helical domain called the C-linker (CL) connects the CNBD to the channel pore. This domain, composed of helices A′–F′, forms extensive contacts between subunits, arranging them in a cytoplasmic gating ring just below the channel pore.

Dynamic Measurements Needed

How does cAMP binding affect the open probability of a pore that is 5-nm away from the CNBD? Attempts to answer this question by solving the crystal structure of the HCN2 C-terminal region in the absence of cAMP produced results that were largely unsatisfying (4). The CL was in the same conformation in the presence and absence of cAMP. In fact, the only differences were in the F′ helix, which adopted a looser coiled conformation and the end of the C-helix, which was absent in the apo structure, indicating a lack of

structure or the ability of the C-helix to access multiple conformations in the absence of ligand. The similarity between the two crystal structures was in contrast to an accumulation of biochemical and electrophysiological evidence that predicted substantial rearrangements of the C-helix and C-linker (2, 5, 6). Therefore, the apo crystal structure was largely dismissed as the victim of crystal packing interactions at the C-helix and rogue Br^- ions bound in place of cAMP.

Saponaro et al. used NMR spectroscopy to solve the solution structure of an HCN2 construct that contains a portion of the CL (helices D′–F′) and the CNBD in the absence of cyclic nucleotides (1). Free from the constraints of the crystal lattice, this structure confirms many of the protein movements expected from years of careful biochemistry and physiology and includes several new features. Strikingly, the apo structure reveals not only rigid body movements of the helical subdomains, but also the stabilization of several of these helices (F′-, P-, and C-helices) by cAMP binding.

In the absence of cAMP, the B- and C-helices are positioned away from the β-roll, allowing for access of ligand to the binding site (Fig. 1C). The P-helix is absent from the apo structure, instead adopting a flexible loop conformation. When bound to ligand, this loop forms a compact, helical structure, making room for the B-helix to swing toward the β-roll, bringing the C-helix along with it. The C-helix forms extensive contacts with cAMP, and the distal end is stabilized from a random coil to a helical conformation. This movement of the C-helix displaces the N-terminal helical bundle (D′–E′ helices) away from the β-roll. The E′ helix moves by more than 5 Å, and the loop between the E′- and A-helices is stabilized to form the F′-helix.

TRIP8b Affects cAMP Action Allosterically

TRIP8b is a brain-specific β-subunit of HCN channels (Fig. 1A) (7). It assembles with
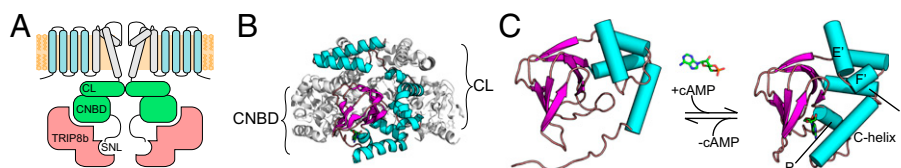

Fig. 1. (*A*) Schematic structure showing the putative transmembrane topology of HCN channels and the organization of the cytoplasmic C-terminal domains. The pore domain is gray and the voltage sensor is cyan. Two of four subunits shown for clarity. (*B*) Crystal structure of the tetrameric C-terminal region of HCN2 (PDB ID code 1Q5O). (*C*) Model of the conformational change in the C-terminal region of HCN2 induced by binding of cAMP.

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HCN in a 1:1 stoichiometry and has profound effects on channel trafficking and gating (8–10). The C-terminal tetratricopeptide repeat (TPR) domain of TRIP8b interacts with the final three amino acids of HCN (SNL in HCN1, -2, and -4; ANM in HCN3) and this interaction affects channel trafficking (9, 11, 12). A core domain of TRIP8b upstream of the TPR domain interacts with the CNBD of HCN, affecting trafficking, but also interfering with the ability of cAMP to activate the channel (11–13). Although the details of the TPR interaction with the distal C terminus of HCN are known at the atomic level, the nature of the interaction between the TRIP8b core domain and the CNBD has not been determined previously (8). It was initially proposed that the core domain inhibited cAMP binding through a direct interaction with the cAMP binding site, but more recent work suggests that both cAMP and TRIP8b can bind simultaneously and TRIP8b exerts its effect allosterically by stabilizing the channel in a closed conformation (11, 13). Saponaro et al. examined the interaction between HCN2 and TRIP8b directly by titrating in TRIP8b and looking for effects on the NMR spectra to map the residues involved in binding (1). The interacting residues on HCN2 formed a continuous surface comprising the loop between the E′- and A-helices and the C-helix. Thus, TRIP8b does not appear to directly compete for cAMP binding, but likely stabilizes these residues, which are known to be involved in the gating conformation transition, in a state that resembles the apo state.

An Emerging Consensus

The model presented here of the conformational change in the C-terminal region of HCN2 is representative of an emerging structural consensus on HCN gating. Transition metal ion FRET was previously used to show that the C-helix undergoes a large translation toward the β-roll subsequent to ligand binding and also suggested that binding of cAMP stabilized the secondary structure of the C-helix (4, 14). Double electron–electron resonance (DEER) and continuous-wave EPR also confirm the cAMP-induced motions of the C-helix and suggest that the distal C-helix is more conformationally heterogeneous when not bound to agonist (15). Finally, a model of the HCN4 C-terminal region in the absence of agonist was recently obtained using homology restraints along with chemical shifts and residual dipolar couplings obtained from NMR (16). This model is consistent with the major findings of Saponaro et al. (1), with cAMP binding causing a movement of the B- and C-helices toward the β-roll, accompanied by a movement of the N-terminal helical bundle away from the β-roll. Helical propensities calculated from chemical-shift data also suggest that binding

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of cAMP orders the P-helix and distal C-helix of HCN4.

Onward and Upward

How does the cAMP binding signal propagate through the CL to the channel pore? Little is known about the dynamics of the CL. It is not even clear whether its conformation in the crystal structures represents an activated or inhibited state (5, 6). To really understand the movements of the CL and its interaction with the pore domain, it will be necessary to examine the structure of full-length HCN channels with an intact connection between the CL and the pore. Although solving this problem using crystallography is a worthy goal, it is still a daunting task to produce high-quality crystal structures of ion channels, let alone structures of channels in multiple states. Furthermore, what has become clear from the dynamic, structural measurements of Saponaro et al. (1) is that HCN is a breathing, malleable molecule. A given functional state of the protein may not be represented by a single structure, but a flexible ensemble of related structures. Therefore, measurements that bring out the dynamic personalities of macromolecules will be crucial to understanding the gating of HCN. To this end, FRET and EPR-based methods like DEER will likely be the key. DEER and FRET look at the interaction between tags attached to specific points on a protein and are effective even in large and impure protein complexes at yielding high-resolution distance constraints (and in the case of DEER, distributions of distances). FRET can even be measured in intact, functioning channels to directly correlate structural and functional channel states. When coupled with the extensive structural information already accumulated for HCN, even a relative few constraints can accurately model gating conformational changes and follow the chain of dominoes all of the way from ligand binding to channel opening (15).

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