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Let It Go and Open Up, an Ensemble of Ion Channel Active States

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plays protective roles against stressed conditions.

Given the fact that Myc is a well-known oncogene for human cancer, a possible protective role of Myc in cancer stem cells is especially intriguing and deserves further investigation. Myc and its downstream metabolic pathways have been extensively tested as targets for potential anticancer therapy. While in many cases those treatments effectively induce apoptosis and regression of tumors, it is largely unknown whether inhibition of the Myc-related metabolic pathway will induce a fraction of tumor cells into a biosynthetic quiescent state similar to the dormant mESCs as revealed in this study. Cells in such a state may be more resistant to the therapeutic treatment and can quickly revert back to their active tumorigenic state upon the removal of the therapeutic agents. This possibility needs to be carefully evaluated in light of the unexpected discovery from this study.

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Let It Go and Open Up, an Ensemble of Ion Channel Active States

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Ligand binding usually moves the target protein from an ensemble of inactive states to a well-defined active conformation. Matthies et al. flip this scheme around, finding that, for the magnesium channel CorA, loss of ligand binding induces an ensemble of conformations that turn the channel on.

How do proteins that act as switches work? Such molecules are at the heart of cellular signaling and can be as simple as a single domain that responds to calcium ion concentration changes or as complex as a multisubunit ion channel. In either case, there are many conformations that will be inactive, whereas the active state will usually be defined by a single or narrowly defined set of conformations. For example, in ion channels for which the switch is driven by ligand binding, ligand binding usually stabilizes formation of an open state that is distinct from a sea of multiple closed states. In this issue of *Cell*, Perozo and colleagues (Matthies et al., 2016), through elegant cryoelectron microscopy studies of a bacterial magnesium channel known as CorA, provide an example of this scenario being inverted, in which an ensemble of active states are counterposed by a well-defined, ligand-bound, and non-conductive state (Figure 1).

CorA is one of the main conduits for magnesium uptake in bacteria (Payandeh et al., 2013) and both conducts and is inhibited by magnesium ions. It is also one of those "special" membrane proteins that likes to crystallize. Consequently, a number of groups have determined CorA structures by X-ray crystallography (Eshaghi et al., 2006; Guskov et al., 2012; Lunin et al., 2006; Payandeh and Pai, 2006; Pfoh et al., 2012). These efforts have provided a fascinating view of CorA architecture, showing a symmetric bell-shaped pentamer that includes a large intracellular domain in which magnesium ions bind at interfacial positions. Because of the importance of intersubunit movements in channel gating, such a binding site location resides in a "sweet spot" on the channel that can drive ligand-gated ion channel (LGIC) function (Unwin, 2013) and seems to support a key role for magnesium ions in driving transitions between open and closed states.

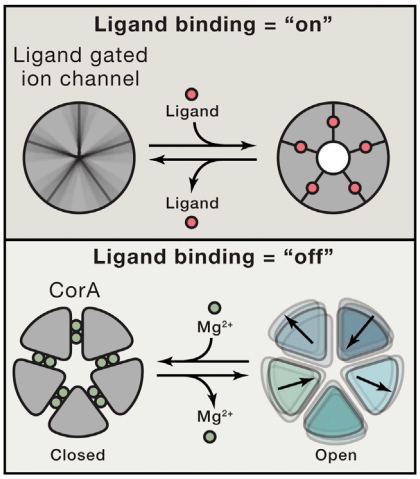

Figure 1. Ligand Gating of Ion Channels
For many ligand-gated ion channels, such as pentameric neurotransmitter receptors (upper; Unwin, 2013), the binding of agonist at intersubunit interfaces stabilizes the open, activated state relative to an ensemble of closed states and turns the channel on. In the case of the CorA pentamer (lower), the ligand is an antagonist. Ligand binding stabilizes subunit-subunit interactions yielding a pore that is too small to permit ion passage, keeping the channel in the "off" state. Loss of ligand binding destabilizes the subunit-subunit interfaces and results in an ensemble of conformations in which the flexibility of the channel assembly is thought to facilitate ion conduction.

For proteins that move between functional states, such as ion channels, answering the question of "which state is captured by a protein crystal structure?" can be particularly challenging (Minor, 2007). With the notable exception of a mutant that can be crystallized in the absence of divalent ions (Pfoh et al., 2012), the magnesium-free state of CorA has resisted crystallization. Oddly, structures of CorA in the presence (Eshaghi et al., 2006; Guskov et al., 2012; Lunin et al., 2006; Payandeh and Pai, 2006) and absence of divalents (Pfoh et al., 2012) have few conformational differences. In both cases the channel has a long, narrow pore that appears to be too narrow to pass a water molecule, let alone an ion. The similarities between the ligand-bound and ligand-free structures, together with the apparently non-conductive state of the pore, raises the possibility that even without ligand stabilization, the non-conductive symmetric conformation may simply be the best fit for a crystal lattice. Hence, the extent of ligand-induced conformational changes in CorA has remained unclear.

Now, taking advantage of the revolution in single particle cryoelectron miscroscopy studies and particle classification algorithms (Nogales and Scheres, 2015), Perozo, Subramaniam, and their team have defined two medium-resolution (7.1 Å) structures of the unliganded form of CorA that reveal surprising large-scale changes in the in CorA structure. In the absence of magnesium, the beautiful five-fold symmetry seen in previous crystallographic studies is broken. Four of the five subunits are displaced relative to the pore central axis, and there are large hinge-bending motions between the transmembrane and intracellular domains. Thus, despite what was seen in the crystal structures, letting go of the ligand does indeed perturb the subunit-subunit interface. Ligand removal from the CorA subunit interfaces frees the channel to explore asymmetric conformations that seem to unleash a flexibility required to conduct ions (Figure 1), and rather than ligand stabilization of an open state as in most ligand-gated channels, the magnesium ligands fix the closed-state conformation. This idea of an ensemble of active states suggested by the multiple conformations of the unliganded form is fascinating and highlights the complex energetic landscapes of multi-subunit systems.

Even though the effects of ligands on activity are opposite to many other ligand-gated channels, some ideas stay the same. Ligand binding energies are used to stabilize a key conformational state. It is just that in CorA, the key conformational state is the "off" state and the ligand is an antagonist rather than an agonist. The picture of an ensemble of ligand-free activate states is an interesting variation on how an ion channel can work. Symmetry breaking and the formation of an open state ensemble of structures offers a solution to the problem posed by the CorA long pore and may shorten and widen the central ion conduction pathway in a way that facilitates hydration and ion passage. Computational studies have suggested that the unliganded state can adopt conformations that favor central cavity hydration (Neale et al., 2015), and neck bending and asymmetric changes in the pore have been suggested previously (Pfoh et al., 2012). It will be important to see what happens in simulations of the new asymmetric states to understand how such large scale conformational changes can shape the central pore hydration and conduction properties.

This study by Perozo and colleagues not only provides new insight into ion channel mechanisms, but also underscores the power of cryoelectron microscopy for visualizing the conformational states that complex macromolecular switches can access. Importantly, the idea of functional ensembles is not restricted to channels. Recent β-adrenergic receptor studies have uncovered a ligand-induced conformational heterogeneity that is thought to be crucial for signaling (Neale et al., 2015). Together with the current results on CorA, such observations emphasize that the initial structure determination of a protein is only the beginning of the quest to unravel how it actually works and that digging deep into mechanism can lead to new general insights into protein function.

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