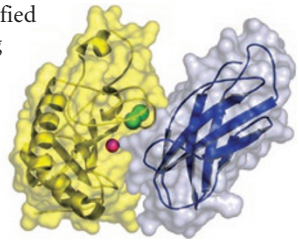
A hedgehog's view



Since hedgehog (Hh) was identified as key to developmental signaling in *Drosophila melanogaster*, and mutations in the vertebrate homologs Sonic hedgehog (Shh) and Indian hedgehog (Ihh) were linked to human developmental abnormalities, the hunt has been on to fully understand its complex receptors interactions. Leahy and colleagues have now found that Shh recognizes one co-receptor, CDO, very differently from how *D. melanogaster* Hh recogizes Ihog (*Drosophila* CDO). The latter interaction occurs in a heparin-dependent manner through fibronectin repeat 1 (Fn1), the third repeat (Fn3) of CDO is now shown to bind vertebrate Shh in a Ca^{2+}-dependent fashion. The structure of the Shh–CDO Fn3 complex reveals a binuclear Ca^{2+} binding site at the interaction interface. Subsequent analyses indicate that this previously unknown Ca^{2+}-dependent binding mode also mediates interaction with other vertebrate Shh receptors. Furthermore, guided mutagenesis of mouse Shh can endow it with fly Ihog binding properties. The newly discovered binding mode seems to be shared by multiple Hh homologs: indeed, mutations in Ihh lie at the calcium binding loop and are shown to disrupt Ihh-receptor interactions. These mutants were based on human mutations that lead to abnormalities in brain and limb development, and the mechanistic basis of these phenotypes is now clearer. (*Nature*, advance online publication 14 September 2008, doi:10.1038/nature07358) *SL*

Sodium switching

Inward-rectifying potassium (Kir) channels require the membrane phospholipid phosphotidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) for activation. Phospholipid sensitivity involves regions in the N and C termini of Kir channels. In addition to PtdIns(4,5)P_2, Kir3 channels require binding by either $G_{\beta\gamma}$ and/or intracellular sodium for activation. Previous studies had identified a C-terminal cytoplasmic domain region proximal to the membrane as important for Na^+ binding. Within this region lie an aspartate residue (Asp223 in Kir3.4) required for Na^+ binding and a highly conserved arginine (Arg225) that affects the channel's sensitivity to PtdIns(4,5)P_2. Logothetis and colleagues now propose how Na^+ binding at Asp223 might contribute to PtdIns(4,5)P_2-sensitive channel gating. Using the crystal structure of the Kir3.1 cytosolic domain determined previously, the authors' model for Kir3.4 suggested that Na^+ was coordinated by Asp223 and His228, and by the backbone carbonyls of two neighboring residues. Molecular dynamic simulations indicated that, in the absence of Na^+, Asp223 is hydrogen-bonded to Arg225. When combined with mutational analysis, the data points to Na^+ as a switch that breaks the interaction between Asp223 and Arg225, freeing up the Arg225 for a direct or allosteric role in PtdIns(4,5)P_2 binding. The Na^+-insensitive Kir3.1 has an arginine residue at the position equivalent to Asp223. Interestingly, the aspartate and histidine binding motif seen here was also identified in the Kir5.1 sequence, and the authors show that heteromeric Kir channels containing this subunit are also Na^+ sensitive. Although this study explains how Na^+ may control Kir channels' sensitivity to phospholipids, the physiological need for Na^+ sensitivity in these channels remains unclear. (*Nat. Chem. Biol.* **4**, 624–631, 2008) *MM*

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Boosting proteostasis

To be functional, proteins must acquire their proper structure. Lysosomal storage diseases (LSDs) such as Gaucher's and Tay-Sachs' are caused by mutations that lead to low folding efficiency in the endoplasmic reticulum (ER). Such misfolded mutant enzymes are degraded by the ER-associated degradation pathway. Some LSDs can be treated by enzyme replacement; another approach is to use pharmacological chaperones—small molecules that bind and stabilize the folded state of the mutated proteins, increasing their concentration. Now, Segatori, Kelly and colleagues explore a more global biological approach to improving the folding of mutant LSD enzymes by boosting the cell's ability to maintain protein homeostasis, or proteostasis, with small molecules which upregulate chaperones that participate in the proteostasis network. They show that two molecules, celastrol (a cellular stresser) and MG-132 (a proteosome inhibitor), can partially restore the enzymatic functions of LSD proteins in patient-derived cell lines. These effects were not due to proteasome inhibition, as lactacystin treatment did not achieve such results. Celastrol and MG-132 were known to induce the heat-shock response and thus enhance the cytoplasmic proteostasis network, but here all three pathways leading to the so-called unfolded protein response (UPR) are activated by these drugs, resulting in the upregulation of components of the ER proteostasis network. Thus, the drugs act as proteostasis regulators, and have a synergistic effect on LSD enzyme activities when used with specific pharmacological chaperones. How celastrol and MG-132 activate the UPR is unclear, but this work provides a proof of principle for the use of proteostasis regulators and opens new avenues for drug development to simultaneously treat multiple LSDs, and potentially other protein-folding diseases. (*Cell* **134**, 769–781, 2008) *IC*

Put a lid on it

Two main models explain how an enzyme reaches the lock-and-key state: the induced-fit and the conformational selection or population-shift model. The induced-fit model features the formation of an encounter complex, where the enzyme is ligand bound but its conformational change has not yet taken place. The second model states that there are multiple enzyme conformational states and the substrate has higher affinity for the lock-and-key state. The finding that even in the absence of ligand some enzymes have been observed to sample multiple conformations, including the ligand-bound (active) state, supports the conformational selection model. However, if active-site formation results in the closure of a lid over the active site, substrate binding directly to this conformer is precluded.

To test whether enzymes with lids use an induced-fit mechanism, Sullivan and Holyoak crystallized structures representing open- and closed-lid conformations of the Michaelis complexes for the forward and reverse reactions catalyzed by phosphoenolpyruvate carboxykinase (PEPCK). They observed the encounter complex where the lid is in its open, or disordered, conformation. In this complex, the location of ligand is identical to that in the binary enzyme–substrate complex previously characterized. This result shows that the open state does represent the encounter complex and that lid closure is necessary for catalysis, because only then are substrates correctly positioned for catalysis (in this case, phosphoryl transfer). Of course, these studies do not address whether enzymes sample multiple conformational states before substrate binding. In the case of PEPCK, the enzyme could sample both the open- and closed-lid states, but the data suggest that the closed-lid population is negligible in the absence of ligand. (*Proc. Natl. Acad. Sci. USA* **105**, 13829–13834, 2008) *BK*