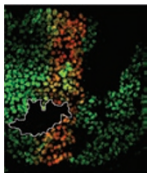
## Smoothened operator

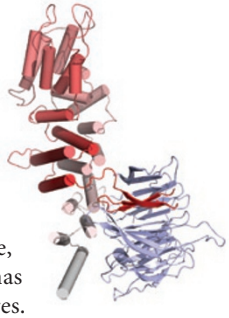


Hedgehog (Hh) signaling is key to developmental patterning across species. Although Hh signaling in *Drosophila melanogaster* depends on the Smoothened (Smo) receptor, which resembles a G protein–coupled receptor, the involvement of G proteins and cAMP regulation in this pathway has been controversial. Indeed, Smo has been proposed to signal through a G protein–independent pathway. Using an RNA interference screen targeting candidate G proteins, Robbins and colleagues have now identified $G\alpha_i$ as a G protein activated by Hh signaling and needed for activation of downstream effectors. Whereas $G\alpha_i$ knockdown leads to decreased activity of downstream components of the pathway, Smo phosphorylation is unaffected, placing $G\alpha_i$ function downstream of receptor activation itself. The placement and involvement of $G\alpha_i$ in the pathway was confirmed using clones of $G\alpha_i$ mutant cells, i.e., generated clones of cells in the developing wing that were either null for or had reduced $G\alpha_i$ function. Such $G\alpha_i$ mutations resulted in small wings, which might result from decreased levels of Dpp, an Hh target, and disrupted closure of the thorax during development, shown here to likely result from decreased Hh signaling. Overexpression of a constitutively activated $G\alpha_i$ mutant in flies resulted in morphological phenotypes such as overgrowth of the wing and alterations in wing vein patterning, which is known to be sensitive to Hh signaling via regulation of Dpp. As well as such phenotypes, constitutively active $G\alpha_i$ resulted in both activation of downstream components of the pathway as well as misexpression of developmental target genes. The authors also measured a decrease in cellular cAMP, the response expected in G protein–mediated signaling, dependent on Hh activation and $G\alpha_i$, as demonstrated through RNA interference targeting these components. The idea that cAMP regulation lies downstream of Hh signaling was further confirmed using an allele of the phosphodiesterase Dunce, reduced function of which should increase cAMP levels, thus antagonizing Hh signaling. Indeed, the authors found that a *dunce* mutant enhances the phenotype of a *smo* mutant, resulting in almost complete loss of wing vein patterning, which depends on Hh signaling. Finally, $G\alpha_i$ association with the downstream effector complex of Hh signaling was observed upon Hh stimulation. Thus, involvement of a canonical G protein in Smo activation by Hh has been shown and implicated in developmental patterning by this pathway. Whether this pathway is widely conserved and whether other Hh family proteins signal in a similar fashion are now questions open for investigation. (*Nature* advance online publication, doi:10.1038/nature07459; 5 November 2008) *SL*

## Remote control

$\sigma^{32}$ is the *Escherichia coli* transcription factor that regulates the cellular response to heat shock. At 30 °C, Hsp70 chaperone DnaK and its cochaperone DnaJ interact with $\sigma^{32}$, leading to its inactivation and rapid degradation by the membrane-bound protease FtsH. At 42 °C, the accumulation of unfolded proteins in the cell titrates DnaK and DnaJ, freeing $\sigma^{32}$ to associate with RNA polymerase and drive gene expression. This elegant system ties the heat shock response to the protein folding state in the cell, but the exact mechanisms involved were not clear. Now, Mayer, Bukau and colleagues reveal the molecular details of $\sigma^{32}$ interactions with DnaK and DnaJ. The authors use a variety of approaches, including hydrogen-deuterium exchange (HDX) experiments followed by protease digestion and mass spectrometry analysis, a powerful technique to map binding sites and to gain information about conformational changes induced across the whole $\sigma^{32}$ protein. They show that DnaK binds to a specific site in $\sigma^{32}$ that seems to be involved in interaction with RNA polymerase, explaining how $\sigma^{32}$ activity can be inhibited. In addition, binding of DnaK to $\sigma^{32}$ leads to conformational changes in two distant sites, one of which shows higher rates of HDX, indicative of destabilization. DnaJ binds to a different site of $\sigma^{32}$ than does DnaK and causes destabilization of distant two segments, which are close to the DnaK binding site. Thus, DnaJ induces conformational changes in $\sigma^{32}$ that may facilitate DnaK binding. In addition, DnaJ should be able to bind $\sigma^{32}$ even in complex with RNA polymerase. Both these events would contribute to the delivery of $\sigma^{32}$ by DnaJ to DnaK. Together, DnaK and DnaJ promote a partial unfolding of $\sigma^{32}$, which may facilitate its degradation by FtsH. This protease is known to be a weak unfoldase capable of processing only substrates with low stability. It will be interesting to explore whether similar mechanisms are used with other substrates of DnaK and DnaJ or by other Hsp70s. (*Mol. Cell* **32**, 347–358; 2008) *IC*

## Common ancestry



Macromolecules cross the nuclear membrane through the nuclear pore complex (NPC). Although the structure of many of the individual protein components are known, the overall structure of the 40–60 MDa NPC is not clearly understood. Cryoelectron microscopy and cryoelectron tomography have provided a view of the NPC structure, but the low resolution of these techniques has prevented the fitting of the crystal structures. Now, the crystal structure of two nucleoporins in complex suggests that the NPC scaffold has properties similar to those of vesicle coats. Of the approximately 30 nucleoporins that make up the NPC, only a few are stably attached to the complex. In the yeast *Saccharomyces cerevisiae*, the core proteins form two essential complexes: the heptameric Nup84 complex and the hetero-oligomeric Nic96-containing complex. Schwartz and colleagues report the structure of two Nup84-complex proteins: residues 1–564 of Nup85 bound to full-length Seh1 at 3.5 Å resolution. These Nup84-complex proteins form distinct units in a tightly associated complex. More striking, though, is the similarity in secondary-structure elements, three-dimensional folds and assembly between Nup85–Seh1 and another member of the Nup84 complex, Nup145C–Sec13. A comparison of Nup85 with nucleoporins Nic96, Nup84 and Nup145C reveals that they share three structural elements: a crown, a trunk and a tail. Further analysis turned up another protein with a similar structure, the coat component of the vesicle COPII, Sec31. The authors named this tripartite element the ancestral coatomer element, ACE1. From this, they propose that the NPC scaffold, in common with vesicle coats, is composed of polygons that form a lattice upon which other NPC proteins can dock. The modular nature of vesicle coats provides flexibility in composition and size; NPCs might also benefit from this versatility. (*Science* published online, doi:10.1126/science.1165886; 30 October 2008) *MH*

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