# Hedgehog Signaling Uses Lipid Metabolism to Tune Smoothened Activation

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**In this issue of *Developmental Cell*, Yavari et al. reveal that membrane phosphatidylinositol-4 phosphate (PI4P) controls the activation of Hh signal transduction. Their data suggest that Ptc functions directly or indirectly to suppress accumulation of PI4P. Binding of Hh to Ptc derepresses PI4P levels, which in turn promotes Smo activation.**

The Hedgehog (Hh) signaling pathway is evolutionarily conserved and plays essential roles in various developmental processes and homeostasis (Jiang and Hui, 2008). Deregulation of this pathway is associated with human congenital defects and leads to the formation of a variety of tumors (Jiang and Hui, 2008). A key step in this signaling pathway occurs at the level of two transmembrane proteins, the 12 transmembrane protein receptor Patched (Ptc) and the 7 transmembrane G-protein-coupled receptor Smoothened (Smo). In the absence of Hh, Ptc acts as an inhibitor to repress Smo activity. Binding of Hh to Ptc inactivates Ptc, relieving the repression of Smo and activating the Hh signaling cascade.

The problem of how Ptc blocks Smo activity in the absence of Hh is a major outstanding issue in the Hh research field. Biochemical studies suggest that Ptc represses Smo through catalytic processes rather than stoichiometric interactions (Taipale et al., 2002). Moreover, Ptc has never been found to be physically associated with Smo and the two proteins do not appear to be enriched at similar subcellular locations in vivo (Jiang and Hui, 2008). Genetic studies in *Drosophila* showed that repression of Smo by Ptc correlates with changes in Smo subcellular localization and reduced stability. Ptc restricts the levels of membrane-localized Smo by promoting Smo endocytosis and degradation, whereas binding of Hh to Ptc induces Smo cell surface accumulation. Similarly, studies in vertebrates showed a strong correlation between Smo localization to primary cilia and Hh pathway activation. In the absence of Hh, Ptc localizes to cilia and prevents Smo from accumulating in the cilia. Binding of Hh to Ptc induces Smo accumulation and reduces Ptc levels in cilia (Rohatgi et al., 2007).

How does Ptc restrict Smo surface levels in the absence of Hh? Because Ptc is homologous to the RND family of prokaryotic proton-driven transporters that act as homotrimeric small molecule pumps (Taipale et al., 2002), one hypothesis is that Ptc may function as a pump to change the concentration of an endogenous small molecule across membranes, leading to changes in Smo subcellular localization (Taipale et al., 2002). In mammalian cells, biochemical experiments showed that specific oxysterols can activate Hh signal transduction (Dwyer et al., 2007) and that treatment of cells with oxysterols can promote accumulation of Smo in cilia (Rohatgi et al., 2007). However, it is currently unknown whether a similar endogenous small molecule is involved in Smo regulation by Ptc in vivo.

In this issue of *Developmental Cell*, Yavari et al. (2010) provide strong evidence that Smo activation is dependent on the levels of phosphatidylinositol-4 phosphate (PI4P). Phosphoinositols (PIs) are lipid constituents of the plasma and organelle membranes of all cells. PI4P is one of seven different phosphorylated PIs (Skwarek and Boulianne, 2009). The interconversion of different PIs is regulated by multiple kinases and phosphatases. In yeast, the *suppressor of actin-1* (*sac1*) phosphatase dephosphorylates PI4P to PI; *sac1* loss causes striking increases in the levels of PI4P (Foti et al., 2001). Conversion of PI into PI4P is controlled by two evolutionarily conserved kinases, PIK1 and STT4, which act in a nonredundant manner to generate distinct, nonoverlapping pools of PI4P in cells (Foti et al., 2001). Studies in yeast showed that PIK1 is primarily present in the nucleus and the Golgi, whereas STT4 is mainly localized to the plasma membrane (Foti et al., 2001).

Yavari et al. first noticed a role for PI4P in Hh signaling when they observed that a loss of Sac1 activity in the *Drosophila* eye disc leads to expression of *decapentaplegic* (*dpp*), a downstream target gene in the Hh signaling pathway. Consistent with a role of Sac1 in Hh signaling, high levels of Ptc and activated Ci protein are also observed in *sac1* mutant cells. Importantly, high levels of membrane-localized Smo accumulate in *sac1* mutant cells irrespective of the presence or absence of Hh ligand. These data argue strongly that loss of *sac1* alone is sufficient to promote both the membrane localization of Smo and its ability to activate downstream components of the Hh pathway. Sac1 acts downstream of the ligand-binding event.

Biochemical studies from yeast established that PI4P is the primary substrate for *sac1*. Indeed, PI4P levels, rather than those of other PIs such as PI(4,5)P and PI(3)P, are strikingly increased in *sac1* mutant imaginal disc cells. To establish a role of PI4P in Hh signaling, the authors examined the involvement of STT4 and *four wheel drive* (*fwd*), the *Drosophila* ortholog of yeast PIK1. In the eye disc, loss of function of STT4 rather than *fwd*

is able to suppress the Hh pathway activation that results from loss of *sacI* activity. Similarly, STT4 activity is required for Hh signaling in wild-type cells and for ectopic Hh signaling activation resulting from Sac1 loss of function in the wing disc. Importantly, loss of Sac1 or STT4 activities in embryos generates defects in cuticle patterns. *sac1* homozygous mutant embryos show a reduction in the width of the denticle belts, a phenotype similar to that seen in *ptc* mutant embryos, whereas loss of *STT4* results in the opposite segment polarity phenotype, similar to that seen in *hh* and *smo* mutant embryos. These data argue that STT4 has a very general function as a positive modulator of Hh signaling. The activity of STT4 in Hh signaling appears to be evolutionarily conserved, because loss of homologous mammalian PI4III kinases $\alpha$ and $\beta$ strongly reduced Shh-stimulated reporter activity in mouse fibroblast cells.

Several lines of evidence provided in the paper likewise argue for a role of Ptc in regulating PI4P levels. First, overexpression of Ptc can override the Smo membrane localization phenotype and PI4P accumulation resulting from loss of *sac1* activity. Second, the loss of Ptc activity causes cell-autonomous increases in PI4P levels in various tissues. Finally, the increase in PI4P levels upon loss of *ptc* function is dependent on STT4 function because simultaneous loss of Ptc and STT4 activities results in the suppression of the phenotype. Based on these data, the authors propose that Ptc may function in keeping low levels of PI4P at the cell surface, possibly by downregulating the STT4 kinase activity. During normal Hh signaling, binding of Hh to Ptc relieves the repression of STT4 kinase by Ptc and causes an increase in PI4P, which in turn promotes Smo activation.

Many questions remain to be resolved. Current data cannot distinguish whether Ptc is directly or indirectly involved in the suppression of STT4 activity. Recent studies in yeast have identified two novel regulatory components of the Stt4 kinase complex, Ypp1 and Efr3 (Baird et al., 2008). Whereas Ypp1 interacts with distinct regions on Stt4 that are necessary for the assembly and recruitment of Stt4 onto cell membranes, Efr3 encodes a multipass transmembrane protein essential for assembly of both Ypp1 and Stt4 on the cell surface (Baird et al., 2008). Because STT4 is a cytoplasmic enzyme anchored to cell membranes by Ypp1 and Efr3, it would be interesting to know whether Ptc can modulate the Ypp1 or Efr3 function, thereby regulating the activity of STT4. It will also be exciting to examine whether oxysterols (Rohatgi et al., 2007) or lipoprotein-derived lipids (Khaliullina et al., 2009) are relevant to this effect of PI4P on Ptc-Smo interactions. Finally, because primary cilia appear to be essential for Smo localization and Hh activation in vertebrate cells, it will be important to determine whether PI4P levels influence Smo translocation in primary cilia.

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