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Smoothened Sensor Places Sodium and Sterols Center Stage

Robert Blassberg[1] and James Briscoe[1,*]
[1]The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK
*Correspondence: james.briscoe@crick.ac.uk
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Regulation of Smoothened by PTCH1 is central to Hedgehog signal transduction. Reporting recently in *PNAS*, Myers et al. (2017) provide evidence that a transmembrane flux of sodium ions drives PTCH1 activity and that cholesterol regulates Smoothened via its transmembrane domain.

The Hedgehog (Hh) signaling pathway modulates a gamut of cellular processes associated with embryonic development and tissue homeostasis, including fate specification, proliferation, survival, and metabolism. Although most of the principal components of the pathway were identified over 20 years ago, fundamental aspects of the molecular mechanism by which Hedgehog signals are transduced remain poorly understood (reviewed in Briscoe and Thérond, 2013). Chief among these is how intracellular signaling is initiated. A defining characteristic of the Hh pathway is the de-repression—double-negative—mechanism of signaling. In the absence of Hh ligand, the transmembrane signal transducer, Smoothened (SMO), a GPCR-like receptor, is repressed by the Hh receptor PTCH1. Binding of Hh protein to PTCH1 inhibits its activity, allowing SMO to signal intracellularly. The mechanism by which PTCH1 represses SMO, and how SMO is activated, has remained unclear. In a recent issue of *PNAS*, Myers et al. (2017) shed light on these questions by developing and employing a SMO biosensor that provides the first direct readout of SMO activity.

To construct the biosensor, Myers et al. (2017) fuse SMO to a $G\alpha_0$ subunit such that $G\alpha_0$ is activated and cAMP levels increase upon SMO de-repression. Then, to assay SMO in intact cells, the authors use the "GloSensor" firefly luciferase variant, which emits light in the presence of cAMP, whereas, in a second assay, GTP binding to the SMO- $G\alpha_0$ fusion is quantified to provide a direct biochemical measurement of activity.

Vertebrate Hedgehog signaling is associated with the primary cilium. PTCH1 is found in and around the cilium, and SMO entry into the cilium is correlated with pathway activation (Rohatgi et al., 2007). Strikingly, assays using the SMO biosensor in human cells lacking primary cilia, in isolated membrane fractions, and in reconstituted lipid bilayers *in vitro* demonstrated that both SMO activation and its repression by PTCH1 are independent of the primary cilium. Moreover, the activity of SMO measured using the biosensor changes within 3–4 minutes in response to Hh binding to PTCH1, whereas accumulation of SMO in the cilium takes several hours (Rohatgi et al., 2007), indicating that the requirement for cilia in vertebrate Hh signaling is likely due to a dependency on cilia for activation of downstream effectors of the pathway, and not SMO itself. Cilium-independent coupling of PTCH1 and SMO raises the possibility that this core mechanism is conserved in *Drosophila*, which lacks primary cilia. In addition, it may offer insight into the evidence that some biological responses to Hedgehog signaling, including cell migration and endothelial tubulogenesis, are cilia independent (Bijlsma et al., 2012; Chinchilla et al., 2010).

Prior work established that PTCH1 repression of SMO is indirect and non-stoichiometric, therefore potentially mediated by a second messenger (Taipale et al., 2002). PTCH1 shares homology with the RND-family bacterial proton-powered efflux pumps, and conserved residues that mediate ion flux are essential for the SMO inhibitory activity of PTCH1. Although this suggested a functional role for ion flux, biochemical evidence had been absent. Unlike bacteria, mammalian cells do not maintain a proton gradient across their plasma membranes, leading Myers et al. (2017) to focus on Na^+ and K^+ ion gradients. They show a rapid and reversible loss of SMO inhibition by PTCH1 when the physiological Na^+ High/K^+ Low extracellular ion composition is switched to Na^+ Low/K^+ High. Although the experiments indicate that PTCH1 activity depends specifically on Na^+ ions, the data also suggest that Na^+ is unlikely to modulate Smo activity directly. Moreover, Ptch1 can still bind Hh if the Na^+ gradient is disrupted. The most likely possibility, therefore, is that the Na^+ gradient is used to power a transport cycle responsible for the inhibition of SMO.

Genetic evidence indicates that PTCH1 inhibits SMO either by removing an activator or delivering an inhibitor (reviewed in Briscoe and Thérond, 2013). Cholesterol and related sterols have been found to either positively or negatively modulate SMO, and SMO harbors several binding sites for sterols. This suggests that PTCH1 might modulate the availability to SMO of one or more of these lipids (reviewed in Blassberg and Jacob, 2017). Myers et al. (2017) assayed SMO biosensor activity *in vitro* in lipid bilayers comprising cholesterol and phospholipids. This revealed that SMO activity was dependent on either cholesterol or cholesterol-derived oxysterols that are known to activate SMO, consistent with prior cell line studies demonstrating a requirement for cholesterol biosynthesis for SMO signaling (Blassberg et al., 2016; Cooper et al., 2003). Importantly, Myers et al. (2017) show that an engineered SMO construct lacking a known extracellular cholesterol-binding domain retained sterol-dependent activity in this minimal system, identifying transmembrane regions of SMO as the likely locus for cholesterol interaction. Because the extracellular sterol-binding domain is

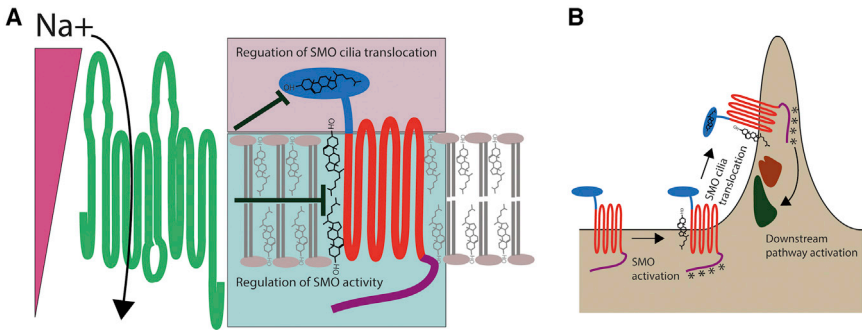

Figure 1. Regulation of SMO Activation and Cilia Localization via Distinct Sterol Interfaces
(A) The GPCR-like receptor SMO contacts lipids via the transmembrane domain (TMD). Myers et al. (2017) demonstrate that interaction between the TMD and sterols is required for SMO activity. Previous studies identified interactions between sterols and the extracellular domain (ECD) of SMO, which regulates SMO cilia localization (reviewed in Blassberg and Jacob, 2017). PTCH1 activity depends upon the Na^+ membrane potential, which may drive repression of SMO activity and cilia localization independently by modulating the availability of cholesterol to distinct interfaces of SMO. (B) Together with previous studies, the data presented by Myers et al. (2017) suggest that engagement of plasma membrane sterols with the TMD activates SMO, whereas engagement of sterols with the ECD promotes localization of SMO to the primary cilium, where it activates downstream signaling components.

essential for SMO cilia localization and downstream signaling in response to both Hh and activating sterols (reviewed in Blassberg and Jacob, 2017), the data presented by Myers et al. (2017) highlight that PTCH1 may regulate SMO activation and cilia localization via distinct sterol interfaces (Figure 1). Although Myers et al. (2017) do not distinguish whether PTCH1 acts by shielding SMO from cholesterol (which comprises up to 50% of the membrane of mammalian cells) or by supplying an inhibitor that blocks cholesterol activity, their findings focus attention on the transmembrane region of SMO as an essential sterol interaction site and establish an *in vitro* activity assay that makes the problem tractable.

As noted by the authors, open questions remain as to how the Na^+ gradient drives the repressive activity of PTCH1 and how PTCH1 inhibits SMO activity. Given the unusual and singular structure of the Hh pathway, we can anticipate that answers to these questions will provide further surprises and perhaps fundamental insights. Beyond the interest to cell and developmental biologists, the regulation of SMO by PTCH1 has attracted attention as a drug target (Frank-Kamenetsky et al., 2002). Unrestrained Hh signaling drives a number of cancers. Conversely, judicious activation of Hh signaling has potential applications in regenerative medicine. Indeed, the identification and development of therapeutic small-molecule modulators of the pathway is an exemplar of successful rational drug design grounded in discoveries in basic research. The development of the SMO biosensor allows direct, rapid assays of SMO activity, broadening the potential for identifying ways to directly target SMO and manipulate the pathway. Taken together, therefore, the work by Myers et al. (2017) advances our understanding of the Hh pathway and raises the prospect of more fully exploiting this knowledge.

REFERENCES

Bijlsma, M.F., Damhofer, H., and Roelink, H. (2012). Hedgehog-stimulated chemotaxis is mediated by smoothened located outside the primary cilium. Sci. Signal. *5*, ra60.

Blassberg, R., and Jacob, J. (2017). Lipid metabolism fattens up hedgehog signaling. BMC Biol. *15*, 95.

Blassberg, R., Macrae, J.I., Briscoe, J., and Jacob, J. (2016). Reduced cholesterol levels impair Smoothened activation in Smith-Lemli-Opitz syndrome. Hum. Mol. Genet. *25*, 693–705.

Briscoe, J., and Thérond, P.P. (2013). The mechanisms of Hedgehog signalling and its roles in development and disease. Nat. Rev. Mol. Cell Biol. *14*, 416–429.

Chinchilla, P., Xiao, L., Kazanietz, M.G., and Riobo, N.A. (2010). Hedgehog proteins activate pro-angiogenic responses in endothelial cells through non-canonical signaling pathways. Cell Cycle *9*, 570–579.

Cooper, M.K., Wassif, C.A., Krakowiak, P.A., Taipale, J., Gong, R., Kelley, R.I., Porter, F.D., and Beachy, P.A. (2003). A defective response to Hedgehog signaling in disorders of cholesterol biosynthesis. Nat. Genet. *33*, 508–513.

Frank-Kamenetsky, M., Zhang, X.M., Bottega, S., Guicherit, O., Wichterle, H., Dudek, H., Bumcrot, D., Wang, F.Y., Jones, S., Shulok, J., et al. (2002). Small-molecule modulators of Hedgehog signaling: identification and characterization of Smoothened agonists and antagonists. J. Biol. *1*, 10.

Myers, B.R., Neahring, L., Zhang, Y., Roberts, K.J., and Beachy, P.A. (2017). Rapid, direct activity assays for Smoothened reveal Hedgehog pathway regulation by membrane cholesterol and extracellular sodium. Proc. Natl. Acad. Sci. USA. 201717891. Published online December 11, 2017. https://doi.org/10.1073/pnas.1717891115.

Rohatgi, R., Milenkovic, L., and Scott, M.P. (2007). Patched1 regulates hedgehog signaling at the primary cilium. Science *317*, 372–376.

Taipale, J., Cooper, M.K., Maiti, T., and Beachy, P.A. (2002). Patched acts catalytically to suppress the activity of Smoothened. Nature *418*, 892–897.