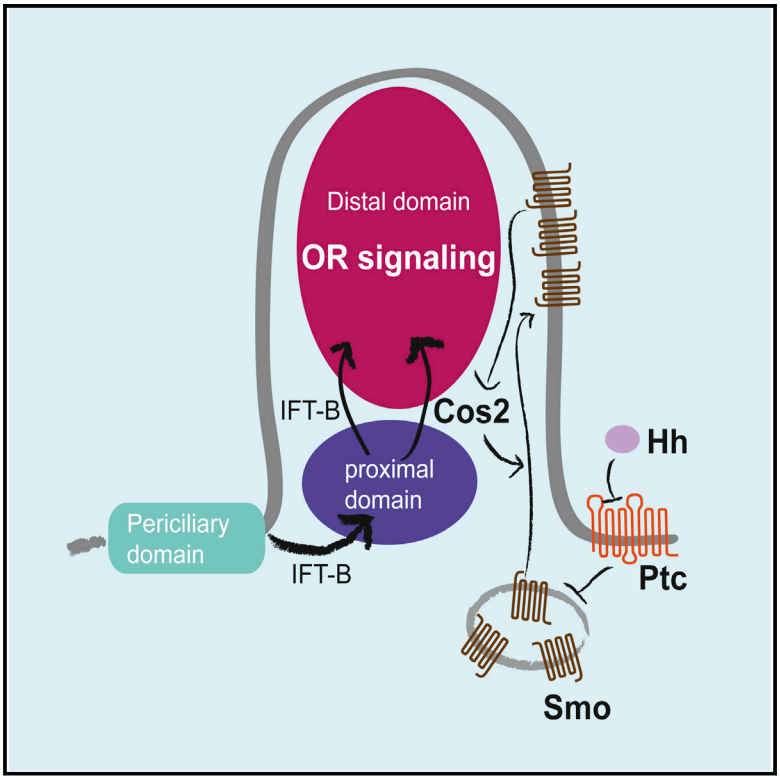
Cell Reports

Hedgehog Signaling Regulates the Ciliary Transport of Odorant Receptors in *Drosophila*

Graphical Abstract



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In Brief

Odor responses are tuned to the ambient odorant environment. Sanchez et al. examine the molecular mechanisms that govern the odorant response, and they find that Hedgehog signaling in *Drosophila* regulates localization of the odorant receptors to the cilium compartment.

Highlights

- Hedgehog signaling regulates the odorant response
- Hedgehog signaling controls OR entry and transport within the cilium compartment
- The regulation of OR transport is a cilium-mediated Hedgehog pathway
- Cos2, a Hedgehog-regulated atypical kinesin, localizes ORs within the cilium

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Hedgehog Signaling Regulates the Ciliary Transport of Odorant Receptors in *Drosophila*

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SUMMARY

Hedgehog (Hh) signaling is a key regulatory pathway during development and also has a functional role in mature neurons. Here, we show that Hh signaling regulates the odor response in adult *Drosophila* olfactory sensory neurons (OSNs). We demonstrate that this is achieved by regulating odorant receptor (OR) transport to and within the primary cilium in OSN neurons. Regulation relies on ciliary localization of the Hh signal transducer Smoothened (Smo). We further demonstrate that the Hh- and Smo-dependent regulation of the kinesin-like protein Cos2 acts in parallel to the intraflagellar transport system (IFT) to localize ORs within the cilium compartment. These findings expand our knowledge of Hh signaling to encompass chemosensory modulation and receptor trafficking.

INTRODUCTION

In both vertebrates and insects, chemical stimuli are detected by odorant receptors (ORs) located on the olfactory sensory neuron (OSN) cilia (DeMaria and Ngai, 2010; Vosshall and Stocker, 2007). Each OSN typically expresses one OR from a large genomic repertoire (Couto et al., 2005; Fishilevich and Vosshall, 2005). The odorant response must be adjusted appropriately to changes in the environment to elicit a suitable behavior and warrant survival of the animal. In *Drosophila*, the type and level of the expressed receptor determine the odorant response (Dobritsa et al., 2003). However, the mechanisms that regulate the receptor level and the level of odorant response are not well understood.

Hedgehog (Hh) signaling regulates nociceptive responsiveness (Babcock et al., 2011). Hh was initially described as a morphogen that defines the segmentation and patterning of the *Drosophila* embryo (Briscoe and Thérond, 2013; Goetz and Anderson, 2010). Hh ligand binding to the inhibitory receptor Patched (Ptc) stabilizes the seven-transmembrane protein Smoothened (Smo) (Denef et al., 2000), which, in vertebrates, translocates to the primary cilium (Corbit et al., 2005) and switches the function of the Gli transcription factors from repression to activation of the Hh target genes (Briscoe and Thérond, 2013; Goetz and Anderson, 2010; Ingham et al., 2011; Rohatgi and Scott, 2007; Teperino et al., 2014). The cells that respond to Hh during *Drosophila* development are non-ciliated, which has led to the general view that *Drosophila* and vertebrates have different Hh pathways (Goetz and Anderson, 2010). However, we have demonstrated recently that cilia do mediate the Hh signal in OSNs, one of the few ciliated cell types in *Drosophila* (Kuzhandaivel et al., 2014).

Here, we examine the function of cilium-mediated Hh signaling in *Drosophila* and show that *Smo* knockdown results in a reduced behavioral response to odors. We demonstrate that the level of Hh pathway activity controls the magnitude of the OSN odorant response and regulates the cilium transport of the ORs. Last, we reveal that Smo and the kinesin-like protein Cos2 control OR transport to and within the cilium compartment.

RESULTS

Hh Signaling Regulates the Odorant Response

To investigate the function of the cilium-mediated Hh pathway in *Drosophila* OSNs, we used RNAi to selectively knock down *Smo* in OSNs. Olfactory performance was measured using a set of T-maze behavioral assays. The results showed that flies with OSNs deficient in Smo function (*peb-Gal4 > Smo-inverted repeat (IR)* were less attracted to vinegar compared with control flies (*Peb-Gal4*) versus *Smo-IR*, Figure 1A). The loss of attraction was not due to a change in motility, as determined by a climbing assay (Figure S1), which indicates that Hh signaling modulates olfaction in *Drosophila*.

To determine whether the change in the behavioral response corresponded to a change in OSN function, we recorded odor-induced changes in intracellular Ca^{2+} concentration in OSNs expressing the genetically encoded fluorescent Ca^{2+} reporter GCAMP5. We initially investigated the response to ethyl acetate, which activates several ORs and OSN classes. In control flies, ethyl acetate triggered robust fluorescence transients that