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Cilia-Mediated Hedgehog Signaling in *Drosophila*

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SUMMARY

Cilia mediate Hedgehog (Hh) signaling in vertebrates and Hh deregulation results in several clinical manifestations, such as obesity, cognitive disabilities, developmental malformations, and various cancers. *Drosophila* cells are nonciliated during development, which has led to the assumption that cilia-mediated Hh signaling is restricted to vertebrates. Here, we identify and characterize a cilia-mediated Hh pathway in *Drosophila* olfactory sensory neurons. We demonstrate that several fundamental key aspects of the vertebrate cilia pathway, such as ciliary localization of Smoothened and the requirement of the intraflagellar transport system, are present in *Drosophila*. We show that Cos2 and Fused are required for the ciliary transport of Smoothened and that cilia mediate the expression of the Hh pathway target genes. Taken together, our data demonstrate that Hh signaling in *Drosophila* can be mediated by two pathways and that the ciliary Hh pathway is conserved from *Drosophila* to vertebrates.

INTRODUCTION

The eukaryotic cilium is a distinct subcellular compartment that mediates mechanical (fluid, flow, touch, and vibration) and chemical (light and odor) signals that are key for sensory input. This subcellular compartment is a microtubule extension surrounded by a specialized membrane that is separate from the rest of the plasma membrane and has a unique composition of proteins that defines the function of the cilium. Most vertebrate cells have a specialized cilium, the primary cilium. Defects in primary ciliary function are the basis of a wide array of human pathologies, the so-called ciliopathies, with manifestations such as cancer, cystic kidney disease, obesity, cognitive disabilities, cerebellar hypoplasia, retinal degeneration, and various developmental malformations (Goetz and Anderson, 2010; Huangfu et al., 2003).

Many of the clinical manifestations of ciliopathies can be attributed to defects in Hedgehog (Hh) signaling (Goetz and Anderson, 2010; Huangfu et al., 2003). The Hh pathway was discovered in one of the first *Drosophila* screens performed, over three decades ago (Nüsslein-Volhard and Wieschaus, 1980). Intense genetic research in *Drosophila* and vertebrate cell culture experiments has revealed a core Hh pathway (Goetz and Anderson, 2010; Roy, 2012). Once Hh binds its receptor Patched (Ptc), the seven-transmembrane protein Smoothened (Smo) is relieved from the tonic repression of Ptc and translocates from the cytoplasm to the membrane. The relocation of Smo initiates an activation sequence resulting in stabilization of the transcription factor Ci (Gli in vertebrates). In the absence of Hh, Ci/Gli is hyperphosphorylated and partially degraded in the cytoplasm. The cleaved form of Ci/Gli represses transcription of the Hh target genes, whereas the stabilized, full-length Ci/Gli functions as a transcriptional activator that relocates to the nucleus, replaces the cleaved, repressive form, and initiates transcription of Hh targets genes.

In vertebrates, Hh binding to its receptor Ptc induces a reciprocal movement of Ptc out of the ciliary compartment and Smo into the ciliary compartment. However, in *Drosophila*, all cells lack cilia during development (Davenport and Yoder, 2005), and instead Smo relocates to the plasma membrane upon Hh stimulation (Jia et al., 2004; Zhu et al., 2003). Therefore, it has been postulated that *Drosophila* and vertebrates have two distinct Hh pathways (Corbit et al., 2005; Goetz and Anderson, 2010; Huangfu et al., 2003; Ingham et al., 2011; Roy, 2012; Wong and Reiter, 2008). Due to the severe phenotypes of misregulated vertebrate Hh signaling, most studies of cilia function have been performed in mammalian cell lines, with often contradictory results in vivo. Thus, the functional difference between the cilia-mediated vertebrate and the *Drosophila* plasma-membrane-mediated Hh pathway remains elusive. Little is known about the possibility that both pathways coexist in one organism.

Here, we demonstrate that ciliated olfactory sensory neurons (OSNs) in *Drosophila* express the Hh components and that Smo in these cells localizes to cilia. We further unravel the role of core Hh signaling components in this cilia-mediated pathway in vivo and show that Hh signaling in *Drosophila* has two pathways: the canonical cilia-independent one and a cilia-mediated one.

RESULTS

Smo Localizes to OSN Cilia

Most cells in *Drosophila* lack cilia, with one of the few exceptions being the ciliated OSNs located in the antenna (Keil, 2012). The

OSN cilia structurally resemble mammalian primary cilia (Jana et al., 2011), but little is known about any functional similarity between the two cilium types (Davenport and Yoder, 2005). Because mammalian primary cilia are an important hub for Hh signaling, we asked whether *Drosophila* OSN cilia can also mediate Hh signaling. We initially found that both Hh and the components of the canonical Hh pathway were expressed at low levels in the *Drosophila* antenna and the mature, ciliated OSNs (Figures 1B–1E). The majority of *Ptc* and *Hh* expression occurred in the support cells, and we found only weak expression in the OSNs (Figures 1D and 1E). To address whether *Smo* is expressed in *Drosophila* OSNs and can localize to cilia, we performed immunohistofluorescence on cryosections of antennae with an antibody against the C-terminal part of Smo (Ogden et al., 2003). The staining revealed that Smo was expressed in OSNs and localized to cell bodies, axons, dendrites, and cilia to various degrees (Figure 1F). The endogenous Smo staining was lost in Smo knockdown antenna (Figure S1). Each OSN expresses one odorant receptor from a large genomic repertoire, and together with the common coreceptor Orco, the odorant receptors localize to the cilia (Benton et al., 2006; Larsson et al., 2004). Double labeling with Orco and Smo showed extensive costaining that demonstrated that Smo localized to OSN cilia (Figures 1G and 1H).

Smo Requires the Intraflagellar Transport System for Ciliary Localization

We only detected weak Smo staining in the OSNs, in accordance with our quantitative PCR results (Figure 1C), implying low *Smo* expression. As this limited our ability to follow Smo localization, we visualized Smo localization with a Smo:GFP fusion protein. Tagged Smo is routinely used both in vivo in *Drosophila* and in vertebrate cell culture experiments, and has been vital in demonstrating the role of Smo transport and localization for Hh signaling (Corbit et al., 2005; Jia et al., 2004; Zhu et al., 2003). We used an inducible Smo:GFP construct (*UAS-Smo:GFP*), whose expression was driven in postmitotic OSNs by *Pebbled-Gal4* (*Peb-Gal4*). Smo:GFP accumulated in OSN cilia to various degrees (Figures 2A and 2B), indicating that the varied ciliary staining of Smo was a result of regulated import and not necessarily expression differences. Cilia lack local protein synthesis and hence have to import proteins via the intraflagellar transport (IFT) system (Berbari et al., 2009). In mice, the IFT component IFT172 is required for targeting Smo to cilia (Ocbina and Anderson, 2008). Knockdown of *IFT172* in the OSNs did not disrupt cilia formation, and α-tubulin staining showed the characteristics of OSN cilia, with microtubules arranged as a cone at the base of the cilium and a thin cilium axoneme in the sensilla (Jana et al., 2011), indicating that our knockdown was not complete. Yet, the knockdown attenuated the transition of Smo:GFP to the cilium (Figures 2A and 2B), which resembles the vertebrate phenotype. Knockdown of a second IFT molecule, IFT88, produced a severe loss of cilia (Figures 2A and 2B). Still, the few remaining cilia were devoid of Smo:GFP, supporting the notion that the IFT machinery is necessary for Smo ciliary transport.

The cytoplasmic tail of vertebrate Smo has a conserved ciliary localization motif that consists of both basic and hydrophobic amino acid residues (WRR; Corbit et al., 2005). This motif is also conserved in *Drosophila Smo* (Figure 2C). Replacement of the first two amino acids in the ciliary localization motif (Smo^{AAR}) with alanine disrupts the ciliary localization in mouse cell culture experiments (Corbit et al., 2005). Introduction of the AAR mutation into *Drosophila Smo* clearly abolished its entry into the OSN ciliary compartment (Figures 2D and 2E). Activated Smo is transported as a multimer complex (Shi et al., 2013; Zhao et al., 2007). The expression of Smo^{AAR} attenuated the ciliary localization of endogenous Smo (Figure 2F), implying that ciliary localization requires the multimerization of Smo. Both Smo^{AAR} and Smo:GFP in the *IFT172* knockdown flies localized at the base of the cilia (Figures 2B, 2E, and 2F), indicating a loss of cilia transport. Together, these results demonstrate that ciliary localization of Smo is conserved from invertebrates to vertebrates.

Ptc Localizes to Cilia and Controls Smo Stability

Expression of Hh was found in both OSNs and support cells (Figures 1B and 1D). Knockdown of *Hh* in OSNs decreased Smo:GFP stability and cilia levels, indicating that OSNs produce and respond to Hh in an autocrine fashion (Figures 3A and 3B). In vertebrates, the Sonic hedgehog receptor Patched1 resides in the cilium, and the binding of Sonic hedgehog has been proposed to trigger movement of Patched1 out of the cilium (Rohatgi et al., 2007). In the OSNs, Ptc:GFP localized to sparse, small puncta in the cilia (Figure 3C). This low occurrence of Ptc:GFP in cilia might have been due to the expression of Hh in the antenna. In the wing disc, Hh binding leads to endosomal internalization and subsequent degradation of the Ptc-Hh complex (Lu et al., 2006; Torroja et al., 2004). To address this possibility, we expressed Ptc^{14}*:GFP*, a mutant that is not endocytosed upon Hh binding (Torroja et al., 2004). Indeed, Ptc^{14}:GFP showed increased localization to cilia compared with Ptc:GFP (Figure 3C), implying that Ptc is removed from the cilia via endocytosis and that endocytosis occurs upon binding of Hh. In vertebrates, Patched1 and Smo are reciprocally transported in the cilium (Rohatgi et al., 2007). In the canonical *Drosophila* Hh pathway, Ptc controls the stability rather than the localization of Smo (Denef et al., 2000; Li et al., 2012; Nakano et al., 2004; Xia et al., 2012; Zhu et al., 2003). To investigate whether Ptc regulates the stability and/or ciliary localization of Smo in OSNs, we manipulated *Ptc* expression. Overexpression of *Ptc* resulted in a uniform loss of Smo in both the cytoplasm and cilia (Figures 3A and 3B). This result implies that Ptc controls Smo stability in OSNs. Knockdown of *Ptc* led to an increase of Smo in axons and cell bodies, but in cilia the Smo level still varied (Figures 3A and 3B), indicating that mechanisms other than Ptc control Smo ciliary transport in OSNs.

Cos2 Functions as a Ciliary Kinesin that Transports Smo

To investigate whether there are other transport systems that control Smo ciliary localization, we turned to the kinesin-like protein Costal 2 (Cos2). *Cos2* is required for Smo transport in the wing disc (Farzan et al., 2008; Liu et al., 2007; Robbins et al., 1997; Ruel et al., 2007; Shi et al., 2011) and has two vertebrate orthologs, Kif7a and Kif27 (Cheung et al., 2009; Endoh-Yamagami et al., 2009; Liem et al., 2009; Varjosalo et al.,

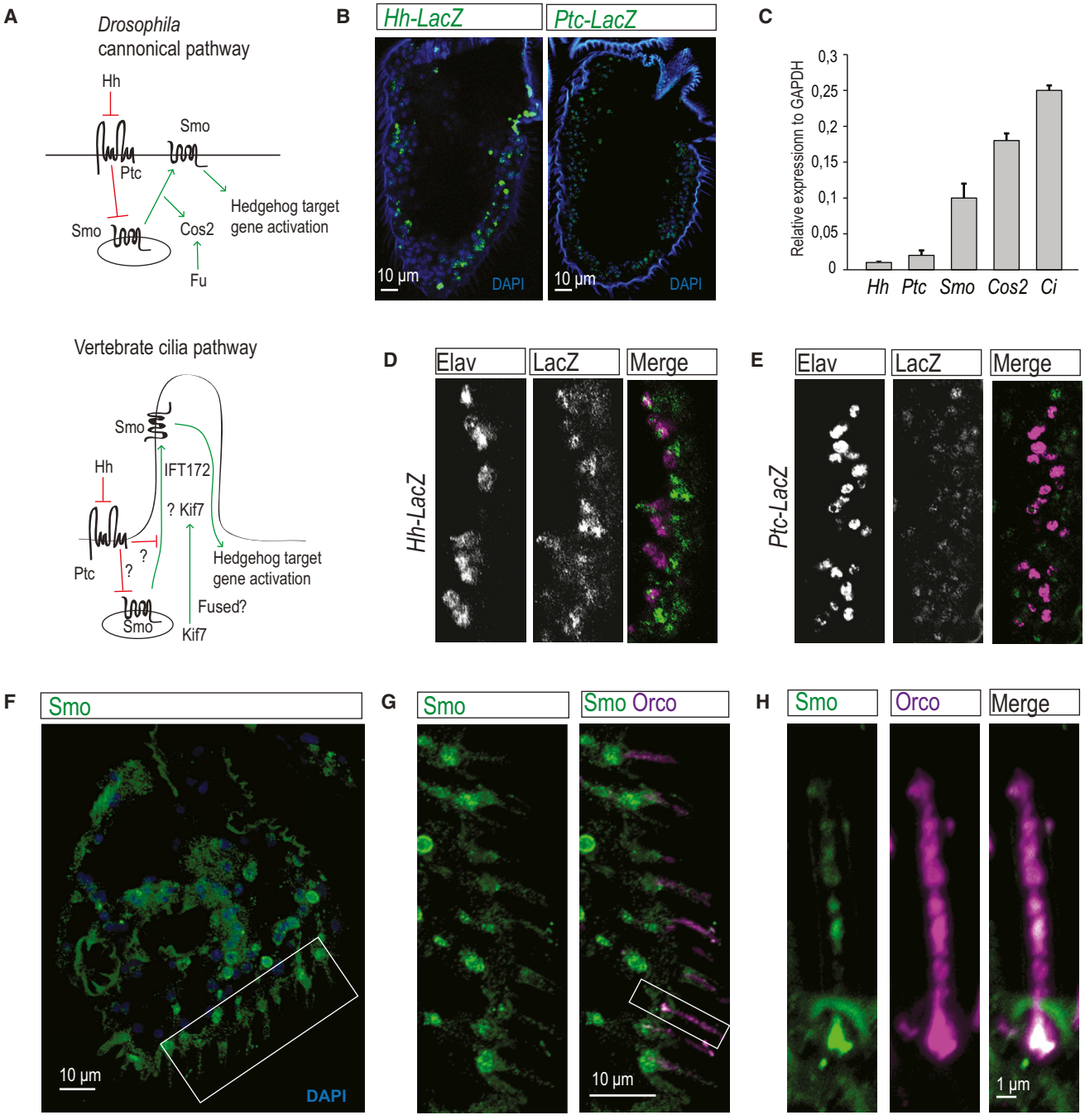

Figure 1. Smo Localizes to OSN Cilia in *Drosophila*

(A) Schematic view of canonical, nonciliated Hh signaling in *Drosophila* and cilia-mediated Hh signaling in vertebrates.

(B) *Hh-lacZ* and *Ptc-lacZ* expression in the antenna (green, lacZ; blue, DAPI and cuticle).

(C) Quantitative PCR of antennae from 4- to 5-day-old flies shows expression of *Hh*, *Ptc*, *Smo*, *Cos2*, and *Ci* relative to *GAPDH*.

(D) OSNs and the surrounding support cells express *Hh-lacZ* (green, lacZ; magenta, elav).

(E) *Ptc-LacZ* is expressed in the OSNs and the surrounding support cells (green, lacZ; magenta, elav).

(F) Endogenous Smo (green) localizes to OSN cell bodies, dendrites, and cilia. The box highlights the OSN cilia. Nuclei are marked by DAPI (blue).

(G) In the cilia, Smo (green) colocalizes with the odorant coreceptor, Orco (magenta).

(H) Magnified view of one OSN sensillum (boxed in G), Smo (green), and Orco (magenta).

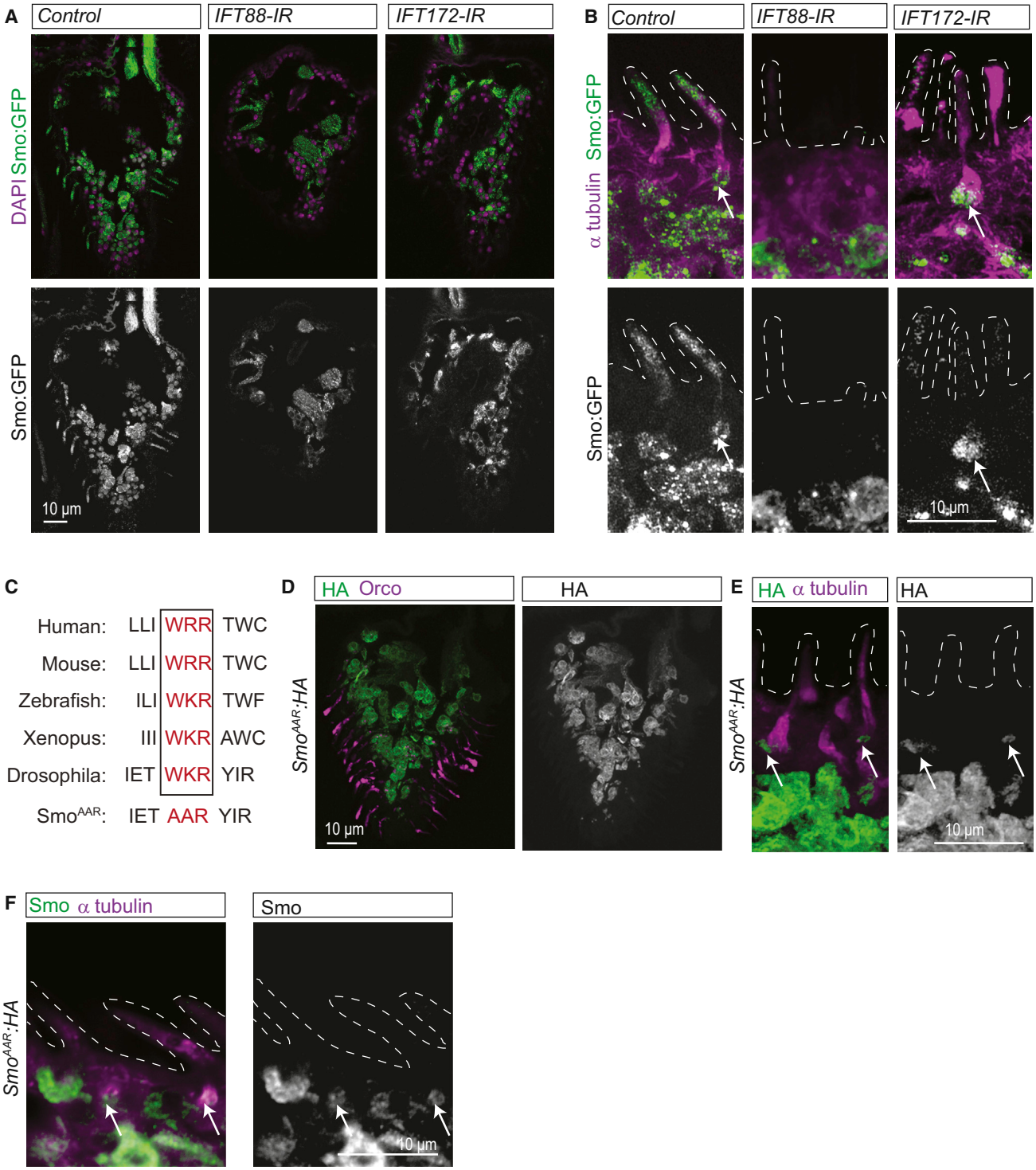

Figure 2. IFT Controls Smo Ciliary Localization

(A) Smo:GFP (green) localizes to cell bodies and cilia in control antenna. RNAi produced by the expression of inverted repeats (-IR) of *IFT88* and *IFT172* attenuate the localization of Smo:GFP to cilia. Nuclei are marked by DAPI (magenta).

(B) Magnified view of cilia marked by α-tubulin (magenta). The dotted line outlines the cilia region that extends into the sensilla. The base of each cilium is characterized by cone-shaped staining of α-tubulin (arrows, in all high-magnification images). Knockdown of *IFT88* reduces the number of cilia, whereas knockdown of *IFT172* causes little change in cilia structure. Smo:GFP (green) shows a marked accumulation at the cilia base in *IFT172-IR* and attenuated dendritic transport in *IFT88-IR* OSNs.

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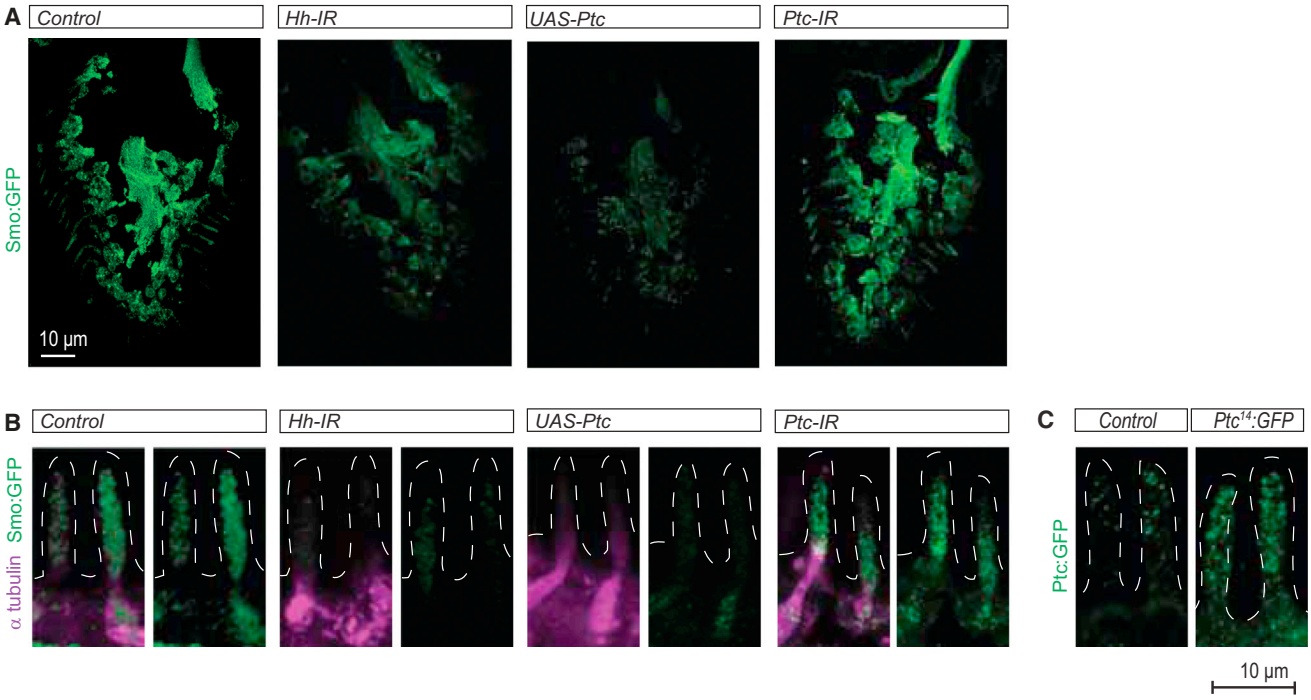


Figure 3. Ptc Localizes to Cilia and Regulates Smo Stability

(A) Smo:GFP levels (GFP, green) in *Hh-IR* flies are reduced compared with control. *Ptc-IR* causes a moderate increase, whereas overexpression of *Ptc* causes a loss of Smo:GFP.

(B) Magnified view of cilia marked by α-tubulin (magenta). The dotted line outlines the cilia region that extends into the sensilla. There was marked loss of Smo:GFP staining in cilia of *Hh-IR* and *UAS-Ptc*. Knockdown of *Ptc* (*Ptc-IR*) showed increased Smo:GFP in cilia.

(C) Ptc:GFP localizes to cilia and the cilia localization is increased in the endocytosis mutant Ptc^{14}:GFP. (Green, GFP).

See also Figure S1 and Table S1.

2006). Genetic experiments in zebrafish and mice have indicated that Kif7a has negative and positive regulatory roles in the Hh pathway (Cheung et al., 2009; Endoh-Yamagami et al., 2009; Liem et al., 2009; Maurya et al., 2013). In vitro studies have shown that Kif7a accumulates in cilia upon Shh stimulation (Endoh-Yamagami et al., 2009; Liem et al., 2009). Similarly to Kif7a, Cos2:GFP localized to cilia in a Hh-dependent manner (Figure 4A). *Cos2* and other kinesins contain ATPase motor domains that are required for their movement and the transport of cargo along microtubules (Farzan et al., 2008; Ho et al., 2005). To investigate whether Cos2 movement is required for cilia transport of Smo, we expressed Cos2 with a deleted motor domain ($Cos2^{\Delta motor}$; Ho et al., 2005) in the OSNs. Upon $Cos2^{\Delta motor}$ expression, the level of Smo:GFP decreased in cilia (Figures 4C–4E). At the same time, the tubulin staining showed that the cilia were thinner in the $Cos2^{\Delta motor}$ OSNs (Figures 4D and 4E). Together, our data show that *Cos2* is required for transport of Smo and likely other cargos in the cilia.

Fu Regulates Cos2 Ciliary Translocation and Smo Transport

In the wing disc, Cos2 forms a complex with the serine/threonine kinase Fused (Fu), which phosphorylates and activates Cos2, an event that is required for the membrane targeting of Smo (Liu et al., 2007; Ranieri et al., 2012; Zhou and Kalderon, 2011). In vertebrates, the function of Fused (Stk36) is unclear, and a second Fused kinase family member, Ulk3, has been proposed to play a redundant role (Maloverjan et al., 2010; Wilson et al., 2009). In the OSNs, hemagglutinin (HA)-tagged Fu did not enter the ciliary compartment (Figure 4B). To investigate whether Fu regulates the ciliary localization of Cos2 in *Drosophila*, we expressed a kinase-dead version of *Fu* (Fu^{G13V}; Liu et al., 2007). Fu^{G13V} prevented the localization of Cos2:GFP to cilia (Figure 4A), which shows that Fu kinase activity is required for Cos2 ciliary localization. In addition, *Fu* knockdown and Fu^{G13V} expression caused a phenotype similar to that of $Cos2^{\Delta motor}$ with decreased Smo:GFP staining in the cilia (Figure 4C). We

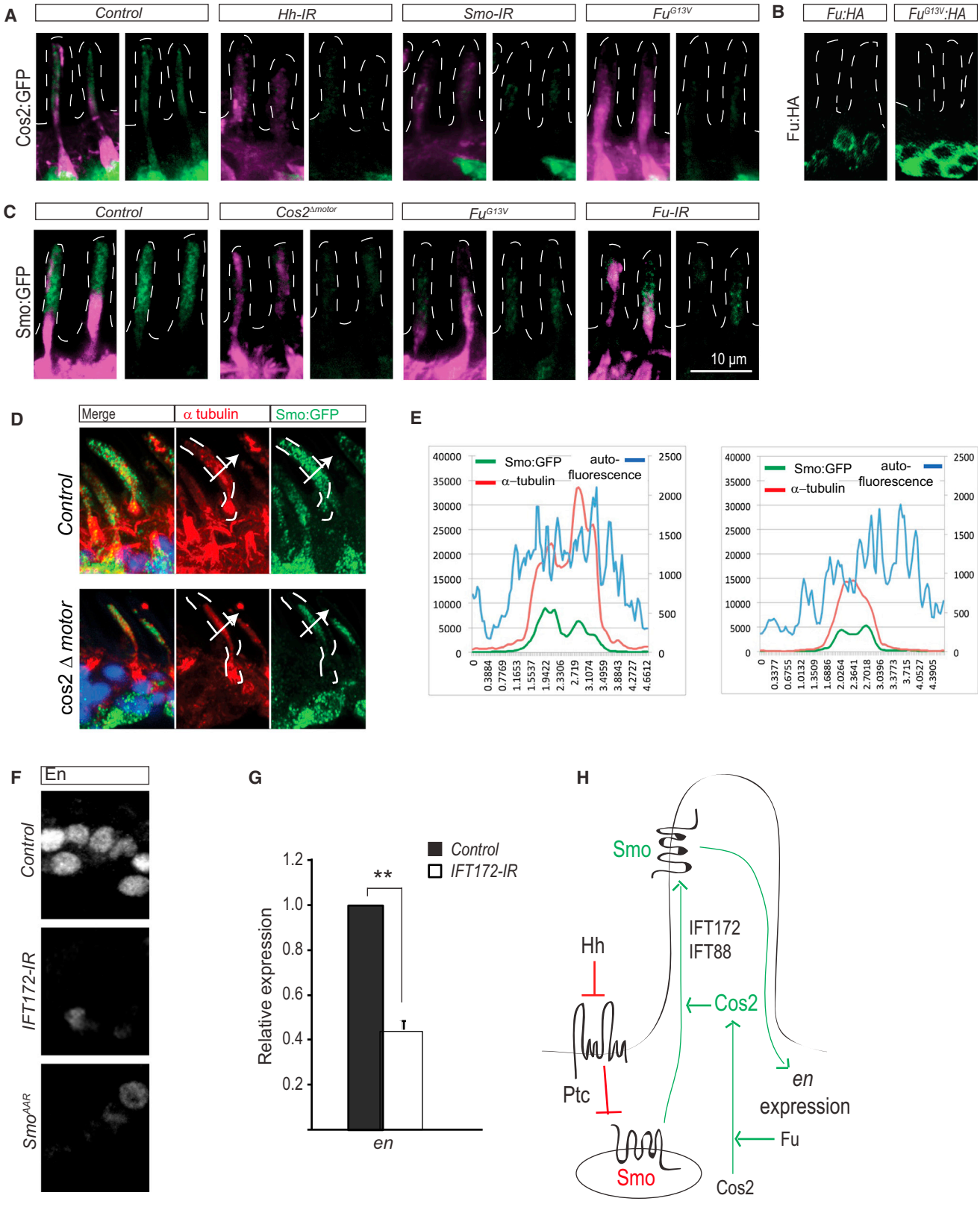
(C) Alignment of the ciliary localization motif in vertebrate and *Drosophila Smo*.

(D) Overexpressed *Drosophila* Smo with a mutated ciliary localization motif (Smo^{AAR}:HA, green) is stable in the OSN soma and dendrites, but fails to enter the ciliary compartment marked by Orco (magenta).

(E) Magnified view of cilia marked by α-tubulin (magenta) shows devoid cilia transport and accumulation at the cilia base of Smo^{AAR}:HA (green, HA).

(F) Smo^{AAR}:HA attenuates ciliary transport of endogenous Smo (green, Smo; magenta, α-tubulin).

See also Table S1.



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therefore propose that Fu regulates Smo transport within cilia by phosphorylating Cos2.

Smo Localization to Cilia Controls the Expression of Hh Target Genes

In the last step of the canonical Hh pathway in both vertebrates and *Drosophila*, the transcription factor Gli/Ci switches from repression to activation of Hh target genes (Huangfu and Anderson, 2006). In vertebrates, the switch involves the localization of Gli factors to the primary cilia (Goetz and Anderson, 2010; Huangfu et al., 2003; Ingham et al., 2011; Zhang et al., 2011). In the OSNs, the Ci level was extremely low, with no detectable staining in cilia (data not shown). In *Drosophila*, the Hh target gene *engrailed* (*en*) is expressed in mature OSNs (Blagburn, 2008). En showed a varied staining pattern in the OSNs (Figure 4F), which is in line with the varying levels of Hh expression and Smo in the cilia (Figures 1B, 1D, and 1F). To measure Ci activity, we monitored the En levels by immunofluorescence. *en* expression levels were reduced in *IFT172-IR* antenna (Figure 4F), which was further confirmed by quantitative PCR (Figure 4G). Because IFT172 is also required for Smo localization to cilia (Figures 2A and 2B), it is tempting to conclude that IFT172 suppresses en expression by inhibiting Smo localization to cilia. This notion is further supported by the finding that expression of the ciliary localization mutant Smo^{AAR} resulted in decreased *en* expression (Figure 4F). Together, these findings suggest that the ciliary localization of Smo regulates Ci function and *en* expression. Thus, our results show that cilia are required for all aspects of Hh signaling in *Drosophila* OSNs (modeled in Figure 4H).

DISCUSSION

Cilia-mediated Hh signaling is involved in several human pathologies and has been thought to not exist outside vertebrates (Corbit et al., 2005; Goetz and Anderson, 2010; Huangfu et al., 2003; Ingham et al., 2011; Roy, 2012; Wong and Reiter, 2008). We demonstrate here that the nonmotile OSN cilia in *Drosophila* are involved in Hh signal transduction and that this ciliary function is conserved from *Drosophila* to vertebrates. The existence of this second cilia-dependent Hh pathway in *Drosophila* shows that Hh signaling can be mediated via two pathways within a single organism. Our results further demonstrate that the core components are shared between the two Hh pathways in *Drosophila*. The function of Cos2 as a putative kinesin in the ciliary compartment indicates that the ancestral Hh signaling pathway may have been cilia specific and that invertebrate cells did not maintain this specialization. Interestingly, not all vertebrate cells have primary cilia (Wheatley, 1995), and different types of tumors react differently to Shh depending on whether they are ciliated (Han et al., 2009; Ho et al., 2013), indicating that there might be a second, overlooked nonciliary pathway in vertebrates.

Our genetic in vivo analysis of Smo ciliary localization revealed that, as in vertebrates (Ocbina and Anderson, 2008), the ciliary IFT system and a ciliary localization signal are required for localization of Smo to cilia in *Drosophila*. Our results further show that the Hh receptor Ptc regulates Smo stability and that ciliary localization depends on the activation of the kinesin-like protein Cos2. In the *Drosophila* wing disc, *Fu* regulates *Cos2* function and is required for most aspects of Hh signaling (Liu et al., 2007; Zhang et al., 2011; Zhou and Kalderon, 2011). Our data show that *Fu* is also required for Cos2 ciliary localization and Smo transport within the cilia. However, Fu is not essential for mammalian Hh signaling, and in zebrafish, loss of Fu results in weak Hh-related morphological phenotypes (Chen et al., 2005; Merchant et al., 2005; Wilson et al., 2009; Wolff et al., 2003). These differences from the *Drosophila* pathway and vertebrate ciliary signaling could be explained by the existence of a second, as yet unidentified kinase with an analogous function. Cell culture and in vivo studies in vertebrates led to the identification of four kinases with phenotypes related to Fu: Ulk3 (Maloverjan et al., 2010), Kif11 (Evangelista et al., 2008), Map3K10, and Dyrk2 (Varjosalo et al., 2008). Further investigation is required to determine whether these kinases control the ciliary transport of Smo and whether Cos2 Smo transport is conserved in vertebrates. Yet, our results demonstrate that cilia-mediated Hh signaling does occur in *Drosophila* and that this pathway is conserved in vertebrates, which makes the *Drosophila* OSN a powerful in vivo model for studying Hh signaling and its ciliary transport regulation.

EXPERIMENTAL PROCEDURES

Drosophila Stocks

The following fly stocks were used: *Pebbled-Gal4* (Jafari et al., 2012); RNAi lines from the VDRC (*Orco-IR* [v13386], *IFT88-IR* [v104419], and *IFT172-IR* [v24795]); RNAi lines from the Transgenic RNAi Project (Bloomington stock number: *Hh-IR* [32489], *Ptc-IR* [28795], *Smo-IR* [27037], *Fu-IR* [35258]); *UAS-Dcr2* (VDRC); *UAS-Smo:GFP* (a gift from J. Jia); *UAS-Ptc:GFP* (a gift from T. Kornberg); $UAS\text{-}Ptc^{14}\text{:}GFP$ (a gift from I. Guerrero); *UAS-Cos2:GFP*, $UAS\text{-}Cos2^{\Delta motor}$ (a gift from M. Scott.); *UAS-Fu:HA*, $UAS\text{-}Fu^{G13V}\text{:}HA$ (a gift

Figure 4. Cos2 and Fu Regulate Smo Ciliary Localization, which Is Required for Hh Pathway Activation

(A) Cilia marked by α-tubulin (magenta). The dotted line outlines the cilia region that extends into the sensilla. The ciliary localization of Cos2:GFP (green, GFP; magenta, α-tubulin) requires *Hh* and *Smo* expression (*Hh-* and *Smo-IR*) and Fu kinase activity (Fu^{G13V}, kinase dead).

(B) Fu:HA and kinase-dead Fu^{G13V}:HA localize to OSN cell bodies.

(C) The ciliary transport of Smo:GFP (GFP green; α-tubulin magenta) requires Cos2 movement ($Cos2^{\Delta motor}$), Fused (*Fu-IR*), and Fu kinase activity ($Fu^{G13V}\text{:}HA$).

(D) $Cos2^{\Delta motor}$ OSNs stained with α-tubulin (magenta) or Smo:GFP (green) show thinner cilia structures compared with control.

(E) Cross-section profiles of deconvoluted z-stack maximum projections show that cilia are thinner in $Cos2^{\Delta motor}$ OSNs compared with control. Each cross-section is marked by an arrow in the images in (D). Cuticle autofluorescence in the DAPI window is shown as a reference to outline the sensillum.

(F) En staining is decreased in antennas that express *IFT172-IR* or Smo^{AAR}.

(G) Quantitative PCR shows attenuated *en* expression in *IFT172-IR* antennae compared with control antennae ($p < 0.0001$; error bars represent SEM).

(H) Model depicting cilia-mediated Hh signaling in *Drosophila*.

See also Figure S1 and Table S1.

from J. Jia); *UAS-Ptc* (Bloomington, 5817); Hh-LacZ (Bloomington, 5530); and Ptc-LacZ (Bloomington, 10514).

Construction of *UAS-Smo^{AAR}*

Smo cDNA with the AAR mutation fused to a 3×HA tag was synthesized by GenScript and cloned into pUAST.

Immunohistochemistry and Antibodies

The following primary antibodies were used: goat anti-Smo (1:50, dC-20; Santa Cruz Biotechnology; Ogden et al., 2003), rabbit anti-Orco (1:20,000; a gift from R. Benton), rabbit anti-GFP (1:2,000, TP-401; Torrey Pines), mouse anti-α-tubulin (1:250, AA4.3c; DSHB), rabbit β-galactosidase (1:1,000, Millipore), rat anti-HA (1:100, Roche), and mouse anti-en (1:10; DSHB). Secondary antibodies were conjugated with Alexa Fluor 488 or Alexa Fluor 568 (1:500; Molecular Probes). Antenna immunohistochemistry was performed as previously described (Couto et al., 2005). Confocal microscopy images were collected on an LSM 700 (Zeiss) and analyzed on an LSM Image Browser. For deconvolution, images were oversampled with a voxel size of 0.05 × 0.05 × 0.140 μm. Deconvolution was performed with Huygens software version 4.4.

Quantitative PCR

To evaluate changes in RNA levels, total RNA from antenna was extracted with TRIzol reagent (Invitrogen) and reverse transcribed with a SuperScript VILO cDNA synthesis kit (Invitrogen). Quantitative PCR was carried out on an Applied Biosystems 7900HT real-time PCR system (Life Technologies) using the Power SYBR Green PCR master mix (Applied Biosystems, Life Technologies) and primer sets designed using Primer Express software v3.0.1 (Integrated DNA Technologies). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or αTub84B was used as an internal control to normalize samples. Quantitative PCR for each primer set was performed on both control and mutant samples for 40 cycles. Following amplification, melt curve analysis and ethidium bromide agarose gel electrophoresis were performed to evaluate the PCR products. The relative quantification of the fold change in mRNA expression was calculated using the $2^{-\Delta\Delta CT}$ threshold cycle method.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.03.052.

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