

Primary Cilia and Mammalian Hedgehog Signaling

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It has been a decade since it was discovered that primary cilia have an essential role in Hedgehog (Hh) signaling in mammals. This discovery came from screens in the mouse that identified a set of genes that are required for both normal Hh signaling and for the formation of primary cilia. Since then, dozens of mouse mutations have been identified that disrupt cilia in a variety of ways and have complex effects on Hedgehog signaling. Here, we summarize the genetic and developmental studies used to deduce how Hedgehog signal transduction is linked to cilia and the complex effects that perturbation of cilia structure can have on Hh signaling. We conclude by describing the current status of our understanding of the cell-type-specific regulation of ciliogenesis and how that determines the ability of cells to respond to Hedgehog ligands.

Hedgehog (Hh) is one of a handful of signaling pathways that is used repeatedly for intercellular communication in development. Hh is critical for the development of nearly every organ in mammals, as well as in homeostasis and regeneration, and Hh signaling is disrupted in several types of cancer. Unlike other core developmental signaling pathways, vertebrate Hh signaling is completely dependent on a highly specialized organelle, the primary cilium.

The *Hh* gene was discovered in *Drosophila* based on the striking phenotype of fly larvae that lack Hh—the mutants do not develop the segmented anterior-to-posterior body plan and have ectopic denticles resembling a Hedgehog (Nüsslein-Volhard and Wieschaus 1980). Mutations in other genes with related phenotypes defined a signaling pathway in which Hh is the

ligand that acts through the membrane receptor Patched (PTCH1) and the seven transmembrane spanning protein Smoothed (SMO) to control the activity of a transcription factor Cubitus interruptus (Ci) (Forbes et al. 1993; Ingham 1998). In the mid-1990s, embryological experiments in chicks and targeted mutations in the mouse homologs of the *Drosophila* Hh pathway genes showed that the Hh signaling pathway is also critical for the development of many tissues and organs in vertebrates. Both the core players and their pathway relationships are conserved (Fig. 1) (Ingham and McMahon 2001). Because of this evolutionary conservation, it was completely unexpected when phenotype-based genetic screens in the mouse identified a set of proteins required for the formation of primary cilia that were also required

Editors: Wallace Marshall and Renata Basto
Additional Perspectives on Cilia available at www.cshperspectives.org

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Cite this article as *Cold Spring Harb Perspect Biol* 2017;9:a028175

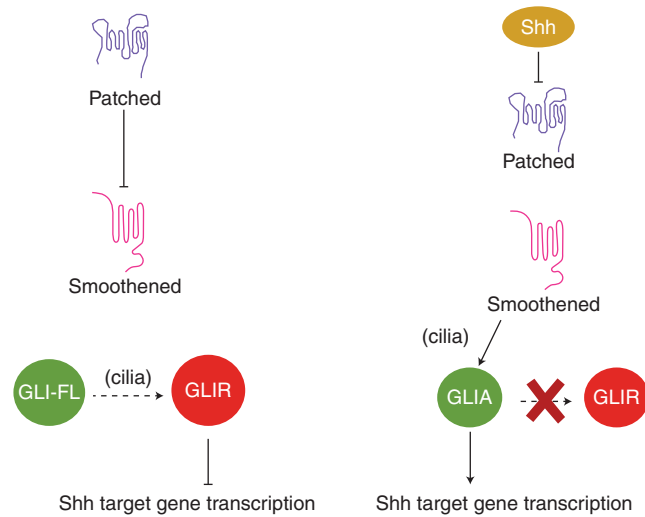


Figure 1. The backbone of the Hedgehog (Hh) signal transduction pathway. The core of the Hh signaling pathway is conserved between *Drosophila* and vertebrates. In the absence of ligand, the Hh receptor Patched (PTCH1) keeps the pathway off by inhibiting the activity of the seven transmembrane-domain protein Smoothed (SMO). When SMO is inactive, the GLI/Ci (glioblastoma/Cubitus interruptus) transcription factors are proteolytically processed to make a transcriptional repressor that binds to Hh target genes and blocks their transcription. Binding of Hh to PTCH1 inhibits its activity, relieving the repression of SMO, which promotes conversion of full-length GLI/Ci into a transcriptional activator. In vertebrates, cilia are required for the production of both GLI-repressor and GLI-activator.

for mammalian (but not *Drosophila*) Hh signaling. The relationship between primary cilia and mammalian Hh signaling has turned out to be fascinating, complex, and directly relevant for specific human diseases, the ciliopathies.

Here, we describe the nature of primary cilia Hh-dependent patterning during mammalian development, with a primary focus on proteins that have been shown to affect Hh signaling in vivo in genetic studies. The combination of genetics, developmental biology, and cell biology has provided overwhelming evidence that mammalian Hh signal transduction absolutely depends on the primary cilium, defined the features of the cilium that are important for signaling, and characterized how different changes in cilia structure have distinct effects on Hh signaling. From these studies, the view emerges that Hh signaling and the primary cilium have coevolved, concentrating critical signaling components in the very small volume at the tip of the cilium to allow efficient responses to low levels of ligand.

HEDGEHOG SIGNALING IN MAMMALIAN DEVELOPMENT

There are three mammalian Hh proteins, Sonic (SHH), Indian (IHH), and Desert (DHH). SHH and IHH have important, and sometimes overlapping, functions in many tissues. SHH has particularly striking roles in specification of cell types in the nervous system and in patterning of the limbs, whereas IHH has critical roles in skeletal development. Many studies have connected both SHH and IHH signaling to the primary cilium (see below). DHH appears to be restricted to the gonads and may also depend on primary cilia for its activity (Nygaard et al. 2015).

Drosophila Hedgehog has important roles in the patterning of adult organs, such as the eye, wing, and leg (Burke and Basler 1997). In these contexts, Hh acts as a short-range inducer of cell fate, inducing target gene expression in a range of 2–3 cell diameters away from the source of the signal. *Drosophila* Hh can activate



expression of secreted proteins, such as Dpp (Bmp4) that can move further in tissues; as a result, *Drosophila* Hh has a profound role in the patterning of entire organs.

In contrast to the short-range signaling of *Drosophila*, the vertebrate Hh proteins can act over a field of cells that are many cell diameters away from the source of the protein. In neural patterning, SHH is first expressed in the notochord, which lies below the neural tube, and acts directly on cells in the ventral half of the neural tube to specify neural progenitors (Fig. 2) (Briscoe and Ericson 2001), and it appears that cells at least 20 cell diameters away from the source respond to Shh. In the limb, Shh is expressed in a classical organizer, the zone of polarizing activity (ZPA), and influences the pattern across most of the developing limb, again at a distance from its source (Ahn and Joyner 2004; Harfe et al. 2004). Indian hedgehog also acts at a distance to control proliferation and differentiation of chondrocytes (Long et al. 2001; Mak et al. 2008). Thus, one significant difference between Hh signaling in vertebrates and *Drosophila* appears to be that Hh ligands act at greater distances from their source in vertebrates; we hypothesize that long-range signaling requires a sensitized signal transduction system dependent on the primary cilium.

INTRAFLAGELLAR TRANSPORT IS REQUIRED FOR SHH SIGNAL TRANSDUCTION IN THE MOUSE EMBRYO

In the developing mouse embryo, perhaps the best-studied function of Shh is to direct patterning of neural progenitors, in which molecular markers make it possible to distinguish six different cell types that are specified by different levels of Shh activity (Briscoe and Ericson 2001; Dessaud et al. 2008). SHH made by the notochord induces formation of the floor plate and then specifies five additional neural cell types—V3 interneuron progenitors are specified adjacent to the floor plate by prolonged high levels of Shh signaling; motor neurons are determined by lower levels of Shh activity; and more dorsal interneurons are specified by yet lower levels of Shh. The final activity of the pathway depends

on both ligand concentration and duration of exposure to ligand, and final cell fate depends on a regulatory network set into motion by SHH (Dessaud et al. 2007; Balaskas et al. 2012). In the absence of SHH or its positive effector SMO, ventral neural cell types fail to be specified, whereas loss of the negative regulator PTCH1 leads to the formation of ventral cell types by all neural progenitors (Fig. 2).

The seminal discovery that linked the primary cilium to Hedgehog signaling in the mouse came from the characterization of chemically induced mutations, such as the *wimple* mutation that lacked Shh-dependent ventral neural cell types and affected genes that encoded intraflagellar transport (IFT) proteins (Huangfu et al. 2003). IFT is the process by which the cilium is assembled and maintained. It is powered by two conserved, dedicated microtubule motors—anterograde transport from the base to the tip depends on a plus-end-directed heterotrimeric kinesin-2 complex, KIF3a, KIF3b, and KIF associated protein 3 (KAP3) (Cole 1999) and retrograde transport from the tip to the base requires the minus-end-directed cytoplasmic dynein2 motor made up of a heavy chain, an intermediate chain, light intermediate chain, and several light chains (Hou and Witman 2015). These motors are coupled to the cargos by large electron-dense IFT trains, made up of two protein complexes, IFT-A and IFT-B (Pigino et al. 2009; Behal and Cole 2013).

Map-based cloning showed that the *wimple* mutation disrupted the gene encoding IFT172, an IFT-B complex protein, resulting in a complete lack of primary cilia. The same screen identified a partial loss-of-function allele of another IFTB complex protein, IFT88, which partially disrupted cilia assembly and showed a milder disruption of neural patterning. Targeted null alleles of *Ifi88* and *Kif3a* also caused the complete absence of cilia accompanied by a loss of Shh-dependent ventral neural cell types. Although *wimple* mutant embryos lacked ventral neural cell types, the mutants still expressed SHH, suggesting that without a cilium these mutants are unable to respond to Shh signaling (Huangfu et al. 2003). It was subsequently discovered that null mutations in other IFT-B

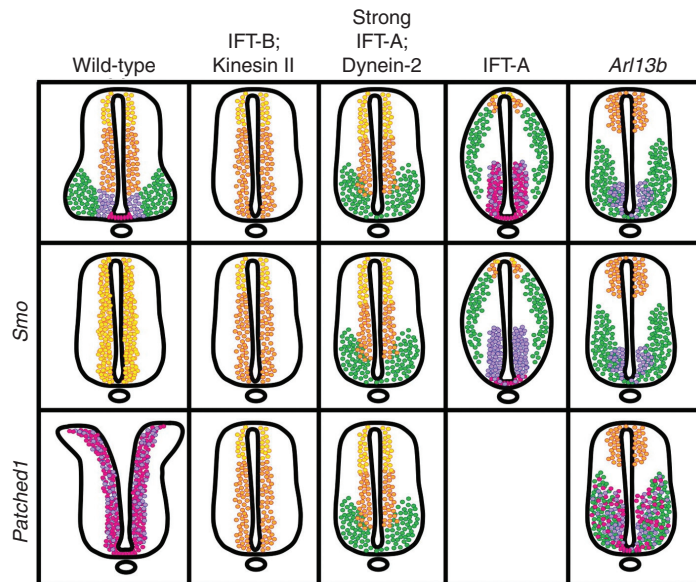


Figure 2. Mutations in cilia genes alter Hedgehog (Hh)-dependent neural patterning. Schematics of the spatial distribution of neural cell types in the developing lumbar neural tube in mutants with abnormal cilia. Dorsal up, ventral down; the notochord is the small oval below the neural tube. SHH ligand released from the notochord and floorplate patterns the dorsoventral axis of the neural tube. The highest levels of Shh signaling specify floorplate cells (pink). Dorsal to the floorplate are V3 neural progenitors, which also require high levels of Shh signaling (magenta). Motor neurons (green) require Shh but are specified at lower concentrations of ligand. V2, V1, and V0 interneurons (orange) require even lower levels of Shh activity. Dorsal progenitors (yellow) are specified by default, and high levels of Shh signaling inhibit the specification of dorsal progenitors. *Smo* mutant embryos fail to specify ventral neural subtypes and all neural progenitors follow a dorsal fate. In *Patched1* mutant embryos, all neural progenitors follow a ventral fate and express markers of the floorplate and V3 neural progenitors. Mouse embryos that lack a core intraflagellar transport (IFT-B) protein or kinesin-II lack primary cilia and therefore are unable to respond to Hh signaling; these mutants lack floorplate, V3 interneurons, and most motor neurons, V0-V2 interneurons and dorsal cell types extend ventrally, but V0-V2 interneurons are specified. *Dync2h1* mutants, which lack the heavy chain of the dynein retrograde IFT motor and strong loss of IFT-A mutant embryos, have very short, bulged primary cilia and cannot transduce Hh signals efficiently; these mutants lack floor plate V3 interneurons and some motor neurons. In combination with Shh, *Smo*, or *Patched1*, double mutant embryos resemble single dynein or strong IFT-A mutants indicating that like IFT-B these components of the cilium assembly machinery are required downstream from SMO and PTCH1. Surprisingly, IFT-A mutant embryos that have a milder effect on cilia morphology (e.g., with cilia of near-normal length and bulged tips) have the opposite effect on neural patterning, with expanded ventral neural cell types. Cilia in *Arl13b^{hmn/hmn}* mutant embryos have structural defects in the microtubule axoneme, and the mutants fail to specify both the most ventral and most dorsal neural cell types. Double mutants that lack both a cilia component and *Smo* or *Patched1* have phenotypes similar to the cilia single mutants, indicating that the cilia machinery is required downstream from *Ptch1* and *Smo*.

genes (IFT52, IFT54, and IFT57) also block cilia formation and Shh-dependent specification of ventral neural cell types (Table 1), providing strong evidence that the primary cilium is required for a cell to receive Shh signals.

Double-mutant analysis defined the step in the Hh signaling pathway that requires

IFT. PTCH1, the Hh receptor, is a negative regulator of Hh signaling. *Ptch1^{-/-}* mutant embryos die at E9.0 and express markers of ventral neural cell types (floor plate and V3 interneuron progenitors) throughout the neural plate, even far from the source of SHH (Fig. 2). In contrast, double mutants that

Table 1. Subtle changes in cilia structure can decrease or increase Hh pathway activity, suggesting that the cilium provides more than just a permissive context for Hh signaling

Mouse gene	Function in cilia	Mouse embryo Hh pathway null phenotype (neural and limb)	Human phenotype
<i>Kif3a</i>	Anterograde IFT motor	Loss of SHH-dependent ventral neural cell types; polydactyly; midgestation lethality (Huangfu et al. 2003)	
<i>Dync2h1</i>	Retrograde IFT motor	Partial loss of ventral neural cell types; polydactyly; midgestation lethality (Huangfu and Anderson 2005; May et al. 2005)	Short rib dysplasia, with or without polydactyly (SRP) (Dagoneau et al. 2009; Merrill et al. 2009)
<i>Dync2li1</i> (<i>D2lic</i>)	Dynein light intermediate chain		SRP (Taylor et al. 2015), Jeune syndrome (JATD) (Kessler et al. 2015)
<i>Wdr34</i>	Dynein light chain		SRP (Huber et al. 2013)
<i>Tctex1d2</i>	Light chain, IFT dynein		Jeune syndrome
<i>Ift25</i> (<i>Hspb11</i>)	IFT-B	Perinatal lethality; weak loss of ventral neural cell types; partially penetrant polydactyly (Keady et al. 2012)	
<i>Ift27</i>	IFT-B	Perinatal lethality; weak loss of ventral neural cell types; partially penetrant polydactyly (Eguether et al. 2014)	
<i>Ift38</i> (<i>Cluap1</i>)	IFT-B	Midgestation lethality; abnormal neural patterning (Botilde et al. 2013)	
<i>Ift52</i>	IFT-B	Loss of SHH-dependent ventral neural cell types; midgestation lethality; polydactyly (Liu et al. 2005)	
<i>Ift54</i> (<i>Traf3ip1</i>)	IFT-B	Loss of SHH-dependent ventral neural cell types; midgestation lethality; polydactyly (Berbari et al. 2011)	Senior-Løken syndrome (Bizet et al. 2015)
<i>Ift57</i> (<i>Hippi</i>)	IFT-B	Loss of SHH-dependent ventral neural cell types; midgestation lethality; polydactyly (Houde et al. 2006)	
<i>Ift80</i> (<i>WDR56</i>)	IFT-B	Hypomorphic allele only: short long bones; polydactyly; late gestation lethality (Rix et al. 2011)	SRP, JATD
<i>IftT88</i> (<i>Polaris</i> ; <i>Tg737</i>)	IFT-B	Loss of SHH-dependent ventral neural cell types; midgestation lethality; polydactyly (Huangfu et al. 2003)	
<i>Ift172</i>	IFT-B	Loss of SHH-dependent ventral neural cell types; midgestation lethality (Huangfu et al. 2003)	Jeune (Halbritter et al. 2013)
<i>Ttc26</i> (<i>hop</i>)	IFT-B	Partial loss of function allele only; abnormal neural patterning (Swiderski et al. 2014)	
<i>Ift121</i> (<i>Wdr35</i>)	IFT-A	Polydactyly (Mill et al. 2011)	SRP (Mill et al. 2011); Ellis-van Creveld (EVC) syndrome (Caparrós-Martín et al. 2015)

Continued

Table 1. *Continued*

Mouse gene	Function in cilia	Mouse embryo Hh pathway null phenotype (neural and limb)	Human phenotype
<i>Ift122</i>	IFT-A	Expansion of SHH-dependent ventral neural cell types; midgestation lethality; polydactyly (Qin et al. 2011)	Sensenbrenner syndrome (Walczak-Sztulpa et al. 2010)
<i>Ift139</i> (<i>Ttc21b</i> / <i>Thm1</i> / <i>alien</i>)	IFT-A	Expansion of SHH-dependent ventral neural cell types; late gestation lethality; polydactyly (Tran et al. 2008)	NPHP12, JATD/SRP (Davis et al. 2011)
<i>Ift140</i>	IFT-A	Polydactyly, neural tube closure defects, (Miller et al. 2013)	SRTD9 (Perrault et al. 2012)
<i>Ift144</i> (<i>Wdr19</i>)	IFT-A	Weak allele: GOF neural; polydactyly. Strong allele: LOF (Liem et al. 2012) (Wicking)	Various: Senior-Løken, Sensenbrenner, Jeune, NPHP, RP (Bredrup et al. 2011; Fehrenbach et al. 2014)
<i>Tulp3</i>	IFT-A-associated	Expansion of SHH-dependent ventral neural cell types; midgestation lethality; polydactyly (Norman et al. 2009; Patterson et al. 2009)	
<i>Gpr161</i>	GPCR	Expansion of SHH-dependent ventral neural cell types; midgestation lethality (Mukhopadhyay et al. 2013)	
<i>C2cd3</i> (<i>chick talpid2</i>)	Distal appendage protein	Loss of SHH-dependent ventral neural cell types; midgestation lethality; polydactyly (Hoover et al. 2008; Ye et al. 2014)	Skeletal dysplasia (Cortés et al. 2016)
<i>Ofd1</i>	Centriole and centriolar satellites	Loss of SHH-dependent ventral neural cell types; midgestation lethality (Ferrante et al. 2006)	Oral-facial-digital syndrome (Ferrante et al. 2001)
<i>Ttbk2</i>	Kinase required for cilia initiation; associates with distal appendages	Loss of SHH-dependent ventral neural cell types; midgestation lethality (Goetz et al. 2012)	Spinocerebellar ataxia type 11 (Houlden et al. 2007)
<i>Talpid3</i>	Associated with distal appendages and PCM	Loss of SHH-dependent ventral neural cell types; midgestation lethality; polydactyly (Bangs et al. 2011)	Ciliopathy spectrum (Alby et al. 2015); Joubert syndrome (Stephen et al. 2015)
<i>Inpp5e</i>	Cilia membrane composition	Bielas et al. 2009; Jacoby et al. 2009	Joubert syndrome (JBTS1) (Bielas et al. 2009); MORM syndrome (Jacoby et al. 2009)
<i>Rab23</i>	Not clear	GOF neural; polydactyly (Eggenchwiler et al. 2001)	Carpenter syndrome (Jenkins et al. 2007)
<i>Kif7</i>	Kinesin-4 protein; controls dynamics of axonemal microtubules	Mild expansion of SHH-dependent ventral neural cell types; polydactyly, defective diaphragm (Cheung et al. 2009; Endoh-Yamagami et al. 2009; Liem et al. 2009; Coles and Ackerman 2013; He et al. 2014)	Joubert syndrome; acrocallosal syndrome

Continued

Table 1. Continued

Mouse gene	Function in cilia	Mouse embryo Hh pathway null phenotype (neural and limb)	Human phenotype
<i>Tectonic</i> (<i>Tctn1</i>)	Transition zone protein	Abnormal patterning of SHH-dependent ventral neural cell types (Reiter and Skarnes 2006)	Joubert (Garcia-Gonzalo et al. 2011)
<i>Mks1</i>	Transition zone protein	Abnormal patterning of SHH-dependent ventral neural cell types (Weatherbee et al. 2009)	Meckel syndrome (Kyttälä et al. 2006)
<i>Fuzzy</i>	CPLANE complex	Loss of SHH-dependent ventral neural cell types; late gestation lethality; polydactyly (Heydeck et al. 2009)	
<i>Inturned</i>	CPLANE complex	Loss of SHH-dependent ventral neural cell types; late gestation lethality; polydactyly (Zeng et al. 2010)	
<i>Jbts17</i>	CPLANE complex	Skeletal and heart defects; no change in neural patterning detected (Damerla et al. 2015)	Joubert syndrome (Srour et al. 2012)
<i>Rsg1</i>	Small GTPase, CPLANE-associated	Loss of SHH-dependent ventral neural cell types; late gestation lethality; polydactyly (S Agbu and KV Anderson, in prep.)	
<i>Evc1/Evc2</i>	Ciliary membrane protein	EVC-like (Ruiz-Perez et al. 2007; Caparrós-Martín et al. 2013)	Ellis–van Creveld syndrome (Ruiz-Perez et al. 2003)
<i>Arl13b</i>	Ciliary membrane GTPase	Loss of both the most dorsal and most ventral neural cell types (Caspary et al. 2007)	Joubert syndrome (Cantagrel et al. 2008)
<i>Sas-4 (p53)</i> (<i>Cpap/Cenpj</i>)	Centriole duplication	Loss of SHH-dependent ventral neural cell types; midgestation lethality (Bazzi and Anderson 2014)	Microcephaly (Bond et al. 2005)
<i>DZip1/Iguana</i>	Cilia base	Loss of SHH-dependent ventral neural cell types; midgestation lethality (Wang et al. 2013)	

References noted in table are for specific mouse and human proteins and phenotypes. This is not a complete list of references, and includes only the earliest mouse references, and is not a complete list of phenotypes.

IFT, intraflagellar transport; GPCR, G-protein-coupled receptor; PCM, pericentriolar material; CPLANE, ciliogenesis and planar polarity effector; SHH, Sonic Hedgehog; GOF, gain of function; LOF, loss of function; MORM, mental retardation, truncal obesity, retinal dystrophy, and micropenis.

lack both *Ptch1* and *Ift172* lack all ventral neural cell types, like the *Ift172* single mutants (Fig. 2) (Huangfu et al. 2003). Thus, the ectopic activation of the Hh pathway caused by loss of *Ptch1* depends on IFT172, arguing that IFT is required at a step in the signal transduction pathway downstream from PTCH1. Similar double mutant analysis showed that IFT172, IFT88, and KIF3A act downstream from both PTCH1 and SMO and upstream of the GLI proteins, at the heart of the signal transduction cascade (Fig. 1).

CENTRIOLE AND BASAL BODY PROTEINS ARE ALSO REQUIRED FOR THE RESPONSE TO Hh

In addition to IFT proteins, other proteins that build cilia are also required for the ability of cells to respond to Hh family ligands (Table 1). The doublet microtubules of the cilium are templated by the triplet microtubules of the mother centriole. Embryos that lack centrioles because of the lack of proteins required for centriole duplication, such as SAS4 (CPAP/CENPJ),



can go through normal segregation at mitosis but fail to assemble cilia. These embryos can survive to midgestation (if a cell-cycle checkpoint is blocked by removal of p53) but Shh-dependent ventral neural cell types are not specified (Bazzi and Anderson 2014). The distal appendages of the mother centriole are required for docking on the plasma membrane (Tanos et al. 2013). The distal appendage protein C2cd3 is required for the centriole to dock on the ciliary vesicle (Ye et al. 2014) and *C2cd3* null mutant embryos lack cilia and lack Hh-dependent ventral neural cell types (Hoover et al. 2008). *Talpid3* and *OFD1* are both centrosome-associated proteins required for ciliogenesis and for the response to SHH in the neural tube (Ferrante et al. 2006; Bangs et al. 2011). Tau tubulin kinase 2 (TTBK2) is required for cilia initiation and mutants lack all cilia and Hh-dependent ventral neural cell types (Goetz et al. 2012). Embryos that lack CP110, a protein that localizes to the distal ends of mother centrioles, also have fewer cilia and mild defects in Hh signaling (Yadav et al. 2016).

The CPLANE (ciliogenesis and planar polarity effector) protein complex is located at the basal body and includes downstream mediators of planar cell polarity in *Drosophila*, *Fuzzy*, *Inturned*, and *Fritz* (*Wdpcp*), and *Jbts17*, a gene responsible for Joubert syndrome in humans (Damerla et al. 2015; Toriyama et al. 2016). The CPLANE complex is required for efficient ciliogenesis as it functions to recruit IFT-A proteins to the cilium RSG1, a small GTPase that appears to be a peripheral member of the CPLANE complex. *Rsg1* mutant mice have fewer cilia of normal morphology, suggesting *Rsg1* has a specific role in cilia initiation (S Agbu and KVAnderson, in prep.). These mutants survive longer than IFT-B mutants, yet still display Hh signaling defects. Thus, diverse classes of proteins are required to build cilia and ensure efficient ciliogenesis. Loss of any type of protein required for normal cilia formation in the mouse prevents or attenuates cells from responding to Shh. Thus, it is the primary cilium, rather than another activity of the IFT machinery, which is required for mammalian cells to respond to Hh ligands.

CILIA ARE REQUIRED FOR RESPONSES TO Hh FAMILY LIGANDS IN ALL VERTEBRATE TISSUES

Conditional genetic deletion of *Ift88* and/or *Kif3a* has been used to test the role of cilia in Hh signaling in other mouse tissues. In every context examined, removal of cilia blocks the response to both SHH and IHH (Table 2). The defects observed include shortened bones of the limb, craniofacial defects, and loss of neural stem and progenitor cells.

Cilia are also required for Hh signaling in other vertebrates. As in the mouse, absence of cilia in the chick blocks the ability of cells to receive Hh signals (Yin et al. 2009). In zebrafish, loss of cilia leads to milder defects in Hh-dependent patterning, in part because of the maternal contribution of cilia proteins or mRNAs to the zebrafish embryo, which masks the phenotype in homozygous embryos, and in part because of the different roles of the zebrafish GLI proteins in Hh responses (Huang and Schier 2009; Tay et al. 2010; Ben et al. 2011).

In humans, it is not possible to directly examine the requirement of cilia for Hh signaling during development, as mutations that completely disrupt IFT genes would presumably result in lethality in the first half of gestation, as in mice, and would not be detected. However, many mutations in human cilia genes do not completely ablate cilia, allowing survival to the end of gestation with comparatively mild Hh pathway defects (Table 1). For example, mutations in the cytoplasmic dynein-2 complex cause short ribs (narrow chest)—short-rib dysplasia, with or without polydactyly (SRP) or Jeune asphyxiating thoracic dystrophy (JATD) (Dagoneau et al. 2009; Merrill et al. 2009).

Further evidence that cilia are important for Hh signaling in humans comes from their impact on two human tumor types, medulloblastoma and basal cell carcinoma, which are both caused by gain of Hh signaling. In mouse medulloblastoma or basal cell carcinoma triggered by activated SMO, conditional removal of cilia blocks tumor formation, consistent with the requirement for cilia at a step downstream from SMO. The same tumors can also be caused by

Table 2. In every context examined, removal of cilia blocks the response to both SHH and IHH

Tissue	Conditional allele	Cre	Phenotype
Cranial neural crest	<i>Kif3a</i>	<i>Wnt1</i>	Craniofacial defects (Liu et al. 2014)
Endochondral bone (Ihh-dependent)	<i>Ift88</i>	<i>Prx1</i>	Short bones (Haycraft et al. 2007)
Postnatal cartilage	<i>Kif3a</i>	<i>Col2a1-Cre</i>	Craniofacial defects (Koyama et al. 2007)
Neural stem cells	<i>Kif3a</i>	<i>hGFAP</i>	Loss of hippocampal stem cells (Han et al. 2008)
Hippocampal stem cells	<i>Ift20</i>	<i>mGFAP</i>	Reduction hippocampal amplifying progenitors (Amador-Arjona et al. 2011)
Postnatal B1 SVZ neural stem cells	<i>Kif3a</i>	<i>Adeno-Cre (injected)</i>	Decreased proliferation of neural stem cells (Tong et al. 2014)
Cerebellar granule cell precursors	<i>Kif3a</i>	<i>hGFAP</i>	Loss of cerebellar granule cell progenitors (Chizhikov et al. 2007; Spassky et al. 2008)
Basal cell carcinoma	<i>Kif3a; Ift88</i>	<i>Keratin14</i>	Removal of cilia inhibited tumors induced by activated Smoothened. Removal of cilia accelerated tumors induced by activated Gli2 (Wong et al. 2009)
Medulloblastoma	<i>Kif3a</i>	<i>hGFAP</i>	Removal of cilia inhibited tumors induced by activated Smoothened. Removal of cilia accelerated tumors induced by activated Gli2 (Han et al. 2009)

SHH, Sonic Hedgehog; IHH, Indian Hedgehog; SVZ, subventricular zone.

activation of GLI2; in these cases, removal of cilia makes the tumors more aggressive because GLI3 repressor is no longer generated resulting in a greater net GLI activation.

TRAFFICKING OF PATHWAY PROTEINS WITHIN THE CILIUM IS REQUIRED FOR Hh SIGNALING

Immunolocalization experiments showed that all the proteins required for transduction of Hh signals are enriched in primary cilia and change their distribution in response to ligand (Fig. 3). The first demonstration of cilia localization came for the membrane protein SMO, which accumulates in the cilium within an hour following stimulation of the Hh pathway (Corbit et al. 2005; Rohatgi et al. 2007). As total SMO protein levels are unaltered, accumulation of SMO in the cilium is a consequence of translocation of a ready-made pool of SMO and not from newly synthesized protein (Rohatgi et al. 2007). A common hydrophobic and basic residue motif following the seventh transmembrane domain at the carboxyl terminus of SMO is required for localization of SMO to the cilium in the presence of SHH (Händel

et al. 1999; Brailov et al. 2000; Dwyer et al. 2001; Corbit et al. 2005; Aanstad et al. 2009). Single-molecule imaging indicates that once in the cilium SMO moves by diffusion within the membrane, rather than by IFT (Milenkovic et al. 2015). Localization of SMO to the cilium is, however, not sufficient for signaling, as SMO accumulates in primary cilia that lack *Dync2h1*, the heavy chain of the retrograde dynein IFT motor, but *Dync2h1* mutants lack ventral neural cell types (Huangfu and Anderson 2005; May et al. 2005).

Even more remarkable, all three transcription factors of the GLI family, the mediators of Hh-regulated transcription, are enriched at cilia tips in the absence of Hh ligand and become further enriched in response to pathway activation (Haycraft et al. 2005). Only full-length GLI proteins localize to cilia, whereas the proteolytically processed repressor forms that lack the carboxy-terminal half of the protein do not (Wen et al. 2010; Santos and Reiter 2014). Deletion analysis identified a 330-amino-acid (out of the 1544 in the full-length protein) central region of GLI2 is required for cilia targeting. SUFU, a key negative regulator of vertebrate Hh signaling, binds to GLI preventing its acti-

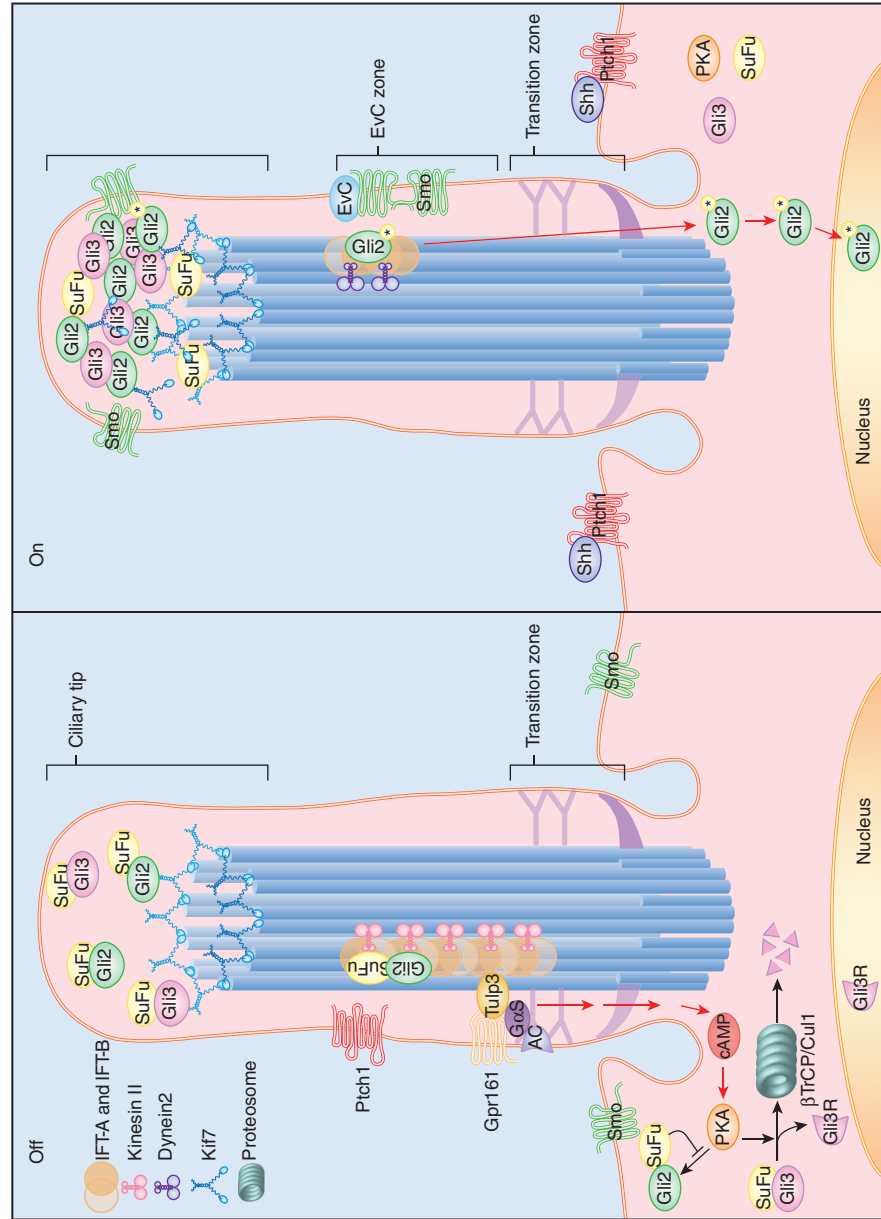


Figure 3. Hedgehog (Hh) signal transduction in the primary cilium. The primary cilium has several spatially distinct regions that promote normal Hh signal transduction. Entry into the cilium is gated at the transition zone; the EVC zone facilitates pathway activation in some cell types; the cilia tip compartment, defined by KIF7, is the site of enrichment and activation of the Gli1/SUFU complex; and proteolytic processing to make Gli1 repressors that may occur at the base where protein kinase A (PKA) is localized and leads to formation of Gli3R. (*Legend continues on following page.*)

vation and is also highly enriched at cilia tips (Haycraft et al. 2005).

PTCH1 has 12 transmembrane domains and the region carboxy-terminal to the last transmembrane domain is required for cilia localization and signaling (Kim et al. 2015). PTCH1 is localized to cilia in the absence of ligand and binding of SHH to PTCH1 triggers its removal from the cilium, although removal of PTCH1 from the cilium is not required for Hh pathway activation. During the same interval after exposure to ligand when PTCH moves out of the cilium, SMO translocates into the cilium (Rohatgi et al. 2007).

CILIA ARE REQUIRED FOR BOTH KEEPING THE PATHWAY OFF AND TURNING THE PATHWAY ON

In mammals, the GLI family of transcription factors, GLI1, GLI2, and GLI3 implement the transcriptional responses to Hh family ligands (Bai et al. 2004). In the absence of ligand, proteolytically processed forms of GLI proteins repress expression of Hh target genes (primarily GLI3, with a minor role for GLI2). In the presence of ligand, processing of the repressor forms is blocked and, instead, full-length GLI proteins are converted (by an unknown mechanism) to GLI activators, principally mediated by GLI2. The formation of both GLI repressor and activator depend on cilia (Huangfu and Anderson 2005; Liu et al. 2005; May et al. 2005).

Proteolytic processing of the GLI proteins depends on phosphorylation by cAMP-dependent PKA, a key evolutionarily conserved com-

ponent of the Hh signal transduction pathway (Wang et al. 2014). In contrast to *Drosophila*, mammalian PKA does not act only by promoting formation of GLI repressor, it is also required to prevent inappropriate activation of GLI2 (Tuson et al. 2011). In addition to the phosphorylation sites on GLI2 required for proteolytic processing, phosphorylation of two additional PKA sites of GLI2 and GLI3 prevents formation of the fully activated form of the transcription factors (Niewiadomski et al. 2014).

Both the catalytic and regulator subunits of PKA are highly enriched at the base of the cilium (Barzi et al. 2010; Tuson et al. 2011). Mutant mouse embryos that lack both genes that encode catalytic subunits (*PKA nulls*) show a very strong activation of the Hh pathway, in which all cells in the neural tube acquire the most ventral fates (Tuson et al. 2011). Compound mutants that lack both PKA catalytic subunits and also lack cilia have a neural phenotype similar to cilia mutants, showing that the action of PKA depends on events that take place in cilia. Although both catalytic and regulatory subunits of PKA are highly enriched at the base of the cilium, recent proximity ligation experiments provide biochemical evidence that regulatory subunits of PKA localize to the cilium (Mick et al. 2015), suggesting that sensing of cAMP by PKA may take place inside cilia.

PKA is activated by cAMP, and several adenylyl cyclases that produce cAMP, including AC3, AC5, and AC6, are enriched in cilia (Bishop et al. 2007; Mick et al. 2015; Vuolo et al. 2015). These, in turn, are regulated by G-protein-coupled receptors (GPCRs), including

Figure 3. (Continued) In the absence of Hh signal, PTCH1 and Gpr161, both negative regulators of the pathway, are present in the cilia membrane. Gpr161 trafficking into the cilium depends on TULP3 and intraflagellar transport (IFT)-A; GPR161 appears to activate G α s, which activates adenylyl cyclase, which increases the levels of cAMP, thereby activating PKA. Activated PKA phosphorylates sites on GLI3 that promote partial proteolysis by β TrCP/Cul1 and the proteasome, generating Gli3 repressor (Gli3R), which moves to the nucleus and represses expression of Hh target genes. GLI2 and GLI3 are trafficked to the tip of the cilium in the absence of ligand in a complex with SuFu, and processing of GLI3 depends on it having transited the cilium. Binding of Hh to PTCH1 triggers its removal from the cilium, allowing Smoothed (SMO) to translocate into the cilium where it can activate downstream signaling. Gpr161 also exits the cilium after exposure to ligand. Binding of EvC to SMO near the base of the cilium promotes SMO activity. In the presence of ligand, the GLI/SUFU complex accumulates to high levels at the tip of the cilium, where dissociation of the complex allows formation of the activator form of GLI2.



Gpr161, which activates ACIII and thereby increases cAMP levels (Mukhopadhyay et al. 2013). Like PTCH1, Gpr161 is present in the cilia membrane in the absence of ligand and moves out of the cilium in response to ligand. This movement is regulated by TULP3, a member of the vertebrate tubby-like family of proteins, which links GPCRs, including Gpr161, to IFT-A, which transports them into the cilium (Nishina et al. 1998; Mukhopadhyay et al. 2010).

Small G proteins, including G α s, mediate the actions of GPCRs. In mouse embryos that lack G α s (Gnas), the Hh pathway is ectopically activated with a phenotype similar to that of PKA or *Ptch1* mutant embryos, indicating that G α s negatively regulates the Hh pathway (REGARD et al. 2013). Thus, the Hh pathway is kept off in the absence of ligand by Gpr161, which is localized to the cilium by TULP3 and activated by G α s. Gpr161 subsequently activates adenylyl cyclase, leading to increased cAMP and increased PKA activity, which promotes GLI repressor formation and prevents GLI activation (Fig. 3) (Mukhopadhyay and Rohatgi 2014).

CHANGES IN AXONEMAL STRUCTURE OR CILIA MEMBRANE COMPOSITION CAN INCREASE OR DECREASE Hh PATHWAY ACTIVITY

The requirement for primary cilia for Hh signaling and the localization of pathway proteins to the cilium would be consistent with the cilium acting as a scaffold for Hh signal transduction or a site for concentration of Hh pathway proteins. However, subtle changes in cilia structure can decrease or increase Hh pathway activity, suggesting that the cilium provides more than just a permissive context for Hh signaling. (References to specific mouse and human proteins and phenotypes are in Table 1.)

Embryos carrying mutations in the gene that encodes the heavy chain of the retrograde motor cytoplasmic dynein 2 (*Dync2h1*) assemble cilia; however, the axoneme is bulged because of accumulation of IFT complexes and other proteins trapped in the cilium as a consequence of defective retrograde transport. Like IFT-B or *Kif3a* mutant embryos, *Dync2h1* mu-

tants lack floor plate and V3 interneuron progenitors indicating these cells cannot respond to high levels of Shh signaling. However, unlike IFT-B or *Kif3a* mutants, motor neurons are specified in the caudal spinal cord of *Dync2h1* mutants in which they are intermingled with V2 interneurons (Fig. 2) (Huangfu and Anderson 2005; May et al. 2005). Thus, low levels of Hh signaling can still be transduced in the bulged *Dync2h1* cilia.

Although null mutations in most IFT-B components block cilia formation, abnormal cilia form in most mutants that lack one of the six proteins of the IFT-A complex. Mutations that strongly disrupt the mouse IFT-A proteins IFT122 or IFT139 cause bulged cilia, similar to mutants that lack Dynein2. However, unlike mutants that disrupt Dynein2, the IFT-A mutants show a gain-of-activity of the Hh pathway in the neural tube, with expanded domains of Shh-dependent ventral neuron cell types (e.g., motor neurons) (Fig. 2) (Tran et al. 2008; Qin et al. 2011). This gain-of-function for Hh pathway activity is independent of ligand, suggesting that IFT-A plays a specific role in keeping the pathway off in the absence of ligand. A similar phenotype is seen in embryos that lack TULP3 (Mukhopadhyay et al. 2010).

The effects of mutations in IFT-A genes on Hh signaling are complex. A weak allele of *Ift144* (WDR19) shows bulged cilia and ventralization of the neural tube similar to that seen in IFT122 or 139 (Table 1). However, a stronger allele that produces only very short bloated cilia with a highly disrupted axoneme causes a loss of Hh signaling in the neural tube. Double mutants that carry both the weak allele of *Ift144* and an allele of *Ift122* show the short bulged cilia and loss-of-function Hh neural phenotype, suggesting that some IFT-A proteins have overlapping functions and that a complete lack of IFT-A complex activity leads to the formation of very short cilia and a loss of Hh activity (Liem et al. 2012). Human mutations in IFT-A genes cause a spectrum of phenotypes, ranging in severity from retinitis pigmentosa in the adult to SRP associated with perinatal lethality (Table 1).

Even small changes in IFT function can have subtle effects on Hh signaling activity. IFT25



and IFT27 form an IFT-B subcomplex. In contrast to other IFT-B proteins, mutants that lack either of these proteins make primary cilia that have apparently normal structure. The mouse mutants survive to birth, when they show mild Hh pathway phenotypes and defects in trafficking of GLI2, SMO, and PTCH1. IFT27 appears to be important for loading of retrograde IFT cargo in trypanosomes (Huet et al. 2014), and given the evolutionary conservation of IFT proteins, it is likely that the Hh defects in mouse *Ift25* and *Ift27* mutants are the result of relatively subtle changes in global IFT. This indicates that Hh signaling is sensitive to even small changes in IFT function.

ARL13B is an ARF-family GTPase that is a component of the ciliary membrane that is required for normal cilia structure—in the absence of ARL13B, cilia are short and the B-tubules of the axoneme are frequently open. The neural tube of *Arl13b* mouse mutants lacks both the most ventral and the most dorsal cell types, indicating that when ciliary structure is disrupted in this way it is not possible to achieve the highest level of Hh signaling or to prevent ectopic low-level activation of the pathway.

It has been supposed that cilia length affects Hh signaling; however, it appears not to be the case. Cilia on *Rfx3* mutant cells are about half the normal length (Bonnafe et al. 2004) and embryos that overexpress ARL13B have cilia that are 50% longer than wild-type (Bangs et al. 2015), but both genotypes are viable and apparently have a normal pattern of Hh-dependent neural cell types.

The ciliary membrane has a distinct lipid composition; the inositol phosphate PI(4)P is enriched in the entire ciliary membrane and PI(4,5)P₂ is enriched at the base of the cilium. *Inpp5e*, a ciliary phosphoinositide 5-phosphatase, is required to maintain this distribution and is one of the genes mutated in Joubert syndrome (Chávez et al. 2015; Garcia-Gonzalo et al. 2015). In *Inpp5e* mutant mice, ciliary PI(4,5)P₂ levels are elevated, causing disrupted Hh signaling, apparently a result of inappropriate accumulation of TULP3, which binds to these phosphoinositides. *Inpp5e* mutant mice survive to birth when they show polydactyly, suggesting

that they have a significant, but relatively mild disruption of Hh signal transduction.

KIF7 ORGANIZES THE CILIA TIP COMPARTMENT

One rationale for why Hh signaling requires the primary cilium is that it concentrates components of the Hh signal transduction cascade in a small volume, promoting their interactions by mass action. It has been calculated that concentrating the entire pool of a protein into the cilium would increase its concentration by two to three orders of magnitude (Nachury 2014). However, certain Hh components, GLI, SuFu, and KIF7, are strongly enriched at the cilia tip. Full-length Gli proteins form complexes with SuFu, and data indicate that separation of SuFu and GLI proteins is critical for GLI activation (Humke et al. 2010); it is likely that this separation occurs or is regulated by events that happen at the tip of the cilium. KIF7 also binds directly to both GLI2 and GLI3 (Cheung et al. 2009; Endoh-Yamagami et al. 2009).

The tip compartment of the primary cilium is organized, at least in part, by KIF7, an evolutionarily conserved core component of the Hh pathway (He et al. 2014). KIF7 and its *Drosophila* homolog Cos2 have both positive and negative roles in Hh signal transduction. KIF7, a member of the Kinesin-4 family, binds microtubule plus-ends at the tip of the cilium to organize microtubule architecture at the tip (He et al. 2014). In the absence of KIF7, axonemal microtubules have variable lengths, and GLI and SuFu are localized in multiple puncta along the ciliary axoneme that appear to correspond to ectopic cilia tip compartments. The mislocalization of the GLI/SUFU complex causes inappropriate, ligand-independent GLI activation and the mild ectopic activation of the pathway seen in *Kif7*^{-/-} mutant embryos.

CELL-TYPE-SPECIFIC DIFFERENCES IN CILIA COMPOSITION CAN MODULATE Hh SIGNALING

Most proteins that have a role in Hh signaling in the cilium act in all cell types. Two exceptions

are EVC and EVC2, transmembrane proteins responsible for Ellis–van Creveld syndrome, a distinctive ciliopathy associated with short limbs, a narrow chest (short ribs), and polydactyly (Ruiz-Perez et al. 2003). Mouse *Evc* is highly expressed in differentiating chondrocytes and all the cartilaginous components of the skeleton. *Evc* mutant mice survive to birth, and some can survive to adulthood; like the affected people, the mice have short bones, short ribs, and abnormal teeth, although the response to Shh in the neural tube appears to be nearly normal (Caparrós-Martín et al. 2013). Specific partial loss-of-function mutations that disrupt the IFT-A protein IFT121/WDR35 also cause EVC syndrome, and both EVC proteins and SMO fail to localize to *Ift121/Wdr35* mutant MEF cilia (Caparrós-Martín et al. 2015). As other IFT-A proteins are also required for the recruitment of transmembrane proteins, including SMO, to cilia (Liem et al. 2012), it appears IFT-A has a general role in recruiting membrane proteins, including the EVC proteins, to the cilium, and chondrocytes appear to be particularly sensitive to the loss of IFT-A.

The Tectonic complex localizes to the transition zones and appears to have complex tissue-specific functions in ciliogenesis (Table 1) (see Vaisse et al. 2016). Mutant fibroblasts assemble cilia but cells in the mutant embryonic node and mesenchymal cells adjacent to the neural tube fail to form cilia. As mutants show both defects in neural patterning and polydactyly, the tissue specificity of the Tectonic complex merits further study.

LINEAGE DETERMINES WHICH CELLS HAVE PRIMARY CILIA IN THE MOUSE EMBRYO

In cell culture, primary cilia are present in the G₀ phase of the cell cycle and can be induced by serum removal (Plotnikova et al. 2009). In contrast, many proliferating cells in the mouse embryo and adult organs in vivo have a primary cilium. Scattered reports indicate that some specific cell types in the whole animal, such as acinar cells of the adult pancreas (Aughsteeen 2001), lack primary cilia. Transgenic mice that

carry fluorescent markers for basal bodies and cilia have been used to systematically identify ciliated and unciliated cells (Bangs et al. 2015). No cilia are present on cells of the pre-implantation embryo; cilia first appear on cells of the epiblast lineage (the embryo proper) shortly after cavitation (E6.0) and all cells derived from the epiblast (ectoderm, mesoderm, and definitive endoderm) are ciliated in the midgestation embryo. In contrast, cells of extra-embryonic lineages, the visceral endoderm and trophoctoderm, have centrioles but lack primary cilia. Cells of both the trophoctoderm lineage and from the embryonic mesoderm contribute to the placenta and cells of the visceral endoderm and mesoderm contribute to the yolk sac. These lineages lack cilia until at least E14.5 (Bangs et al. 2015).

Stem cell lines that correspond to the different embryonic lineages (Ralston and Rossant 2005) recapitulate the cilia status of the lineages in the embryo—all nondividing epiblast stem cells (EpiSCs) (which recapitulate the status of the E6.5 epiblast) are ciliated, whereas extraembryonic endoderm stem cells (XEN cells) and trophoctoderm stem cells (TS cells) lack cilia (Bangs et al. 2015). For all three stem cell types, the presence or absence of primary cilia on embryo-derived stem cells is independent of the presence of serum.

The absence of primary cilia on cells of extraembryonic origin implies that these cells cannot respond to Hh ligands. The first time that Hh signaling is active in the mouse embryo is at the beginning of gastrulation, when *Ihh* expressed in the extraembryonic visceral endoderm signals to the adjacent epiblast derived extraembryonic mesoderm, which is ciliated. This is necessary to promote blood island formation (Fig. 4) (Farrington et al. 1997; Dyer et al. 2001). In the placenta, the only cells that respond to Hh signaling are embryo-derived ciliated cells that surround the fetal blood vessels (Fig. 4) (Jiang and Herman 2006). It is interesting to speculate that normal development of the placenta and yolk sac depends on preventing extraembryonic lineages from responding to Hh signals and this is achieved by blocking ciliogenesis in these cells.

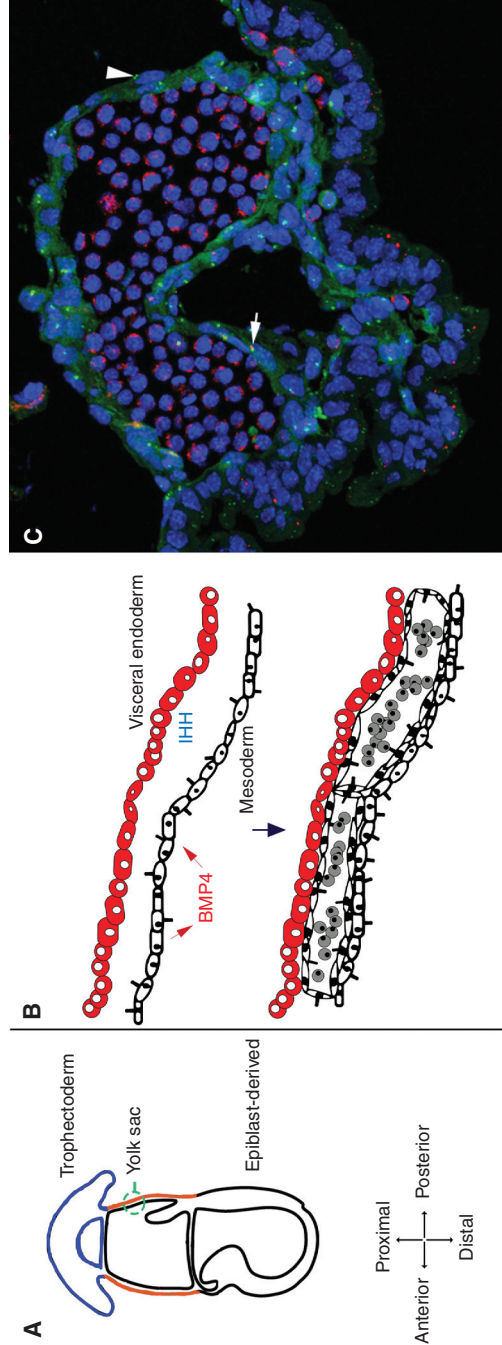


Figure 4. Lineage-dependent cilia formation in the early mouse embryo. (A) Three lineages defined in the preimplantation embryo persist in the postimplantation embryo (E8.0 embryo shown). Two extraembryonic lineages, the trophectoderm (blue) and visceral endoderm (red) contribute to the placenta and yolk sac respectively; these two lineages lack primary cilia. Nearly all nonmitotic cells of the third lineage, the epiblast (black), are ciliated; this lineage gives rise to almost all cells of the embryo proper, as well as to mesoderm-derived components of the placenta and yolk sac. (B) Paracrine Hh signaling in the yolk sac. *Upper* drawing represents the two layers of the E8.0 yolk sac (lassoed in panel A). The unciliated visceral endoderm layer produces IHH, but does not activate Hh target genes. The adjacent ciliated extraembryonic mesoderm responds to the IHH produced by the visceral endoderm and produces BMP4, which leads to formation of the blood islands (*lower* panel). (Image based on data in Baron 2001.) (C) A blood vessel in the E14.5 yolk sac from an embryo expressing a *Centrin2-GFP*, a marker for the basal body, and *ARL13b-mCherry*, a marker of the cilia membrane. The lineage relationships are preserved from earlier development—the visceral endoderm (cell layer at the top of the image) has GFP^+ centrioles, but lacks cilia (arrowhead), whereas the extraembryonic mesoderm that surrounds the vessel filled with round blood cells is ciliated (arrow). (Image from Bangs et al. 2015; modified, with permission, from the authors.)

These cases of Hh ligand made by extraembryonic epithelia signaling to adjacent Hh-responsive ciliated cells reflect one of the common modes of Hh signaling in other organs. Paracrine signaling from ligand-producing cells to adjacent Hh responsive cells has been seen in a number of organs, including the digestive tract and the prostate (Yu et al. 2009; Mao et al. 2010). Thus, it will be interesting to see whether tissue-specific absence of primary cilia enforces paracrine, rather than autocrine Hh signaling.

CONCLUDING REMARKS

Vertebrate Hedgehog signal transduction, which is essential for the development and maintenance of most organs, takes place in the primary cilium. The responses to Hh ligands are exquisitely sensitive to alterations in cilia structure—disruptions of many cilia components reduce the response to Hh ligands, but disruption of specific cilia proteins can also cause inappropriate activity of the pathway, and some proteins can be altered in different ways that enhance or interfere with Hh signal transduction. Dozens, and probably hundreds, of proteins are required to build the primary cilium; because of the many essential roles of Hh signaling, mutations in cilia genes collectively have a broad impact on human health.

The central effectors of Hh signaling, GLI proteins and the negative regulator SUFU, are regulated in the tiny compartment at the tip of the primary cilium. A single core conserved component of the Hh pathway, the kinesin-family protein KIF7, has an essential role in organizing the cilia tip. The dual roles of KIF7 in Hh signal transduction and primary cilia structure argue for an ancient intertwining of Hh signal transduction with the structure of the primary cilium.

The distribution of primary cilia determines the ability of tissues to respond to Hh ligands, but the tissue distribution of primary cilia and the mechanisms that regulate cilia assembly and disassembly in vivo are poorly understood. A deeper understanding of the mechanisms that control cilia formation in vivo is likely to pro-

vide the foundation for therapeutic interventions in both ciliopathies and cancer.

ACKNOWLEDGMENTS

We thank Meg Distinti for valuable assistance with the manuscript. Work in the Anderson laboratory in this area is supported by the National Institutes of Health (NIH) Grants R37 HD03455 and R01 NS044385 to K.V.A. and the MSKCC Cancer Center Support Grant (P30 CA008748).

REFERENCES

*Reference is also in this collection.

- Aanstad P, Santos N, Corbit KC, Scherz PJ, Trinh le A, Salvenmoser W, Huisken J, Reiter JF, Stainier DY. 2009. The extracellular domain of Smoothened regulates ciliary localization and is required for high-level Hh signaling. *Curr Biol* **19**: 1034–1039.
- Ahn S, Joyner AL. 2004. Dynamic changes in the response of cells to positive Hedgehog signaling during mouse limb patterning. *Cell* **118**: 505–516.
- Alby C, Piquand K, Huber C, Megarbané A, Ichkou A, Legendre M, Pelluard F, Encha-Ravazi F, Abi-Tayeh G, Besières B, et al. 2015. Mutations in *KIAA0586* cause lethal ciliopathies ranging from a hydroletharus phenotype to short-rib polydactyly syndrome. *Am J Hum Genet* **97**: 311–318.
- Amador-Arjona A, Elliott J, Miller A, Ginbey A, Pazour GJ, Enikolopov G, Roberts AJ, Terskikh AV. 2011. Primary cilia regulate proliferation of amplifying progenitors in adult hippocampus: Implications for learning and memory. *J Neurosci* **31**: 9933–9944.
- Aughstee AA. 2001. The ultrastructure of primary cilia in the endocrine and excretory duct cells of the pancreas of mice and rats. *Eur J Morphol* **39**: 277–283.
- Bai CB, Stephen D, Joyner AL. 2004. All mouse ventral spinal cord patterning by hedgehog is Gli dependent and involves an activator function of Gli3. *Dev Cell* **6**: 103–115.
- Balaskas N, Ribeiro A, Panovska J, Dessaud E, Sasai N, Page KM, Briscoe J, Ribes V. 2012. Gene regulatory logic for reading the Sonic Hedgehog signaling gradient in the vertebrate neural tube. *Cell* **148**: 273–284.
- Bangs F, Antonio N, Thongnuek P, Welten M, Davey MG, Briscoe J, Tickle C. 2011. Generation of mice with functional inactivation of *talpid3*, a gene first identified in chicken. *Development* **138**: 3261–72.
- Bangs FK, Schrode N, Hadjantonakis AK, Anderson KV. 2015. Lineage specificity of primary cilia in the mouse embryo. *Nat Cell Biol* **17**: 113–122.
- Baron MH. 2001. Molecular regulation of embryonic hematopoiesis and vascular development: A novel pathway. *J Hematother Stem Cell Res* **10**: 587–594.
- Barzi M, Berenguer J, Menendez A, Alvarez-Rodriguez R, Pons S. 2010. Sonic-Hedgehog-mediated proliferation



- requires the localization of PKA to the cilium base. *J Cell Sci* **123**: 62–69.
- Bazzi H, Anderson KV. 2014. Acentriolar mitosis activates a p53-dependent apoptosis pathway in the mouse embryo. *Proc Natl Acad Sci* **111**: E1491–E1500.
- Behal RH, Cole DG. 2013. Analysis of interactions between intraflagellar transport proteins. *Methods Enzymol* **524**: 171–94.
- Ben J, Elworthy S, Ng AS, van Eeden F, Ingham PW. 2011. Targeted mutation of the *talpid3* gene in zebrafish reveals its conserved requirement for ciliogenesis and Hedgehog signalling across the vertebrates. *Development* **138**: 4969–4978.
- Berbari NF, Kin NW, Sharma N, Michaud EJ, Kesterson RA, Yoder BK. 2011. Mutations in *Traf3ip1* reveal defects in ciliogenesis, embryonic development, and altered cell size regulation. *Dev Biol* **360**: 66–76.
- Bielas SL, Silhavy JL, Brancati F, Kisseleva MV, Al-Gazali L, Sztrihai L, Bayoumi RA, Zaki MS, Abdel-Aleem A, Rosti RO, et al. 2009. Mutations in *INPP5E*, encoding inositol polyphosphate-5-phosphatase E, link phosphatidyl inositol signaling to the ciliopathies. *Nat Genet* **41**: 1032–1036.
- Bishop GA, Berbari NF, Lewis J, Mykytyn K. 2007. Type III adenylyl cyclase localizes to primary cilia throughout the adult mouse brain. *J Comp Neurol* **505**: 562–571.
- Bizet AA, Becker-Heck A, Ryan R, Weber K, Filhol E, Krug P, Halbritter J, Delous M, Lasbennes MC, Linghu B, et al. 2015. Mutations in *TRAF3IP1/IFT54* reveal a new role for IFT proteins in microtubule stabilization. *Nat Commun* **6**: 8666.
- Bond J, Roberts E, Springell K, Lizarraga SB, Scott S, Higgins J, Hampshire DJ, Morrison EE, Leal GF, Silva EO, et al. 2005. A centrosomal mechanism involving *CDK5RAP2* and *CENPJ* controls brain size. *Nat Genet* **37**: 353–355.
- Bonnafe E, Touka M, AitLounis A, Baas D, Barras E, Ucla C, Moreau A, Flamant F, Dubruille R, Couble P, et al. 2004. The transcription factor *RFX3* directs nodal cilium development and left–right asymmetry specification. *Mol Cell Biol* **24**: 4417–4427.
- Botilde Y, Yoshida S, Shinohara K, Hasegawa T, Nishimura H, Shiratori H, Hamada H. 2013. *Cluap1* localizes preferentially to the base and tip of cilia and is required for ciliogenesis in the mouse embryo. *Dev Biol* **381**: 203–212.
- Brailov I, Bancila M, Brisorgueil MJ, Miquel MC, Hamon M, Vergé D. 2000. Localization of 5-HT₆ receptors at the plasma membrane of neuronal cilia in the rat brain. *Brain Res* **872**: 271–275.
- Bredrup C, Saunier S, Oud MM, Fiskerstrand T, Hoischen A, Brackman D, Leh SM, Midtbø M, Filhol E, Bole-Feysot C, et al. 2011. Ciliopathies with skeletal anomalies and renal insufficiency due to mutations in the *IFT-A* gene *WDR19*. *Am J Hum Genet* **89**: 634–643.
- Briscoe J, Ericson J. 2001. Specification of neuronal fates in the ventral neural tube. *Curr Opin Neurobiol* **11**: 43–49.
- Burke R, Basler K. 1997. Hedgehog signaling in *Drosophila* eye and limb development—Conserved machinery, divergent roles? *Curr Opin Neurobiol* **7**: 55–61.
- Cantagrel V, Silhavy JL, Bielas SL, Swistun D, Marsh SE, Bertrand JY, Audollent S, Attié-Bitach T, Holden KR, Dobyns WB, et al. 2008. Mutations in the cilia gene *ARL13B* lead to the classical form of Joubert syndrome. *Am J Hum Genet* **83**: 170–179.
- Caparrós-Martín JA, Valencia M, Reytor E, Pacheco M, Fernandez M, Perez-Aytes A, Gean E, Lapunzina P, Peters H, Goodship JA, et al. 2013. The ciliary *Evc/Evc2* complex interacts with *Smo* and controls Hedgehog pathway activity in chondrocytes by regulating *Sufu/Gli3* dissociation and *Gli3* trafficking in primary cilia. *Hum Mol Genet* **22**: 124–139.
- Caparrós-Martín JA, De Luca A, Cartault F, Aglan M, Temtamy S, Otaify GA, Mehrez M, Valencia M, Vázquez L, Alessandri JL, et al. 2015. Specific variants in *WDR35* cause a distinctive form of Ellis–van Creveld syndrome by disrupting the recruitment of the *EvC* complex and *SMO* into the cilium. *Hum Mol Genet* **24**: 4126–4137.
- Caspary T, Larkins CE, Anderson KV. 2007. The graded response to Sonic Hedgehog depends on cilia architecture. *Dev Cell* **12**: 767–778.
- Chávez M, Ena S, Van Sande J, de Kerchove d’Exaerde A, Schurmans S, Schiffmann SN. 2015. Modulation of ciliary phosphoinositide content regulates trafficking and Sonic Hedgehog signaling output. *Dev Cell* **34**: 338–350.
- Cheung HO, Zhang X, Ribeiro A, Mo R, Makino S, Puviindran V, Law KK, Briscoe J, Hui CC. 2009. The kinesin protein *Kif7* is a critical regulator of *Gli* transcription factors in mammalian hedgehog signaling. *Sci Signal* **2**: ra29.
- Chizhikov VV, Davenport J, Zhang Q, Shih EK, Cabello OA, Fuchs JL, Yoder BK, Millen KJ. 2007. Cilia proteins control cerebellar morphogenesis by promoting expansion of the granule progenitor pool. *J Neurosci* **27**: 9780–9789.
- Cole DG. 1999. Kinesin-II, coming and going. *J Cell Biol* **147**: 463–466.
- Coles GL, Ackerman KG. 2013. *Kif7* is required for the patterning and differentiation of the diaphragm in a model of syndromic congenital diaphragmatic hernia. *Proc Natl Acad Sci* **110**: E1898–E1905.
- Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, Reiter JF. 2005. Vertebrate smoothened functions at the primary cilium. *Nature* **437**: 1018–1021.
- Cortés CR, McInerney-Leo AM, Vogel I, Rondón Galeano MC, Leo PJ, Harris JE, Anderson LK, Keith PA, Brown MA, Ramsing M, et al. 2016. Mutations in human *C2CD3* cause skeletal dysplasia and provide new insights into phenotypic and cellular consequences of altered *C2CD3* function. *Sci Rep* **6**: 24083.
- Dagoneau N, Goulet M, Geneviève D, Sznajder Y, Martinovic J, Smithson S, Huber C, Baujat G, Flori E, Tecco L, et al. 2009. *DYNC2H1* mutations cause asphyxiating thoracic dystrophy and short rib–polydactyly syndrome, type III. *Am J Hum Genet* **84**: 706–711.
- Damerla RR, Cui C, Gabriel GC, Liu X, Craig B, Gibbs BC, Francis R, Li Y, Chatterjee B, San Agustin JT, et al. 2015. Novel *Jbts17* mutant mouse model of Joubert syndrome with cilia transition zone defects and cerebellar and other ciliopathy related anomalies. *Hum Mol Genet* **24**: 3994–4005.
- Davis EE, Zhang Q, Liu Q, Diplas BH, Davey LM, Hartley J, Stoetzel C, Szymanska K, Ramaswami G, Logan CV, et al. 2011. *TTC21B* contributes both causal and modifying



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- alleles across the ciliopathy spectrum. *Nat Genet* **43**: 189–196.
- Dessaud E, Yang LL, Hill K, Cox B, Ulloa F, Ribeiro A, Mynett A, Novitch BG, Briscoe J. 2007. Interpretation of the Sonic Hedgehog morphogen gradient by a temporal adaptation mechanism. *Nature* **450**: 717–720.
- Dessaud E, McMahon AP, Briscoe J. 2008. Pattern formation in the vertebrate neural tube: A sonic hedgehog morphogen-regulated transcriptional network. *Development* **135**: 2489–503.
- Dwyer ND, Adler CE, Crump JG, L'Etoile ND, Bargmann CI. 2001. Polarized dendritic transport and the AP-1 μ 1 clathrin adaptor UNC-101 localize odorant receptors to olfactory cilia. *Neuron* **31**: 277–287.
- Dyer MA, Farrington SM, Mohn D, Munday JR, Baron MH. 2001. Indian hedgehog activates hematopoiesis and vasculogenesis and can respecify prospective neuroectodermal cell fate in the mouse embryo. *Development* **128**: 1717–1730.
- Eggenchwiler JT, Espinoza E, Anderson KV. 2001. Rab23 is an essential negative regulator of the mouse Sonic hedgehog signalling pathway. *Nature* **412**: 194–198.
- Eguether T, San Agustin JT, Keady BT, Jonassen JA, Liang Y, Francis R, Tobita K, Johnson CA, Abdelhamed ZA, Lo CW, et al. 2014. IFT27 links the BBSome to IFT for maintenance of the ciliary signaling compartment. *Dev Cell* **31**: 279–290.
- Endoh-Yamagami S, Evangelista M, Wilson D, Wen X, Theunissen JW, Phamluong K, Davis M, Scales SJ, Solloway MJ, de Sauvage FJ, et al. 2009. The mammalian Cos2 homolog Kif7 plays an essential role in modulating Hh signal transduction during development. *Curr Biol* **19**: 1320–1326.
- Farrington SM, Belaousoff M, Baron MH. 1997. *Winged-helix, Hedgehog* and *Bmp* genes are differentially expressed in distinct cell layers of the murine yolk sac. *Mech Dev* **62**: 197–211.
- Fehrenbach H, Decker C, Eisenberger T, Frank V, Hampel T, Walden U, Amann KU, Krüger-Stollfuß I, Bolz HJ, Häffner K, et al. 2014. Mutations in *WDR19* encoding the intraflagellar transport component IFT144 cause a broad spectrum of ciliopathies. *Pediatr Nephrol* **29**: 1451–1456.
- Ferrante MI, Giorgio G, Feather SA, Bulfone A, Wright V, Ghiani M, Selicorni A, Gammaro L, Scolari F, Woolf AS, et al. 2001. Identification of the gene for oral–facial–digital type I syndrome. *Am J Hum Genet* **68**: 569–576.
- Ferrante MI, Zullo A, Barra A, Bimonte S, Messaddeq N, Studer M, Dollé P, Franco B. 2006. Oral–facial–digital type I protein is required for primary cilia formation and left–right axis specification. *Nat Genet* **38**: 112–117.
- Forbes AJ, Nakano Y, Taylor AM, Ingham PW. 1993. Genetic analysis of hedgehog signalling in the *Drosophila* embryo. *Dev Suppl* **1993**: 115–124.
- García-Gonzalo FR, Corbit KC, Sirerol-Piquer MS, Ramaswami G, Otto EA, Noriega TR, Seol AD, Robinson JE, Bennett CL, Josifova DJ, et al. 2011. A transition zone complex regulates mammalian ciliogenesis and ciliary membrane composition. *Nat Genet* **43**: 776–784.
- García-Gonzalo FR, Phua SC, Roberson EC, García G III, Abedin M, Schurmans S, Inoue T, Reiter JF. 2015. Phosphoinositides regulate ciliary protein trafficking to modulate Hedgehog signaling. *Dev Cell* **34**: 400–409.
- Goetz SC, Liem KF Jr, Anderson KV. 2012. The spinocerebellar ataxia-associated gene *Tau tubulin kinase 2* controls the initiation of ciliogenesis. *Cell* **151**: 847–858.
- Halbritter J, Bizet AA, Schmidts M, Porath JD, Braun DA, Gee HY, McInerney-Leo AM, Krug P, Filhol E, Davis EE, et al. 2013. Defects in the IFT-B component IFT172 cause Jeune and Mainzer–Saldino syndromes in humans. *Am J Hum Genet* **93**: 915–925.
- Han YG, Spassky N, Romaguera-Ros M, Garcia-Verdugo JM, Aguilar A, Schneider-Maunoury S, Alvarez-Buylla A. 2008. Hedgehog signaling and primary cilia are required for the formation of adult neural stem cells. *Nat Neurosci* **11**: 277–284.
- Han YG, Kim HJ, Dlugosz AA, Ellison DW, Gilbertson RJ, Alvarez-Buylla A. 2009. Dual and opposing roles of primary cilia in medulloblastoma development. *Nat Med* **15**: 1062–1065.
- Händel M, Schulz S, Stanarius A, Schreff M, Erdtmann-Vourliotis M, Schmidt H, Wolf G, Höllt V. 1999. Selective targeting of somatostatin receptor 3 to neuronal cilia. *Neuroscience* **89**: 909–926.
- Harfe B, Scherz PJ, Nissim S, Tian H, McMahon AP, Tabin CJ. 2004. Evidence for an expansion-based temporal Shh gradient in specifying vertebrate digit identities. *Cell* **118**: 517–528.
- Haycraft CJ, Banizs B, Aydin-Son Y, Zhang Q, Michaud EJ, Yoder BK. 2005. Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. *PLoS Genet* **1**: e53.
- Haycraft CJ, Zhang Q, Song B, Jackson WS, Detloff PJ, Serra R, Yoder BK. 2007. Intraflagellar transport is essential for endochondral bone formation. *Development* **134**: 307–316.
- He M, Subramanian R, Bangs F, Omelchenko T, Liem KF Jr, Kapoor TM, Anderson KV. 2014. The kinesin-4 protein Kif7 regulates mammalian Hedgehog signalling by organizing the cilium tip compartment. *Nat Cell Biol* **16**: 663–672.
- Heydeck W, Zeng H, Liu A. 2009. Planar cell polarity effector gene *Fuzzy* regulates cilia formation and Hedgehog signal transduction in mouse. *Dev Dyn* **238**: 3035–3042.
- Hoover AN, Wynkoop A, Zeng H, Jia J, Niswander LA, Liu A. 2008. C2cd3 is required for cilia formation and Hedgehog signaling in mouse. *Development* **135**: 4049–4058.
- Hou Y, Witman GB. 2015. Dynein and intraflagellar transport. *Exp Cell Res* **334**: 26–34.
- Houde C, Dickinson RJ, Houtzager VM, Cullum R, Montpetit R, Metzler M, Simpson EM, Roy S, Hayden MR, Hoodless PA, et al. 2006. Hippo is essential for node cilia assembly and Sonic hedgehog signaling. *Dev Biol* **300**: 523–533.
- Houlden H, Johnson J, Gardner-Thorpe C, Lashley T, Hernandez D, Worth P, Singleton AB, Hilton DA, Holton J, Revez T, et al. 2007. Mutations in *TTBK2*, encoding a kinase implicated in tau phosphorylation, segregate with spinocerebellar ataxia type 11. *Nat Genet* **39**: 1434–1436.
- Huang P, Schier AF. 2009. Dampened Hedgehog signaling but normal Wnt signaling in zebrafish without cilia. *Development* **136**: 3089–3098.



- Huangfu D, Anderson KV. 2005. Cilia and Hedgehog responsiveness in the mouse. *Proc Natl Acad Sci* **102**: 11325–11330.
- Huangfu D, Liu A, Rakeman AS, Murcia NS, Niswander L, Anderson KV. 2003. Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature* **426**: 83–87.
- Huber C, Wu S, Kim AS, Sigaudy S, Sarukhanov A, Serre V, Baujat G, Le Quan Sang KH, Rimoin DL, Cohn DH, et al. 2013. *WDR34* mutations that cause short-rib polydactyly syndrome type III/severe asphyxiating thoracic dysplasia reveal a role for the NF- κ B pathway in cilia. *Am J Hum Genet* **93**: 926–931.
- Huet D, Blisnick T, Perrot S, Bastin P. 2014. The GTPase IFT27 is involved in both anterograde and retrograde intraflagellar transport. *eLife* **3**: e02419.
- Humke EW, Dorn KV, Milenkovic L, Scott MP, Rohatgi R. 2010. The output of Hedgehog signaling is controlled by the dynamic association between Suppressor of Fused and the Gli proteins. *Genes Dev* **24**: 670–682.
- Ingham PW. 1998. Transducing Hedgehog: The story so far. *EMBO J* **17**: 3505–3511.
- Ingham PW, McMahon AP. 2001. Hedgehog signaling in animal development: Paradigms and principles. *Genes Dev* **15**: 3059–3087.
- Jacoby M, Cox JJ, Gayral S, Hampshire DJ, Ayub M, Blockmans M, Pernot E, Kisseleva MV, Compère P, Schiffmann SN, et al. 2009. *INPP5E* mutations cause primary cilium signaling defects, ciliary instability and ciliopathies in human and mouse. *Nat Genet* **41**: 1027–1031.
- Jenkins D, Seelow D, Jehee FS, Perlyn CA, Alonso LG, Bueno DF, Donnai D, Josifova D, Mathijssen IM, Morton JE, et al. 2007. *RAB23* mutations in Carpenter syndrome imply an unexpected role for hedgehog signaling in cranial-suture development and obesity. *Am J Hum Genet* **80**: 1162–1170.
- Jiang F, Herman GE. 2006. Analysis of *Nsdhl*-deficient embryos reveals a role for Hedgehog signaling in early placental development. *Hum Mol Genet* **15**: 3293–3305.
- Keady BT, Samtani R, Tobita K, Tsuchya M, San Agustin JT, Follit JA, Jonassen JA, Subramanian R, Lo CW, Pazour GJ. 2012. IFT25 links the signal-dependent movement of Hedgehog components to intraflagellar transport. *Dev Cell* **22**: 940–951.
- Kessler K, Wunderlich I, Uebe S, Falk NS, Gießl A, Brandstätter JH, Popp B, Klinger P, Ekici AB, Sticht H, et al. 2015. *DYNC2L1* mutations broaden the clinical spectrum of dynein-2 defects. *Sci Rep* **5**: 11649.
- Kim J, Hsia EY, Brigui A, Plessis A, Beachy PA, Zheng X. 2015. The role of ciliary trafficking in Hedgehog receptor signaling. *Sci Signal* **8**: ra55.
- Koyama E, Young B, Nagayama M, Shibukawa Y, Enomoto-Iwamoto M, Iwamoto M, Maeda Y, Lanske B, Song B, Serra R, et al. 2007. Conditional *Kif3a* ablation causes abnormal hedgehog signaling topography, growth plate dysfunction, and excessive bone and cartilage formation during mouse skeletogenesis. *Development* **134**: 2159–2169.
- Kyttälä M, Tallila J, Salonen R, Kopra O, Kohlschmidt N, Paavola-Sakki P, Peltonen L, Kestilä M. 2006. *MKS1*, encoding a component of the flagellar apparatus basal body proteome, is mutated in Meckel syndrome. *Nat Genet* **38**: 155–157.
- Liem KF Jr, He M, Ocbina PJ, Anderson KV. 2009. Mouse *Kif7/Costal2* is a cilia-associated protein that regulates Sonic hedgehog signaling. *Proc Natl Acad Sci* **106**: 13377–13382.
- Liem KF Jr, Ashe A, He M, Satir P, Moran J, Beier D, Wicking C, Anderson KV. 2012. The IFT-A complex regulates Shh signaling through cilia structure and membrane protein trafficking. *J Cell Biol* **197**: 789–800.
- Liu A, Wang B, Niswander LA. 2005. Mouse intraflagellar transport proteins regulate both the activator and repressor functions of Gli transcription factors. *Development* **132**: 3103–3111.
- Liu B, Chen S, Johnson C, Helms JA. 2014. A ciliopathy with hydrocephalus, isolated craniosynostosis, hypertelorism, and clefting caused by deletion of *Kif3a*. *Reprod Toxicol* **48**: 88–97.
- Long F, Zhang XM, Karp S, Yang Y, McMahon AP. 2001. Genetic manipulation of hedgehog signaling in the endochondral skeleton reveals a direct role in the regulation of chondrocyte proliferation. *Development* **128**: 5099–5108.
- Mak KK, Kronenberg HM, Chuang PT, Mackem S, Yang Y. 2008. Indian hedgehog signals independently of PTHrP to promote chondrocyte hypertrophy. *Development* **135**: 1947–1956.
- Mao J, Kim BM, Rajurkar M, Shivdasani RA, McMahon AP. 2010. Hedgehog signaling controls mesenchymal growth in the developing mammalian digestive tract. *Development* **137**: 1721–1729.
- May SR, Ashique AM, Karlen M, Wang B, Shen Y, Zarbalis K, Reiter J, Ericson J, Peterson AS. 2005. Loss of the retrograde motor for IFT disrupts localization of Smo to cilia and prevents the expression of both activator and repressor functions of Gli. *Dev Biol* **287**: 378–389.
- Merrill AE, Merriman B, Farrington-Rock C, Camacho N, Sebald ET, Funari VA, Schibler MJ, Firestein MH, Cohn ZA, Priore MA, et al. 2009. Ciliary abnormalities due to defects in the retrograde transport protein *DYNC2H1* in short-rib polydactyly syndrome. *Am J Hum Genet* **84**: 542–549.
- Mick DU, Rodrigues RB, Leib RD, Adams CM, Chien AS, Gygi SP, Nachury MV. 2015. Proteomics of primary cilia by proximity labeling. *Dev Cell* **35**: 497–512.
- Milenkovic L, Weiss LE, Yoon J, Roth TL, Su YS, Sahl SJ, Scott MP, Moerner WE. 2015. Single-molecule imaging of Hedgehog pathway protein Smoothed in primary cilia reveals binding events regulated by Patched1. *Proc Natl Acad Sci* **112**: 8320–8325.
- Mill P, Lockhart PJ, Fitzpatrick E, Mountford HS, Hall EA, Reijns MA, Keighren M, Bahlo M, Bromhead CJ, Budd P, et al. 2011. Human and mouse mutations in *WDR35* cause short-rib polydactyly syndromes due to abnormal ciliogenesis. *Am J Hum Genet* **88**: 508–515.
- Miller KA, Ah-Cann CJ, Welfare MF, Tan TY, Pope K, Caruana G, Freckmann ML, Savarirayan R, Bertram JF, Dobbie MS, et al. 2013. *Cauli*: A mouse strain with an Ift140 mutation that results in a skeletal ciliopathy modelling Jeune syndrome. *PLoS Genet* **9**: e1003746.



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- Mukhopadhyay S, Rohatgi R. 2014. G-protein-coupled receptors, Hedgehog signaling and primary cilia. *Semin Cell Dev Biol* **33**: 63–72.
- Mukhopadhyay S, Wen X, Chih B, Nelson CD, Lane WS, Scales SJ, Jackson PK. 2010. TULP3 bridges the IFT-A complex and membrane phosphoinositides to promote trafficking of G-protein-coupled receptors into primary cilia. *Genes Dev* **24**: 2180–2193.
- Mukhopadhyay S, Wen X, Ratti N, Loktev A, Rangell L, Scales SJ, Jackson PK. 2013. The ciliary G-protein-coupled receptor Gpr161 negatively regulates the Sonic hedgehog pathway via cAMP signaling. *Cell* **152**: 210–223.
- Nachury MV. 2014. How do cilia organize signalling cascades? *Philos Trans R Soc Lond B Biol Sci* **369**: 20130465.
- Niewiadomski P, Kong JH, Ahrends R, Ma Y, Humke EW, Khan S, Teruel MN, Novitch BG, Rohatgi R. 2014. Gli protein activity is controlled by multisite phosphorylation in vertebrate Hedgehog signaling. *Cell Rep* **6**: 168–181.
- Nishina PM, North MA, Ikeda A, Yan Y, Naggert JK. 1998. Molecular characterization of a novel tubby gene family member, *TULP3*, in mouse and humans. *Genomics* **54**: 215–220.
- Norman RX, Ko HW, Huang V, Eun CM, Abler LL, Zhang Z, Sun X, Eggenschwiler JT. 2009. Tubby-like protein 3 (*TULP3*) regulates patterning in the mouse embryo through inhibition of Hedgehog signaling. *Hum Mol Genet* **18**: 1740–1754.
- Nüsslein-Volhard C, Wieschaus E. 1980. Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**: 795–801.
- Nygaard MB, Almstrup K, Lindbæk L, Christensen ST, Svingsen T. 2015. Cell context-specific expression of primary cilia in the human testis and ciliary coordination of Hedgehog signalling in mouse Leydig cells. *Sci Rep* **5**: 10364.
- Patterson VL, Damrau C, Paudyal A, Reeve B, Grimes DT, Stewart ME, Williams DJ, Siggers P, Greenfield A, Murdoch JN. 2009. Mouse *hitchhiker* mutants have spina bifida, dorso-ventral patterning defects and polydactyly: Identification of *Tulp3* as a novel negative regulator of the Sonic hedgehog pathway. *Hum Mol Genet* **18**: 1719–1739.
- Perrault I, Saunier S, Hanein S, Filhol E, Bizet AA, Collins F, Salih MA, Gerber S, Delphin N, Bigot K, et al. 2012. Mainzer-Saldino syndrome is a ciliopathy caused by *IFT140* mutations. *Am J Hum Genet* **90**: 864–870.
- Pigino G, Geimer S, Lanzavecchia S, Paccagnini E, Cantele F, Diener DR, Rosenbaum JL, Lupetti P. 2009. Electron-tomographic analysis of intraflagellar transport particle trains in situ. *J Cell Biol* **187**: 135–148.
- Plotnikova OV, Pugacheva EN, Golemis EA. 2009. Primary cilia and the cell cycle. *Methods Cell Biol* **94**: 137–160.
- Qin J, Lin Y, Norman RX, Ko HW, Eggenschwiler JT. 2011. Intraflagellar transport protein 122 antagonizes Sonic Hedgehog signaling and controls ciliary localization of pathway components. *Proc Natl Acad Sci* **108**: 1456–1461.
- Ralston A, Rossant J. 2005. Genetic regulation of stem cell origins in the mouse embryo. *Clin Genet* **68**: 106–112.
- Regard JB, Malhotra D, Gvozdenovic-Jeremic J, Josey M, Chen M, Weinstein LS, Lu J, Shore EM, Kaplan FS, Yang Y. 2013. Activation of Hedgehog signaling by loss of *GNAS* causes heterotopic ossification. *Nat Med* **19**: 1505–1512.
- Reiter JF, Skarnes WC. 2006. Tectonic, a novel regulator of the Hedgehog pathway required for both activation and inhibition. *Genes Dev* **20**: 22–27.
- Rix S, Calmont A, Scambler PJ, Beales PL. 2011. An *Ift80* mouse model of short rib polydactyly syndromes shows defects in hedgehog signalling without loss or malformation of cilia. *Hum Mol Genet* **20**: 1306–1314.
- Rohatgi R, Milenkovic L, Scott MP. 2007. Patched1 regulates hedgehog signaling at the primary cilium. *Science* **317**: 372–376.
- Ruiz-Perez VL, Tompson SW, Blair HJ, Espinoza-Valdez C, Lapunzina P, Silva EO, Hamel B, Gibbs JL, Young ID, Wright MJ, et al. 2003. Mutations in two nonhomologous genes in a head-to-head configuration cause Ellis-van Creveld syndrome. *Am J Hum Genet* **72**: 728–732.
- Ruiz-Perez VL, Blair HJ, Rodriguez-Andres ME, Blanco MJ, Wilson A, Liu YN, Miles C, Peters H, Goodship JA. 2007. *Evc* is a positive mediator of *Ihh*-regulated bone growth that localises at the base of chondrocyte cilia. *Development* **134**: 2903–2912.
- Santos N, Reiter JF. 2014. A central region of *Gli2* regulates its localization to the primary cilium and transcriptional activity. *J Cell Sci* **127**: 1500–1510.
- Spassky N, Han YG, Aguilar A, Strehl L, Besse L, Laclef C, Ros MR, Garcia-Verdugo JM, Alvarez-Buylla A. 2008. Primary cilia are required for cerebellar development and *Shh*-dependent expansion of progenitor pool. *Dev Biol* **317**: 246–259.
- Srouf M, Schwartzentruber J, Hamdan FF, Ospina LH, Patry L, Labuda D, Massicotte C, Dobrzyniecka S, Capo-Chichi JM, Papillon-Cavanagh S, et al. 2012. Mutations in *C5ORF42* cause Joubert syndrome in the French Canadian population. *Am J Hum Genet* **90**: 693–700.
- Stephen LA, Tawamie H, Davis GM, Tebbe L, Nürnberg P, Nürnberg G, Thiele H, Thoenes M, Boltshauser E, Uebe S, et al. 2015. *TALPID3* controls centrosome and cell polarity and the human ortholog *KIAA0586* is mutated in Joubert syndrome (*JBTS23*). *eLife* **4**: e08077.
- Swiderski RE, Nakano Y, Mullins RF, Seo S, Bánfi B. 2014. A mutation in the mouse *tte26* gene leads to impaired hedgehog signaling. *PLoS Genet* **10**: e1004689.
- Tanos BE, Yang HJ, Soni R, Wang WJ, Macaluso FP, Asara JM, Tsou MF. 2013. Centriole distal appendages promote membrane docking, leading to cilia initiation. *Genes Dev* **27**: 163–168.
- Tay SY, Yu X, Wong KN, Panse P, Ng CP, Roy S. 2010. The iguana/DZIP1 protein is a novel component of the ciliogenic pathway essential for axonemal biogenesis. *Dev Dyn* **239**: 527–534.
- Taylor SP, Dantas TJ, Duran I, Wu S, Lachman RS, University of Washington Center for Mendelian Genomics Consortium, Nelson SF, Cohn DH, Vallee RB, Krakow D. 2015. Mutations in *DYNC2LI1* disrupt cilia function and cause short rib polydactyly syndrome. *Nat Commun* **6**: 7092.
- Tong CK, Han YG, Shah JK, Obernier K, Guinto CD, Alvarez-Buylla A. 2014. Primary cilia are required in a unique



- subpopulation of neural progenitors. *Proc Natl Acad Sci* **111**: 12438–12443.
- Toriyama M, Lee C, Taylor SP, Duran I, Cohn DH, Bruel AL, Tabler JM, Drew K, Kelly MR, Kim S, et al. 2016. The ciliopathy-associated CPLANE proteins direct basal body recruitment of intraflagellar transport machinery. *Nat Genet* **48**: 648–656.
- Tran PV, Haycraft CJ, Besschetnova TY, Turbe-Doan A, Stottmann RW, Herron BJ, Chesebro AL, Qiu H, Scherz PJ, Shah JV, et al. 2008. THM1 negatively modulates mouse sonic hedgehog signal transduction and affects retrograde intraflagellar transport in cilia. *Nat Genet* **40**: 403–410.
- Tuson M, He M, Anderson KV. 2011. Protein kinase A acts at the basal body of the primary cilium to prevent Gli2 activation and ventralization of the mouse neural tube. *Development* 2011 **138**: 4921–4930.
- * Vaisse C, Reiter JF, Berbari NF. 2016. Cilia and obesity. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a028217.
- Vuolo L, Herrera A, Torroba B, Menendez A, Pons S. 2015. Ciliary adenylyl cyclases control the Hedgehog pathway. *J Cell Sci* **128**: 2928–2937.
- Walczak-Sztulpa J, Eggenschwiler J, Osborn D, Brown DA, Emma F, Klingenberg C, Hennekam RC, Torre G, Garshasbi M, Tzschach A, et al. 2010. Cranioectodermal dysplasia, Sensenbrenner syndrome, is a ciliopathy caused by mutations in the *IFT122* gene. *Am J Hum Genet* **86**: 949–956.
- Wang C, Low WC, Liu A, Wang B. 2013. Centrosomal protein DZIP1 regulates Hedgehog signaling by promoting cytoplasmic retention of transcription factor GLI3 and affecting ciliogenesis. *J Biol Chem* **288**: 29518–29529.
- Wang H, Kane AW, Lee C, Ahn S. 2014. Gli3 repressor controls cell fates and cell adhesion for proper establishment of neurogenic niche. *Cell Rep* **8**: 1093–1104.
- Weatherbee SD, Niswander LA, Anderson KV. 2009. A mouse model for Meckel syndrome reveals Mks1 is required for ciliogenesis and Hedgehog signaling. *Hum Mol Genet* **18**: 4565–4575.
- Wen X, Lai CK, Evangelista M, Hongo JA, de Sauvage FJ, Scales SJ. 2010. Kinetics of hedgehog-dependent full-length Gli3 accumulation in primary cilia and subsequent degradation. *Mol Cell Biol* **30**: 1910–1922.
- Wong SY, Seol AD, So PL, Ermilov AN, Bichakjian CK, Epstein EH Jr, Dlugosz AA, Reiter JF. 2009. Primary cilia can both mediate and suppress Hedgehog pathway-dependent tumorigenesis. *Nat Med* **15**: 1055–1061.
- Yadav SP, Sharma NK, Liu C, Dong L, Li T, Swaroop A. 2016. Centrosomal protein CP110 controls maturation of the mother centriole during cilia biogenesis. *Development* **143**: 1491–1501.
- Ye X, Zeng H, Ning G, Reiter JF, Liu A. 2014. C2cd3 is critical for centriolar distal appendage assembly and ciliary vesicle docking in mammals. *Proc Natl Acad Sci* **111**: 2164–2169.
- Yin Y, Bangs F, Paton IR, Prescott A, James J, Davey MG, Whitley P, Genikhovich G, Technau U, Burt DW, et al. 2009. The *Talpid3* gene (*KIAA0586*) encodes a centrosomal protein that is essential for primary cilia formation. *Development* **136**: 655–664.
- Yu M, Gipp J, Yoon JW, Iannaccone P, Walterhouse D, Bushman W. 2009. Sonic Hedgehog-responsive genes in the fetal prostate. *J Biol Chem* **284**: 5620–5629.
- Zeng H, Hoover AN, Liu A. 2010. PCP effector gene *Inturned* is an important regulator of cilia formation and embryonic development in mammals. *Dev Biol* **339**: 418–428.