The Primary Cilium: A Signaling Center During Vertebrate Development

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Abstract

The primary cilium has recently stepped into the spotlight, as a flood of data demonstrate that this organelle has crucial roles in vertebrate development and human genetic diseases. Cilia are required for the response to developmental signals, and evidence is accumulating that the primary cilium is specialized for Hedgehog (Hh) signal transduction. Formation of cilia, in turn, is regulated by other signaling pathways, possibly including the planar cell polarity pathway. The cilium therefore represents a nexus for signaling pathways during development. The connections between cilia and developmental signaling have begun to clarify the basis of human diseases associated with ciliary dysfunction.

The primary cilium, a slim microtubule-based organelle that projects from the surface of vertebrate cells, has been the focus of intensive studies transforming it from a poorly understood curiosity into a structure recognized for its importance in development, inherited human disease and cancer. Cilia and flagella are ancient structures present in organisms as diverse as single celled eukaryotes and humans. The evolutionarily conserved mechanism of Intraflagellar Transport (IFT), first described in the alga *Chlamydomonas*, is essential for the construction and maintenance of these structures in all species[1, 2].

In the past decade, the function of mammalian primary cilia has been revealed by both developmental genetic analyses and human genetic studies. Disruptions of the primary cilium have been associated with the common disorder human cystic kidney disease[3–6]. In addition, rare recessive human disorders known as ciliopathies, with complex syndromes that include cystic kidneys, obesity, mental retardation, blindness and various developmental malformations, have been shown to be caused by mutations in proteins localized to cilia and ciliary basal bodies (reviewed in[7–10]). In parallel, genetic studies in the mouse demonstrated that cilia are essential for signaling through the Hh pathway, a crucial signaling pathway for organizing the body plan, organogenesis and tumorigenesis[11].

The importance of primary cilia in vertebrate development was first revealed in genetic experiments that demonstrated that cilia are required for survival and patterning of the mouse embryo[11]. Phenotypic, genetic and biochemical analysis then showed that embryonic phenotypes of the cilia mutants were caused by disruption of Hh signal transduction. This unexpected finding raised many questions, including why the cilium is a good locale for signal transduction, why cilia are required for vertebrate but not invertebrate Hh signaling, and whether primary cilia are important in regulating other developmental signaling pathways.

Other recent experiments have suggested that additional developmental signaling pathways help regulate the formation of cilia. The most complete studies have implicated components

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of the planar cell polarity (PCP) pathway in the regulation of the position and formation of cilia. These processes, which could indirectly regulate the activity of Hh signaling, appear to be particularly important during organogenesis.

Here we review the relationships between primary cilia and signaling pathways during vertebrate embryonic development. After describing the evolutionarily conserved mechanism of IFT, we review the evidence that Hh signaling requires IFT and cilia. We then describe recent work suggesting that the primary cilium in vertebrate embryos is specialized such that Hh signaling is restricted to the cilium. After considering whether additional developmental signaling pathways require cilia, we discuss the evidence that other signaling pathways regulate ciliogenesis. We conclude with a discussion of how the findings on the relationship between cilia and developmental signals are beginning to explain the syndromes seen in cilia-related human diseases, focusing on the formation of kidney cysts, a hallmark of disorders caused by abnormal primary cilia.

Intraflagellar Transport

The cilium is extended and maintained by the transport of particles along the axoneme mediated by the IFT machinery (Fig. 1). IFT trafficking from the base to the tip of the cilium depends on the microtubule plus-end directed motor Kinesin-2, which associates with two IFT protein complexes, IFT-A and IFT-B. IFT-B is essential for anterograde trafficking, while IFT-A and the minus-end directed motor cytoplasmic Dynein-2 (Dync2h1) are required for retrograde trafficking[1]. In all organisms studied, disruption of the Kinesin-2 motor or the IFT-B complex blocks cilia formation. Perturbation of retrograde trafficking by disruption of the Dynein or the IFT-A complex results in short, bulged cilia[1, 12–15] (Table1).

Cilia are nucleated by the basal body, which is made up of the mother centriole and associated pericentriolar proteins. Some basal body proteins are required for cilia formation; evidence suggests some may recruit cargo from the Golgi to the nascent ciliary membrane and others may promote loading of cargo into the axoneme[16, 17] (Fig. 1).

Evidence Linking Hh Signaling to Cilia

Vertebrate Hh signaling requires IFT

The first evidence that vertebrate Hh signaling depends on cilia came from a phenotype-based screen for mutations that alter patterning of the mouse embryo. This screen identified several mutants displaying morphological and patterning phenotypes consistent with altered Hh signaling, including loss of the ventral cell types in the neural tube specified by high levels of Sonic hedgehog (Shh)[11]. The genes disrupted in these mutants encode several components of the IFT machinery, including the IFT-B complex components Ift172 and Ift88, as well as Dync2h1, the IFT-dedicated retrograde motor[11, 18, 19] (Fig. 1). Disruption of the Kinesin-2 motor in *Kif3a* null embryos also caused similar defects in Shh-dependent neural patterning (Fig. 2)[5]. Genetic studies showed that IFT proteins act at the heart of the Shh pathway, downstream of the membrane proteins Patched (Ptch) and Smoothened (Smo) and upstream of the Gli transcription factors that implement the pathway[11, 18] (Fig. 3, Table 1).

The role of IFT proteins in Hh signaling is complex, partly because of the complex output of the Hh pathway. In the absence of Hh ligand, Gli transcription factors, which function as effectors of the pathway, are proteolytically processed to Gli repressor forms (GliR) that keep Hh target genes off (Fig. 3). In response to Hh ligand, processing of GliR is blocked and activated Gli transcription factors (GliA) activate the expression of Hh target genes. IFT is required both for production of GliA and Gli3R[18–21]; as a result, IFT mutants show loss of

Hh phenotypes in some cell types and gain of Hh phenotypes in others. For example, GliA plays a central role in neural patterning, and IFT mutant embryos show a loss of Hh signaling in the neural tube. In contrast, GliR plays a central role in limb development and IFT mutants that survive to later stages of embryogenesis show pre-axial polydactyly, characteristic of loss of GliR (Fig. 2)[18, 19, 21].

Recent experiments demonstrated that IFT is also required for Hh signaling in zebrafish. Zebrafish lacking both maternal and zygotic *Ift88* exhibit Hh signaling defects in the neural tube and somites[22], however the patterning defects caused by loss of IFT in zebrafish are slightly different than those seen in mammals. Mouse *Ift88* mutants lack Shh-dependent ventral neural cell fates[11], and zebrafish maternal/zygotic *Ift88* mutants lack cell fates requiring the highest levels of Hh, such as V3 interneuron progenitors and muscle pioneer cells. However, cell types within both the neural tube and somites normally specified by lower levels of Hh are expanded[22]. This difference may be due to a different balance of Gli activators and repressors in the fish compared to the mouse[22].

Basal body proteins required for Hh signaling

Additional evidence that cilia are required for Hh signaling came from analysis of basal body protein mutations. Dozens of proteins are localized to centrosomes and the pericentriolar material, and a subset of these proteins has been shown to be required for cilia formation (Table 1). In every case examined thus far, these proteins are required for Hh signaling. For example, the chick *talpid3* mutation was first identified based on polydactyly[23, 24] and causes developmental defects consistent with disrupted Hh signaling[25, 26]. *Talpid3* mutant embryos fail to form cilia, and the affected gene was shown to encode a centrosomal protein[27].

Mutations disrupting other basal body proteins, including OFD1, FTM, MKS1 and EVC, cause human ciliopathies and affect mammalian Hh signaling. Mice mutant for *Ofd1*, *Ftm/Rpgrip1l*, or *Mks1* have abnormal or absent cilia, and exhibit Hh signaling defects corresponding to the severity of the cilia disruption[28–32] (Table 1). EVC also localizes to the basal body and is mutated in the human skeletal disorder Ellis-van Creveld syndrome[33, 34]. Unlike other basal body proteins, expression of *Evc* in the mouse is limited to developing skeletal structures, and Evc does not appear to be required for the formation of cilia in chondrocytes. Nevertheless, *Evc−/−* mice exhibit reduced Indian hedgehog (Ihh) signaling specifically within skeletal structures. Thus Evc apparently does not affect ciliogenesis, but is required for Hh signaling in a specific cell type.

Enrichment of Hh pathway components in cilia

Based on the genetic studies associated Hh signaling with cilia, several groups have tested whether the proteins that mediate Hh signal transduction are localized to cilia. Remarkably, all the key components of the Hh pathway are enriched in cilia (Fig. 3b).

Two transmembrane proteins, Ptch1 (the Hh receptor) and Smo (which acts downstream of Ptch1), show dynamic, Hh-dependent trafficking in cilia[35, 36]. In the absence of Hh ligand, Ptch1 is localized to the base of the cilium and Smo is not associated with cilia. Upon exposure to ligand, Ptch1 exits and Smo moves into the cilium[35, 36]. Activating mutations in Smo, as well as pathway agonists, cause Smo to localize constitutively to the cilium[36]. Despite the enrichment of Smo in the cilium in response to Shh, Smo also accumulates in cilia in cells that lack the Dync2h1 retrograde IFT motor in the absence of ligand[37, 38], suggesting that Smo traffics through the cilium in the absence of ligand, and that Shh increases ciliary accumulation of Smo.

Gli transcription factors are also enriched in cilia. Both Gli2, which functions primarily as a transcriptional activator in mammalian Hh signaling, and Gli3, which can be processed into a repressor, localize to the tips of cilia[39], and recent reports indicate that pathway activation increases the amount of Gli2 and Gli3 at the tips of cilia in fibroblasts[37, 40]. Ciliary enrichment of Gli2 depends on the presence of activated Smo[37]. Like Smo, Gli2 accumulates at high levels in Dync2h1 mutant cilia[37], suggesting that Gli2 trafficks continuously through the cilium, and activated Smo increases the accumulation of Gli2 at the tip.

Sufu, an important negative regulator of mammalian Hh signaling, also localizes to the primary cilia tip[3940]. Genetic and biochemical data have shown that Sufu can inhibit Hh signaling even in the absence of cilia,[41, 42] however partial knockdown of Sufu results in pathway activation only if cilia are present, suggesting a complex role for Sufu in the cilium[20] (M. Tuson and K. Anderson, unpub. data). While the relationship between Sufu and cilia remains to be defined, the data are consistent with a model in which Smo activates the pathway at the cilia tip by antagonizing the activity of Sufu, thereby promoting activation of Gli transcription factors[40] (Fig. 3b). Thus, Gli2 activation requires cilia, however the precise mechanism by which this occurs is not defined. In addition to suppression of Sufu, it may require post-translational modifications to Gli2 and the presence of yet-unidentified Hh pathway components within cilia.

Trafficking within the Cilium Regulates Hh Signaling

The finding that vertebrate Hh signaling requires primary cilia has raised the question of why this organelle is particularly suited to this critical pathway. The simplest explanation is that the cilium provides an environment where pathway components are enriched to facilitate their interactions. However, the dynamic relocalization of pathway components in response to ligand suggests that trafficking of Hh pathway proteins is crucial for pathway activation, and it is likely that IFT proteins are important in this trafficking.

IFT depends on two protein complexes, IFT-A and IFT-B, that form large platforms transporting cargo between the base and tip of the cilium[13] (Fig. 1). Mutants lacking components of the IFT-B protein complex (*Ift172*, *Ift88*, *Ift52* and *Ift57*)[11, 21, 43] lack cilia and all response to Hh ligands, precluding analysis of the role of IFT-mediated transport within the cilium. In contrast, mutations in IFT-A proteins allow the formation of cilia (with abnormal morphology) and cause very different developmental phenotypes from mutants that prevent cilia formation: Hh signaling is activated rather than decreased (Fig. 2, Table 1). Studies in *Chlamydomonas* argue that the IFT-A complex cooperates with dynein to mediate retrograde transport[1], as the rate of anterograde IFT is normal in these mutants, while retrograde trafficking is slowed[12, 14, 44]. Mutants in two mouse IFT-A complex proteins have been characterized, THM-1 (aka Ttc1b; IFT-139) and IFT-122. These mutants show an expansion of Hh-dependent neural cell types, as well as increased expression of direct Hh target genes[15, 45, 46].

The opposing phenotypes of IFT-A and IFT-B mouse mutants are surprising, as IFT-A and -B were originally identified as subcomplexes of a single large complex[47], and appear to move coordinately in the same particle[48, 49]. Both IFT-A and Dync2h1 are important for retrograde IFT, but mutations in IFT-A proteins increased Hh pathway activity[15, 45] while mutations in Dync2h1 block the response to Hh ligands[18, 19]. These findings suggest that disruption of IFT-A may differentially disrupt trafficking of Hh pathway components, thereby causing phenotypes distinct from those observed in mutants in which cilia or absent or the dynein motor is disrupted. Recent data suggests that Smo may be trafficked laterally from the plasma membrane into the cilium, presumably an IFT-independent mechanism[50]. Given that the IFT machinery functions downstream of Smo but upstream of the Gli

transcription factors, it will be particularly informative to examine trafficking of Smo and the Gli proteins in IFT-A mutants.

Why is Hh Signaling Tied to Cilia?

Kif7 as a link between Hh signaling and cilia

Despite the evolutionary conservation of the Hh pathway and the importance of primary cilia in vertebrate Hh signaling, cilia are not required for Hh signaling in *Drosophila*. This raises the question of why vertebrate Hh signaling is coupled to cilia. Recent data suggest that Kif7, a kinesin that is the vertebrate homolog of *Drosophila* Costal2 (Cos2), may tether the vertebrate Hh pathway to cilia.

Cos2, a key component of the *Drosophila* Hh pathway, is a kinesin-related protein that serves as a scaffold for Hh signaling complexes. Cos2 has dual functions in the pathway: it promotes formation of the repressor form of Ci (the Gli homolog) in the absence of Hh ligand by recruiting kinases that prime Ci for processing; and it permits high levels of pathway activation upon Hh stimulation by antagonizing Sufu[51–53] (Fig. 3). Although Cos2 can bind microtubules, amino acids in its motor domain have diverged from those of other kinesins such that its ability to bind ATP is disrupted[54].

Several recent papers demonstrated that zebrafish and mouse Kif7 proteins, like *Drosophila* Cos2, both positively and negatively regulate the Shh pathway[40, 55–57]. Unlike Cos2, the vertebrate Kif7 motor domain retains all the motifs typical of kinesin motors, suggesting that it should act as a motor protein. In the absence of ligand, Kif7 localizes to the base of the primary cilium and moves to the tip of the cilium in response to pathway activation[40, 55] (Fig. 3). This translocation depends on the Kif7 motor domain, suggesting that Kif7, like Kinesin-II, acts as an anterograde motor within the cilium[55].

Conventional kinesins, such as Kif7, carry cargo towards the plus end of microtubules, and the minus ends of both axonemal and cytoplasmic microtubules are located at the base of the cilium. Because Kif7-eGFP is enriched at the base of the cilium, we proposed that Kif7 may traffic Gli2 protein away from the cilium in the absence of ligand to prevent Gli activation[55] (Fig. 3b). The positive role of Kif7 is presumably coupled to its movement to the cilia tip after pathway activation, where Sufu and the Gli transcription factors are enriched. Kif7 may promote Gli activation at the tip, perhaps by antagonizing the activity of Sufu[55]. Kif7 appears to be required for the increase of both Gli2 and Gli3 at the cilia tip after exposure to ligand[40], suggesting both Gli proteins may be cargos of Kif7 within the cilium. Thus, the dual roles of Kif7 as a Shh pathway component and ciliary motor could explain why mammalian Shh signaling depends on the primary cilium (Fig. 3b).

Fused is a cilia-associated protein in vertebrates

Fused (Fu) is an important component of the *Drosophila* Hh pathway: it is a serine/threonine kinase that phosphorylates Cos2, Sufu and perhaps other components of the pathway. and is required for activation of Ci in response to Hh ligand[53]. Fu is also important for Hh signaling in zebrafish,[58] but Hh signaling is normal in mice lacking Fu[59,60]. Recent work has shown that mammalian and zebrafish Fu are required for the construction of specialized motile cilia[61], and *Fu* mutant mice die postnatally with hydrocephalus, presumably due to dysfunction of motile cilia in brain ventricles[59,60]. Thus Fu, like Kif7/Cos2, links Hh signaling with cilia, although this connection appears to have been lost in mammals. It has been proposed that another unidentified kinase may substitute for Fu in mammalian Hh signaling, and several human kinome screens have been undertaken to identify kinases required for Hh signaling[62, 63]. While the kinases identified in these screens have yet to be

characterized *in vivo*, it will be interesting to determine whether a protein that is functionally homologous to Fu also links Shh signaling to cilia in mammals.

The evolution of Kif7 and Fused

Recent work in planaria supports the view that some conserved components of the Hh pathway were associated with cilia before they were associated with Hh signaling. Planaria homologues of the Hh pathway components Kif7/Cos2, Fu, and Iguana are required in planaria for formation of motile cilia but not Hh signaling[64, 65]. Planaria represent a lineage of animals distinct from both that of insects and of vertebrates. Thus, the finding that Kif7 and Fu function in cilia in two independent metazoan lineages suggests the ancestral role of these proteins was in cilia. The requirement for these cilia-associated proteins in *Drosophila* Hh signaling suggests that Hh signaling was associated with cilia in the common ancestor of *Drosophila* and vertebrates.

Are Cilia Dedicated to Hh Signaling?

Most cells in the mouse embryo have primary cilia, while a relatively small number of cells respond to Hh at any particular stage. This has raised interest in the possibility that other developmental signaling pathways may also depend on cilia. However, the disruption of other developmental signaling pathways, including canonical and non-canonical Wnt, TGFβ, Notch and FGF signaling, causes developmental abnormalities that do not overlap with the IFT mutant phenotypes. Nevertheless, cilia could have more subtle roles in other signaling pathways or might be important for signaling at later embryonic stages, after IFT mutants arrest.

Wnt signaling

Most attention has focused on the relationship between cilia and Wnt signaling. Several groups reported that knockdown of cilia-associated proteins in cultured cells or zebrafish embryos elevates canonical Wnt signaling and/or disrupts processes that depend on non-canonical Wnt signaling, such as convergent extension[66–72]. The primary cilium was therefore proposed to act as a switch between canonical and non-canonical Wnt signaling pathways[68, 69].

However, this connection between cilia and Wnt signaling is controversial. Mouse IFT mutants do not show the phenotypes characteristic of Wnt pathway mutants. For example, reduced canonical Wnt signaling disrupts gastrulation and early patterning[73] and inappropriate activation of the Wnt pathway can cause axis duplications and failure to form anterior structures[74–77]. Although mammalian non-canonical Wnt pathway mutants fail to close the entire neural tube caudal to the forebrain, neural patterning in these mutants is relatively normal[78, 79]. Similarly, zebrafish mutants that lack both maternal and zygotic activity of the *Ift88* gene have defects in Hh signaling, but do not show the defects in convergent extension defects associated with disruption of non-canonical Wnt signaling[22].

Recent work examined the expression of canonical Wnt reporters *in vivo* in *Kif3a*, *Ift88*, *Ift172* and *Dync2h1* mutant mice and failed to find any alteration in either the domain or levels of Wnt activity[38]. Similarly, mouse embryos homozygous for a mutation in the IFTA protein Tct21b (IFT-139) have a neural patterning phenotype consistent with activation of Hh signaling[15, 46], but do not show altered canonical Wnt signaling[46]. Thus it appears that cilia are not required for canonical or noncanonical Wnt signaling in the first half of vertebrate embryogenesis.

Pdgfra signaling

Cilia have been found to be important for signaling by PDGF receptor alpha (Pdgfrα) in cultured fibroblasts[80], as well as for PDGF-dependent directed cell migration in these cells[81]. Additionally, the receptor is localized to primary cilia in vivo in neural stem cells of the adult rat subventricular zone (SVZ)[82]. Loss of Pdgfrα signaling does not produce any striking phenotypes in early mouse embryos, but is critical for development of later tissues, including oligodendrocytes and neural crest-derived craniofacial structures[83, 84]. It will be important to test whether loss of cilia in the second half of embryogenesis affects Pdgfrα signaling in these cell types in vivo.

Hedgehog signaling in adult tissues

After birth, Shh signaling continues to play important roles in the growth of the brain and the maintenance of neural progenitors[85]. Conditional deletion of *Ift88* or *Kif3a* within the brain results in severe hypoplasia of the cerebellum due to a failure of granule cell progenitor proliferation[86], a process that depends on Shh signaling[85, 87]. Primary cilia are also required to modulate the Shh-dependent formation and maintenance of hippocampal granule neuron precursors, important for maintaining neurogenesis in the adult[88]. Based on the tight association between IFT/cilia mutants and specific defects on Hh signaling, we propose that cilia are essential for Hedgehog signaling in all cell types and that, at least in early development, primary cilia in vertebrate embryos are dedicated to Hh signal transduction. The data do not rule out the possibility that cilia may have important roles in other signaling pathways later in development or in specific cell types. For example, although a number of the defects observed in human ciliopathies can be attributed to abnormal Hh signaling, the molecular bases of other features of these diseases remain unknown (Box 1).

Signaling Pathways that Regulate Ciliary Development

Because of the importance of primary cilia in embryonic patterning, there is considerable interest in identifying signaling pathways that regulate cilia formation. Several transcription factors are known to be required for formation of motile cilia and node cilia (reviewed by[89]). However, only recently has evidence emerged about signaling pathways that regulate the formation and position of primary cilia.

Fgf and inositol signaling

Recent evidence from zebrafish implicates FGF signaling in the regulation of cilia length. Knockdown of Fgfr1 or Fgf ligands results in shortened cilia in Kupffer's vesicle and randomized organ laterality[90–92]. The expression of ciliogenic transcription factors and *Ift88* is reduced in these embryos [91]. It will be interesting to test whether Fgf pathway mutations in the mouse also affect ciliogenesis, and if these effects on ciliogenesis alter Hh signal transduction.

Components of the phosphatidylinositol signaling cascade also appear to regulate cilia length. In zebrafish, morpholino knockdown of the inositol kinase Ipk1 reduces the frequency of cilia beating and decreased cilia length[93]. In humans, the inositol phosphatase INPP5E is mutated in one form of Joubert syndrome, a ciliopathy. INPP5E is enriched in the ciliary axoneme, and in fibroblasts from patients with Joubert syndrome, cilia were more labile than wild type[94].

The mechanisms by which the Fgf and phosphatidylinositol pathways regulate cilia formation or maintenance remain to be elucidated. It will be informative to investigate

whether these pathways have a general role in primary cilia formation or act in a subset of specialized cilia.

Planar cell polarity signaling and cilia

Recent experiments argue that there is a close connection between components of planar cell polarity (PCP) signaling and cilia positioning. An excellent example of this connection is found in the mechanosensory hair cells in the organ of Corti in the cochlea. The primary cilium of the hair cell, called the kinocilium, is always oriented on the lateral side of the developing cell. The position of the kinocilium determines the polarity of the chevron of stereocilia (actin-based sensory organelles) on the hair cell. Core components of the non-canonical Wnt pathway, a PCP pathway, are required for the polarity of these hair cells[95–97,98].

When primary cilia are removed by conditional deletion of *Ift88*, the polarity of the hair cells is disrupted[99], similar to the phenotype seen in non-canonical Wnt mutants[97]. This finding indicated that the presence of the kinocilium is important for the correct orientation of the stereocilia and the organization of the hair cells, and raised the possibility that the primary cilium might regulate the non-canonical Wnt pathway in this tissue; however the relationship between the kinocilium and planar polarity is more complex. As in *Drosophila*, components of the non-canonical Wnt pathway are planar polarized in hair cells, and that polarity is both required for PCP signaling and provides a readout of effective PCP signaling[97, 100]. In the hair cells of *Ift88* conditional mutants, the polarity of PCP proteins is not disrupted. This indicates that IFT88, and presumably cilia, are not required for the activity of the core PCP pathway in this tissue, as in early embryos. Instead, it appears that one output of the non-canonical Wnt signaling is to control the position of the basal body and thereby cilia position[99] (Fig. 4a). In addition, the findings indicate that IFT88 itself must be required to reposition the basal body to a polarized position. The mechanisms by which the position of the basal body is regulated by IFT88 are not known.

Studies on the motile cilia on the epidermis of *Xenopus* embryos support the hypothesis that components of the planar polarity pathway control polarized organization of cilia. These cells are multiciliated and the cilia on each cell share a common polarity[101]. Disruption of the activity of the PCP protein Disheveled (Dvl) disrupts the polarity of the cilia on these cells. Dvl localizes to the basal bodies of epidermal cells and Dvl morphants have a reduced number of short cilia[101]; this finding indicates that apical docking of basal bodies, and therefore the ability to form cilia, depends on Dvl and possibly other components of the PCP pathway (Fig. 4b).

While changes in the position of cilia are unlikely to influence their ability to transduce Hh signals, some PCP components do affect Hh signaling. Inturned and Fuzzy are downstream effectors of the non-canonical Wnt pathway in *Drosophila*, and morpholino knockdown of *Xenopus* Inturned or Fuzzy disrupts both the apical actin network and cilia formation[102]. These morphants fail to undergo normal convergent extension due to defects in PCP and also display defects consistent with a loss of Shh signaling[102]. Similarly, mouse *Fuz* and *Inturned* mutants have short cilia and disrupted Hh signaling[103–105]. Thus, components of the planar polarity pathway can be important for both formation and polarity of cilia (Fig. 4b). There is no evidence, however, that other components of the non-canonical Wnt pathway are required for ciliogenesis, suggesting that the roles of Inturned and Fuzzy in cilia formation may be unrelated to non-canonical Wnt signaling.

Cilia, signaling and disease

Numerous human disorders have now been linked to defects in cilia structure or in cilia localized proteins. These include autosomal dominant polycystic kidney disease (PKD), and recessive pleiotropic disorders such as Bardet-Biedl syndrome, Joubert syndrome, Meckel syndrome, and Ellis-van Creveld syndrome. Some aspects of these disorders, such as polydactyly and skeletal abnormalities, are likely due to misregulated Hh signaling, but the molecular basis of other defects, such as cystic kidneys, are not well understood (Box 1). The dysregulated Hh signaling associated with several types of human cancers also depends on cilia. Thus studies on the relationship between cilia and signaling during development have direct implications for human disease.

Hedgehog Signaling in Tumors

Inappropriate activation of Shh signaling can cause medulloblastoma and rhabdomyosarcoma, pediatric tumors of the cerebellum and muscle, and is found in all cases of basal cell carcinoma[106–108]. In addition, growing evidence indicates that Shh signals promote the growth of other types of tumors[109, 110]. Recent studies demonstrate that cilia regulate Hh signaling in tumors, and that the role of cilia in tumors depends on how the pathway is activated. Expression of activated Smo in the postnatal mouse brain can cause medulloblastoma, but removal of cilia prevents tumor formation[111], consistent with the earlier genetic experiments indicating that cilia are required for activity of the pathway at a step downstream of Smo. Constitutively active Gli2 can also cause in medulloblastoma in mice, but only when cilia are removed[111]. This suggests that Gli2 alone cannot activate tumorigenesis in this cellular context when cilia-dependent Gli3 repressor is also present. Similar results were observed in basal cell carcinomas in mice harboring similar activating Hh pathway mutations: activated Smo caused tumors only in the presence of cilia whereas removal of cilia enhanced tumorigenesis caused by expression of activated Gli2[112]. Thus, in tumors as in development, cilia have both positive and negative effects on the Hh pathway.

Cilia and polycystic kidney disease

A hallmark of many human ciliopathies is the formation of kidney cysts, which often begins during fetal life, and is a developmental, rather than physiological, defect[3, 113]. This raises the question of whether kidney cysts result from a disruption of cilia-dependent developmental signals. Hh signaling is required for normal kidney development[114], but kidney cysts have not been reported in mutants that lack either positive or negative Hh regulators[115, 116]. Although a role for Hh signaling in PKD cannot be ruled out, some data suggest that cilia might modulate Wnt signaling in this tissue[117].

The connection between cilia, cystic kidneys and Wnt signaling was first raised by analysis of the *inversin (inv)* gene. Inv binds microtubules, and localizes to the basal body[118] and cilium[119]. Mutations in *inv* cause kidney disease in humans[120], and renal cysts in mice[118]. Inv interacts with Dvl, targeting membrane-bound Dvl for destruction. Inv has been proposed to act as a switch between canonical and non-canonical Wnt signaling, based on cell culture and morpholino knock-down experiments[68]. However, altered canonical Wnt signaling has not been reported in the kidneys of *inv−/−* mice.

Mouse overexpression experiments show that increased Wnt signaling can cause kidney cysts, and increased nuclear β-catenin is observed in the cystic kidney tubules of mice in which cilia have been conditionally ablated[121, 122]. However, reduced Wnt signaling has been observed in mice lacking Jouberin (Jbn), a cilia-localized protein mutated in a form of Joubert syndrome: mice that lack Jbn (*Ahi1−/−*) have cystic kidneys with reduced expression of a Wnt reporter and reduced nuclear β-catenin[123]. Thus additional experiments

that examine Wnt signaling during kidney development will be required to reconcile the conflicting data.

Recent theories of PKD have focused on the importance of the plane of cell division, under the control of PCP signaling, as a possible underlying defect in kidney cysts[124]. The elongation of kidney tubules is thought to depend on oriented cell divisions, and this is disrupted in kidney tubule cells of mice with cystic kidneys[117, 124]. Supporting this hypothesis, mice lacking the PCP protein Fat4 exhibit polycystic kidneys beginning at E16 associated with misorientation of mitotic spindles within the renal tubules[125]. This cyst formation is enhanced by removal of one or both copies of a core PCP pathway component *Vangl2*. Moreover, Fat4 localizes to cilia within the kidney, implicating the cilium in the modulation of the kidney PCP pathway[125]. *Drosophila* Fat, however, acts in a PCP pathway that does not depend on non-canonical Wnt signaling[126]. Moreover, mouse mutants of other PCP pathway components such as Vangl2 and Fuzzy have not been reported to have cystic kidneys, and the polarity of PCP component localization in kidney tubule cells has not been assessed in mice lacking renal cilia. Recent results suggest that misoriented cell division is neither necessary nor sufficient for the formation of kidney cysts[113]. Thus the pathway (or pathways) downstream of primary cilia that are misregulated to cause renal cysts remain to be determined.

Conclusions and Perspectives

Non-motile primary cilia play vital roles in vertebrate development from early stages of embryonic patterning, when they regulate the activity of the Hh pathway, to organogenesis, when they are important in the development and homeostasis of numerous tissues. The recent resurgence in interest in primary cilia has raised many new questions about the roles of cilia.

We know very little about the events of Hh signal transduction that occur within cilia. The mechanisms that traffic Smo to cilia, Ptch out of cilia, and that modulate the trafficking of Gli proteins within cilia in response to Hh pathway activation are all unknown. The opposing effects of different IFT components upon the regulation of the Hh pathway suggest that, in addition to providing a compartment where Hh pathway components are enriched, the IFT machinery plays a more complex role in regulating the pathway, but those roles have not been defined. In order to address these questions, it will be necessary to examine the trafficking of Hh pathway components in real time as well as probe their physical associations with the IFT machinery in wild-type cells as well as in cells mutant for various IFT components.

The dual roles of Kif7 in intraciliary trafficking and in the Hh pathway suggest a reason why vertebrate Hh signaling is tied to cilia, and other proteins may also have dual roles. For example: is there a mammalian kinase that performs functions analogous to those of *Drosophila* Fu and, if so, does it play roles in both ciliogenesis and Hh signaling? Do other components of the Hh signaling pathway affect the dynamics of ciliary trafficking?

Based on the phenotypes of the numerous IFT mutants characterized to date, it appears that during early vertebrate development, the cilium functions as a Hh-dedicated organelle. However, this does not preclude a requirement for cilia in modulating other signaling pathways in specific tissues later in development. These may include Pdgfrα signaling, signaling through G-protein coupled receptors in specific neurons[127], and PCP signaling in the kidney. A particularly interesting question is whether, in specific cell types, cilia are sites where Hh and other signaling pathways are integrated. As complex cross-talk between pathways is vital in regulating cellular responses during development and in disease states like cancer, understanding the function of the cilium as a signaling center will be critical.

Acknowledgments

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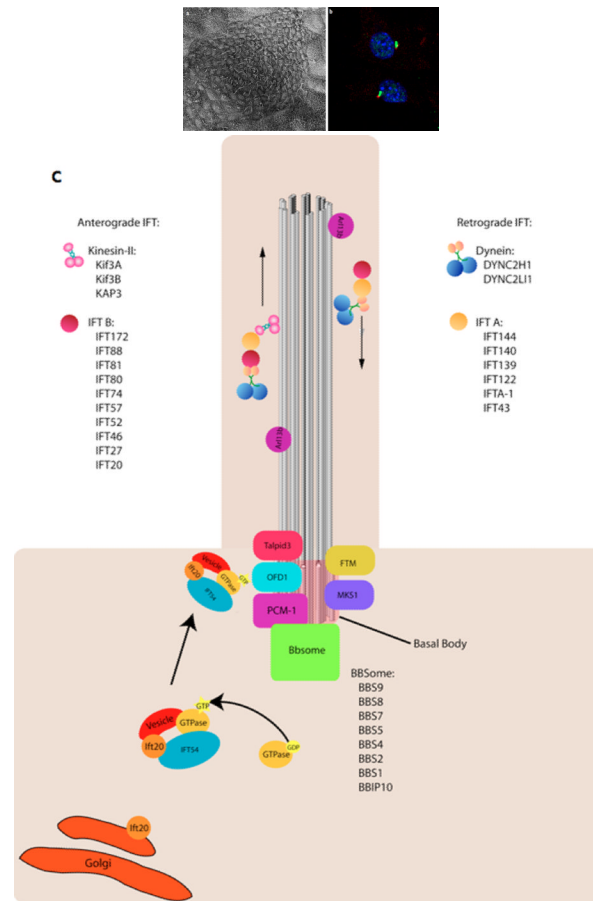
**Figure 1. Cilia structure and IFT**

a–b | Examples of types of mammalian cilia. **a** | The long cilia of the node at E7.5 are required for left-right asymmetry. **b** | Primary cilia in embryonic fibroblasts in green with the basal body in red.

c | Cargo is transported from the base to the tip of the cilium along the microtubule axoneme by Kinesin-2 together with the IFT-A and IFT-B complexes. Dynein mediates the return of IFT cargo to the base of the cilium[1, 14]. IFT-B proteins IFT-20 and IFT-54 may also participate in the trafficking of membrane vesicles from the Golgi to the ciliary membrane together with small GTPases[128]. Other small GTPases, including Arl13b also localize to cilia. While its precise trafficking role is not known, Arl13b is required for axoneme structure[129]. Certain basal body proteins also influence ciliary trafficking. Among these are components of the Bbsome, named their association with Bardet-Biedl syndrome (BBS). The precise functions of BBS proteins in cilia formation are unclear as they are not individually required for primary cilia formation, however they may function to promote loading of cargo to the ciliary axoneme[17]. Other basal body associated protein such as MKS1, FTM, OFD1, and Talpid3 are required for cilia formation, though how they regulate ciliogenesis has not been defined (For a review, see[10]).
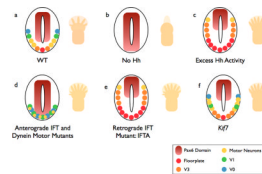
Figure 2. Summary of neural and limb patterning phenotypes in Hh pathway and cilia mutants
a | In wild-type (WT) embryos, ventral neural cell fates are specified by a gradient of Shh. The number and identity of digits in the limb is established by a Shh gradient from the posterior limb bud opposed by anterior Gli3 repressor (Gli3R). **b** | In the absence of Hh, (e.g. *Smo −/−*), ventral neural cell fates are lost. The limbs of Shh mutants lack digits. **c** | If the pathway is hyperactive (e.g. *Ptch −/−*), ventral cell types expand in the neural tube. Activation of the pathway within the limb, such as in Gli3 mutants (which lack Gli3R), causes the formation of extra digits. **d** | Anterograde IFT mutants (e.g. the IFT-B mutants *Ift88* or *Ift172*) lack cilia: Hh signaling is reduced, and the neural tube is dorsalized. This phenotype is milder than in (**b**) because cilia are also required for cilia Gli3R processing, and cell types that require low levels of Hh signaling are specified. Reduced Gli3R results in polydactyly. Dynein mutants display a similar phenotype, however they retain motor neurons in the caudal neural tube. **e** | IFT-A mutants (ie *Ift139*) exhibit phenotypes consistent with excess Hh signaling. **f** | Mutations that disrupt the kinesin *Kif7* cause a partial activation of the Hh pathway, with a modest expansion of cells that require intermediate levels of Hh.
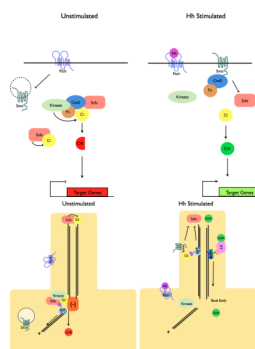
Figure 3. Localization of Hh pathway complexes in *Drosophila* and mammals

a | Modulation of protein complex structure and localization in *Drosophila* by Hh. In the absence of ligand, Ptch prevents translocation of Smo to the plasma membrane. A microtubule-associated complex including Cos2, Fu, Sufu and Ci recruits kinases including PKA, CK1, GSK3β that promote the processing of Ci into its repressor form (CiR)[130–133]. Sufu may also associate with full length Ci to prevent its translocation to the nucleus[130]. Upon activation of the pathway, Smo moves to the plasma membrane, and the Fu-Cos2 complex associates with the C-terminal tail of Smo resulting in the release of Ci. Pathway activation also inactivates the negative regulator[130–133]. **b** | In vertebrate Hh signaling, signal transduction takes place within cilia, but the behavior of protein complexes may parallel that in *Drosophila*. In the absence of ligand, Ptch localizes to the cilium, and is thought to block the entry of Smo into cilia[35]. Kif7 (the Cos2 homologue) localizes to the base of the cilium[55] where it may form a complex Gli proteins and other pathway components. Kif7 at the cilium base prevents Gli enrichment within the cilium and promotes processing of GliR. Upon activation of the pathway, Smo moves to the ciliary membrane and Kif7 translocates into the cilium thereby promoting Gli2 accumulation at the cilium tip[40, 55]. Kif7 at the cilia tip may also block the function of Sufu. Activated Gli is transported out of the cilium by Dynein and IFT particles.
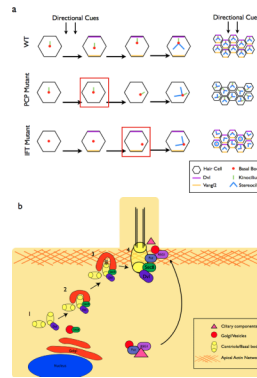
Figure 4. The role of the planar cell polarity pathway in cilia formation

a | In the WT cochlea, directional cues establish localization of core PCP pathway components. The kinocilium then directs the basal body towards the medial side of the cells. This in turn directs the orientation of the stereocilia bundles, resulting in the correct orientation of the bundles within the cochlea. In PCP mutants, the initial cell polarity is never established, resulting in the improper positioning of the basal body and stereocilia. In IFT mutants, planar polarity is established, however the basal body is not repositioned in the absence of the IFT-dependent kinocilium[99]. Red boxes depict the step in the establishment of ppolarity that is defective in each category of mutants.

b | Components of the PCP pathway are implicated in cilia formation. The centrioles are initially located away from the cell surface (1)[1] where they associate with Dvl, a PCP protein. Sec8, a component of vesicle trafficking machinery is recruited to the basal body in (2)[101]. At the cell surface in (3), Dvl mediates the fusion of the basal body-associated membrane with the cell membrane[101, 134]. The axoneme of the cilium extends in (4). *In vivo* mouse experiments indicate that the PCP effector Fuzzy, together with the small GTPase RGS1, also plays a role in trafficking membrane vesicles and ciliary components to the basal body[103].
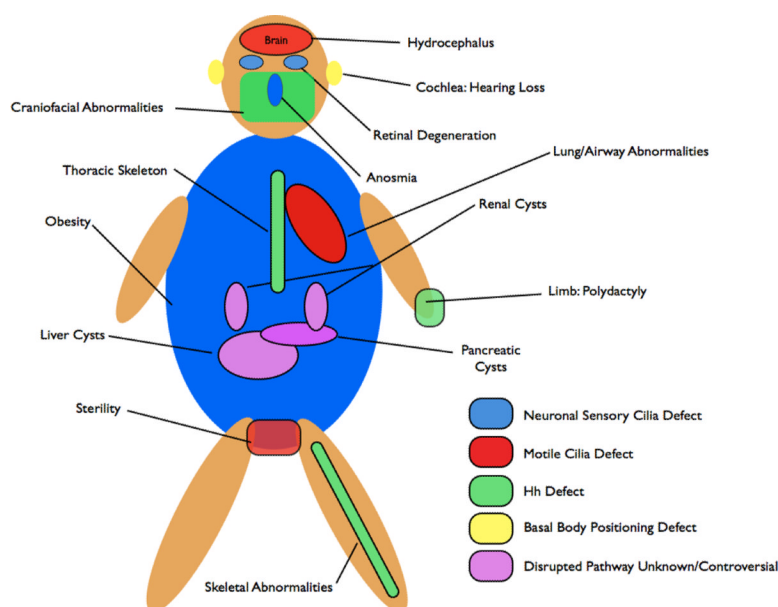
Box 1. Organs affected in human ciliopathies

Numerous pleiotropic human disorders have been attributed to defects in cilia formation[10, 135]. Some aspects of these syndromes, such as the polydactyly in patients with Bardet-Biedl syndrome (BBS) and Meckel Syndrome (MKS) and the skeletal abnormalities affecting the limbs in Ellis-van Creveld are attributed to defective Hh signaling. Polydactyly results from a loss of Gli3R and skeletal abnormalities resemble those observed in mutants that lack Ihh signaling[136]. Shh signaling is also required for craniofacial development, and defects in craniofacial structures, such as those observed in *Mks1* mutant mice, are also likely due to mis-regulated Hh signaling[32]. In addition, patients with a Joubert syndrome-like disorder exhibit ataxia due to cerebellar hypoplasia[137]. Growth of this tissue is Hh dependent[85]. Other attributes of human disorders result from defective specialized cilia. Retinal degeneration results from defects to photoreceptor cilia, which connect the outer light-responsive segment to the cell body. Detection of odorants depends on the primary cilia of sensory neurons in the olfactory epithelium and BBS patients often exhibit anosmia[138–140]. Another sensory deficit, hearing loss, is due to a requirement for the specialized primary cilia of the cochlea downstream of the PCP pathway in establishing the correct polarity of sensory hair cells[99]. Infertility observed in patients with ciliopathies is the result of defective motile cilia of spermatids or oviducts[139].

For some of the most severe and common abnormalities associated with ciliopathies, such as cyst formation in the kidneys, liver, biliary duct and pancreas, the underlying molecular causes downstream of the cilium remain unclear. Cyst formation is thought to result from defects in cell proliferation or mis-orientation of the mitotic spindle, however, whether and how these processes are regulated by cilia remain the subject of active investigation[124]. In addition, BBS patients often exhibit obesity and cognative impairments thought to be due to neuronal defects, however the specific pathways responsible for these attributes in BBS patients have not been clearly identified[141, 142].

Table 1

Roles of Ciliary and Basal Body Genes in Development and Disease

Mouse Gene	Function	Mutant Phenotype	Primary Cilia Phenotype	Human Disorder
Arl13b	Small GTPase	Hh signaling defects[129]	Abnormal microtubule structure[129]	Joubert syndrome[143]
Bbs1	Basal body protein, Bbsome component	Sensory defects, obesity[138]	Defects in specialized cilia only[138]	Bardet-Biedl syndrome (BBS)[144]
Bbs10	Chaperonin-like	ND	ND	BBS[145]
Bbs11	E3 ubiquitin ligase	Muscle defects[146]	ND	BBS[147], Muscular dystrophy[148]
Bbs12	Chaperonin-like	ND	ND	BBS[149]
Bbs2	Basal body protein, Bbsome component	Sensory defects, obesity[140]	Defects in specialized cilia only[140]	BBS[150]
Bbs3	Small GTPase	ND	ND	BBS[151]
Bbs4	Basal body protein, Bbsome component	Sensory defects, male infertility, obesity[138, 139]	Defects in specialized cilia only[138, 139]	BBS[152]
Bbs5	Basal body protein, Bbsome component	ND	ND	BBS[153]
Bbs6	Chaperonin-like	Sensory defects, male infertility, obesity	Defects in specialized cilia only	BBS, McKusick-Kaufman syndrome
Bbs7	Basal body protein, Bbsome component	ND	ND	BBS[154]
Bbs8	Basal body protein, Bbsome component	ND	ND	BBS[155]
Bbs9	Basal body protein, Bbsome component	ND	ND	BBS[156]
Dync2h1	Dynein retrograde motor subunit	Reduced Hh signaling[18, 19]	Bulged[18]	Jeune asphyxiating thorasic dystrophy (JATD)[157]
Evc	Basal body protein- skeletal specific	Ihh signaling defects[136]	Normal[136]	Ellis-van Creveld syndrome[33]
Ftm/Rgrip1	Basal body protein	Reduced Hh signaling[31]	Short[31]	Joubert syndrome type B[28] Meckel syndrome[158]
Fuz	PCP effector	Hh signaling defects[103, 104]	Short cilia[103, 104]	ND
Ift122	IFT Complex A	Increased Hh signaling[45]	Bulged[45]	ND
Ift139	IFT Complex A	Increased Hh signaling[15]	Bulged, short[15]	ND
Ift172	IFT Complex B	Reduced Hh signaling[11]	Absent[11]	ND
Ift52	IFT Complex B	Reduced Hh signaling[21]	ND	ND
Ift57	IFT Complex B	Reduced Hh signaling[43]	Absent[43]	ND
Ift80	IFT Complex B	ND	ND	JATD[159]
Ift88	IFT Complex B	Reduced Hh signaling[11]	Absent[160]	ND
Inturned	PCP effector	Hh signaling defects[105]	Short cilia[105]	ND
Kif3a	Kinesin2 subunit-anterograde	Reduced Hh signaling[11]	Absent[161]	ND
Kif3b	Kinesin2 subunit	ND	Absent[162]	ND
Kif7	Kinesin-like; Cos2 homolog	Hh signaling defects[40, 55, 56]	Normal[40, 55]	ND

Mouse Gene	Function	Mutant Phenotype	Primary Cilia Phenotype	Human Disorder
Mks1	Basal body protein	Hh defects, skeletal defects, cystic kidneys[32]	Sparse, short[32]	Meckel syndrome[163]
Ofd1	Basal body protein	Skeletal defects, reduces Hh signaling[29]	Short[29]	Oral-facial-digital syndrome[30]
Stil	Centrosomal protein	Hh signaling defects[164]	ND	Primary microcephaly[165]

ND, Not determined