The primary cilium: a signalling centre during vertebrate development

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Abstract | The primary cilium has recently stepped into the spotlight, as a flood of data show 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 signal transduction. The formation of cilia, in turn, is regulated by other signalling pathways, possibly including the planar cell polarity pathway. The cilium therefore represents a nexus for signalling pathways during development. The connections between cilia and developmental signalling have begun to clarify the basis of human diseases associated with ciliary dysfunction.

Ciliopathies
Human disorders affecting diverse organ systems in which the underlying cellular defect has been found to be structural or functional abnormalities of cilia.

Basal bodies
Cylindrical, microtubule-based structures at the base of cilia from which ciliary axonemes are nucleated. They are derived from mother centrioles.

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The primary cilium, a slim microtubule-based organelle that projects from the surface of vertebrate cells, has been the focus of intensive studies that have transformed 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 that are present in organisms as diverse as single-celled eukaryotes and humans. The evolutionarily conserved mechanism of intraflagellar transport (IFT), which was first described in the alga *Chlamydomonas reinhardtii*, is essential for the construction and maintenance of these structures in all species[1,2].

Over the past decade, the functions of mammalian primary cilia have been revealed through 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 — complex syndromes that can involve 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[7–10]. In parallel, genetic studies in mice showed that cilia are essential for signalling through the hedgehog (Hh) pathway, a crucial signalling 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 showed that cilia are required for survival and patterning of the mouse embryo[11]. Phenotypic, genetic and biochemical analyses 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 location for signal transduction, why cilia are required for vertebrate but not invertebrate Hh signalling, and whether primary cilia are important for regulating other developmental signalling pathways.

Other recent experiments have suggested that additional developmental signalling pathways help to regulate the formation of cilia. The most complete studies have implicated components 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 signalling, seem to be particularly important during organogenesis.

Here, we review the relationships between primary cilia and signalling pathways during vertebrate embryonic development. After describing the evolutionarily conserved mechanism of IFT, we review the evidence that Hh signalling requires IFT and cilia. We then describe recent work suggesting that the primary cilium in vertebrate embryos is specialized such that Hh signalling is restricted to the cilium. After considering whether additional developmental signalling pathways require cilia, we discuss the evidence that other signalling 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, and we focus on the formation of kidney cysts, a hallmark of disorders caused by abnormal primary cilia.

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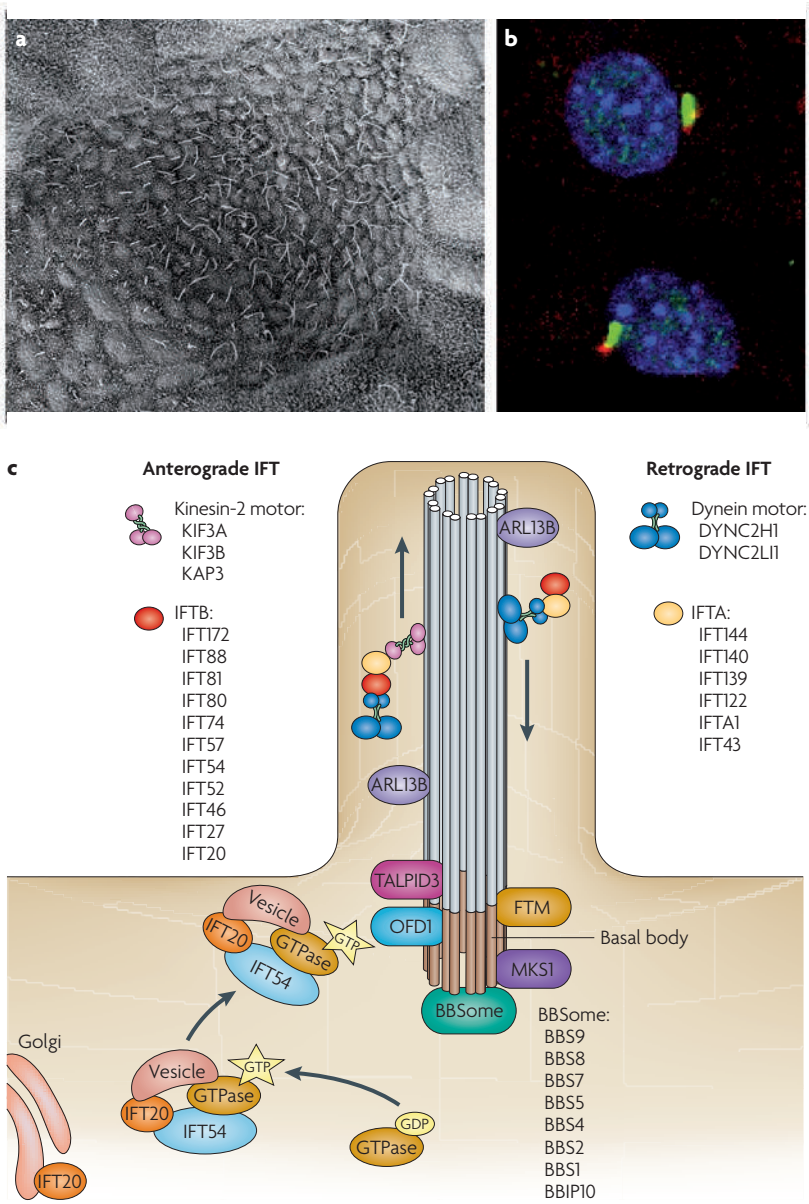

Figure 1 | **Cilia structure and intraflagellar transport. a** | Mammalian nodal cilia at embryonic day 7.75. The long cilia of the mouse node are required for left–right asymmetry. **b** | Primary mammalian cilia (green) in embryonic fibroblasts. The basal bodies are shown in red and nuclei are stained blue. **c** | Cargo is transported from the base to the tip of the cilium along the microtubule axoneme by the kinesin-2 motor together with the intraflagellar transport A (IFTA) and IFTB complexes. The dynein motor (which consists of cytoplasmic dynein 2 heavy chain 1 (DYNC2H1) and cytoplasmic dynein 2 light intermediate chain 1 (DYNC2LI1)) mediates the return of IFT cargo to the base of the cilium[1,14]. The IFTB proteins IFT20 and IFT54 may also participate in the trafficking of membrane vesicles from the Golgi complex to the ciliary membrane together with small GTPases[136]. Other small GTPases, including ADP-ribosylation factor-like 13B (ARL13B), also localize to cilia. Although its precise trafficking role is not known, ARL13B is required for axoneme structure[137]. Certain basal body proteins also influence ciliary trafficking. Among these are components of the BBSome, which are named after 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 proteins, such as Meckel syndrome type 1 (MKS1), fantom (FTM, also known as RPGRIP1L), oral-facial-digital syndrome 1 (OFD1) and TALPID3, are required for cilia formation, although how they regulate ciliogenesis has not been defined[10]. BBIP10, BBSome-interacting protein of 10 kDa; KAP3, KIF-associated protein 3 (also known as KIFAP3).

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 kinesin-2 motor (also known as the KIF3 motor complex), which consists of two kinesin-2 family proteins (KIF3A and KIF3B) and KIF-associated protein 3 (KAP3, also known as KIFAP3) and associates with two IFT protein complexes, IFTA and IFTB. IFTB is essential for anterograde trafficking, whereas IFTA and the minus-end-directed cytoplasmic dynein motor (which consists of cytoplasmic dynein 2 heavy chain 1 (DYNC2H1) and cytoplasmic dynein 2 light intermediate chain 1 (DYNC2LI1)) are required for retrograde trafficking[1]. In all organisms studied, disruption of the kinesin-2 motor or IFTB blocks cilia formation. Perturbation of retrograde trafficking by disruption of the dynein motor or IFTA results in short, bulged cilia[1,12–15] (TABLE 1).

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 that some may recruit cargo from the Golgi complex to the nascent ciliary membrane and others may promote loading of cargo into the axoneme[16,17] (FIG. 1).

Evidence linking hedgehog signalling to cilia

Vertebrate hedgehog signalling requires intraflagellar transport. The first evidence that vertebrate Hh signalling depends on cilia came from a phenotype-based screen for mutations that alter the patterning of the mouse embryo. This screen identified several mutants showing morphological and patterning phenotypes that were consistent with altered Hh signalling: these phenotypes included 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 IFTB complex components IFT172 and IFT88, as well as DYNC2H1, which is a subunit of 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[5] (FIG. 2). Genetic studies showed that IFT proteins act at the heart of the SHH pathway, downstream of the membrane proteins patched 1 (PTCH1) 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 signalling 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 (GliRs) that keep Hh target genes switched off (FIG. 3). In response to Hh ligand, processing of GliRs is blocked and activated Gli transcription factors (GliAs) activate the expression of Hh target genes. IFT is required for the production of GliAs and GliRs[18–21], and as a result IFT mutants show loss of Hh phenotypes in some cell

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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 signalling defects[137]	Abnormal microtubule structure[137]	Joubert syndrome[143]
Bbs1	Basal body protein, BBSome component	Sensory defects, obesity[131]	Defects in specialized cilia only[131]	BBS[144]
Bbs2	Basal body protein, BBSome component	Sensory defects, obesity[133]	Defects in specialized cilia only[133]	BBS[145]
Bbs3	Small GTPase	ND	ND	BBS[146]
Bbs4	Basal body protein, BBSome component	Sensory defects, male infertility, obesity[131,132]	Defects in specialized cilia only[131,132]	BBS[147]
Bbs5	Basal body protein, BBSome component	ND	ND	BBS[148]
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[149]
Bbs8	Basal body protein, BBSome component	ND	ND	BBS[150]
Bbs9	Basal body protein, BBSome component	ND	ND	BBS[151]
Bbs10	Chaperonin-like	ND	ND	BBS[152]
Bbs11	E3 ubiquitin ligase	Muscle defects[153]	ND	BBS[154], muscular dystrophy[155]
Bbs12	Chaperonin-like	ND	ND	BBS[156]
Dync2h1	Dynein retrograde motor subunit	Reduced Hh signalling[18,19]	Bulged[18]	JATD[157]
Evc	Basal body protein, skeletal specific	IHH signalling defects[129]	Normal[129]	EVC[33]
Ftm	Basal body protein	Reduced Hh signalling[31]	Short[31]	Joubert syndrome type B[28], MKS[158]
Fuz	PCP effector	Hh signalling defects[103,104]	Short[103,104]	ND
Ift122	IFT complex A	Increased Hh signalling[45]	Bulged[45]	ND
Ift139	IFT complex A	Increased Hh signalling[15]	Bulged, short[15]	ND
Ift172	IFT complex B	Reduced Hh signalling[11]	Absent[11]	ND
Ift52	IFT complex B	Reduced Hh signalling[21]	ND	ND
Ift57	IFT complex B	Reduced Hh signalling[43]	Absent[43]	ND
Ift80	IFT complex B	ND	ND	JATD[159]
Ift88	IFT complex B	Reduced Hh signalling[11]	Absent[160]	ND
Intu	PCP effector	Hh signalling defects[105]	Short[105]	ND
Kif3a	Kinesin-2 motor subunit, anterograde	Reduced Hh signalling[11]	Absent[161]	ND
Kif3b	Kinesin-2 motor subunit	ND	Absent[162]	ND
Kif7	Kinesin-like, COS2 homologue	Hh signalling defects[40,55,56]	Normal[40,55]	ND
Mks1	Basal body protein	Hh defects, skeletal defects, cystic kidneys[32]	Sparse, short[32]	MKS[163]
Ofd1	Basal body protein	Skeletal defects, reduces Hh signalling[29]	Short[29]	OFD[30]
Stil	Centrosomal protein	Reduced Hh signalling[164]	ND	Primary microcephaly[165]

Arl13b, ADP-ribosylation factor-like 13B; BBS, Bardet–Biedl syndrome; COS2, Costal 2; *Dync2h1*, cytoplasmic dynein 2 heavy chain 1; EVC, Ellis–van Creveld syndrome; *Ftm*, fantom (also known as *Rpgrip1l*); *Fuz*, fuzzy; Hh, hedgehog; IFT, intraflagellar transport; IHH, Indian hedgehog; *Intu*, inturned; JATD, Jeune asphyxiating thorasic dystrophy; *Kif*, kinsein superfamily protein; MKS, Meckel syndrome; ND, not determined; OFD, oral-facial-digital syndrome; PCP, planar cell polarity; *Stil*, Scl/Tal1 interrupting locus.

Axoneme
The long projection of the cilium into the extracellular space. It is composed of a circular array of nine microtubule doublets.

Anterograde trafficking
Transport towards the microtubule plus end (the cilia tip).

Retrograde trafficking
Transport towards the microtubule minus end (the cilia base).

Mother centriole
Centrioles are tube-shaped structures composed of nine triplets of microtubules. One of the two centrioles is the mother centriole, which forms the basal body.

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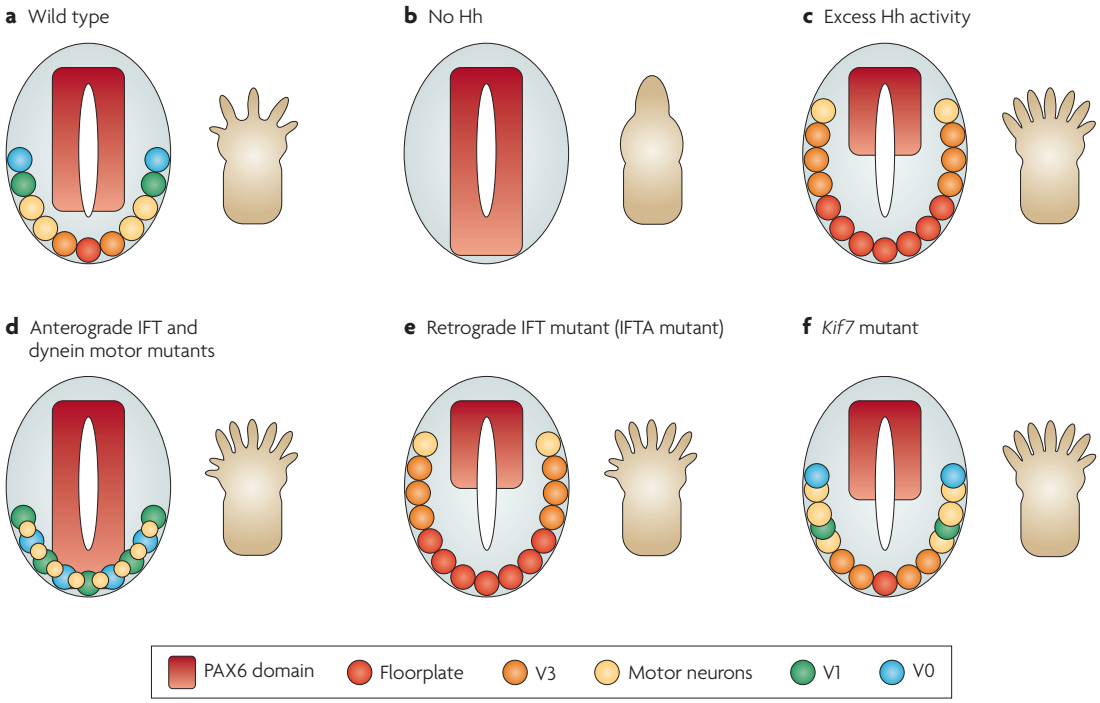

Figure 2 | **Neural and limb patterning phenotypes in hedgehog pathway and cilia mutants. a** | In wild-type embryos, ventral neural cell fates are specified by a gradient of sonic hedgehog (SHH). The number and identity of digits in the limb is established by an SHH gradient from the posterior limb bud to the anterior limb bud, where the repressor form of GLI3 (GLI3R) inhibits SHH. **b** | In the absence of hedgehog (Hh) (for example, in smoothened (*Smo*)$^{-/-}$ embryos) ventral neural cell fates are lost. The limbs of *Shh*$^{-/-}$ mutants lack digits. **c** | If the pathway is hyperactive (for example, in patched 1 (*Ptch1*)$^{-/-}$ embryos), 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 intraflagellar transport (IFT) mutants (for example, *Ift88*$^{-/-}$ or *Ift172*$^{-/-}$ embryos) lack cilia: Hh signalling is reduced, and the neural tube is dorsalized. This phenotype is milder than in **b** because cilia are also required for GLI3R processing, and cell types that require low levels of Hh signalling are specified. Reduced GLI3R results in polydactyly. Dynein motor mutants display a similar phenotype; however, they retain motor neurons in the caudal neural tube. **e** | IFTA complex mutants (for example, *Ift139*$^{-/-}$ embryos) exhibit phenotypes consistent with excess Hh signalling. **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. PAX6, paired box 6.

types and gain of Hh phenotypes in others. For example, GliAs have a central role in neural patterning, and IFT mutant embryos show a loss of Hh signalling in the neural tube. By contrast, GliRs have a central role in limb development, and IFT mutants that survive to later stages of embryogenesis show preaxial polydactyly, which is characteristic of loss of GliRs[18,19,21] (FIG. 2).

Recent experiments showed that IFT is also required for Hh signalling in zebrafish. Zebrafish that lack both maternal and zygotic *ift88* have Hh signalling defects in the neural tube and somites[22]; however, the patterning defects caused by loss of IFT in zebrafish are slightly different from those seen in mammals. Mouse *Ift88* mutants lack SHH-dependent ventral neural cell fates[11], and zebrafish maternal and zygotic *ift88* mutants lack cell fates, such as V3 interneuron progenitors and muscle pioneer cells, that require the highest levels of Hh. However, cell types in the neural tube and somites that are normally specified by lower levels of Hh are expanded[22]. This difference may be due to a different balance of GliAs and GliRs in zebrafish compared with mice[22].

Basal body proteins required for hedgehog signalling. Additional evidence that cilia are required for Hh signalling came from the 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 so far, these proteins are required for Hh signalling. For example, the chick *talpid3* mutation was first identified based on polydactyly[23,24] and causes developmental defects consistent with disrupted Hh signalling[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, such as oral-facial-digital syndrome 1 (OFD1), fantom (FTM, also known as RPGRIP1L), Meckel syndrome type 1 (MKS1) and Ellis–van Creveld syndrome protein (EVC), cause human ciliopathies and affect mammalian Hh signalling. Mice mutant for *Ofd1*, *Ftm* or *Mks1* have abnormal or absent cilia and exhibit Hh signalling defects corresponding to the severity of the cilia disruption[28–32] (TABLE 1). EVC also localizes to the basal body and is

Polydactyly
The formation of additional digits on the limbs. Additional posterior digits are referred to as postaxial, and additional anterior digits are referred to as preaxial.

Pericentriolar material
A network of fibres and associated proteins that surround the centriole and contain the microtubule-organizing activity of the centrosome.

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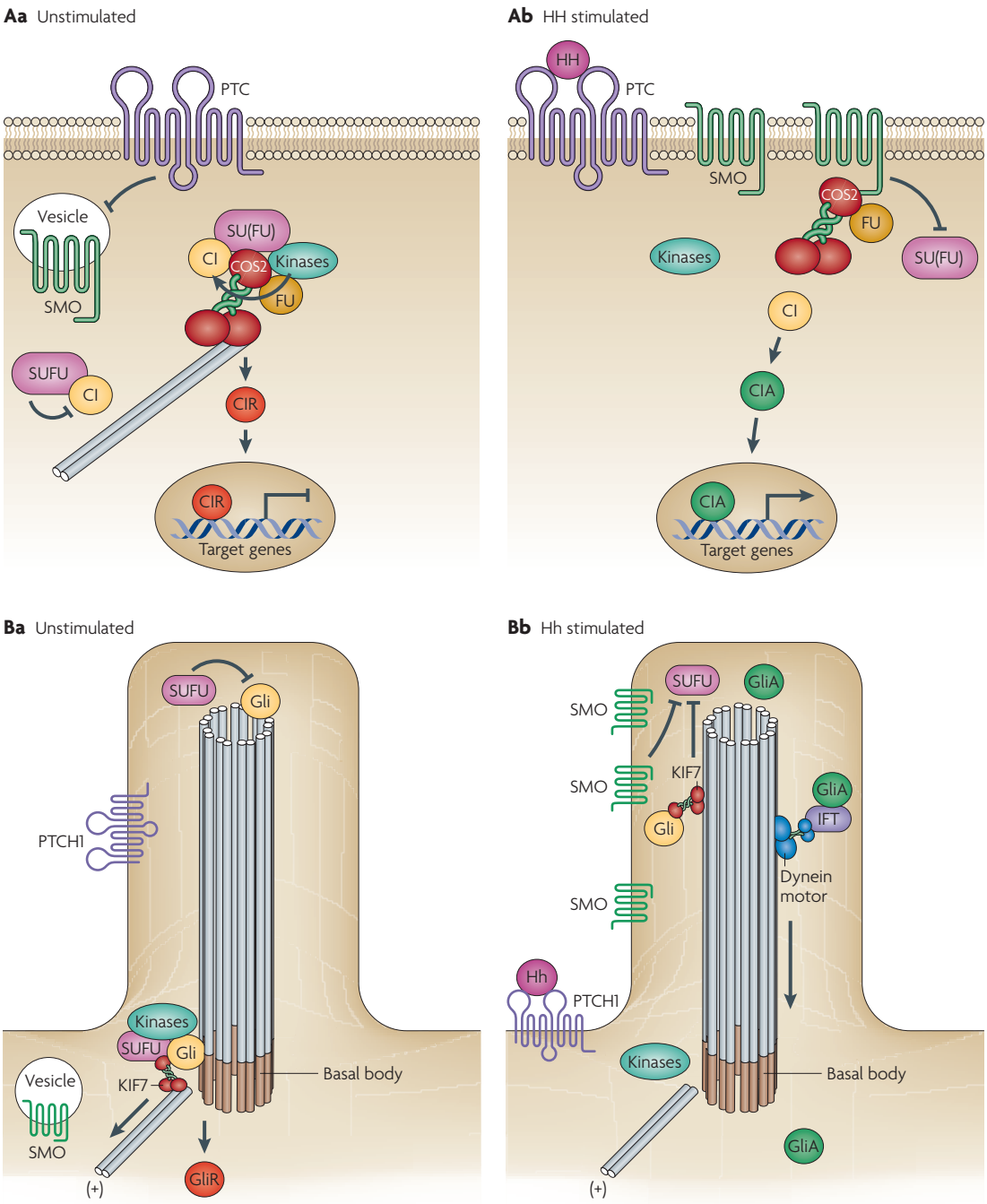

Figure 3 | **Localization of hedgehog pathway complexes in *Drosophila melanogaster* and mammals.** **A** | Modulation of protein complex structure and localization in *D. melanogaster* by Hedgehog (HH). **Aa** | In the absence of ligand, Patched (PTC) prevents translocation of Smoothened (SMO) to the plasma membrane. A microtubule-associated complex including Costal 2 (COS2), Fused (FU), Suppressor of fused (SU(FU)) and Cubitus interruptus (CI) recruits kinases, including Protein kinase A (PKA), Casein kinase I (CKI) and Glycogen synthase kinase 3β (GSK3β), that promote the processing of CI into its repressor form (CIR)[138–141]. SU(FU) may also associate with full-length CI to prevent its translocation to the nucleus[138]. **Ab** | Upon activation of the pathway, SMO moves to the plasma membrane, and the FU–COS2 complex associates with the carboxy-terminal tail of SMO, resulting in the release of CI. Pathway activation also inactivates the negative regulator[138–141]. **B** | In vertebrate Hh signalling, signal transduction takes place within cilia, but the behaviour of protein complexes may parallel that of protein complexes in *D. melanogaster*. **Ba** | In the absence of ligand, PTCH1 localizes to the cilium and is thought to block the entry of SMO into cilia[35]. The kinesin KIF7 (the COS2 homologue) localizes to the base of the cilium[55], where it may form a complex with Gli proteins and other pathway components. KIF7 at the cilium base prevents Gli enrichment within the cilium and promotes processing of GliRs. **Bb** | After 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 the dynein motor and intraflagellar transport (IFT) particles.

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mutated in the human skeletal disorder Ellis–van Creveld syndrome[33,34]. Unlike other basal body proteins, expression of *Evc* in mice is limited to developing skeletal structures, and EVC does not seem to be required for the formation of cilia in chondrocytes. Nevertheless, *Evc*$^{-/-}$ mice show reduced Indian hedgehog (IHH) signalling specifically within skeletal structures. Therefore EVC apparently does not affect ciliogenesis but is required for Hh signalling in a specific cell type.

Enrichment of hedgehog pathway components in cilia. Based on the genetic studies that associated Hh signalling with cilia, several groups have tested whether the proteins that mediate Hh signal transduction are localized to cilia. Remarkably, all of 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, in the absence of ligand SMO also accumulates in cilia in cells that lack the dynein retrograde IFT motor[37,38], which suggests 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 signalling, 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], which suggests that GLI2 traffics continuously through the cilium and activated SMO increases the accumulation of GLI2 at the tip.

Suppressor of fused (SUFU), an important negative regulator of mammalian Hh signalling, also localizes to the primary cilia tip[39,40]. Genetic and biochemical data have shown that SUFU can inhibit Hh signalling even in the absence of cilia[41,42]; however, partial knockdown of SUFU results in pathway activation only if cilia are present, which suggests a complex role for SUFU in the cilium[20] (M. Tuson and K.V.A., unpublished data). Although 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). Therefore, GLI2 activation requires cilia, but 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 as-yet-unidentified Hh pathway components in cilia.

Trafficking in the cilium regulates hedgehog signalling. The finding that vertebrate Hh signalling requires primary cilia has raised the question of why this organelle is particularly suited to this crucial pathway. The simplest explanation is that the cilium provides an environment in which 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, IFTA and IFTB, which form large platforms for transporting cargo between the base and tip of the cilium[13] (FIG. 1). Mutants lacking components of the IFTB 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 in the cilium. By contrast, mutations in IFTA proteins allow the formation of cilia (with abnormal morphology) and cause very different developmental phenotypes from mutants that prevent cilia formation: Hh signalling is activated rather than decreased (FIG. 2; TABLE 1). Studies in *C. reinhardtii* argue that the IFTA complex cooperates with the dynein motor to mediate retrograde transport[1], as the rate of anterograde IFT is normal in these mutants, whereas retrograde trafficking is slowed[12,14,44]. Mutants in two mouse IFTA complex proteins — IFT139 (also known as THM1 and TTC21B) and IFT122 — have been characterized. 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 IFTA and IFTB mouse mutants are surprising, as IFTA and IFTB were originally identified as subcomplexes of a single large complex[47] and seem to move coordinately[48,49]. Both IFTA and DYNC2H1 are important for retrograde IFT, but mutations in IFTA proteins increased Hh pathway activity[15,45], whereas mutations in DYNC2H1 block the response to Hh ligands[18,19]. These findings suggest that disruption of IFTA may differentially disrupt trafficking of Hh pathway components, thereby causing phenotypes distinct from those observed in mutants in which cilia are absent or the dynein motor is disrupted. Recent data suggest that SMO may be trafficked laterally from the plasma membrane into the cilium[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 IFTA mutants.

Why is hedgehog signalling tied to cilia?

KIF7 as a link between hedgehog signalling and cilia. Despite the evolutionary conservation of the Hh pathway and the importance of primary cilia in vertebrate Hh signalling, cilia are not required for Hh signalling in *Drosophila melanogaster*. This raises the question of why vertebrate Hh signalling is coupled to cilia. Recent data suggest that KIF7, a kinesin that is the vertebrate homologue of *D. melanogaster* Costal 2 (COS2), may tether the vertebrate Hh pathway to cilia.

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COS2, a key component of the *D. melanogaster* Hh pathway, is a kinesin-related protein that serves as a scaffold for Hh signalling complexes. COS2 has dual functions in the pathway: it promotes formation of the repressor form of Cubitus interruptus (CI, the *D. melanogaster* Gli homologue) in the absence of Hh ligand by recruiting kinases that prime CI for processing, and it permits high levels of pathway activation after Hh stimulation by antagonizing SU(FU)[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 motor function is disrupted[54].

Several recent papers showed that zebrafish and mouse KIF7 proteins, like *D. melanogaster* COS2, both positively and negatively regulate the SHH pathway[40,55–57]. Unlike COS2, the vertebrate KIF7 motor domain retains all of the motifs that are 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, which suggests that KIF7, like the kinesin-2 motor, acts as an anterograde motor in the cilium[55].

Conventional kinesins, such as KIF7, carry cargo towards the plus end of microtubules. The minus ends of axonemal and cytoplasmic microtubules are located at the base of the cilium. Because KIF7–enhanced GFP (eGFP) is enriched at the base of the cilium, we proposed that KIF7 might traffic GLI2 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, after pathway activation, to the cilia tip, 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]. Therefore, the dual roles of KIF7 as a SHH pathway component and ciliary motor could explain why mammalian SHH signalling depends on the primary cilium (FIG. 3B).

Fused is a cilia-associated protein in vertebrates. Fused (FU) is an important component of the *D. melanogaster* Hh pathway: it is a serine/threonine kinase that phosphorylates COS2, SU(FU) and perhaps other components of the pathway, and it is required for activation of CI in response to Hh ligand[53]. Fu is also important for Hh signalling in zebrafish[58], but Hh signalling 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]. Therefore FU, like KIF7 or COS2, links Hh signalling with cilia, although the connection to Hh seems to have been lost in mammals. It has been proposed that another unidentified kinase may substitute for FU in mammalian Hh signalling, and several human kinome screens have been undertaken to identify kinases that are required for Hh signalling[62,63]. Although 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 signalling 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 signalling. Planaria homologues of the Hh pathway components KIF7, FU and iguana are required in planaria for formation of motile cilia but not Hh signalling[64,65]. Planaria are a distinct lineage of animals from both insects and vertebrates. Therefore, the finding that KIF7 and FU function in cilia in two independent metazoan lineages suggests that the ancestral role of these proteins was in cilia. The requirement for these cilia-associated proteins in *D. melanogaster* Hh signalling suggests that Hh signalling was associated with cilia in the common ancestor of *D. melanogaster* and vertebrates.

Are cilia dedicated to hedgehog signalling?

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

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

However, this connection between cilia and Wnt signalling is controversial. Mouse IFT mutants do not show the phenotypes that are characteristic of Wnt pathway mutants. For example, reduced canonical Wnt signalling 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 signalling but do not show the defects in convergent extension that are associated with disruption of non-canonical Wnt signalling[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 IFT139 have a neural patterning phenotype that is consistent with the activation of Hh signalling[15,46] but do

Hydrocephalus
The build-up of cerebrospinal fluid within the ventricles of the brain.

Kinome
The set of protein kinases in the genome of a given organism.

Metazoan
Multicellular organisms that, with the exception of sponges, have specialized cell types. Historically referred to as the kingdom 'Animalia'.

Convergent extension
A morphogenetic movement characterized by the intercalation and elongation of cells, which causes a structure to generally become longer and thinner.

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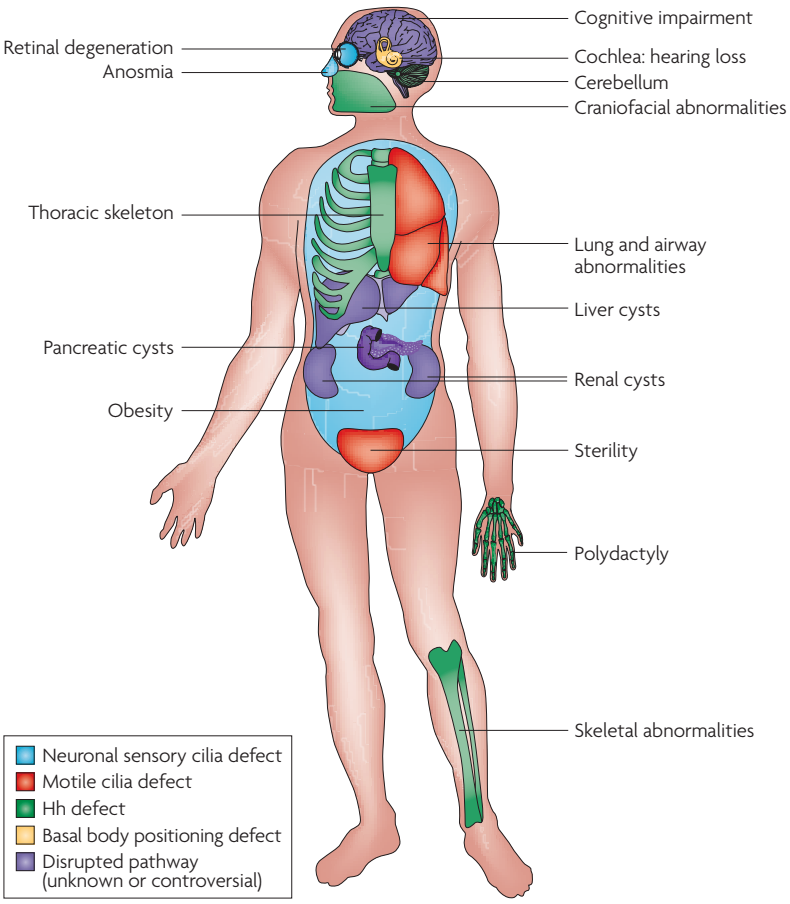
Box 1 | **Organs affected in human ciliopathies**

Numerous pleiotropic human disorders have been attributed to defects in cilia formation[10,128] (see the figure). Some aspects of these syndromes, such as the polydactyly in patients with Bardet–Biedl syndrome (BBS) and Meckel syndrome and the skeletal abnormalities that affect the limbs of patients with Ellis–van Creveld syndrome, have been attributed to defective hedgehog (Hh) signalling. Polydactyly results from a loss of the repressor form of GLI3 (GLI3R) and skeletal abnormalities resemble those observed in mutants that lack Indian hedgehog (IHH) signalling[129]. Sonic hedgehog (SHH) signalling is also required for craniofacial development, and defects in craniofacial structures, such as those observed in Meckel syndrome type 1 (*Mks1*) mutant mice, are also likely to be due to misregulated Hh signalling[32]. In addition, patients with a Joubert syndrome-like disorder exhibit ataxia due to cerebellar hypoplasia[130]. Growth of this tissue is Hh dependent[85].

Other attributes of human disorders result from defective specialized cilia. Retinal degeneration results from defects in photoreceptor connecting 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 patients with BBS often exhibit anosmia[131–133]. Another sensory deficit, hearing loss, is due to a requirement for the specialized primary cilia of the cochlea downstream of the planar cell polarity pathway in establishing the correct polarity of sensory hair cells[99]. Infertility observed in patients with ciliopathies is the result of defective sperm flagella and motile oviduct cilia[132].

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 misorientation of the mitotic spindle. However, whether and how these processes are regulated by cilia remain the subject of active investigation[124]. In addition, patients with BBS often show obesity and cognitive impairments that are thought to be due to neuronal defects; however, the specific pathways responsible for these attributes in patients with BBS have not been clearly identified[134,135].



not show altered canonical Wnt signalling[46]. Therefore it seems that cilia are not required for canonical or non-canonical Wnt signalling in the first half of vertebrate embryogenesis.

Platelet-derived growth factor receptor-α signalling. Cilia have been found to be important for signalling by platelet-derived growth factor receptor-α (PDGFRA) in cultured fibroblasts[80], as well as for PDGF-dependent directed 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[82]. Loss of PDGFRA signalling does not produce any striking phenotypes in early mouse embryos but is crucial for the 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 PDGFRA signalling in these cell types *in vivo*.

Hedgehog signalling in adult tissues. After birth, SHH signalling continues to have important roles in the growth of the brain and the maintenance of neural progenitors[85]. Conditional deletion of *Ift88* or *Kif3a* in the brain results in severe hypoplasia of the cerebellum due to the failure of granule cell progenitor proliferation[86], a process that depends on SHH signalling[85,87]. Primary cilia are also needed to modulate the SHH-dependent formation and maintenance of hippocampal granule neuron precursors, which are important for maintaining neurogenesis in adults[88]. Based on the tight association between cilia mutants and specific defects of Hh signalling, we propose that cilia are essential for Hh signalling 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 signalling 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 signalling, the molecular bases of other features of these diseases remain unknown (BOX 1).

Signalling pathways that regulate ciliogenesis

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

Fgf and inositol signalling. Recent evidence from zebrafish implicates Fgf signalling 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 mice also affect ciliogenesis, and whether these effects on ciliogenesis alter Hh signal transduction.

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Components of the phosphatidylinositol signalling cascade also seem to regulate cilia length. In zebrafish, morpholino knockdown of inositol-pentakisphosphate 2-kinase (Ipk1, also known as Ippk) reduced the frequency of cilia beating and decreased cilia length[93]. In humans, inositol polyphosphate 5-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 are more labile than wild-type cilia[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 signalling and cilia. Recent experiments argue that there is a close connection between components of PCP signalling 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 *D. melanogaster*, components of the non-canonical Wnt pathway are planar polarized in hair cells, and that polarity is required for PCP signalling and provides a read-out of effective PCP signalling[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 seems that one output of non-canonical Wnt signalling 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 needed 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 of the motile cilia on the epidermis of *Xenopus laevis* embryos support the hypothesis that components of the PCP 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 proteins dishevelled 1 (dvl1), dvl2 and/or dvl3 disrupts the polarity of the cilia on these cells — based on morpholino data targeting, all three dvl proteins localize 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).

Although changes in the position of cilia are unlikely to influence their ability to transduce Hh signals, some PCP components do affect Hh signalling. Inturned and Fuzzy (FUZ) are downstream effectors of the non-canonical Wnt pathway in *D. melanogaster*, and morpholino knockdown of *X. laevis* inturned (*intu*) or *fuz* disrupts the apical actin network and cilia formation[102]. These morphants fail to undergo normal convergent extension due to defects in PCP and also show defects that are consistent with a loss of shh signalling[102]. Similarly, mouse *Fuz* and *Intu* mutants have short cilia and disrupted Hh signalling[103–105]. Therefore, components of the planar polarity pathway can be important for both formation and polarity of cilia (FIG. 4b). However, there is no evidence that other components of the non-canonical Wnt pathway are required for ciliogenesis, which suggests that the roles of INTU and FUZ in cilia formation may be unrelated to their roles in non-canonical Wnt signalling.

Cilia, signalling 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 to be due to misregulated Hh signalling, but the molecular bases of other defects, such as cystic kidneys, are not well understood (BOX 1). The deregulated Hh signalling associated with several types of human cancers also depends on cilia. Therefore studies on the relationship between cilia and signalling during development have direct implications for human disease.

Hedgehog signalling in tumours. Inappropriate activation of SHH signalling can cause medulloblastomas and rhabdomyosarcomas (paediatric tumours of the cerebellum and muscle, respectively) 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 tumours[109,110]. Recent studies show that cilia regulate Hh signalling in tumours and that the role of cilia in tumours depends on how the pathway is activated. Expression of activated SMO in the postnatal mouse brain can cause medulloblastomas, but removal of cilia prevents tumour formation[111], consistent with earlier genetic experiments indicating that cilia are required for the activity of the pathway at a step downstream of SMO[11]. Constitutively active GLI2 can also cause medulloblastomas in mice, but only when cilia are removed[111]. This suggests that GLI2 alone cannot activate tumorigenesis in this cellular context when the cilia-dependent GLI3 repressor is also present. Similar results were observed in basal cell carcinomas in mice harbouring similar activating Hh pathway

Subventricular zone
A layer of cells lining the ventricles of the brain in which neurogenesis takes place in adult mammals.

Kupffer's vesicle
A ciliated organ of the fish embryo that serves to generate asymmetry during development, functioning analogously to the mammalian node.

Hair cells
The sensory cells in the vertebrate auditory system. They are contained within the cochlea.

Organ of Corti
The organ in the mammalian inner ear that contains the hair cells.

Cochlea
The portion of the inner ear that contains the sensory organs of hearing.

Kinocilium
The specialized primary cilium of the hair cells of the cochlea.

Stereocilia
The actin-based sensory organelles that form a polarized chevron-shaped structure in the hair cell.

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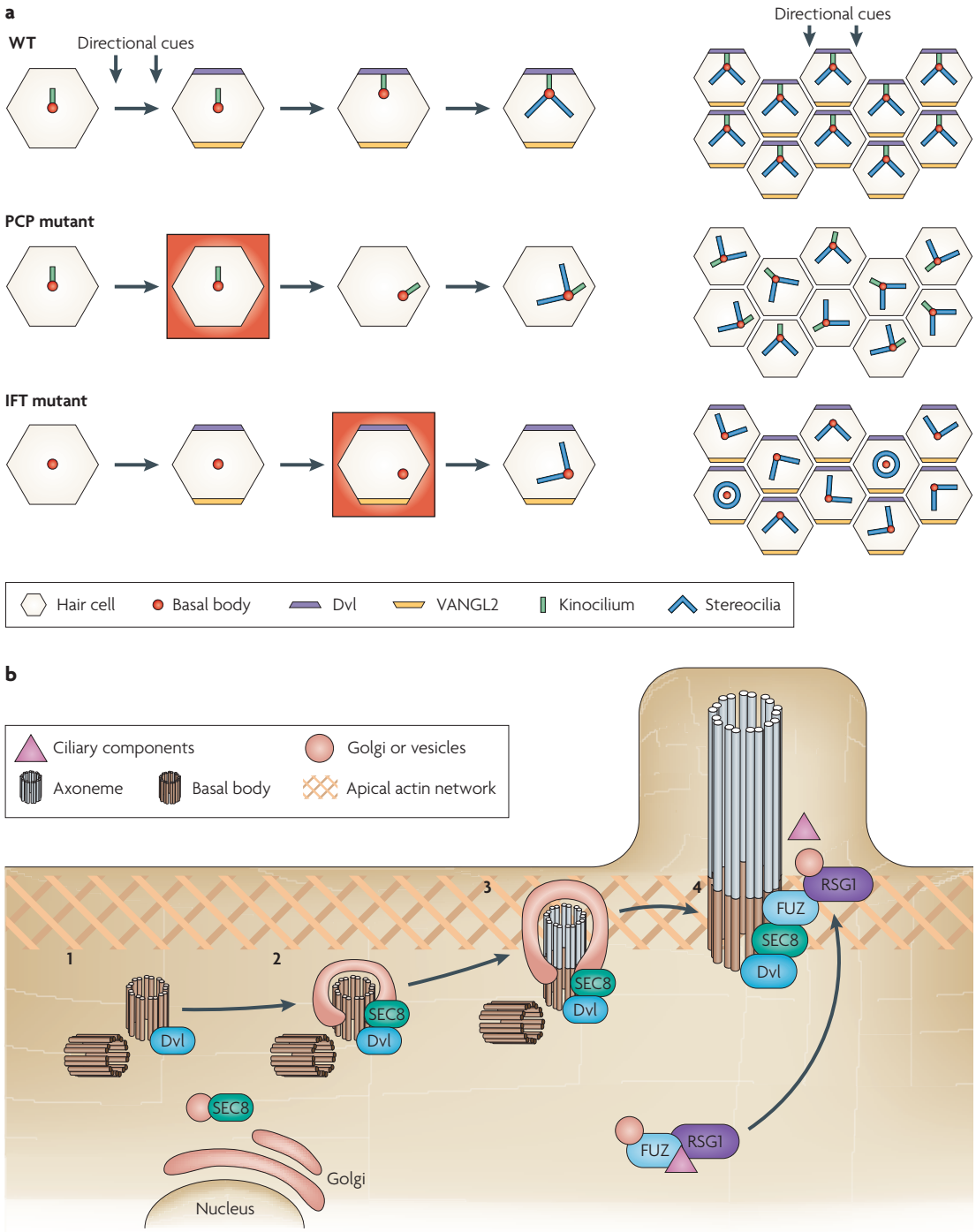

Figure 4 | **The role of the planar cell polarity pathway in cilia formation. a** | In the wild-type (WT) cochlea, directional cues establish the localization of core planar cell polarity (PCP) pathway components. The kinocilium then directs the basal body towards the medial side of the cells. This in 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 intraflagellar transport (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 polarity that is defective in each category of mutants. **b** | Components of the PCP pathway are implicated in cilia formation. The centrioles — one of which will become the basal body — are initially located away from the cell surface (1)[1], where they associate with PCP proteins from the dishevelled (Dvl) family (DVL1, DVL2 or DVL3). A component of the vesicle trafficking machinery, SEC8 (also known as EXOC4), is then recruited to the basal body (2)[101]. At the cell surface (3), DVL mediates the fusion of the basal-body-associated membrane with the cell membrane[101,142], and the axoneme then extends through IFT (4). *In vivo* mouse experiments indicate that the PCP effector fuzzy (FUZ) and Rem/Rab-similar GTPase 1 (RSG1, also known as C1orf89) also have roles in trafficking membrane vesicles and ciliary components to the basal body[103]. VANGL2, vang-like 2.

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mutations: activated SMO caused tumours only in the presence of cilia, whereas removal of cilia enhanced tumorigenesis due to expression of activated GLI2 (REF. 112). Therefore, in tumours 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 signalling 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 signalling in PKD cannot be ruled out, some data suggest that cilia might modulate Wnt signalling in this tissue[117].

The connection among cilia, cystic kidneys and Wnt signalling was first raised by analysis of the inversin *(Invs)* gene. INVS binds microtubules and localizes to the basal body[118] and cilium[119]. Mutations in *INVS* cause kidney disease in humans[120] and renal cysts in mice[118]. INVS interacts with DVL1, targeting membrane-bound DVL1 for destruction. INVS has been proposed to act as a switch between canonical and non-canonical Wnt signalling, based on cell culture and morpholino knockdown experiments[68]. However, altered canonical Wnt signalling has not been reported in the kidneys of *Invs*$^{-/-}$ mice.

Mouse overexpression experiments show that increased Wnt signalling 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 signalling has been observed in mice lacking Jouberin (AHI1), a cilia-localized protein mutated in a form of Joubert syndrome. *Ahi1*$^{-/-}$ mice have cystic kidneys with reduced expression of a Wnt reporter and reduced nuclear β-catenin[123]. Therefore additional experiments that examine Wnt signalling during kidney development will be needed 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 signalling, 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 embryonic day 16 associated with misorientation of mitotic spindles within the renal tubules[125]. This cyst formation is enhanced by removal of one or both copies of the core PCP pathway component vang-like 2 (*Vangl2*). Moreover, FAT4 localizes to cilia within the kidney, implicating the cilium in the modulation of the kidney PCP pathway[125]. *D. melanogaster* FAT, however, acts in a PCP pathway that does not depend on non-canonical Wnt signalling[126]. Moreover, mouse mutants of other PCP pathway components, such as VANGL2 and FUZ, 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]. Therefore it remains to be determined which pathway (or pathways) downstream of primary cilia is the underlying cause of renal cysts.

Conclusions and perspectives

Non-motile primary cilia have 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, traffic PTCH1 out of cilia and modulate the trafficking of Gli proteins within cilia in response to Hh pathway activation are largely unknown. The opposing effects of different IFT components upon the regulation of the Hh pathway suggest that, in addition to providing a compartment in which Hh pathway components are enriched, the IFT machinery has a more complex role in regulating the pathway, but these roles have not been defined. To address these questions, it will be necessary to examine the trafficking of Hh pathway components in real time and to probe their physical associations with the IFT machinery in wild-type cells, as well as in cells that are mutated for various IFT components.

The dual roles of KIF7 in intraciliary trafficking and in the Hh pathway suggest a reason why vertebrate Hh signalling 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 *D. melanogaster* FU? And if so, does it have roles in both ciliogenesis and Hh signalling? Do other components of the Hh signalling pathway affect the dynamics of ciliary trafficking?

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

Note added in proof

Recent work by Singla *et al.*[166] describes the mechanism by which the centrosomal protein OFD1 functions to promote ciliogeneis. This paper shows that OFD1 controls the length of the centriole and is required for microtubule stability. OFD1 is also important in recruiting IFT88 to the centrosome.

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Competing interests statement
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