

# The roles of cilia in developmental disorders and disease

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Cilia are highly conserved organelles that have diverse motility and sensory functions. Recent discoveries have revealed that cilia also have crucial roles in cell signaling pathways and in maintaining cellular homeostasis. As such, defects in cilia formation or function have profound effects on the development of body pattern and the physiology of multiple organ systems. By categorizing syndromes that are due to cilia dysfunction in humans and from studies in vertebrate model organisms, molecular pathways that intersect with cilia formation and function have come to light. Here, we summarize an emerging view that in order to understand some complex developmental pathways and disease etiologies, one must consider the molecular functions performed by cilia.

## Introduction

Cilia and flagella, which are distinguished by distinctive patterns of movement, are identical in structure and composition. Cilia project from most eukaryotic cell surfaces with the notable exceptions of cells of higher plants and fungi (Mitchell and Nakatsugawa, 2004). Although cilia are almost ubiquitously present in vertebrate cells, in invertebrates they are restricted to sensory neurons (Wheatley et al., 1996). Ciliary motility is important for moving fluids and particles over epithelial surfaces, and for the cell motility of vertebrate sperm and unicellular algae *Chlamydomonas*. Sensory cilia function in the detection of light, odor and fluid flow in vertebrates; and in sensing osmolarity changes, chemoattractants and repellants, and sound in invertebrates (Evans et al., 2006).

Recent discoveries have revealed that cilia have crucial roles in the signal transduction pathways that regulate intracellular  $Ca^{2+}$  levels, as well as in the Hedgehog (Hh) and planar cell polarity (PCP) pathways. Studies that link cilia/basal body/centrosome proteins to human genetic disorders have also shown that compromised ciliary function can have profound consequences for cellular homeostasis. Dysfunction of ciliary proteins gives rise to phenotypes that range from being organ specific (e.g. some polycystic kidney diseases) to broadly pleiotropic (e.g. Bardet-Biedl syndrome). Emerging from this complex spectrum of disease and developmental mutant phenotypes are a set of phenotypic indicators of ciliary dysfunction, including such seemingly disparate phenotypes as cystic disease of the kidney, liver and pancreas, neural tube defects, postaxial polydactyly, situs inversus, and retinal degeneration. The affected genes and the molecular and cellular basis for these phenotypes are now beginning to be elucidated.

In this review, we focus on recent reports that have greatly expanded our appreciation of the diverse functional roles cilia play in cell signaling pathways and in maintaining cellular homeostasis, and discuss how this new information provides new insights into developmental and disease phenotypes.

## Cilia structure and function

### Structure of the ciliary apparatus

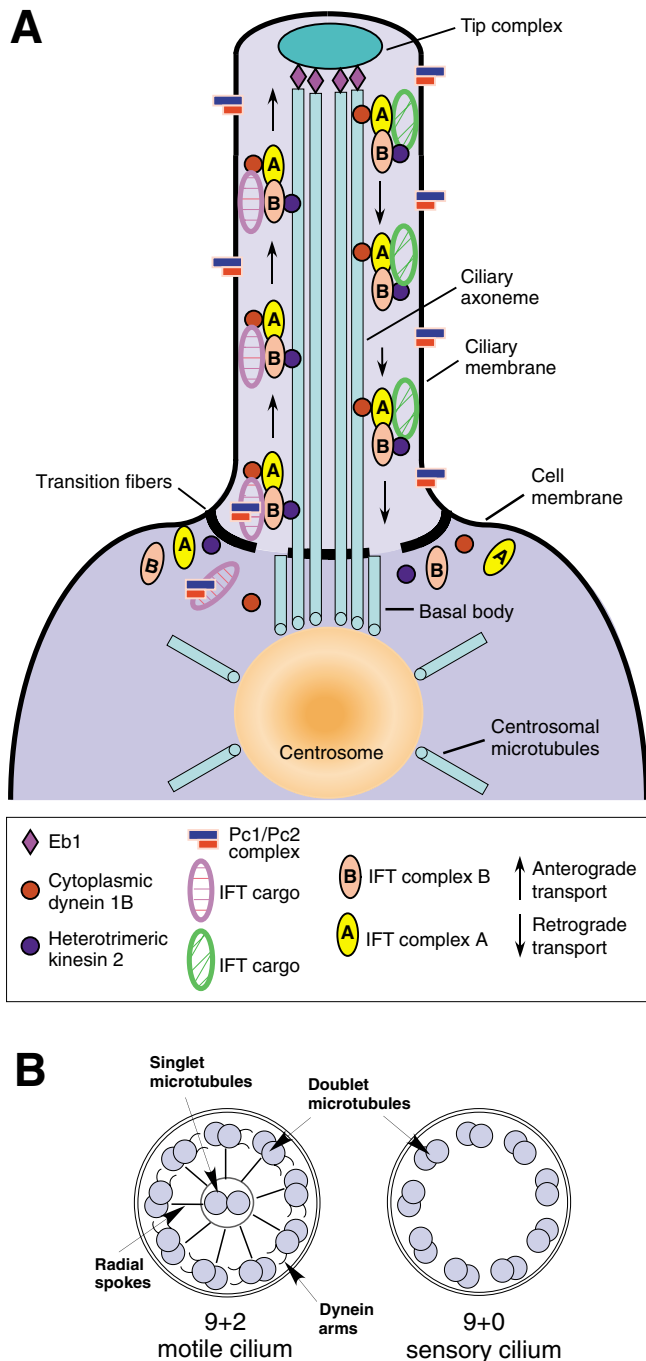
The cilium consists of a microtubule-based axoneme covered by a specialized plasma membrane that extends from the cell surface into the extracellular space (Fig. 1A). The axoneme is a highly ordered structure of nine peripheral microtubule doublets arranged around a central core that may or may not contain two central microtubules (9+2 or 9+0 axoneme, respectively) (Fig. 1B). 9+2 cilia usually have dynein arms that link the microtubule doublets and are motile, while most 9+0 cilia lack dynein arms and are non-motile. Ciliary motility is accomplished by dynein motor activity, which slides the microtubule doublets relative to one another. At its base, the ciliary axoneme extends from the nine triplet microtubules of the basal body, a microtubule-organizing center (MTOC) derived from the older of the pair of centrioles. The transition zone at the junction of the basal body and the ciliary axoneme is demarcated by Y-shaped fibers, which extend from the microtubule outer doublets to the ciliary membrane. The transition zone, in combination with the internal structure of the basal body, is thought to function as a filter for the cilium, regulating the molecules that can pass into or out of the cilium. The distal tips of cilia are structurally and functionally complex microtubule-capping structures, which link the ends of the axonemal microtubules to the ciliary membrane (Sloboda, 2005).

### Intraflagellar transport

During ciliogenesis, cilia elongate from the basal body by the addition of new axonemal subunits to the distal tip. As protein synthesis does not occur in cilia, axonemal and membrane components are conveyed in non-membrane-bound macromolecular particles by intraflagellar transport (IFT) along the axonemal doublet microtubules (Fig. 1) (Kozminski et al., 1993). IFT particles are organized into two complexes: complex A contains four or five polypeptides, whereas complex B contains at least 12 polypeptides (Cole et al., 1998; Piperno et al., 1998). Because axonemal microtubules are oriented with their plus-ends at the tip of the cilia, and microtubule motors typically move in only one direction, two types of motors are needed for IFT. Anterograde transport is driven by heterotrimeric kinesin 2, which is composed of motor subunits Kif3a and Kif3b and a non-motor subunit (Cole et al., 1993). In *C. elegans*, anterograde IFT to the proximal and middle part of the cilium is accomplished cooperatively by heterotrimeric kinesin 2 and homodimeric kinesin 2 (OSM-3), while IFT to the distal cilium relies only on OSM-3 (Snow et al., 2004). In addition to moving axonemal components, these kinesins also move ciliary membrane proteins and help form signaling complexes within the ciliary membrane (Qin et al., 2005; Wang et al., 2006). It is unknown whether the vertebrate ortholog of OSM-3 (Kif17) functions in axonemal construction, although it is localized to the cilia of cultured canine kidney (MDCK) cells and functions in the transport of membrane proteins (Jenkins et al., 2006). Retrograde transport back to the cell body is accomplished by cytoplasmic dynein 1B (Pazour et al., 1998; Signor et al., 1999). The transition between anterograde and retrograde transport of IFT particles occurs at the ciliary tip. IFT cargo loading and unloading, and motor protein regulation, at the tip of the cilium are poorly understood but likely

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**Fig. 1. Cilia structure and intraflagellar transport.** (A) A typical cilium consists of an axoneme of nine doublet microtubules (two are shown in B). Each doublet arises from the inner two microtubules of the basal body microtubule triplets. The axoneme is surrounded by a specialized ciliary membrane that is separated from the cell membrane by a zone of transition fibers. (B) A cross-section of 9+2 and 9+0 cilium. Cilia are broadly divided into two types based on the presence or absence of a central pair of microtubule singlets in the axoneme (9+2 or 9+0 structure, respectively). Inner and outer dynein arms, which are usually associated with 9+2 cilia, can be present in either type of cilium and are important for ciliary motility. Ciliary assembly and maintenance is accomplished by intraflagellar transport (IFT), which relies on the microtubule motor proteins kinesin 2 and cytoplasmic dynein to transport IFT protein complexes and their associated cargo up and down the length of the cilium (depicted in A). Abbreviations: Eb1, end-binding protein 1; Pc1 and Pc2, polycystin 1 and polycystin 2.

include proteins that are involved in the control of microtubule stability, such as the plus-end-binding protein, Eb1 (Pedersen et al., 2003).

The basal body-centrosome complex also plays a crucial role in coordinating IFT and the formation of cilia. The centrosome is surrounded by pericentriolar material (PCM), which serves as a nucleation site for microtubules. In mammalian cells, RNAi knockdown of a protein important for PCM organization, pericentrin, inhibits ciliogenesis and reduces the abundance of IFT components near the centrioles (Jurczyk et al., 2004). Mutations in a *Drosophila* pericentrin-like-homolog also cause malformations in sensory neuron cilia and sperm, indicating that the pericentrin-mediated interaction between centrosomal and IFT proteins is evolutionarily conserved (Martinez-Campos et al., 2004).

### Types of cilia

In mammals, motile 9+2 cilia normally concentrate in large numbers on the cell surface, beat in an orchestrated wavelike fashion, and are involved in fluid and cell movement. In contrast to motile cilia, primary cilia (monocilia) project as single immotile organelles from the cell surface. Primary cilia are found on nearly all cell types in mammals (Wheatley et al., 1996) (see also <http://members.global2000.net/browser/cilia.html>), and many are highly adapted to serve specialized sensory functions. For example, photoreceptor proteins in the vertebrate retina are housed in a highly elaborated 9+0 cilium connected to the cell body by a second 'connecting' cilium that emerges from the basal body (Rohlich, 1975). Although cilia are broadly classified as 9+2 motile cilia or 9+0 immotile sensory cilia, there are examples of 9+2 sensory cilia and 9+0 motile cilia (reviewed by Afzelius, 2004; Ibanez-Tallon et al., 2003). For example, odorant receptors have been found on the immotile 9+2 sensory cilia of specialized olfactory neurons (Menco, 1994), and 9+0 motile cilia, which have dynein arms associated with the outer microtubule doublets and rotate, are present on mouse embryonic node cells (Nonaka et al., 1998; Sulik et al., 1994).

### Disorders of motile cilia

#### Primary cilia dyskinesia

A link between ciliary function and human disease was discovered when individuals suffering from syndromes with symptoms including respiratory infections, anosmia, male infertility and situs inversus, were shown to have defects in ciliary structure and function (primary cilia dyskinesia or PCD) (Afzelius, 1976; Eliasson et al., 1977). Individuals with PCD also occasionally suffer from retinal degeneration, cystic kidney disease and hydrocephalus. The ciliary structural defects observed in such individuals include abnormal or missing dynein arms, radial spokes and central microtubule doublets. Dynein arm defects are associated with mutations in the genes encoding the dynein heavy chain subunits DNAH5, and DNAH11, and the intermediate chain subunit DNAI1 (Table 1) (Bartoloni et al., 2002; Olbrich et al., 2002; Pennarun et al., 1999).

The clinical symptoms of PCD reflect the distribution of motile cilia in affected tissues and organs (Afzelius, 2004). Loss of ciliary function in the respiratory tract results in impaired mucociliary transport that leads to chronic respiratory infections. Male infertility results when immature sperm fail to move to the vas deferens, owing to loss of ciliary motility in the efferent ductules, or from a loss of sperm flagellar motility. Hydrocephalus has been proposed to result from impaired fluid flow in the brain ventricles because of loss of ependymal cilia motility (Eley et al., 2005). This has been confirmed from studies of mice with mutations in the axonemal dynein heavy chain Mdnah5, or a hypomorphic mutation in the IFT protein polaris

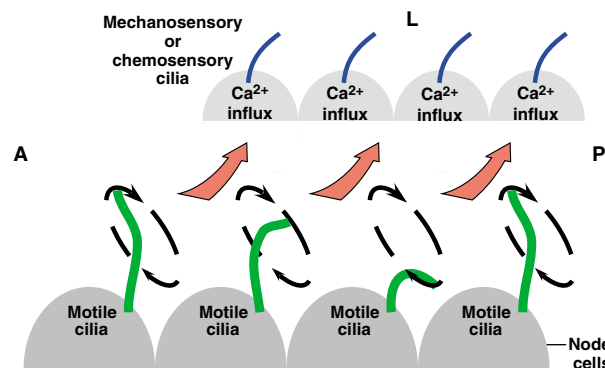
(Ift88) as well as in zebrafish larvae in which IFT proteins including polaris are disrupted by mutation or antisense morpholinos (Banizs et al., 2005; Bisgrove et al., 2005; Ibanez-Tallon et al., 2004; Kramer-Zucker et al., 2005). In mice with hypomorphic mutations in *Ift88*, increased cerebrospinal fluid  $\text{Cl}^-$  levels, arising from altered cellular functions caused by loss of primary cilia, may also be a contributing factor for hydrocephalus (Banizs et al., 2005).

### Cilia and left-right asymmetry

A link between ciliary motility and the regulation of left-right (LR) asymmetry was proposed when it was observed that some individuals with PCD also have situs inversus (Afzelius, 1976). This link was strengthened by the discovery that the mouse *iv* (inversus viscerum) mutant results from a mutation in a ciliary dynein gene, left-right dynein (*lrd*; *Dnahc11*), which is expressed specifically in monociliated cells of the embryonic node (Supp et al., 1997), the region that organizes the primary anteroposterior body axis during gastrulation. In normal mouse embryos, node monocilia rotate in a clockwise direction and generate an asymmetric flow of extracellular fluid that moves across the node in a leftward direction (Nonaka et al., 1998) (reviewed by Shiratori and Hamada, 2006). In *lrd* embryos, node cilia are immotile and there is no nodal fluid flow, indicating that nodal flow is necessary for establishing the LR axis (Okada et al., 1999; Supp et al., 1999). Studies of mouse embryos in flow chambers have shown that leftward fluid flow across the node correctly specifies the LR axis of *iv* mice, whereas rightward flow can reverse the LR axis in wild-type embryos, suggesting that fluid flow per se is sufficient for specifying the LR axis (Nonaka et al., 2002). In other vertebrates, rotational ciliary beating also causes leftward flow across structures that are analogous to the mouse node including the posterior notochordal plate in rabbits and the Kupffer's vesicle (KV) in zebrafish and medaka fish (Essner et al., 2005; Kramer-Zucker et al., 2005; Okada et al., 2005).

Left-right asymmetry provides an important paradigm for both the challenges and rewards of studying the multiple roles of cilia in development. Studies of cilia mutants have revealed, for example, that a correlation exists between loss of monocilia structure or movement, alterations in nodal flow and aberrant LR development, as is seen in mice with mutations in microtubule motors and IFT proteins, including Kif3a, Kif3b, dynein 2 light intermediate chain, polaris, wimple and Rfx3, a regulator of ciliogenic genes (Bonnafant et al., 2004; Huangfu et al., 2003; Marszałek et al., 1999; Murcia et al., 2000; Nonaka et al., 1998; Rana et al., 2004; Takeda et al., 1999). LR defects are also caused by mutations in other ciliary or basal body proteins including polycystin 2 (*Pc2*) and inversin (*Invs*) (Mochizuki et al., 1998; Pennekamp et al., 2002). In zebrafish embryos, interfering with KV cilia structure or function through knockdown of polaris, *Invs*, *Lrd* or *Pc2* causes alterations in LR patterning similar to those reported for the corresponding mouse mutants (Bisgrove et al., 2005; Essner et al., 2005; Kramer-Zucker et al., 2005; Otto et al., 2003; Sun et al., 2004). The challenge is to determine which phenotypes are due to loss of cilia structure, motility and flow, and loss of mechanosensory and/or signal transduction functions. It is likely that some LR phenotypes are compound defects that eliminate not only the function of the targeted protein, but also functions of other cilia proteins. In addition, some proteins that have functions in cilia might also have functions in other cellular compartments that are important for LR development (Wagner and Yost, 2000).

How do rotating nodal cilia cause laminar leftward flow of fluid and not local vortices? Fluid dynamics studies suggest a linear directional flow might be achieved if the rotation axis of the cilia has



**Fig. 2. Primary cilia in the embryonic node and left-right axis specification.**

The illustration represents the ventral surface of the mouse embryonic node, viewed from the center of the node toward the left. Motile cilia (green) in the center of the node rotate in a clockwise direction. Because the cilia are positioned at an angle at the posterior end of the node cells, when clockwise-rotating cilia stroke toward the right they are close to the cell surface and flow is impeded; when they stroke to the left at the top of the arc, they are away from the cell surface and flow is unimpeded. This produces an asymmetric flow of fluid (red arrows) towards the left periphery of the node. At the left periphery of the node, fluid flow is sensed by mechanosensory cilia (blue), and/or secreted signaling molecules are concentrated by the flow and received by chemosensory cilia. These events, alone or in combination, cause intracellular  $\text{Ca}^{2+}$  levels to increase in cells on the left side of the node, which triggers a signal transduction pathway that controls the asymmetric expression of genes that establish the left-right axis. A, anterior; L, left; P, posterior; R, right.

a posterior tilt (Fig. 2) (Buceta et al., 2005; Cartwright et al., 2004) (reviewed by Shiratori and Hamada, 2006). Microscopy studies confirmed that nodal cilia in mouse and rabbit embryos are tilted posteriorly and that laminar flow moves particles in a leftward direction (Nonaka et al., 2005; Okada et al., 2005). Cilia on the dorsal surface of KV in zebrafish embryos also appear to be tipped toward the posterior, indicating that the mechanism of creating laminar flow is evolutionarily conserved (Kramer-Zucker et al., 2005).

Two models have been proposed to explain how fluid flow confers LR asymmetry (Fig. 2). The 'morphogen flow' model proposes that signaling proteins, such as sonic hedgehog (Shh), fibroblast growth factor (Fgf) or Nodal are swept to the left side of the node where they initiate downstream signaling pathways (Nonaka et al., 1998; Okada et al., 1999). Although flow in rabbit and mouse embryos can concentrate fluorescently labeled proteins on the left side of the node (Okada et al., 2005), and small extracellular vesicles (so-called 'nodal vesicular particles') have been reported to be swept to the left periphery of the node where they fragment, releasing their cargo and triggering downstream signaling events including an elevation of  $\text{Ca}^{2+}$  levels (Tanaka et al., 2005), asymmetric distribution of endogenous proteins has not been detected. The mechanosensory or 'two cilia' model proposes that two populations of primary cilia exist in the mouse node (McGrath et al., 2003): central *Lrd*-expressing motile cilia and peripheral non-motile cilia. The model proposes that leftward fluid flow generated by motile cilia is detected by mechanical bending of non-motile mechanosensory cilia, which initiate downstream  $\text{Ca}^{2+}$  mediated signaling events. In support of this, asymmetric  $\text{Ca}^{2+}$  transients are not detected in *Pkd2* (*Pc2*) mouse mutant embryos, which lack functional ciliary mechanosensitive cation channels (see below), or in zebrafish in which *Ipk1*, an important mediator of intercellular  $\text{Ca}^{2+}$  signaling, is

**Table 1. Ciliary proteins essential for cellular function**

Gene and protein	Ciliary localization	Proposed function	Human disease	Disease pathology or developmental defect
<b>Intraflagellar transport</b>				
<i>Kif3a</i> , Kif3a	Cilia	Subunit of the anterograde IFT motor protein kinesin 2	Unknown	Kidney cysts, retinal dystrophy, situs inversus (M)
<i>Kif3b</i> , Kif3b	Cilia	Subunit of the anterograde IFT motor protein kinesin 2	Unknown	Situs inversus (M)
<i>Dnchc2</i> (M)/ cytoplasmic dynein	Cilia?	Heavy chain subunit of the retrograde IFT dynein motor complex	Unknown	Neural tube defects, polydactyly, situs inversus (M)
<i>Tg737</i> , Polaris/Ift88	Cilia, basal bodies	IFT complex B protein	Unknown	Renal, hepatic and pancreatic cysts, hydrocephalus, polydactyly, situs inversus (M)
Wimble (M), Ift172	Cilia	IFT complex B protein	Unknown	Neural tube defects, polydactyly, situs inversus (M)
<i>Ngd</i> (M), Ift52	Cilia	IFT complex B protein	Unknown	Neural tube defects, polydactyly, situs inversus (M)
<i>hippi</i> (Z), Ift57	Cilia?	IFT complex B protein	Unknown	Hydrocephalus, kidney cysts, situs inversus (Z)
<b>Ciliary motility</b>				
<i>Dnah5</i> , axonemal dynein	Cilia	Heavy chain subunit of ciliary outer dynein arms	PCD	Sinusitis, bronchiectasis, infertility, hydrocephalus, situs inversus
<i>Dnai1</i> , axonemal dynein	Cilia	Intermediate chain subunit of ciliary outer dynein arms	PCD	Sinusitis, bronchiectasis, infertility, hydrocephalus, situs inversus
<i>Dnah11</i> ( <i>ivlrd</i> )(M), left-right dynein	Cilia	Dynein motor protein subunit; required for motility in nodal cilia	PCD?	Situs inversus
<b>Transcription of ciliary genes</b>				
<i>Rfx3</i> (M), Rfx3	Nucleus	X-box transcription factor; regulates ciliogenic genes	Unknown	Situs inversus (M)
<i>Hfh4/Foxj1</i> (M), Foxj1	Nucleus	Forkhead transcription factor; regulates ciliogenic genes	Unknown	Situs inversus (M)
<b>Polycystic kidney diseases</b>				
<i>Pkd1</i> , polycystin 1	Cilia, basal bodies	Mechanosensitive and G protein-coupled receptor	ADPKD	Kidney, liver and pancreatic cysts
<i>Pkd2</i> , polycystin 2	Cilia	Mechanosensitive, nonselective cation channel permeable to Ca <sup>2+</sup>	ADPKD	Kidney, liver and pancreatic cysts; situs inversus (M, Z)
<i>Pkhd1</i> , fibrocystin/polyductin	Cilia, basal body	Unknown; transmembrane protein	ARPKD	Kidney cysts, liver fibrosis
<i>Cys1</i> , cystin	Cilia, basal body	Unknown	Unknown	Kidney cysts, <i>cpk</i> mouse model of PKD
<i>Nphp1</i> , nephrocystin	Cilia, basal bodies, centrosomes	Unknown; interacts with focal adhesion signaling complex elements and nephroretinin	NPHP type I (juvenile)	Kidney cysts, liver fibrosis, retinal dysplasia
<i>Nphp2</i> , <i>Invs</i> (M), inversin	Cilia, basal bodies, centrosomes	Interacts with Apc2, N-cadherin calmodulin, nephrocystin, Dvl; functions in regulation of cell cycle and Wnt pathways	NPHP type II (infantile)	Kidney cysts, situs inversus
<i>Nphp3</i> , <i>Pcy</i> (M), nephrocystin 3	Cilia, basal bodies, photoreceptor connecting cilia	Unknown; interacts with nephrocystin	NPHP type III (adolescent)	Kidney cysts and fibrosis
<i>Nphp4</i> , nephroretinin	Cilia, basal bodies	Unknown; interacts with nephrocystin	NPHP type IV	Kidney cysts, retinitis pigmentosa
<i>Nphp5/Iqcb1i</i> , nephrocystin 5	Cilia, photoreceptor connecting cilium	Unknown; interacts with retinitis pigmentosa GTPase regulator and calmodulin	Senior-Loken syndrome	Kidney cysts, retinitis pigmentosa
<i>Nphp6/Cep290</i> , nephrocystin 6	Centrosomes, photoreceptor connecting cilium	Unknown; interacts with transcription factor Atf4	Joubert syndrome	Kidney cysts, retinitis pigmentosa, cerebellar vermis aplasia



Table 1. Continued

Gene and protein	Ciliary localization	Proposed function	Human disease	Disease pathology or developmental defect
<b>Cell cycle regulation</b>				
<i>Pdgfra</i> , <i>Pdgfra</i>	Cilia	Platelet-derived growth factor receptor; involved in cell-cycle regulation	Malignancies	Mutations associated with gastrointestinal, lung and ovarian tumors
<i>Nek1</i> ( <i>kat</i> ), <i>Nek1</i>	Cilia, basal bodies, centrosomes	NIMA kinase family member; interacts with PKD proteins, cell cycle regulation?	Unknown	Kidney cysts, male infertility; mouse model of progressive PKD
<i>Nek8</i> , <i>jck</i> (M), <i>Nek8</i>	Cilia	NIMA kinase family member; cell cycle, cytoskeletal regulation?	Unknown	Kidney cysts; mouse model of juvenile cystic kidney disease
<b>Intercellular signaling</b>				
<i>Smo</i> , <i>smoothened</i>	Cilia, cytoplasm	Transmembrane hedgehog receptor, ciliary localization necessary for processing of Gli transcription factors	Unknown; gain of function in some basal cell carcinomas	Neural tube defects, polydactyly (M)
<b>BBS and other disease syndromes</b>				
<i>Bbs1-Bbs11</i> , <i>Bbs1-Bbs11</i>	Basal bodies, centrosome, photoreceptor connecting cilium	Involved in IFT in photoreceptor and olfactory cilia and in intracellular microtubule transport processes	Bardet-Biedl syndrome (BBS)	Kidney cysts, obesity, anosmia, retinal dystrophy, male infertility, situs inversus, diabetes
<i>Alms1</i> , <i>Alms1</i>	Cilia, centrosomes	Unknown	Alstrom syndrome	Retinal degeneration, obesity, diabetes
<i>Ofd1</i> , <i>Ofd1</i>	Basal bodies	Implicated in IFT and intracellular transport processes	Oral-facial-digital syndrome I	Kidney cysts, malformations of the oral cavity, face, and digits
<i>Mks1</i> and <i>Mks3</i> , <i>Mks1</i> and <i>Mks3</i>	Cilia?	Unknown	Meckel-Gruber syndrome	Kidney and liver cysts, CNS malformations, polydactyly, hydrocephalus

Genes and phenotypes specific to mice (M), zebrafish (Z) are indicated.

ADPKD, autosomal polycystic kidney disease; *Alms1*, Alstrom syndrome 1; *Cys1*, cystin 1; *Hfh4*, hepatocyte nuclear factor/forkhead homolog 4; IFT, intraflagellar transport; *Kif3*, kinesin superfamily 3; *Mks1*, Meckel syndrome 1; *Nek*, NIMA-related kinase; PCD, primary cilia dyskinesia; *Pdgfra*, platelet-derived growth factor  $\alpha$ ; PKD, polycystin; NPHP, nephrocystin; *Ofd1*, oral-facial-digital type 1 syndrome.

knocked down by morpholinos (McGrath et al., 2003; Sarmah et al., 2005). Further studies will be necessary to clarify the mechanism that determines how LR axis patterning information is conveyed by fluid flow and, perhaps, sensed by specialized cilia.

### Disorders of primary cilia

The immotile primary cilia that are associated with many cell types were thought to be of little physiological importance until recently when homologs of ciliary proteins in *Chlamydomonas* and *C. elegans* were linked to polycystic kidney diseases (PKDs) in humans and mice, and were also found to localize to renal primary cilia (Barr and Sternberg, 1999; Pazour et al., 2002b; Yoder et al., 2002a; Yoder et al., 2002b). The list of proteins that localize to the primary cilium/basal body/centrosome complex and are linked to PKDs, nephronophthisis (NPHP) and several other human syndromes such as Bardet-Biedl Syndrome (BBS) continues to grow (see Table 1, Fig. 3). Primary cilia also play essential roles as signal transducers in the Shh and non-canonical Wnt developmental pathways (see Huangfu et al., 2003; Simons et al., 2005), as discussed in more detail below. In the following sections we describe recent advances in the study of development and human disease that shed light on the importance of the ciliary apparatus and its role in controlling cell fate and homeostasis.

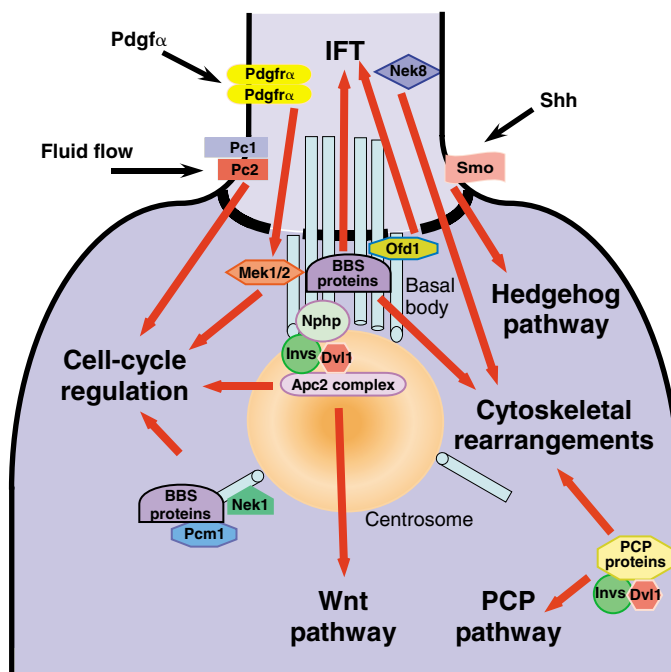
### Cilia as mechanosensors and PKD

In humans, inherited PKDs include autosomal dominant PKD (ADPKD), autosomal recessive PKD (ARPKD) and NPHP. During disease progression, renal epithelial cells proliferate to form fluid-

filled cysts that replace normal renal tissue. The mechanisms responsible for cyst development are unclear, but cysts appear to arise due to a combination of factors, including increased cell proliferation, loss of cell polarity and failure of cell differentiation. Intracellular  $Ca^{2+}$  levels, which are important for the regulation of cell proliferation and apoptosis, and for other properties of nephron function (including ion reabsorption rates), probably play a central role in cyst formation (Boletta and Germino, 2003).

The gene disrupted in ARPKD encodes a transmembrane protein known as fibrocystin or polyductin, which localizes to renal primary cilia and basal bodies, the function of which remains enigmatic (Wang et al., 2004; Ward et al., 2002). By contrast, ADPKD, which results from mutations in *PKD1* or *PKD2* (encoding polycystin 1 (Pc1) and polycystin 2 (Pc2), respectively), is better understood. Pc1 and Pc2 are multi-pass integral membrane proteins that interact to form  $Ca^{2+}$  permeable cation channels at the plasma membrane and in primary cilia of human and mouse renal epithelial cells (Hanaoka et al., 2000; Pazour et al., 2002b; Yoder et al., 2002a). Bending the primary cilia of MDCK cells in culture causes  $Ca^{2+}$  to influx through these channels in the ciliary membrane (Praetorius and Spring, 2001). These  $Ca^{2+}$  transients can be blocked by antibodies that block the function of Pc1 or Pc2 (Nauli et al., 2003), indicating that these proteins function in detecting mechanical stress and can probably detect fluid flow.

Several additional studies indicate that ciliary localization is important for the function of the Pc1/Pc2 channel and that IFT and Pc2 functions are linked. In mammals, polaris localizes to the basal body and axoneme of motile and immotile cilia (Taulman et al.,



**Fig. 3. Functions of primary cilia.** The primary cilium acts as a sensory organelle that transfers information from the extracellular environment to the cell interior. For example, cation channels composed of the polycystin proteins Pc1 and Pc2 in the ciliary membrane sense mechanical stress, while receptors such as the platelet-derived growth factor receptor  $\alpha$  (Pdgfr $\alpha$ ) sense extracellular ligands. The processing and transfer of signaling information to the cell is mediated by specialized proteins, including smoothened (Smo), and several microtubule-associated protein complexes that include members of the nephronophthisis protein family [Nphp, inversin (Invs)], and proteins associated with Bardet-Biedl syndrome (BBS). Signals from primary cilia ultimately are involved in regulating crucial cellular processes, including the cell cycle, cytoskeletal organization, intraflagellar transport and signaling pathways, such as the hedgehog, canonical Wnt and non-canonical Wnt/planar cell polarity (PCP) pathways. Abbreviations: Apc2, anaphase promoting complex protein 2; Dvl1, dishevelled; Nek8, NIMA-related kinase 8; Ofd1, oral-facial-digital type 1 protein; Pcm1, pericentriolar material protein 1.

2001) and colocalizes with Pc1 and Pc2 and a novel murine PKD protein, cystin, in the primary cilia of kidney cells (Hou et al., 2002; Pazour et al., 2002b; Yoder et al., 2002a). In cultured renal epithelial cells from polaris mutant mice, high levels of Pc2 accumulate in stunted cilia and in the apical membrane, concomitant with elevated sub-apical  $Ca^{2+}$  levels (Pazour et al., 2002b; Siroky et al., 2006). Similarly, in *C. elegans*, Pc1, Pc2 and polaris homologs colocalize in sensory neuron cilia and accumulate in the stunted cilia of *osm-5* (*polaris*) mutants (Barr et al., 2001; Barr and Sternberg, 1999; Haycraft et al., 2001; Qin et al., 2001). Together, these results indicate that polaris-dependant IFT is required to regulate ciliogenesis and Pc2 levels in cilia, and that misregulation of Pc2 levels might perturb  $Ca^{2+}$  entry and contribute to cyst development.

In addition to forming cation channels with Pc2, Pc1 also forms complexes with proteins involved in Wnt signaling, heterotrimeric G protein signaling and activation of the JAK-STAT pathway (Delmas, 2004; Low et al., 2006). Because Pc1 is localized to multiple sites in the cell, it is unclear whether any of these processes are related to ciliary function. Cessation of renal fluid flow, however, has been found to induce intramembrane proteolysis of Pc1. This

generates a Pc1 C-terminal fragment that can translocate to the nucleus in a complex with STAT6 and P100 and activate the AP-1 pathway (Chauvet et al., 2004). These data suggest that transduction of ciliary mechanosensory information might also involve changes in gene transcription mediated through proteolysis of Pc1.

Cilia-mediated mechanoreception of fluid flow may also play a role in the development and function of other organ systems. Individuals with ADPKD and ARPKD, and several murine models of PKD that are associated with ciliary proteins also frequently have cysts in the liver and pancreas (Cano et al., 2004; Igarashi and Somlo, 2002; Lager et al., 2001). Although the direct causes of these cystic diseases have not been determined, it is tempting to speculate that a loss of ciliary mechanosensory functions in hepatic and pancreatic ducts may be involved in these pathologies.

### NPHP: PKD proteins that link mechanoreception and cell cycle regulation?

Nephronophthisis (NPHP) encompasses a polygenic group of autosomal recessive cystic kidney diseases in children and young adults. Individuals with this disease can also have pancreatic and hepatic fibrosis, situs inversus, retinal degeneration (in Senior-Loken syndrome and Joubert syndrome), complex brainstem malformation and mental retardation (Joubert syndrome). Mutations in six genes (*NPHP1*-*NPHP6*) have been linked to NPHP (Hildebrandt et al., 1997; Mollet et al., 2002; Olbrich et al., 2003; Otto et al., 2002; Otto et al., 2005; Otto et al., 2003; Saunier et al., 1997; Sayer et al., 2006; Valente et al., 2006). NPHP proteins contain multiple protein-protein interaction domains and appear to function as a complex in the primary cilia/basal body/centrosome apparatus (Morgan et al., 2002a; Olbrich et al., 2003; Otto et al., 2005; Otto et al., 2003; Watanabe et al., 2003). Disruption of *Nphp2* (*Invs*) in mice or zebrafish does not alter cilia structure (Okada et al., 1999; Otto et al., 2003). *C. elegans* homologs of human NPHP1 and NPHP4 (*NPH-1* and *NPH-4*) localize to the transition zone of ciliated sensory neurons. Although *C. elegans nph-4* mutants have no ciliary structural defects, cilia-mediated sensory functions, including chemotaxis and male mating behavior are abnormal, suggesting a role for the proteins in ciliary signaling (Winkelbauer et al., 2005; Wolf et al., 2005). NPHP proteins could also provide a link between sensory cilia and cell cycle regulation. *Invs* (*Nphp2*) contains two predicted calmodulin-binding domains and can bind  $Ca^{2+}$  (Morgan et al., 2002b). It also associates with the anaphase promoting complex protein Apc2 and has a dynamic pattern of expression during mitosis, cycling between localization at the centrosomes, mitotic spindle poles and the midbody between cells (Morgan et al., 2002a; Nurnberger et al., 2002). *Nphp6* also has a dynamic pattern of expression, alternating between the nucleus and centrosome in a cell cycle-dependant manner (Sayer et al., 2006). From these observations, it is tempting to speculate that NPHP proteins provide a link between Pc1/Pc2,  $Ca^{2+}$  and the cell cycle, and that primary cilia serve as environmental sensors for the centrosome in the regulation of the cell cycle.

### Cilia dependent signaling and cell cycle coordination

Centrioles play dual roles in the cell: as basal bodies, they influence cilia function; as centrioles in centrosomes, they mediate cell division. Centriole duplication is coordinated with the cell cycle. Thus, entry into the cell cycle is preceded by cilium resorption, whereas exit from mitosis and cellular differentiation is accompanied by cilium assembly. If cilia provide information that helps cells remain in a differentiated state, then defects in cilia-dependent signaling would be predicted to cause proliferative disorders and alterations in cellular differentiation.

Cell proliferation, migration and apoptosis in mammalian tissues are controlled, in part, by platelet-derived growth factor receptor  $\alpha$  (Pdgfr $\alpha$ ), which localizes to primary cilia in primary cultures of mouse embryonic fibroblasts (Schneider et al., 2005). In response to ligand, Pdgfr $\alpha$  homodimerizes and activates the Akt and Mek1/2-Erk1/2 pathways. Mek1/2 phosphorylation occurs within the cilium and basal body. Fibroblasts derived from a hypomorphic polaris mutant mouse (polaris functions in IFT) form abnormal stunted cilia and fail to activate the Mek1/2-Erk1/2 pathway in response to ligand, indicating that activation of Pdgfr $\alpha$  and its cellular functions depend on its localization to normal cilia (Schneider et al., 2005).

Studies of mouse PKD models also implicate two members of a cell-cycle kinase family (NIMA-related kinases or Nek kinases) as links between ciliary function and cell cycle control. *kat* (*Nek1*) mutant mice have autosomal recessive PKD (Upadhyaya et al., 2000). Nek1 interacts with several proteins, including Kif3a, and localizes to the centrosomes (Mahjoub et al., 2005; Surpili et al., 2003). *Nek8* mutant mice have juvenile cystic kidney (JCK) disease (Liu et al., 2002). Nek8 localizes to the proximal region of the primary cilia during interphase and is undetectable during mitosis (Mahjoub et al., 2005). A kinase domain mutation of Nek8 affects cell cycle progression and overexpression of mutant forms of Nek8 lead to multinucleate cells (Bowers and Boylan, 2004; Liu et al., 2002). As Nek8 is not required for cilia assembly (Mahjoub et al., 2005), these observations suggest that it functions to link cilia and cycle regulation.

### Cilia-dependent signaling and developmental disorders

#### Wnt signaling

Several recent studies implicate cilia in two important developmental signaling pathways: Wnt signaling and Shh signaling. During early kidney development, canonical Wnt signaling is required for metanephric mesenchyme induction and cell proliferation during branching morphogenesis (Perantoni, 2003). Later in development, signaling through the non-canonical Wnt, or PCP, pathway (see Box 1) (Park et al., 2005; Veeman et al., 2003; Wallingford and Habas, 2005) is required to align the mitotic orientation of proliferating cells of the renal tubules to allow the tubules to lengthen without substantially increasing their diameter (Fischer et al., 2006).

Observations that the kidney phenotype of *Invs* mutants resembled that of mice with dysregulated canonical Wnt signaling suggested that *Invs* (*Nphp2*) functions in Wnt signaling (Simons et al., 2005). In MDCK cells, *Invs* forms a protein complex with dishevelled (*Dvl1*) and *Apc2*. This complex targets *Dvl1* for proteosomal degradation, resulting in a loss of  $\beta$ -catenin stabilization and inhibition of the canonical Wnt pathway (Simons et al., 2005). Subsequently, as MDCK cells become confluent and polarized, *Invs* and *Dvl1* translocate to the plasma membrane, where they are in a position to associate with other PCP proteins and activate the non-canonical Wnt pathway. Although *Invs*-mediated PCP signaling in the kidney remains to be formally demonstrated, morpholino-mediated knockdown of *Invs* impairs PCP-dependant convergent extension movements in frog embryos, indicating that *Invs* functions in the PCP pathway (Simons et al., 2005). *Invs* has also been implicated in PCP processes that orient ciliated cells of the mouse node along the AP axis (Okada et al., 2005).

In PKD, cyst formation is associated with increased numbers of cells in the circumference of renal tubules. In mice with renal-specific inactivation of *Tcf2*, and in the *pck* rat, which has reduced expression of *Pkd2* and/or *Pkhd1*, mitotic alignments along the axis of the tubules are significantly distorted, indicating a loss of PCP

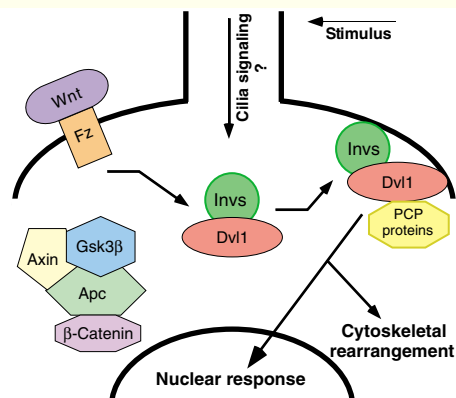
(Fischer et al., 2006). In the *pck* rat, this distortion precedes tubular distension, suggesting a correlation between PCP loss and PKD development. As several proteins implicated in PKD, including *Invs*, fibrocystin, *Pc1* and *Pc2* localize to the cilium and/or basal body, one could speculate that cilia sense primary tubular urine flow and terminate canonical Wnt signaling, which facilitates non-canonical Wnt signaling necessary for tubulogenesis. Ciliary bending might also provide a vectorial cue that orients cell division. Consistent with this model, when ciliated inner medullary collecting duct cells are exposed to flow, *Invs* levels increase and  $\beta$ -catenin levels decrease (Simons et al., 2005).

Structural defects in ciliogenesis are correlated with defective PCP. In *Xenopus*, the PCP proteins inturned (*Xint*) and fuzzy (*Xfy*) accumulate at the apical surface of ciliated cells (Park et al., 2006). *Xint* and *Xfy* morphants have convergent extension defects that correlate with a loss of cilia from cells of the ventral neural tube. In epidermal cells of morphant embryos, the apical actin network is less dense and contains fewer actin foci than in control embryos, and microtubules are not organized into apically projecting cilia. Thus, *Xint* and *Xfy* control an apical actin network essential for the orientation of microtubules and their assembly into cilia.

#### Shh signaling

In vertebrates, cilia also function in the Shh-dependent patterning of the developing neural tube and limb (see Box 2) (reviewed by Huangfu and Anderson, 2006). Mouse mutants with defects in

#### Box 1. Ciliary involvement in Wnt signaling pathways



The cilium might act as a switch between canonical Wnt signaling and the non-canonical Wnt/planar cell polarity (PCP) pathway. Canonical and non-canonical Wnt pathways both require Wnt ligands, frizzled (Fz) receptors and dishevelled (*Dvl1*) but they diverge downstream of *Dvl1*. In the canonical pathway, Wnt binding to Fz leads to the activation of *Dvl1* and the stabilization of cytoplasmic  $\beta$ -catenin, through inhibition of the *Apc2/axin/Gsk3 $\beta$*  degradation complex.  $\beta$ -Catenin translocates to the nucleus, where it activates *Lef/Tcf*-mediated gene transcription. In response to an environmental stimulus such as fluid flow, a ciliary signal mediates a switch from canonical to noncanonical Wnt signaling. *Dvl1* and possibly *inversin* (*Invs*) localize to the plasma membrane, where they interact with PCP proteins;  $\beta$ -catenin is degraded by the *Apc/axin/Gsk3 $\beta$*  complex. PCP signaling influences gene transcription and regulates cytoskeletal rearrangements. The PCP pathway in vertebrates directs several processes, including mitotic spindle orientation, epithelial cell polarization and convergent extension movements during gastrulation and neurulation.

ciliogenesis resulting from mutations in IFT protein-encoding genes, such as *polaris*, *wimple* (*Ift172*), *Ngd5* (*Ift52*) and the gene encoding the retrograde motor *Dnchc2*, have neural tube defects and preaxial polydactyly phenotypes similar to mutants with defects in Shh pathway proteins (Haycraft et al., 2005; Huangfu et al., 2003; Liu et al., 2005; May et al., 2005; Zhang et al., 2003). Cilia are present in the forebrain neuroectoderm and in ectodermal and mesenchymal cells of the limb, but are aberrant or absent from these tissues in IFT mutants (Haycraft et al., 2005; May et al., 2005).

Shh expression in the notochord and limb buds of IFT mutants is normal, but expression of downstream targets, including patched 1 (*Ptch1*) and *Gli1*, in responding tissues (the neural tube and limb bud, respectively) is reduced, consistent with a loss of Shh signal transduction. Interestingly, IFT mutants display a loss-of-function Shh phenotype in the neural tube, where Gli activators (*Gli1*, *Gli2*) have a major role in pattern formation, and a gain-of-function Shh phenotype in the limb, where the Gli3 repressor (*Gli3R*) plays the main role (Huangfu and Anderson, 2005). A series of elegant studies (including epistasis analyses of mouse IFT and Shh pathway protein mutants, and analysis of Gli3 activator to Gli3R in IFT mutants) and studies of *Gli1* and *Gli3R* construct expression in cells derived from IFT mutants indicate that IFT is required downstream of *Ptch1* and *smoothed* (*Smo*). These studies also show that IFT proteins are required for Gli activation and for the proteolytic processing of *Gli3* into *Gli3R*, but not for the trafficking of Gli proteins to the nucleus (Haycraft et al., 2005; Huangfu and Anderson, 2005; Huangfu et al., 2003; Liu et al., 2005; May et al., 2005).

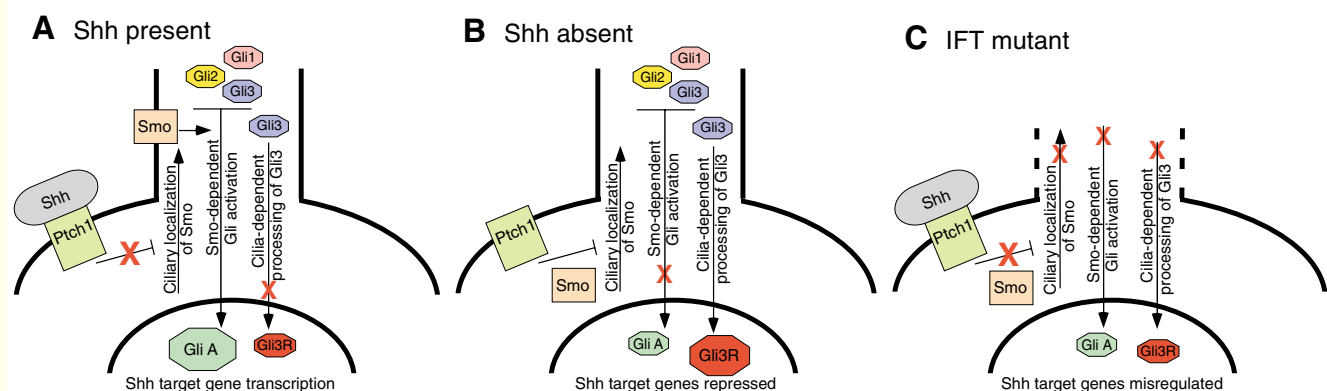
*Smo* plays a crucial role in the Shh pathway controlling both Gli activation and, in the absence of Shh signaling, the proteolytic processing of *Gli3* to *Gli3R*, making it a likely candidate for the component of the pathway directly affected in IFT mutants. *Smo* accumulates in the cytoplasm and also on the primary cilia of MDCK cells and of mouse embryonic node cells (Corbit et al., 2005;

May et al., 2005). In MDCK cells, the ciliary localization of *Smo* increases in response to Shh; blocking the Shh pathway with cyclopamine eliminates the localization of an activated *Smo* protein (Corbit et al., 2005). Cyclopamine treatment also reduces *Smo* levels in mouse nodal cilia (Corbit et al., 2005). Deletion of a C-terminal hydrophobic motif, common to other cilia-localized proteins (Brailov et al., 2000; Dwyer et al., 2001; Handel et al., 1999) prevents the truncated *Smo* protein from localizing to cilia, even in the presence of Shh, and abolishes *Smo* activity (Corbit et al., 2005). These observations indicate that mobilization of *Smo* to primary cilia is a key event in the activation of the Shh pathway (see Box 2). However, it is unclear whether IFT function is directly required in Shh signaling to localize *Smo* to the ciliary membrane or whether it is required indirectly for the formation of a structurally and functionally intact cilium. Several other components of the Shh pathway are also localized to cilia, including *suppressor of fused* and the three full-length Gli transcription factors (Corbit et al., 2005; Haycraft et al., 2005; May et al., 2005). How *Smo* activates Shh signaling at the cilium is unclear, but the cilium might provide a specialized microtubule-associated domain that coordinates *Smo* and other components of the pathway to facilitate the activation of Gli transcription factors and the proteolytic processing of *Gli3* to generate *Gli3R*.

#### Bardet-Biedl syndrome: microtubule transport connecting ciliary and cellular function?

Bardet-Biedl syndrome (BBS) is a genetically heterogeneous pleiotropic disorder with symptoms including kidney abnormalities, retinal degeneration, mental retardation, obesity, diabetes, polydactyly and situs inversus. The syndrome has been linked to mutations in 11 loci that produce clinically indistinguishable phenotypes, indicating that the encoded proteins participate in a common cellular process. With the exceptions of BBS4 and BBS8, which contain multiple protein-protein interaction domains, BBS6

### Box 2. Role of cilia in vertebrate hedgehog signaling



Primary cilia play a crucial role in mediating hedgehog (Hh) signaling in vertebrates. The transcription of sonic hedgehog (Shh) target genes is regulated by the nuclear ratio of Gli activators (GliA) to Gli3 repressors (Gli3R). (A) Upon Shh ligand binding to patched 1 (*Ptch1*), inhibition of *smoothed* (*Smo*) is eliminated and it localizes to the cilium, where it controls the activation of Gli transcription factors and their translocation to the nucleus where they activate transcription of Shh target genes. (B) In the absence of Shh, the cilium-mediated proteolytic processing of *Gli3* generates a N-terminal fragment that functions in the nucleus as a transcriptional repressor (*Gli3R*). (C) In intraflagellar transport (IFT) mutants with aberrant or absent cilia, the formation of both GliA and GliR is impaired, which can lead to Shh loss- or gain-of-function phenotypes in different tissues. Interestingly, in *Drosophila*, Hh-responsive tissues lack cilia, and activation of the Hh pathway is associated with *Ptch1*-mediated mobilization of *Smo* from intracellular stores to the cell surface [for a review comparing vertebrate and invertebrate Hh signaling, see Huangfu and Anderson (Huangfu and Anderson, 2006)].



and BBS10, which encode chaperonin-like proteins, and BBS11, which encodes an E3 ubiquitin ligase, most show limited homology to other proteins of known function (Ansley et al., 2003; Badano et al., 2003; Chiang et al., 2006; Chiang et al., 2004; Fan et al., 2004; Katsanis et al., 2000; Kim et al., 2005; Li et al., 2004; Mykytyn et al., 2001; Mykytyn et al., 2002; Nishimura et al., 2001; Nishimura et al., 2005; Slavotinek et al., 2000; Stoetzel et al., 2006; Stone et al., 2000).

BBS proteins localize to the cilia/basal body/centrosome complex. In *C. elegans*, BBS::GFP fusion proteins localize to the base of cilia and move along the ciliary axoneme in sensory neurons (Ansley et al., 2003; Blacque et al., 2004; Fan et al., 2004; Li et al., 2004). In mammals, BBS4, BBS5, BBS6 and BBS8 localize to the centrosome, pericentriolar region and basal body, but are absent from the ciliary axoneme in most cell types, except in the photoreceptor connecting cilium (Ansley et al., 2003; Kim et al., 2004; Kim et al., 2005; Li et al., 2004).

Studies in humans and several model systems indicate that BBS proteins function in microtubule-based cellular processes. In *C. elegans*, mutations in *bbs-7* or *bbs-8* result in loss of the distal ciliary segment, and dissociation of IFT particles into A and B complexes, as seen in *osm-3* mutants (Blacque et al., 2004; Ou et al., 2005), indicating that BBS-7 and BBS-8 are required to keep IFT particles intact and may regulate their association with the kinesin motors. In humans, BBS4, BBS6 and BBS8 associate with PCM1, a centrosomal protein involved in centriolar replication (Ansley et al., 2003; Kim et al., 2004; Kim et al., 2005). BBS4 also interacts with dynactin, a protein that modulates cargo binding to dynein. RNAi silencing of BBS4 causes PCM1 mislocalization to the cytosol, the de-anchoring of microtubules at the centrosome and arrested cell division, indicating that BBS4 functions in association with dynein to transport PCM1 to the centrosome (Kim et al., 2004). RNAi knockdown of BBS6, which assembles at the centrosome in a microtubule-independent manner, also causes cytokinesis defects. Furthermore, patient-derived mutant variants of BBS4 and BBS6 fail to associate with centrosomes, suggesting that loss of this function may cause BBS (Kim et al., 2004; Kim et al., 2005). BBS6 and PCM1 do not colocalize in all cell types, which may explain the cell-type-specific phenotypes associated with this disease.

Loss of BBS function in humans often leads to retinal degeneration and anosmia. *Bbs2* and *Bbs4* mutant mice have normal retinas and normal photoreceptor-connecting cilia early in life, but subsequently undergo progressive retinal degeneration (Mykytyn et al., 2004; Nishimura et al., 2004). Retinal degeneration results from reduced anterograde transport of rhodopsin across the connecting cilium of the photoreceptors, which leads to rhodopsin accumulation in the cell body, triggering cell death (Nishimura et al., 2004). Similar defects are seen in photoreceptors of mice with mutant IFT proteins (Marszalek et al., 1999; Pazour et al., 2002a), indicating that BBS proteins interact with IFT proteins in some cell types. Like retinal degeneration, anosmia in *Bbs1* and *Bbs4* mutant mice, results from the depletion of olfactory proteins from the ciliary layer of the olfactory neurons and the accumulation of these proteins in the cell bodies (Kulaga et al., 2004). Olfactory cilia of the mutant mice are also depleted of stable microtubules. Interestingly, the neighboring respiratory epithelium has normal cilia, indicating that BBS proteins are not required in all ciliated cells. In zebrafish, morpholino knockdown of ubiquitously expressed BBS genes delays retrograde intracellular transport of melanosomes within melanophores (Yen et al., 2006), consistent with a role for these proteins in microtubule-based transport processes.

In addition to characteristic BBS phenotypes, some *Bbs4* mutant mice also exhibit anterior neural tube defects similar to those seen in mouse PCP gene mutants (Ross et al., 2005). Cochlear stereocilia bundles (actin-enriched microvilli) are misoriented in *Bbs1*, *Bbs4* and *Bbs6* mouse mutants and in double heterozygotes of *Bbs6* and the PCP mutant looptail/*Vangl2*, indicating that BBS proteins function in the PCP pathway. In zebrafish embryos, disruption of *Bbs4* or *Bbs6* enhances the convergent extension defects in the zebrafish PCP mutant *trilobite* (*vangl2*). In mammalian epithelial cells, *Vangl2* localizes to the ciliary axoneme and basal bodies. Although it is unclear whether this localization is necessary for its function, it is consistent with a link between BBS and PCP protein function, and provides additional evidence that cilia or basal bodies are intrinsically involved in PCP processes.

In conclusion, although BBS proteins do not appear to be required for the function of the ciliary/basal body/centrosome complex in all cell types, several cellular functions are compromised by mutations in these genes. Present studies indicate that these proteins play crucial roles in other microtubule-based protein transport processes associated with the centrosomes and other intracellular microtubule arrays.

### Other cilia-related human disease syndromes

Alstrom syndrome (ALMS) is caused by mutations in a novel gene of unknown function (ALMS1) (Collin et al., 2002; Hearn et al., 2002) and is characterized by several phenotypes reminiscent of BBS, including retinal degeneration, obesity and diabetes. ALMS1 protein localizes to centrosomes and to the base of cilia. In fibroblasts with disrupted ALMS1, primary cilia and the microtubule cytoskeleton appear to be normal, suggesting that the ALMS phenotype results from impaired ciliary function rather than from abnormal ciliary structure (Hearn et al., 2005).

Affected individuals with oral-facial-digital type I syndrome (OFD1) have craniofacial abnormalities, postaxial polydactyly and polycystic kidneys. *Ofd1* localizes to the centrosome and basal body of primary cilia (Ferrante et al., 2001; Romio et al., 2004). Mouse *Ofd1* mutants lack cilia on the luminal surface of kidney glomerular and tubular cells, lack nodal cilia and have LR patterning defects. In addition, they have morphological and *Shh* target gene expression defects in the neural tube and limb bud that recapitulate those seen in *Ift172*, *Kif3a* and *polaris* mutants (Ferrante et al., 2006). Protein localization data and mutant phenotypes indicate that *Ofd1* is associated with the IFT machinery and that the human disease syndrome results from a loss of primary cilia function.

Meckel-Gruber syndrome (MKS) is associated with mutations in at least three loci and is characterized by renal and hepatic cysts, CNS malformations, polydactyly and occasionally hydrocephalus. MKS1 has little homology to other proteins and no known function. Its phenotypes and the fact that a *Chlamydomonas* ortholog is present in the flagella/basal body proteomes suggest a ciliary function (Keller et al., 2005; Kytala et al., 2006; Li et al., 2004). MKS3 encodes a seven-transmembrane receptor protein and is syntenic to the *wpk* locus in rat, which is associated with PKD and neural tube defects, including hydrocephalus (Smith et al., 2006). The lack of a *Chlamydomonas* ortholog suggests that it is not part of the basal IFT machinery, but the existence of an ortholog in the *C. elegans* ciliary data set (Blacque et al., 2005) implicates it in ciliary function. Continued research into the mechanistic basis for diseases such as those mentioned above will greatly expand our understanding of the many essential roles played by ciliary function in developmental disorders and disease.

## Conclusions

Ciliary function has recently been shown to be crucial in an increasingly diverse range of biological functions. The location of the cilium on the cell surface effectively positions it to function as an environmental sensor and vehicle to transfer information that affects important developmental decisions. The ciliary membrane and cytoplasm are relatively isolated from the cell body, which offers the advantage of compartmentalization, while the IFT machinery allows for rapid, and presumably regulated, transport of proteins between the cilium and the cell body. Recent discoveries indicate that cilia provide both inside-out signaling, such as the positioning of cilia to control the direction of extracellular fluid flow, and outside-in signaling, which modulates hedgehog signaling, Pdg $\alpha$  signaling and intracellular Ca<sup>2+</sup> levels.

We now have considerable insight into the molecular and cellular basis of several ciliopathies, including cystic kidney disease, polydactyly and situs inversus. In others, such as the formation of hepatic and pancreatic cysts associated with some forms of PKD, the role of cilia is less well understood but may reflect similar mechanisms as those seen in the generation of renal cysts. A role for ciliary dysfunction in other pathologies, such as obesity and diabetes seen in BBS and ALMS remain enigmatic, but raises the intriguing possibility that ciliary/basal body function is involved in the regulation of glucose metabolism.

Almost all mammalian cells have a primary cilium, and although the functions of these cilia in some organs are now understood, the roles of cilia on many cell types and organs remain obscure. Although it is clear that the primary cilium has multiple functions, there does not yet seem to be a common denominator for all tissues. Ciliary functions are partially overlapping, but different phenotypes present in PKD, BBS, NPHP and other diseases indicates that many roles of cilia are compartmentalized and that specific diseases are a result of defects in specific aspects of cilia function. The diverse phenotypes caused by ciliary dysfunction could reflect variable expression of ciliary proteins in different cell and tissue types. Some ciliary proteins also appear to play multiple functional roles in cells. For example, Invs has been implicated in the coordination of the cell cycle, and as a molecular switch that regulates the canonical and non-canonical Wnt signaling pathways. Much remains to be carried out in order to understand fully the contribution of each of these factors to the spectrum of ciliary function. One of the challenges is to understand which of the many functions of cilia, or which combinations of functions are responsible for each developmental process. Many additional roles for ciliary proteins are likely to be uncovered and will greatly contribute to our understanding of developmental mechanisms and disease.

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