An incredible decade for the primary cilium: a look at a once-forgotten organelle

James R. Davenport and Bradley K. Yoder
Department of Cell Biology, University of Alabama at Birmingham, Birmingham, Alabama

Davenport, James R., and Bradley K. Yoder. An incredible decade for the primary cilium: a look at a once-forgotten organelle. Am J Physiol Renal Physiol 289: F1159–F1169, 2005; doi:10.1152/ajprenal.00118.2005.—Since the discovery that numerous proteins involved in mammalian disease localize to the basal bodies and cilia, these organelles have emerged from relative obscurity to the center of intense research efforts in an expanding number of disease- and developmental-related fields. Our understanding of the association between cilia and human disease has benefited substantially from the use of lower organisms such as Chlamydomonas and Caenorhabditis elegans and the availability of murine models and cell culture. These research endeavors led to the discovery that loss of normal ciliary function in mammals is responsible for cystic and noncystic pathology in the kidney, liver, brain, and pancreas, as well as severe developmental patterning abnormalities. In addition, the localization of proteins involved in rare human disorders such as Bardet-Biedl syndrome has suggested that cilia-related dysfunction may play a role in modern human epidemics such as hypertension, obesity, and diabetes. Although we have made great advances in demonstrating the importance of cilia over the past decade, the physiological role that this organelle plays in most tissues remains elusive. Research focused on addressing this issue will be of critical importance for a further understanding of how ciliary dysfunction can lead to such severe disease and developmental pathologies.

AN INTRODUCTION TO MOTILE AND IMMOTILE CILIA

Cilia are common organelles present on nearly every cell in the mammalian body (for a complete list of cells that have been reported to have cilia, see http://www.bowserlab.org/primarycilium/ciliumpage2.htm). Structurally, the cilium consists of a microtubule-based axoneme covered by a specialized plasma membrane. The cilia axoneme emerges from the basal body, a centriole-derived, microtubule-organizing center, and extends from the cell surface into the extracellular space (5, 78). Found in large groups on a single cell or as solitary structures (Fig. 1 A and B), cilia are classified into three basic categories: motile, primary, or nodal.

Historically, motile cilia have been the most studied of all cilia and are generally found on the epithelial cells of the trachea, ependymal cells in the brain, and on cells lining the ovudct and epididymis of the reproductive tracts. Normally concentrated in large numbers on the cell surface, motile cilia beat in an orchestrated wavelike fashion and are involved in fluid and cell movement such as mucociliary clearance in the lung, cerebrospinal fluid movement in the brain, and ovum and sperm transport along the respective reproductive tracts. These cilia are constructed on what is referred to as a 9+2 microtubule pattern, where nine separate microtubule doublets surround a central pair of microtubules (Fig. 1 C). This central pair is important for the generation of forces needed to induce motility (67).

In contrast, primary cilia are solitary organelles projecting from the surface of cells (Fig. 1B). These cilia lack the central pair of microtubules needed to generate motile force and are thus described as having a 9+0 pattern (Fig. 1C) (61). While the function of the primary cilia on most cells remains elusive, in the mammalian kidney and Caenorhabditis elegans neurons, the cilia function primarily as either chemo- or mechano-sensors.

A third class of cilia, known as nodal cilia, has been localized to the node in gastrulation-stage embryos. As in the case of the primary cilia, the nodal cilia are solitary organelles that contain a 9+0 microtubule architecture; however, these cilia are distinct, as they possess the ability to move in a propeller-like fashion (51). Mutations that disrupt nodal cilia in mice indicate that they play essential roles in establishing signaling events required for specification of the left-right body axis in mammals (8, 38, 45, 51, 85).

CONSTRUCTING A CILIUM: DISCOVERY OF INTRAFLAGELLAR AND INTRACILIARY TRANSPORT

Both cilia and its structurally similar cousin organelle, the flagellum, are found on most eukaryotic cells. They are constructed through an evolutionarily conserved process termed intraflagellar transport (IFT) (29, 33, 63). IFT was first described in Chlamydomonas as the bidirectional movement of particles along the flagellar axoneme on raftlike transport.
structures located between the axoneme membrane and the outer doublet of microtubules (Fig. 2) (33). IFT particles have now been detected along the ciliary axoneme in *C. elegans* as well as in certain mammalian cells. The process of assembling these particles occurs at the base of the cilia near the transition fibers. At this point, they are transported in an anterograde manner toward the tip of the axoneme by the action of the heterotrimeric kinesin II motor complex (Kif3a, Kif3b, and Kap3 in mammalian systems). Recently, OSM-3 was identified in *C. elegans* as an additional kinesin involved in the anterograde movement of the IFT particle, specifically in distal segments of the cilia on sensory neurons (77). It is still uncertain as to whether a similar OSM-3-like kinesin exists in other higher eukaryotic organisms. Once the particle reaches the tip of the cilia axoneme, it undergoes a poorly understood transition, possibly mediated by mitotic spindle-associated proteins (58). The modification at the tip of the axoneme results in the inactivation of the kinesin and facilitates the retrograde return of the raft to the base of the cilium via a cytoplasmic dynein (17, 56, 57, 62, 66, 74). Because cilia and flagella are devoid of protein synthesis, IFT is thought to be the sole mechanism for transporting proteins required for cilia as-
assembly, maintenance, and function to their location in the axoneme.

Analysis of isolated IFT rafts in *Chlamydomonas* revealed that it consists of >17 different proteins. These proteins are organized into two associating complexes, complex A (550 kDa) and complex B (710 kDa) (12). Through biochemical, genetic, and comparative genomic studies, several proteins involved in IFT have been identified. In addition, our understanding of IFT has been enhanced greatly from the availability of a large number of mutants in *C. elegans* and *Chlamydomonas* that contain defects in cilia or flagella formation. Generally, mutations that affect complex A proteins exhibit shortened cilia with the accumulation of IFT particles along the axoneme. This is similar to the phenotype of mutants lacking the IFT dynein, such as CHE-3 in *C. elegans*, and it has led to the proposal that complex A is important in retrograde transport (64, 74, 76). Mutations in genes encoding complex B proteins generally result in truncated cilia but lack the accumulation of IFT particles along the axoneme that is typical of complex A or IFT dynein mutants. The cilia phenotype of complex B mutants is similar to that seen with organisms containing mutations in the IFT kinesin motor proteins, suggesting that complex B is required for anterograde movement along the ciliary axoneme (36, 55). These predictions are supported by analysis in *C. elegans* utilizing IFT::green fluorescent protein (GFP) fusion proteins (23). In mutants affecting complex A, the complex B GFP fusion proteins could be detected along the axoneme moving only in the anterograde direction. However, in complex B mutants, these fusion proteins are inefficient in entering the ciliary axoneme. Although the discovery of IFT as the transport mechanism in the cilia and identification of many of the IFT particle proteins are major advances, our understanding of how the particle assembles and what cargo IFT is transporting has yet to be fully understood.

**PRIMARY CILIA AND THEIR ROLE IN RENAL PATHOLOGY AND DISEASE**

While it is well accepted that motile cilia are of critical importance in human health, the presence of primary cilia on nearly every cell in the mammalian body had previously produced speculation that their role in mammalian homeostasis is of little consequence. This assertion has recently been challenged by the observation that several proteins associated with human cystic kidney disorders localize to the primary cilia (Fig. 2). These include polycystin-1 (PC-1) and polycystin-2 (PC-2), two proteins that are responsible for autosomal dominant polycystic kidney disease (ADPKD), and fibrocystin, a newly discovered protein that is involved in the autosomal recessive form of polycystic kidney disease (ARPKD) (84, 88, 92). While the role of fibrocystin within the cilia is still not well understood, it has been shown that fluid flow-mediated deflection of the cilia axoneme results in calcium mobilization that requires both polycystin proteins (Fig. 2) (47). These data have led to the conclusion that the primary cilia is a mechanosensor, measuring flow rates through the lumen of the collecting duct tubule. The physiological consequence of this calcium signal in the functioning of the renal epithelium remains to be uncovered.

In addition to calcium signaling, recent data have indicated that PC-1 is a component of a signaling pathway with similarities to that of the Notch pathway. In response to a lack of fluid flow or tubule obstruction, the COOH-terminal region of PC-1 undergoes regulated intramembrane proteolysis (RIP) (11). The COOH-terminal region of PC-1 translocates to the nucleus, where it regulates AP-1 activity (Ref. 11 and reviewed in Ref. 20). This finding opens the possibility that cilia are important in mechanosensory reception that results in gene transcription changes in response to proteolysis of PC-1; however, because PC-1 is localized to multiple sites in the cell, it remains to be determined whether this occurs in the cilia.

Intriguingly, the homologs of polycystin-1 (*lov-1*) and polycystin-2 (*pkd-2*) in *C. elegans* have also been localized to cilia of sensory neurons in males (2). Whereas the cilia in *lov-1* or *pkd-2* mutants appear normal, the mutants exhibit abnormal male mating behavior that is thought to be due to defects in a mechanosensory role of the cilia (2, 25). What is not known is whether these defects in mating behavior are associated with altered calcium signaling or PC-1 processing as proposed for fluid flow-mediated signaling in the mammalian collecting duct.

In addition to the proteins involved in PKD, proteins associated with cysts in non-PKD disorders such as nephrophosphthisis (nephrocystin-1, nephrocystin-2, and nephrocystin-4) and Bardet-Biedl syndrome (BBS 1–8) have also been reported to localize to the cilia and/or basal body (Fig. 2). Although the function of these proteins in the renal epithelium of mammals remains to be fully evaluated, in *C. elegans* the BBS proteins, BBS-7 and BBS-8, appear to be involved in regulating rates of IFT movement along the cilia axoneme, as defects in these genes result in abnormal ciliogenesis (7). In contrast, the cilia of *C. elegans* nephrocystin-1 and nephrocystin-4 mutants appear morphologically normal; however, these mutants display behavioral abnormalities typical of cilia-mediated sensory defects (86a, 87). These include suppressed male mating efficiency, defects in chemotaxis toward volatile attractants, and extended lifespan. Interestingly, in *C. elegans* the nephrocystin-1 and nephrocystin-4 proteins are not found in the cilia but rather at the transition zone (analogous to the mammalian basal body) at the base of the cilia. This further raises the possibility that both the primary renal cilium and basal body have important sensory functions that may be impaired in human nephrophosphthisis and cystic kidney disease patients.

Additional evidence supporting the direct involvement of cilia in the development of cystic kidneys has resulted from the characterization of a large number of cystic kidney disease mouse models (Table 1; see Ref. 19 for a recent review). Many of these models have now been shown to be associated with mutations in proteins residing in the cilia. One of the murine models that helped to uncover the connection between cilia and cystic kidney disease was the Oak Ridge Polycystic Kidney (*Tg737* ) mutant mouse. *Tg737* is a hypomorphic allele of the *Tg737* gene encoding the IFT protein Polaris (82). Analysis of the Polaris homolog in *Chlamydomonas* and *C. elegans* indicated that it is a complex B protein involved in anterograde movement of the IFT raft (23, 55). Due to defects in IFT, *Tg737* mice develop cystic lesions in the kidney that resemble the pathology seen in human ARPKD patients. Analysis of their renal epithelium reveals that the primary cilia are severely stunted (55, 82) and, furthermore, that the mutant cells possess an abrogated calcium signal in response to fluid flow.
<table>
<thead>
<tr>
<th>Mouse Gene of Interest (Protein)</th>
<th>Pathology Related to Disease</th>
<th>Protein Function</th>
<th>Basal Body and/or Cilia Localization</th>
<th>Human Disease Caused by Mutation</th>
<th>Chlamydamonas/C. elegans Homolog</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pkd1</strong> (polycystin-1)</td>
<td>Renal, biliary, and pancreatic cysts, cardiac conotruncal defects, hydrops fetalis, chondrocyte pathology (mouse)</td>
<td>Sensory organelle, G protein-coupled receptor, activator of transcription via regulated intramembrane proteolysis (COOH-terminal)</td>
<td>Basal body and cilia</td>
<td><strong>PKD1</strong></td>
<td>ADPKD</td>
</tr>
<tr>
<td><strong>Pkd2</strong> (polycystin-2)</td>
<td>Renal, biliary and pancreatic cysts, cardiac defects, situs inversus (mouse)</td>
<td>Transient receptor potential (TRP)-like nonselective cation channel permeable to Ca^{2+}; thought to be involved with mechanosensory and chemosensory pathways</td>
<td>Cilia</td>
<td><strong>PKD2</strong></td>
<td>ADPKD</td>
</tr>
<tr>
<td><strong>Pkdhd1</strong> (fibrocystin/ polyductin)</td>
<td>Renal and biliary cysts, biliary tract fibrosis, bile duct hyperplasia</td>
<td>Unknown; thought to be involved with the sensory aspects of determining bile and collecting duct size during terminal differentiation</td>
<td>Basal body and cilia</td>
<td><strong>PKHD1</strong></td>
<td>ARPKD</td>
</tr>
<tr>
<td><strong>NPHP1</strong> (nephrocystin)</td>
<td>Renal cysts primarily located at corticomedullary border, growth retardation, salt wasting, anemia, polyuria, tubulointerstitial inflammation, ocular motor apraxia (mouse)</td>
<td>Unknown; interacts with focal adhesion signaling complex elements and nephroretinin in mouse, chemosensory elements in C. elegans</td>
<td>Cilia</td>
<td><strong>NPHP1</strong></td>
<td>Type I nephronophthisis (juvenile form)</td>
</tr>
<tr>
<td><strong>INVS/NPHP2</strong> (Inversin)</td>
<td>Renal cysts primarily located at corticomedullary border, tubulointerstitial inflammation, situs inversus cardiovascular defects (mouse), hepatobiliary anomalies (mouse)</td>
<td>Unknown</td>
<td>Cilia</td>
<td><strong>NPHP2</strong></td>
<td>Type II nephronophthisis (infantile form)</td>
</tr>
<tr>
<td><strong>NPHP4</strong> (nephroretinin)</td>
<td>Renal cysts primarily located at corticomedullary border, growth retardation, salt wasting, anemia, polyuria, tubulointerstitial inflammation, retinitis pigmentosa, ocular motor apraxia (mouse)</td>
<td>Unknown; associates with nephrocystin; possibly involved in similar focal adhesion signaling pathway; chemosensory elements in C. elegans</td>
<td>Cilia</td>
<td><strong>NPHP4</strong></td>
<td>Type IV nephronophthisis (juvenile form)</td>
</tr>
<tr>
<td><strong>IQCB1/I/NPHP5</strong> (nephrocystin-5)</td>
<td>Renal cysts primarily located at corticomedullary border, tubulointerstitial inflammation, Leber congenital amaurosis</td>
<td>Unknown; interacts with retinitis pigmentosa GTPase regulator and calmodulin within the photoreceptor cilia</td>
<td>Cilia</td>
<td><strong>IQCB1</strong></td>
<td>Senior-Loken syndrome type 5</td>
</tr>
<tr>
<td><strong>OFD1</strong> (OFD1)</td>
<td>Renal cysts, malformations in the oral cavity, face, and digits, cognitive defects</td>
<td>Unknown</td>
<td>Basal body</td>
<td><strong>OFD1</strong></td>
<td>Orul-facial-digital syndrome type I</td>
</tr>
<tr>
<td><strong>Bbs1-8</strong> (BBS1-8)</td>
<td>Renal cysts, obesity, anemia, renal dystrophy, male infertility, social dominance disorders, learning disorders, cardiac malformations</td>
<td>Several BBS proteins may be related to regulation of IFT; BBS3: related to ARL6; a small G protein related to ADP ribosylation; BBS6: chaperonin activity</td>
<td>Basal body and cilia</td>
<td><strong>BBS1-8</strong></td>
<td>Bardet-Biedl syndrome</td>
</tr>
<tr>
<td><strong>TgN737Rpw</strong> (Polaris)</td>
<td>Mouse: renal cysts resembling ARPKD, hydrocephalus, growth retardation, polydactyly, acinar cell atrophy, inability to regulate blood glucose, hepatobiliary anomalies, situs inversus</td>
<td>Component of complex B in the IFT raft used to build and maintain cilia and flagella</td>
<td>Basal body and cilia</td>
<td><strong>Tg737</strong></td>
<td>Unknown</td>
</tr>
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</table>
Intriguingly, mutations in the \textit{Tg737} homolog in \textit{C. elegans} (\textit{osm-5}) result in the loss of cilia and produce a similar male mating behavior defect seen in both \textit{lov-1} and \textit{pkd-2} mutant males (69). These results support the idea that the polycystins require cilia for at least some of their activity.

Arguably, the most compelling data supporting a role for cilia in cyst development come from mice with mutations in \textit{Kif3a}. Mice lacking \textit{Kif3a} result in embryonic lethality; however, conditional disruption of the floxed allele of \textit{Kif3a} in the kidney resulted in viable offspring with cystic lesions in the distal nephron, specifically where cre recombinase was expressed (35, 38, 80). The cystic phenotype was directly correlated with the loss of cilia, whereas adjacent proximal tubules expressing normal cilia remained noncystic. Similar findings were obtained using morpholino antisense oligonucleotides to disrupt several of the IFT proteins in the pronephros of zebrafish (79). Collectively, these data indicate that there is an essential role for the cilia in normal renal physiology; however, whether this function is as a mechano- and/or chemosensor remains to be fully explored.

**PRIMARY CILIA AND THEIR ROLE IN EXTRARENAL PATHOLOGY: SENSORY INVOLVEMENT IN MANY TISSUES**

Human patients with cystic kidney disease and murine models often exhibit pathologies beyond the kidney now thought to result from ciliary abnormalities. In human PKD patients, this includes cysts in the pancreas and liver, intrahepatic biliary duct dilatation with marked fibrosis, and vascular defects (reviewed in Ref. 86). Nephronophthisis is associated with retinitis pigmentosa, diabetes insipidus, sensorineural hearing loss, and congenital hepatic fibrosis (6, 52, 75). In addition, human BBS patients possess a variety of symptoms that include diabetes, hypertension, asthma, retinal dystrophy, polydactyly, obesity, cardiac malformations, and learning disabilities (4, 14, 18, 31). With the knowledge that basal body- and cilia-localizing proteins are at least partially responsible for kidney pathology in these disorders, it suggests that this organelle will have critical functions in a large number of tissues that need further investigation.

Accordingly, an example where this is clearly demonstrated is in the complex extrarenal phenotypes observed in the \textit{Tg737} mutant. In addition to kidney cysts, \textit{Tg737} mutants develop biliary and bile duct hyperplasia in the liver, acinar cell atrophy and duct hyperplasia in the pancreas, defects in glucose homeostasis, hydrocephalus, retinal degeneration, and male infertility, as well as skeletal patterning defects that include polydactyly, cleft palate, and supernumerary teeth (10, 54, 93, 94). In contrast, complete loss of \textit{Tg737} function in the \textit{Tk737} mutant results in embryonic lethality with severe neural tube closure and patterning defects, extensive polydactyly, and random determination of the left-right body axis (45). Thus the generation of multiple \textit{Tg737} alleles in the mouse is uncovering novel research arenas that will need to be further explored to evaluate the relationship among cilia, development, and disease.
PRIMARY CILIA AND CHOLANGIOCYTES: A ROLE FOR CILIA IN THE LIVER

Apart from the kidney, the liver is the most commonly affected organ in both human ADPKD and ARPKD. Symptoms tend to worsen with age, as the presence of cysts within the liver increases from 20% of patients in their third decade to 70% in their seventh decade (65). In addition, liver dysfunction is a primary cause of morbidity in ARPKD patients that survive pulmonary and renal complications in their first 2 yr of life (91). These patients often develop extensive hepatic fibrosis characteristic of Caroli’s disease, which also is characterized by intrahepatic biliary duct dilatations and liver cysts (73, 81). In addition, several murine models of PKD that are related to mutations in cilia-localizing proteins, such as cpk, orpk, and inv mice and the pck rat, all display distinct liver pathology (see Ref. 19 for a detailed description of the murine models). Until recently, the role of the immotile cilia on cholangiocytes of the bile ducts remained obscure. New insights into the function of the cholangiocyte cilia have been generated from studies of the liver pathology in the pck rat. The pck rat has been shown to contain a mutation in Pkhd1, the homolog of the gene responsible for human ARPKD (32, 34, 40, 73). Similar to human ARPKD, pck rats develop congenital hepatic fibrosis in addition to liver cysts (34, 40). Even in noncystic regions, the volume of the entire biliary tree of the PCK liver was expanded and there was marked proliferation of cholangiocytes (39). Intriguingly, the cilia on mutant pck cholangiocytes were found to be significantly shorter and malformed, often with aberrant bulbs extending from the ciliary axoneme. The liver phenotypes of the cpk, inv, and Tg737opk mouse models of PKD suggest that a similar mechanism is involved in these mutants; however, more detailed analysis is needed to evaluate this hypothesis (24, 41, 71, 89).

CILIUM AND THE PANCREAS: IMPORTANT COMPONENTS IN ENDOCRINE AND EXOCRINE FUNCTION?

Another tissue that is severely affected in the mouse models of PKD is the pancreas; however, pancreatic defects are not a prominent feature of PKD in humans. Around 10% of ADPKD patients develop pancreatic cysts, whereas rare cases of pancreatic fibrosis have been identified in ARPKD patients (15, 27, 30, 48, 83). The importance of cilia in pancreatic function is supported by studies in Tg737opk mice. In these mutants, their pancreatic ducts start to dilate and/or form cysts shortly after birth, whereas the acini undergo massive cell death (10, 93). Intriguingly, even though the acini are severely disturbed, cilia and Tg737 expression are detected only within islets and ductal epithelial cells. The cilia in the pancreatic ducts and islets possess the distinct 9+0 microtubule pattern characteristic of primary cilia, suggesting that they are immotile and unable to direct fluid flow through mechanical means. Therefore, it is possible that these cilia function as chemo- or mechanosensors, as proposed in the liver and kidney. The current hypothesis is that the cilia are required for sensing or regulating ion transport and fluid flow and that the loss of this organelle results in abrogated fluid secretion into the duct lumen (93). The paucity of fluid could be responsible for premature enzyme activation and autodigestion of the exocrine component of the pancreas, similar to what occurs during pancreatic duct obstruction or in the pancreatic phenotype of the cystic fibrosis mouse model. Other hypotheses have suggested that cilia function might be involved in sensing luminal pressure within the duct, because it has been noted that their length increases dramatically with luminal pressure induced by duct obstruction (93).

While the morphology of the endocrine tissue in Tg737opk mutants appeared overtly normal, these animals were shown to have idiosyncrasies in regard to their glucose homeostasis. Tg737opk mutants were hypoglycemic after short-term fasting and had marked delay in their return to baseline blood glucose levels following glucose challenge (93). The mechanism behind the glucose homeostasis defects is still being evaluated; however, this analysis is hindered by the fact that other tissues also display severe pathology that may influence glucose utilization, and mutants on the FVB/N background experience early lethality that hinders the ability to test glucose tolerance beyond the first few weeks. Subsequent studies will require the analysis of mice with conditional mutations in IFT genes to specifically disrupt islet cilia.

THE CILIUM WITHIN THE RETINA: FUNCTIONS IN PHOTORECEPTION

The retina is one of the most common tissues involved in non-PKD forms of cystic kidney disorders in both human patients and murine models. Pathological changes relating to the retina, including retinal degeneration and retinitis pigmentosa, have been reported in patients with Bardet-Biedl syndrome and nephronophthisis (9, 75). Retinal disease is also present in several of the murine models of PKD, most notably Kif3a and Tg737opk (37, 54). Analysis of the connection in these models demonstrates that IFT plays an important role in initially forming and maintaining photoreceptors in both rods and cones. Previous work has shown that the outer segment (OS) of the photoreceptor is a highly modified primary cilium that is continually extruded and regenerated. The OS is connected to the inner segment (IS) by a connecting cilium that emerges directly from a basal body (72). Disruption of IFT in the Kif3a conditional mutants and Tg737opk mice results in an accumulation of opsin within the IS, followed shortly by degeneration of the OS (37, 54). Thus IFT is thought to mediate the transport of proteins from the IS to the OS of the photoreceptor and to be required for regeneration of the membrane disks in the OS of the photoreceptor.

Similar to the IFT mutants, BBS2−/− and BBS4−/− mice both display retinal degeneration, and recent data indicate that BBS2−/− mice exhibit an accumulation of mislocalized rhodopsin (46, 49). Some insight into the possible mechanism involved in retinal degeneration in human BBS patients has come from analysis of two C. elegans homologs of the BBS proteins. These studies demonstrate that loss of bbs-7 or bbs-8 impacts the rate of IFT movement along the axoneme of sensory cilia (7). Thus the photoreceptor defects in human BBS patients and certain BBS murine models may be due to impaired IFT movement along the connecting cilium. The connection between cilia and the retinal pathology observed in nephronophthisis patients is less clear. Unlike the IFT or BBS mutants, the analyses of the nephronophthisis homologs (nephrocystin-1 and nephrocystin-4) in C. elegans have shown no significant cilia morphological defects, although changes in sensory function have been noted (86a, 87). It will be interest-
ing to determine whether these proteins are playing a similar sensory role in mammals and how their disruption leads to retinitis pigmentosa.

CRITICAL ROLES FOR CILIA IN DEVELOPMENT

In addition to their involvement with disease pathology, defects in cilia function have been associated with developmental abnormalities. One of the first indications that cilia may play a part in development came from human patients with Kartagener’s syndrome. Due to loss of ciliary motility, Kartagener’s patients develop recurrent respiratory infections, sinusitis, male infertility, inner ear infections, and situs inversus, where the visceral organs are reversed across the left-right body axis (1, 16). Recent discoveries have indicated that the causes of Kartagener’s syndrome are mutations in the dynein intermediate and heavy chains within the ciliary axoneme (3, 21, 53, 59). This led Afzelius (1) to propose that motile cilia in the early embryo acted to position the heart in its correct location required for normal situs.

Insights into the connection between situs determination and cilia have been obtained from analysis of mouse mutants that disrupt cilia structure (Tg737, Kif3a, and Kif3b) or function (Pkd2). Mice homozygous for mutations in the IFT motor kinesin II complex (Kif3a or Kif3b) or the complex B protein Polaris suffer midgestation lethality (38, 45, 51). These embryos are characterized by pericardial sac ballooning, neural tube patterning and closure abnormalities, and randomized left-to-right asymmetry. The randomization of situs in these animals is attributed to the loss of cilia on cells of the ventral surface of the embryonic node, a gastrulation stage-organizing center involved in asymmetric expression of genes such as lefty1, lefty2, nodal, and pitx2 required for normal axis specification (13, 43, 44, 90). Ultrastructural analysis of nodal cilia indicates that they contain 9+0 microtubule architecture and thus were originally thought to be immotile. However, subsequent characterization of the cilia found that their motion is rotational, driving leftward-directed fluid flow over the node surface (50, 51). These findings led to the “nodal flow” hy-

![Fig. 3. Mutations in Tg737 and IFT172 have revealed an unexpected role for IFT in the hedgehog signaling pathway. In the absence of the ligand (left), Patched (Ptc) functions to inhibit Smoothened (Smo) signaling and results in the proteolytic processing of Gli (Ci in Drosophila) to a repressor (GliR) form. GliR translocates to the nucleus and represses transcription of downstream targets, such as Gli1 and Ptc. In the presence of ligand (Shh, right), Ptc repression of Smo is alleviated, unprocessed forms of Gli (GliA) transcription factors accumulate, and this leads to induction of target genes. Suppressor of fused (Sufu) has been shown to interact with the Gli proteins and negatively regulate their activity. While both Costal2 (Cos2) and Fused (Fu) are required for hedgehog pathway regulation in Drosophila, their importance in the mammalian hedgehog pathway is unclear. Whether there is a direct role for cilia in this pathway (i.e., localization of Patched, Smoothened, Sufu, or Gli proteins in the axoneme) or a cytosolic function for the IFT proteins (i.e., transport of Gli proteins to the nucleus or to a site of processing) remains to be determined; however, genetic studies have indicated that IFT function is required in the hedgehog-responding cell downstream from Ptc and Smo but upstream of the direct targets of the pathway.](http://ajprenal.physiology.org/)

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hothesis where rotation of the cilia and subsequent fluid flow result in transport of morphogens toward the left side of the node, where they bind receptors and trigger asymmetric gene expression that determine the left-to-right axis (51). However, recently it was demonstrated that the node has two forms of cilia: motile cilia on cells found in the central region of the node and immotile cilia on cells in the periphery. A newly proposed hypothesis of nodal flow predicts that the axoneme of the immotile cilia in the peripheral node bends in response to fluid flow, eliciting a calcium signal on the left side of the node (47, 60, 68). This model is supported by that fact that the calcium signal is not observed on the left side of the node in conditions where the nodal cilia are paralyzed, such as in the left-right dynein (lrD) mouse mutant (42). Intriguingly, the calcium signal requires polycystin-2, the cilia protein involved in human PKD that is necessary for the fluid flow response in the kidney (42, 60). These findings help explain why loss of Pkd2 in mice results in situs defects even though nodal cilia appear morphologically normal and retain normal rotational beating.

In later stages of development, cilia function also appears to have an essential role for normal patterning of tissues. This is evident in IFT mouse mutants such as Tg737Δ2–3pdk1, IFT172(wmple), and Kif3a, which exhibit abnormalities in both neural tube development and limb bud formation (26). The neural tube defects are characterized by the loss of the floor plate and abnormalities in dorsal ventral patterning. Analysis of these murine models suggests that the developmental defects in the IFT mutants are associated with the abnormalities in the hedgehog (Hh) signaling pathway that was first described in Drosophila (22, 70). In contrast to the data in mice, IFT mutations in Drosophila do not disrupt the Hh pathway. This suggests that IFT may have been commandeered by higher eukaryotic organisms to function in this pathway. It has been shown that Hh signals through the Patched (pIcch) receptor to cause the derepression of Smoothened (Smo), leading to the modification of a large microtubule-associated complex consisting of Suppressor of Fused (SuFu), Costal-2 (Cos2), Cubitus interruptus (Ci), and Fused (Fu) (Fig. 3; see Ref. 28 for an in-depth review of the pathway). As a result, Ci accumulates in the nucleus and induces transcription of Hh target genes, one of which is the inhibitor of the Patched pathway. Ci can also act as a transcriptional repressor depending on its proteolytic cleavage state, an event that is inhibited by Hh signaling. A similar but more complex pathway has been described in mammals, where there are three Hedgehog homologs (Shh, Ihh, Dhh), two Patched homologs, Smoothened, and three Ci homologs (Gli1, Gli2, and Gli3), whereas several components identified as essential in Drosophila, such as Cos2 and the kinase activity of Fused, appear to be dispensable for the mammalian pathway. While the immediate steps following Smo activation in mammals remain unclear, the consequences are similar to that in Drosophila, where the Gli proteins are activated and, in the case of Gli2 and Gli3, processing to the repressor form is inhibited. The role that IFT and possibly cilia play in this pathway is still being investigated. However, recent data indicate that the loss of Tg737 results in abnormal processing and activity of Gli2 and Gli3. Whether these data reflect a role for cilia in Hh signaling, or an unidentified cytosolic role for the IFT proteins in the Hh pathway, has yet to be determined.

PHYSIOLOGY AND THE PRIMARY CILIAl A LOOK AT THE NEXT DECADE OF DISCOVERY

Although research over the past decade has provided us with a significant amount of data regarding cilia and the components required for their assembly, defining the physiological roles of this organelle has been extremely challenging. Although it has been well established for decades that motile cilia are important in cell and fluid movement, and loss of this organelle leads to pathology related to several human diseases, the significance of primary and nodal cilia has only recently been demonstrated. Much of this has been obtained from research conducted utilizing model organisms such as Chlamydomonas and C. elegans, where investigators made the surprising observation that proteins involved in mammalian cystic kidney disease are also required for cilia assembly and sensory activity in lower eukaryotes. These original findings have resulted in an explosion of research activity aimed at uncovering the importance of cilia in multiple tissues as well as in mammalian development. Studies of the complex phenotypes in Tg737Δpdk2 and Kif3a mutant mice now have strongly established that the primary cilium is not just a vestigial remnant carried over from our evolutionary past. Instead, the cilium is an organelle with chemos-, photo-, and mechanosensory functions in multiple tissues of the body that also play important roles in normal mammalian development. Despite the impressive amount of progress made over the past decade, we are left with even more challenging and critical questions. These questions include how extracellular stimuli perceived by the cilium result in changes in cell behavior and physiology and how functional defects in this organelle result in such a wide spectrum of pathologies, including cystic kidneys, hydrocephaly, ducatal abnormalities in the liver and pancreas, embryonic and skeletal patterning abnormalities, and modern day epidemic diseases such as obesity, hypertension, and diabetes.

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