Primary Cilia and Signaling Pathways in Mammalian Development, Health and Disease

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Key Words
Primary cilia • Cellular GPS • Signal transduction • Development • Tissue homeostasis • Ciliopathies

Abstract
Although first described as early as 1898 and long considered a vestigial organelle of little functional importance, the primary cilium has become one of the hottest research topics in modern cell biology and physiology. Primary cilia are nonmotile sensory organelles present in a single copy on the surface of most growth-arrested or differentiated mammalian cells, and defects in their assembly or function are tightly coupled to many developmental defects, diseases and disorders. In normal tissues, the primary cilium coordinates a series of signal transduction pathways, including Hedgehog, Wnt, PDGFRα and integrin signaling. In the kidney, the primary cilium may function as a mechano-, chemo- and osmosensing unit that probes the extracellular environment and transmits signals to the cell via, e.g., polycystins, which depend on ciliary localization for appropriate function. Indeed, hypomorphic mutations in the mouse ift88 (previously called Tg737) gene, which encodes a ciliogenic intraflagellar transport protein, result in malformation of primary cilia, and in the collecting ducts of kidney tubules this is accompanied by development of autosomal recessive polycystic kidney disease (PKD). While PKD was one of the first diseases to be linked to dysfunctional primary cilia, defects in this organelle have subsequently been associated with many other phenotypes, including cancer, obesity, diabetes as well as a number of developmental defects. Collectively, these disorders of the cilium are now referred to as the ciliopathies. In this review, we provide a brief overview of the structure and function of primary cilia and some of their roles in coordinating signal transduction pathways in mammalian development, health and disease.

Structure and Diversity of Cilia

Cilia and flagella (the terms are equivalent) are antenna-like organelles that emanate from the surface of many growth-arrested or differentiated eukaryotic cells. They consist of a microtubule (MT)-based axoneme ensheathed by a bilayer lipid membrane that is continuous with the plasma membrane of the cell body, but contains a distinct subset of receptors and other proteins involved

This review is based on an invited lecture given during the Takis Anagnostopoulos Symposium on Renal and Epithelial Physiology and Physiopathology at Faculté de Médecine Necker in Paris, June 26–27, 2008.
in signaling. The axoneme grows out from the distal end of a modified centriole, the basal body, which provides a template for the formation of the 9-fold symmetry of the ciliary axoneme, and also serves to anchor the axoneme in the cell. Separating the ciliary and plasma membrane compartments is a region known as the ciliary necklace [1]. The ciliary necklace is connected via fibers to the transition zone of the basal body, and these fibers are thought to be part of a ‘ciliary pore complex’ through which only selected proteins are allowed to enter the ciliary compartment [2].

In general, cilia are classified as motile (9 + 2) or nonmotile (9 + 0), where ‘9’ refers to the number of outer doublet MTs present in the ciliary axoneme and ‘2’ or ‘0’ refers to the number of central MTs present. Motility requires the presence of axoneme-associated dynein arms to generate power, and for most motile cilia additional accessory structures, e.g. radial spokes and central pair projections, are involved in regulating dynein-mediated motility [3]. Some motile cilia, however, contain an extra central pair or lack the central pair entirely. For example, cilia with 9 + 4, 9 + 2, and 9 + 0 axonemes have been observed on the notochordal plate of rabbit embryos and these axonemes all contain dynein arms, indicating that they are motile [4]. Consistent with this, 9 + 0 monocilia on the embryonic mouse node, as well as 9 + 0 cilia on Kupffer’s vesicle in medaka fish, were observed to perform rotational beating in a manner that generates a directional flow across the cell surface, which is required for establishment of the left-right axis [5–7].

In mammals, numerous motile 9 + 2 cilia are present on the surface epithelial cells lining the airways, brain ventricles, and oviducts. The main function of these 9 + 2 cilia is to promote the movement of fluids or substances, e.g. airway surface liquid, cerebrospinal fluid, or egg cells, across the epithelial surface; failure to do so may result in airway disease, hydrocephalus, or sterility [8]. A single motile flagellum is present on the mammalian sperm cell, whereas the green alga *Chlamydomonas*, a commonly used model organism for studying ciliary assembly and function, contains two motile flagella that propel the cell towards or away from a light source [8, 9]. In addition to their motile functions, 9 + 2 cilia also have important sensory functions, which may in part play a role in regulating motility [3, 10]. However, in terms of ciliary-mediated signaling, it is the nonmotile 9 + 0 cilia that have attracted the most attention in recent years.

Nonmotile 9 + 0 cilia, also known as primary cilia, are present on most cells in the mammalian body (see http://www.bowserlab.org/primarycilium/ciliumpage2.htm for a comprehensive list of cells known to possess primary cilia). Like motile 9 + 2 cilia, the axoneme of 9 + 0 cilia consists of 9 outer doublet MTs that are nucleated by the basal body, but the central MT pair and structures involved in motility (e.g. dynein arms, radial spokes) are lacking. The primary ciliary membrane is enriched for a number of receptors and ion channels, including platelet-derived growth factor receptor-α (PDGFRα), somatostatin receptor 3, serotonin receptor 5, melanin-concentrating hormone receptor 1, polycystins 1 and 2, as well as components of the Hedgehog (Hh) and Wnt signaling pathways [10]. Therefore, the primary cilium is considered to function mainly as a sensory organelle ([11]; see also below). Some of the best examples of primary cilia that act as sensory organelles are the sensory cilia present in vertebrate olfactory organs and the outer segments of vertebrate photoreceptors. The latter are initially formed from primary cilia during development of the eye and remain connected to the inner segment in adult retina by a short ‘connecting cilium’ that is functionally and structurally equivalent to the transition zone of other types of cilia. The outer segment of photoreceptors turn over at a high rate and therefore large quantities of photo transduction proteins are continuously being transported from the inner to the outer segment, mainly via a process known as intraflagellar transport (IFT), which will be described in more detail below. Defects in IFT impair transport of photo transduction proteins from the inner to the outer segment and lead to degeneration of the outer segments, ultimately leading to blindness [12]. Likewise, the dysfunction of the cilium on olfactory neurons leads to anosmia and results in their degeneration [13].

In addition to differentiated cells of olfactory and visual organs, cells of many other organs and tissues in our body (e.g. kidney, liver, pancreas, brain, and oviduct) also display 9 + 0 primary cilia on their surface when the cells are in growth arrest. While these primary cilia are thought to serve as sensory ‘antennae’ that detect and transmit signals from the surrounding environment to the cell body in order to regulate embryonic development and tissue homeostasis in the adult [10, 11], there is a growing body of evidence suggesting that primary cilia also play a crucial role in cell cycle control. Since the primary cilium is subtended by the basal body, which is equivalent to one of the mitotic centrioles of the centrosome, a prerequisite for cell cycle entry is disassembly of the primary cilium, a tightly regulated and still not well understood process that appears to involve mitotic kinases such as Aurora and NIMA-related kinases [11, 14, 15]. Consistent with a role for primary cilia in growth
control, defective primary cilia were hypothesized to be associated with cancers resulting from abnormal mitogenic signaling or von Hippel-Lindau tumor suppressor signaling [16, 17].

Assembly of the Primary Cilium

Assembly of the primary cilium begins in G1 when Golgi-derived (primary) vesicles attach to the distal end of the older (mother) centriole of the centrosome. As ciliogenesis progresses, axonemal subunits are added directly onto the distal end of the mother centriole, and additional vesicles fuse with the primary vesicles eventually forming a membrane sheath around the nascent ciliary axoneme. In addition, the mother centriole acquires accessory structures and appendages that promote docking and attachment of the mother centriole to the apical plasma membrane of the cell [14, 18–20]. Following docking of the mother centriole to the apical membrane, the axoneme continues to elongate within the membrane-enclosed compartment by addition of axonemal subunits to the distal end of the growing ciliary axoneme. Because the ciliary compartment lacks the capacity for de novo protein synthesis, axonemal assembly depends on transport of ciliary precursors from the base of the cilium to its distal tip. This transport is carried out by IFT, which is essential for the assembly and maintenance of almost all eukaryotic cilia and flagella [2].

IFT is a highly conserved process initially discovered in Chlamydomonas as a bidirectional movement of groups of large protein complexes (IFT particles) along the ciliary axoneme [21]. Movement in the anterograde (base to tip) direction is mediated via kinesin-2 motors (Kif3a/Kif3b/KAP complex in vertebrates), whereas movement in the retrograde (tip to base) direction is mediated via cytoplasmic dynein 2 [2, 22–24]. These motors attach to the IFT particles, which in turn are thought to be associated with axonemal cargo proteins entering and leaving the cilium [25]. The IFT particles and motors as well as their cargo proteins accumulate near the site where transition fibers contact the ciliary membrane at the base of the cilium prior to entry into the ciliary compartment, and kinesin-2 then transports IFT particles, cargo proteins, and inactive cytoplasmic dynein 2 to the ciliary tip. At the tip, the IFT particles are remodeled, cargo is presumably unloaded, and kinesin-2 becomes inactive while cytoplasmic dynein 2 becomes active and transports the IFT particles and ciliary turnover products back to the cell body for recycling [2, 22, 23, 26]. The mechanisms that regulate IFT at the ciliary base and tip are not well understood, although some key proteins involved, e.g. MAP kinases and IFT172, have been identified [23, 24].

The IFT particles are composed of approximately 16 different polypeptides, which in Chlamydomonas can be separated biochemically into two distinct complexes termed complex A and B [27]. Almost all of the genes encoding IFT particle polypeptides have been cloned and sequenced, and bioinformatic analyses of IFT polypeptide sequences have revealed that many of them contain motifs and domains known to be involved in transient protein-protein interactions, similar to components of coat protein I and clathrin-coated vesicles [28, 29]. Functional studies of individual IFT particle proteins in diverse ciliated organisms have confirmed a requirement for these proteins in ciliary assembly, and have further indicated that components of IFT complex B are associated with anterograde IFT while components of IFT complex A primarily function during retrograde IFT [23, 26, 28]. For example, inactivation of the complex B polypeptide IFT88/Polaris, which is encoded by the ift88 (previously called Tg737) gene, impairs primary cilium formation in the mouse, presumably because ciliary building blocks fail to enter the ciliary compartment via anterograde IFT [30]. In contrast, inactivation of the complex A polypeptide IFT139/THM1 in the mouse leads to the formation of stunted, bulbous cilia, presumably due to defective retrograde IFT resulting in accumulation of IFT particles within the cilium [31].

Because of their essential role in building the primary cilium, IFT proteins are required for appropriate functioning of a variety of cilium-mediated signaling pathways such as Hh [32] and PDGFRα [33] signaling. However, there is growing evidence that IFT also plays a more direct role in signaling, both in Chlamydomonas and in vertebrates, although the exact mechanisms involved are still somewhat obscure [34, 35].

Introduction to Primary Cilia and Ciliopathies

The finding that the gene whose function was disrupted in the Oak Ridge Polycystic Kidney mouse (ORPK mouse, ift88<sup>orpk</sup>, or ift88<sup>G737N</sup>) encodes an ortholog of Chlamydomonas IFT88 was the eye opener for many cell and developmental biologists. The IFT88 mutant mouse revealed primary cilium function as important sensory devices that probe the extracellular environment and send on information to the cell body in order to control developmental processes and tissue homeostasis. Mutations in
ifth88 in mice were known to cause a series of developmental defects [36], including cystic kidney disease, but the function of the gene was mysterious until its involvement in IFT and ciliary assembly was demonstrated. Thus in 2000, Pazour et al. [30] first showed that Chlamydomonas IFT88 is an ortholog of the previously characterized mouse Tg737 gene and demonstrated a role for IFT88 in flagellar assembly. Consistent with these findings it was shown that primary cilia in the renal tubules [30] and cilia of the node [37] of tg737/ift88 mutant mice were abnormally short or missing, which suggested that PKD and defects in the establishment of left-right asymmetry during embryonic development could be ciliary diseases. Another key finding that further substantiated the link between cilia and PKD was the observation that GFP-tagged versions of the Caenorhabditis elegans orthologs of mammalian polycystin 1 (PKD1) and polycystin 2 (PKD2) localize to ciliated endings of male sensory neurons [38]. This prompted other investigators to reexamine the subcellular localization of mammalian PKD1 and PKD2, leading to the demonstration that both of these proteins indeed localize to primary cilia in cultured human and mouse kidney cells [39, 40]. While Chlamydomonas, C. elegans, and mice were crucial in establishing the first link between primary cilia and PKD, additional model systems have subsequently proven to be of great benefit for investigation of ciliary assembly, ciliopathies, and ciliary signaling. These other important model organisms include Tetrahymena, Drosophila, trypanosomes and zebrafish, which are easy to manipulate and analyze in the laboratory. Zebrafish has become an excellent genetic tool in the study of ciliary genes involved in vertebrate embryogenesis and human disease since its transparent embryo develops outside the mother’s body and it has many of the same organ systems that are affected in human ciliopathies. Collectively, studies on several model organisms as well as cultures and tissues of mouse and human origin have not only provided important insight into the molecular mechanisms of PKD, but have also revealed a link between primary cilia and a growing list of other diseases and syndromes, including nephronophthisis (NPHP) and Bardet-Biedl, orofacial-digital type 1, Meckel-Gruber, and von Hippel-Lindau syndromes (fig. 1). Doubtless, many more ciliopathies will be added to this list, since limited information is available on primary cilia in many organs and tissues during development and in the adult.

Great attention has been given to the nephronal disorders arising from ciliary dysfunction such as PKD, due to which a connection between ciliary malfunctions and aberrant tissue homeostasis was first hypothesized [30, 40, 41]. Both the autosomal dominant (ADPKD) and recessive (ARPKD) variants of PKD are characterized by the formation of large fluid-filled cysts and greatly enlarged kidneys that are associated with increased proliferation late in the disease process [42]. ADPKD is mainly due to mutations in the PKD1 and PKD2 genes, whereas ARPKD is caused by mutations in the gene encoding fibrocystin [42]. Another complex of recessive cystic kidney diseases is NPHP, resulting from defects in the genes encoding nephrocystin (Nphp) 1–9, and being the main genetic cause of end-stage renal failure within the first three decades of life. As opposed to PKD, tubular cyst

**Fig. 1.** Overview of primary cilia in signaling pathways and ciliopathies. a Scanning electron microscopy of a primary cilium (arrow) emanating from the surface of an hESC. The inset shows a cross-section of the hESC cilium with a 9 + 0 microtubule ultrastructure analyzed by transmission electron microscopy (TEM). Reprinted from Kiprilov et al. [43] with permission from J.C.B. 

b TEM analysis of the structural relationship between the primary ciliary axoneme (Ax), the distal (Dc) and proximal (Pc) centrioles and the Golgi apparatus (G) in a chicken chondrocyte. Reprinted from Jensen et al. [47] with permission from Elsevier. 

**c** Immunofluorescence microscopy analysis of primary cilia [anti-acetylated α-tubulin (tb), red, arrows] in cultures of growth-arrested NIH3T3 cells. The nucleus was stained with DAPI (blue).

**Inset:** An NIH3T3 primary cilium costained with anti-centrin-2 (green) that marks the two centrioles (asterisks). d List of various signal transduction systems being coordinated by the primary cilium.

e Gross morphology (left panels) and histological sections (right panels) of a normal (top panels) and an ifth88<sup>em210k</sup> cystic kidney mutant (bottom panels) at 3 weeks of age. Tissue sections (right panels) were stained with haematoxylin and eosin. f List of proposed ciliopathy phenotypes and human syndromes caused by defects in assembly or function of primary cilia in mammals.

g Schematic overview of signal transduction systems being coordinated by the primary cilium and the centrosome in regulation of cell proliferation, survival, polarity, migration and differentiation. Activation of transmembrane receptors [e.g. by interaction with ECM, by binding to soluble ligands such as PDGF-AA (see also fig. 2a) and morphogens, or due to mechanostimulation (see also fig. 2c)] (1) leads to activation of effector molecules in the cilium (2) or at the centrosome (3) followed by activation of specific transcription factors for de novo gene expression (4). Effector molecules may also activate downstream components in signal transduction independent of the nucleus in regulation of cellular processes. As part of this, there is a continuous turnover of transmembrane receptors in the cilium, partly regulated by effector molecules, that controls the trafficking of signaling components into and out of cilium (see also fig. 2b) (5), such as the concerted movement of Ptc out of and Smo into the cilium in response to Hh stimulation. PM = Plasma membrane.
formation in NPHP does not result in enlarged kidneys, but phenotypic characteristics include degradation of tubular basement membranes, tubular collapse, and interstitial fibrosis [43–45]. The cystic kidney phenotype in NPHP is frequently combined with other defects such as cerebellar hypoplasia and ataxia in Joubert syndrome, or retinitis pigmentosa in Senior-Løken syndrome [43–45]. Interestingly, the phenotype in NPHP-2 patients with mutations in the INVS gene that encodes inversin has an earlier onset than the other NPHP types, and combines the phenotypic characteristics of NPHP, such as renal interstitial and glomerular fibrosis and tubular cysts, with features of ADPKD, including enlargement of the kidneys due to cysts outside of the medullary region [46]. Moreover, microarrays and immunoblotting analysis of kidneys from mice containing a homozygous carboxy-terminal deletion of invs showed increased proliferation and cell cycle progression as compared to wild-type mice [47].

Many proteins whose functions are disturbed in cystic diseases have been localized to the cilium or the ciliary basal body, where they might contribute to regulating kidney development and function. Included herein are the polycystins [39, 40], the nephrocystins [46, 48–57], fibrocystin [58], and proteins regulating Wnt signaling and planar cell polarity [59–62], which in different ways coordi-
nate a series of signal transduction pathways in the kidney. It was proposed that PKD1 and PKD2 form a protein complex in the primary cilium to function as a mechanosensor to elicit a calcium signal in response to fluid movement through the renal tubules [63] (fig. 2), where loss of cilia or mutations in the polycystins lead to cyst formation. In line with this, earlier studies showed that bending of primary cilia in cultures of renal epithelial duct cells by fluid shear or mechanical stimulation causes intracellular Ca²⁺ to increase [64–66]. This suggests that ciliary mechanotransduction is important for normal function of the kidney epithelium and that loss of the cilium leads to PKD.

More recent studies in mice have revealed that the rate of cyst formation and cystic disease severity are dependent on the time point when cilia or polycystin function is disrupted [67, 68]. Using conditional alleles of ift88, kif3a (component of the heterotrimeric IFT kinesin-2 motor complex), or PKD1, and inducible Cre deleter mouse lines, it was shown that disruption in early postnatal life (P1–P12) results in rapid cyst formation within 3 weeks of loss of the gene. In contrast, if cilia or polycystin-1 function is disrupted after P12, cyst formation requires 6 months to a year to form. Another surprising finding from these studies was that there was no marked increase in proliferation rates between the mutant and

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**Fig. 2.** Simplistic models of different signaling systems coordinated by the primary cilium. **a** Ciliary PDGFRαα signaling. Upon growth arrest expression of PDGFRαα is up-regulated and the receptor is targeted to the primary cilium. Stimulation with PDGF-AA leads to PDGFRαα homodimerization in the cilium followed by activation of PI3K/Akt and Mek1/2-Erk1/2 pathways, leading to cell migration and transcriptional control of cell cycle entry. **b** Ciliary Hh signaling. Binding of Hh ligands to Ptc-1 in the primary cilium results in translocation of Ptc-1 out of the cilium and targeting of Smo into the cilium. This favors the formation of active forms of Gli transcription factors which may occur in the cilium followed by translocation of the active Gli forms to the nucleus for activation of the Hh response. **c** Bending of the primary cilium by fluid flow leads to activation of the PKD1/2 complex in the cilium and influx of Ca²⁺ into the cilium followed by activation of Ca²⁺ channels in endoplasmic reticulum (ER). Fibrocystin may assist PKD2 function. In the absence of ciliary bending, PKD1 is proteolytically cleaved and the C-terminus translocates to the nucleus in a protein complex with p100 and STAT6 to activate transcriptional activity in cystic diseases. Please see text for references.
control kidneys, even in the cystic animals where function was disrupted in perinatal periods. Together these data indicate that there is a critical time point at which cilium dysfunction causes a rapid- or slow-progressing cystogenic phenotype and raised concern about the simple pathogenic model whereby loss of cilium-mediated mechanosensation is the cause of cyst formation. In further studies, it was revealed that this switch point might be associated with completion of renal differentiation, which occurs at about 2 weeks of age in mice, changes in the proliferative environment, and a large change in the gene expression profile that occurs at around P12.

The importance of having a proliferative environment for cyst formation in the cilium mutants was analyzed by inducing cilium loss in adult mice followed by renal injury through obstruction or ischemic reperfusion. This injury reinitiates proliferation in the adult kidney as part of the repair process and was found to result in rapid cyst formation similar to that seen in the perinatally induced cilium mutants. Cysts were not present in the contralateral noninjured cilium mutant kidney. A possible mechanism connecting ciliary dysfunction, cell proliferation, and cyst formation was further defined by studies by Fischer et al. [69]. They showed that mitotic spindles in the perinatal kidney normally align along the axis of the nephron such that cell divisions increase nephron length. In contrast, in cystic disease mouse and rat models the orientation of cells divisions occurs randomly, with many resulting in expansion of the diameter of the nephron. Deletion of the ciliary protocadherin and planar cell polarity (PCP) protein Fat4 was recently shown to elicit a similar disruption of spindle orientation during renal tubular elongation in mice [61]. Thus, these data suggest that cilia or the basal body are needed for normal orientation of the spindle. Whether this is in response to fluid flow and the polycystin-generated calcium signal, and how this is coupled to PCP has yet to be fully explained.

**Signaling Pathways Coordinated by the Primary Cilium**

In normal tissues, the primary cilium coordinates a series of signal transduction pathways, including the Hh, Wnt, and PDGFβRs pathways, and also functions as a phototransductance unit that probes and relays information from the extracellular environment into the cell. In many cases, proper signaling is tightly coupled to the correct translocation of receptors and downstream effector molecules involved in signal transduction to the cilium. We still know very little about the mechanisms that control trafficking of signal components into and out of the cilium, although IFT is likely to be involved in several aspects of receptor trafficking [23, 34, 35]. Further, a single primary cilium may contain many different signal transduction systems in order to carry out diverse signaling processes in tissue development and homeostasis. Therefore, it is likely that the composition of signal systems closely reflects the functionality of the cell type in different tissues, i.e. that some ciliary signal systems are tissue specific. This may be particularly relevant when comparing cilia situated deep inside various tissues and organs versus cilia that protrude from the apical surface into the lumen of e.g. tubular structures as seen on epithelial and endothelial cells (ECs). Also, during development, the composition of ciliary signal systems may change as part of the dynamic process that controls cell differentiation, allocating different signal systems to different cell types to determine cell fate and function.

In the following we will present a brief overview of some of the signal transduction pathways that have been shown to be coordinated by the primary cilium to control cellular processes during development and in tissue homeostasis.

**Signaling in Primary Cilia on Epithelial and Endothelial Cells**

As outlined above, PKD1 and PKD2 may form a mechanosensory complex that coordinates a flow-sensing response in kidney primary cilia. However, ciliary polycystins may well have other functions in relaying this response to control kidney development and homeostasis [42]. The channel activity and positioning of PKD2 in the cilium is regulated by fibrocystin, which is indirectly linked to the N-terminus of PKD2 through Kif3a/b of the kinesin 2 complex [70–73]. In addition to their role in Ca2+ signaling, the polycystins contribute to maintaining homeostasis through p53 and JNK [74] and also negatively regulate the JAK/STAT [75, 76] and the mTOR pathways [77]. Alterations in mechanostimulation induce cleavage of PKD1, releasing tuberin and mTOR from their flow-dependent inhibition by PKD1 [77], and allowing the transcription factor STAT6 and cofactor P100 to translocate from the cilium to the nucleus [76]. By itself, the PKD1 C-terminal fragment may influence gene transcription and perhaps modulate Wnt signaling [76, 78, 79], all processes that promote dedifferentiation and proliferation [for a review, see 80].

The nephrocystins are also assumed to exert their function through the primary cilium in renal develop-
ment and maintenance, although the exact mechanisms remain elusive [43–45]. More than half of the Nphps (1, 4, 5, 6 and 8) have been localized specifically to the ciliary transition zone at the base of the cilium, suggesting a role in ciliary assembly and/or transport of specific proteins into the ciliary compartments [48, 50, 52–54, 56, 57]. As such, Nphp-1 and Nphp-4 have also been proposed to play a role in axonemal or IFT modeling [81], whereas Nphp-9/Nek8 is necessary for expression and ciliary positioning of PKD1 and PKD2 [82, 83]. Nphp-3 may also impact on ciliary length in mammalian cells [81, 84], and was recently demonstrated to interact directly and genetically with Nphp-2/inversin in the establishment of bilateral asymmetry and promotion of tissue polarity [84, 85]. The latest hypothesis proposes the existence of one or more nephrocystin complexes at the ciliary transition zone, in line with the BBSome, which may interact with or be part of a ciliary pore complex [43, 45, 56, 86]. In addition, the activation of the transcription factor ATF4/CREB-2 by Nphp-6/CEP290 [55] indicates that the actions of the nephrocystins are complex and may affect ciliary functions on several levels. Furthermore, studies of Nphp-2 and Nphp-3 [84, 85] as well as recent findings with ift88, kif3a, and bbs mutants [62, 87] suggest an important role for the primary cilium in regulating Wnt signaling, which will be described in further detail below.

Primary cilia on cholangiocytes that extend from the epithelium into the bile duct lumen also possess a series of receptors and signaling molecules that control tissue homeostasis. These include PKD1, PKD2, fibrocystin, TRPV4, and G-protein-coupled purinergic receptor, P2Y12, that ultimately coordinate mechanos-, osmo-, and chemo-sensory functions, which when defective cause, e.g., cystic and fibrotic liver diseases [88]. Primary cilia on endothelial cells (EC) of the blood vessels and in endocardium were proposed to function as shear stress sensors. In cultured human umbilical vein ECs, cilia were found to disassemble in response to laminar shear stress [89]. PKD1 strongly localized to EC cilia in embryonic mouse aorta. In cultures of embryonic ECs, fluid shear stress cleaves PKD1, and ultimately leads to changes in Ca2+ signaling and nitric oxide synthesis [90] as well as expression of shear-responsive genes such as Krüppel-like factor-2 [91]. Consequently, dysfunctional cilia in the cardiovascular system may increase the risk for arterosclerosis and hypertension [90, 92].

**PDGFRα Signaling in Cycle Control and Migration**

Signaling via PDGFs and PDGFRs plays an essential role in cell survival, growth control and cell migration during gastrulation, fetal development and in maintenance of tissues in the adult, with defects causing a range of diseases, including cancer, vascular disorders and fibrosis [93]. Recently, it was shown that PDGFRα signaling is coordinated by the primary cilium in mouse embryonic fibroblasts (MEFs), i.e., expression of PDGFRα is upregulated during growth arrest and targeted to the cilium where PDGF-AA-dependent activation of the receptor and its initial downstream effectors such as Mek1/2 and Akt occurs [33, 94] (fig. 2). In wild-type cells these signaling events stimulate cell cycle entrance, which is blocked in ift88orpk (ift88Tg737NRpw) MEFs that lack the primary cilium [33]. Consequently, PDGFRα signaling through the fibroblast primary cilium may be important in tissue homeostasis while perturbations in this pathway could lead to oncogenesis.

The fibroblast primary cilium may function as a cellular GPS that coordinates directional migration and PDGFRα-mediated chemotaxis. Using micropipettes to generate a PDGF-AA gradient wild-type growth-arrested MEFs respond immediately to PDGF-AA injection, and migrate uniformly towards the pipette, while ift88orpk MEFs do not respond to PDGF-AA and move around randomly. In vitro wound-healing assays, primary cilia in wild-type MEFs orient parallel to one another, perpendicular to the wound, and incubation with PDGF-AA increases the migration speed and the directional movement of the cells. In contrast, in ift88orpk cells, the migration speed is unaffected by PDGF-AA incubation, and cells have decreased directionality [94]. PDGFRα-mediated migration is associated with activation of the ubiquitous plasma membrane Na+ /H+ exchanger, NHE1, and inhibition of NHE1 reduces PDGF-AA-mediated cell migration speed and directionality of wild-type MEFs, whereas this inhibition is markedly reduced in ift88orpk MEFs [95]. These results support the conclusion that the primary cilium represents an alternative mechanism of sensing chemotactic gradients and is part of the positioning machinery that coordinates directed migration in wound healing and developmental processes.

Primary cilia may also directly interact with extracellular matrix (ECM) proteins as the cell moves, transmitting mechanical information from the outside milieu to the cell. In vascular smooth muscle cells, primary cilia that contain PKD1, PKD2 and integrins are critical for cell-ECM interaction and mechanosensing that allow the cilium to project into the ECM and potentially control wound healing [96]. Also, in chondrocytes primary cilia make direct physical contact with ECM components via specific ECM receptors, suggesting that mechanical stim-

Nephron Physiol 2009;111:p39–p53

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ultrami may be transmitted through the cilium to control tissue development and to construct a mechanically robust skeletal system [97, 98]. Although speculative at this point, the directional migration of fibroblasts may also be similarly regulated through interactions between the cilium and ECM, and potentially in concert with chemotractants in embryonic patterning and adult tissue reorganization.

Wnt Signaling

As indicated above, the primary cilium has been proposed to have a role in Wnt signaling that regulates cell proliferation, cell fate determination, and migration. Recently, Wnt signaling was described as a network of interacting rather than individual pathways [99]. Traditionally, Wnt signals are divided into at least three distinct pathways, all initiated by binding of a ligand of the Wnt family to a 7-transmembrane Frizzled (Fz) receptor. Canonical Wnt signaling involves Dishevelled (Dvl)-mediated stabilization of β-catenin, which serves as a transcriptional coactivator and in turn induces cell cycle progression, proliferation, differentiation, and growth in addition to migration and regulation of embryonic development [100]. Noncanonical, or β-catenin-independent Wnt signaling pathways act through aPKC, CamK and JNK to control cellular polarity, migration and PCP, necessary for convergence extension during, e.g., gastrulation and neurulation [for a review, see 101].

The primary cilium and basal body have been assigned a role in regulating both the canonical and the noncanonical Wnt signaling pathways due to the ciliary/basal body localization of the PCP proteins inversin [49, 85], Vangl-2 [102], and Fat4 [61], in addition to members of the degradation complex, GSK-3β [59] and APC [62]. Vangl-2 appears to interact with Prickle-1 and inversin [85], both of which can suppress Wnt/β-catenin signaling through Dvl degradation [85, 103], the latter in concert with another ciliary protein, nephrocystin-3, as mentioned above [84]. PCP-like phenotypes have been observed in the inner ear of mice where ift88 has been disrupted [104], and in Bbs4-deficient mice and zebrafish [102]. Further, loss of primary cilia in the ift88orpk mouse causes a series of abnormalities in the pancreas, including extensive cyst formation in ducts associated with defects in cell cycle control [105, 106]. In the dilated ducts and cysts, the cytosolic localization of β-catenin is increased and there is an increased expression of Tcf/Lef [105], which activates transcription of Wnt target genes [107–109]. These observations support the conclusion that primary cilia are associated with the regulation of Wnt signaling in the pancreas.

More recently, two different approaches were used to disrupt cilia/basal body function and demonstrated the importance of these structures for the regulation of canonical Wnt signaling [62, 87]. RNAi-induced basal body disruption impaired gastrulation in zebrafish due to obstructed convergence extension movements, and was accompanied by moderately increased canonical signaling. The latter was also the response to shRNA knockdown of either BBS4 or BBS6/MKKS in HEK293 cells, which impaired the ability of noncanonical WNT5a to suppress the canonical Wnt3a activity. An identical effect was seen in cells where cilia were ablated by knockdown of KIF3A with shRNA [87]. A corresponding hyperresponse to Wnt3a was observed in kif3a or ift88orpk MEFs using a BATgal canonical Wnt reporter as well as in MEFs and Ofd1-deficient murine embryonic stem cells (mESCs), albeit not in the absence of Wnt3a [62]. In vivo there was a general increase in canonical Wnt signaling activity in kif3a mutants, although spatially the activity was normal. The phenocopying of these effects by specific inhibition of the proteasomal subunit RPN10, which associates with BBS4 [87], suggests that proteasomal targeting of β-catenin is a process that requires the basal body. Notably, whereas basal body disruption impaired noncanonical signaling and convergence extension [87], the noncanonical Wnt5a was still able to induce cytoskeletal rearrangements indicative of PCP equally well in heterozygous and kif3a–/– MEFs [62], suggesting that repression of the canonical pathway and activation of noncanonical Wnt signal are two independent processes involving the cilium or basal body. Further insights into the connection between cilia/basal body and Wnt signaling have been revealed by Bergmann et al. [84], who demonstrated that Nph3 binds to inversin and can inhibit inversin-mediated canonical Wnt signaling.

Hh Signaling

Another critical signal transduction pathway that is coordinated by the primary cilium is the Hh pathway. In addition to its general roles in tissue homeostasis, this pathway is crucial in tissue differentiation during embryonic development, and dysfunction of the Hh pathway is responsible for, e.g., basal cell carcinoma, the most common form of cancer in humans.

In mammals, the Hh ligand comes in three different varieties [sonic (Shh), Indian (Ihh) and desert (Dhh)] that are spatially and temporally regulated and whose concentration gradient ultimately helps determine the eventual cell fate and proliferation rate [for a review, see 110]. The ligands work through two transmembrane proteins,
Patched (Ptc) and Smoothened (Smo), that in turn regulate the activity of three transcription factors of the Gli family (Gli1, 2, 3) [111]. Ptc is the receptor for Hh ligands, and in the absence of Hh ligands it negatively regulates Hh signaling by suppressing the activity of Smo. Upon binding of Hh ligand to Ptc, the inhibition of Smo is relieved, preventing processing of Gli3 to a repressor and activating the Gli-2 transcription factor, which in turn induces the Hh pathway through their nuclear transcriptional targets [112].

A series of observations have now shown that primary cilia are critical regulators of the Hh pathway where the regulated concerted movement of Ptc out of and Smo into the cilium may create a switch by which cells can turn Hh signaling on and off during development and in control of tissue homeostasis [for reviews, see 111, 113]. In this scenario, binding of ligands to Ptc in the cilium activates the Hh pathway by removal of Ptc from the cilium [114] in a process that is associated with ciliary enrichment of Smo [115] (fig. 2). In vitro activation of Smo in cells exposed to Shh is shown to be blocked in MEFs lacking IFT172 or the dynein retrograde motor, Dync2h1 [116]. The translocation of Smo into the primary cilium upon Shh stimulation can also be blocked by knocking down Kif3A or β-arrestins, which are thought to be adapter proteins for the Smo protein [117].

Mutations in IFT proteins required for ciliary assembly results in dysfunctional Hh signaling and severe developmental disorders in mammals [for a review, see 111]. Removal of Kif3A causes aberrant Hh signaling which has been shown to have an impact on skeletogenesis [118, 119], neural tube formation [120], and cerebellar development [121, 122]. Mutations in the IFT139 homologue (Thm1) specifically result in abnormal Gli3 activator/repressor ratios which in turn result in defects in neural tube formation [31]. Similarly, mutations in a basal body protein (Ftm1) also resulted in abnormal ratios of Gli3 activator/repressor which lead to defects in left-right symmetry, neural tube formation and limb development [123]. The inhibition of Gli3 cleavage for subsequent activation of the Hh pathway was shown to also require other ciliary proteins: the retrograde IFT dynein motor subunit, Dnchc2 [124], the IFT72 protein [125] and the ciliary Arl13b (a small GTPase of the Arf/Arl family) [126]. Recently, a siRNA screen identified different mediators of the Hh pathway, among the genes controlling ciliogenesis: Nek1 (NIMA-like kinase) and Prka (a kinase participating in miRNA processing and thought to localize at the base of the cilium) [127]. Further, deletion of ift88 in ovary using Cre-Lox recombination in mice resulted in a severe delay in mammary gland development and defects in ovarian function [128]. Further, in IFT57-deficient mice, there were defects in ventral neural tube formation (exencephaly) due to aberrant Shh signaling [129]. These findings highlight the importance of IFT proteins in the Hh pathway.

One example of the in vivo changes in the levels of Hh molecules in the cilium comes from studies on the development of the pancreas, which is controlled by the graded response to Hh signaling [130–133]. Interestingly, Smo and Gli2 are absent from pancreatic primary cilia at human embryonic stage week 7.5, i.e. before the formation of the endocrine system, but highly concentrated in cilia in 14- and 18-week-old fetuses [134]. This increase in ciliary localization of Smo and Gli2 is accompanied by loss of Gli3 in ductal epithelium, suggesting that a graded Hh signaling response coordinated by the primary cilium regulates the development of the human pancreas. Therefore, disruption of pancreatic development in mice with defects in primary cilia [105, 106, 135] may be due to loss of both coordinated Hh and Wnt signaling during genesis of the pancreas. The canonical Wnt and Ihh pathways may also help to coordinate osteoblast and chondrocyte differentiation during bone development [136, 137; for reviews, see 138, 139]. One known disorder resulting from developmental skeletal problems is chondroectodermal dysplasia in Ellis-van Creveld syndrome, which arises from mutations in Evc, which localizes to the base of the chondrocyte primary cilium [140].

**Stem Cells and Primary Cilia**

Stem cells hold great promises for their potential therapeutic abilities. Starting off in the pluripotent states, they have the potential to give rise to all three germinal layers and can differentiate to form specific cell types dependent on the environment and specific factors present. Thus far, it is thought that they could be used for therapies directed against Alzheimer’s disease, Parkinson’s disease, diabetes, and a host of other conditions [141]. Also, a credible paradigm has stem cells as important targets against cancer since cancer stem cells are the true progenitors of cancers [142].

Recently, human embryonic stem cells (hESCs) were shown to possess primary cilia with the classic 9 + 0 architecture. Furthermore, it was shown that the Hh pathway was functional through the primary cilium as evidenced by the movement of Smo into and Ptc out of the primary cilium upon treatment with an agonist [143].
This implies that primary cilia could play pivotal roles in the earliest stages of development in terms of cell differentiation and proliferation. In addition, PDGFβR, which helps maintain hESCs in an undifferentiated state \([144]\), and components of the Wnt signaling cascade, which maintains the self-renewal in both mESCs and hESCs \([145]\), have been shown to localize to the primary cilia and/or in the centrosomal region at the base of the cilium of hESCs \([146]\). Further, it was reported that the traditional stem cell markers (Oct4, Sox2, and Nanog) localize to the primary cilia in a subpopulation of cultured hESCs \([146]\). While their exact function in the cilium is not known, one hypothesis is that processing of these transcription factors could occur in the primary cilium analogous to that suggested for the Gli proteins. These data further hint at the critical role of the primary cilium in coordinating pathways determining cell differentiation and proliferation.

In later stages of development, it has now been reported that the primary cilia are critical for the development of neural stem cells needed for proper development of the hippocampal region. In mice lacking the Ki67a or the Smo proteins, there is a failure in the maturation of radial astrocytes, which would normally develop in the dentate gyrus and be responsible for maintenance of adult neurogenesis \([147]\). Similarly, removal of either of these proteins from the cerebellar granule cell precursors results in the improper development of the cerebellum \([121]\). A similar effect is seen when the IFT88 protein is knocked down \([122]\). Cilia are also important for proper neurogenesis in the hippocampal region as shown in mutants lacking the stumpy protein (Gli3 processing was altered). Knocking down the Smo or Shh proteins was also shown to lead to a failure in the development of the neocortex \([148]\). Postnatally, this leads to a lack of a specific subtype of neural stem cells \([149]\).

**Conclusions**

Recent research in primary cilia and their function in coordinating cellular signal transduction pathways, developmental processes and tissue homeostasis have moved this organelle to a central position in human pathophysiology. While PKD was one of the first diseases to be linked to dysfunctional primary cilia, defects in these organelles have subsequently been associated with many other phenotypes, including cystic pancreatic and liver diseases, retinitis pigmentosa, anosmia, defective neurogenesis, polydactyly, and other developmental defects now referred to as the ciliopathies, and there are also indications that the primary cilium is important in behavioral and mental disorders and oncogenesis. Recent work has further shown that stem cells possess primary cilia with signal transduction components that control maintenance of stem cell pluripotentiality and regulate early differentiation and proliferation. These findings imply that primary cilia play pivotal roles in the earliest stages of embryonic development, which could be important in regenerative medicine. Future studies on the mechanisms in ciliary assembly, translocation of signal components in and out of the cilium and coordination of ciliary signaling transduction pathways in developmental processes and tissue homeostasis will add further and important insight into the intrinsic biology of primary cilia in human health and disease.

**Acknowledgements**

This work was supported by the Lundbeck Foundation (No. R9-A969), the Novo Nordisk Foundation, the Danish Natural Science Research Council (No. 272-07-0530 and No. 272-07-0411 to S.T.C. and L.B.P.), a Novo Nordisk/Novozymes Scholarship (I.R.V.), the Lundbeck Foundation (No. 150/05 to A.A.), and NIH RO1 DK065655 and HD050327 (B.K.Y.). We apologize to those whose work is not described in this review owing to restricted space.

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Nephron Physiol 2009;111:p39–p53

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