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The Primary Cilium as a Complex Signaling Center

Review

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Respect for the primary cilium has undergone a remarkable renaissance over the past decade, and it is now thought to be an essential regulator of numerous signaling pathways. The primary cilium's functions range from the movement of cells and fluid, to sensory inputs involved with olfaction and photoreception. Disruption of cilia function is involved in multiple human syndromes collectively called 'ciliopathies'. The cilium's activities are mediated by targeting of receptors, channels, and their downstream effector proteins to the ciliary or basal body compartment. These combined properties of the cilium make it a critical organelle facilitating the interactions between the cell and its environment. Here, we review many of the recent advances contributing to the ascendancy of the primary cilium and how the extraordinary complexity of this organelle inevitably assures many more exciting future discoveries.

Introduction

Cilia are microtubule-based organelles found on most eukaryotic cells [1]. Historically, they have been classified as either motile or immotile (primary cilia). While motile cilia generate extracellular fluid flow or propel individual cells, the immotile primary cilium was long thought to be vestigial. Cilia are assembled by the conserved process of intraflagellar transport (IFT); interestingly, it appears that this organelle has evolved into a complex signaling center in mammals, providing important sensory functions [2,3]. This realization occurred with the discovery that cilia dysfunction causes several genetic diseases [4]. Fortunately, analysis of cilia in model systems has given insights into their roles in signaling and potential disease mechanisms.

Cilia and flagella contain a microtubule core called an 'axoneme'. The axoneme of all cilia is composed of nine peripheral microtubule doublets. In motile cilia, these doublets surround a central pair of microtubules and thus this ultra-structure is referred to as '9+2' (Figure 1A). The axoneme of the primary cilium lacks the central pair and has a 9+0 arrangement (Figure 1A). Cilia extend from a basal body derived from the mother centriole that serves as a microtubule-organizing center just beneath the cell membrane (Figure 1B) [5]. The basal body and its associated transition-fiber proteins are thought to regulate protein entry and exit from the cilia compartment [6,7].

With the exception of sperm flagella, 9+2 motile cilia are found in large numbers at the apical surfaces of epithelial cells. Typical examples are the epithelia of the trachea and ependymal cells lining the brain ventricles. These cilia beat in a rhythmic manner to propel mucus or cerebrospinal fluid. In contrast, the primary cilium is present on most mammalian cells as a solitary, immotile organelle.

Although, cilia have been historically classified as either motile or immotile sensory cilia (primary), these are inaccurate definitions. Many protists possess flagella that function in both motility and sensory reception [8]. Additionally, vertebrates possess motile 9+0 cilia on the embryonic node that generate fluid movement vital for left-right body axis specification [9–11]. Also, cilia of olfactory sensory neurons violate the classification as they are immotile 9+2 structures.

Despite the ultra-structural diversity of cilia, ciliogenesis is evolutionarily conserved. A microtubule motor-based transport system called 'intraflagellar transport' (IFT) is responsible for their formation and maintenance (reviewed in [12–14]). Most of our understanding of IFT has emerged from the study of the biflagellated green alga, *Chlamydomonas reinhardtii*, and the nematode *Caenorhabditis elegans*. The IFT particle consists of two distinct subcomplexes (complexes A and B). Complex B functions in anterograde transport from the base to the tip of the cilium, while complex A is needed for retrograde movement from the tip back to the base of the cilium (reviewed in [14]). The study of components of the IFT machinery has not only shed light on cilia assembly, but also led to a better understanding of the biological functions of cilia themselves.

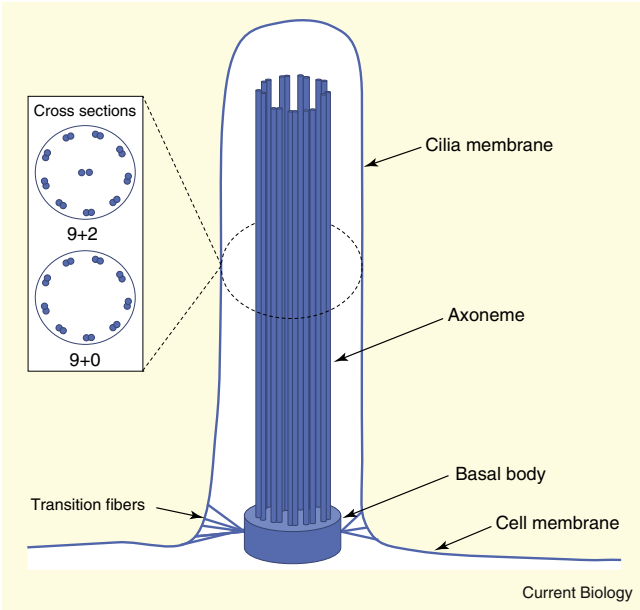

Figure 1. Cilia structure.

The nine peripheral microtubule doublets of the axoneme form the backbone of the appendage while the basal body at the base is utilized as a template. The axoneme is sheathed in the cilia membrane, which is distinct from the cell membrane. Structures at the base of the cilium, such as the transition fibers and the basal body, are important for regulating the protein content of the cilia membrane. The inset shows a cross-section of the microtubule arrangement of two axoneme microtubule ultra-structures: 9+2, found in most motile cilia, and 9+0, found in primary cilia.

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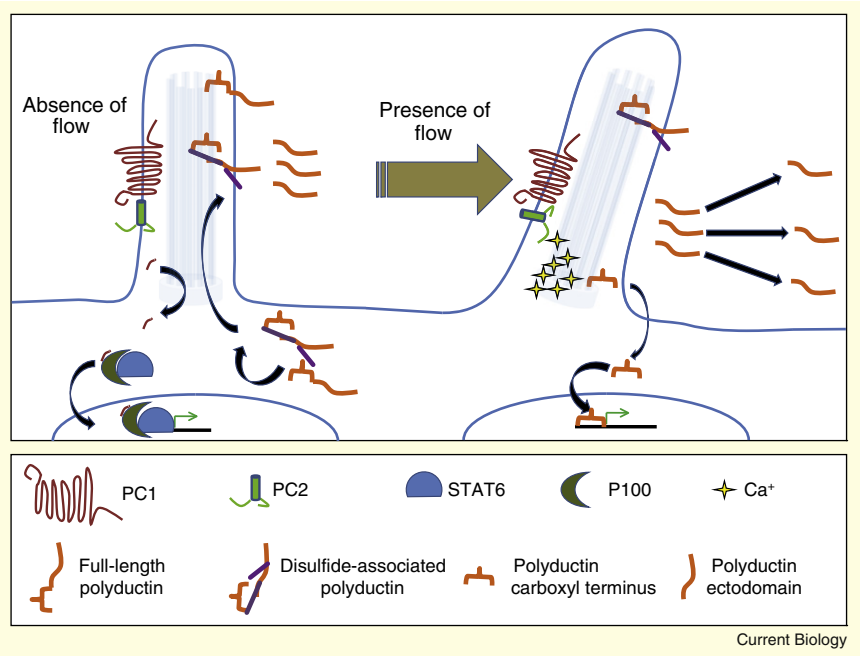

Figure 2. Mechanosensory renal cilia.

Primary cilia of renal tubule cells act as mechanosensors of fluid flow and possibly facilitate cell–cell communication. In the absence of flow (left), the carboxyl terminus of polycystin-1 (PC1) is cleaved. The PC1 carboxy-terminal peptide has been detected in the nucleus where it is associated with STAT6 and P100 transcriptional regulation. Proteolytic processing of PC1 (right) does not occur in the presence of fluid flow. Polyductin/fibrocystin (left) also undergoes proteolytic processing where the amino-and carboxy-terminal regions remain attached. The amino-terminal ectodomain of polyductin possibly also undergoes shedding. Thus, secretion from the cilium may have important functions in paracrine signaling (right). The carboxy-terminal region of PD has been detected in the nucleus, but its function is not known. In the presence of flow (right) calcium enters the cell in response to deflection of the cilium. This calcium signal is dependent on both PC1 and PC2 in the cilium; however, it remains unclear how dysregulation of this calcium signaling may lead to cyst formation.

Cilia as Mechanosenors

The discovery that perturbation of primary cilia function in the *ift88*^{TGN737Rpw} mouse (hereafter called ORPK) caused cyst formation in the kidney was a seminal finding that revealed the cilium as a clinically relevant organelle [15,16]. Subsequent data showed that primary cilia on renal epithelium function as mechanosensors and that deflection of these cilia in response to fluid movement initiated an intracellular calcium signal [17,18]. The connection between the cilium and renal cystic disease was initially shown by Barr *et al.* [19] who demonstrated that LOV-1 and PKD-2 function in the cilium of the *C. elegans* male sensory neurons [19,20]. These proteins are homologs of human polycystin-1 (PC1) and polycystin-2 (PC2), mutations in which cause human autosomal dominant polycystic kidney disease [21,22]. PC1 and PC2 are transmembrane proteins present in the cilium. Additionally, PKHD1 polyductin/fibrocystin (PD) is disrupted in autosomal recessive polycystic kidney disease and also localizes to the cilium. While the functions of PC1 and PD are unknown, PC2 is a transient receptor potential (TRP)-like cation channel. PC1, PC2, and PD appear to function in the cilium as part of a mechanosensory system. This is supported by the loss of a calcium (Ca^{2+}) signal induced by cilia deflection in the absence of these proteins [23], despite normal cilia structure (Figure 2). Similar roles for cilia have been reported in tissues such as the endothelium and the biliary duct [24,25].

The association of cilia dysfunction and cyst formation is further established through genetic mutations in mouse models and from the identification of genes involved in human cystic kidney disorders, many of whose corresponding proteins localize to primary cilia or basal bodies. How the loss of cilia-mediated mechanosensation within the tubule leads to cyst formation remains to be determined. One model proposes that cilia dysfunction alters the orientation of mitotic spindles [26]. In tubules where cilia function has been perturbed, the orientation of division is randomized and results in expansion (cyst formation) of the tubule. This could represent defects in mechanotransduction, a role for ciliary proteins in proper centriole migration, or an unrecognized connection between the cilium and centrioles during spindle formation.

In addition to mediating mechanosensitive calcium flux, PC1 and PD undergo post-translational processing that results in cleavage of their carboxy-terminal regions in the absence of flow (Figure 2). In the case of PC1, the carboxy-terminal domain translocates to the nucleus and associates with the transcription factor STAT6 and the co-activator p100 to stimulate gene expression (Figure 2) [27]. PC1 cleavage is important, as mice expressing a non-cleavable form of PC1 develop cysts [28]. PD goes through a complicated cleavage process to produce a large amino-terminal extracellular domain that remains tethered to its carboxyl terminus by di-sulfide bridges (Figure 2). Intriguingly, the amino terminus of PD can be detected in cultured renal cell media, indicating that it may undergo ectodomain shedding. The carboxyl terminus of PD is present in the nucleus; however, the consequences of its localization are unknown.

Fluid movement through the tubules and mechanosensory activities of the cilium may have an important impact on cellular responses. The activity of the amino-terminal domain of PC1 and the effect of ectodomain shedding of PD is unknown; however, these findings raise the possibility that the release of PC1 and PD signaling peptides into the renal lumen may affect communication between cells. One area of cilia biology that is largely unexplored is the potential for cilia to not only receive but also to transmit signals. For example, both secretion of vesicular particles from ciliated cells of the embryonic node [29] as well as the presence of PKD-protein containing exosomes that may be derived from the cilium have been reported [30]. However, the relevance of these observations remains to be determined.

All of the above data led to the ‘ciliary hypothesis of polycystic kidney disease’, which views a mechanosensitive cilium as crucial for the etiology of cyst formation [31]. However, recent findings suggest that the renal cilium and its role in kidney development and homeostasis may be more complex: several groups have disrupted cilia or PC1

function in mice at different postnatal time points and found that cyst formation is dependent on when cilia function is impaired. If induced prior to postnatal day 12 (P12), a severe cystic phenotype ensues within three weeks. In contrast, if cilia function is disrupted after P14, cysts are not evident for six months and progression occurs slowly [32,33]. This suggests a critical time period for cilia function that is needed to prevent rapid cyst formation and challenges the idea that loss of mechanosensory input in itself results in cysts. Thus, a combination of factors is likely to be needed for cystogenesis. One additional factor may be that rapid cyst formation requires a proliferative environment, such as the perinatal kidney. Under these conditions, the randomized orientation of cell divisions associated with the cilia defects could contribute to tubule expansion and cysts. This model of cyst formation is supported by studies that show rapid cyst formation in adult cilia mutants after proliferation and repair is induced by renal injury [34].

Another putative mechanosensory role for the cilium is in the establishment of left-right asymmetry during development. In mice, left-right asymmetry is established by the node, a cup-like structure found at the ventral tip of the embryo, each cell of which has a cilium. Some of the nodal cilia rotate counterclockwise, creating a directed fluid flow. The immotile cilia on the node detect this fluid flow and, in a PC2 dependent manner, initiate an asymmetric Ca^{2+} influx on the future left side of the embryo.

In addition to responses induced by fluid shear, cilia have important functions in pressure, touch and vibration sensation. This is exemplified in invertebrates such as *Drosophila melanogaster* and *C. elegans*. In *Drosophila*, neurons of a sensory organ known as the 'chordotonal organ' [35,36] extend a cilium into a cavity, the scolopale, generated by support cells. The tip cilium is attached to the cap of the scolopale. The sensory neurons respond to vibrations when the cap is displaced, stretching the cilium. The deformation of the cilium is thought to initiate a rapid electrical response via an ion channel in the axoneme. Mutations in IFT proteins result in short cilia, altering the organs structure and resulting in loss of sensory reception [35]. Similar to *Drosophila*, cilia in *C. elegans* extend from dendrites of mechanosensory neurons beneath the cuticle. It is unknown if these cilia connect to the cuticle, but the enclosed space does contain extracellular matrix (ECM) material, and mutations disrupting this ECM result in impaired mechanosensory functions [36]. Analogous to the chordotonal organ of insects, mechanosensation in *C. elegans* involves movement of a mechanosensory complex relative to the ECM and the underlying cytoskeleton. This stimulus regulates the activity of sodium channels mec-4 and mec-10 (ENaC superfamily) and converts the stimuli into a rapid electrical response.

These studies of the chordotonal organ in fly and mechanosensation in *C. elegans* raise intriguing ideas for how cilia could be involved in sensory reception in mammals. Although cilia have not been reported in association with mechanosensitive structures such as Meissner and Pacinian corpuscles in mammalian skin, it would not be too surprising if a similar mechanism is operative as Bardet-Biedl syndrome (BBS) patients have defects in mechanosensation and thermosensation [37]. Furthermore, this may be a paradigm of how cilia function on cells tightly embedded in the ECM. For example, the axonemes of cilia that are present on cells in cartilage or tendons are thought to make direct connections with the surrounding extracellular matrix [38–40]. The functional importance of these embedded cilia is unexplored. Based on the above model, it is possible that responses to stress placed on joints or tendons through muscle activity, or pressure placed on the skin, could be initiated by deformation of the cilium as a consequence of its association with the ECM. In the context of chondrocytes and cartilage, studies have revealed that cilia defects alter cortical actin and microtubule architecture, which may be associated with osteoarthritis [39,41].

Cilia as Environmental Sensors

In addition to its roles as a mechanosensor, the primary cilium has important functions in sensing the external environment and is essential to photoreception and olfaction. Defects in cilia-mediated signaling are associated with blindness and anosmia. As discussed below, these cilia have undergone specialization in order to facilitate their sensory roles.

Light Detection

In the mammalian retina, photoreceptors utilize a modified primary cilium for sensing light, and degeneration of photoreceptors is commonly associated with cilia disorders. The photoreceptors contact both bipolar and horizontal neurons and their dendrites extend toward the retinal surface. These dendrites end in a structure called the outer segment. The outer segment consists of an array of elaborate discs derived from the plasma membrane (Figure 3A). The cilium functions as a backbone connecting the outer segment and the cell body. Outer segment proteins pass through the connecting cilium and this region is thus thought to be a key regulator of entry into the outer segment. It appears that the outer segment utilizes at least two mechanisms for the transport of proteins through the connecting cilium: one relies on membrane protein diffusion while another is an active process requiring IFT (reviewed in [42]).

The outer segment is highly dynamic and undergoes continual turnover [43–45], with its construction and maintenance being dependent on IFT [12]. Thus, in the absence of IFT, the outer segment collapses, resulting in blindness. This is observed in both *kif3a* (a kinesin subunit required for IFT) and in the ORPK mutant mice. Furthermore, defects in photoreceptor maintenance are also present in many of the ciliopathies such as BBS [46] and nephronophthisis [47].

Odorant Detection

Olfactory sensory neurons extend their axons through the cribiform plate and their dendritic knobs end in clusters of 15–20 sensory cilia embedded within the olfactory epithelium (Figure 3B). The importance of cilia in olfaction is exemplified by mouse cilia mutants and BBS patients, which both display anosmia [48]. Once odorants come into contact with the epithelium, olfactory signaling is initiated (Figure 3C). This begins with the odorants acting as ligands to the odorant G-protein coupled receptors in the sensory neuron's cilia. Once activated, the receptors induce adenylyl cyclase type III (ACIII) through a stimulatory G-protein (G_{olf}). ACIII then generates an increase in cAMP that causes the opening of cyclic nucleotide gated ion channels. This response results in depolarization of the neuron, which is amplified via a Ca^{2+}-activated chloride channel and produces the sensation of smell. This entire olfaction signaling cascade takes place within the olfactory sensory cilia (reviewed in [49]).

Utilizing olfaction as a model for how cilia on other cell types may transduce signals could prove quite useful in

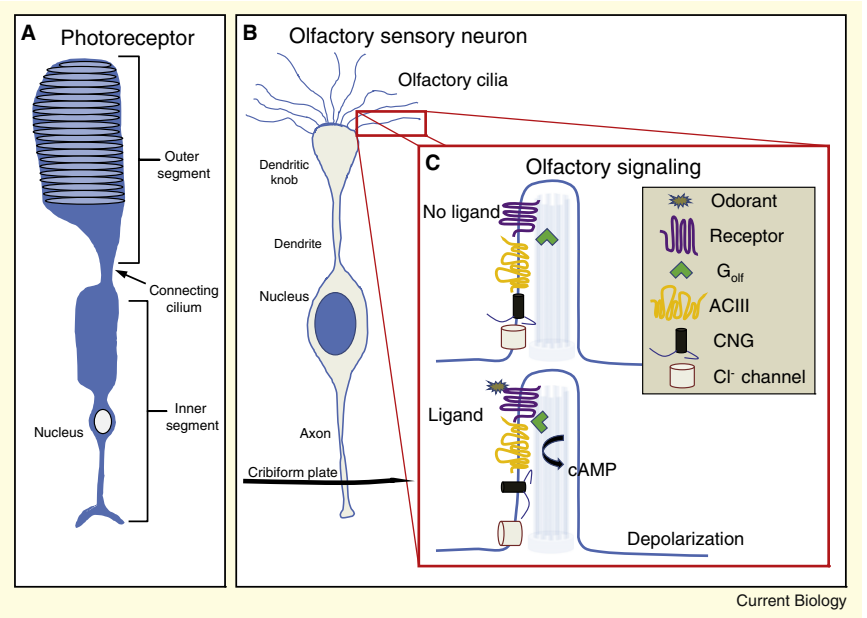

Figure 3. Modified sensory cilia.

The outer segment of photoreceptors (A), an elaborate array of membrane discs in which light detection takes place, is connected to the photoreceptor cell by a connecting cilium. The light detecting protein machinery must pass through the connecting cilium on its way to the outer segment. (B) In the olfactory sensory neuron of vertebrates, a dendrite ends in a dendritic knob from which olfactory cilia originate. There are several cilia per neuron which protrude into the mucus layer where odorants bind to receptors. (C) Upon odorant receptor binding to the olfactory G-protein coupled receptor (receptor) the G-protein (G_{olf}) is activated, producing cAMP. The cAMP then causes the cyclic nucleotide gated ion channels to open and subsequently affect Cl^- channels, then potentiating a depolarization of the OSN.

expanding our understanding of this complex organelle. For example, it is now known that cultured neurons and most regions of the rodent central nervous system (CNS) possess primary cilia that contain ACIII [37,50,51]. Even more compellingly, a rapidly increasing number of G-protein coupled receptors (GPCRs) have been reported in cilia, not only in neurons but also in other cell types [52–56]. Although the physiological relevance of these observations remains unknown, it is enticing to speculate that cilia in other regions of the body will utilize signaling processes similar to those found in the olfactory system.

The Primary Cilium in Development

The primary cilium also plays critical roles in cell-to-cell communication by sensing extracellular signals during development. Two extensively studied pathways where *in vivo* and *in vitro* data support a role for cilia are the Hedgehog (Hh) and Wingless (Wnt) pathways.

Hedgehog Signaling

Hedgehog (Hh) is a secreted protein regulating a vast number of developmental processes in both vertebrates and invertebrates [57,58]. The connection between Hh and cilia emerged from a mouse mutagenesis screen for neural-tube closure and patterning phenotypes — processes regulated by one of the mouse Hh proteins, Shh. Surprisingly, it was found that several mutant lines had mutations in IFT genes [59]. Subsequent data showed that components of the Hh pathway dynamically localize in cilia [60] and that cells lacking cilia are unable to induce the pathway in response to exogenous Shh ligand [61,62].

In mammals, the Hh pathway is initiated by binding of Hh to the transmembrane receptor Patched-1 (Ptch) in the cilia membrane [63]. In the absence of Hh, Ptch represses the activity of a seven transmembrane protein, Smoothened (Smo), through an unknown mechanism (Figure 4). Once Hh binding occurs, Ptch is internalized from the cilia membrane while Smo translocates into the cilium [60,63]. This alleviates Smo repression and results in pathway activation. Interestingly, cilia are not required for Hh pathway regulation in *Drosophila*, suggesting evolutionary divergence. However, as in mammals, *Drosophila* Patched and Smoothened are targeted to different cytosolic vesicles or to the cell membranes in response to Hh binding [64]. This may indicate that although cilia are not involved in regulating *Drosophila* Hh signaling, analogous mechanisms may operate. In addition to Ptch and Smo, several other Hh pathway regulators, including Suppressor of Fused (SuFu) and the Gli transcription factors (Gli1, Gli2, and Gli3), have been reported to be localized in the cilium [62,65,66].

Transcriptional activation in response to Hh is largely driven by Gli1 and Gli2, while Gli3 is a pathway repressor. In the absence of Hh, Gli2 is inactive, Gli3 is in a repressor (Gli3R) form, and Gli1 is not expressed. The processing of the Gli2 and Gli3 proteins is dependent on the E3 ubiquitin ligase βTrcp and the proteasome [67]. Induction of Hh signaling results in the increased activity of Gli1 and Gli2 and inhibition of Gli3 processing, thus leading to the accumulation of the full-length Gli3 protein. Data suggest that the processing of Gli3 to its repressor and the activation of Gli2 in response to Hh binding are dependent on cilia function [62,68]; however, the importance of cilia in Gli2 activity has been called into question recently [69].

The genetic data suggest that IFT plays multiple roles in Shh responsiveness, as some IFT mutants display Shh gain-of-function phenotype and others a loss-of-function phenotype. These seemingly contradictory roles of the IFT proteins have been partly teased out through analysis of two Shh dependent developmental events, neural tube and limb patterning. In the developing embryo, neuronal precursor cell populations are specified in the neural tube by Shh signaling. Shh is secreted from the notochord and floor plate, diffuses dorsally, and creates a signaling gradient throughout the neural tube. Both the concentration and length of Shh exposure result in the expression of unique transcription factor complements that define neural cell types [70]. In a wild-type animal, the more ventral cell populations in the neural tube receive higher concentrations of Shh for a longer time and express ventral-specific genes through Gli1 and Gli2 activator functions. These ventral cell

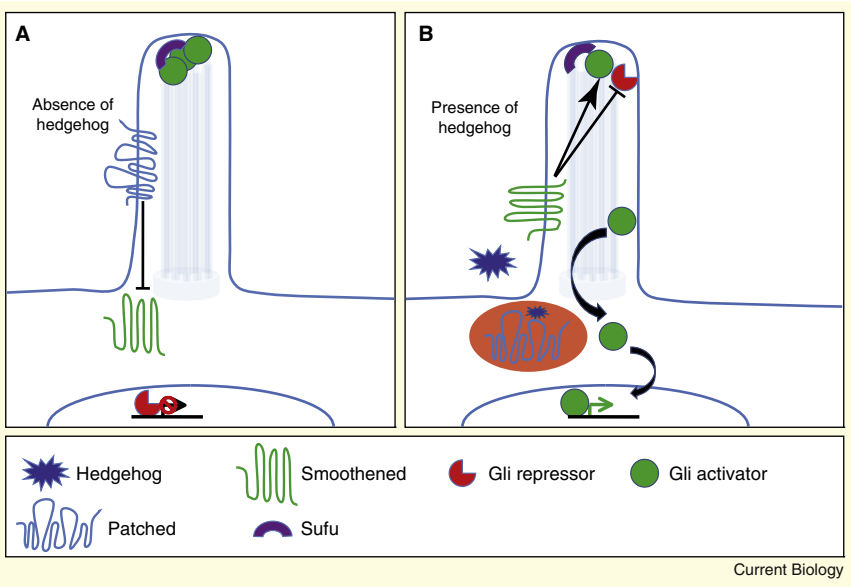

Figure 4. Hedgehog signaling and the cilium.
The Hedgehog (Hh) pathway in vertebrates utilizes the cilium as a signaling compartment. In the absence of the Hh ligand (A), the receptor Ptch resides in the cilium and through an unknown mechanism inhibits the transmembrane protein Smo. In the absence of ligand, the repressor forms of the Gli transcription factors inhibit Hh responsive gene transcription, while Gli activator is maintained in the cilia via Sufu binding. (B) Upon Hh binding, Ptch translocates out of the cilia membrane and its inhibition of Smo is alleviated. Activated Smo translocates into the cilia membrane and through unknown mechanisms the Gli activators translocate to the nucleus and activate Hh responsive gene expression.

types are lost in the neural tube of most IFT complex B mutants [68]. However, recent studies have suggested that the situation is more complex, with one IFT mutant, *Alien* (IFT139), having a dorsal expansion of ventral cell types [71]. In contrast to IFT complex B mutants affecting Hh signaling, IFT139 is part of IFT complex A. Thus, the difference in neural tube patterning and Hh response may reflect whether anterograde or retrograde cilia transport is altered. Another possibility is that some IFT proteins may have different effects on Gli2 versus Gli3 activities. In the region of the neural tube analyzed in the IFT mutants, Gli2 activator is the predominant factor regulating patterning along the dorsal ventral axis with minor roles for the repressor forms of Gli2 or Gli3. Thus, in the neural tube, most IFT mutants present with what appears to be a Hh pathway loss-of-function phenotype due to the lack of functional Gli activators.

Similarly, limb development depends on Hh signaling through the formation of a gradient of activity across the limb field [72,73]. Shh is secreted from a small mesenchymal population of cells, called the 'zone of polarizing activity', located in the posterior region of the limb. This localized region of Shh expression and secretion creates high levels of pathway activity mediated by Gli activators in the posterior versus low pathway activity and high Gli3 repressor formation in the anterior. The number of digits and their pattern along the anterior-posterior axis is determined by the ratio of Gli3 activator to Gli3 repressor [74,75].

Mutations disrupting IFT in mice alter the Gli3 activator-to-repressor ratio and result in severe polydactyly [62,71,76]. These digits lack identity with regard to their anterior-posterior position, all resembling digit one. Interestingly, the limb phenotype in the cilia mutants resembles those of Gli3 or Shh;Gli3 double mutants [62]. Several laboratories have addressed the limb phenotype in the IFT mutants and found abnormalities in the production of the Gli3 repressor [62,76]. These data point to the cilium as necessary for both Gli activation and repressor formation. Consequently, the Hh pathway appears deregulated in cilia mutants.

These data indicate that the cilium plays a crucial role in Hh signaling. While this has led to several insights into this well studied pathway, many questions remain. One is whether dynamic localization of Hh signaling proteins in the cilium is important for pathway regulation. Furthermore, it is possible that the phenotypes observed in IFT mutants are not due to loss of the cilium but rather an unidentified role for IFT proteins in the cell. Determining the molecular mechanisms governing localization and processing of Hh signaling components and the effects on pathway activity requires further investigation.

Wnt Signaling

Another developmentally important pathway that may require IFT for normal regulation is the Wnt pathway [77] (Figure 5). The Wnt pathway can be divided into canonical and non-canonical arms. The downstream effects of the canonical Wnt pathway are mediated by β-catenin. In the absence of a canonical Wnt signal, cytosolic levels of β-catenin are kept low due to its degradation by the β-catenin destruction complex consisting of casein kinase I (CKI), glycogen synthase kinase 3β (GSK3β), adenomatous polyposis coli (APC), and axin 1 [77]. While part of this complex, β-catenin is phosphorylated, promoting its degradation by the proteasome, which may occur near the basal body. In the absence of β-catenin, the nuclear transcription factors T cell factor (TCF) and lymphocyte enhancer factor (Lef) are associated with transcriptional suppressors, such as Groucho, to keep the canonical Wnt pathway inactive.

The non-canonical Wnt pathway is less well understood than the canonical pathway. Like the canonical pathway, it involves binding of Wnt ligand to a Frizzled receptor; however, it does not require LRP co-receptors and is β-catenin independent. The non-canonical Wnt pathway mediates its effects through intracellular Ca^{2+} levels and by regulating RhoA, Rock, and Jnk kinase. These factors have major effects on the cytoskeleton and are important for regulating planar cell polarity (PCP). PCP involves intercellular communication that coordinates the organization of cells in order to orient structures within the plane of a tissue (i.e. stereocilia bundle orientation in the inner ear). PCP effects are regulated through the cytoskeleton and by asymmetric distribution of proteins within the cell [78].

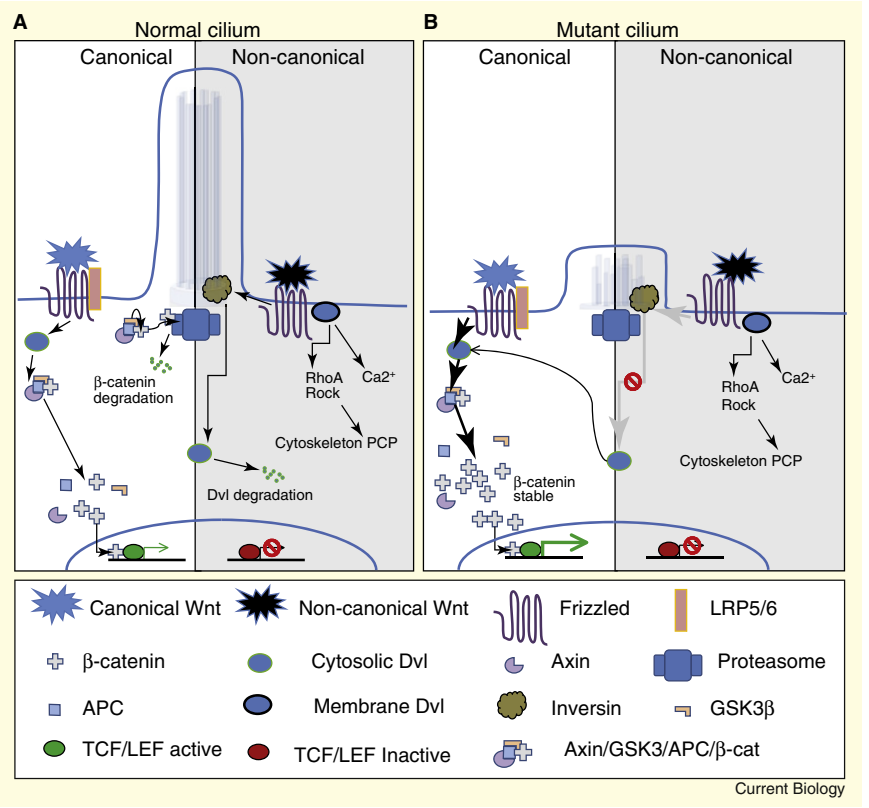

Figure 5. Wnt signaling and the cilium.

The cilium/basal body may function as a regulatory switch to control the balance between the canonical and non-canonical Wnt pathways. In the canonical pathway (A, left), a Wnt ligand binds to the co-receptors Frizzled and LRP. This inhibits the activity of the β-catenin destruction complex possibly through the Dishevelled (Dvl) protein and leads to stabilization of β-catenin, which accumulates in the nucleus and with LEF and TCF activates target genes. In the non-canonical pathway (A, right), Wnt binds to a frizzled receptor, independent of LRP. This activates a membrane form of Dvl which regulates downstream targets. The non-canonical Wnt signal activates Inversin, which resides in multiple locations in the cell, including the cilium or at the base of the cilium. Inversin induces the degradation of cytoplasmic but not the membrane form of Dvl. In a ciliated cell (A), both the canonical and non-canonical pathways are operative and the strength of the canonical pathway (A, right) is thought to be influenced by the non-canonical Wnt pathway (A, left). In the absence of cilia (B), the model would suggest that the non-canonical pathway is unable to efficiently antagonize the activity of the canonical pathway.

A possible connection between the cilium and the Wnt pathway may be the protein inversin (Inv). Inv localizes to several sites, including the base of the cilium [79]. Mutations in Inv cause cilia-related phenotypes, including left-right axis abnormalities and cystic kidney disease seen in human patients with nephronophthisis type 2 [80]. One function of Inv is to regulate Dishevelled (Dvl), which is thought to be at the crossroads between the canonical and non-canonical pathways. Early models suggested that the canonical Wnt pathway inactivates the β-catenin destruction complex through the cytosolic form of Dvl, while the non-canonical Wnt pathway regulates the membrane localized form. Inv is thought to exert its effects on the Wnt pathway by reducing only the cytoplasmic levels of Dvl. Thus, cilia-mediated signaling through Inv could regulate the switch between canonical and non-canonical pathways due to its disparate effects on membrane versus cytosolic Dvl. However, recent reports suggest a need to revise this model. Manipulation of the subcellular distribution of Dvl in *Xenopus laevis* revealed that the plasma membrane form of Dvl is indeed required for convergent extension movement and cell shape changes associated with the non-canonical PCP pathway [81], but membrane-anchored Dishevelled was also found to be a potent activator of canonical Wnt signals. Thus, the simple model whereby Inv can suppress the canonical Wnt pathway by selectively degrading cytosolic Dvl is likely to be overly simplified and needs to be further evaluated.

Additional support for cilia function in regulating canonical Wnt signaling comes from the analyses of mutant mice with cilia defects. In both the kidney and pancreas, the levels of β-catenin are markedly increased in mutants, presumably in association with elevated canonical Wnt activity [82,83]. Furthermore, data comparing the *in vitro* response of ciliated and non-ciliated cells to the addition of canonical and non-canonical Wnts demonstrated that the response is abnormally regulated [84]. In cultured wild-type cells, non-canonical Wnt signaling was shown to restrain the canonical pathway in response to a canonical stimulus. This inhibition failed to occur in cells lacking cilia. Thus, cilia might modulate the strength of the canonical signal rather than functioning as a key factor needed for reception of Wnt ligand or induction of the pathway. However, *in vivo* analyses of cilia function in Wnt signaling remain controversial. In one study using a canonical Wnt reporter, cilia mutant embryos were found to have a marked elevation in the canonical Wnt signals, although the spatial pattern of Wnt activity in the embryo was normal [84]. In contrast, a second study using another Wnt-reporter line in a different cilia mutant did not observe increased reporter activity [2].

The non-canonical Wnt pathway may also require the cilium as evidenced by the PCP related phenotypes seen in cilia mutants. As discussed above, recent data suggest that renal cysts do not arise simply as a consequence of the loss of mechanosensation. Rather, this must occur in the context of a proliferative environment where cilia loss is associated with alterations in the direction of cell division that could be regulated through the PCP pathway. This connection was recently strengthened by showing that disruption of *Fat4*, a gene with an established role in PCP in multiple organisms, caused a cystic kidney phenotype involving abnormal orientation of mitotic spindles [85]. How Fat4 and the cilium are connected is unknown.

An additional connection between cilia and non-canonical PCP signaling is evident by the convergent-extension defects observed during zebrafish gastrulation. During convergent extension, cells converge along the midline and extend the

embryo in the rostral-caudal axis. However, several studies in zebrafish where cilia function has been abrogated using morpholino oligonucleotides to knockdown BBS genes resulted in embryos with a greatly expanded lateral axis and a reduced rostral-caudal axis [86,87]. Although the BBS proteins do not appear to be directly required for cilia formation in vertebrates, they do localize to the basal body and the cilium and mutations in these proteins do cause phenotypes seen in the cilia mutants [88,89].

While gastrulation defects have not been reported in mouse cilia mutants, there is an additional example where cilia dysfunction results in PCP phenotypes. This is evident in the development of the inner ear where the PCP pathway directs the organization of the actin-based stereocilia (unrelated to primary cilia) in the Organ of Corti [78]. The stereocilia normally align as four parallel rows of "V" shaped bundles with a true microtubule based cilium (kinocilium) located at the apex. In mice with mutations in either *bbs-4*, *bbs-6*, or *ift88*, the coordinated alignment of the stereocilia is severely affected [86,90]. Current data suggest that the inner ear defect, at least in the *ift88* mutants, arises through abnormalities in positioning or transport of the basal body to the correct subcellular location.

The connection between cilia and the PCP proteins remains poorly defined. Analyses of core PCP proteins, such as VANGL2, during inner ear development in mice have shown that their disruption does not cause ciliary defects [90]. Furthermore, loss of cilia due to conditional mutations in *ift88* or *Kif3a* did not have an overt effect on the subcellular distribution of any of the PCP proteins analyzed. On the other hand, there appears to be a connection as shown by the genetic interaction observed with mice having a mutation in a cilia gene and a heterozygous mutation in the core PCP protein Vangl2 which enhanced the inner ear phenotype [86,90]. These data suggest that independent or converging pathways regulate PCP involving the cilium and the PCP proteins.

In contrast to the results obtained with the *ift88* and *vangl2* mutations in mice, there have been several studies using knockdown approaches that suggest that the PCP pathway does regulate ciliogenesis. In *Xenopus*, the PCP effectors Fuzzy and Inturned are needed for normal convergent extension movements and were found to disrupt formation of cilia [91]. In addition, knockdown of the Duboraya (dub) protein, a non-canonical Wnt signaling mediator, in zebrafish results in defects in convergent extension and left-right axis formation, the latter being associated with cilia abnormalities on cells in Kupffer's vesicle, a structure similar to the murine node [92]. Furthermore, the function of dub in ciliogenesis was regulated by phosphorylation requiring the non-canonical Wnt receptor Frizzled-2. These models are often used as evidence for PCP regulation of ciliogenesis, but it should be noted that these genes have major roles in regulation of the cytoskeleton. Thus, the defects in ciliogenesis may be due to the cytoskeleton abnormalities that impair movement of centrioles/basal bodies to the apical surface.

The Cilium in Human Health

The recent intense interest in cilia stems from the finding that mutations in cilia proteins are the basis for many human genetic disorders collectively termed 'ciliopathies'. The list of ciliopathies and their associated genes continues to grow. Remarkably, the clinical features associated with these disorders are extremely diverse and include randomization of the left-right body axis, abnormalities in neural tube closure and patterning, polydactyly, cystic kidney, liver, and pancreatic diseases, retinal degeneration, anosmia, cognitive defects, and obesity [4]. Several groups have pursued *in silico*, biochemical, and comparative genomics approaches to further identify the components of the cilia proteome [93–99] The subsequent mining and meta-analyses of the data have begun to reveal the complexity of the cilium (For some of the publicly available databases, see: http://www.sfu.ca/~leroux/ciliome_database.htm and http://www.ciliaproteome.org). Furthermore, these datasets have contributed to a dramatic increase in the rate of identification of human ciliopathy disease loci [100].

Some interesting observations are emerging from the study of ciliopathies: while they are clinically distinct syndromes, their genetics suggest a different story. In fact, a specific mutation in one cilia gene may present as one clinical disorder while a different mutation in the same gene will give a different clinical outcome, suggesting that the severity of the mutations may have important implications for clinical presentation.

In addition, ciliopathies such as BBS may also arise through a triallelic mode of inheritance. BBS is a rare disorder characterized by polydactyly, retinal degeneration, obesity, and renal anomalies that can result from mutations in any of the 14 known BBS genes. Some BBS cohorts were identified that are homozygous for mutations in one BBS gene and also heterozygous for a mutation at another BBS gene. This triallelic status was found to affect clinical outcome [101–103]. It is not known whether this phenomenon will be seen in the other ciliopathies [104–106]. Secondary mutations or modifier loci could also account for the diverse phenotypes of ciliopathies. How these various mutations in cilia genes or background modifier loci alter the signaling pathways mentioned above is only beginning to be assessed. Due to the complexity of this question, these analyses are being conducted using model organisms such as zebrafish and *C. elegans*. These malleable systems have the advantage of knockdown approaches in which different mutant cilia genes can be analyzed in different combinations and their subsequent effects on phenotype severity or presentation determined.

Although ciliopathies are generally rare, their clinical features offer important opportunities to glean insights into more common disease processes. Obesity is one example of a common disease process associated with some ciliopathies. Specific disruption of cilia in adult mice leads to hyperphagia-induced obesity [32] and this effect could be narrowed to disruption of *ift88* in proopiomelanocortin (POMC) neurons in the hypothalamus. POMC neurons are responsive to several neuropeptides, including leptin, which elicits an anorexigenic response to reduce feeding behavior. These data raise the possibility that cilia are involved in the leptin pathway. This was recently supported by data showing that the BBS proteins are required for leptin receptor signaling in BBS mouse models [107].

Another possible connection between neuronal cilia and obesity came from studies demonstrating that the BBS proteins are required for normal localization of GPCRs in the cilium [108]. One such GPCR was the melanin-concentrating hormone receptor 1 (Mchr1), which has known roles in feeding behavior. The relevance of Mchr1 cilia localization remains to be determined. The work in all of these studies points to a role for neuronal cilia in reception or regulation of satiation signals; however, the mechanism of how cilia

regulate feeding behavior will be an exciting avenue of future research.

Despite the importance of cilia on specialized sensory neurons in chemosensation and mechanosensation in invertebrates such as *C. elegans* and *Drosophila*, mammalian neuronal cilia have been relatively ignored. It will be interesting to determine not only how cilia are specifically involved in neural development but whether they play diverse roles in the adult CNS. Some data already suggest that cilia are important for adult neurogenesis and indeed cilia are found on neural precursor cells [109]. One puzzling question is why neurons, which possess intricate processes such as axons and dendrites, need a primary cilium. The answer is not immediately intuitive, but it is enticing to speculate that the cilium ultimately plays a role in neuronal activity. In *Drosophila* and *C. elegans*, neuronal cilia often extend into specialized domains with distinct ECM environments created by support cells. It is not known whether similar structures are present in mammals. It will be of great interest to evaluate if cilia on mammalian CNS neurons have effects on behaviors such as addiction, stress responses, learning, memory, anxiety, activity, and aggression. Many of these possibilities are supported by phenotypes observed in human ciliopathy patients or mouse models. Whether these phenotypes arise due to developmental-related defects or as a direct role of cilia in regulating neuronal activity needs further assessment.

Conclusions

It has become apparent that the once underappreciated primary cilium is integrated with the activities of multiple cellular pathways. In certain contexts, it acts as a mechanosensor, in others it performs highly specialized sensory functions, and in yet other cells, the cilium acts as a signaling center that mediates cell-to-cell communication. How the cilium fulfills these diverse roles is not understood. Furthermore, not all cilia are created equal: their roles differ depending on the cell type and the signaling machinery expressed by that particular cell.

The complexity of this organelle and the wide array of sensory and signaling activities that it facilitates have raised many questions. For example, can our current understanding of cilia in olfactory signaling in mammals or mechanosensory signaling in lower eukaryotes serve as a guide for studying other cilia signaling events? Furthermore, signaling-protein localization to the cilium appears to be dynamic, as seen in the case of the Hh pathway. Will this emerge as a general theme in other cilia-mediated pathways? Also, it is not known what other signaling effectors and pathways are associated with cilia. The spectrum of phenotypes observed in the human ciliopathies and in model organisms argues that there are a large number of signaling pathways yet to be identified. Even in contexts where the roles of cilia are somewhat better defined, major questions remain; such as, how do changes in mechanical forces detected by the cilium result in changes in cell physiology and behavior? Pervading ideas in the field view the cilium as a sensory antenna, but it has yet to be determined if the cilium can also serve to transmit signaling information. One thing is certain: as research on the primary cilium progresses there will be more exciting and unexpected findings. Much work is required to fully appreciate the complex nature of this organelle and how it regulates the spectrum of events with which it is now known to be associated.

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