Intraflagellar Transport Proteins as Regulators of Primary Cilia Length

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Primary cilia are small, antenna-like organelles that detect and transduce chemical and mechanical cues in the extracellular environment, regulating cell behavior and, in turn, tissue development and homeostasis. Primary cilia are assembled via intraflagellar transport (IFT), which traffics protein cargo bidirectionally along a microtubular axoneme. Ranging from 1 to 10 μm long, these organelles typically reach a characteristic length dependent on cell type, likely for optimum fulfillment of their specific roles. The importance of an optimal cilia length is underscored by the findings that perturbation of cilia length can be observed in a number of cilia-related diseases. Thus, elucidating mechanisms of cilia length regulation is important for understanding the pathobiology of ciliary diseases. Since cilia assembly/disassembly regulate cilia length, we review the roles of IFT in processes that affect cilia assembly/disassembly, including ciliary transport of structural and membrane proteins, ectocytosis, and tubulin posttranslational modification. Additionally, since the environment of a cell influences cilia length, we also review the various stimuli encountered by renal epithelia in healthy and diseased states that alter cilia length and IFT.

Keywords: IFT-B, IFT-A, ciliogenesis, cilia disassembly, ectocytosis, posttranslational modification, kidney

INTRODUCTION

Cilia or flagella are evolutionarily conserved organelles that protrude from a wide range of eukaryotic cells, from single-celled protists, like *Chlamydomonas reinhardtii*, to almost every vertebrate cell. Cilia are classified by function and structure into two general categories: motile and non-motile. Motile cilia not only generate motion but also contain receptors that provide sensory function (Jain et al., 2012). Motile cilia propel *C. reinhardtii*, as well as sperm, and sweep fluid and particles along the mammalian brain ventricles and respiratory and reproductive tracts. Non-motile cilia, also termed primary cilia, have chemo- and mechanosensory roles and are present on the sensory neurons of *Caenorhabditis elegans* and on most vertebrate cells. Primary cilia transduce light and mechanical and chemical cues (Poole et al., 1985; R Ferreira et al., 2019), mediate signaling pathways (Huangfu et al., 2003; Corbit et al., 2008; Wheway et al., 2018), and regulate cell cycle (Pan et al., 2013), cell differentiation (Ezratty et al., 2011; Forcioli-Conti et al., 2015), cell–cell communication (Viau et al., 2018), and autophagy (Pampliega et al., 2013; Orhon et al., 2016). The multiplicity and importance of these functions render primary cilia vital for organismal development and homeostasis (Badano et al., 2006).

A cilium is comprised of a microtubule-based axoneme that extends from a modified centriole, the basal body, and is ensheathed by a ciliary membrane (**Figure 1**). In most motile cilia, nine doublet peripheral microtubules are arranged in a circle around two single central microtubules, while in motile cilia of the embryonic node as well as in non-motile cilia, only nine doublet peripheral microtubules comprise the axoneme, forming "9 + 2" and "9 + 0" conformations, respectively (Satir, 2005). Distal to the basal body is the ciliary gate, consisting of transition fibers that join the basal body to the base of the ciliary membrane, and the transition zone, comprised of modules and has Y-links that tether the most proximal part of the axoneme to the ciliary membrane. The transition fibers and transition zone regulate ciliary entry and exit of proteins, allowing for compartmentalization and formation of a unique environment, such that the cilioplasm and ciliary membrane are composed of proteins, second messengers, and phospholipids, distinct from that of the cytosol and plasma membrane (Deane et al., 2001; Stephan et al., 2007; Garcia-Gonzalo et al., 2011; Williams et al., 2011; Garcia-Gonzalo and Reiter, 2017; Gonçalves and Pelletier, 2017).

Primary cilia are dynamic structures that assemble and disassemble in coordination with the cell cycle. Cilia form when cells become quiescent, in G1 and G0, and begin disassembly before cells re-enter the cell cycle (Nigg and Stearns, 2011; Paridaen et al., 2013; Malicki and Johnson, 2017). The assembly and maintenance of cilia require intraflagellar transport (IFT), which was first observed in *C. reinhardtii* (Kozminski et al., 1993) and mediates the bidirectional transport of structural and signaling molecules along the microtubular axoneme. IFT is mediated by multiprotein complexes that can be dissociated biochemically into IFT complexes B (IFT-B) and A (IFT-A), consisting of 10 and 6 subunits, respectively. These IFT complexes form linear arrays or "trains" that are transported from the base to the tip of the cilium in anterograde IFT, powered by the kinesin-2 motor (Cole et al., 1998), then returned to the ciliary base in retrograde IFT, driven by cytoplasmic dynein-2 (Pazour et al., 1998, 1999; Porter et al., 1999; Signor et al., 1999). Another multiprotein complex, the BBSome, acts like an adaptor connecting IFT complexes to signaling molecules and is required for the ciliary export of activated signaling receptors (Nachury, 2018; Ye et al., 2018).

Primary cilia typically obtain a characteristic length for a cell type (**Table 1**), likely to achieve optimal function. In humans, mutation of ciliary genes results in disease syndromes, termed ciliopathies, which can manifest craniofacial defects, skeletal dysplasia, brain and cognitive defects, retinal degeneration, obesity, and fibrocystic disease of the liver, pancreas, and kidney (Badano et al., 2006; Hildebrandt et al., 2011). These mutations can cause signaling defects as well as cilia length differences (Bredrup et al., 2011; Halbritter et al., 2013; Alazami et al., 2014; Zhang et al., 2016; Duran et al., 2017; Shaheen et al., 2020). Additionally, in complex diseases and conditions not caused by a primary cilia genetic lesion, such as in obesity and type 2 diabetes and kidney injury, cilia lengths have also been reported to be shortened or lengthened on affected cells (Verghese et al., 2008; Han et al., 2014; Ritter et al., 2018; Yu et al., 2019). Thus,

understanding cilia length regulation is critical to understanding the pathobiology of cilia-related disease.
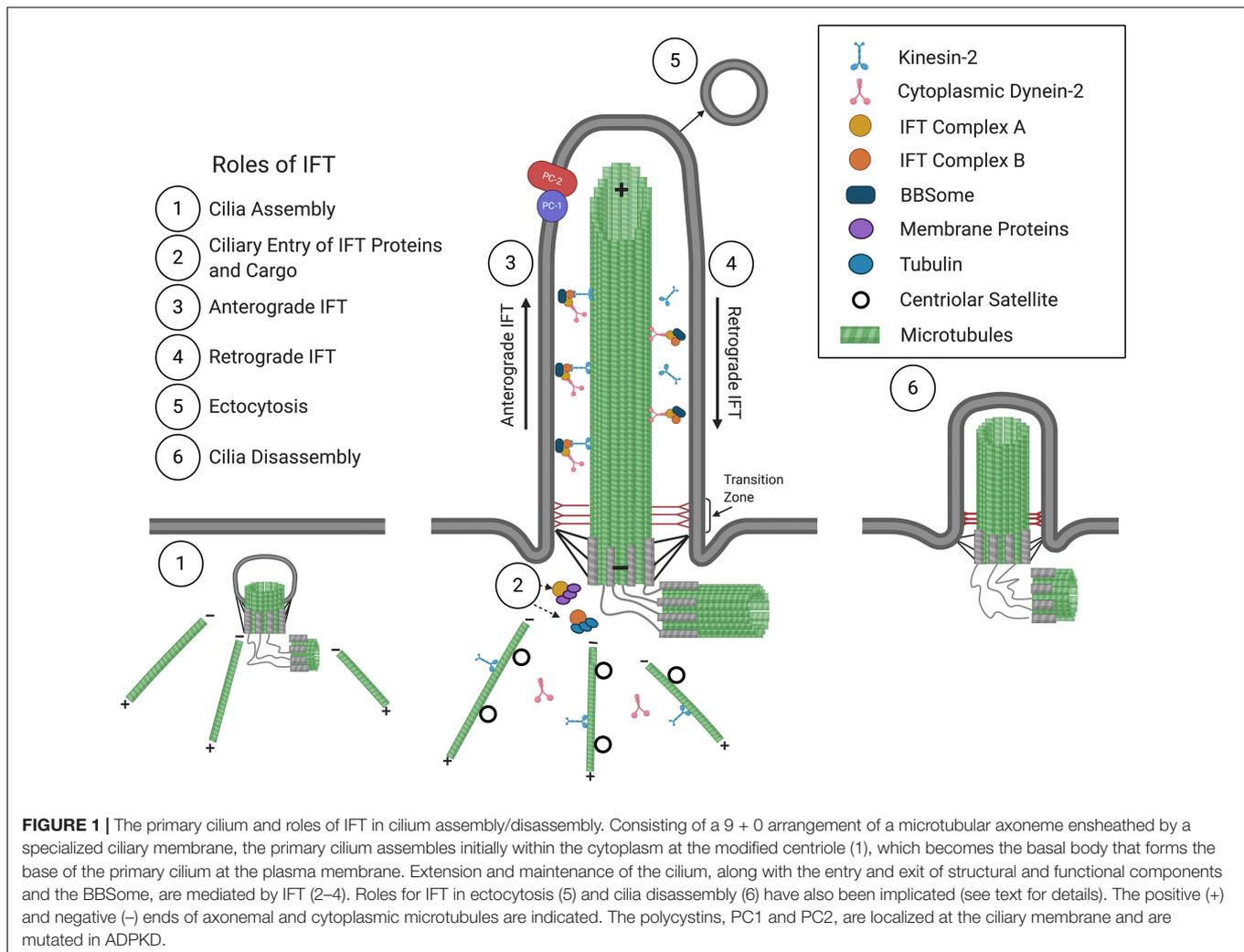
Cilia length is determined by the balance of cilia assembly and disassembly (Marshall and Rosenbaum, 2001; Marshall et al., 2005). In *C. reinhardtii*, live imaging has revealed that frequency of IFT train ciliary entry, IFT train size and speed, and cargo loading vary with cilia length, which has led to various models of cilia length control (Marshall et al., 2005; Wren et al., 2013; Chien et al., 2017; Fai et al., 2019; Wemmer et al., 2020). However, primary cilia length regulation in mammalian cells has been much less studied. Here, we review the primary cilia phenotypes of IFT-B and IFT-A mammalian mutants to glean mechanisms by which IFT proteins influence cilia assembly/disassembly. This includes roles of IFT in ciliary trafficking of tubulin and membrane-associated proteins and in influencing ectocytosis. Since posttranslational modification of axonemal tubulin can promote cilia assembly or disassembly, we also review the effects of glutamylation and O-GlcNAcylation on IFT. In addition to these intrinsic ciliary factors, primary cilia lengths are modulated by changes in the extracellular environment. In the kidney, cilia lengths change in healthy and diseased states, including in polycystic kidney disease and during kidney injury and repair. Thus, we also review the effects of chemical and mechanical signals in the renal environment on cilia length and IFT.

IFT IN CILIARY IMPORT OF STRUCTURAL AND MEMBRANE PROTEINS AFFECTING CILIOGENESIS

To initiate ciliogenesis, the mother centriole matures into the basal body and migrates and docks at the plasma membrane. During migration of the mother centriole, preciliary vesicles derived from the Golgi and recycling endosome attach to the subdistal appendages of the maturing mother centriole and fuse into a larger ciliary vesicle (Sorokin, 1962). Centriolar coiled coil protein 110 (CP110) localizes to the distal end of the mother centriole and regulates the start of cilium extension (Chen et al., 2002; Yadav et al., 2016). Rab8a is recruited to the mother centriole and activated by Rab11 and Rabin8 to enable ciliary membrane assembly (Westlake et al., 2011). This, together with the recruitment of IFT and transition zone proteins to the cilia base, allows for cilium extension (Deane et al., 2001; Rosenbaum and Witman, 2002; Wang and Dynlacht, 2018).

Ciliary Import of Tubulin

To extend the axoneme, α- and β-tubulin are imported into primary cilia and are added to the distal plus ends of microtubules at the cilia tip (Witman, 1975; Johnson and Rosenbaum, 1992). Live imaging of green fluorescent protein (GFP)-tagged tubulin in *C. reinhardtii* demonstrates that both diffusion and IFT allow for tubulin ciliary import (Craft et al., 2015; Craft Van De Weghe et al., 2020). In cells with growing cilia, anterograde transport of tubulin was increased, and in flagella length mutants, tubulin transport was dysregulated (elevated and reduced in *long flagella2-1* and *short flagella2* mutants, respectively, relative to steady-state wild-type cilia), suggesting a possible link between

FIGURE 1 | The primary cilium and roles of IFT in cilium assembly/disassembly. Consisting of a 9 + 0 arrangement of a microtubular axoneme ensheathed by a specialized ciliary membrane, the primary cilium assembles initially within the cytoplasm at the modified centriole (1), which becomes the basal body that forms the base of the primary cilium at the plasma membrane. Extension and maintenance of the cilium, along with the entry and exit of structural and functional components and the BBSome, are mediated by IFT (2–4). Roles for IFT in ectocytosis (5) and cilia disassembly (6) have also been implicated (see text for details). The positive (+) and negative (–) ends of axonemal and cytoplasmic microtubules are indicated. The polycystins, PC1 and PC2, are localized at the ciliary membrane and are mutated in ADPKD.

IFT-mediated transport of tubulin and regulation of cilia length (Craft et al., 2015; Wemmer et al., 2020).

In most IFT-B mutants, cilia are shortened or even lost (**Table 2**), indicating that the IFT-B complex is essential for ciliogenesis. The IFT-B complex consists of a 10-subunit core subcomplex and a 6-subunit peripheral subcomplex (IFT38, IFT57, IFT80, IFT20, IFT172, IFT54) (Katoh et al., 2016; Taschner et al., 2016). The core can be separated further into Core 1 (IFT25, IFT27, IFT74, IFT81, IFT22) and Core 2 (IFT56, IFT46, IFT52, IFT88, IFT70) subcomplexes (Nakayama and Katoh, 2020). Except for IFT74, all IFT-B components have been knocked out or mutated in mammalian cells with primary cilia (**Table 2**). Generally, loss of most Core 2 or peripheral subunits, with exception of IFT56, results in severely shortened or absent primary cilia. In contrast, deletion of Core 1 subunits has much milder effects, with many mutants lacking overt cilia length defects, although ciliary localization of signaling molecules and membrane-associated proteins is aberrant. Mammalian IFT70A and IFT70B constitute the orthologs of *C. reinhardtii* IFT70. Loss of both IFT70A and IFT70B in retinal pigment epithelial (RPE) cells causes the absence of cilia, while re-expression of either

IFT70A or IFT70B restores ciliogenesis, revealing redundancy between IFT70A and IFT70B (Takei et al., 2018). In certain cases, cilia phenotypes are more severe in *C. reinhardtii* than in mammalian cells. For instance, IFT56-deficient *C. reinhardtii* have shortened cilia (Ishikawa et al., 2014), but *Ift56*-null mice do not (Xin et al., 2017). This may reflect greater functional redundancy among mammalian IFT proteins. Furthermore, deletion versus deficiency of an IFT protein can result in different ciliary phenotypes. Depletion of IFT80 in C3H10T1/2 mesenchymal cells causes a lack of cilia (Yang and Wang, 2012), while hypomorphic mutation of *Ift80* in mice results in normal cilia morphology (Rix et al., 2011), suggesting a threshold of IFT deficiency which can be tolerated.

Given that axonemal elongation requires anterograde transport of tubulin (Craft et al., 2015), the ciliogenesis defects of IFT-B mutants could result from the lack of IFT-based transport of tubulin. In *C. reinhardtii*, the N-termini of IFT74 and IFT81 dimerize and bind α- and β-tubulin as cargo of anterograde IFT (Bhogaraju et al., 2013; Kubo et al., 2016). Additionally, IFT proteins are designed to form protein–protein interactions, and the loss of an IFT-B subunit can cause destabilization of the

TABLE 1 | Mammalian cilia lengths.

Cell type			Average cilia length, mm (range)	References
Renal epithelia	Collecting ducts, 6 wks		~4	Tran et al., 2014
	Tubules distal to the proximal tubule, P7		3.5 ± 1.7	Pazour et al., 2000
Cholangiocyte	5–12 months		3.26 ± 1.29	Stroope et al., 2010
Neuron	Hypothalamic arcuate nucleus, 21–30 weeks		~3.5	Lee et al., 2020
	Hypothalamus	E12.5, E15.5	~0.5	
		E18.5 P1	~1	
		P7	~1.5	
		P14	~2	
		P28, P60	~3.5	
	Hippocampal dentate gyrus, P14		~2.8	
	Hippocampus, P15		~3.2	
	Cerebellum, 3–5 months		~3.8 (1–8)	Diaz et al., 2020
Neural tube	E9.5		~1 (0.5–2)	Nandadasa et al., 2019
Osteocyte	*In vivo*	1 month	2.3 (1.5–3.4)	Lim et al., 2020
	In vitro	MLO-Y4 cells	~2.8	Spasic and Jacobs, 2017
Osteoblast	*In vivo*	1 month	2.9 (1.4–4.3)	Lim et al., 2020
	In vitro	Primary osteoblasts, P0	~2.6	Teves et al., 2015
		MC3T3-E1	3 ± 0.8 (1–5)	Li et al., 2020
Chondrocyte	*In vivo*	E16.5	1.20 ± 0.01	Martin et al., 2018
		P1	1.19 ± 0.02 (1–1.7)	Kunova Bosakova et al., 2018
		P3	~1.6 (1–3)	
		P5	~1.9 (1–3)	
	In vitro	Primary chondrocytes, E16.5	2.82 ± 0.05	Martin et al., 2018
		Primary chondrocytes, P0, P7	~3	Teves et al., 2015; Liu et al., 2020
Endothelia	*In vitro*	Primary endothelial cells, E15.5	~0.85	Abdul-Majeed and Nauli, 2011

Values are of mouse tissues or cells. ~ Indicates values were obtained from a graph within the publication. P, postnatal day; E, embryonic day; wks, weeks of age.

IFT-B complex. The molecular architecture of the IFT-B complex appears conserved between *C. reinhardtii* and mammalian cells (Katoh et al., 2016). IFT88, together with IFT52, connects the IFT-B core and peripheral complexes (Taschner et al., 2016). In fibroblasts of a ciliopathy patient with short rib polydactyly, mutation of *IFT52* greatly reduced IFT52 protein levels, leading to a destabilized anterograde IFT complex, demonstrated by reduced cellular levels of IFT88, IFT74, IFT81, and ADP ribosylation factor like GTPase 13B (ARL13B), a ciliary membrane protein, as well as reduced IFT88 in cilia (Zhang et al., 2016). This anterograde IFT defect caused the presence of less ciliated cells and irregular distribution of ciliary lengths in patient cells.

Beyond the role of IFT-B subunits to form the IFT-B complex, IFT-B proteins also connect the IFT-B and IFT-A complexes, as well as anterograde and retrograde IFT. In *C. reinhardtii*, IFT74 was shown to associate IFT-B and IFT-A particles at the flagellar base and to be essential for flagellar import of IFT-A. Loss of the IFT74 residues required to bind IFT-A caused stunted cilia, thus revealing a role for the interdependence between IFT-B and IFT-A in ciliogenesis (Brown et al., 2015). Additionally, in mammalian retinal pigment epithelial (RPE) cells, expression of a truncated form of IFT88 on a CRISPR/Cas9-mediated IFT88 knockout background produced a ciliary phenotype similar to IFT-A knockout cells (Kobayashi et al., 2021). In *C. reinhardtii* and in mice, *Ift54*-null mutants lack cilia (Berbari et al., 2011; Zhu

et al., 2017b), and a recent study showed that in *C. reinhardtii* and mammalian cells, IFT54 interacts with both the kinesin-2 and dynein motors (Zhu et al., 2020). Deletion of the IFT54 residues required to bind kinesin-2 reduced anterograde IFT, causing IFT motors and proteins to accumulate in the proximal region of cilia, while deletion of the residues that bind dynein impaired retrograde IFT, causing accumulation of IFT proteins at the distal tip (Zhu et al., 2020). Thus, IFT-B and IFT-A as well as anterograde and retrograde transport are interconnected, and these interconnections are integral to ciliogenesis.

Ciliary Import of Membrane-Associated Proteins

The IFT-A complex consists of three core subunits (IFT122/IFT140/IFT144) and three peripheral subunits (IFT42/IFT121/IFT139) (Nakayama and Katoh, 2020). In mammals, the IFT139 homolog consists of two paralogs, THM1/TTC21B and THM2/TTC21A (Tran et al., 2008; Wang et al., 2020). With the exception of THM2, loss of any IFT-A core or peripheral subunit can result in shortened cilia with bulbous distal tips (**Table 3**). The severity of cilia phenotypes varies with cell type and/or *in vitro* or *in vivo* contexts. Additionally, as observed with IFT56, the occurrence of a more severe phenotype – loss of cilia – in *ift140*-null *C. reinhardtii* mutants (Picariello et al., 2019) compared to shortened cilia in

TABLE 2 | Mammalian IFT-B ciliary phenotypes.

IFT-B	Cilia structure	Cell type	Cilia localization of proteins	References
Core 1				
Ift25 ko	No cilia length defects	MEF	Less GLI2 at ciliary tip, increased ciliary PTCH1 and SMO	Keady et al., 2012
Ift27/Rabl4/Bbs19 ko	No cilia length defects	MEF, primary dermal fibroblasts	Increased ciliary SMO; diminished GLI2 at ciliary distal tip; decreased BBS and Arl6	Eguether et al., 2014; Liew et al., 2014; Yang et al., 2015
Ift81 (deficiency)	Increased cilia length	Patient chondrocytes	None reported	Duran et al., 2016
Ift81 loss-of-STOP mutation	Reduced ciliated cells and reduced number of cilia longer than 3 mm	Patient fibroblasts	No abnormalities in IFT or Hh protein ciliary localization but increased mRNA expression of *Gli2*	Perrault et al., 2015
Ift22/Rabl5 ko	No cilia length defects	RPE		Takei et al., 2018
Core 2				
Ift56[hop/hop] (null) or ko	No cilia length defects but reduced numbers of microtubule doublets and disrupted circular arrangement of microtubules	Neural tube, RPE	Reduced GLI2 and GLI3 at ciliary tip; no KIF17 at ciliary tip	Funabashi et al., 2017; Xin et al., 2017
Ift46 ko	Loss of nodal cilia	Mouse node		Lee et al., 2015
Ift52 (deficiency)	Shortened cilia but wider range of cilia lengths	Patient fibroblasts	Reduced IFT88	Zhang et al., 2016
Ift88 ko	No cilia	Mesenchymal cells, renal epithelial cells, bone cells		Pazour et al., 2000; Haycraft et al., 2007
Ift70A;Ift70B dko	No cilia	RPE		Takei et al., 2018
Peripheral				
Ift38/Cluap1 ko	No cilia	MEF, node		Botilde et al., 2013
Ift57 deficiency	No cilia length defects	Patient fibroblasts	Altered ciliary distribution pattern of Ift57	Thevenon et al., 2016
Ift80 ko	No cilia	C3H10T1/2 mesenchymal cells		Yang and Wang, 2012
Ift80[gt/gt] hypomorph	No cilia length defects	MEF, renal epithelial cells	None reported but decreased *Gli1* and *Ptch1* mRNA expression in response to Hh agonist	Rix et al., 2011
Ift20 strong kd/ko; mild kd	No cilia/less ciliated cells; no abnormalities	RPE, NIH/3T3	In mild *Ift20* kd, reduced ciliary polycystin 2	Follit et al., 2006
Ift172[wim/wim]; ko	No cilia or severely shortened	Mouse node, embryonic neuroepithelium		Huangfu et al., 2003; Gorivodsky et al., 2009
Ift54/Trap3ip1[GT/GT]	No cilia	MEF, neural tube		Berbari et al., 2011

ko, knockout; dko, double ko; kd, knockdown; gt or GT, gene trap; MEF, mouse embryonic fibroblasts; RPE, retinal pigment epithelial cells; Hh, Hedgehog.

mammalian cells (Liem et al., 2012; Hirano et al., 2017) supports that there may be greater redundancy among IFT proteins in mammalian cells or that other compensatory mechanisms exist.

Live imaging of short hairpin RNA (shRNA)-mediated *Thm1* knockdown inner medullary collecting duct (IMCD) cells expressing IFT88-eYFP demonstrates that a mammalian IFT-A subunit is required for retrograde IFT (Tran et al., 2008). Consistent with a general role for IFT-A in retrograde IFT, bulbous distal tips with sequestered IFT-B, IFT-A, BBS, and signaling proteins in mutants of both the IFT-A core and peripheral complexes indicate defective retrograde IFT (**Table 3**). Since retrograde IFT is required to bring back IFT proteins to the base for their recycling and re-entry into cilia, this could be a contributing mechanism by which IFT-A loss or deficiency decreases cilia assembly and, in turn, cilia length.

A comprehensive study examining ciliogenesis in *Ift121/Wdr35* knockout RPE cells showed that cilia assembly was reduced and delayed due to disruption of the ciliary import and export of the various cargoes of IFT-A (Fu et al., 2016). Rab8 localization near centrioles was reduced, suggesting that early formation of cilium membrane assembly is disrupted. Additionally, the localization of centriolar satellite proteins was misregulated. Centriolar satellites regulate protein composition of cilia and are essential for efficient ciliogenesis (Odabasi et al., 2019). Ciliary entry of ciliary membrane proteins, ARL13B and inositol polyphosphate-5-phosphatase E (INNP5E), was also impeded. This could contribute to impaired ciliogenesis, since ARL13B is essential for ciliary membrane extension, which is coupled to axoneme elongation (Lu et al., 2015). Ciliary ARL13B is also lost in *Ift144* knockout and *Ift43* knockdown cells and reduced in *Thm1*-null mouse embryonic fibroblasts (MEFs) (Liem et al., 2012; Fu et al., 2016; Wang et al., 2020). Furthermore, ARL13B was shown to bind to IFT43, IFT122, and IFT139/THM1,

TABLE 3 | Mammalian IFT-A ciliary phenotypes.

IFT-A	Cilia structure	Cell type	Cilia localization of proteins	References
Core				
$Ift144^{dmhd/dmhd}$ (null)	Extremely short cilia	Neural tube, MEF	No Arl13B, ACIII, or SMO entry; IFT-B accumulation	Liem et al., 2012
$Ift144$ ko or kd	Slightly shortened cilia with bulbous distal tip	RPE	Loss of IFT-A around basal body, no entry of SMO or ARL13B; IFT-B, BBS4 accumulation	Fu et al., 2016; Hirano et al., 2017
$Ift144^{twt/twt}$ hypomorph	No obvious length defects	Neural tube, MEF	Decreased ACIII; slightly increased GLI2 at ciliary distal tip	Liem et al., 2012
$Ift122^{sopb/sopb}$ (null)	Short with bulbous distal tip	Mouse node, MEF	Increased IFT-B, Gli2, Gli3 at distal tip; no TULP3	Qin et al., 2011
$Ift122$ ko	No cilia	RPE	Loss of IFT-A and GPR161 around basal body, normal IFT-B around basal body; no SMO entry	Takahara et al., 2018
$Ift140^{cauli/cauli}$	Short with bulbous distal tip	Limb buds		Miller et al., 2013
Peripheral				
$Ift121/Wdr35$ mutant and ko	Shortened cilia with bulbous distal tip	Patient fibroblasts; RPE	Increased BBS4 and BBS5, Gli2, IFT-B, IFT-A, Kif3A; no ARL13B, INNP5E, SSTR3, MCHR1, serotonin receptor; reduced SMO	Fu et al., 2016; Duran et al., 2017
$Ift43$ kd	Shortened cilia with bulbous distal tip	RPE	Increased IFT88, BBS4; no ARL13B	Fu et al., 2016
$Ift43$ deficiency	Shortened cilia	Patient fibroblasts		Duran et al., 2017
$Thm1/Ttc21b^{aln/aln}$ (null) or kd	Shortened with bulbous distal tip	Limb bud, MEF, 3T3-LT1, renal epithelial	IFT-B, IFT-A, BBS, SMO accumulation at distal tip; decreased Arl13b, INNP5E, IFT-A	Tran et al., 2008; Tran et al., 2014; Jacobs et al., 2020; Wang et al., 2020
$IFT139/Ttc21b$ ko	Normal cilia length with bulbous distal tip	RPE	IFT-B, IFT-A, SMO, GPR161 accumulation at distal tip	Hirano et al., 2017
$Thm2/Ttc21a$-null	Normal	MEF	Normal	Wang et al., 2020
Adaptor				
$Tulp3$ kd; cko and K407I (deficiency)	No length defects	RPE; renal epithelia	Decreased SSTR3 and MCHR1; severely reduced ARL13B, reduced polycystin 2	Mukhopadhyay et al., 2010; Hwang et al., 2019; Legue and Liem, 2019
$Tulp3$ ko	Shortened	RPE	No ARL13B or INNP5E	Han et al., 2019

SSTR3, somatostatin receptor 3; Mchr1, melanin-concentrating receptor 1; ACIII, adenylate cyclase type III.

suggesting that ARL13B is a cargo passenger of IFT-A (Fu et al., 2016).

Fu et al. (2016) proposed a unifying mechanism for the IFT-A mutant defects in ciliary entry of membrane proteins and in retrograde IFT: that IFT-A mutation causes a defect in trafficking cargo to the minus ends of both axonemal and cytoplasmic microtubules. In *Ift122* knockout RPE cells, IFT-A localization around the basal body was lost, suggesting that IFT122 transports IFT-A proteins to the cilium base (Takahara et al., 2018). Similarly, in *C. reinhardtii*, loss of IFT43 resulted in reduced IFT proteins in the peri-basal body region, and both IFT43 and IFT140 were demonstrated to transport ciliary proteins from the cytosol to the peri-basal body region (Zhu et al., 2017a). These data support a defect in transporting cargo to the minus ends of microtubules with loss of IFT-A.

Depletion of IFT-A also causes ciliary localization defects of the transmembrane Hedgehog transducer, Smoothened (SMO). Loss of IFT-A core components, IFT144 and IFT122, results in absence of SMO in cilia (Liem et al., 2012; Hirano et al., 2017; Takahara et al., 2018), while loss of peripheral subunit IFT121 results in reduced SMO in cilia (Fu et al., 2016). In contrast, loss of THM1 causes increased ciliary SMO and its accumulation at the distal tip (Wang et al., 2020). While ciliary localization defects of SMO do not overtly affect cilia length, these differential phenotypes may reflect differences in cargoes or protein interactions of core versus peripheral subcomplexes or of individual IFT proteins. Like IFT121, THM1 is part of the peripheral subcomplex, and except for the opposing SMO ciliary localization defect, *Thm1*-null MEF show ciliary protein localization defects as well as reduced and delayed ciliogenesis (Wang et al., 2020), similar to *Ift121*-depleted RPE cells (Fu et al., 2016). However, while contrasting from other IFT-A mutants, the increased ciliary SMO in *Thm1*-null MEF could be due to a similar mechanism that causes increased ciliary SMO in *Ift25*- and *Ift27*-null IFT-B Core 1 mutants. IFT25 and IFT27 form a heterodimer and connect IFT to the BBSome, which exports

signaling molecules out of primary cilia (Keady et al., 2012; Eguether et al., 2014; Liew et al., 2014).

In mammalian cells, the IFT-A core also binds to the tubby-related protein 3 (TULP3), which acts like an adaptor to import a subset of G protein-coupled receptors (GPCRs) into primary cilia. These GPCRs include melanin-concentrating hormone receptor (MCHR1), somatostatin receptor subtype 3 (SSTR3), GPR161, neuropeptide Y receptor 2 (NPY2R), and free fatty acid receptor 4 (FFAR4) (Mukhopadhyay et al., 2010, 2013; Badgandi et al., 2017; Hilgendorf et al., 2019). Consistent with the requirement of the IFT-A core for ciliary entry of TULP3, TULP3 was absent from cilia of an *Ift122*-null mutant mouse (Qin et al., 2011). *Tulp3* knockdown RPE cells, a *Tulp3* kidney-specific knockout mouse and a *Tulp3* hypomorphic mutant mouse showed a lack of ciliary ARL13B but normal cilia length (Mukhopadhyay et al., 2010; Hwang et al., 2019; Legue and Liem, 2019). However, *Tulp3* knockout RPE cells showed absent ciliary ARL13B as well as shortened cilia lengths (Han et al., 2019). Thus, cell specificity, extent of *Tulp3* depletion, and *in vitro* versus *in vivo* contexts, which would expose cilia to various extracellular factors, likely contribute to affecting cilia length. Unlike the loss of IFT-A core and peripheral subunits, *Tulp3* knockdown did not cause accumulation of ciliary IFT-B proteins (Mukhopadhyay et al., 2010), indicating Tulp3 is not required for retrograde IFT.

Since loss of many IFT-B proteins results in absence of primary cilia, the role of IFT-B in ciliary entry of membrane-associated proteins is comparatively less explored. IFT25 and IFT27, which are components of IFT-B Core 1, are dispensable for cilia formation but are required for ciliary removal of the BBsome and associated cargo (Keady et al., 2012; Eguether et al., 2014; Liew et al., 2014). Conversely, since ciliary entry of SSTR3 or SMO is not impeded in *Ift25*- or *Ift27*-null mutants, this may indicate that ciliary import of these receptors may not require IFT-B (Eguether et al., 2018; Ye et al., 2018). However, the role of other IFT-B proteins in SSTR3 and SMO ciliary import has not been investigated. Knockdown of *Ift57* and *Ift172* in IMCD cells severely reduced ciliogenesis, but in cells retaining primary cilia, D1-type dopaminergic receptors in the ciliary membrane was reduced, suggesting that IFT-B is required for ciliary import of certain membrane-associated signaling receptors (Leaf and Von Zastrow, 2015). This requirement for IFT-B in ciliary membrane import of D1-type dopaminergic receptors also involves Rab23 and Kif17. Supporting a role for IFT172 in associating with the membrane, *C. reinhardtii* IFT172 was shown to interact with and remodel membrane, and in mammalian (RPE) cells, IFT172 localized to the ciliary membrane (Wang et al., 2018).

IFT IN MODULATING ECTOCYTOSIS

Primary cilia can release small vesicles or ectosomes, containing components from the ciliary membrane in a process termed ectocytosis (Long and Huang, 2019). This secretion function of cilia was first discovered in *C. reinhardtii* and now has been observed in multiple species, including in *C. elegans*, *Trypanosoma brucei*, and in mammalian cells (Wood et al., 2013; Wang et al., 2014; Long et al., 2016; Szempruch et al.,

2016; Nager et al., 2017; Phua et al., 2017). Shedding of ciliary ectosomes or extracellular vesicles regulates ciliary compartmentalization and homeostasis, signaling, and organismal and intercellular communication.

There is some variation in the release of ciliary vesicles and their contents across organisms. In *C. reinhardtii*, ectosomes are released from the ciliary tip in a process that requires the endosomal sorting complex required for transport (ESCRT) pathway. *C. reinhardtii* ectosomes contain ciliary membrane proteins, enzymes, ubiquitinated proteins, and ESCRT proteins (Long et al., 2016). In *C. elegans*, sensory neurons release extracellular vesicles from the cilia base in a process that is ESCRT independent but requires IFT-B and IFT-A (Wang et al., 2014). These extracellular vesicles contain LOV-1 and PKD-2, the *C. elegans* polycystin orthologs, and regulate communication and mating-related behavior. In mammalian cells, ectocytosis occurs from the ciliary tip upon stimulation with growth factors, which changes the phospholipid content of the ciliary membrane via INNP5E (Phua et al., 2017). Mammalian ectosomes contain ciliary membrane proteins, GPCRs (Nager et al., 2017), as well as IFT-B proteins, which are not present in ciliary vesicles of *C. reinhardtii* and *C. elegans* (Phua et al., 2017). Ectocytosis is linked to cilia resorption and disassembly in *C. reinhardtii* and mammalian cells (Long et al., 2016; Phua et al., 2017), and the containment of IFT-B proteins in mammalian ectosomes may be a mechanism by which primary cilia dispose of IFT-B proteins and become primed for disassembly (Phua et al., 2017).

Studies with BBS and IFT mutants shed additional light on the regulation of ectocytosis. In IMCD cells null for regulators or subunits of the BBSome, such as IFT27, ARL6, or BBS2, failure of BBSome-mediated ciliary removal of activated GPCRs caused accumulation of active GPCRs at the ciliary tip, which was followed by ectocytosis (Nager et al., 2017). In *Thm1*-null MEF, which sequester proteins at the ciliary tip, serum stimulation following starvation caused an increased presence of IFT-B foci that were separate from and distal to the cilia tip (Wang et al., 2020). Such observations likely reflect ectocytosis. In RPE cells depleted of mitogen-activated protein kinase-like kinase, ICK, which binds to IFT-B and localizes at ciliary tips, cilia were lengthened with an accumulation of IFT and signaling proteins at the distal tip, indicative of impaired retrograde IFT. Furthermore, these accumulated proteins were released in extracellular vesicles at the distal tip, indicative of ectocytosis (Nakamura et al., 2020). Collectively, these studies suggest that defective retrograde IFT promotes ectocytosis.

Ectocytosis precedes cilium resorption and disassembly (Phua et al., 2017). Consistent with this pattern, *Thm1*-null MEF, which exhibit a phenomenon consistent with increased ectocytosis also show enhanced serum-induced cilia loss (Wang et al., 2020). Cilia disassembly can occur via cilia resorption, which shortens cilia length gradually; complete cilia shedding, which severs the entire cilium; and a combination of both. Complete cilia shedding was found to be the predominant mechanism of cilia disassembly in wild-type mammalian cells (Mirvis et al., 2019). Future investigation into the role of IFT in regulating cilia disassembly could deepen our understanding of why one mechanism prevails over another.

TUBULIN POSTTRANSLATIONAL MODIFICATION AND IFT

Posttranslational modification (PTM) of the axonemal microtubules affects their stability, promoting either cilia assembly or disassembly. Acetylation, glutamylation, and glycylation stabilize the axoneme, favoring cilia assembly (Pugacheva et al., 2007; Shida et al., 2010; Gadadhar et al., 2017; He et al., 2018). In contrast, ubiquitination destabilizes the axoneme, leading to cilia disassembly (Huang et al., 2009; Wang et al., 2019).

Studies also suggest that PTMs regulate IFT. In shortening flagella of *Chlamydomonas*, IFT139 was identified to be ubiquitinated and to interact with ubiquitylated α-tubulin, suggesting that the IFT-A complex may traffic ubiquitylated axonemal proteins out of flagella, thus contributing to cilia disassembly (Wang et al., 2019). In *C. elegans*, a *ccpp-1* deglutamylase mutant altered stability of the axonemal β-tubules and increased velocity of the kinesin 3 KSP-6 and kinesin 2 SOM-3/KIF17 accessory motors (O'hagan et al., 2011). Also in *C. elegans*, starvation activated tubulin glutamate ligase 4 (TTLL4), which increased glutamylation, as well as the speed of the kinesin II canonical motor and, hence, of anterograde IFT (Kimura et al., 2018).

Recently, N-acetylglucosamine (GlcNAc), a nutrient sensor and product of the hexosamine biosynthetic pathway that can be linked enzymatically to serine/threonine residues of a multitude of intracellular proteins (Hart et al., 2011), has been shown to regulate cilia length (Tian and Qin, 2019; Yu et al., 2019, 2020). Both tubulin and histone deacetylase 6 (HDAC6, which regulates cilia disassembly) (Pugacheva et al., 2007), are O-GlcNAcylated (Tian and Qin, 2019). In one study, pharmacological and genetic inhibition of O-GlcNAc transferase (OGT), which transfers the GlcNAc moiety onto protein substrates, increased cilia length in RPE and IMCD cells (Tian and Qin, 2019). In another study, tissues (retinal photoreceptors, trachea) of *Ogt* knockout mice and RPE cells treated with an OGT inhibitor displayed shortened primary cilia with an accumulation of IFT proteins in bulbous distal tips, suggesting impaired retrograde IFT (Yu et al., 2020). While more investigations are required to reconcile these opposing results, these studies indicate that O-GlcNAcylation is another PTM affecting cilia length and IFT.

Together, these studies hint at a role for IFT in mediating the cilium assembly/disassembly associated with a particular PTM. Live imaging of IFT in mammalian cells following a PTM event is required to establish that PTMs affect IFT and to explore how PTMs regulate the IFT machinery.

MODULATION OF RENAL EPITHELIAL CILIA LENGTH AND RENAL FUNCTION

In a context-dependent manner, primary cilia demonstrate multiple roles. These organelles sense and transduce light, chemical, and mechanical cues and are hubs for multiple signaling pathways, including Hedgehog, Wnt, Notch, transforming growth factor beta (TGFβ), platelet-derived growth factor receptor (PDGFR), and G protein coupled receptor (GPCR) (Huangfu et al., 2003; Schneider et al., 2005; Eggenschwiler and Anderson, 2007; Corbit et al., 2008; Ezratty et al., 2011; Clement et al., 2013; Anvarian et al., 2019). Activation or downregulation of these signaling pathways regulates cell behavioral response, including proliferation, differentiation, and tissue organization. Mutation of ciliary genes reveals importance of primary cilia in multiple organ systems. The presence or absence as well as severity of phenotypes or clinical manifestations reflect the cell-specific and developmental and homeostatic roles of primary cilia.

Among the first revelations that primary cilia are essential to mammalian health was the discovery that mutation of IFT causes renal cysts (Yoder et al., 1995, 2002; Pazour et al., 2000). In renal tubules, cilia extend from the apical surface of most epithelial cells, suggesting that the role of renal tubular epithelial cilia is to sense the environment of the renal tubular lumen and transmit this information into the epithelium and possibly to "downstream" tubule segments (via ectosomes). Analyses of human kidneys reveal that cilia lengths change with nephron segment, as well as with development, being shortest in renal vesicles (0.59 μm) and lengthening as fetal nephrons mature (3.04 μm) (Saraga-Babic et al., 2012). Additionally, mutation of genes required for ciliogenesis usually causes shortened or absent cilia, and less commonly lengthened cilia, and often results in fibrocystic renal disease (Davis et al., 2011; Srivastava et al., 2017). In mice, the mutation and/or deletion of IFT genes models this phenomenon (Yoder et al., 1995, 2002; Pazour et al., 2000; Jonassen et al., 2008, 2012; Patel et al., 2008; Tran et al., 2014). Together, these findings indicate that the presence and architecture of primary cilia regulate kidney development, morphology, and function.

In autosomal dominant polycystic kidney disease (ADPKD), the majority of mutations occur in *PKD1* and *PKD2*, which encode polycystin 1 and polycystin 2, which form a complex and function at the primary cilium (Freedman et al., 2013; Cai et al., 2014; Walker et al., 2019). In renal tissue of ADPKD patients as well as of orthologous mouse models, cilia lengths are increased (Hopp et al., 2012; Liu et al., 2018; Shao et al., 2020). Similarly, in the *jck* non-orthologous mouse model of ADPKD, renal primary cilia are also lengthened (Smith et al., 2006). Interestingly, deletion of *Kif3a*, *Ift20*, and *Ift88* in *Pkd1* and *Pkd2* conditional knockout mice ablated or shortened primary cilia and markedly attenuated the ADPKD cystic phenotype (Ma et al., 2013; Viau et al., 2018; Shao et al., 2020). Similarly, pharmacological shortening of primary cilia (via a CDK5 inhibitor) in *jck* mice ameliorated the cystic disease (Husson et al., 2016). Conversely, genetic and pharmacological inhibition of cilia disassembly in *Pkd1* conditional knockout mice increased renal cilia lengths and worsened ADPKD severity (Nikonova et al., 2014). These data suggest that cilia length may be an important modifier of ADPKD severity.

Renal epithelial cilia lengths also change during acute and chronic kidney injury and repair. A variety of methods have been employed to induce renal injury in both *in vitro* and *in vivo* model systems (**Table 4**). Acute injury, or the exposure to hypoxia to model chronic injury, appears to cause deciliation

TABLE 4 | Modulators of renal cilia length.

	Cilia length	Cell type	Mechanism or cellular effect	References
In vitro				
↓ intracellular Ca^{2+}	↑	IMCD, MEK, BME	Increased anterograde IFT speed	Besschetnova et al., 2010
↑ intracellular cAMP	↑	IMCD, MEK, BME	Activated PKA; increased anterograde IFT speed	Besschetnova et al., 2010
Fluid flow	↓	IMCD, MEK, BME	↓ cAMP; decreased cAMP and cilia length response abolished by *Pkd1* and *Pkd2* kd or mutation	Besschetnova et al., 2010
H_2O_2 (reactive oxygen species)	Deciliation	MDCK		Kim et al., 2013
Dopamine	↑	LLC-PK1	Increased cilia length increases intracellular Ca^{2+} response to fluid shear stress	Upadhyay et al., 2014
Cobalt chloride (hypoxia)	↑	MDCK	Increased HIF1-alpha	Verghese et al., 2011
aldosterone	↑	mCCD	Reduced degradation of IFT88	Komarynets et al., 2020
In vivo				
Pkd1^{RC/RC}; *Pkd1* ko or *Pkd2* ko; *Pkd1^{RC/RC}*:*Pkhd1* ko	↑	Collecting duct; cyst-lining renal epithelia		Hopp et al., 2012; Liu et al., 2018; Olson et al., 2019
Nek8^{jck/jck} mutant	↑	Cyst lining renal epithelia		Smith et al., 2006
Ischemia	↓ 1 day after; ↑ 8 days after	Bowman's capsule parietal epithelium, proximal tubule, collecting duct	Deciliation	Verghese et al., 2008; Kim et al., 2013
RBPj ko (Notch signaling downregulation)	↑	Cystic renal epithelia of developing kidney	Increased Akap12, a scaffolding protein that interacts with PKA, cyclins, and protein kinase C	Mukherjee et al., 2020
Hypoxia (chronic renal injury)	↑	Fetal ovine renal epithelia	Increased cilia length increased intracellular Ca^{2+} response to fluid shear stress	Shamloo et al., 2017
Mineralocorticoid receptor ko	↓	Distal nephron	Inability to respond to aldosterone	Komarynets et al., 2020

IMCD, mouse inner medullary collecting duct cells; MEK, mouse embryonic kidney cells; BME, bone mesenchymal cells; MDCK, Madin–Darby Canine Kidney cells; LLC-PK1, Lilly Laboratories Cell-Porcine Kidney 1 cells; mCCD, murine cortical collecting duct cells.

followed by elongation of cilia and eventual return to normal cilia length. Such alterations in cilia length are proposed to alter cilium sensing function and to form part of an epithelial repair mechanism whereby cilia regulate changes in signaling pathways appropriate for normal repair of injured renal tubules (Verghese et al., 2019). This dynamic modulation of renal cilia lengths during renal injury and repair suggests that cilia lengths are tuned to maintain normal renal tubular structure and function (Verghese et al., 2008, 2009; Han et al., 2017; Park, 2018).

Cellular signaling pathways and signals from the local environment modulate cilium length. As renal primary cilia protrude into the tubular lumen, cilia are exposed to filtrate flow. In response to fluid flow, primary cilia of cultured renal epithelial cells deflect, and intracellular calcium (Ca^{2+}) increases (Praetorius and Spring, 2001, 2003). In cells that are stimulated resulting in lengthened cilia, the increase in intracellular Ca^{2+} induced by fluid flow is elevated, indicating that increased cilia length increases sensitivity to fluid flow (Upadhyay et al., 2014; R Ferreira et al., 2019). Conversely, in cells mutant for *Pkd1* or *Pkd2*, the increased intracellular Ca^{2+} response to fluid flow is abrogated (Nauli et al., 2003). In ADPKD cells, intracellular Ca^{2+} is decreased, and intracellular cyclic AMP (cAMP) is increased (Yamaguchi et al., 2004).

Treatments to decrease intracellular Ca^{2+} and to elevate intracellular cAMP of cultured IMCD cells increased primary cilium lengths (Besschetnova et al., 2010). Using live microscopy, the increase in cilia length coincided with increased velocity of anterograde IFT. Additionally, fluid shear-mediated deflection reduced cilia length, decreased intracellular cAMP, and reduced the intracellular Ca^{2+} response to fluid flow, indicating the presence of a negative feedback loop. In *Pkd1-* or *Pkd2*-deficient cells, the Ca^{2+} response to fluid flow was abolished, and so was the negative feedback loop. In the absence of fluid flow, cultured *Pkd1* MEF and renal epithelia also displayed reduced cilia disassembly via disruption of a centrosomal integrity pathway mediated by p53 (Gerakopoulos et al., 2020). In contrast, Streets et al. reported shortened primary cilia in *Pkd1*-mutant renal epithelial cells via increased actin polymerization mediated by RhoA (Streets et al., 2020). These varied results may reflect that the altered cilia lengths are captured at a particular time point during dynamic modulation of cilia length by these individual pathways. Together, these studies demonstrate that regulation of cilia length and cilia-mediated function is tightly connected. Furthermore, the disruption of this connection in ADPKD suggests that this connection may be critical to staving off disease.

Extrinsic factors that regulate kidney homeostasis have also been shown to alter cilia length. Dopamine activates the intrarenal dopaminergic pathway to reduce salt and water reabsorption by the kidney, and dysregulation of intrarenal dopaminergic signaling increases risk for essential hypertension (Harris and Zhang, 2012). Treatment of LLC-PK1 porcine kidney epithelial cells with dopamine and fenoldopam to activate ciliary-localized dopamine receptor type-5 increased cilia length (Upadhyay et al., 2014). Fenoldopam caused a greater increase in cilia length than dopamine, and increased cilia length correlated with the sensitivity of the cells to fluid shear stress as measured by the increase in intracellular Ca^{2+}, substantiating the interconnection between cilia length modulation and ciliary signaling. Aldosterone, a mineralocorticoid steroid hormone that is produced by the adrenal cortex, conserves sodium in the kidney. In a mineralocorticoid knockout mouse, cilia of multiple tubule types were shortened. Explaining this result, treatment of cultured cortical collecting duct cells with aldosterone increased primary cilia length, and this increase correlated with intensity of the transepithelial Na+ transport and was demonstrated to occur via reduced degradation of IFT88 (Komarynets et al., 2020). Thus, modulation of cilia length is inherent to kidney homeostasis and physiology.

DISCUSSION

Primary cilia lengths differ with cell type, developmental stage, disease state, and in response to injury or repair. Multiple intrinsic (IFT and ciliary proteins) and extrinsic factors contribute to modulating cilia length, and these factors are likely intertwined to control cilia length and function. More analyses in mammalian cells are required to determine the extent to which mechanisms discovered in *C. reinhardtii* and other ciliated organisms are similar or diverge in mammalian cells. The redundancy of IFT proteins in mammalian cells suggests a means of additional species- or cell-specific roles for these proteins in regulating cilia length and function. High-resolution live microscopy of IFT and associated cargo in mammalian cells will be instrumental in obtaining new mechanistic insights.

Signaling events can affect cilia length, which in turn influences sensitivity to external factors, such as fluid flow in the kidney. Expanding our knowledge of the signaling pathways that influence cilia lengths and those that are affected by cilia length would contribute to a regulatory network of cilia length control. Since studies suggest that cell type and *in vitro* versus *in vivo*

contexts can produce differential results, expansion of cell types and development of *in vitro* models that more closely mimic the *in vivo* setting, such as 3D models and cultures that implement multiple cell types, may help to reconcile these differences.

Given that rescue of the increased cilia length in ADPKD mouse models correlates with attenuation of the disease, could cilia length in certain cases be a therapeutic target? In a developmental study, the rescue of cilia length and structure of *Dync2h1*-null embryos via deletion of one allele of *Ift172* mitigated the Hh signaling defects and early embryonic lethality, suggesting that *Ift* mutations cause their phenotypes primarily by affecting cilia architecture rather than by directly regulating signaling (Ocbina et al., 2011). Since *Ift* genes have differential roles, determining how to rescue cilia architecture for each *Ift* gene deletion would require expanding digenic analyses to multiple *Ift* genes. Additionally, cancer studies show that drugs can have differential effects on cilia lengths of different cell types within a tissue, emphasizing cell-specific control of ciliogenesis (Kiseleva et al., 2019).

However, in ciliopathy patient cells, cilia length is not always altered, or inconsistencies exist in whether a gene mutation causes cilia to be lengthened or shortened. This could suggest that clinical manifestations are due to signaling defects and that cilia length alterations *per se* may not be the primary cause of perturbed signaling. Rather, IFT proteins may regulate both cilia architecture and signaling pathways. Technologies to target a signaling pathway of a specific cell (Shillingford et al., 2012) or cilia (Pala et al., 2019a,b) of a particular cell type may further help provide the molecular tools to treat cilia-related disease.

AUTHOR CONTRIBUTIONS

WW, RM, and PT wrote the first draft. HW constructed **Figure 1**. WW, BJ, HW, MK, RM, and PT edited the manuscript and approved the final version.

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REFERENCES

Abdul-Majeed, S., and Nauli, S. M. (2011). Dopamine receptor type 5 in the primary cilia has dual chemo- and mechano-sensory roles. *Hypertension* 58, 325–331. doi: 10.1161/hypertensionaha.111.172080

Alazami, A. M., Seidahmed, M. Z., Alzahrani, F., Mohammed, A. O., and Alkuraya, F. S. (2014). Novel IFT122 mutation associated with impaired ciliogenesis and cranioectodermal dysplasia. *Mol. Genet. Genomic Med.* 2, 103–106. doi: 10.1002/mgg3.44

Anvarian, Z., Mykytyn, K., Mukhopadhyay, S., Pedersen, L. B., and Christensen, S. T. (2019). Cellular signalling by primary cilia in development, organ function and disease. *Nat. Rev. Nephrol.* 15, 199–219. doi: 10.1038/s41581-019-0116-9

Badano, J. L., Mitsuma, N., Beales, P. L., and Katsanis, N. (2006). The ciliopathies: an emerging class of human genetic disorders. *Annu. Rev. Genomics Hum. Genet.* 7, 125–148. doi: 10.1146/annurev.genom.7.080505.115610

Badgandi, H. B., Hwang, S. H., Shimada, I. S., Loriot, E., and Mukhopadhyay, S. (2017). Tubby family proteins are adapters for ciliary trafficking of integral membrane proteins. *J. Cell Biol.* 216, 743–760. doi: 10.1083/jcb.201607095

Berbari, N. F., Kin, N. W., Sharma, N., Michaud, E. J., Kesterson, R. A., and Yoder, B. K. (2011). Mutations in Traf3ip1 reveal defects in ciliogenesis, embryonic development, and altered cell size regulation. *Dev. Biol.* 360, 66–76. doi: 10.1016/j.ydbio.2011.09.001

Besschetnova, T. Y., Kolpakova-Hart, E., Guan, Y., Zhou, J., Olsen, B. R., and Shah, J. V. (2010). Identification of signaling pathways regulating primary cilium length and flow-mediated adaptation. *Curr. Biol.* 20, 182–187. doi: 10.1016/j.cub.2009.11.072

Bhogaraju, S., Cajanek, L., Fort, C., Blisnick, T., Weber, K., Taschner, M., et al. (2013). Molecular basis of tubulin transport within the cilium by IFT74 and IFT81. *Science* 341, 1009–1012. doi: 10.1126/science.1240985

Botilde, Y., Yoshiba, S., Shinohara, K., Hasegawa, T., Nishimura, H., Shiratori, H., et al. (2013). Cluap1 localizes preferentially to the base and tip of cilia and is required for ciliogenesis in the mouse embryo. *Dev. Biol.* 381, 203–212. doi: 10.1016/j.ydbio.2013.05.024

Bredrup, C., Saunier, S., Oud, M. M., Fiskerstrand, T., Hoischen, A., Brackman, D., et al. (2011). Ciliopathies with skeletal anomalies and renal insufficiency due to mutations in the IFT-A gene WDR19. *Am. J. Hum. Genet.* 89, 634–643. doi: 10.1016/j.ajhg.2011.10.001

Brown, J. M., Cochran, D. A., Craige, B., Kubo, T., and Witman, G. B. (2015). Assembly of IFT trains at the ciliary base depends on IFT74. *Curr. Biol.* 25, 1583–1593. doi: 10.1016/j.cub.2015.04.060

Cai, Y., Fedeles, S. V., Dong, K., Anyatonwu, G., Onoe, T., Mitobe, M., et al. (2014). Altered trafficking and stability of polycystins underlie polycystic kidney disease. *J. Clin. Invest.* 124, 5129–5144. doi: 10.1172/jci67273

Chen, Z., Indjeian, V. B., Mcmanus, M., Wang, L., and Dynlacht, B. D. (2002). CP110, a cell cycle-dependent CDK substrate, regulates centrosome duplication in human cells. *Dev. Cell* 3, 339–350. doi: 10.1016/s1534-5807(02)00258-7

Chien, A., Shih, S. M., Bower, R., Tritschler, D., Porter, M. E., and Yildiz, A. (2017). Dynamics of the IFT machinery at the ciliary tip. *Elife* 6:e28606.

Clement, C. A., Ajbro, K. D., Koefoed, K., Vestergaard, M. L., Veland, I. R., Henriques De Jesus, M. P., et al. (2013). TGF-beta signaling is associated with endocytosis at the pocket region of the primary cilium. *Cell Rep.* 3, 1806–1814. doi: 10.1016/j.celrep.2013.05.020

Cole, D. G., Diener, D. R., Himelblau, A. L., Beech, P. L., Fuster, J. C., and Rosenbaum, J. L. (1998). *Chlamydomonas* kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in *Caenorhabditis elegans* sensory neurons. *J. Cell Biol.* 141, 993–1008. doi: 10.1083/jcb.141.4.993

Corbit, K. C., Shyer, A. E., Dowdle, W. E., Gaulden, J., Singla, V., Chen, M. H., et al. (2008). Kif3a constrains beta-catenin-dependent Wnt signalling through dual ciliary and non-ciliary mechanisms. *Nat. Cell Biol.* 10, 70–76. doi: 10.1038/ncb1670

Craft, J. M., Harris, J. A., Hyman, S., Kner, P., and Lechtreck, K. F. (2015). Tubulin transport by IFT is upregulated during ciliary growth by a cilium-autonomous mechanism. *J Cell Biol* 208, 223–237. doi: 10.1083/jcb.201409036

Craft Van De Weghe, J., Harris, J. A., Kubo, T., Witman, G. B., and Lechtreck, K. F. (2020). Diffusion rather than intraflagellar transport likely provides most of the tubulin required for axonemal assembly in *Chlamydomonas*. *J. Cell Sci.* 133:jcs249805.

Davis, E. E., Zhang, Q., Liu, Q., Diplas, B. H., Davey, L. M., Hartley, J., et al. (2011). TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum. *Nat. Genet.* 43, 189–196. doi: 10.1038/ng.756

Deane, J. A., Cole, D. G., Seeley, E. S., Diener, D. R., and Rosenbaum, J. L. (2001). Localization of intraflagellar transport protein IFT52 identifies basal body transitional fibers as the docking site for IFT particles. *Curr. Biol.* 11, 1586–1590. doi: 10.1016/s0960-9822(01)00484-5

Diaz, J., Gerard, X., Emerit, M. B., Areias, J., Geny, D., Degardin, J., et al. (2020). YIF1B mutations cause a post-natal neurodevelopmental syndrome associated with Golgi and primary cilium alterations. *Brain* 143, 2911–2928. doi: 10.1093/brain/awaa235

Duran, I., Taylor, S. P., Zhang, W., Martin, J., Forlenza, K. N., Spiro, R. P., et al. (2016). Destabilization of the IFT-B cilia core complex due to mutations in IFT81 causes a Spectrum of Short-Rib Polydactyly Syndrome. *Sci. Rep.* 6:34232.

Duran, I., Taylor, S. P., Zhang, W., Martin, J., Qureshi, F., Jacques, S. M., et al. (2017). Mutations in IFT-A satellite core component genes IFT43 and IFT121 produce short rib polydactyly syndrome with distinctive. *Cilia* 6:7.

Eggenschwiler, J. T., and Anderson, K. V. (2007). Cilia and developmental signaling. *Annu Rev Cell Dev Biol* 23, 345–373.

Eguether, T., Cordelieres, F. P., and Pazour, G. J. (2018). Intraflagellar transport is deeply integrated in hedgehog signaling. *Mol. Biol. Cell* 29, 1178–1189. doi: 10.1091/mbc.e17-10-0600

Eguether, T., San Agustin, J. T., Keady, B. T., Jonassen, J. A., Liang, Y., Francis, R., et al. (2014). IFT27 links the BBSome to IFT for maintenance of the ciliary signaling compartment. *Dev. Cell.* 31, 279–290. doi: 10.1016/j.devcel.2014.09.011

Ezratty, E. J., Stokes, N., Chai, S., Shah, A. S., Williams, S. E., and Fuchs, E. (2011). A role for the primary cilium in Notch signaling and epidermal differentiation during skin development. *Cell* 145, 1129–1141. doi: 10.1016/j.cell.2011.05.030

Fai, T. G., Mohapatra, L., Kar, P., Kondev, J., and Amir, A. (2019). Length regulation of multiple flagella that self-assemble from a shared pool of components. *Elife* 8:e42599.

Follit, J. A., Tuft, R. A., Fogarty, K. E., and Pazour, G. J. (2006). The intraflagellar transport protein IFT20 is associated with the Golgi complex and is required for cilia assembly. *Mol. Biol. Cell* 17, 3781–3792. doi: 10.1091/mbc.e06-02-0133

Forcioli-Conti, N., Lacas-Gervais, S., Dani, C., and Peraldi, P. (2015). The primary cilium undergoes dynamic size modifications during adipocyte differentiation of human adipose stem cells. *Biochem. Biophys. Res. Commun.* 458, 117–122. doi: 10.1016/j.bbrc.2015.01.078

Freedman, B. S., Lam, A. Q., Sundsbak, J. L., Iatrino, R., Su, X., Koon, S. J., et al. (2013). Reduced ciliary polycystin-2 in induced pluripotent stem cells from polycystic kidney disease patients with PKD1 mutations. *J. Am. Soc. Nephrol.* 24, 1571–1586. doi: 10.1681/asn.2012111089

Fu, W., Wang, L., Kim, S., Li, J., and Dynlacht, B. D. (2016). Role for the IFT-A complex in selective transport to the primary cilium. *Cell Rep.* 17, 1505–1517. doi: 10.1016/j.celrep.2016.10.018

Funabashi, T., Katoh, Y., Michisaka, S., Terada, M., Sugawa, M., and Nakayama, K. (2017). Ciliary entry of KIF17 is dependent on its binding to the IFT-B complex via IFT46-IFT56 as well as on its nuclear localization signal. *Mol. Biol. Cell* 28, 624–633. doi: 10.1091/mbc.e16-09-0648

Gadadhar, S., Dadi, H., Bodakuntla, S., Schnitzler, A., Bieche, I., Rusconi, F., et al. (2017). Tubulin glycylation controls primary cilia length. *J. Cell Biol.* 216, 2701–2713. doi: 10.1083/jcb.201612050

Garcia-Gonzalo, F. R., Corbit, K. C., Sirerol-Piquer, M. S., Ramaswami, G., Otto, E. A., Noriega, T. R., et al. (2011). A transition zone complex regulates mammalian ciliogenesis and ciliary membrane composition. *Nat. Genet.* 43, 776–784. doi: 10.1038/ng.891

Garcia-Gonzalo, F. R., and Reiter, J. F. (2017). Open sesame: how transition fibers and the transition zone control ciliary composition. *Cold Spring Harb Perspect Biol.* 9:a028134. doi: 10.1101/cshperspect.a028134

Gerakopoulos, V., Ngo, P., and Tsiokas, L. (2020). Loss of polycystins suppresses deciliation via the activation of the centrosomal integrity pathway. *Life Sci. Alliance* 3:e202000750. doi: 10.26508/lsa.202000750

Gonçalves, J., and Pelletier, L. (2017). The ciliary transition zone: finding the pieces and assembling the gate. *Mol. Cells* 40, 243–253. doi: 10.14348/molcells.2017.0054

Gorivodsky, M., Mukhopadhyay, M., Wilsch-Braeuninger, M., Phillips, M., Teufel, A., Kim, C., et al. (2009). Intraflagellar transport protein 172 is essential for primary cilia formation and plays a vital role in patterning the mammalian brain. *Dev. Biol.* 325, 24–32. doi: 10.1016/j.ydbio.2008.09.019

Halbritter, J., Bizet, A. A., Schmidts, M., Porath, J. D., Braun, D. A., Gee, H. Y., et al. (2013). Defects in the IFT-B component IFT172 cause Jeune and Mainzer-Saldino syndromes in humans. *Am. J. Hum. Genet.* 93, 915–925. doi: 10.1016/j.ajhg.2013.09.012

Han, S., Miyoshi, K., Shikada, S., Amano, G., Wang, Y., Yoshimura, T., et al. (2019). TULP3 is required for localization of membrane-associated proteins ARL13B and INPP5E to primary cilia. *Biochem. Biophys. Res. Commun.* 509, 227–234. doi: 10.1016/j.bbrc.2018.12.109

Han, S. J., Jang, H. S., Seu, S. Y., Cho, H. J., Hwang, Y. J., Kim, J. I., et al. (2017). Hepatic ischemia/reperfusion injury disrupts the homeostasis of kidney primary cilia via oxidative stress. *Biochim. Biophys. Acta Mol. Basis Dis.* 1863, 1817–1828. doi: 10.1016/j.bbadis.2017.05.004

Han, Y. M., Kang, G. M., Byun, K., Ko, H. W., Kim, J., Shin, M. S., et al. (2014). Leptin-promoted cilia assembly is critical for normal energy balance. *J. Clin. Invest.* 124, 2193–2197. doi: 10.1172/jci69395

Harris, R. C., and Zhang, M. Z. (2012). Dopamine, the kidney, and hypertension. *Curr. Hypertens. Rep.* 14, 138–143. doi: 10.1007/s11906-012-0253-z

Hart, G. W., Slawson, C., Ramirez-Correa, G., and Lagerlof, O. (2011). Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. *Annu. Rev. Biochem.* 80, 825–858. doi: 10.1146/annurev-biochem-060608-102511

Haycraft, C. J., Zhang, Q., Song, B., Jackson, W. S., Detloff, P. J., Serra, R., et al. (2007). Intraflagellar transport is essential for endochondral bone formation. *Development* 134, 307–316. doi: 10.1242/dev.02732

He, K., Ma, X., Xu, T., Li, Y., Hodge, A., Zhang, Q., et al. (2018). Axoneme polyglutamylation regulated by Joubert syndrome protein ARL13B controls ciliary targeting of signaling molecules. *Nat. Commun.* 9:3310.

Hildebrandt, F., Benzing, T., and Katsanis, N. (2011). Ciliopathies. *N. Engl. J. Med.* 364, 1533–1543.

Hilgendorf, K. I., Johnson, C. T., Mezger, A., Rice, S. L., Norris, A. M., Demeter, J., et al. (2019). Omega-3 fatty acids activate ciliary FFAR4 to control Adipogenesis. *Cell* 179:e1221.

Hirano, T., Katoh, Y., and Nakayama, K. (2017). Intraflagellar transport-A complex mediates ciliary entry and retrograde trafficking of ciliary G protein-coupled receptors. *Mol. Biol. Cell* 28, 429–439. doi: 10.1091/mbc.e16-11-0813

Hopp, K., Ward, C. J., Hommerding, C. J., Nasr, S. H., Tuan, H. F., Gainullin, V. G., et al. (2012). Functional polycystin-1 dosage governs autosomal dominant polycystic kidney disease severity. *J. Clin. Invest.* 122, 4257–4273. doi: 10.1172/jci64313

Huang, K., Diener, D. R., and Rosenbaum, J. L. (2009). The ubiquitin conjugation system is involved in the disassembly of cilia and flagella. *J. Cell Biol.* 186, 601–613. doi: 10.1083/jcb.200903066

Huangfu, D., Liu, A., Rakeman, A. S., Murcia, N. S., Niswander, L., and Anderson, K. V. (2003). Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature* 426, 83–87. doi: 10.1038/nature02061

Husson, H., Moreno, S., Smith, L. A., Smith, M. M., Russo, R. J., Pitstick, R., et al. (2016). Reduction of ciliary length through pharmacologic or genetic inhibition of CDK5 attenuates polycystic kidney disease in a model of nephronophthisis. *Hum. Mol. Genet.* 25, 2245–2255. doi: 10.1093/hmg/ddw093

Hwang, S. H., Somatilaka, B. N., Badgandi, H., Palicharla, V. R., Walker, R., Shelton, J. M., et al. (2019). Tulp3 regulates renal cystogenesis by trafficking of cystoproteins to cilia. *Curr. Biol.* 29:e795.

Ishikawa, H., Ide, T., Yagi, T., Jiang, X., Hirono, M., Sasaki, H., et al. (2014). TTC26/DYF13 is an intraflagellar transport protein required for transport of motility-related proteins into flagella. *Elife* 3:e01566.

Jacobs, D. T., Allard, B. A., Pottorf, T. S., Silva, L. M., Wang, W., Al-Naamani, A., et al. (2020). Intraflagellar-transport A dysfunction causes hyperphagia-induced systemic insulin resistance in a pre-obese state. *FASEB J.* 34, 148–160. doi: 10.1096/fj.201900751r

Jain, R., Javidan-Nejad, C., Alexander-Brett, J., Horani, A., Cabellon, M. C., Walter, M. J., et al. (2012). Sensory functions of motile cilia and implication for bronchiectasis. *Front. Biosci.* 4:1088–1098. doi: 10.2741/s320

Johnson, K. A., and Rosenbaum, J. L. (1992). Polarity of flagellar assembly in *Chlamydomonas*. *J. Cell Biol.* 119, 1605–1611. doi: 10.1083/jcb.119.6.1605

Jonassen, J. A., San Agustin, J., Follit, J. A., and Pazour, G. J. (2008). Deletion of IFT20 in the mouse kidney causes misorientation of the mitotic spindle and cystic kidney disease. *J. Cell Biol.* 183, 377–384. doi: 10.1083/jcb.200808137

Jonassen, J. A., Sanagustin, J., Baker, S. P., and Pazour, G. J. (2012). Disruption of IFT complex A causes cystic kidney without mitotic spindle misorientation. *J. Am. Soc. Nephrol.* 23, 641–651. doi: 10.1681/asn.2011080829

Katoh, Y., Terada, M., Nishijima, Y., Takei, R., Nozaki, S., Hamada, H., et al. (2016). Overall architecture of the intraflagellar transport (IFT)-B complex containing cluap1/IFT38 as an essential component of the IFT-B peripheral subcomplex. *J. Biol. Chem.* 291, 10962–10975. doi: 10.1074/jbc.m116.713883

Keady, B. T., Samtani, R., Tobita, K., Tsuchya, M., San Agustin, J. T., Follit, J. A., et al. (2012). IFT25 links the signal-dependent movement of Hedgehog components to intraflagellar transport. *Dev. Cell* 22, 940–951. doi: 10.1016/j.devcel.2012.04.009

Kim, J. I., Kim, J., Jang, H. S., Noh, M. R., Lipschutz, J. H., and Park, K. M. (2013). Reduction of oxidative stress during recovery accelerates normalization of primary cilia length that is altered after ischemic injury in murine kidneys. *Am. J. Physiol. Renal. Physio.l* 304, F1283–F1294.

Kimura, Y., Tsutsumi, K., Konno, A., Ikegami, K., Hameed, S., Kaneko, T., et al. (2018). Environmental responsiveness of tubulin glutamylation in sensory cilia is regulated by the p38 MAPK pathway. *Sci. Rep.* 8:8392.

Kiseleva, A. A., Korobeynikov, V. A., Nikonova, A. S., Zhang, P., Makhov, P., Deneka, A. Y., et al. (2019). Unexpected activities in regulating ciliation contribute to off-target effects of targeted drugs. *Clin. Cancer Res.* 25, 4179–4193. doi: 10.1158/1078-0432.ccr-18-3535

Kobayashi, T., Ishida, Y., Hirano, T., Katoh, Y., and Nakayama, K. (2021). Cooperation of the IFT-A complex with the IFT-B complex is required for ciliary retrograde protein trafficking and GPCR import. *Mol. Biol. Cell* 32, 45–56. doi: 10.1091/mbc.e20-08-0556

Komarynets, O., Chassot, A., Bernabeu, E., Czogalla, J., Roth, I., Liaudet, N., et al. (2020). Aldosterone controls primary cilium length and cell size in renal collecting duct principal cells. *FASEB J.* 34, 2625–2640. doi: 10.1096/fj.201901947r

Kozminski, K. G., Johnson, K. A., Forscher, P., and Rosenbaum, J. L. (1993). A motility in the eukaryotic flagellum unrelated to flagellar beating. *Proc. Natl. Acad. Sci. U.S.A.* 90, 5519–5523. doi: 10.1073/pnas.90.12.5519

Kubo, T., Brown, J. M., Bellve, K., Craige, B., Craft, J. M., Fogarty, K., et al. (2016). Together, the IFT81 and IFT74 N-termini form the main module for intraflagellar transport of tubulin. *J Cell Sci* 129, 2106–2119. doi: 10.1242/jcs.187120

Kunova Bosakova, M., Varecha, M., Hampl, M., Duran, I., Nita, A., Buchtova, M., et al. (2018). Regulation of ciliary function by fibroblast growth factor signaling identifies FGFR3-related disorders achondroplasia and thanatophoric dysplasia as ciliopathies. *Hum. Mol. Genet.* 27, 1093–1105. doi: 10.1093/hmg/ddy031

Leaf, A., and Von Zastrow, M. (2015). Dopamine receptors reveal an essential role of IFT-B, KIF17, and Rab23 in delivering specific receptors to primary cilia. *Elife* 4:e06996.

Lee, C. H., Song, D. K., Park, C. B., Choi, J., Kang, G. M., Shin, S. H., et al. (2020). Primary cilia mediate early life programming of adiposity through lysosomal regulation in the developing mouse hypothalamus. *Nat. Commun.* 11:5772.

Lee, M. S., Hwang, K. S., Oh, H. W., Ji-Ae, K., Kim, H. T., Cho, H. S., et al. (2015). IFT46 plays an essential role in cilia development. *Dev. Biol.* 400, 248–257. doi: 10.1016/j.ydbio.2015.02.009

Legue, E., and Liem, K. F. Jr. (2019). Tulp3 Is a ciliary trafficking gene that regulates polycystic kidney disease. *Curr. Biol.* 29:e805.

Li, Y. H., Zhu, D., Cao, Z., Liu, Y., Sun, J., and Tan, L. (2020). Primary cilia respond to intermittent low-magnitude, high-frequency vibration and mediate vibration-induced effects in osteoblasts. *Am. J. Physiol. Cell Physiol.* 318, C73–C82.

Liem, K. F. Jr., Ashe, A., He, M., Satir, P., Moran, J., Beier, D., et al. (2012). The IFT-A complex regulates Shh signaling through cilia structure and membrane protein trafficking. *J. Cell Biol.* 197, 789–800. doi: 10.1083/jcb.201110049

Liew, G. M., Ye, F., Nager, A. R., Murphy, J. P., Lee, J. S., Aguiar, M., et al. (2014). The intraflagellar transport protein IFT27 promotes BBSome exit from cilia through the GTPase ARL6/BBS3. *Dev. Cell* 31, 265–278. doi: 10.1016/j.devcel.2014.09.004

Lim, J., Li, X., Yuan, X., Yang, S., Han, L., and Yang, S. (2020). Primary cilia control cell alignment and patterning in bone development via ceramide-PKCzeta-beta-catenin signaling. *Commun. Bio.l* 3:45.

Liu, M., Alharbi, M., Graves, D., and Yang, S. (2020). IFT80 is required for fracture healing through controlling the regulation of TGF-beta signaling in chondrocyte differentiation and function. *J. Bone Miner Res.* 35, 571–582. doi: 10.1002/jbmr.3902

Liu, X., Vien, T., Duan, J., Sheu, S. H., Decaen, P. G., and Clapham, D. E. (2018). Polycystin-2 is an essential ion channel subunit in the primary cilium of the renal collecting duct epithelium. *Elife* 7:e33183.

Long, H., and Huang, K. (2019). Transport of ciliary membrane proteins. *Front. Cell Dev. Biol.* 7:381.

Long, H., Zhang, F., Xu, N., Liu, G., Diener, D. R., Rosenbaum, J. L., et al. (2016). Comparative analysis of ciliary membranes and ectosomes. *Curr. Biol.* 26, 3327–3335. doi: 10.1016/j.cub.2016.09.055

Lu, H., Toh, M. T., Narasimhan, V., Thamilselvam, S. K., Choksi, S. P., and Roy, S. (2015). A function for the Joubert syndrome protein Arl13b in ciliary membrane extension and ciliary length regulation. *Dev. Biol.* 397, 225–236. doi: 10.1016/j.ydbio.2014.11.009

Ma, M., Tian, X., Igarashi, P., Pazour, G. J., and Somlo, S. (2013). Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. *Nat. Genet.* 45, 1004–1012. doi: 10.1038/ng.2715

Malicki, J. J., and Johnson, C. A. (2017). The cilium: cellular antenna and central processing unit. *Trends Cell Biol.* 27, 126–140. doi: 10.1016/j.tcb.2016.08.002

Marshall, W. F., Qin, H., Rodrigo Brenni, M., and Rosenbaum, J. L. (2005). Flagellar length control system: testing a simple model based on intraflagellar transport and turnover. *Mol. Biol. Cell* 16, 270–278. doi: 10.1091/mbc.e04-07-0586

Marshall, W. F., and Rosenbaum, J. L. (2001). Intraflagellar transport balances continuous turnover of outer doublet microtubules: implications for flagellar length control. *J. Cell Biol.* 155, 405–414. doi: 10.1083/jcb.200106141

Martin, L., Kaci, N., Estibals, V., Goudin, N., Garfa-Traore, M., Benoist-Lasselin, C., et al. (2018). Constitutively-active FGFR3 disrupts primary cilium length and IFT20 trafficking in various chondrocyte models of achondroplasia. *Hum. Mol. Genet.* 27, 1–13. doi: 10.1093/hmg/ddx374

Miller, K. A., Ah-Cann, C. J., Welfare, M. F., Tan, T. Y., Pope, K., Caruana, G., et al. (2013). Cauli: a mouse strain with an Ift140 mutation that results in a skeletal ciliopathy modelling Jeune syndrome. *PLoS Genet.* 9:e1003746. doi: 10.1371/journal.pgen.1003746

Mirvis, M., Siemers, K. A., Nelson, W. J., and Stearns, T. P. (2019). Primary cilium loss in mammalian cells occurs predominantly by whole-cilium shedding. *PLoS Biol.* 17:e3000381. doi: 10.1371/journal.pbio.3000381

Mukherjee, M., Ratnayake, I., Janga, M., Fogarty, E., Scheidt, S., Grassmeyer, J., et al. (2020). Notch signaling regulates Akap12 expression and primary cilia length during renal tubule morphogenesis. *FASEB J.* 34, 9512–9530. doi: 10.1096/fj.201902358rr

Mukhopadhyay, S., Wen, X., Chih, B., Nelson, C. D., Lane, W. S., Scales, S. J., et al. (2010). TULP3 bridges the IFT-A complex and membrane phosphoinositides to promote trafficking of G protein-coupled receptors into primary cilia. *Genes. Dev.* 24, 2180–2193. doi: 10.1101/gad.1966210

Mukhopadhyay, S., Wen, X., Ratti, N., Loktev, A., Rangell, L., Scales, S. J., et al. (2013). The ciliary G-protein-coupled receptor Gpr161 negatively regulates the Sonic hedgehog pathway via cAMP signaling. *Cell* 152, 210–223. doi: 10.1016/j.cell.2012.12.026

Nachury, M. V. (2018). The molecular machines that traffic signaling receptors into and out of cilia. *Curr. Opin. Cell Biol* .51, 124–131. doi: 10.1016/j.ceb.2018.03.004

Nager, A. R., Goldstein, J. S., Herranz-Perez, V., Portran, D., Ye, F., Garcia-Verdugo, J. M., et al. (2017). An actin network dispatches ciliary gpcrs into extracellular vesicles to modulate signaling. *Cell* 168:e214.

Nakamura, K., Noguchi, T., Takahara, M., Omori, Y., Furukawa, T., Katoh, Y., et al. (2020). Anterograde trafficking of ciliary MAP kinase-like ICK/CILK1 by the intraflagellar transport machinery is required for intraciliary retrograde protein trafficking. *J. Biol. Chem.* 295, 13363–13376. doi: 10.1074/jbc.ra120.014142

Nakayama, K., and Katoh, Y. (2020). Architecture of the IFT ciliary trafficking machinery and interplay between its components. *Crit. Rev. Biochem. Mol. Biol.* 55, 179–196. doi: 10.1080/10409238.2020.1768206

Nandadasa, S., Kraft, C. M., Wang, L. W., O'donnell, A., Patel, R., Gee, H. Y., et al. (2019). Secreted metalloproteases ADAMTS9 and ADAMTS20 have a non-canonical role in ciliary vesicle growth during ciliogenesis. *Nat. Commun.* 10:953.

Nauli, S. M., Alenghat, F. J., Luo, Y., Williams, E., Vassilev, P., Li, X., et al. (2003). Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* 33, 129–137. doi: 10.1038/ng1076

Nigg, E. A., and Stearns, T. (2011). The centrosome cycle: centriole biogenesis, duplication and inherent asymmetries. *Nat. Cell Biol* .13, 1154–1160. doi: 10.1038/ncb2345

Nikonova, A. S., Plotnikova, O. V., Serzhanova, V., Efimov, A., Bogush, I., Cai, K. Q., et al. (2014). Nedd9 restrains renal cystogenesis in Pkd1-/- mice. *Proc. Natl. Acad. Sci. U.S.A.* 111, 12859–12864. doi: 10.1073/pnas.1405362111

Ocbina, P. J., Eggenschwiler, J. T., Moskowitz, I., and Anderson, K. V. (2011). Complex interactions between genes controlling trafficking in primary cilia. *Nat Genet* 43, 547–553. doi: 10.1038/ng.832

Odabasi, E., Gul, S., Kavakli, I. H., and Firat-Karalar, E. N. (2019). Centriolar satellites are required for efficient ciliogenesis and ciliary content regulation. *EMBO Rep.* 20, e47723.

O'hagan, R., Piasecki, B. P., Silva, M., Phirke, P., Nguyen, K. C., Hall, D. H., et al. (2011). The tubulin deglutamylase CCPP-1 regulates the function and stability of sensory cilia in C. elegans. *Curr. Biol.* 21, 1685–1694. doi: 10.1016/j.cub.2011.08.049

Olson, R. J., Hopp, K., Wells, H., Smith, J. M., Furtado, J., Constans, M. M., et al. (2019). Synergistic genetic interactions between *Pkhd1* and *Pkd1* result in an ARPKD-Like phenotype in murine models. *J. Am. Soc. Nephrol.* 30, 2113–2127. doi: 10.1681/ASN.2019020150.

Orhon, I., Dupont, N., Zaidan, M., Boitez, V., Burtin, M., Schmitt, A., et al. (2016). Primary-cilium-dependent autophagy controls epithelial cell volume in response to fluid flow. *Nat. Cell Biol.* 18, 657–667. doi: 10.1038/ncb3360

Pala, R., Mohieldin, A. M., Shamloo, K., Sherpa, R. T., Kathem, S. H., Zhou, J., et al. (2019a). Personalized nanotherapy by specifically targeting cell organelles to improve vascular hypertension. *Nano Lett.* 19, 904–914. doi: 10.1021/acs.nanolett.8b04138

Pala, R., Mohieldin, A. M., Sherpa, R. T., Kathem, S. H., Shamloo, K., Luan, Z., et al. (2019b). Ciliotherapy: remote control of primary cilia movement and function by magnetic nanoparticles. *ACS Nano* 13, 3555–3572.

Pampliega, O., Orhon, I., Patel, B., Sridhar, S., Diaz-Carretero, A., Beau, I., et al. (2013). Functional interaction between autophagy and ciliogenesis. *Nature* 502, 194–200. doi: 10.1038/nature12639

Pan, J., Seeger-Nukpezah, T., and Golemis, E. A. (2013). The role of the cilium in normal and abnormal cell cycles: emphasis on renal cystic pathologies. *Cell Mol. Life Sci.* 70, 1849–1874. doi: 10.1007/s00018-012-1052-z

Paridaen, J. T., Wilsch-Brauninger, M., and Huttner, W. B. (2013). Asymmetric inheritance of centrosome-associated primary cilium membrane directs ciliogenesis after cell division. *Cell* 155, 333–344. doi: 10.1016/j.cell.2013.08.060

Park, K. M. (2018). Can tissue cilia lengths and urine cilia proteins be markers of kidney diseases? *Chonnam. Med. J.* 54, 83–89. doi: 10.4068/cmj.2018.54.2.83

Patel, V., Li, L., Cobo-Stark, P., Shao, X., Somlo, S., Lin, F., et al. (2008). Acute kidney injury and aberrant planar cell polarity induce cyst formation in mice lacking renal cilia. *Hum. Mol. Genet.* 17, 1578–1590. doi: 10.1093/hmg/ddn045

Pazour, G. J., Dickert, B. L., Vucica, Y., Seeley, E. S., Rosenbaum, J. L., Witman, G. B., et al. (2000). Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *J. Cell Biol.* 151, 709–718. doi: 10.1083/jcb.151.3.709

Pazour, G. J., Dickert, B. L., and Witman, G. B. (1999). The DHC1b (DHC2) isoform of cytoplasmic dynein is required for flagellar assembly. *J. Cell Biol.* 144, 473–481. doi: 10.1083/jcb.144.3.473

Pazour, G. J., Wilkerson, C. G., and Witman, G. B. (1998). A dynein light chain is essential for the retrograde particle movement of intraflagellar transport (IFT). *J. Cell Biol.* 141, 979–992. doi: 10.1083/jcb.141.4.979

Perrault, I., Halbritter, J., Porath, J. D., Gerard, X., Braun, D. A., Gee, H. Y., et al. (2015). IFT81, encoding an IFT-B core protein, as a very rare cause of a ciliopathy phenotype. *J. Med. Genet.* 52, 657–665. doi: 10.1136/jmedgenet-2014-102838

Phua, S. C., Chiba, S., Suzuki, M., Su, E., Roberson, E. C., Pusapati, G. V., et al. (2017). Dynamic remodeling of membrane composition drives cell cycle through primary cilia excision. *Cell* 168:e215.

Picariello, T., Brown, J. M., Hou, Y., Swank, G., Cochran, D. A., King, O. D., et al. (2019). A global analysis of IFT-A function reveals specialization for transport of membrane-associated proteins into cilia. *J. Cell Sci.* 132, jcs220749.

Poole, C. A., Flint, M. H., and Beaumont, B. W. (1985). Analysis of the morphology and function of primary cilia in connective tissues: a cellular cybernetic probe? *Cell Motil.* 5, 175–193. doi: 10.1002/cm.970050302

Porter, M. E., Bower, R., Knott, J. A., Byrd, P., and Dentler, W. (1999). Cytoplasmic dynein heavy chain 1b is required for flagellar assembly in *Chlamydomonas*. *Mol. Biol. Cell* 10, 693–712. doi: 10.1091/mbc.10.3.693

Praetorius, H. A., and Spring, K. R. (2001). Bending the MDCK cell primary cilium increases intracellular calcium. *J. Membr. Biol.* 184, 71–79. doi: 10.1007/s00232-001-0075-4

Praetorius, H. A., and Spring, K. R. (2003). Removal of the MDCK cell primary cilium abolishes flow sensing. *J. Membr. Biol.* 191, 69–76. doi: 10.1007/s00232-002-1042-4

Pugacheva, E. N., Jablonski, S. A., Hartman, T. R., Henske, E. P., and Golemis, E. A. (2007). HEF1-dependent Aurora A activation induces disassembly of the primary cilium. *Cell* 129, 1351–1363. doi: 10.1016/j.cell.2007.04.035

Qin, J., Lin, Y., Norman, R. X., Ko, H. W., and Eggenschwiler, J. T. (2011). Intraflagellar transport protein 122 antagonizes Sonic Hedgehog signaling and controls ciliary localization of pathway components.

Proc. Natl. Acad. Sci. U.S.A. 108, 1456–1461. doi: 10.1073/pnas.10114 10108

R Ferreira, R., Fukui, H., Chow, R., Vilfan, A., and Vermot, J. (2019). The cilium as a force sensor-myth versus reality. *J. Cell Sci.* 132, jcs213496.

Ritter, A., Friemel, A., Kreis, N. N., Hoock, S. C., Roth, S., Kielland-Kaisen, U., et al. (2018). Primary cilia are dysfunctional in obese adipose-derived mesenchymal stem cells. *Stem Cell Rep.* 10, 583–599. doi: 10.1016/j.stemcr.2017.12.022

Rix, S., Calmont, A., Scambler, P. J., and Beales, P. L. (2011). An Ift80 mouse model of short rib polydactyly syndromes shows defects in hedgehog signalling without loss or malformation of cilia. *Hum. Mol. Genet.* 20, 1306–1314. doi: 10.1093/hmg/ddr013

Rosenbaum, J. L., and Witman, G. B. (2002). Intraflagellar transport. *Nat. Rev. Mol. Cell Biol.* 3, 813–825.

Saraga-Babic, M., Vukojevic, K., Bocina, I., Drnasin, K., and Saraga, M. (2012). Ciliogenesis in normal human kidney development and post-natal life. *Pediatr. Nephrol.* 27, 55–63. doi: 10.1007/s00467-011-1941-7

Satir, P. (2005). Tour of organelles through the electron microscope: a reprinting of Keith R. Porter's classic harvey lecture with a new introduction. *Anat. Rec. A Discov. Mol. Cell Evol. Biol.* 287, 1184–1185. doi: 10.1002/ar.a.20222

Schneider, L., Clement, C. A., Teilmann, S. C., Pazour, G. J., Hoffmann, E. K., Satir, P., et al. (2005). PDGFRalphaalpha signaling is regulated through the primary cilium in fibroblasts. *Curr. Biol.* 15, 1861–1866. doi: 10.1016/j.cub.2005.09.012

Shaheen, R., Alsahli, S., Ewida, N., Alzahrani, F., Shamseldin, H. E., Patel, N., et al. (2020). Biallelic mutations in tetratricopeptide repeat domain 26 (Intraflagellar Transport 56) cause severe biliary ciliopathy in humans. *Hepatology* 71, 2067–2079. doi: 10.1002/hep.30982

Shamloo, K., Chen, J., Sardar, J., Sherpa, R. T., Pala, R., Atkinson, K. F., et al. (2017). Chronic hypobaric hypoxia modulates primary cilia differently in adult and fetal ovine kidneys. *Front. Physiol.* 8:677.

Shao, L., El-Jouni, W., Kong, F., Ramesh, J., Kumar, R. S., Shen, X., et al. (2020). Genetic reduction of cilium-length by targeting intraflagellar transport 88 protein impedes kidney and liver cysts formation in mouse models of autosomal polycystic kidney disease. *Kidney Int.* 98, 1225–1241. doi: 10.1016/j.kint.2020.05.049

Shida, T., Cueva, J. G., Xu, Z., Goodman, M. B., and Nachury, M. V. (2010). The major alpha-tubulin K40 acetyltransferase alphaTAT1 promotes rapid ciliogenesis and efficient mechanosensation. *Proc. Natl. Acad. Sci. U.S.A.* 107, 21517–21522. doi: 10.1073/pnas.1013728107

Shillingford, J. M., Leamon, C. P., Vlahov, I. R., and Weimbs, T. (2012). Folate-conjugated rapamycin slows progression of polycystic kidney disease. *J. Am. Soc. Nephrol.* 23, 1674–1681. doi: 10.1681/asn.2012040367

Signor, D., Wedaman, K. P., Orozco, J. T., Dwyer, N. D., Bargmann, C. I., Rose, L. S., et al. (1999). Role of a class DHC1b dynein in retrograde transport of IFT motors and IFT raft particles along cilia, but not dendrites, in chemosensory neurons of living *Caenorhabditis elegans*. *J. Cell Biol.* 147, 519–530. doi: 10.1083/jcb.147.3.519

Smith, L. A., Bukanov, N. O., Husson, H., Russo, R. J., Barry, T. C., Taylor, A. L., et al. (2006). Development of polycystic kidney disease in juvenile cystic kidney mice: insights into pathogenesis, ciliary abnormalities, and common features with human disease. *J. Am. Soc. Nephrol.* 17, 2821–2831. doi: 10.1681/asn.2006020136

Sorokin, S. (1962). Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. *J. Cell Biol.* 15, 363–377. doi: 10.1083/jcb.15.2.363

Spasic, M., and Jacobs, C. R. (2017). Lengthening primary cilia enhances cellular mechanosensitivity. *Eur. Cell Mater.* 33, 158–168. doi: 10.22203/ecm.v033a12

Srivastava, S., Ramsbottom, S. A., Molinari, E., Alkanderi, S., Filby, A., White, K., et al. (2017). A human patient-derived cellular model of Joubert syndrome reveals ciliary defects which can be rescued with targeted therapies. *Hum. Mol. Genet.* 26, 4657–4667. doi: 10.1093/hmg/ddx347

Stephan, A., Vaughan, S., Shaw, M. K., Gull, K., and Mckean, P. G. (2007). An essential quality control mechanism at the eukaryotic basal body prior to intraflagellar transport. *Traffic* 8, 1323–1330. doi: 10.1111/j.1600-0854.2007.00611.x

Streets, A. J., Prosseda, P. P., and Ong, A. C. (2020). Polycystin-1 regulates ARHGAP35-dependent centrosomal RhoA activation and ROCK signaling. *JCI Insight* 5:e135385.

Stroope, A., Radtke, B., Huang, B., Masyuk, T., Torres, V., Ritman, E., et al. (2010). Hepato-renal pathology in pkd2ws25/- mice, an animal model of autosomal

dominant polycystic kidney disease. *Am. J. Pathol.* 176, 1282–1291. doi: 10.2353/ajpath.2010.090658

Szempruch, A. J., Sykes, S. E., Kieft, R., Dennison, L., Becker, A. C., Gartrell, A., et al. (2016). Extracellular vesicles from trypanosoma brucei mediate virulence factor transfer and cause Host Anemia. *Cell* 164, 246–257. doi: 10.1016/j.cell.2015.11.051

Takahara, M., Katoh, Y., Nakamura, K., Hirano, T., Sugawa, M., Tsurumi, Y., et al. (2018). Ciliopathy-associated mutations of IFT122 impair ciliary protein trafficking but not ciliogenesis. *Hum. Mol. Genet.* 27, 516–528. doi: 10.1093/hmg/ddx421

Takei, R., Katoh, Y., and Nakayama, K. (2018). Robust interaction of IFT70 with IFT52-IFT88 in the IFT-B complex is required for ciliogenesis. *Biol. Open* 7:bio033241.

Taschner, M., Weber, K., Mourao, A., Vetter, M., Awasthi, M., Stiegler, M., et al. (2016). Intraflagellar transport proteins 172, 80, 57, 54, 38, and 20 form a stable tubulin-binding IFT-B2 complex. *EMBO J.* 35, 773–790. doi: 10.15252/embj.201593164

Teves, M. E., Sundaresan, G., Cohen, D. J., Hyzy, S. L., Kajan, I., Maczis, M., et al. (2015). Spag17 deficiency results in skeletal malformations and bone abnormalities. *PLoS One* 10:e0125936. doi: 10.1371/journal.pone.0125936

Thevenon, J., Duplomb, L., Phadke, S., Eguether, T., Saunier, A., Avila, M., et al. (2016). Autosomal recessive IFT57 hypomorphic mutation cause ciliary transport defect in unclassified oral-facial-digital syndrome with short stature and brachymesophalangia. *Clin. Genet.* 90, 509–517. doi: 10.1111/cge.12785

Tian, J. L., and Qin, H. (2019). O-GlcNAcylation regulates primary ciliary length by promoting microtubule disassembly. *iScience* 12, 379–391. doi: 10.1016/j.isci.2019.01.031

Tran, P. V., Haycraft, C. J., Besschetnova, T. Y., Turbe-Doan, A., Stottmann, R. W., Herron, B. J., et al. (2008). THM1 negatively modulates mouse sonic hedgehog signal transduction and affects retrograde intraflagellar transport in cilia. *Nat. Genet.* 40, 403–410. doi: 10.1038/ng.105

Tran, P. V., Talbott, G. C., Turbe-Doan, A., Jacobs, D. T., Schonfeld, M. P., Silva, L. M., et al. (2014). Downregulating hedgehog signaling reduces renal cystogenic potential of mouse models. *J. Am. Soc. Nephrol.* 25, 2201–2212. doi: 10.1681/asn.2013070735

Upadhyay, V. S., Muntean, B. S., Kathem, S. H., Hwang, J. J., Aboualaiwi, W. A., and Nauli, S. M. (2014). Roles of dopamine receptor on chemosensory and mechanosensory primary cilia in renal epithelial cells. *Front. Physiol.* 5:72.

Verghese, E., Martelotto, L. G., Cain, J. E., Williams, T. M., Wise, A. F., Hill, P. A., et al. (2019). Renal epithelial cells retain primary cilia during human acute renal allograft rejection injury. *BMC Res. Notes* 12:718.

Verghese, E., Ricardo, S. D., Weidenfeld, R., Zhuang, J., Hill, P. A., Langham, R. G., et al. (2009). Renal primary cilia lengthen after acute tubular necrosis. *J. Am. Soc. Nephrol.* 20, 2147–2153. doi: 10.1681/asn.2008101105

Verghese, E., Weidenfeld, R., Bertram, J. F., Ricardo, S. D., and Deane, J. A. (2008). Renal cilia display length alterations following tubular injury and are present early in epithelial repair. *Nephrol. Dial. Transplant* 23, 834–841. doi: 10.1093/ndt/gfm743

Verghese, E., Zhuang, J., Saiti, D., Ricardo, S. D., and Deane, J. A. (2011). In vitro investigation of renal epithelial injury suggests that primary cilium length is regulated by hypoxia-inducible mechanisms. *Cell Biol. Int* .35, 909–913. doi: 10.1042/cbi20090154

Viau, A., Bienaime, F., Lukas, K., Todkar, A. P., Knoll, M., Yakulov, T. A., et al. (2018). Cilia-localized LKB1 regulates chemokine signaling, macrophage recruitment, and tissue homeostasis in the kidney. *EMBO J.* 37:e98615.

Walker, R. V., Keynton, J. L., Grimes, D. T., Sreekumar, V., Williams, D. J., Esapa, C., et al. (2019). Ciliary exclusion of Polycystin-2 promotes kidney cystogenesis in an autosomal dominant polycystic kidney disease model. *Nat. Commun.* 10:4072.

Wang, J., Silva, M., Haas, L. A., Morsci, N. S., Nguyen, K. C., Hall, D. H., et al. (2014). C. elegans ciliated sensory neurons release extracellular vesicles that function in animal communication. *Curr. Biol.* 24, 519–525. doi: 10.1016/j.cub.2014.01.002

Wang, L., and Dynlacht, B. D. (2018). The regulation of cilium assembly and disassembly in development and disease. *Development* 145:dev151407.

Wang, Q., Peng, Z., Long, H., Deng, X., and Huang, K. (2019). Polyubiquitylation of alpha-tubulin at K304 is required for flagellar disassembly in *Chlamydomonas*. *J. Cell Sci.* 132:jcs229047.

Wang, Q., Taschner, M., Ganzinger, K. A., Kelley, C., Villasenor, A., Heymann, M., et al. (2018). Membrane association and remodeling by intraflagellar transport protein IFT172. *Nat. Commun.* 9:4684.

Wang, W., Allard, B. A., Pottorf, T. S., Wang, H. H., Vivian, J. L., and Tran, P. V. (2020). Genetic interaction of mammalian IFT-A paralogs regulates cilia disassembly, ciliary entry of membrane protein, Hedgehog signaling, and embryogenesis. *FASEB J.* 34, 6369–6381. doi: 10.1096/fj.201902611r

Wemmer, K., Ludington, W., and Marshall, W. F. (2020). Testing the role of intraflagellar transport in flagellar length control using length-altering mutants of *Chlamydomonas*. *Philos. Trans. R. Soc. Lond B Biol. Sci.* 375:20190159. doi: 10.1098/rstb.2019.0159

Westlake, C. J., Baye, L. M., Nachury, M. V., Wright, K. J., Ervin, K. E., Phu, L., et al. (2011). Primary cilia membrane assembly is initiated by Rab11 and transport protein particle II (TRAPPII) complex-dependent trafficking of Rabin8 to the centrosome. *Proc. Natl. Acad. Sci. U.S.A.* 108, 2759–2764. doi: 10.1073/pnas.1018823108

Wheway, G., Nazlamova, L., and Hancock, J. T. (2018). Signaling through the primary cilium. *Front. Cell Dev. Biol.* 6:8.

Williams, C. L., Li, C., Kida, K., Inglis, P. N., Mohan, S., Semenec, L., et al. (2011). MKS and NPHP modules cooperate to establish basal body/transition zone membrane associations and ciliary gate function during ciliogenesis. *J. Cell Biol.* 192, 1023–1041. doi: 10.1083/jcb.201012116

Witman, G. B. (1975). The site of in vivo assembly of flagellar microtubules. *Ann. N. Y. Acad. Sci.* 253, 178–191. doi: 10.1111/j.1749-6632.1975.tb19199.x

Wood, C. R., Huang, K., Diener, D. R., and Rosenbaum, J. L. (2013). The cilium secretes bioactive ectosomes. *Curr. Biol.* 23, 906–911. doi: 10.1016/j.cub.2013.04.019

Wren, K. N., Craft, J. M., Tritschler, D., Schauer, A., Patel, D. K., Smith, E. F., et al. (2013). A differential cargo-loading model of ciliary length regulation by IFT. *Curr. Biol.* 23, 2463–2471. doi: 10.1016/j.cub.2013.10.044

Xin, D., Christopher, K. J., Zeng, L., Kong, Y., and Weatherbee, S. D. (2017). IFT56 regulates vertebrate developmental patterning by maintaining IFTB complex integrity and ciliary microtubule architecture. *Development* 144, 1544–1553. doi: 10.1242/dev.143255

Yadav, S. P., Sharma, N. K., Liu, C., Dong, L., Li, T., and Swaroop, A. (2016). Centrosomal protein CP110 controls maturation of the mother centriole during cilia biogenesis. *Development* 143, 1491–1501. doi: 10.1242/dev.130120

Yamaguchi, T., Wallace, D. P., Magenheimer, B. S., Hempson, S. J., Grantham, J. J., and Calvet, J. P. (2004). Calcium restriction allows cAMP activation of the B-Raf/ERK pathway, switching cells to a cAMP-dependent growth-stimulated phenotype. *J. Biol. Chem.* 279, 40419–40430. doi: 10.1074/jbc.m405079200

Yang, N., Li, L., Eguether, T., Sundberg, J. P., Pazour, G. J., and Chen, J. (2015). Intraflagellar transport 27 is essential for hedgehog signaling but dispensable for ciliogenesis during hair follicle morphogenesis. *Development* 142, 2194–2202. doi: 10.1242/dev.115261

Yang, S., and Wang, C. (2012). The intraflagellar transport protein IFT80 is required for cilia formation and osteogenesis. *Bone* 51, 407–417. doi: 10.1016/j.bone.2012.06.021

Ye, F., Nager, A. R., and Nachury, M. V. (2018). BBSome trains remove activated GPCRs from cilia by enabling passage through the transition zone. *J. Cell Biol.* 217, 1847–1868. doi: 10.1083/jcb.201709041

Yoder, B. K., Richards, W. G., Sweeney, W. E., Wilkinson, J. E., Avener, E. D., and Woychik, R. P. (1995). Insertional mutagenesis and molecular analysis of a new gene associated with polycystic kidney disease. *Proc. Assoc. Am. Physicians* 107, 314–323.

Yoder, B. K., Tousson, A., Millican, L., Wu, J. H., Bugg, C. E. Jr., Schafer, J. A., et al. (2002). Polaris, a protein disrupted in orpk mutant mice, is required for assembly of renal cilium. *Am. J. Physiol. Renal. Physiol.* 282, F541–F552.

Yu, F., Guo, S., Li, T., Ran, J., Zhao, W., Li, D., et al. (2019). Ciliary defects caused by dysregulation of O-GlcNAc modification are associated with diabetic complications. *Cell Res.* 29, 171–173. doi: 10.1038/s41422-018-0114-7

Yu, F., Li, T., Sui, Y., Chen, Q., Yang, S., Yang, J., et al. (2020). O-GlcNAc transferase regulates centriole behavior and intraflagellar transport to promote ciliogenesis. *Protein Cell* 11, 852–857. doi: 10.1007/s13238-020-00746-2

Zhang, W., Taylor, S. P., Nevarez, L., Lachman, R. S., Nickerson, D. A., Bamshad, M., et al. (2016). IFT52 mutations destabilize anterograde complex assembly, disrupt ciliogenesis and result in short rib polydactyly syndrome. *Hum. Mol. Genet.* 25, 4012–4020. doi: 10.1093/hmg/ddw241

Zhu, B., Zhu, X., Wang, L., Liang, Y., Feng, Q., and Pan, J. (2017a). Functional exploration of the IFT-A complex in intraflagellar transport and ciliogenesis. *PLoS Genet.* 13:e1006627. doi: 10.1371/journal.pgen.1006627

Zhu, X., Liang, Y., Gao, F., and Pan, J. (2017b). IFT54 regulates IFT20 stability but is not essential for tubulin transport during ciliogenesis. *Cell Mol. Life Sci.* 74, 3425–3437. doi: 10.1007/s00018-017-2525-x

Zhu, X., Wang, J., Li, S., Lechtreck, K., and Pan, J. (2020). IFT54 directly interacts with kinesin-II and IFT dynein to regulate anterograde intraflagellar transport. *EMBO J.* 40, e105781.