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INVITED REVIEW

Sperm Biology

The roles of intraflagellar transport (IFT) protein 25 in mammalian signaling transduction and flagellogenesis

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Cilium, an organelle with a unique proteome and organization, protruding from the cell surface, generally serves as a force generator and signaling compartment. During ciliogenesis, ciliary proteins are synthesized in cytoplasm and transported into cilia by intraflagellar transport (IFT) particles, where the inner counterparts undergo reverse trafficking. The homeostasis of IFT plays a key role in cilial structure assembly and signaling transduction. Much progress has been made on the mechanisms and functions of IFT; however, recent studies have revealed the involvement of IFT particle subunits in organogenesis and spermatogenesis. In this review, we discuss new concepts concerning the molecular functions of IFT protein IFT25 and how its interactions with other IFT particle subunits are involved in mammalian development and fertility.

INTRODUCTION

Intracellular transport is driven by molecular motors that move along cytoskeletal tracks, which is responsible for delivering organelles, vesicles, and macromolecular complexes.[1] Although specific motors of individual cargos are crucial for executing correct and robust cargo deliveries, generally, they work in concert with distinct protein linkers resulting in multi-subunit complexes.[2]

Intraflagellar transport (IFT) is a well-studied bidirectional transport of a multi-subunit complex (often referred to as IFT particles or trains) along microtubule-based axoneme between the axoneme and the ciliary membrane.[3] This process was initially observed in flagella of green alga *Chlamydomonas reinhardtii* (*C. reinhardtii*)[4] and since then, it has been shown to be conserved in multiple species, including mammals. IFT plays important roles in the assembly and function of cilia by contributing to several essential processes in motility, sensory reception, and signaling. Defects in IFT and ciliogenesis have been linked to multisystem human disorders such as Bardet–Biedl syndrome (BBS), Joubert syndrome (JBTS), nephronophthisis (NPHP), Meckel syndrome (MKS), and short-rib thoracic dysplasia (SRTD). These disorders are generally referred to as the ciliopathies (**Figure 1**).[5]

IFT particles were first purified from the flagellar extracts of *C. reinhardtii*. These particles were dissociated into two distinct subunit complexes, namely IFT-A and IFT-B, under high ionic strength conditions. A much more complex picture of IFT subunits has been produced through many years of biochemical characterization in ciliate organisms, including mammals.

The IFT-A complex comprises six components (namely IFT43, IFT121, IFT122, IFT139, IFT140, and IFT144) plus the adaptor protein

TUB-like protein 3 (TULP3). In a predicted IFT-A model using the visible immunoprecipitation assay, IFT122, IFT140, and IFT144 form the core subcomplex, while the IFT43-IFT121 dimer interacts with IFT139 to form a noncore subcomplex.[6] The core and noncore subcomplexes are connected by IFT121. In the mammalian IFT-A complex, TULP3, a member of the Tubby family proteins, is associated with the core subcomplex and probably connects membrane proteins to the IFT-A complex.[7,8] This macromolecular complex is believed to mediate dynein-based delivery of IFT particles from the ciliary tip to the basal body (retrograde trafficking). It is also thought to be involved in the import of membrane proteins across the ciliary gate.[9,10] This diffusion and permeability barrier contains transition fibers and the transition zone.

The IFT-B complex is divided into two subcomplexes: the core subcomplex and the peripheral subcomplex. The core subcomplex can be further divided into two parts: the core-1 (B1-1) subgroup composed of five subunits (IFT22, IFT25, IFT27, IFT74, and IFT81) and the core-2 (B1-2) subgroup composed of another five subunits (IFT46, IFT52, IFT56, IFT70, and IFT88). The peripheral subcomplex is composed of six subunits (IFT20, IFT38, IFT54, IFT57, IFT80, and IFT172). Several subunits (*i.e.*, IFT20, IFT38, IFT46, IFT80, and IFT88) were shown to play a crucial role in IFT-B assembly and thereby in ciliary protein trafficking, whereas other subunits (IFT22, IFT25, IFT27, and IFT56) located peripherally in the IFT-B architecture were shown to be dispensable for IFT-B assembly at least in mammalian cells.[11–15] IFT-B complex plays an essential role in kinesin-based trafficking of IFT particles from the basal body to the ciliary tip (anterograde trafficking; **Table 1**).

In addition to the IFT-A and IFT-B complexes, the BBSome, a macromolecular complex, plays an essential role in IFT by contributing

to ciliary development and signaling.[16,17] This involves membrane protein trafficking, IFT particle integrity maintenance, and regulation of extracellular signaling vesicle shedding.[18]

In this review, we discuss the canonical functions of the IFT complex and introduce the novel functions of IFT25 in the mouse model. We outline how IFT25 interacts with other IFT subunits (IFT27 and IFT20). We focus on two main topics: recently emerged molecular functions of IFT25 in hedgehog signaling and the main roles of IFT25 during sperm flagellogenesis.

IFT25

Human ortholog of IFT25, C1orf41, was first purified from saline extracts of human term placentas and characterized as a member of small heat shock protein (sHSP), accordingly named as Hspb11, Hsp16.2, and HSPC034.[19] However, this characterization was not supported by structural chemistry analysis due to lack of canonical HSP feathers in HSPC034, including low percentage of consequence similarity, absence of an alpha-crystallin domain, distinct topology, and aggregation activity, which provide an argument concerning the main biological function of HSPC034.[20] The key clue to this issue came from systematic mapping of human protein interactome networks. In the yeast two-hybrid system, HSPC034 was found to interact with a known IFT subunit, IFT27.[21] HSPC034 orthologue (FAP232) also appeared in flagellar proteome of *C. reinhardtii*.[22] Both studies prompted the characterization of the HPS1.6 orthologue in mammal model organisms. In kidney epithelial cells (IMCD3), green fluorescent protein (GFP)-fused FAP232 was distributed at the ciliary basal body and along axoneme as punctual dots, which is a typical distribution pattern of IFT complex.[23] In addition, two independent studies concluded that FAP232 orthologue in *C. reinhardtii* was part of IFT-B complex and named it IFT25 after its real molecular weight in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).[24,25] Most recently, several high-resolution approaches delineated the peripheral location of IFT25 in the IFT-B model.[6]

The process of characterization established a unique association between IFT25 and another IFT-B1 core subunit, IFT27. This protein serves as an Rab-like small GTPase, the activity of which is modulated upon nucleotide-binding state.[26] As being lack of typical prenylation motif of Rab proteins, IFT27 is not allowed for membrane association upon GTP or GDP binding. This structural characteristic defines a distinct cellular distribution pattern compared with canonical Rab proteins Rab8 and Rab11, despite high structural similarity. Immunofluorescent microscopy assay showed that IFT27 is located at the ciliary basal body and translocation zone, significantly overlapped with IFT25. In addition, in mammal models, IFT27 was co-immunoprecipitated by IFT25 antibody in the reciprocal pull-down.[27] The most compelling evidence of tight association between these two IFT-B subunits came from X-ray crystallographic analyses, in which IFT25/27 heterodimer was found to interact through a conserved interface.[28] Notable, IFT25 in *C. reinhardtii* was found to stabilize IFT27 by preventing its aggression, but this requirement for stability is not mutual, as the abundance of IFT25 is not affected by the loss of IFT27.[11] These data have shown physical and functional interaction between IFT25 and IFT27. Given the specific module of heterodimer, it is not surprised to find that both specific *Ift25* and *Ift27* mutant mice manifest highly similar phenotypes.

IFT25 AND HEDGEHOG SIGNALING

The phylogenetic distributions of IFT25 among a variety of model organisms suggest a biological function besides in ciliogenesis. Its gene orthologs are present in ciliate species genome including

Table 1: Components of intraflagellar transport complexes and their activities

| IFT complex	IFT-A	IFT-B
Components	Core subcomplex	Core subcomplex
	IFT122	Core-1 groups
	IFT140	IFT22
	IFT144	IFT25
		IFT27
		IFT74
		IFT81
		Core-2 groups
		IFT46
		IFT52
		IFT56
		IFT70
		IFT88
	Noncore subcomplex	Peripheral subcomplex
	IFT43	IFT20
	IFT121	IFT38
	IFT139	IFT54
		IFT57
		IFT80
		IFT172
Activities	Retrograde trafficking; import of membrane protein across the ciliary gate	Anterograde trafficking

IFT: intraflagellar transport

C. reinhardtii and various vertebrates, but not in *Drosophila melanogaster* (*D. melanogaster*) and *Caenorhabditis elegans* (*C. elegans*). These flies and worms (invertebrates) assemble normal cilia in the absence of IFT25. However, this subunit of the IFT complex has been now thought to be involved in hedgehog signaling of mammals. *Ift25*-deficient mice died shortly after birth, accompanied by a series of phenotypes due to hedgehog signaling defects. These include polydactyly, cleft palate, lung isomerism, and structural heart defects, which cannot completely be explained by ciliary structural defects. Photochemistry analysis of embryonic fibroblast (MEF) from *Ift25* mutant mice revealed that Gli2, the transcription factor of the hedgehog pathway, was not trafficked to the ciliary tip in response to pathway activation. Moreover, other hedgehog components, Patched-1 (Ptch1) and Smoothened (Smo), were also found trapped in cilia, thus keeping the pathway in an attenuated state.[29] These data revealed the involvement of IFT25 in coupling hedgehog components to IFT particles, which suggested a separable role of IFT25 in signal transduction upon stimulation. Recently, this suggestion was supported by duplex kidney conformation of *Ift25* and *Ift27* mutant mice, which phenocopy urogenital system phenotypes caused by a *Gli3* mutation in murine.[30]

The molecular interaction of hedgehog components and the IFT25/27 heterodimer remain poorly understood. However, these may involve coupling of the BBSome to IFT-B complex. The BBSome has been proven to associate with trafficking of specific receptors, including Pach1 and Smo, into cilia and exporting membrane proteins from cilia.[17] This macromolecular complex undergoes dynamic transports between cell body and cilia, which is regulated by BBS-binding protein/regulator leucine zipper transcription factor like 1 (Lztfl1) and the small GTPase ADP ribosylation factor like GTPase 6 (Arl6). In IFT27-deficient fibroblast cells, Smo abnormally accumulated in cilia, accompanied with ciliary BBSome and Lztfl1 aggregation. In addition, mutations in *Lztfl1* resulted in accumulation of BBSome proteins in cilia, without disturbing the IFT27 distribution. These data indicate that Lztfl1 functions the downstream of the IFT25/27 heterodimer in coupling the BBSome to the IFT particle, which regulates the removal of hedgehog
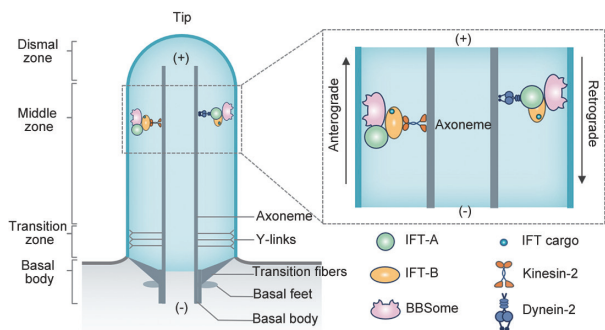
Figure 1: Schematic diagram of a cilium and intraflagellar transport. The anterograde IFT, driven by kinesin-2, moves along the axoneme from the basal body to the ciliary tip. The retrograde IFT is powered by dynein-2 and moves in the opposite direction of the anterograde IFT. IFT: intraflagellar transport.
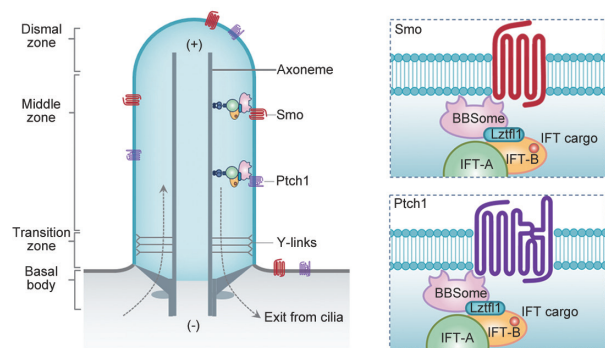
Figure 2: During hedgehog signaling, the hedgehog components, Ptch1 and Smo, are coupled to retrograde IFT particles by binding BBSome, which interact with Lztfl1 and IFT-B (*i.e.*, IFT25/27 heterodimer). IFT: intraflagellar transport; Ptch1: Patched-1; Smo: Smoothened; Lztfl1: leucine zipper transcription factor like 1.
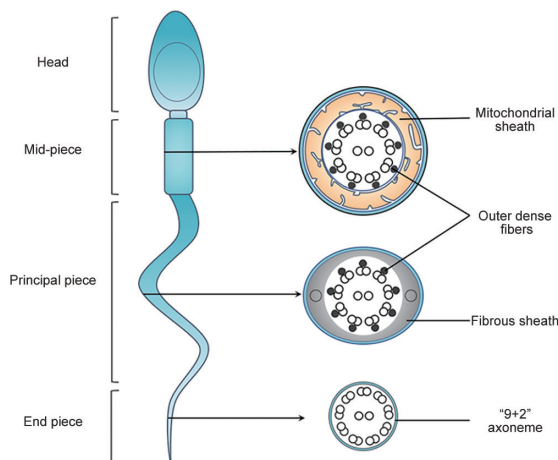
Figure 3: Schematic representation of mammalian spermatozoa and flagellum structure. Left panel, overall view of the spermatozoa showing the main head and flagellum structures and compartments. Right panel, cross section from the principal piece of the flagellum showing the organization of periaxonemal structures: mitochondrial sheath, fibrous sheath, outer dense fiber, and "9+2" axoneme.

components, Ptch1 and Smo, from cilia during hedgehog signaling (**Figure 2**).

IFT25 AND CILIOGENESIS

The redundancy of IFT25 in IFT complex assembly and ciliogenesis is well documented. As noted, genes encoding IFT25 homologs are absent from the genomes of *D. melanogaster* and *C. elegans*, both of which are ciliate organisms. RNA interference (RNAi)-mediated knockdown of *IFT25* exerted no effects on IFT-B complex assembly and flagellar length in *C. reinhardtii*. Moreover, global *Ift25* knockout (KO) mice survived to birth with normal abundance of cilia in tracheal epithelial cells,[14] and no detectable changes were identified in ciliary conformation. However, our study showed that conditional deletion of *Ift25* in mouse male germ cells resulted in reduced sperm number and multiple sperm defects, including round heads; short and bent tails, with some tails showing branched flagella and others with frequent abnormal thicknesses; as well as swollen tips of the tail.[31] Transmission electron microscope (TEM) analysis revealed disordered axoneme and microtubule doublets accompanied by disrupted accessory structures, which indicates the crucial role of IFT25 in sperm flagellogenesis.[31] Given the conserved conformation and composition of axoneme across species, IFT25 may play different roles in the development of cilia/flagella in somatic cells and male germ cells. Although the mechanisms underlying this new concept of IFT25 function remain elusive, these may involve retrograde trafficking in sperm flagella. To support this concept, in *Trypanosoma brucei* (*T. brucei*), another flagellar model organism, knockdown of *IFT25* using RNAi led to a phenotype similar to that in mouse male germ cells.[32] Notably, depletion of IFT25 induced dilation of unknown molecular composition at the distal flagellar end in both mouse sperm and *T. brucei*, which is typical for retrograde trafficking defects.[31,32] Immunofluorescence assay analysis, subsequently, revealed a disruption of IFT-B complex cycling between the cilia and the cell body. This deregulated process prevents IFT-A entry into cilia, which is essential for IFT complex integrity and retrograde IFT.[32]

The distinct function of IFT25 in somatic ciliogenesis and sperm flagellogenesis can be highly associated with protein variants among species and cells. For example, *C. reinhardtii* IFT25 shares 30%–35% similarity with the human homolog. IFT complex comprises at least 22 proteins, which can result in various compositions and architectures of IFT particles. Furthermore, the general regulation of protein expression at the transcription level and posttranscriptional level produces homologous proteins associated with cell types. These molecular diversities necessitate the structural studies of IFT machinery to reconcile such discrepancies in mammals.

In addition, both IFT25/27 and BBSome are involved in sperm flagellogenesis. The phylogenetic data demonstrate the high conservation of BBSome components (BBS1, BBS4, and BBS8) in multiple species.[33] Mice with global knockout of *BBS4* showed normal primary cilia and motile cilia, which is evidenced by the intact structure of cilia in kidney epithelial cells and trachea cells.[16] However, the disruption of *BBS4* resulted in sperm flagellogenesis failure, despite the normal sperm head and acrosome. This phenotype indicates that BBSome is involved in sperm flagellogenesis. Given that IFT25/27 complex is linked to BBSome by Lztfl1, depletion of IFT25 in spermatozoa can disrupt recruitment of cargos to the IFT complex, which will phenocopy the BBSome knockout mouse.

IFT25 AND IFT27, DIFFERENT ROLES IN SPERM ASSEMBLE

The popular architecture of IFT machinery is mainly based on the studies of model organisms, especially *C. reinhardtii*. Whether this architecture and binary protein interactions work in mammalian cells is still uncertain.

The cell type- and species-specific protein isoforms make it more complicated to study interactions between IFT subunits.[34] One example is the functions of IFT25 and IFT27. It has been shown that the two proteins form a complex in *C. reinhardtii*. Even though similar phenotypes were reported in the germ-cell-specific *Ift25* and *Ift27* knockout mice, differences were discovered. Decreased testicular expression level of IFT20 was found in the germ-cell-specific *Ift25* knockout mice, but not in the *Ift27* mutants. Similarly, sperm lipid raft was missing in the *Ift25* mutants, but not in the *Ift27* mutants.[35] Given that IFT25 has not been proven to regulate IFT20 expression, this is more possible due to the variable stability, which is reminiscent of the fact that IFT27 is stabilized by combination with IFT25. These data raise a question whether IFT25 stabilizes IFT20 by direct combination, or an indirect approach. To further explore this, structural and biochemistry studies will expand our knowledge on the molecular mechanism of IFT in mammals. IFT25 might also be involved in lipid trafficking, but not IFT27.

IFT25 AND PERIAXONEMAL STRUCTURES

IFT25 participates in the assembly of flagellar periaxonemal structures in mouse spermatozoa. The difference between mobile cilia and sperm flagella is the existence of highly organized periaxonemal structures, including mitochondrial sheath, outer dense fiber (ODF), and fiber sheath (FS), as shown in **Figure 3**.[36,37]

Components of ODF and FS are assembled in the cytoplasm and transported by IFT into flagella, where they are integrated into the flagellar structure.[38] Thus, it can be concluded that defects in IFT may disrupt axoneme assembly due to disordered paraflagellar structure. Our studies supported this conclusion since conditional disruption of IFT25 expression in germ cells led to abnormal "9+2" axoneme (a cylindrical structure that was composed of 9 fused pairs of microtubules on the outside of the cylinder and 2 unfused microtubules at the center), accompanied by disappearance of ODF and central apparatus, as well as disordered FS in mutant sperm flagella.[31] Therefore, the abnormal axoneme can be the secondary phenotype caused by defects in periaxonemal structures, as IFT25 depletion disrupts the integrity of IFT complex. The mechanism underlying this process remains largely unknown.

CONCLUSION

In this review, we discussed the discovery of novel functions of IFT25 with important roles in signaling and spermatogenesis, which opens new avenues of inquiry to study the IFT complex. These are essential for understanding this physiological process and especially for infertility in mammals. Further investigation aimed at clarifying phenotypic and mechanistic contributions of IFT proteins in mammals will be key to understanding their physiological importance and therapeutic value.

AUTHOR CONTRIBUTIONS

MYH and WI contributed to the literature review and drafting of the manuscript. ZL and ZZB revised the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

REFERENCES

1   Hafner AE, Santen L, Rieger H, Shaebani MR. Run-and-pause dynamics of cytoskeletal motor proteins. *Sci Rep* 2016; 6: 37162.
2   Nachury MV, Mick DU. Establishing and regulating the composition of cilia for signal transduction. *Nat Rev Mol Cell Biol* 2019; 20: 389–405.
3   Hao L, Scholey JM. Intraflagellar transport at a glance. *J Cell Sci* 2009; 122: 889–92.
4   Kozminski KG, Johnson KA, Forscher P, Rosenbaum JL. A motility in the eukaryotic flagellum unrelated to flagellar beating. *Proc Natl Acad Sci U S A* 1993; 90: 5519–23.
5   Toriyama M, Lee C, Taylor SP, Duran I, Cohn DH, *et al*. The ciliopathy-associated CPLANE proteins direct basal body recruitment of intraflagellar transport machinery. *Nat Genet* 2016; 48: 648–56.
6   Nakayama K, Katoh Y. Architecture of the IFT ciliary trafficking machinery and interplay between its components. *Crit Rev Biochem Mol Biol* 2020; 55: 179–96.
7   Han S, Miyoshi K, Shikada S, Amano G, Wang Y, *et al*. TULP3 is required for localization of membrane-associated proteins ARL13B and INPP5E to primary cilia. *Biochem Biophys Res Commun* 2019; 509: 227–34.
8   Mukhopadhyay S, Wen X, Chih B, Nelson CD, Lane WS, *et al*. TULP3 bridges the IFT-A complex and membrane phosphoinositides to promote trafficking of G protein-coupled receptors into primary cilia. *Genes Dev* 2010; 24: 2180–93.
9   Badgandi HB, Hwang SH, Shimada IS, Loriot E, Mukhopadhyay S. Tubby family proteins are adapters for ciliary trafficking of integral membrane proteins. *J Cell Biol* 2017; 216: 743–60.
10   Hirano T, Katoh Y, Nakayama K. Intraflagellar transport – a complex mediates ciliary entry and retrograde trafficking of ciliary G protein-coupled receptors. *Mol Biol Cell* 2017; 28: 429–39.
11   Dong B, Wu S, Wang J, Liu YX, Peng Z, *et al*. Chlamydomonas IFT25 is dispensable for flagellar assembly but required to export the BBSome from flagella. *Biol Open* 2017; 6: 1680–91.
12   Eguether T, San Agustin JT, Keady BT, Jonassen JA, Liang Y, *et al*. IFT27 links the BBSome to IFT for maintenance of the ciliary signaling compartment. *Dev Cell* 2014; 31: 279–90.
13   Funabashi T, Katoh Y, Michisaka S, Terada M, Sugawa M, *et al*. 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* 2017; 28: 624–33.
14   Keady BT, Samtani R, Tobita K, Tsuchya M, San Agustin JT, *et al*. IFT25 links the signal-dependent movement of Hedgehog components to intraflagellar transport. *Dev Cell* 2012; 22: 940–51.
15   Takei R, Katoh Y, Nakayama K. Robust interaction of IFT70 with IFT52–IFT88 in the IFT-B complex is required for ciliogenesis. *Biol Open* 2018; 7: bio033241.
16   Mykytyn K, Mullins RF, Andrews M, Chiang AP, Swiderski RE, *et al*. Bardet-Biedl syndrome type 4 (BBS4)-null mice implicate Bbs4 in flagella formation but not global cilia assembly. *Proc Natl Acad Sci U S A* 2004; 101: 8664–9.
17   Wingfield JL, Lechtreck KF, Lorentzen E. Trafficking of ciliary membrane proteins by the intraflagellar transport/BBSome machinery. *Essays Biochem* 2018; 62: 753–63.
18   Ye F, Nager AR, Nachury MV. BBSome trains remove activated GPCRs from cilia by enabling passage through the transition zone. *J Cell Biol* 2018; 217: 1847–68.
19   Bohn H, Winckler W. Isolation and characterization of five new soluble placental tissue proteins (PP22, PP23, PP24, PP25, PP26). *Arch Gynecol Obstet* 1991; 248: 111–5.
20   Kappe G, Boelens WC, de Jong WW. Why proteins without an alpha-crystallin domain should not be included in the human small heat shock protein family HSPB. *Cell Stress Chaperones* 2010; 15: 457–61.
21   Rual JF, Venkatesan K, Hao T, Hirozane-Kishikawa T, Dricot A, *et al*. Towards a proteome-scale map of the human protein-protein interaction network. *Nature* 2005; 437: 1173–8.
22   Pazour GJ, Agrin N, Leszyk J, Witman GB. Proteomic analysis of a eukaryotic cilium. *J Cell Biol* 2005; 170: 103–13.
23   Follit JA, Xu F, Keady BT, Pazour GJ. Characterization of mouse IFT complex B. *Cell Motil Cytoskeleton* 2009; 66: 457–68.
24   Lechtreck KF, Luro S, Awata J, Witman GB. HA-tagging of putative flagellar proteins in Chlamydomonas reinhardtii identifies a novel protein of intraflagellar transport complex B. *Cell Motil Cytoskeleton* 2009; 66: 469–82.
25   Wang Z, Fan ZC, Williamson SM, Qin H. Intraflagellar transport (IFT) protein IFT25 is a phosphoprotein component of IFT complex B and physically interacts with IFT27 in Chlamydomonas. *PLoS One* 2009; 4: e5384.
26   Qin H, Wang Z, Diener D, Rosenbaum J. Intraflagellar transport protein 27 is a small G protein involved in cell-cycle control. *Curr Biol* 2007; 17: 193–202.
27   Mizuno N, Taschner M, Engel BD, Lorentzen E. Structural studies of ciliary components. *J Mol Biol* 2012; 422: 163–80.
28   Bhogaraju S, Taschner M, Morawetz M, Basquin C, Lorentzen E. Crystal structure of the intraflagellar transport complex 25/27. *EMBO J* 2011; 30: 1907–18.
29   Yang N, Li L, Eguether T, Sundberg JP, Pazour GJ, *et al*. Intraflagellar transport 27 is essential for hedgehog signaling but dispensable for ciliogenesis during hair follicle morphogenesis. *Development* 2015; 142: 2860.
30   Desai PB, San Agustin JT, Stuck MW, Jonassen JA, Bates CM, *et al*. *Ift25* is not a

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cystic kidney disease gene but is required for early steps of kidney development. *Mech Dev* 2018; 151: 10–7.

31 Liu H, Li W, Zhang Y, Zhang Z, Shang X, *et al*. IFT25, an intraflagellar transporter protein dispensable for ciliogenesis in somatic cells, is essential for sperm flagella formation. *Biol Reprod* 2017; 96: 993–1006.

32 Huet D, Blisnick T, Perrot S, Bastin P. IFT25 is required for the construction of the trypanosome flagellum. *J Cell Sci* 2019; 132: jcs228296.

33 Nachury MV, Loktev AV, Zhang Q, Westlake CJ, Peranen J, *et al*. A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell* 2007; 129: 1201–13.

34 Kornblihtt AR, Schor IE, Allo M, Dujardin G, Petrillo E, *et al*. Alternative splicing: a pivotal step between eukaryotic transcription and translation. *Nat Rev Mol Cell Biol* 2013; 14: 153–65.

35 Zhang Y, Liu H, Li W, Zhang Z, Shang X, *et al*. Intraflagellar transporter protein (IFT27), an IFT25 binding partner, is essential for male fertility and spermiogenesis in mice. *Dev Biol* 2017; 432: 125–39.

36 Escalier D. New insights into the assembly of the periaxonemal structures in mammalian spermatozoa. *Biol Reprod* 2003; 69: 373–8.

37 Zhao W, Li Z, Ping P, Wang G, Yuan X, *et al*. Outer dense fibers stabilize the axoneme to maintain sperm motility. *J Cell Mol Med* 2018; 22: 1755–68.

38 Young SA, Miyata H, Satouh Y, Aitken RJ, Baker MA, *et al*. CABYR is essential for fibrous sheath integrity and progressive motility in mouse spermatozoa. *J Cell Sci* 2016; 129: 4379–87.