Ciliogenesis: building the cell's antenna

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Abstract | The cilium is a complex organelle, the assembly of which requires the coordination of motor-driven intraflagellar transport (IFT), membrane trafficking and selective import of cilium-specific proteins through a barrier at the ciliary transition zone. Recent findings provide insights into how cilia assemble and disassemble in synchrony with the cell cycle and how the balance of ciliary assembly and disassembly determines the steady-state ciliary length, with the inherent length-dependence of IFT rendering the ciliary assembly rate a decreasing function of length. As cilia are important in sensing and processing developmental signals and directing the flow of fluids such as mucus, defects in ciliogenesis and length control are likely to underlie a range of cilium-related human diseases.

Leftward flow
Flow of extraembryonic fluid across the node surface that moves to the left side of the animal.

Node
The anterior end of the primitive streak. Leftward flow in the node is important to determine the left–right axis of the body.

Axoneme
The insoluble microtubule-based structural scaffold of a cilium.

Basal body
A centriole that is acting to nucleate a cilium.

B tubule
The incomplete second microtubule that, together with the A tubule, forms the outer doublet of the ciliary axoneme.

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Cilia are present on almost every cell type in the human body (FIG. 1a–d). After years of scientific disregard, the cilium has emerged as a key organelle in numerous physiological and developmental processes (BOX 1). Cilia generate mucus flow and cerebrospinal-fluid flow, as well as leftward flow in the mouse node. Cilia also act as 'antennae' to sense extracellular signals, such as growth factors, hormones, odorants and developmental morphogens[1,2]. Defects in ciliary assembly and function lead to a wide range of human disease symptoms, including polycystic kidney disease, hydrocephalus and retinal degeneration (BOX 1). The recent explosion of interest in cilia-related diseases has prompted systematic analyses of the protein composition of cilia; however, although hundreds of ciliary proteins have been discovered in the past few years[3], in most cases their functions remain unclear. Here, we consider the pathways that direct the assembly of this complex set of building blocks into a functional cilium. We focus on the trafficking machinery required for ciliogenesis and the mechanisms that regulate ciliogenesis, particularly the control of ciliary length.

The cilium as a dynamic structure

The cilium is composed of a microtubule-based core structure called the axoneme, which is surrounded by a ciliary membrane that is continuous with the plasma membrane (FIG. 1e–g). The axoneme is constructed from nine parallel doublet microtubules known as outer doublets, which elongate from the basal body. At the ultrastructural level, the most distinctive feature of axonemal microtubules is the doublet structure, which consists of one complete microtubule (the A tubule) connected to an incomplete second microtubule (the B tubule) composed of fewer protofilaments (FIG. 1f,g). The tubulin of the outer doublets is subject to numerous post-translational modifications, including acetylation, glutamylation and glycylation[4], which seem to alter ciliary assembly and motility[5–10]. In addition to tubulin, structural components of the doublet include tektins[11] and the protofilament (pf) ribbon proteins[12], which contribute to accessory structures that are visible in the doublet by electron microscopy[13–15]. This doublet structure presumably underlies the high stability of axonemal microtubules, which do not disassemble spontaneously *in vitro*.

Cilia are conventionally classified into two categories, primary and motile cilia. A single primary cilium is found on the apical surface of the majority of cells in the human body. Primary cilia are immotile but they can sense physical and biochemical extracellular signals[1,2]. Motile cilia are present in large numbers on the epithelial cell surfaces of the trachea and oviduct, and cooperatively beat in wave-like patterns to generate fluid movement. To drive their bending motion, motile cilia have two extra microtubules in the centre of the axoneme, termed the central pair, as well as radial spokes and dynein arms attached to the microtubule doublets (FIG. 1f). Flagella are found on single-celled eukaryotes and sperm, and primarily function in cell locomotion. As the structure of the flagellum is identical to the motile cilium, the two names are often used interchangeably.

Cilia assemble through an ordered pathway of distinct steps[16,17]. First, basal bodies form (either from pre-existing centrioles or *de novo*), migrate to the cell surface and dock onto the actin-rich cortex. En route to the cortex, basal bodies associate with membrane vesicles, the subsequent fusion of which to the plasma membrane is likely to establish the ciliary membrane compartment. The positioning and orientation of the basal bodies dictates the alignment of the resulting cilia. Next, the basal body nucleates outgrowth of axonemal microtubules,

which protrude beneath an extension of membrane, giving rise to the cilium. The distal region of the basal body, where the outer doublets begin to form, is called the transition zone. The ciliary pocket, an invagination of the plasma membrane at the root of cilium, is found on some types of mammalian cells and trypanosomatids[18]. Assembly of the outer doublets occurs exclusively at the distal end of the cilium[19]. Because all protein synthesis is restricted to the cytoplasm, continued elongation of the cilium requires the selective import and transport

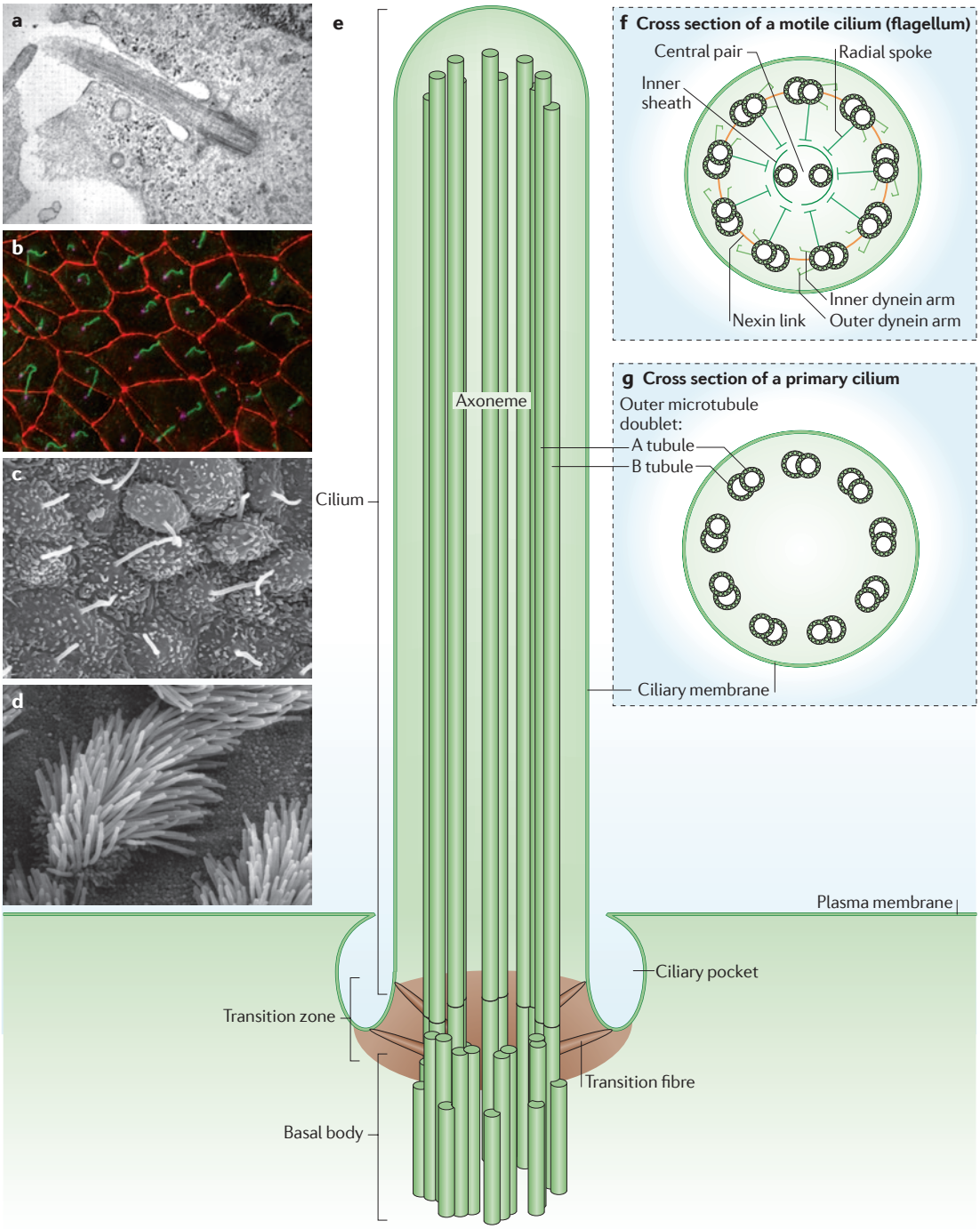

Figure 1 | **The architecture of cilia. a** | Transmission electron micrograph of the primary cilium of retinal pigment epithelial (RPE1) cells. **b** | Immunofluorescence image of primary cilia in inner medullary collecting duct (IMCD3) cells. The primary cilium (green) is produced once per cell and extends from the basal body (magenta). Cell–cell junctions are shown in red. **c**,**d** | Scanning electron micrographs of mouse nodal cilia (**c**) and mouse tracheal motile cilia (**d**). **e** | Schematic diagram of the primary cilium. **f**,**g** | Cross-section diagrams of a typical motile cilium (which is identical to a flagellum) (**f**) and a non-motile primary cilium (**g**). Image in part **a** is modified, with permission, from REF. 18 © (2010) The Company of Biologists. Image in part **c** is courtesy of S. Nonaka, The National Institute for Basic Biology, Japan. Image in part **d** is courtesy of Dartmouth College, Electron Microscope Facility, USA.

Primary cilium
A cilium, the basal body of which is the mother centriole that the cell inherited during the previous mitosis. The term is meant to contrast with 'secondary cilia', which refers to any other cilia that form later in the cell cycle.

Centriole
A cylindrical array of nine microtubule triplets that is found in the core of the centrosome.

of ciliary proteins out to the tip by intraflagellar transport (IFT), which is a molecular motor-driven process discussed below. The only exception to the requirement for IFT in ciliogenesis is found in certain species, such as *Plasmodium falciparum*[20], which assemble axonemes in the cytoplasm and subsequently push them out beneath a protrusion of the plasma membrane. A specialized system for transporting proteins from the cytoplasm to the tip is not required in this rare example, as the axoneme tip is bathed in cytoplasm.

When ciliary growth is completed, the cilium remains highly dynamic. New tubulin is continually incorporated at the tip of cilia at steady-state[21], but the cilium does not elongate further because the assembly is balanced by ongoing turnover[21–23]. Disassembly of microtubules at the tip does not seem to occur through spontaneous depolymerization but instead requires an active mechanism, which is likely to involve a Kinesin-13 molecular motor[24–26]. The fact that cilia are constantly turning over means that elements of the assembly machinery are also required for the maintenance of cilia length (see below). Because the assembly mechanisms outlined above appear to be shared by motile and non-motile cilia, defects in IFT or other aspects of ciliary assembly impair diverse physiological functions, including motility-related and sensory-related cilia functions.

Intraflagellar transport

As mentioned above, the transport of ciliary proteins from the cytoplasm to the ciliary tip is mediated by IFT, the bidirectional movement of multiprotein complexes (named IFT particles or IFT trains) along the axoneme[27,28] (FIG. 2).

IFT was first described, through differential interference contrast (DIC) microscopy, as the bidirectional movement of granule-like particles along the flagella of the green alga *Chlamydomonas reinhardtii*[29]. IFT trains have been observed, through transmission electron microscopy, to consist of a varying number of electron-opaque particles assembled into linear arrays that seem to contact the outer doublet B tubules and the overlying ciliary membrane[29,30]. Recent electron tomographic analysis of *C. reinhardtii* flagella revealed the detailed ultrastructure of IFT trains and grouped them into two different classes[31]. One class of IFT train is relatively long (around 700 nm) and less electron-opaque, with a particle periodicity of ~40 nm. The other class is around 250 nm in length, with ~16 nm periodicity. These long and short IFT trains were hypothesized to contribute to anterograde (from base to tip) and retrograde (from tip to base) transport, respectively, based on the observation that short trains disappear in a retrograde IFT mutant[31]. It has also been suggested that changes in particle periodicity could reflect differences in cargo loading.

IFT motors. Movement of cargo proteins along microtubules is catalysed by kinesin and dynein motor proteins, with any particular kinesin or dynein protein being specific for motion in a single direction, either towards the plus end or towards the minus end. IFT is driven by opposing motors: members of the Kinesin-2 family and cytoplasmic dynein 2 (previously known as cytoplasmic dynein 1b). IFT trains are carried to the ciliary tip (anterograde) by Kinesin-2 and returned to the cell body (retrograde) by cytoplasmic dynein 2 (REFS 27,32).

Two Kinesin-2 motors, heterotrimeric and homodimeric, are known to contribute to anterograde IFT. The canonical anterograde IFT motor is heterotrimeric Kinesin-2, which consists of two heterodimerized Kinesin-2 motor subunits and an accessory subunit, kinesin-associated protein (KAP)[33]. Heterotrimeric Kinesin-2 is indispensable for the assembly and maintenance of cilia and flagella in most ciliated organisms[30,34–36]. For example, in *C. reinhardtii*, the null mutant of flagellar-assembly impaired 10 (*fla10*) (one of the motor subunits of heterotrimeric Kinesin-2) cannot assemble flagella, and the temperature-sensitive *fla10* mutant undergoes flagellar resorption at restrictive temperatures[30]. However, *Caenorhabditis elegans* that harbour a mutation in the *kinesin-like protein 11* (*klp-11*) or *kap-1* heterotrimeric Kinesin-2 subunits have full-length sensory cilia because the homodimeric Kinesin-2 motor osmotic avoidance abnormal 3 (OSM-3) shares a partially redundant function with heterotrimeric Kinesin-2 (REFS 37,38). Genetic studies in *C. elegans* suggest that OSM-3 transports cell-specific proteins that contribute to differences in cilia morphology and function[37,39,40].

Cytoplasmic dynein 2 is a multiprotein complex that returns IFT trains from the ciliary tip. Knockdown or mutation of cytoplasmic dynein 2 components in *C. reinhardtii*, *C. elegans,* and mice produces short or stumpy cilia and flagella that are swollen with accumulated IFT particles[41–47]. This accumulation of proteins in cilia and flagella upon disruption of cytoplasmic dynein 2 is consistent with a role for the motor in retrograde IFT.

Box 1 | **Cilia in physiology and disease**

We typically think of cilia as motile organelles, and indeed this is one of their major functions. Arrays of dynein arms in motile cilia slide the outer doublets past one another, resulting in a large-scale bending motion. Cilium-driven motility is important for clearing mucus out of airways, moving eggs along oviducts, and circulating cerebrospinal fluid in brain ventricles. Cilium-driven flow in the node of the developing embryo is critical for determining left–right asymmetry of the viscera[35]. Furthermore, sperm move using bending motions of their flagella. In addition to these motile functions, cilia are important sensory organelles, owing to the fact that the ciliary membrane is rich in receptors and channels[1,2]. One key sensory function of cilia is mechanosensation; for example, cilia in the kidney can sense the flow of urine to modulate duct morphogenesis. Olfactory receptors are all located on cilia, and the rods and cones of the retina are merely highly modified sensory cilia. Cilia also have a key role in transducing Hedgehog signalling during development[65].

The wide range of ciliary functions means that ciliary defects can cause a wide range of disease symptoms. Defects in ciliary motility produce failures in mucus clearance, leading to bronchiectasis and chronic sinusitis[152]. Defects in ciliary motility in the node can cause situs inversus, which is an inversion of the left–right asymmetry of the viscera[152], and defects in ciliary motion in the brain can cause hydrocephalus (water on the brain)[153]. Sensory defects are equally deleterious. Defects in sensory cilia function in the kidney lead to polycystic kidney disease and nephronophthisis[61,66]. Defects in primary cilia in other tissues can cause anosmia, retinal degeneration and obesity. Because of the role of cilia in transducing Hedgehog signalling, which is involved in short- and long-range patterning processes during embryogenesis, ciliary defects can also lead to polydactyly.

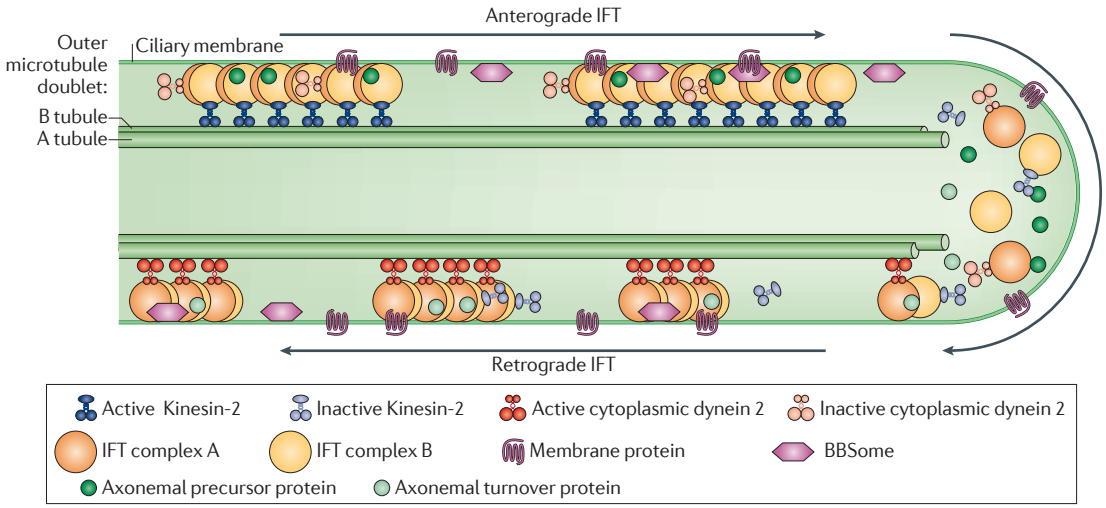

Figure 2 | **Intraflagellar transport machinery.** The canonical anterograde intraflagellar transport (IFT) motor, heterotrimeric Kinesin-2, transports IFT complexes A and B, axonemal proteins and cytoplasmic dynein 2 (previously known as cytoplasmic dynein 1b) to the tip of cilium. During this anterograde motion, Kinesin-2 is active and the retrograde motor, cytoplasmic dynein 2, is somehow kept inactive to allow smooth processive anterograde movement. At the tip of cilium, anterograde IFT trains release axonemal proteins and rearrange their conformation for retrograde IFT. Cytoplasmic dynein 2 is activated and transports retrograde IFT trains to the cell body. Subsets of IFT trains are involved in transporting membrane proteins and the BBSome (a complex comprised of at least seven Bardet–Biedl syndrome proteins).

IFT complex proteins. IFT particles were first isolated from *C. reinhardtii* flagella and were found to consist of two large, biochemically distinct complexes, termed IFT complex A and complex B[48,49]. IFT complex A and complex B seem to move together within the cilium, but they can be dissociated from each other *in vitro*. Subsequent studies have identified additional IFT protein constituents of these complexes. IFT particles are constructed from at least 20 proteins; IFT complex A contains six known proteins (IFT43, IFT121, IFT122, IFT139, IFT140 and IFT144) and IFT complex B contains 14 known proteins (IFT20, IFT22, IFT25, IFT27, IFT46, IFT52, IFT54, IFT57, IFT70, IFT74 (also known as IFT72), IFT80, IFT81, IFT88 and IFT172) (TABLE 1 and reviewed in REFS 27,28). The majority of these IFT proteins are conserved among ciliated organisms and are enriched in protein–protein interaction domains[50,51]. The phosphoprotein IFT25 was recently identified[52–54] and shown to interact with IFT27, a small GTPase of the RAB family[55]. In *C. elegans*, the IFT22 homologue (IFT-associated 2 (IFTA-2)) is a RAB-like protein that is not required for ciliogenesis, but it was recently shown to be required for signalling by the insulin-like growth factor pathway[56]. IFT70, a homologue of *C. elegans* abnormal dye-filling 1 (DYF-1) (REF. 57) and zebrafish Fleer (also known as Ttc30a)[6], was recently identified as an integral component of IFT complex B in *C. reinhardtii*[58]. IFT70 is also required for tubulin polyglutamylation and the formation of the outer doublet B tubule in *C. elegans*, zebrafish and *Tetrahymena*[6,59].

The two IFT complexes play complementary but distinct parts in the transport of ciliary proteins. IFT complex B contributes to anterograde transport and is essential for the assembly and maintenance of cilia and flagella (FIG. 2). In most cases, loss of any IFT complex B protein results in short or absent cilia[55,60–67]. By contrast, IFT complex A is required for retrograde transport that returns proteins to the cell body for turnover, but it does not appear to be necessary for ciliary assembly[68–73]. For example, *C. reinhardtii* with mutations in *fla15* and *fla17* (that encode IFT144 and IFT139, respectively) do assemble flagella, but these have abnormal bulges that contain accumulated IFT complex B proteins[68,73,74]. It is currently unknown whether IFT complexes A and B carry distinct sets of cargo proteins, and the specific roles of most IFT proteins remain uncharacterized.

IFT complex accessory proteins. Although IFT proteins have been conventionally defined as proteins that were identified through the biochemical analysis of purified IFT particles[48–50], some studies have identified putative IFT particle components independently of this type of analysis. In this Review, we classify these additional IFT proteins as IFT complex accessory proteins. The protein products of *C. elegans* dye-filling-defective mutants *dyf-3* and *dyf-13* were identified as IFT complex B accessory proteins by genetic and bioinformatic analyses[75–78]. DYF-13 may be required to activate the OSM-3 Kinesin-2 by docking this motor onto IFT complex B[57]. The *dyf-3* mutation was also mapped to the *qilin* (also known as *cluap1*) zebrafish cystic kidney mutant[66].

Tubby-like protein 3 (TULP3) interacts with IFT complex A and promotes the ciliary localization of a subset of G protein-coupled receptors (GPCRs), such as somatostatin receptor 3 (SSTR3) and melanin-concentrating hormone receptor 1 (MCHR1)[79].

Some Bardet–Biedl syndrome (BBS) proteins, products of *BBS* genes, have been shown to undergo IFT-like movement along the ciliary axoneme[57,78,80,81]. In *C. elegans*, loss-of-function mutations in *bbs-7* and *bbs-8* lead to structural and functional ciliary defects. This might be because

Polyglutamylation
Modification by addition of multiple glutamate residues onto a protein.

Bardet–Biedl syndrome
A ciliopathy that is characterized by obesity, retinitis pigmentosa, polydactyly and cognitive disability.

Table 1 | **Components of the intraflagellar transport system***

IFT system component	General protein name	*Chlamydomonas reinhardtii*	*Caenorhabditis elegans*	*Homo sapiens*	Other
Kinesin-2	Heterotrimeric	FLA10	KLP-20	KIF3A	
		FLA8	KLP-11	KIF3B	
		FLA3	KAP-1	KAP3, KIFAP3	
Kinesin-2	Homodimeric	–	OSM-3, KLP-2	KIF17	Kin5
Cytoplasmic dynein 2‡	Heavy chain	DHC1B	CHE-3	DYNC2H1	
	Intermediate chain	FAP133	DYCI-1	WDR34	
	Light intermediate chain	D1BLIC	D2LIC, XBX-1	DYNC2LI1	
	Light chain	LC8, FLA14	DLC-1	DYNLL1	
IFT complex A	IFT144	IFT144	DYF-2	WDR19	
	IFT140	IFT140	CHE-11	IFT140	
	IFT139	IFT139	ZK328.7	THM1, TTC21B	
	IFT122, IFT122A	IFT122, FAP80	DAF-10	IFT122, WDR10	
	IFT121, IFT122B	IFT121	IFTA-1	WDR35	
	IFT43	IFT43	–	IFT43, C14orf179	
IFT complex B	IFT172	IFT172	OSM-1	IFT172	
	IFT88	IFT88	OSM-5	IFT88	Tg737, Polaris
	IFT81	IFT81	IFT-81	IFT81	
	IFT80	IFT80	CHE-2	IFT80, WDR56	
	IFT74, IFT72	IFT74, IFT72	IFT-74	IFT74, IFT72	
	IFT70	IFT70, FAP259	DYF-1	TTC30A, TTC30B	Fleer
	IFT57	IFT57	CHE-13	IFT57	Hippi
	IFT54	IFT54, FAP116	DYF-11	IFT54, TRAF3IP1, MIPT3	Elipsa
	IFT52	IFT52, BLD1	OSM-6	IFT52, NGD5	
	IFT46	IFT46	DYF-6	IFT46, C11orf60	
	IFT27	IFT27	–	IFT27, RABL4	
	IFT25	IFT25, FAP232	–	IFT25, HSPB11	
	IFT22	IFT22, FAP9	IFTA-2	RABL5	
	IFT20	IFT20	Y110A7A.20	IFT20	
IFT complex A accessory		TLP1	TUB-1	TULP3	
IFT complex B accessory		FAP22	DYF-3	CLUAP1	Qilin
		DYF13	DYF-13	TTC26	
BBSome	BBS1	BBS1	BBS-1	BBS1	
	BBS2	–	BBS-2	BBS2	
	BBS4	BBS4	F58A4.14	BBS4	
	BBS5	BBS5	BBS-5	BBS5	
	BBS7	BBS7	BBS-7	BBS7	
	BBS8	BBS8	BBS-8	BBS8	
	BBS9	BBS9	BBS-9	BBS9	
	BBIP10	–	–	BBIP10	

BBIP10, BBSome-interacting protein of 10 kDa; BBS, Bardet–Biedl syndrome; CHE, chemotaxis abnormal; CLUAP1, clusterin-associated protein 1; DAF-10, abnormal dauer formation 10; DYF, abnormal dye-filling; FAP, flagellar-associated protein; FLA, flagellar-assembly impaired; HSPB11, heat shock protein β11; IFT, intraflagellar transport; IFTA, IFT-associated; KAP, kinesin-associated protein; KIF, kinesin family; KIFAP3, KIF-associated protein 3; KLP, kinesin-like protein; MIPT3, microtubule-interacting protein associated with TRAF3; OSM, osmotic avoidance abnormal; RABL, RAB-like; THM1, TTC-containing Hedgehog modulator; TLP1, tubby-like protein 1; TRAF3IP1, TRAF3-interacting protein 1; TTC, tetratricopeptide repeat; TUB-1, tubby protein homologue 1; TULP3, tubby-like protein 3; WDR, WD repeat-containing; XBX-1, X-Box promoter element-regulated 1.*In many cases, a single IFT protein may have several different names, as listed, depending on which organism it was discovered in. ‡Previously known as cytoplasmic dynein 1b.

these mutations cause combined IFT trafficking particles to fragment into separate IFT complexes A and B, which are then transported separately by heterotrimeric and homodimeric Kinesin-2, respectively[57,80]. Although it remains unclear why separate transport of complexes A and B would cause ciliary defects, it suggests that interaction between the two complexes may regulate some aspects of cargo transport. In *Bbs*-knockout mice, cilia persist in numerous cell types, although the ciliary tips are sometimes swollen or tapered[82–85]. In cultured epithelial cells, no significant differences have been observed between control and BBS-knockdown cells, with the exception of BBS1 and BBS5 small interfering RNA-treated cells, which fail to form cilia[81,86]. Although perhaps not required for ciliogenesis, BBS proteins may facilitate the transport of cell type-specific membrane proteins, such as SSTR3, MCHR1 and OSM-9, which fail to localize to primary cilia in *Bbs*-knockout mice and *C. elegans bbs* mutants[87,88]. Recently, *bbs* mutants were identified in *C. reinhardtii*. These mutants assemble motile, normal-length flagella that abnormally accumulate several putative signalling proteins, such as phospholipase D and a Ser/Thr protein kinase[89]. This observation agrees with the hypothesis that BBS proteins transport specific ciliary cargo other than indispensable axonemal components.

Ciliary trafficking

As the molecular composition of the IFT system gradually becomes defined, the key areas of interest will be how this system functions as a whole to mediate the assembly of cilia and how IFT interacts with other transport systems in the cell to get the correct cargo to the cilium in the first place. In the following sections, the current picture of ciliary trafficking is discussed.

Cargo transport by IFT. The requirement for IFT, discussed above, is thought to result from the need to move cargo, including axonemal components, ciliary membrane proteins and signal transduction proteins, along the length of the cilium. Several lines of evidence support this hypothesis. Axonemal proteins, such as the dynein arms that produce the force to drive ciliary motility, co-immunoprecipitate with IFT particles in extracts of *C. reinhardtii* flagella[90], suggesting a direct association between these cargo proteins and the IFT machinery. When IFT is inhibited by shifting *C. reinhardtii* temperature-sensitive *fla10*-mutant cells to the non-permissive temperature, flagella slowly resorb and cannot grow without restoring IFT[30,91]. Thus, at the very least, IFT must carry axonemal components into flagella, and it is likely to transport this cargo to the site of axoneme assembly at the flagellar tip. Certain membrane proteins tagged with green fluorescent protein (GFP) (including the transient receptor potential vanilloid (TRPV) channels OSM-9 and OCR-2 in *C. elegans* and polycystic kidney disease 2 (PKD2) in *C. reinhardtii*) display IFT-like movement[92,93]. As a major function of cilia is to sense the environment, the fact that important ciliary membrane-localized receptors and channels undergo active movement is of great interest, but at this point the functional consequences of this transport remain unclear. One possibility is that the active movement is important to achieve a uniform distribution of receptors and channels on the ciliary membrane surface.

Insight into the role of IFT in ciliary maintenance was obtained through experiments on radial spoke protein trafficking in *C. reinhardtii*. Radial spokes are multi-protein complexes that are involved in regulating flagellar motility (FIG. 1f). Radial-spoke complexes are partially assembled in the cell body and trafficked into flagella, where they fully assemble upon incorporation into the axoneme[90]. In normal cells, fully assembled radial spoke complexes can be seen in the cytoplasm but only if flagella are present, indicating that spoke complexes assembled in the flagellum can be returned to the cytoplasm. However, when IFT is shut off in a temperature-sensitive *fla10* mutant, partially assembled radial spoke complexes are depleted from flagella and fully assembled radial spoke turnover products accumulate in the soluble fraction of the flagellum, arguing that IFT normally brings these assembled radial spoke complexes out of the flagellum and that assembled radial spokes accumulate in the flagellum when IFT is abrogated[90].

A partial suppressor mutation of IFT46 produces cells that cannot recruit outer dynein arms into flagella, providing further evidence for the role of IFT in cargo transport[94,95]. However, the requirement for IFT to localize specific axonemal proteins may depend on the particular proteins involved, as inner dynein arms seem to have a stronger requirement for IFT than outer dynein arms[96]. Given the large number of proteins that must be assembled into the growing cilium, it would be surprising if the IFT train itself bound selectively to each one of them. Indeed, there is now evidence for specific adaptor proteins that link defined cargos to the IFT system. For example, outer dynein arm 16 (ODA16) is specifically required for the IFT-mediated transport of outer dynein arms[95,97].

Recently, dual-colour live-imaging of BBS4–GFP and IFT20–mCherry in *C. reinhardtii* flagella demonstrated that BBS4 is transported with IFT trains. This interaction is transient, as BBS4 sporadically 'boards' and 'falls off' moving trains[89]. Such transient interactions, with fast off-rates, may be desirable for IFT–cargo interactions, as they would facilitate the release of cargo when the IFT train reaches the cilium tip.

Membrane trafficking to cilia. Ciliogenesis requires the transport of membrane proteins to construct the specialized ciliary membrane (FIG. 3). *C. reinhardtii* IFT20 and IFT54 (a homologue of *C. elegans* DYF-11, mammalian MIPT3 and zebrafish elipsa) are involved in vesicle transport from the Golgi apparatus to cilia[53,67,98]. IFT20 is currently the only IFT protein that has been localized to the Golgi in addition to cilia and basal bodies[67]. IFT54 was recently identified as an IFT complex B protein that directly interacts with IFT20 (REFS 53,98,99). IFT54 also interacts with RAB8 through rabaptin 5 (also known as RABEP1), an endocytosis regulator. The small GTPase RAB8 promotes vesicle trafficking and plays a part in

Endocytosis
The pathway by which cells take up molecules from the plasma membrane by forming invaginations that close off to become intracellular vesicles.

Guanine nucleotide exchange factor
A protein that stimulates exchange of GDP for GTP on GTP-binding proteins, including G-proteins and GTPases.

cilia formation[81,100]. Moderate knockdown of IFT20 in mammalian cells decreases the amount of the calcium-permeable cation channel PKD2 in cilia, perhaps owing to reduced transport from the Golgi[67]. Moreover, in *C. elegans dyf-11* mutants, OSM-9 mislocalizes to the distal dendritic ends of ciliated neurons[98].

BBS proteins may also be involved in vesicle transport from the Golgi to basal bodies and cilia. At least seven BBS proteins have been shown to form a complex (termed the BBSome; see TABLE 1) that associates with ciliary membrane and binds Rabin8, a guanine nucleotide exchange factor (GEF) for RAB8 (REF. 81). The BBSome also interacts

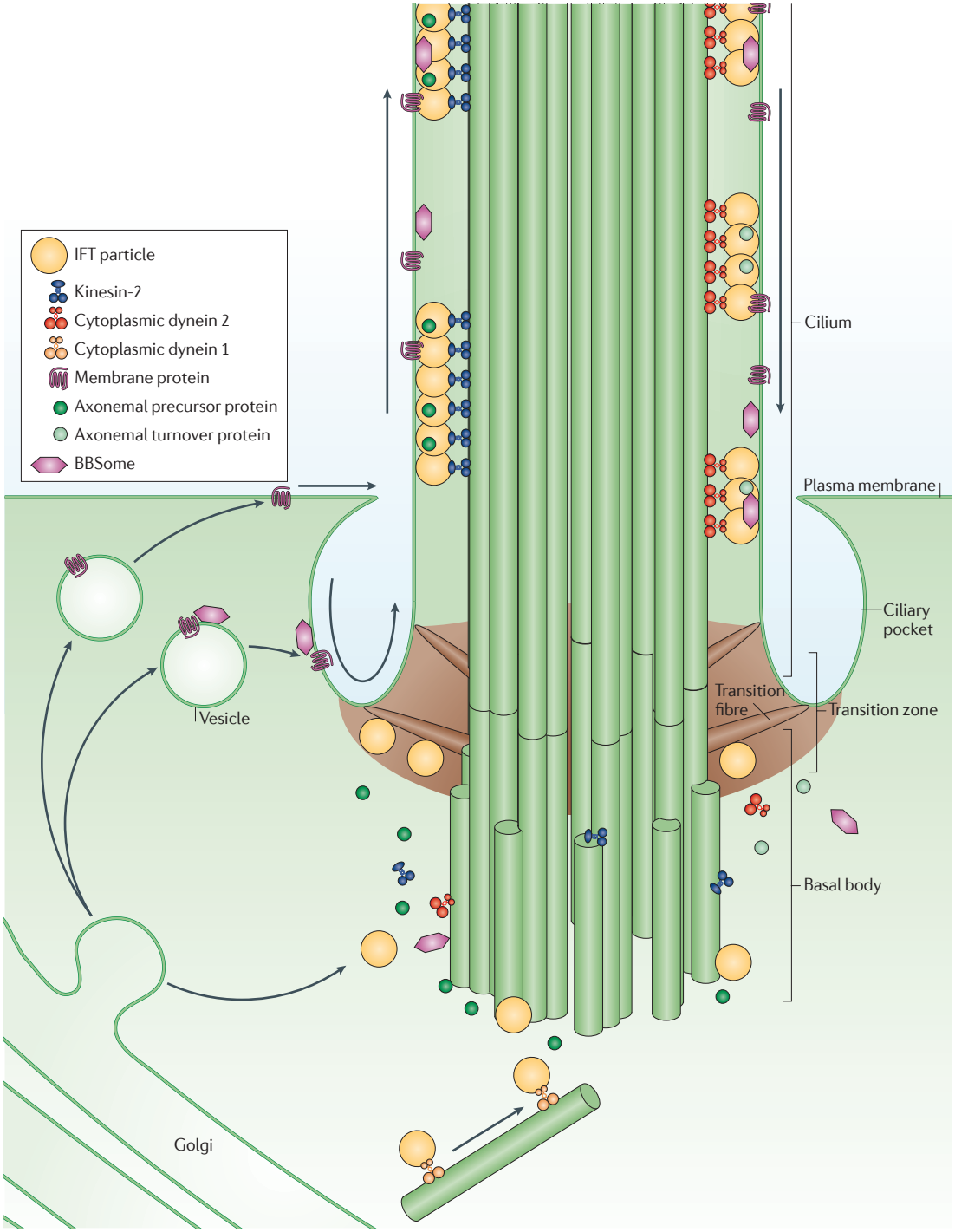

Figure 3 | **Ciliary trafficking pathways.** The transition zone forms a selectivity barrier at the base of the cilium. Proteins pass through this barrier either directly from the cytoplasm, in conjunction with intraflagellar transport (IFT) particles that have active motility, or via lateral motion in the membrane following the docking and fusion of Golgi-derived vesicles near the ciliary base, which could create a high local concentration of cilium-targeted proteins.

with ARF-like 6 (Arl6; also known as BBS3), a GTPase, to form a coat complex that sorts membrane proteins, such as SSTR3, to cilia[101]. Moreover, several IFT and BBS proteins share common structural components with clathrin–adaptin and coat protein I (COPI), which form transient scaffolds that drive the formation of transport vesicles. This similarity indicates that IFT and BBS proteins and vesicle trafficking proteins may share similar evolutionary roots and perform analogous functions[51,101,102]. Furthermore, certain IFT proteins are expressed in non-ciliated cells[103,104] and are required for the polarized recycling of the T-cell receptor–CD3 complex to the immune synapse[103]. These findings suggest that IFT proteins have a more general role in intracellular membrane transport.

Gating access to the cilium. The protein content of cilia differs markedly from the general cytoplasm, indicating the presence of a selectivity barrier at the ciliary base. Electron microscopy shows that the ciliary membrane is tightly associated with axonemal microtubules at the transition zone[105] (FIG. 1e), potentially impeding the free entry of soluble proteins. Direct analysis of the diffusion of ciliary membrane proteins confirmed the existence of a diffusion barrier at the base of the cilium. A role for septins[106] — membrane-associated proteins that form barriers to lateral diffusion during cytokinesis — in this barrier was implicated by further genetic analysis. Furthermore, the cilia disease-related centrosomal protein of 290 kDa (CEP290) was recently shown to be a component of the membrane–microtubule links at the transition zone, and mutations in *CEP290* led to a reduction in barrier function[105].

In addition to a barrier, there must be mechanisms that allow the correct subset of proteins to selectively pass the barrier and enter the cilium. It has been hypothesized that such a selective gate may resemble the nuclear pore (the transport machinery for moving cargo between the nucleus and the cytoplasm), at least at a functional level[32]. RAN is present in the cilium, and recent experiments expressing mutated RAN in the cytoplasm suggest that a RAN–GTP gradient may function in ciliary protein import, in much the same way that it does for nuclear import[107]. In nuclear import, the nuclear pore itself does not possess selectivity determinants, which are instead conferred by the importins and associated machinery that recognize nuclear import signals. For the cilium, it is possible that the IFT particles perform selective cargo recognition and then use their active motility to transfer this cargo through the diffusion barrier at the transition zone.

In a further analogy to the nuclear pore, there has been extensive effort to identify the ciliary equivalent of a nuclear localization signal (NLS). Such studies have revealed ciliary targeting signals from a number of different ciliary proteins, including PKD2, fibrocystin and rhodopsin[88,108–112]. However, at present there is no clear consensus sequence that is predictive of ciliary localization, and the protein machinery that detects these individual targeting sequences also remains undetermined. More detailed discussion of ciliary protein targeting can be found in the recent review by Nachury *et al.*[113].

Regulation of ciliogenesis

As ciliogenesis requires a complex programme of macromolecular synthesis and assembly, it must be carefully regulated. In dividing cells, the cilium is disassembled before cell division and the centrioles are inherited by the daughter cells, in which they act as templates for the next generation of cilia. Ciliogenesis is also regulated in response to cell confluence, fluid flow[114,115] and cell spreading[116].

The cycle of cilia birth, growth, and death is poorly understood in mechanistic detail. However, for dynamic structures such as cilia, there are three potential control points at which their assembly and disassembly can be regulated: synthesis of precursors; transport and assembly of precursors onto the growing structure; and turnover of this structure. Cells exploit all three control points to regulate the presence or absence of cilia.

Controlling the timing of ciliogenesis. Cilia typically form during G1 or G0 and disassemble around the time of mitosis (FIG. 4). This cycle does not occur in multiciliated cells, which are terminally differentiated and do not undergo division. The reasons for the cycle of assembly and disassembly remain unclear, although one probable explanation is that disassembly ensures proper formation of the mitotic spindle. As the centrioles that specify spindle pole location are attached to the ciliary base, repositioning of the centrioles to the cell interior usually requires the removal of cilia. Removal of cilia before division can be achieved by two mechanisms, which may be employed simultaneously. One mechanism involves cleavage of the cilium away from the centriole, a process that employs katanin-mediated microtubule severing of the doublet microtubules at the junction between the basal body and the transition zone[117]. Alternatively, cilia can be resorbed through disassembly from the ciliary tip. This active disassembly mechanism is regulated by its own signal transduction pathway[26,118,119]. Two proteins that have recently been found to regulate ciliary disassembly are the basal body-associated protein Pitchfork[120] and the scaffolding protein human enhancer of filamentation 1 (HEF1)[121], which mediate ciliary resorption through an interaction with Aurora A kinase. Aurora A, in turn, may exert some of its effect on ciliary disassembly by positively regulating the tubulin deacetylase histone deacetylase 6 (HDAC6)[121,122]; a decrease in tubulin acetylation reduces microtubule stability. The timing of cilia regrowth is also tightly regulated by cell cycle stage and by centriole age, with older centrioles forming cilia sooner than younger centrioles[123].

The regulated synthesis of cilium-specific proteins is another key regulator of ciliogenesis[124], the molecular basis of which is incompletely understood. Several hundred genes are upregulated when cells construct cilia[125]. This regulation apparently involves the regulatory factor X (RFX) transcription factor abnormal dauer formation 19 (DAF-19) in *C. elegans*[76] and forkhead box J1 (FOXJ1) in vertebrate cells with motile cilia[126].

IFT control of ciliogenesis. After ciliary precursors such as tubulin are synthesized, they must be transported into the cilium from the cytoplasm and moved to the site of

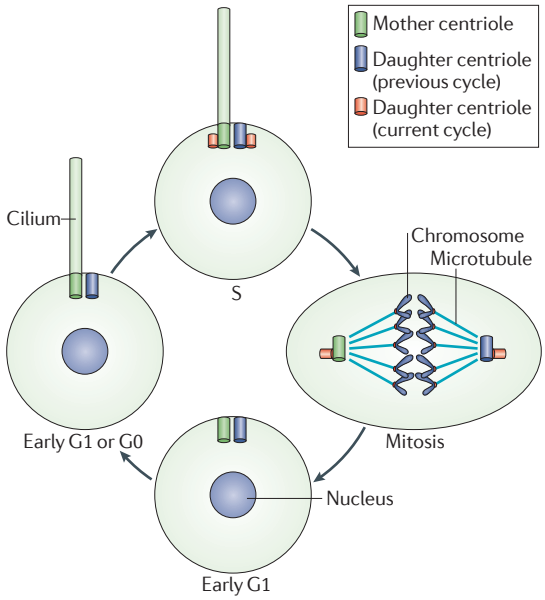

Figure 4 | **Synchronization of ciliary assembly and disassembly with the cell cycle.** Newly divided cells lack cilia. During the course of G1, the mother centriole docks at the cell cortex and nucleates a cilium, whereas the associated daughter centriole from the previous division generally does not form a cilium. During S-phase, the mother and daughter centrioles each duplicate to form new daughter centrioles. Before mitosis in most cells, the cilium is resorbed, allowing the centriole pairs to detach from the cortex and move to the spindle poles, which they help organize and position. Cilia are re-formed in the two daughter cells after re-entry into G1 phase.

assembly at the ciliary tip by the IFT system. The complexity of the IFT machinery thus presents numerous potential points for ciliogenesis control; indeed, various IFT parameters, such as IFT train size and speed, periodicity of train entry, and cargo selection, are likely to be regulated by uncharacterized mechanisms.

Growing cilia require greater quantities of axonemal precursors at their tip than cilia at steady-state[91], implying a need for regulation of transport. During flagellar regeneration in *C. reinhardtii*, IFT train size decreases as flagellar length increases (FIG. 5a), whereas IFT frequency remains independent of flagellar length[127]. Thus, the tips of growing flagella receive more building materials, delivered by larger IFT trains with greater cargo-carrying capacity. However, it is not yet known how IFT train size is regulated. As most IFT proteins and axonemal proteins accumulate at the ciliary base[53,90], it is probable that the assembly of anterograde IFT trains and the selective loading of cargo takes place at the transition zone, which is a morphologically distinct region of the axoneme that joins the flagellum with the basal body.

Regulation of IFT trains is also likely to occur at the ciliary tip, where trains turn around and switch from Kinesin-2- to cytoplasmic dynein 2-powered locomotion. In *C. reinhardtii,* IFT172 is involved in mediating the tip transition between anterograde and retrograde IFT[128,129]. A model for IFT turnaround has been proposed, in which the removal of Kinesin-2 from trains at the flagellar tip relieves the inhibition of cytoplasmic dynein 2, allowing retrograde transport[127]. However, this model requires additional investigation and is supported only by the observations in *C. reinhardtii* that KAP–GFP exhibits very few retrograde transport events and that Kinesin-2 can exit flagella in mutant cells lacking retrograde IFT[130,131]. The ability to differentiate between anterograde and retrograde IFT trains by electron tomography[31] may prove to be useful for testing models of IFT regulation at the flagellar tip.

Among the IFT proteins, IFT27 (REF. 55) seems to be a probable target of IFT regulation because it is a small GTPase of the RAB family, other members of which have key regulatory roles in various cellular membrane trafficking processes. Understanding how the nucleotide state of IFT27 regulates IFT, and what upstream factors modulate IFT27 nucleotide hydrolysis, is therefore a high priority for appreciating the regulation of ciliogenesis at the level of transport.

Molecular pathways controlling ciliary length. The same three control points that can be switched on and off to form or remove cilia (precursor synthesis, IFT and turnover at the ciliary tip) can also be modulated, in a less extreme manner, to control the steady-state length of cilia.

Ciliary length appears to be subject to biological regulation, as distinct cell types possess cilia of different average lengths. For motile cilia, it is likely that the generation of the ciliary waveform, as well as the hydrodynamic interactions of the cilium with the surrounding fluid, will be strong functions of length. Hence, a given cell type may have an optimal ciliary length to generate the desired type of ciliary beat and fluid flow. At any given beat frequency, the velocity of fluid flow generated by a cilium is predicted to be a decreasing function of ciliary length, such that cilia that are too long would be unable to generate an effective flow[132]. For sensory cilia, it is less obvious how ciliary length may affect function, but this may be due to our present ignorance of the teleological purpose of using cilia for sensory functions. Several cilium-related diseases and disease models seem to involve alterations in ciliary length; this provides insight into the molecular pathways that might control ciliary length. For example, tuberous sclerosis 1 (*Tsc1*)- or *Tsc2*-knockout mice and *tsc1a*-knockdown zebrafish develop kidney cysts and have cilia that are longer than the wild-type length[133–135]. Retinitis pigementosa 1 (RP1) has also been shown to affect ciliary length control[136], as have the Meckel–Gruber syndrome related proteins Meckel syndrome 1 (MKS1) and MKS3 (also known as meckelin and TMEM67) and the nephronophthisis-related protein nephrocystin 4 (REFS 137,138). Until the mechanisms that determine ciliary length are clarified, it will be difficult to disentangle the direct impacts of length alterations from other effects of these disease mutations.

Genetic screens in *C. reinhardtii* have identified a set of mutations that alter the length of flagella and cilia, which have been grouped into 'short flagella' (*shf*) and 'long flagella' (*lf*) mutants (for a review on length mutants see REF. 139). Several genes, the mutation of which leads to

Ciliary waveform
A type of ciliary motility that is characterized by large asymmetrical bending motions, as opposed to the flagellar waveform which is characterized by a symmetrical sine-wave-like bending pattern.

Nephronophthisis
A paediatric kidney cyst disease that is characterized by normal-sized kidneys with abnormally dilated ducts.

long flagella, have been cloned, and two of them encode kinases. One of these is a cyclin-dependent kinase (CDK) family member called LF2 and the other is a mitogen-activated protein (MAP) kinase family member called LF4 (REFS 140,141), but their targets are unknown. Many elements of this genetic length control pathway are apparently also conserved in mammals, including a mammalian orthologue of LF4 called male germ cell-associated kinase (MAK), a vertebrate orthologue of LF2 called cell cycle-related kinase (CCRK) and its binding partner, broad-minded[136,142]. Despite many years of genetic analysis studies and a constantly growing list of genes, such studies have not revealed the actual mechanism by which length is regulated. Biochemical and inhibitor studies have implicated other kinases, especially glycogen synthase kinase 3β (GSK3β), and calcium-mediated signalling pathways in length control[115,119,143,144], but their functional targets are unclear and, again, such studies have not directly revealed a length-control mechanism. It is tempting to speculate that such signalling molecules may be part of a feedback-control pathway regulating ciliary length (FIG. 5b), but at present there is no evidence that the activity of any of these molecules is length-dependent.

Theoretical models for ciliary length control. The lack of mechanistic information about individual gene function has prompted the development of theoretical models for length control in terms of cellular processes, rather than specific molecules[21,91,145–147]. Current models for length control are based on the observation that ciliary assembly at the tip not only occurs when the cilium first grows but also continues even after the cilium has reached its final length[21–23,148]. This steady-state assembly is balanced by the continuous removal of subunits from the tip of the cilium. The continuous assembly and disassembly of cilia at their tip results in their continuous turnover at steady-state. Steady-state assembly requires IFT to provide new axonemal subunits[21,96], and when IFT is turned off in full-length cilia, the cilium immediately begins to resorb[30]. The resorption that occurs when IFT is halted takes place at a constant rate throughout the entirety of the resorption process. This implies that the rate of disassembly at the tip is independent of ciliary length[21].

Because assembly and disassembly occur continually at steady-state, ciliary length can only be maintained when the two rates are equal to each other. As disassembly is length-independent, whatever mechanism exists within the cilium to regulate length must act by modulating the assembly process. Assembly appears to be at least partially determined by IFT, based on the observation that partial reduction in the IFT rate yields cilia of reduced length[21]. Therefore, if length is set by the balance of assembly and disassembly, disassembly is length-independent, and assembly is dictated by transport, the problem of length control reduces to the problem of how IFT is regulated as a function of length in order to achieve a particular steady-state equilibrium length. One simple model for length control is based on the finding that the total amount of IFT protein in the cilium is independent of ciliary length[21]. This implies that the number of IFT particles in the cilium

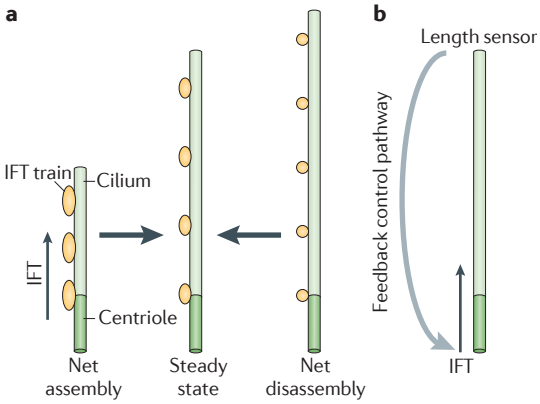

Figure 5 | **Ciliary length-control mechanisms. a** | The 'balance-point' mechanism. In this model, the transport efficiency of intraflagellar transport (IFT) scales inversely with the length of the cilium. The total amount of IFT protein and the frequency of IFT train traffic are length-independent, whereas the size of individual IFT trains scales as a decreasing function of ciliary length[127]. IFT-dependent assembly is balanced by continuous length-independent disassembly from the tip[21]. In short cilia, assembly predominates over disassembly, leading to growth, as large IFT trains carry more axonemal cargo to the ciliary tip (left). In long cilia, disassembly predominates over assembly, leading to shrinkage, as small IFT trains carry less cargo to the tip (right). In theory, the length-dependent assembly rate could also be modified by changing other parameters of IFT, such as transport speed or the efficiency of cargo loading on IFT trains. The system is stable at a unique steady-state length, at which the arrival of axonemal proteins at the ciliary tip drives assembly at exactly the same rate as disassembly. **b** | The feedback-control pathway, in which a length sensor (the mechanism of which is currently unknown) activates a signalling pathway that modulates ciliary assembly in response to changes in ciliary length.

is length-independent, although we have shown that whether these particles are grouped into small or large IFT trains is correlated with ciliary length[127]. Clearly, the time it takes a single IFT particle to travel with its cargo from the cell body out to the tip, deliver its cargo, and then travel back to the cell body to receive more cargo is proportional to the length — the farther the particle has to travel, the longer it takes. Hence, the frequency with which a given particle can deliver cargo to the tip decreases as ciliary length increases. If the number of particles is constant, then the net rate of cargo transport by IFT will decrease as the length of the cilium increases. The frequency of IFT train arrival at the tip remains constant, but IFT particles will be redistributed into smaller trains with reduced cargo-carry capacity. Assuming that transport becomes rate-limiting as the flagellum grows, we conclude that at steady-state the assembly rate will be a decreasing function of length. There will only be a single value for the length at which the length-dependent assembly rate and the length-independent disassembly rate will balance perfectly, resulting in a unique stable steady-state solution (FIG. 5a). This 'balance-point' is determined by

the rate of transport and also by the rate of turnover. Several specific predictions of this model, for example the dependence of length on the number of flagella per cell, have been confirmed in experimental tests[91].

This model conflicts with reports that cells containing the *fla10-1* mutation in a motor subunit of Kinesin-2 can, under certain conditions, have normal-length flagella despite having reduced levels of Kinesin-2 in the flagella[149]. However, this initial report was based on the quantity of FLA10 that was bound to isolated axonemes, whereas we now appreciate that the kinesin motor is not an axonemal component but rather a part of the soluble 'membrane and matrix fraction'[150]. Subsequent analyses of the *fla10-1* mutant at permissive temperature have shown that, in fact, there is little or no reduction of IFT protein levels in these mutant flagella compared with the wild type[30,36,52]. Moreover, live-cell imaging showed that neither the speed nor the frequency of IFT trains was significantly altered in *fla10-1* mutants at permissive temperature[68]. Finally, it was shown that partial reduction in FLA10 function, obtained by growing conditional *fla10* alleles at intermediate temperatures between fully permissive and fully restrictive, yields flagella of intermediate length[91], thus confirming the role of IFT in regulating flagellar length.

This balance-point model based on IFT and turnover leaves open the crucial question of what systems maintain a constant quantity of IFT proteins per cilium. We propose that many of the genes identified in screens for length mutants may be involved in the regulation of IFT quantity. One such length-altering mutant, *lf3*, identified in *C. reinhardtii* genetic screens, has been proposed to affect IFT protein levels in the flagella[151], based on the detection of increased IFT protein levels relative to total flagellar protein levels in isolated *lf3*-mutant flagella. However, this particular allele of the *LF3* gene causes cells to form abnormally short flagella, which means that when an equal total level of flagellar proteins is loaded on a gel, the wells loaded with *lf3* will contain proteins from a much larger number of flagella than the control wells. As IFT protein content per flagellum is length-independent, wells loaded with a sample from *C. reinhardtii* with shorter flagella will contain more IFT protein, owing to the greater number of flagella that were loaded. Such results cannot, therefore, be taken as evidence that the *LF3* gene acts through the control of IFT. In addition to keeping IFT quantity constant, IFT speed does not seem to vary dramatically with length, and how this is achieved even when the cargo-loading states may change substantially during ciliary growth remains unclear. In some systems, the interaction between different Kinesin-2 motors may have a role in speed regulation[38].

In considering length-control mechanisms, we should also ask whether the mechanisms that regulate steady-state length in mature cilia need to be the same as those that regulate length increases during ciliary growth. When a cilium has reached its target length, it would, in principle, be possible to shutdown axonemal microtubule turnover, thereafter 'locking-in' the ciliary length. In such a case, depletion of the IFT machinery or reductions in axonemal precursor synthesis might have substantially less effect on ciliary length. It will therefore be interesting to conduct detailed measurements of the rates of ciliary turnover and transport in both the assembling and mature states to see if there are any measurable differences.

Conclusions

The assembly of cilia is a complex and fascinating process that is only now starting to be understood. The field has made great progress in enumerating the list of parts of the cilium itself and of the components of the IFT system that promotes ciliary assembly. By contrast, still very little is known about how these parts function and work together. A major goal of research on IFT must now be to determine what the individual proteins are actually doing, in terms of enzymatic functions and regulated interactions. At the same time, there is a clear need for integrative models to understand how the huge, and still-increasing, number of trafficking components and pathways all work together to produce the highly regulated assembly and disassembly of cilia.

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