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Intraflagellar transport: from molecular characterisation to mechanism

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1. ABSTRACT

Research from a wide range of model systems such as *Chlamydomonas*, *C. elegans* and mice have shown that intraflagellar transport (IFT) is a bidirectional motility of large protein complexes along cilia and flagella that is essential for building and maintaining these organelles. Since its discovery in 1993, much progress has been made in uncovering the molecular and functional basis of IFT. Presently, many components of the core IFT machinery are known, including the anterograde kinesin 2 motor(s), the IFT-dynein retrograde motor and the collection of at least 17 proteins that makes up the IFT particle. Most significantly, discoveries linking IFT to polycystic kidney disease and other developmental phenotypes have broadened the context of IFT research by demonstrating that primary cilia and IFT are required for processes such as kidney tubule and retinal tissue development, limb bud morphogenesis and organ patterning. Central to the functional basis of IFT is its ability to traffic various ciliary protein cargos, which include structural ciliary subunits, as well as non-structural proteins such as transmembrane channels/receptors and sensory signalling molecules. Indeed, exciting data over the past 3-4 years, linking IFT and primary cilia to developmental and growth factor signalling, as well as the cell cycle, indicates that the current repertoire of IFT cargos is likely to expand. Here we present a comprehensive review of IFT, with particular emphasis on its molecular composition and mechanism of action.

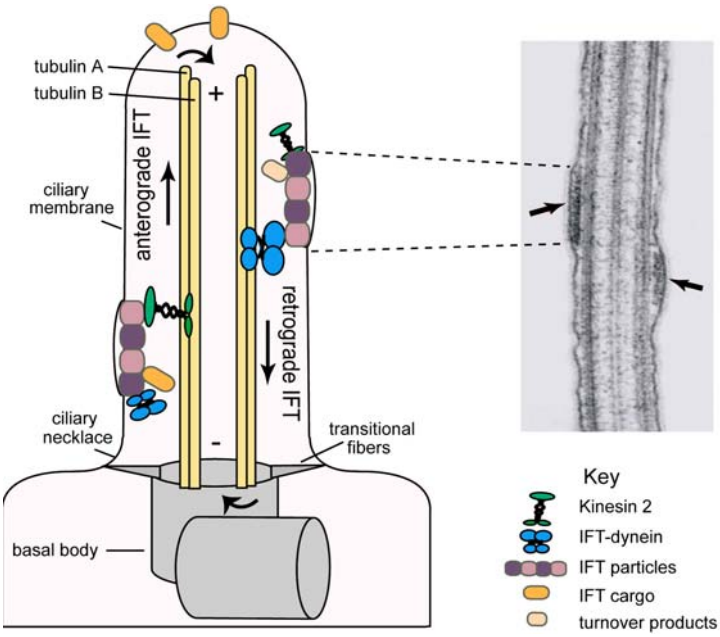

Figure 1. Intraflagellar transport is a microtubule-dependent bidirectional motility along ciliary axonemes. Schematic depicts kinesin 2 (towards the microtubule plus end) and IFT-dynein (towards the microtubule minus end) driven IFT along the outer doublet microtubules of ciliary axonemes (for simplicity, only two of the nine outer doublets are shown). Also indicated is the anchoring basal body, the transitional fibers and the position of the membrane-associated ciliary necklace, all of which may form a pore that regulates entry of ciliary proteins into the organelle. The electron micrograph of a *Chlamydomonas* flagellum shows the linear arrangement of electron dense IFT granules between the outer doublet microtubules and the ciliary membrane. Image reprinted from Pedersen *et al*. (ref. 41), with permission from Elsevier. Kinesin 2 drives anterograde transport (ciliary base to tip; towards the microtubule plus end) of IFT particles (and presumed cargo such as IFT-dynein), whereas IFT-dynein (cDHC1b) recycles the IFT machinery (and potentially ciliary turnover products) back to the ciliary base. Remodelling of IFT assemblies occurs at the turnaround phases at the tip and base of cilia (denoted by curved arrows).

2. INTRODUCTION

2.1. Scope of Review

In this review, we mostly discuss findings from three principal models of IFT – the unicellular alga *Chlamydomonas reinhardtii*, the nematode *Caenorhabditis elegans*, and the mouse. Specific topics include the molecular basis of IFT, IFT regulation/modulation, IFT and the cell cycle, and the role of IFT in cilium-based signalling. The review concludes with a mechanistic model for IFT.

2.2. Overview of cilia and flagella

2.2.1. Two classes of cilia: motile and non-motile

Motile cilia beat in a whip-like fashion, moving cells through fluids (e.g., sperm flagella and the cilia of many unicellular protists), or in higher organisms, propelling fluids across a layer of tissue (e.g., respiratory tract epithelial cell cilia). Dysfunction of motile cilia has long been known to cause prevalent mammalian disorders such as primary ciliary dyskinesia (defects in mucociliary clearance, male infertility, *situs inversus*) and hydrocephalus (abnormalities in cerebrospinal fluid movement). Many cell types also possess non-motile cilia such as the primary cilium, which is found on most mammalian cell surfaces. Examples include kidney tubule epithelial cell cilia, which protrude into fluid-filled kidney tubules, and the photoreceptor cell outer segment, which is a highly modified primary cilium. An excellent resource, found at http://www.bowserlab.org/primarycilia/cilialist.html, provides a comprehensive list of cells with primary cilia. Until recently, with the exception of certain sensory cilia, the cellular role(s) of most mammalian primary cilia was not known and it had even been suggested that these organelles were mostly vestigial in function. However, exciting data over the past decade or so has disproved this notion by showing that abnormalities in primary cilia function underlie numerous pathologies, including polycystic kidney disease, retinal degeneration, and organ laterality defects, as well as pleiotropic phenotypes such as Bardet-Biedl syndrome. The prevailing view is that most (or all) primary cilia (and motile cilia) serve as sensory organelles, acting as chemo-, mechano- or photo-sensors to transduce biochemical (e.g., odorants, developmental ligands) or physical (e.g., light, fluid flow) signals (1-3).

2.2.2. Cilium structure

Cilia are microtubule-based structures, enveloped by an extension of the plasma membrane, and anchored by a basal body. Aside from various cilia subtype-specific variations, some of which are discussed later on in this review, the basic structure of a cilium is shown in Figure 1. Ciliary axonemes consist of a ring of nine outer

microtubule doublets (heterodimers of tubulin A and B), which surround a varying number (normally 0-2) of central microtubule fibers. Motile ciliary axonemes usually possess two central microtubules (9+2 axonemes), whereas the axonemes of primary cilia typically possess no central microtubules (9+0 axonemes) (1, 3). Anchoring the ciliary axoneme at the cell periphery is the basal body, a centriole-based structure consisting of nine outer triplet microtubules, with no central microtubules. The transition zone defines the distal end of the basal body where the outer microtubule structure changes from centriolar triplets to axonemal doublets, and connecting the transition zone to the ciliary membrane are transitional fibers, which may serve as part of a ciliary pore, allowing regulated entry of proteins into the cilium (1). Finally, also at the base of cilia is the ciliary necklace, which comprises rows of membrane-associated protein particles that delineate the ciliary membrane from the main cell body membrane (4). Additional structural features found only in motile cilia include the force-generating dynein arms, radial spokes (connect outer doublets to central pair) and the flagellar tip complex (may function in flagellar rotation and/or cilium biogenesis) (1, 3, 5).

2.2.3. The 'ciliome'

Given the structural complexity and functional diversity of cilia, it is no surprise that these organelles are comprised of hundreds (if not thousands) of different proteins. Over the past 5 years, numerous proteomic, genomic and bioinformatics based studies of cilia from a diverse range of organisms have sought to identify the full complement of cilia-related genes and proteins (6-16). Indeed, very recently, Liu and colleagues presented the first proteomic analysis of a mammalian primary cilium (mouse photoreceptor sensory cilia) (17). The 'ciliome' contains a myriad of different proteins, including structural axonemal components, membrane-associated proteins (e.g., ion channels and membrane receptors), intraflagellar transport proteins and regulatory proteins such as kinases, phosphatases and G-proteins (to view the 'ciliome', go to: http://www.ciliome.com and http://www.ciliaproteome.org) (10, 18).

2.3. Discovery of IFT

A landmark finding for cilia research was the discovery in 1993 of intraflagellar transport (IFT). Using video-enhanced differential interference contrast (DIC) microscopy to observe *Chlamydomonas* flagella, Joel Rosenbaum's laboratory first observed IFT as discrete granule-like particles (rafts) moving bidirectionally between the base and distal tips of flagella (19). Anterograde IFT (base to tip) was found to occur at ~2.0 μms^{-1}, whereas retrograde IFT (tip to base) occurred at the faster rate of ~3.5 μms^{-1} (19). Electron micrographs of *Chlamydomonas* flagella revealed that the IFT rafts correspond to linear arrays of single non-vesicular electron dense particles (IFT particles), which exist between the outer microtubule doublets and the ciliary membrane (Figure 1) (19). As will be discussed in this review, the discovery of IFT paved the way for other important discoveries including: (i) IFT is an evolutionarily conserved process essential for building and maintaining ciliary structures, (ii) proper development of vertebrate tissues and organs requires IFT and primary cilia, and (iii) various intracellular signalling pathways (e.g., sonic Hedgehog signalling during vertebrate development) is dependent upon cilia and IFT.

2.4. IFT in outline

In broad strokes, the molecular basis of IFT can be described as follows (see also Figure 1): (i) kinesin 2 motor(s) drive anterograde IFT (base to ciliary tip), which delivers proteins (as IFT cargos) to the organelle. (ii) IFT-dynein powers the return retrograde trip, which serves to recycle the IFT machinery and potentially remove turnover products. (iii) Also essential for IFT is the IFT particle, which consists of at least 17 proteins that associate with the IFT motors and serve as potential docking points for IFT cargos. (iv) IFT cargos constitute the 'delivered' proteins and since IFT is required for building and maintaining ciliary structures, these cargos may include many different structural and functional ciliary components. (v) IFT regulators control and modulate IFT, particularly during the turnaround phases at the base and tip of cilia, where significant remodelling of IFT assemblies occurs. Taken together, IFT is a cycling process, consisting of coordinated anterograde and retrograde steps, which enables the assembly of functional ciliary structures.

3. THE IFT MACHINERY: LESSONS FROM *CHLAMYDOMONAS*

In this section we describe the core IFT machinery (Table 1). Since many of the initial discoveries were made in *Chlamydomonas*, most of the presented data stems from work with this unicellular green alga.

3.1. Kinesin 2: the anterograde IFT motor

The first kinesin 2 motor to be biochemically isolated and characterised was sea-urchin kinesin-II, which was found to exist as a microtubule plus end-directed heterotrimeric complex, consisting of two closely related motor subunits (KRP85 and KRP95) and a kinesin-associated protein (KAP) (20). The first evidence that kinesin 2 is required for IFT came from studies of temperature-sensitive flagellar assembly (*fla*) mutants in *Chlamydomonas*, where it was shown that FLA10 (*Chlamydomonas* KRP95 homologue) is essential for IFT and flagellar assembly (21). In *fla10*^{ts} mutants, growth at the restrictive temperature (32^{o}C) causes IFT to cease and cells become bald (i.e., flagella-less) 5-24 hours later (21). In *fla10* null mutants, flagellar structures are never observed (22). Similar to KRP95, mutations in *Chlamydomonas* KAP (*fla3-1*) and KRP85 (*fla-8*) also cause defective flagellar assembly and maintenance (23-25). In agreement with the *Chlamydomonas* data, studies in numerous other models, including sea urchins, *Tetrahymena* and mice all show that kinesin-II is required for normal cilia formation and function (26-30).

That kinesin-II is indeed the motor that actually drives transport of IFT particles from the base to tip of cilia came from careful investigation of IFT motor and IFT particle motility rates, conducted both *in vivo* (e.g.,

Table 1. Ciliogenic IFT proteins.

	Chlamydomonas	*C. elegans*	*H. sapiens*	**Domain structure**
Kinesin 2	FLA8	KLP-20	KIF3A	kinesin-II motor
	FLA10	KLP-11	KIF3B	kinesin-II motor
	FLA3	KAP-1	KAP3	kinesin-II associated
		OSM-3	KIF17	homodimeric kinesin 2
IFT-dynein	cDHC1b	CHE-3	DHC2	dynein heavy chain
	D1bLIC	XBX-1	D2LIC	coiled-coil
IFT Particle Complex B	IFT172	OSM-1	IFT172	WD40, TPR [1]
	IFT88	OSM-5	IFT88/Polaris	TPR [1]
	IFT81	F32A6.2	IFT81/CDV-1	coiled-coil
	IFT80	CHE-2	IFT80	WD40
	IFT74/72	C18H9.8	IFT74/72	coiled-coil
	IFT57/55	CHE-13	IFT57/Hippi	coiled-coil
	IFT52	OSM-6	IFT52/NGD5	GIFT domain
	IFT46	DYF-6	CAB66868	
	IFT27		RABL4	GTPase
	IFT20	Y110A7A.20	IFT20	coiled-coil
IFT Particle Complex A	IFT144	?	?	WD40 (see ref. 51)
	IFT140	CHE-11	IFT140	WD40, TPR [1]
	IFT139	?	?	
	IFT122A	DAF-10	IFT122/WDR10	WD40, TPR [1]
	IFT122B	?	?	WD40 (see ref. 51)
	IFT43	?	?	
Other putative IFT proteins	FAP259	DYF-1	TPR30A	TPR, coiled-coil
	FAP66	DYF-2	WDR19	WD40, TPR [1]
	FAP22	DYF-3	CLUAP1	coiled-coil
	81760	DYF-13	FLJ12571	
	FAP118	IFTA-1	WDR35	WD40, TPR [1]
IFT regulators	132537	BBS-1	BBS1	
	190054	BBS-7	BBS7	coiled-coil
	140113	BBS-8	BBS8	TPR [1]

Shown are homologs of ciliogenic IFT proteins in *Chlamydomonas*, *C. elegans* and *humans*. Also included are a number of nematode IFT proteins (i.e., DYF-1, -2, -3. -13, IFTA-1, BBS-1, BBS-7 and BBS-8), all of which undergo IFT and are required for sensory cilia assembly. The BBS proteins are listed as IFT regulators since their function is not generally required for IFT to occur, but rather is essential for proper regulation of kinesin 2 motor association (33, 77). Note that most ciliogenic IFT proteins are enriched for protein-protein interaction motifs (e.g., WD40, TPR, coiled coils). Note also that for four of the *Chlamydomonas* complex A proteins (IFT144, 139, 122B and 43), sequence information is not yet available. Accordingly, their likely homologs are denoted by question marks. Abbreviations: tetratricopeptide repeat [1]

examination of fluorescence tagged IFT motors and particles along *C. elegans* sensory cilia) and *in vitro* (e.g., the transport properties of purified IFT motors) (31-35). Interestingly, although kinesin-II is the sole anterograde IFT motor in *Chlamydomonas*, other organisms, including *Tetrahymena*, *C. elegans* and humans (but evidently not *Chlamydomonas*) possess a second kinesin 2 holoenzyme, termed OSM-3 in worms or KIF17 in mammals (Table 1). KIF17/OSM-3 exists as a homodimeric motor, which may also serve evolutionarily conserved roles in anterograde IFT, since in *C. elegans*, OSM-3 functions cooperatively and redundantly with kinesin-II (discussed in detail in section 4.2 below) (32, 36).

3.2. IFT-dynein: the retrograde IFT motor

Dynein was first indirectly implicated as the retrograde IFT motor when it was found that *Chlamydomonas* LC8 dynein light chain mutants (*fla14*) assemble short immotile bulging flagella, display highly reduced levels of retrograde but not anterograde IFT, and possess abnormal flagellar accumulations of IFT-particle-containing rafts (37). The actual identity of the retrograde IFT motor was subsequently revealed to be the heavy chain of cytoplasmic dynein 1b (cDHC1b) (Table 1), which possesses overlapping and even more severe flagellar phenotypes to those of *fla14* (LC8) mutants (38, 39). Similarly, *C. elegans* cDHC1b mutants (*che-3*) also assemble short sensory cilia with a bulge at the tip, associated with inhibited retrograde, but not anterograde, IFT (40). The anterograde transport of cDHC1b and D1bLIC (see below) is dependent on kinesin-II, suggesting that IFT-dynein is a cargo of kinesin-II (24, 41).

Studies in *Chlamydomonas*, *C. elegans*, and mammals revealed that the IFT-dynein motor complex includes a light intermediate chain, D1bLIC (Table 1), which co-immunoprecipitates and co-localises with cDHC1b, and is also required for retrograde IFT and cilia assembly (42-45). Interestingly, in *Chlamydomonas* D1bLIC mutants, cDHC1b does not accumulate within flagella, whereas IFT particle proteins do (41, 42). This is similar to what is observed in *C. elegans* D1bLIC mutants (*xbx-1*) (44), indicating that D1bLIC does not directly regulate the ability of cDHC1b to exit ciliary structures, but rather could serve other functions such as cargo binding (42, 44). Other components of IFT-dynein may include a conserved tctex-type dynein light chain (DYLT-2), which was found in *C. elegans* to undergo IFT and is required for sensory cilia assembly (9). Since dyneins typically exist as multi-subunit complexes, consisting of varying numbers of heavy chains, intermediate chains, light intermediate chains and light chains, it seems likely that additional components of IFT-dynein remain uncharacterised. Interestingly, the functional relationship between LC8 (the first dynein

component linked to retrograde IFT) and cDHC1b/LIC is unclear, since LC8 does not appear to co-purify with cDHC1b/LIC, and LC8 mutant flagella do not accumulate cDHC1b/LIC proteins (43).

3.3. The IFT Particle

Conventional and immunogold EM showed that *Chlamydomonas* IFT particles appear as electron-opaque granules beneath the flagellar membrane (21, 41, 46, 47). Typically, *Chlamydomonas* kinesin-II-driven anterograde particles are 120 nm in diameter and IFT-dynein-driven retrograde particles are 60 nm in diameter, with the former moving at 1.9-2.0 μms^{-1} and the latter at 2.7-3.5 μms^{-1} (19, 47). IFT particles were first biochemically isolated from *Chlamydomonas* flagella and found to be composed of two salt-stable complexes, IFT particle complex A (hereafter referred to as complex A) and IFT particle complex B (hereafter referred to as complex B) (Table 1) (48, 49). In *Chlamydomonas*, complex A consists of at least 6 proteins (IFT43, IFT122A, IFT122B, IFT139, IFT140 and IFT144), with a minimum of 10 or 11 proteins (IFT20, IFT27, IFT46, IFT52, IFT57/55, IFT74/72, IFT80, IFT81, IFT88 and IFT172) comprising complex B (48, 49). Complex A and B proteins are readily co-immunoprecipitated and immunodepleted from *Chlamydomonas* flagellar extracts, indicating that these complexes associate with each other during IFT (50). Direct evidence that the biochemically-isolated IFT particle complexes actually correspond to the electron-dense granules under the flagellar membrane is provided by immunogold EM studies, which show that kinesin-II and IFT particle proteins colocalise with IFT granules (41, 46). As expected, anterograde IFT of complexes A and B is dependent on kinesin-II (FLA10) function (48).

Most IFT particle proteins are enriched for predicted protein-protein interaction motifs such as WD-repeats, tetratricopeptide repeats (TPR) and coiled coils (Table 1) (51). Such motifs may facilitate formation of the multi-subunit IFT particle complexes and/or provide a mechanistic basis for tethering various IFT cargos. Although many IFT particle proteins have now been identified and characterised to some extent, how they interact and are assembled into functional IFT particles is poorly understood. To shed light on these questions, Lucker *et al.* employed chemical cross-linking and yeast two-hybrid approaches to investigate the structural hierarchy of *Chlamydomonas* IFT particles and found that complex B possesses a 500 kDa core, consisting of IFT88, IFT81, IFT74/72, IFT52, IFT46 and IFT27 (52). In addition, IFT81 and IFT74/72 were found to form higher-order oligomers, possibly $(IFT81)_2(IFT74/72)_2$ tetramers, which may act as a scaffold for the assembly of complex B (52).

Like the IFT motors, IFT particle proteins are also required for assembling fully functional ciliary structures. This was first shown in *C. elegans*, since at the time of cloning the *Chlamydomonas* IFT particle genes, mutant alleles of the corresponding gene homologues were already available in nematodes, and in each case, sensory cilia structure and function was severely affected (53, 54). Subsequent studies, principally in *Chlamydomonas* (55-60), *C. elegans* (61-66) and mice (56, 67-69), but also in organisms such as *Tetrahymena* (70, 71), *Drosophila* (72) and zebrafish (73, 74), demonstrated that the ciliogenic role of IFT particle genes is evolutionarily conserved.

3.3.1. IFT particle complexes A and B are functionally distinct

An important question is whether complexes A and B, initially described biochemically in *Chlamydomonas*, possess distinct and physiologically relevant functions *in vivo*. Early evidence that complexes A and B are functionally distinct came from the analysis of several uncloned *Chlamydomonas* flagellar assembly mutants (*fla15*^{ts}, *fla16*^{ts}, *fla17*^{ts}), which are defective in retrograde IFT. In these mutants, flagella were found to possess large accumulations of complex B proteins, yet complex A protein levels were severely depleted (24, 75). Similarly, mutations in *Chlamydomonas* IFT172 (*fla11*^{ts}) and D1bLIC (*yh43*^{ts}; dynein light intermediate chain) cause flagellar tip accumulation of complex B proteins, but not complex A proteins (24, 41, 58). Furthermore, within the *Chlamydomonas* peri-basal body region, IFT172 (complex B) and IFT139 (complex A) only partially colocalise, suggesting that complexes A and B are not always physically associated (60).

Further evidence for distinct complex A and B functions has also been obtained from *C. elegans*. In complex B mutants (*che-13*/*ift57* and *osm-6*/*ift52*), all examined IFT proteins are excluded from cilia, whereas in a complex A mutant (*che-11*/*ift140*), complex B proteins (OSM-5/IFT88) accumulate within cilia (44, 64). Furthermore, in *C. elegans* Bardet-Biedl syndrome (BBS) gene mutants (*bbs-1*, *-7*, *-8*), complexes A and B are uncoupled from each other, with each complex displaying distinct ciliary localisation patterns and rates of IFT motility (discussed below in section 4.4.1) (33, 76, 77). Finally, electron micrographs of IFT mutant cilia demonstrate that complex B mutants possess very short ciliary axonemes (< 2 μm), whereas the ciliary axonemes of complex A mutants are considerably longer (4-6 μm) (53).

Taken together, the data indicates that complexes A and B play physiologically distinct functions. Presently, the data suggests a model whereby complex B proteins are generally required for entry of IFT proteins into the ciliary axoneme via kinesin 2 driven anterograde IFT, whereas complex A proteins facilitate the removal/recycling of IFT proteins from cilia via IFT-dynein-driven retrograde IFT.

4. IFT IN METAZOANS: THE *C. ELEGANS* MODEL

Investigation of IFT in *C. elegans* sensory cilia has contributed significantly to our understanding of the molecular basis of IFT. In addition to corroborating many of the findings in *Chlamydomonas*, studies in *C. elegans* have uncovered new ciliogenic IFT components and have also indicated that modulation of IFT generates structural and functional ciliary diversity.

4.1. Structure/function of *C. elegans* sensory cilia

In *C. elegans* adult hermaphrodites, 60 neuronal cells possess sensory cilia (non-motile primary cilia) that extend from dendritic tips. Grouped into distinct specialised sensory organs called sensillae (e.g., the 12 sensory neurons that make up the amphid sensillum), where cilia are directly or indirectly exposed to the environment via openings in the nematode cuticle, sensory cilia play important roles in chemo-, mechano- and osmo-sensation (78). Unlike the structure of *Chlamydomonas* flagella, *C. elegans* sensory cilia possess many different shapes and various microtubule arrangements. For example, most amphid and phasmid cilia possess a bipartite microtubule structure, consisting of a 3-4 μm middle segment (9 outer doublet microtubules) and a ~3 μm distal segment (9 outer singlet microtubules due to the termination of the tubulin B subfiber at the distal end of the middle segment) (Figure 2) (53). Ciliary distal segments are likely of general functional importance, since they are found in many organisms and cells, including *Chlamydomonas* gametes engaged in mating and various vertebrate cell types (79-81). In nematodes, distal segments are clearly functional, since animals specifically lacking this part of the cilium (e.g., *osm-3* mutants) possess sensory abnormalities.

As expected, mutations in *C. elegans* IFT genes cause cilia assembly defects and sensory behavioural phenotypes, including dye uptake abnormalities (Dyf), chemosensation (Che) defects, high osmolarity avoidance abnormalities (Osm) and dauer entry/exit deficiencies (53, 78, 82). Complex B mutants (*che-2*, *che-13*, *osm-1*, *osm-5*, *osm-6*, *dyf-6*) typically possess severely stunted cilia (< 2μm) (53, 61-65), whereas complex A (*che-11*, *daf-10*) and IFT-dynein subunit (*che-3*, *xbx-1*) mutants possess moderately truncated ciliary axonemes that are enlarged and swollen near the tip (44, 53, 63). Interestingly, deletion mutants of *C. elegans ift-72* and *ift-81* possess only moderately truncated cilia, which suggests that IFT-74 and IFT-81 proteins may be somewhat less important for nematode sensory cilia biogenesis than other complex B proteins (66). Unlike *Chlamydomonas*, *C. elegans* heterotrimeric kinesin-II (*klp-11*, *klp-20*, *kap-1*) mutants possess cilia of wild-type length, with no obvious morphological abnormalities (32, 53, 83). As discussed below (section 4.2), the reason for this is because of a second kinesin 2 motor, OSM-3-kinesin, which acts redundantly with kinesin-II to build ciliary structures. Finally, the transcriptional regulation of most *C. elegans* IFT genes is driven by the RFX transcription factor DAF-19 (84), a process that appears to be evolutionarily conserved (85-88).

4.2. Not one, but two kinesin 2 motors drive *C. elegans* anterograde IFT

In *C. elegans*, two kinesin 2 motors drive anterograde IFT (Figure 2) (32, 36). By observing the movement of GFP-tagged IFT proteins along amphid channel cilia, it was found that both heterotrimeric kinesin-II (KLP-11/KLP-20/KAP-1) and homodimeric OSM-3 co-transport IFT particles along the middle segments at an average rate of ~0.70 μms^{-1} (Figure 2A) (32). At the distal ends of middle segments, kinesin-II disengages from moving IFT assemblies, whereas OSM-3 continues to drive distal segment transport at a faster average rate of ~1.30 μms^{-1} (Figure 2A) (32). Since on their own, *C. elegans* OSM-3 motors moves IFT particles at ~1.3 μms^{-1} (Figure 2B) and kinesin-II motors moves IFT particles at ~0.5 μms^{-1} (Figure 2C), an intermediary rate of ~0.7 μms^{-1} in middle segments suggests that these two motors cooperate to drive middle segment anterograde IFT (Figure 2A). One model to explain these observations is that the two motors are tethered to the same IFT particles, and that the observed intermediate motility rate (~0.7 μms^{-1}) arises due to the faster motor (OSM-3; ~1.3 μms^{-1}) being retarded by the slower motor (kinesin-II; ~0.5 μms^{-1}) (32). The observation that kinesin-II motors do not enter distal segments provides an explanation as to why the amphid channel cilia of *osm-3* mutants specifically lack distal segments (Figure 2C).

The above findings also demonstrate that kinesin 2 function is partially redundant in building amphid channel cilia, since either motor alone can compensate for the absence of the other to successfully drive the assembly of middle (but not distal) segments (32). Similar observations were also made in *C. elegans* AWC amphid cilia, which have a winged structure and appear to lack typical distal segments. Here, kinesin 2 motor function is completely redundant, since OSM-3 function can fully compensate for loss of kinesin-II function and *vice versa* (83). From the amphid channel and AWC cilium studies, it has been suggested that kinesin-II acts as a 'canonical' anterograde IFT motor, whereas OSM-3 serves as an 'accessory' motor, which can substitute for kinesin-II loss, as well as extend the canonical pathway in certain ciliary structures (e.g., build distal segments of amphid channel cilia) (83).

A very recent report has shown that assembly of the branched AWB cilium structure requires cell-specific regulation of IFT (89). Although AWB ciliary middle segments are built via the cooperative and mostly redundant functions of kinesin-II and OSM-3, the specific IFT mechanism employed for AWB cilia assembly differs from that of amphid channel cilia assembly. For example, in AWB cilia, OSM-3 is not required to build the distal segments. Furthermore, unlike amphid channel cilia, a pool of OSM-3 moves independently of kinesin-II in the AWB ciliary middle segments and OSM-3 (as well as OSM-5/IFT88 and DAF-10/IFT122A) rarely enters the corresponding distal segments (89). The latter observation raises the intriguing question that if OSM-3 is generally excluded from the distal segments of AWB cilia, how is the distal segment assembled? Possible answers could include, (i) distal segment assembly is IFT-independent, or (ii) after being assembled during embryogenesis, the maintenance and function of AWB distal segments requires only transient levels of IFT (89). Taken together, these findings indicate that cell-specific regulation of *C. elegans* IFT contributes to the assembly of morphologically distinct and functionally specialised cilium structures. In a wider context, differential regulation of IFT may also contribute to the formation of discrete ciliary subtypes in vertebrates and mammals. Indeed, in *Chlamydomonas* engaged in mating, the tubulin A subfiber of outer doublet microtubules elongates to create an extended singlet

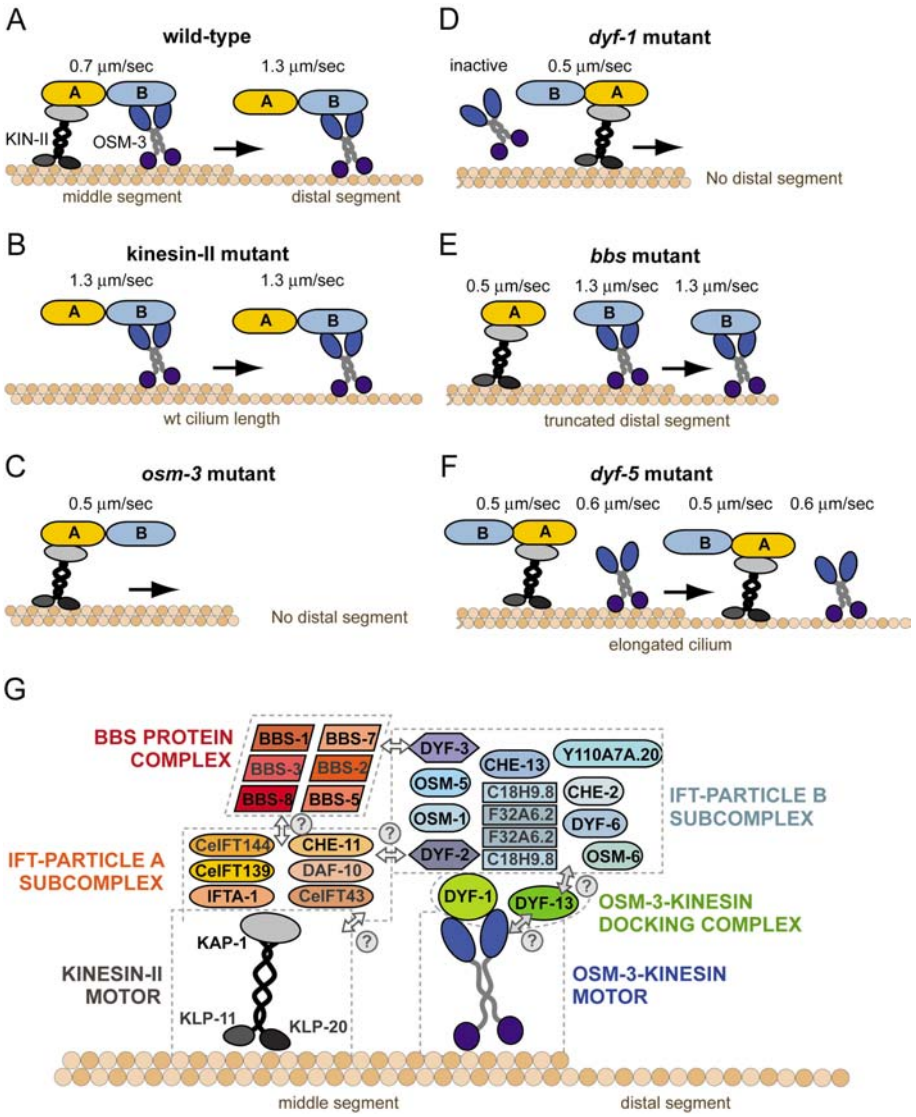

Figure 2. Modulation of anterograde IFT in *C. elegans* sensory cilia. A) In wild-type amphid channel cilia, heterotrimeric kinesin-II (KIN-II; slow motor) and homodimeric OSM-3 (fast motor) cooperate to drive middle segment (composed of outer doublet microtubules) anterograde transport at an intermediary rate (0.7 μms^{-1}). At the distal ends of middle segments, kinesin-II disengages from IFT particles and OSM-3 alone takes over distal segment (composed of singlet outer microtubules) transport at a fast rate (1.3 μms^{-1}). B) In kinesin-II mutants, OSM-3 drives middle segment IFT at a fast rate (1.3 μms^{-1}). C) In *osm-3* mutants, kinesin-II drives middle segment IFT at a slow rate (0.5 μms^{-1}). Since kinesin-II cannot move along singlet microtubules, distal segments are not assembled in *osm-3* mutants. D) DYF-1 regulates OSM-3 processivity and tethering to IFT particles, since in a *dyf-1* mutant, OSM-3 is inactive and uncoupled from moving IFT assemblies. Like *osm-3* mutants, *dyf-1* mutants do not assemble distal segments. E) BBS proteins functionally coordinate kinesin 2 motors. In *bbs* mutant cilia, which are partially truncated, the anterograde motors are uncoupled and moving separately along middle segments. Furthermore, complexes A and B are also uncoupled along *bbs* mutant cilia, with complex A travelling with kinesin-II and complex B travelling with OSM-3, suggesting that in wild type anterograde IFT assemblies, complex A is closely associated with kinesin-II, whereas complex B is closely associated with OSM-3. F) DYF-5 regulates kinesin 2 motor association with IFT particles. In *dyf-5* mutant cilia (which are elongated), OSM-3 is at least partially uncoupled from IFT particles and kinesin-II in middle segments, OSM-3 anterograde motility is reduced, and kinesin-II is not restricted to middle segments. G) Modular architecture of *C. elegans* anterograde IFT assemblies based on cilia morphology and IFT motility phenotypes. Ou *et al.* (ref. 77) proposes six modules: kinesin-II and OSM-3 motor modules, an OSM-3 docking module, complexes A and B modules and a BBS protein module. Notably, DYF-1 and DYF-13 form an accessory motor module, which activates OSM-3 and serves to dock this motor onto complex B. DYF-2 and DYF-3 form part of complex B, with DYF-2 possibly linking complexes A and B, and DYF-3 possibly linking complex B and the BBS protein complex. IFTA-1 is closely associated with complex A and is required for retrograde IFT. Question marks denote that the module connection points are unknown. Also shown for complex B is an $(F32A6.2/IFT81)_2(C18H9.8/IFT74)_2$ tetramer, which in *Chlamydomonas* is proposed to act as a scaffold for complex B assembly (ref. 52).

microtubule structure that resembles *C. elegans* distal segments (81). Although the mechanism underlying this process in *Chlamydomonas* is unknown, it could involve modulation of IFT.

4.3. Novel IFT genes

Using genetics-, cell biology- and bioinformatics-based approaches in *C. elegans*, five novel and evolutionarily conserved ciliogenic genes have been identified: *dyf-1*, *dyf-2*, *dyf-3*, *dyf-13* and *ifta-1* (8, 33, 90-93). A number of pieces of evidence indicate that these genes encode *bona fide* IFT components, required for mediating and/or modulating IFT pathways. Firstly, each gene encodes a protein that localises to basal bodies and sensory cilia, and undergoes IFT. Secondly, like known IFT particle proteins, anterograde ciliary movement of these five proteins along amphid channel cilia is biphasic (i.e., middle segment transport via kinesin-II *and* OSM-3; distal segment transport via OSM-3 alone). Thirdly, mutations in these genes affect the structure and functions of sensory cilia, as well as disrupting the normal localisation and/or function of other known IFT proteins (8, 33, 77, 90-93). Below follows a short description of these five proteins, all of which are represented by likely orthologues in mammals.

dyf-1 encodes a TPR domain-containing protein, required for OSM-3 activation and for docking this motor onto IFT particles (33). Consistent with a role in modulating OSM-3 function, DYF-1 is also specifically required to build the distal segments of amphid channel cilia (Figure 2D, G) (33). Interestingly, no physical interactions were detected between OSM-3 and DYF-1, indicating that DYF-1 may indirectly regulate OSM-3 function (33).

Loss of DYF-2, a WD repeat and TPR domain-containing protein, results in very short cilia, similar to those of *C. elegans* complex B mutants (91). However, *dyf-2* mutants phenocopy the retrograde IFT defects of complex A mutants *and* the anterograde IFT abnormalities of complex B mutants. Taken together, it is proposed that DYF-2 forms part of complex B, but in ways that are not understood, can modulate specific functions of complex A and retrograde IFT (Figure 2G) (91). This function may be similar to *Chlamydomonas* IFT172 (a complex B protein), which is required for proper transition of anterograde to retrograde IFT at the flagellar tip (58).

dyf-3 mutants possess severely truncated sensory cilia, suggesting that this gene is also part of complex B (Figure 2G) (92, 93). Consistent with this notion is the observation that in *bbs* mutant cilia (see section 4.4.1 below), the localisation and IFT behaviour of DYF-3 is identical to that of other complex B proteins (77). Interestingly, like other IFT proteins, mutation of vertebrate *DYF3*/*qilin* results in cystic kidney formation (73).

dyf-13 encodes a TPR domain-containing protein, which like OSM-3 and DYF-1, is required specifically for building ciliary distal segments (8). Together with the observation that all three proteins display similar localisation patterns and IFT behaviour along *bbs* mutant cilia, it is proposed that DYF-13 and DYF-1 may form part of a discrete IFT complex, which functions as an accessory motor module to dock OSM-3 onto IFT particles (Figure 2G) (77).

ifta-1 (IFT-associated protein 1) encodes a WD repeat domain-containing protein, which when mutated results in the assembly of moderately truncated cilia (90). Similar to complex A mutants, *ifta-1* mutant cilia also display accumulations of other IFT proteins (90). Together with the observation that IFTA-1 localisation and IFT behaviour along *bbs* mutant cilia is identical to CHE-11/IFT140 (complex A protein), IFTA-1 is proposed to function within the retrograde phase of IFT, perhaps via a close association with complex A (Figure 2G) (90).

4.4. Regulation of *C. elegans* anterograde IFT

4.4.1. *bbs* gene function coordinates kinesin 2 motor association

BBS proteins have been associated with intracellular trafficking in both zebrafish and mammalian cells (94). In *C. elegans*, BBS proteins are also implicated in IFT-related functions. Data supporting this latter viewpoint include the following: (i) GFP-tagged BBS proteins accumulate at the ciliary base and undergo IFT (76, 77, 95, 96). (ii) The anterograde movement of BBS::GFP proteins along amphid channel cilia is biphasic and dependent on the cooperative activities of kinesin-II and OSM-3 (33). (iii) *bbs* mutants possess moderately truncated ciliary structures, chemosensory abnormalities and abnormal ciliary localisation and IFT motility of known IFT components (8, 33, 76, 77). Further evidence that BBS proteins play functions related to IFT (and other intracellular trafficking pathways) is provided by the phenotypic nature of human BBS, which is characterised by overlapping symptoms with mouse models of IFT (e.g., retinal degeneration, cystic kidney development and *situs inversus*) (94).

In the middle segments of *bbs* mutant amphid channel cilia, kinesin 2 motors are uncoupled and move separately (Figure 2E). Specifically, unlike wild-type middle segments where kinesin-II and OSM-3 both move at ~0.7 μms^{-1}, these motors move at their respective solo rates of ~0.5 μms^{-1} and ~1.3 μms^{-1} in *bbs* mutants (33, 77). Similarly, complexes A and B are also uncoupled, with complex A traveling only along middle segments at kinesin-II-associated speeds (i.e., ~0.5 μms^{-1}) and complex B traveling along middle and distal segments at OSM-3-associated speeds (i.e., 1.1-1.3 μms^{-1}) (33, 77). Taken together, these observations indicate that *C. elegans* BBS proteins are required to coordinate the association of two different kinesin 2 motors. Importantly, these data also suggest that in wild-type anterograde IFT assemblies, complex A is more strongly tethered to kinesin-II, whereas complex B is more strongly tethered to OSM-3 (all panels in Figure 2) (33, 77).

The precise mechanism by which *C. elegans* BBS proteins functionally coordinate kinesin 2 motors is unclear. One model favoured by us is that BBS proteins

form a heteromeric complex, which stabilises moving IFT assemblies during anterograde IFT, perhaps by acting as a bridging adaptor between complexes A and B (Figure 2G) (33, 77). Recent findings contribute to and support this model. (i) Numerous observations support the notion that BBS proteins form heteromeric complexes. For example, almost all mammalian BBS proteins co-immunoprecipitate (97) and *C. elegans bbs* genes possess almost identical overlapping functions (94). A BBS heteromeric complex could therefore function as an individual functional module of IFT, similar to complexes A and B (77). (ii) In individual *C. elegans bbs* mutants, other BBS proteins fail to enter cilia and undergo IFT, and instead are found only at the ciliary base (77). This important observation indicates that in order to coordinate kinesin 2 motor association, BBS proteins must enter cilia and undergo IFT and cannot perform this function solely from the base of cilia. (iii) Analysis of IFT in *bbs;kinesin 2* double mutants provides evidence that kinesin-II and OSM-3-kinesin associate via mechanical competition. In this tug-of-war model, both motors simultaneously engage and move along microtubules, which due to the differing processivity of the individual motors, produce a tension on moving IFT assemblies (98). It is proposed that a BBS protein module would stabilise this tension, thereby preventing the dissociation of moving anterograde IFT assemblies into kinesin-II/complex A and OSM-3/complex B sub-assemblies. How exactly a BBS module would interact with other IFT modules is unclear; however, a putative 2-hybrid interaction between BBS-7 and DYF-3 (99) may represent a connection point between the BBS and complex B modules (Figure 2G).

An important remaining question is how BBS proteins perform non-ciliary trafficking roles such as melanosome transport in zebrafish or PCM-1 trafficking in mammalian cells (100-102). Indeed, there is now accumulating evidence that other IFT proteins play trafficking roles in the main cell body (67). Consequently, it will be interesting to see whether BBS protein function overlaps with that of the core IFT machinery in extra-ciliary sites.

4.4.2. *dyf-5* regulates the docking/undocking of kinesin 2 motors

In addition to BBS proteins, *C. elegans* kinesin 2 motors are also regulated by a MAP kinase, DYF-5, which serves to restrict kinesin-II to ciliary middle segments (Figure 2F) (35). Furthermore, DYF-5 modulates the processivity of OSM-3 and is required for tethering this motor to IFT particles (Figure 2F) (35). Finally, similar to homologs in *Chlamydomonas* (LF4) and *Leishmania* (LmxMPK9), DYF-5 regulates cilium length (103, 104). To explain these findings, Burghoorn and colleagues propose that DYF-5 regulates IFT at the distal tips of middle segments, where kinesin-II disengages from anterograde IFT assemblies and IFT particles are switched from one kinesin 2 motor (i.e., kinesin-II) to the other (i.e., OSM-3). It is suggested that DYF-5 regulates this process by modulating the affinities of kinesin 2 motors for their target molecules (e.g., IFT particles or microtubules) (35).

5. VERTEBRATE IFT

Although most of our current knowledge regarding the molecular basis of IFT has been garnered from research in lower eukaryotes (e.g., *Chlamydomonas* and *C. elegans*), mouse studies have uncovered key functions for IFT and primary cilia in embryonic tissue/organ patterning and developmental signalling. Discussed below and in section 6 are the principal findings.

5.1. IFT and disease

IFT was first linked to vertebrate tissue development and disease, when it was realised that IFT88 is the protein product of *Tg737/Polaris*, the gene mutated in the oak ridge mouse model of polycystic kidney disease (105). *Tg737* mutants are at least partially defective in cilia assembly and present with a pleiotropy of pathologies, including bilateral cystic kidneys, liver lesions, hydrocephalus, polydactyly, pancreatic exocrine/endocrine defects and cyst formation, post-natal growth plate development and organ laterality defects (56, 68, 105-114). Similar associations between primary cilia dysfunction and vertebrate pathologies have been found for mouse mutants of kinesin 2 (26, 28), IFT-dynein (115, 116), IFT52 (110), IFT57 (69) and IFT172 (68, 115). In zebrafish, mutations in *ovl* (IFT88), *curly* (IFT57), *larry* (IFT81) and *moe* (IFT172) cause glomerular tubular kidney cysts and retinal degeneration (73, 74). In addition, *ovl* mutants and knockdown morphants of IFT52 and IFT57 also display a loss of sensory cilia structures (74).

Very recently, Beales *et al.* showed that mutations in human IFT80 are associated with Jeune asphyxiating thoracic dystrophy (JATD), a condrodysplasia characterised by a constricted thoracic cage, respiratory insufficiency, frequent infantile death, and possibly retinal degeneration, cystic kidney formation and polydactyly (71). Additional functional analyses showed that IFT80 is required for ciliogenesis in *Tetrahymena* and zebrafish (71). This study is the first to link the dysfunction of an IFT particle protein to a human disease and demonstrates that like in the mouse, IFT in humans is an essential process, required for life.

5.2. Investigation of mammalian IFT

A significant technical impediment to dissecting the molecular basis of IFT in mammals has been the inability to observe IFT in live cells. However, a recent study in cultured mammalian cells has overcome this obstacle (67). Using advanced time-lapse video microscopy, GFP-tagged IFT20 was observed to move bidirectionally between the cell body and the ciliary tips of Llc-pk1 cells, at speeds of ~0.6 μms^{-1} (anterograde) and ~0.7 μms^{-1} (retrograde) (67). Notably, these rates are somewhat similar to those measured in *C. elegans* sensory cilia but significantly slower than those observed in *Chlamydomonas* flagella (19, 77), suggesting comparable regulation of IFT in nematode sensory cilia and mammalian renal epithelial cells. Although not evident from this study, it will be interesting to see if a biphasic pattern of anterograde IFT exists in mammalian cells, similar to that observed in *C. elegans*. Follit *et al.* also show that a pool of IFT20 is localised at

the Golgi region and that IFT20 is required for maintaining ciliary levels of the transmembrane protein polycystin-2. From these data, it is suggested that IFT20 functions to deliver ciliary proteins from the Golgi complex to the cilium (67). Indeed, this raises intriguing questions as to whether other IFT proteins also function at the Golgi apparatus and whether they too traffic various cargos (membranous or non-membranous) from the cell body to the base of cilia.

6. IFT AND CILIUM-BASED SIGNALLING

As sensory organelles, cilia serve to relay physico-chemico signals, presumably via various signalling molecules (e.g., membrane receptors and channels, signal amplifiers etc.), which localise within ciliary structures. Although it has long been known that cilia play important roles in certain types of sensory signalling (e.g., olfactory signal transduction in *C. elegans*), recent research has shown that IFT and cilia are important for other types of signalling processes, including developmental and growth factor signalling in vertebrates and mating-induced signalling in *Chlamydomonas*.

6.1. IFT and sonic hedgehog (Shh) signalling

Undoubtedly the most exciting recent development in vertebrate IFT research has been the discovery that IFT plays important roles in Shh signalling. Shh is a secreted lipoprotein, which signals through the Patched (Ptch) receptor and downstream Gli transcription factors to orchestrate numerous important developmental processes such as neural tube closure, limb formation, and body patterning (117). Defects in Shh signalling cause a wide range of developmental abnormalities including mid-gestation lethality, neural tube patterning defects, polydactyly and randomised organ laterality (117). Over the past four years, numerous studies have shown that IFT is required for Shh signalling in mice. These observations have been the subject of several excellent reviews (118-120), so here we will only briefly discuss the principal and latest findings.

The first evidence connecting IFT with mammalian developmental signalling came from observations that mouse mutations in *Ift88*, *Ift172* and *Kif3a* phenotypically overlap with mutations of Shh signalling components (68). Numerous studies subsequently showed that Shh signalling requires the function of at least seven IFT machinery genes - five complex B genes (*Ift52*, *Ift57*, *Ift80*, *Ift88*, *Ift172*), one kinesin-II subunit gene (*Kif3a*) and two IFT-dynein subunit genes (*DnCHc2*, *D2lic*) (68, 69, 71, 109, 110, 115, 116, 121-123). Genetic analyses showed that IFT functions downstream of Ptch and Smo (Smoothened) membrane receptors, but upstream of the Gli transcription factors (68, 115). Furthermore, IFT is required for the transcriptional activator *and* repressor roles of Gli proteins, indicating that IFT is critical for both the 'on' and 'off' states of Shh signalling (110, 115, 116, 121).

Consistent with a role for IFT in Shh signalling, cilia are present in Shh responsive tissues such as the mesenchymal ectoderm of the developing limb, the apical surface of neuroectodermal cells and cells of the embryonic node (27, 116). Furthermore, a number of Shh signalling proteins, namely Smo, Gli1, Gli2, Gli3 and Sufu, have all been found to localise within primary cilia of the embryonic node and limb bud, with Gli1-3 and Sufu being found specifically at the ciliary tips (116, 121, 124). Most interestingly, stimulation of the Shh pathway (via Shh ligand) promotes the ciliary localisation of Smo, whereas disruption of Smo ciliary translocation blocks Shh signal transduction (124). Taken together, the available data strongly implicates a role for primary cilia and IFT in transducing Shh signals during development. Although the precise function of IFT in this process is unclear, it has been proposed that IFT serves to traffic and concentrate Shh components to discrete regions of primary cilia (e.g., the distal tips) where ciliary Shh signal transduction is mediated and regulated (121).

6.2. IFT and PDGF-AA signalling

In addition to developmental signalling, there is now evidence that IFT may play roles in growth factor signalling. In 2006, Schneider and colleagues showed that PDGF-AA growth factor signalling in quiescent (growth arrested) fibroblasts requires primary cilia and IFT (118, 125). A number of observations support this notion: (i) during growth arrest, PDGFα receptor and Mek1/2 (downstream signal transducer) are upregulated and localised to fibroblast primary cilia (125). (ii) In dividing wild-type cells or in growth-arrested cells from IFT88/Tg737^{ORPK} mice, PDGFα accumulates at the base of truncated cilia and PDGF-AA signal transduction through Erk1/2 and Akt/PKB is blocked (125). (iii) Unlike wild-type quiescent fibroblast cells, those derived from IFT88/Tg737^{ORPK} mice fail to upregulate PDGFα levels (125). Taken together this data indicates that the pleiotropic phenotype of IFT mutant mice may in part be caused by defective PDGF−AA signalling. Consistent with this notion is the fact that abrogated PDGF−AA signalling causes multiple developmental phenotypes, as well as a wide range of carcinomas.

6.3. IFT directly mediates *Chlamydomonas* signalling during mating

Since most available models of IFT have severe cilium assembly defects, it has been difficult to investigate whether IFT plays indirect or direct roles in cilium-based signalling. Recently, research from William Snell's laboratory has provided strong evidence that IFT *directly* mediates signalling events in *Chlamydomonas* engaged in mating (126). In this study, *fla10*^{ts} (kinesin-II subunit) mutants were employed, which upon switching to the restrictive growth temperature for ~ 1 hour, present with full length flagella that are devoid of IFT assemblies. Consequently, there is a short time window in this mutant where IFT is disrupted but flagella are essentially intact (126). Using this system, it was found that CrPKG (cGMP-dependent protein kinase), a flagellar signalling protein activated upon flagellar adhesion during mating and essential for fertilisation, requires IFT for its proper targeting to a discrete flagellar compartment (126). Furthermore, upon flagellar activation, IFT particles were also found to be targeted to the same flagellar compartment

as CrPKG (126). From these observations, it is postulated that IFT directly traffics CrPKG to a specific region within *Chlamydomonas* flagella. In addition, since the biochemical properties of IFT molecules alter following flagellar adhesion during mating (126), this study is perhaps another example of how IFT can be modulated to serve specific roles, similar to that described above (section 4.2) for the assembly of certain *C. elegans* sensory cilia.

7. IFT CARGO

Although significant strides have been made in understanding the molecular basis of the core IFT machinery (i.e., IFT motors, IFT particle proteins, etc.), relatively little is known about the various protein cargos that are presumably trafficked by this transport system. In theory, any ciliary protein is a candidate for IFT cargo, which could include both structural (e.g., axonemal subunits) and functional (e.g., membrane receptors/channels) ciliary components. Below follows a description of likely and putative cargos identified to date.

Using co-immunoprecipitation techniques, numerous ciliary proteins associate biochemically with the core IFT machinery. In *Chlamydomonas*, these proteins include radial spoke proteins (50), inner and outer dynein arms (50), acetylated α-tubulin (50), EB1 (58) and a cGMP dependent protein kinase (126). In ciliated mammalian cells, olfactory CNG membrane channels co-immunoprecipitate with OSM-3/KIF17 (127), polycystin 2 and fibrocystin/polyductin associate with kinesin-II and IFT proteins (128-130), RPGR (retinitis pigmentosa GTPase regulator) co-immunoprecipitates with IFT88 (131), and pericentrin (basal body component) associates with IFT88, IFT57 and IFT20 (130).

In *C. elegans*, 3 putative cargo proteins have been observed to undergo IFT along sensory cilia (132, 133). In each case, these proteins are not required for cilia assembly or maintaining an intact IFT process and so are considered *bona fide* cargo. Two of the proteins are transient receptor potential vanilloid (TRPV) membrane receptors (OSM-9, OCR-2) (132), which likely play sensory transduction roles, whereas the third is a Rab-like G-protein (IFTA-2), which serves to regulate life-span determination (133). For each of these proteins, IFT motility rates are similar to those of core IFT machinery proteins (77, 132). Together with the findings that the normal ciliary localization and IFT motility of these cargo proteins requires an intact IFT process (77, 132, 133), it is concluded that OCR-2, OSM-9 and IFTA-2 are tethered to the core IFT machinery. Interestingly, in *bbs* mutant cilia where moving IFT assemblies are destabilised and uncoupled (see section 4.4.1 above), IFTA-2 moves with uncoupled OSM-3/complex B sub-assemblies, suggesting that in wild-type cilia, IFTA-2 cargo is closely tethered to complex B (77).

An increasing number of ciliary proteins are candidate IFT cargo based on a genetic association with the core IFT machinery. For example, in *Chlamydomonas*, kinesin-II is required for transporting an aurora protein kinase (CALK) into flagellar structures (134), and in mice, IFT-dynein is required for proper ciliary localisation of Smo at embryonic nodal cilia (116). Furthermore, in *Chlamydomonas ift46* mutants, outer dynein arms are not transported into flagella (60). Similar to *Chlamydomonas*, tubulin is also likely to be an IFT cargo in metazoans since in *Drosophila*, the ciliary localisation of GFP-tagged α-tubulin is dependent on *IFT172* and kinesin-II gene function (6). Other candidate mammalian IFT cargo (based only on genetic evidence) include the PDGFRα receptor (125), opsin (135) and other Shh signalling proteins such as Sufu and Gli transcription factors (121). To confirm whether these proteins are *bona fide* IFT cargos or not, more direct associations with the IFT machinery will need to be demonstrated (e.g., biochemical interactions or direct visualisation of IFT).

8. IFT AND THE CELL CYCLE

In mammalian cells, primary cilia assemble from the mother centriole during resting G_0 phase. However, as most cells prepare for cell division (during G1/S-phase), primary cilia are lost, most likely because the centriolar structure of anchoring basal bodies must now serve as part of the mitotic apparatus (136). There is now accumulating evidence that cilia disassembly/assembly and cell cycle entry/exit are coordinately regulated (136). For example, in mammalian models of IFT, loss of cilia causes pathologies such as polycystic kidney disease, which is suggestive of abnormal cell proliferation and defects in cell cycle regulation.

A number or reports have linked kinesin 2 functions to cell cycle progression. Firstly, double knockout of two *Tetrahymena thermophila* kinesin-II subunits (*KIN1* and *KIN2*) causes cytokinesis arrest (e.g., cells are multi-nucleated and large in size) (30). Secondly, over-expression of the KIF3B tail in cultured mammalian cells (COS-7, HeLa) also produced multi-nucleated cells, indicative of cytokinesis defects (137). Thirdly, in cultured mouse NIH3T3 fibroblast cells, dominant negative expression of a KIF3B fragment (lacks C-terminal 33% of KIF3B) fused to GFP caused chromosomal aneuploidy and abnormal spindle formation (138). Consistent with these data are findings which show that kinesin-II subunits associate with the mitotic machinery (centrioles, mitotic spindles and cytokinesis mid-body) (137, 139-141), and redistribute to the nuclear compartment during mitosis (22, 25, 29). However, in contrast to the above data, knockout or knockdown of kinesin-II function in *Chlamydomonas* and sea-urchins does not appear to specifically affect cell cycle progression (22, 25, 29).

Cell cycle-associated defects have also been linked to disruption of IFT particle and IFT-dynein gene function. In *Trypanosoma brucei*, RNAi knockdown of either IFT88 or DHC1b gene function results in small cell size, slower growth rates and defects in cytokinesis (142). Furthermore, in mouse quiescent fibroblasts, loss of IFT88 gene function prevents PDGFα induced cell cycle entry (125). Similarly, overexpression of IFT88 in cultured mammalian cells (HeLa) prevents entry into S-

phase, and loss of IFT88 gene function promotes cell cycle progression to S, M and G2 phases, indicating that IFT88 can regulate G1-S transition (143). Most recently, it was found that RNAi knockdown of the GTPase IFT27 in *Chlamydomonas* causes an elongation of the cell cycle (slow growth), cytokinesis abnormalities (multi-nucleated cells) and in some cases, cell death (perhaps via apoptosis) (55). Interestingly, loss of IFT52 or IFT88 gene function in *Chlamydomonas* does not alter cell cycle progression, indicating that cell cycle regulation is not a general function of all *Chlamydomonas* complex B proteins, but is specific for IFT20 (56).

Loss of mammalian BBS protein function is also linked to defects in cell cycle progression. RNAi knockdown of BBS4 in HeLa cells resulted in cell cycle defects and increased numbers of apoptotic cells (101). Loss of BBS6 gene function in ciliated cultured mammalian cells (COS7, NIH3T3) caused cytokinesis abnormalities, as determined by the high frequency of bi- or multi- nucleated cells, an abnormal centrosomal number and non-resolved cytoplasmic bridges during telophase (144). Consistent with a cell cycle-linked function, BBS6 protein is found at the centrosome and at the cytokinesis mid-body (144).

Taken together, the above findings suggest that at least in certain cell types, specific IFT proteins are required for normal cell cycle progression / regulation. Consistent with this notion are findings which show that putative cell cycle regulators such as NIMA kinases, EB1 and the von-Hippel Lindau tumour suppressor protein (pVHL) localise within the basal body/centrosomal region and along ciliary axonemes (145-148). In addition, by modulating microtubule dynamics, these proteins are also required for cilia assembly and/or disassembly (145-148).

9. MECHANISM OF IFT: A CURRENT MODEL

The IFT cycle consists of multiple stages, which at the very least includes the accumulation of IFT proteins within the basal body region, anterograde and retrograde movement along the ciliary axoneme, and turnaround steps at the tips and base of cilia where significant remodelling and reorganisation of IFT assemblies occurs (24, 41, 48, 77). Drawing from observations in *Chlamydomonas* IFT tip turnaround mutants and from important findings in other studies (principally in *Chlamydomonas* and *C. elegans*), Pedersen and colleagues recently outlined a detailed 6-phase model of IFT (41), which we describe and discuss below, and present in Figure 3A.

Phase 1: Gathering of IFT machinery (motors, IFT particles) within the basal body region

Although little is known about how IFT proteins are targeted from their site of synthesis to the base of cilia, there is evidence that partial pre-assembly of IFT complexes/cargo in the cell body may be an important prerequisite for trafficking to the ciliary base (50, 64). In *Chlamydomonas*, IFT52 localises at the transitional fibers, indicating that this region of the basal body may serve as a docking site for the IFT machinery (59). While the mechanism by which IFT complexes are assembled and subsequently loaded onto the microtubule tracks prior to anterograde IFT is unknown, it is clear that IFT particles can be regulated with respect to their rate of entry into the cilium and their ability to bind cargo. For example, during flagellar shortening in *Chlamydomonas*, IFT particles enter flagella at an increased rate and appear to possess empty cargo binding sites (149). Furthermore, another study in *Chlamydomonas* has observed that IFT particle entry into flagella is periodic and frequently pauses, suggesting that the loading/release of IFT assemblies at the ciliary base is highly regulated (47).

Phase 2: Anterograde transport from base to flagellar tip

Kinesin-II transports IFT particles and inactive IFT-dynein to the distal ends of outer doublet microtubules. Data from *Chlamydomonas* and *C. elegans* indicates that within these moving anterograde IFT assemblies, kinesin-II is closely associated with complex A (33, 77), which in turn is associated with complex B (50). It is also proposed that inactive cDHC1b is tethered to complex B independent of complex A or D1bLIC (41). The latter observation might explain why the flagellar localisation of *Chlamydomonas* cDHC1b is unaffected in D1bLIC mutants, and why *C. elegans* IFT-dynein motility is independent of complex A gene function (41, 42, 44). Although unproven, the association of cDHC1b with complex B may serve to keep IFT-dynein in an inactive state until it can be activated at the ciliary tip (41). Interestingly, in *Chlamydomonas* flagella, anterograde IFT assemblies sometimes pause, or even reverse direction (47). The functional significance of this behaviour is unknown.

Phase 3: Dissociation of anterograde IFT assemblies in the flagellar tip compartment

From biochemical and genetic analyses of IFT protein distribution/localisation in the flagella of various *Chlamydomonas* tip turnaround mutants (e.g., *fla11*/*ift172*, *fla15-17*, *fla24*) (24, 41, 43, 58, 148), the next step is proposed to be the release of anterograde IFT assemblies into the 'flagellar tip compartment', followed by their dissociation (i.e., complex A dissociates from complex B and inactive cDHC1b dissociates from complex B). From EM analysis in *Chlamydomonas*, the 'flagellar tip compartment' is proposed to extend from the distal end of the outer doublet microtubule structure and consist of very short (~2 μm) singlet A-tubules linked to the flagellar tip membrane via short filaments (41). Since *C. elegans* kinesin-II does not appear to move within ciliary segments consisting of singlet microtubules (32), it is suggested that kinesin-II is not released into the tip compartment (41).

Phase 4: Formation of active retrograde IFT assemblies

Since complex B cannot undergo retrograde IFT in the absence of complex A (44, 54) or D1bLIC (42, 44), it is proposed that complex A first associates with active cDHC1b, and then complex B binds to complex A (41). Exactly when and how IFT-dynein is activated is unclear. cDHC1b activation does not seem to depend on complex A

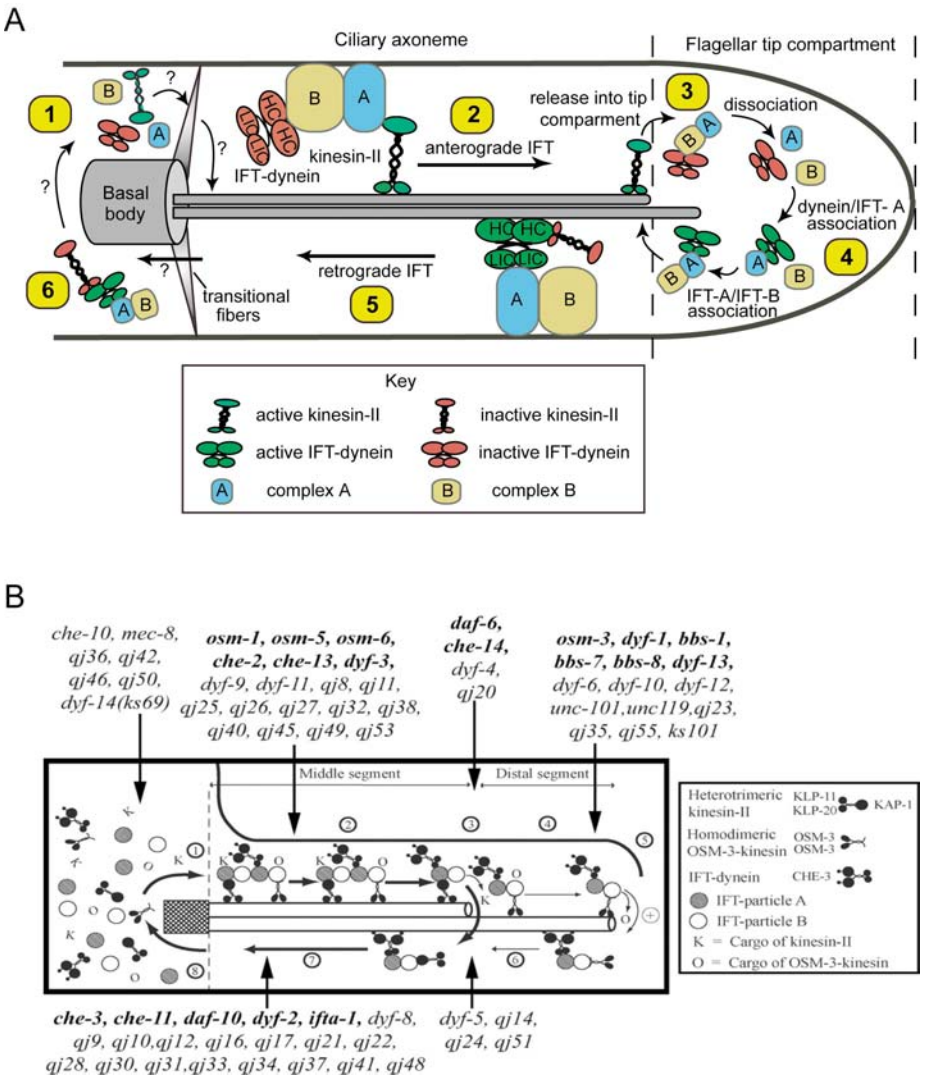

Figure 3. Molecular mechanism of IFT. A) Shown is an adaptation of a schematic by Pedersen *et al.* (see ref. 41). Based on a large number of findings from *Chlamydomonas* and *C. elegans*, IFT along *Chlamydomonas* flagella is proposed to consist of 6 phases: (1) IFT machinery accumulates within the basal body region, with the transitional fibers possibly serving as a docking point. (2) Active kinesin-II transports IFT particles and inactive IFT-dynein to ciliary tips. Kinesin-II associates with complex A (A), and DHC1b (HC) associates with complex B (B) independent of D1bLIC (LIC). (3) Release of anterograde IFT assemblies into the flagellar tip compartment (FTC), followed by dissociation of assemblies. Kinesin-II does not enter the FTC. (4) Active IFT-dynein first associates with complex A, which then associates with complex B. Retrograde IFT assemblies exit the FTC, where inactive kinesin-II now associates with DHC1b (HC). (5) IFT-dynein returns the IFT machinery to the ciliary base. (6) Retrograde assemblies are reorganised/remodelled in preparation for another IFT cycle. Question marks denote that although IFT turnaround at the ciliary base may occur similarly to phases 3 and 4, little is currently known about this process. Note that active IFT motors are coloured green and inactive IFT motors are coloured red. B) In *C. elegans* amphid channel cilia, 8 phases of transport are proposed (schematic reprinted from Ou *et al*. (ref. 77), with permission from The American Society for Cell Biology). IFT machinery and cargo accumulate at the base of cilia (Phase 1). OSM-3-kinesin and kinesin-II cooperatively transport IFT particles and cargo along middle segments (Phase 2). At the tip of the middle segment, kinesin-II (and associated IFT particles) disengages from anterograde assemblies (Phase 3) and are returned to the ciliary base via IFT-dynein powered retrograde IFT (phase 7). OSM-3-kinesin alone drives transport along distal segments (Phase 4). At the tips of distal segments, assemblies are once again remodelled (Phase 5) and the IFT machinery is subsequently returned to the ciliary base by IFT-dynein-driven retrograde IFT (Phases 6 and 7). Also shown are known (denoted in bold) and 49 novel uncloned genes, all of which are required for normal cilia biogenesis and for a fully intact IFT system (ref. 77). Based on cilia morphology and the pattern of ciliary mislocalisation observed for a GFP-tagged reporter of IFT (namely, OSM-6/IFT52), the steps at which these genes are predicted to function are indicated.

because in *C. elegans* complex A mutants (*che-11*), D1bLIC can still exit the cilium (44). As *Chlamydomonas* kinesin-II can exit flagella independent of complex A, complex B and D1bLIC, it is suggested that the anterograde motor associates with active cDHC1b (41).

Phase 5: Retrograde transport from flagellar tip to base of cilia

Active cDHC1b drives the transport of retrograde IFT assemblies back to the ciliary base.

Phase 6: Recycling of IFT proteins to the cell body

Little, if anything, is known about how this would occur, although it is possible/likely that a similar disassembly of motor-IFT particles would occur at or near the transition zone before active anterograde IFT particles are re-assembled.

The model presented above is simply a blueprint for IFT and is subject to cilia-specific variations in different cells and organisms. This is perhaps best illustrated in *C. elegans* where as discussed above (section 4), two kinesin 2 motors are differentially and redundantly employed to drive anterograde IFT in various sensory cilia subtypes, thereby providing a possible means for generating cilia diversity and specialisation (32, 83, 89). In a recent study of *C. elegans* IFT, Ou and colleagues presented a comprehensive genetic model of IFT, which shows that middle segment IFT assemblies in amphid channel cilia consist of at least 6 modules (two kinesin-2 motor modules, complex A and B modules, a BBS protein module, and an OSM-3 docking module) (Figure 2G) (77). Whether all of these modules play similar functions in the assembly of cilia subtypes in other organisms remains to be determined.

Based on the transport and phenotypic profiles of known IFT components, as well as 49 novel and uncloned cilia assembly mutants, Ou *et al.* also propose that IFT along bipartite amphid channel cilia consists of 8 phases (Figure 3B) (77). In essence, the extra phases over the 'canonical' model (Figure 3A) arise due to there being two anterograde and two retrograde phases along amphid channel cilia. Within this cycle, turnaround of IFT assemblies occurs at three locations: (i) at the ciliary tips, (ii) at the base of cilia, and (iii) at the distal end of middle segments where kinesin-II disengages from anterograde IFT assemblies and presumably returns to the ciliary base (Figure 3B) (77). Once the 49 new cilia assembly mutations are cloned, it will be of great interest to more closely investigate how the corresponding genes mediate and/or regulate the various phases of *C. elegans* IFT. In particular, like the recent findings for *dyf-5*, which acts as a docking/undocking (remodelling) factor for the anterograde kinesins (35), these new genes should shed further light on the mechanisms underlying the turnaround phases of IFT. Also, these new components may illuminate how IFT cargos are tethered to the IFT machinery, a process which remains very poorly understood.

10. PERSPECTIVE

As detailed above, since its initial discovery 1993, much progress has been made in elucidating the molecular basis of IFT. Many of the IFT machinery components have been identified and mechanistic data regarding the mediation, regulation and modulation of IFT is now beginning to emerge. In the context of mammalian pathologies such as cystic kidney disease, organ laterality defects and limb patterning abnormalities, the uncovering of key functional links between IFT and developmental signalling (e.g., sonic hedgehog) now provide a fresh and very exciting opportunity for understanding the role of cilia in mediating mammalian tissue and organ development. Furthermore, tantalising evidence towards a cell cycle-associated role for cilia and IFT raises the possibility that ciliary dysfunction may even contribute to cancer progression. Why mammals utilise cilia and IFT for important developmental signalling processes is unclear. Possible answers could include: (i) cilia provide a large membranous surface area for signalling. (ii) Cilia are compartmentalised, meaning that most proteins cannot simply diffuse into the organelle. This property could provide an additional means for regulating signalling cascades. (iii) IFT may provide a mechanism by which signalling molecules are delivered and clustered within specific regions of the cilium.

Many intriguing questions regarding the mechanistic basis of IFT remain and some of these are outlined as follows: (i) what is the full complement of IFT machinery? Although many of these proteins have already been identified, the steady and continuous discovery of new IFT proteins indicates that more remain undiscovered. (ii) What is the quaternary structure of IFT assemblies and their individual complexes/modules? This key question will provide critical insight into the proposed hierarchical nature of IFT complex assembly/disassembly. (iii) What are the cargos transported by IFT and how are these ciliary proteins loaded and unloaded onto IFT assemblies? (iv) How are the turnaround phases of IFT regulated? (v) How exactly does regulation of IFT generate structural and functional ciliary diversity? (vi) How does IFT regulate cilium length? Although not discussed in this review, various lines of evidence from *Chlamydomonas* indicate that IFT plays critical roles in this process (150). (vii) What is the mechanism by which IFT regulates developmental signalling in vertebrates and do IFT proteins play extra-ciliary roles? Indeed, there is gathering evidence that IFT proteins do indeed function within the cell body. For example, IFT20 localises at the golgi complex and may be required for targeting polycystin to ciliary structures, and the mammalian homologue of OSM-3-kinesin (i.e., KIF17) serves to target post-synaptic components to distal regions of dendrites (67, 151). (viii) What is the interaction, if any, between IFT and other types of intracellular trafficking systems? Interestingly, based on sequence similarities, components of IFT and coated vesicle transport (i.e., COP1 and clathrin) share remarkable homology (e.g., TPR *and* WD repeats), suggesting that IFT evolved as a specialised form of vesicular transport (152). Consequently, we might expect direct functional links between these different intracellular transport processes.

Through the continuing use of genetics, biochemistry and cell biology in multiple eukaryotic

systems, answers to the above questions will be forthcoming. If the previous 14 years is anything to go by, exciting times lay ahead.

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