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Intraflagellar Transport: Mechanisms of Motor Action, Cooperation and Cargo Delivery

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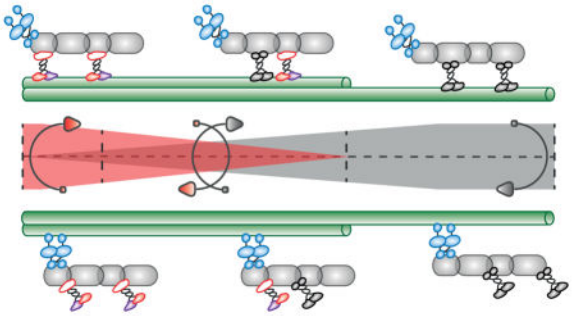
Abstract

Intraflagellar transport (IFT) is a form of motor-dependent cargo transport that is essential for the assembly, maintenance and length-control of cilia, which play critical roles in motility, sensory reception and signal transduction in virtually all eukaryotic cells. During IFT, anterograde kinesin-2 and retrograde IFT-dynein motors drive the bidirectional transport of IFT trains that deliver cargo, for example axoneme precursors such as tubulins as well as molecules of the signal transduction machinery, to their site of assembly within the cilium. Following its discovery in *Chlamydomonas,* IFT has emerged as a powerful model system for studying general principles of motor-dependent cargo transport and we now appreciate the diversity that exists in the mechanism of IFT within cilia of different cell-types. The absence of heterotrimeric kinesin-2 function, for example, causes a complete loss of both IFT and cilia in *Chlamydomonas* but following its loss in *C. elegans,* where its primary function is loading the IFT machinery into cilia, homodimeric kinesin-2-driven IFT persists and assembles a full-length cilium. Generally, heterotrimeric kinesin-2 and IFT-dynein motors are thought to play widespread roles as core IFT-motors whereas homodimeric kinesin-2 motors are accessory motors that mediate different functions in a broad range of cilia, in some cases contributing to axoneme assembly or the delivery of signaling molecules but in many other cases their ciliary functions, if any, remain unknown. In this review, we focus on mechanisms of motor action, motor cooperation and motor-dependent cargo delivery during IFT.

Graphical abstract

Intraflagellar transport is a form of motor-dependent cargo transport that is essential for the assembly, maintenance and length-control of cilia, which play critical roles in motility, sensory reception and signal transduction in virtually all eukaryotic cells. In this review of intraflagellar transport, we focus on mechanisms of motor action, motor cooperation and motor-dependent cargo delivery.



Keywords

Intraflagellar transport; kinesin-2; IFT dynein; motor cooperation

1. Introduction: Basic Mechanism and Functions of IFT

Cilia are microtubule (MT)-based organelles that protrude from the surface of virtually all eukaryotic cells [1]. Motile cilia (also called flagella) are used for cell locomotion or for the generation of fluid flow over a cell surface, whereas non-motile (aka sensory or primary) cilia sense extracellular signals and transmit signals from the cilium to the cytoplasm and nucleus in order to control gene expression and cell behavior, thereby playing several important roles in cell and developmental biology. Cilia consist of a cylindrical, MT-based axoneme, which projects, MT plus ends distal, from a basal body located at the cell surface, surrounded by a specialized membrane that contains various cilium-based signaling molecules (Fig. 1A). Cilia can be found in all branches of the eukaryotic tree indicating that the last eukaryotic common ancestor (LECA) was already equipped with this intriguing cellular antenna [2–4]. The mechanism of cilium assembly (aka cilium biogenesis or ciliogenesis) is currently a cutting-edge topic in cell and molecular biology research [5].

The discovery of intraflagellar transport as a critical component of the mechanism of ciliogenesis was catalyzed by the observation that polystyrene beads could move along the motile cilia of *Chlamydomonas* in a bidirectional fashion, independent of ciliary beating [6, 7]. This paved the way for a series of pioneering experiments which revealed that ciliary precursors that form in the cell body, e.g. tubulin subunits and pre-assembled motility-related complexes, assemble onto the distal tips of the 10 μm long, motile *Chlamydomonas* cilium [8]. To test the hypothesis that a mechanism exists for transporting these precursors from the cytoplasm and along the cilium to the distal tip, Rosenbaum and colleagues used differential interference contrast (DIC) microscopy of paralyzed ciliary mutants to visualize the bidirectional movement, i.e. “intraflagellar transport” or “IFT”, of large particles beneath the ciliary membrane [9]. The subsequent purification of these particles, now named IFT trains, opened up the field for molecular analysis [10, 11] [and the discovery of several cilia-related diseases [12]], while the application of GFP-tagging allowed the direct visualization of specific IFT components moving along the cilium in living cells [13]. The rapid progress made by multiple laboratories in understanding the mechanism of IFT has been covered in several reviews [14–23]. The current view is that anterograde IFT trains, which deliver a

variety of cargo molecules for incorporation into the ciliary axoneme, membrane and matrix, are moved from the base to the distal tip of the cilium by heterotrimeric and, in some cases, homodimeric motors of the kinesin-2 family, whereas retrograde IFT trains, which recycle turnover products from the tip to the base of the cilium are moved by IFT-dynein (aka dynein-2 or class dhc1b dynein) (Fig. 1B–D) [24, 25].

The elucidation of the cellular functions of IFT, which depends upon the delivery of cargo molecules by the IFT machinery, is an exciting problem in cell-biology research. It has long been recognized that IFT is required for ciliogenesis because it delivers cargo consisting of axonemal precursors such as tubulin subunits, pre-assembled radial-spoke complexes and dynein arms to their site of assembly at the tip of the cilium [17]. The delivery of these same cargo molecules is also relevant to the interesting topic of cilium length control, a topic that has been pioneered by Marshall and others [26–30]. IFT is also thought to be involved in the delivery of specific molecules required for the compartmentalization of the cilium [31] and, via cooperation with the BBSome, to contribute to the biogenesis of the ciliary membrane [32, 33]. Finally, it is now well established that cilia play critical roles in the intracellular signaling that underlies several critical cell and developmental processes, for example in Hedgehog, Notch, Wnt, Planar cell polarity, TGF-β and opsin-dependent signaling, as well as in DNA damage repair pathways, to name but a few [34–38]. A cilium can plausibly do so by serving as a “cellular antenna” that detects extracellular signals from the environment, by serving as a signaling platform that concentrates signaling molecules, and by transmitting signals to the cytoplasm and nucleus to control gene expression and cell function. IFT is thought to play key roles in cilium-based signaling, not only by building the foundation of the ciliary antenna, but also by delivering signaling molecules as cargo to the ciliary membrane and matrix and it possibly also plays a more direct, yet poorly understood role in the mechanism of signal transduction itself. This topic has been nicely covered in several recent reviews e.g. [39, 40].

2. Structure of Cilia and IFT trains

To put in context the topic of IFT as a model system for studying mechanisms of motor-dependent cargo transport, it may be useful to consider the contribution of IFT to the overall process of ciliogenesis and the generation of the complex architecture of cilia, features of which can significantly influence the process of IFT (Fig. 1A).

The basal body and the initiation of ciliogenesis

IFT is thought to begin significantly after the actual initiation of ciliogenesis which begins with the formation and positioning of the basal body (BB), a cylindrical structure consisting of 9 symmetrically arranged triplet MTs (named A, B and C tubules) associated with a complex network of membranes (see fig. 1 in [41]). The BB is derived from the mother centriole which resides at the mitotic spindle pole during M-phase, then translocates to the site of ciliogenesis at the cell surface following mitotic exit [42, 43] in a process that may involve kinesin-2 function [44]. The initial stages of ciliogenesis are characterized by the docking of small “distal appendage vesicles” (DAVs) onto the distal end of this centriole followed by their fusion into a single ciliary vesicle [45, 46]. In a complex, poorly-

understood sequence of events, the BB elaborates lateral transitional fibers as its A- and B-tubules elongate, possibly by an IFT-independent mechanism, to form the transition zone doublets, which are crosslinked to the overlying membrane via Y-shaped linkers (Fig 1A) [47–49]. While the exact timing is unclear, it is plausible to think that this is the stage at which IFT engages and begins to assemble the axoneme proper by further elongating the A and B tubules of the transition zone, as the ciliary vesicle fuses with the plasma membrane to form the ciliary membrane, which now surrounds the protruding axoneme (see Fig. 2 of [41]). It should be noted that, in *C. elegans*, the BB undergoes substantial degeneration after ciliogenesis, leaving behind only the transition fibers (which, based on recent electron microscopy (EM), might actually be flaring MT doublets) [50, 51].

The ciliary axoneme

The axoneme core of the cilium, consisting of 9 doublet MTs extending with their plus ends distal from the A and B tubules of the BB, is assembled and maintained by IFT [52]. The A-tubule of each doublet is a complete MT consisting of 13 protofilaments (pf), whereas the B-tubule is an incomplete MT often consisting of only 10 pfs [53, 54]. Near the ciliary tip, the B-tubules generally terminate before the A-tubules, generating distal singlet MTs that make up the distal segment (ds) whose length varies substantially; in trypanosomes it is non-existent [55], in *C. elegans* it can be several μm long [50], while in frog olfactory cilia it extends over a hundred microns [56]. It is unclear how the length of the ds relates to ciliary function but it has been hypothesized that it might define a differentiated membrane responsible for specific sensory function [57].

In motile cilia, 9 symmetrically arranged doublet MTs often surround a pair of single MTs, in a so-called 9 + 2 configuration [58]. The doublets in these cilia are linked together via nexin links, and the central MT pair is linked to the A-tubules of the doublets via radial spokes [59, 60]. Outer dynein arms and inner dynein arms are located on the A-tubules of the doublets, allowing the dynein motor domains to contact the neighboring B-tubule in order to generate the forces required for cilium motility [61]. In non-motile cilia, most components required for force generation, such as the single MT pair, are lacking, resulting in a 9 + 0 configuration. In these cilia, the radial spokes, the nexin links and the inner- and outer-arm dynein complexes are also absent. Although the 9 + 2 and the 9 + 0 configurations are the two most commonly found MT structural arrangements, a wide variety of other arrangements exist for both motile and non-motile cilia [62]. In *C. elegans*, for example, the non-motile chemosensory cilia often have a variable number of singlet MTs at the core of the axoneme [50], which could have associated transport functions although this is unclear. At the tip of the axoneme, ciliary-tip complexes have been observed that might regulate and stabilize axoneme length, and also regulate turnaround of IFT, although further work is required to learn their detailed structure and functions [57].

The axoneme is very stable with only the tips of the A-tubule and B-tubule displaying continuous tubulin turnover [29, 63]. The stability of the axoneme could be the result of various tubulin post-translation modifications (PTMs), which are also known to influence intracellular transport and to facilitate the accumulation of microtubule-inner proteins (MIPs) and outer microtubule-associated proteins (MAPs) [64–66]. Furthermore, the A- and

B-tubules can be composed of different tubulin isotypes and can also acquire different post-translation modifications [67]. As described below, these modifications can affect IFT, thereby tuning ciliary function.

Transition zone and the ciliary membrane

The transition zone (TZ) characterized by the Y-shaped linkers between the MT doublets and ciliary membrane, together with the transition fibers and the so-called ciliary necklace at the base of the cilium, form the so-called ciliary gate that provides a physical barrier between the cytoplasm and the ciliary compartment [33, 68]. The TZ contains several protein modules, mutations in which are linked to ciliary diseases [69]. The gate provides structural integrity to the cilium and is thought to influence ciliary entry and exit by physically restricting the size of proteins that can diffuse across it (to about ~10 nm) and by regulating IFT [70–73]. Larger cargoes accumulate in front of the gate before entering the cilium, at the ciliary pocket, which is thought to function as a docking side for ciliary-cargo containing vesicles, and at the transition fibers that can function as docking sites for IFT particles [41, 74]. Interestingly, in the cilia of *Tetrahymena,* there exists a different physical barrier named the ciliary pore complex (CPC) which consists of a plate containing openings for the MT doublets, the IFT machinery and associated cargo [75].

The ciliary gate also provides a physical barrier between the ciliary membrane and the plasma membrane, and together with the high curvature of the ciliary pocket, it prevents rapid exchange between the two membranes and maintains their distinct lipid and protein compositions thereby permitting their functional differentiation [41, 76]. For example, the dynamic regulation of ciliary membrane composition by the ciliary gate is critical for cilium-specific signaling and recent work suggests that this also depends upon a pathway that utilizes the retrograde IFT-dynein motor in the cytoplasm and the shedding of membrane-bounded ectosomes at the cilium tip [77]. Finally, in addition to its role as a regulator of ciliary membrane composition, the ciliary gate also substantially influences IFT dynamics, as described further below [78].

Structure of IFT trains

IFT trains were first observed in *Chlamydomonas* cilia using light and electron micoroscpy [9] and similar structures were soon observed in EM images of other types of cilia such as retinal photoreceptor cells [17]. Surprisingly, IFT-train size varies dramatically, with the longest trains being about ~0.7 μm long and displaying a 40-nm periodicity, while the shortest are only ~0.25 μm long and exhibit a 16-nm periodicity [79]. In *Chlamydomonas*, the size of IFT trains has been shown to scale inversely with ciliary length; trains are longer when the cilium is shorter and *vice versa*, indicating that regulation of the IFT-train length might represent a crucial ciliary-length control mechanism [80, 81]. More recent work using correlative light and electron microscopy (CLEM) has provided more detailed insight into IFT-train structure [82]. Anterograde- and retrograde IFT-trains appeared to have a similar length of ~0.2 μm, however, anterograde IFT trains, which moved primarily along B-tubules, consisted of more electron-dense structures compared to retrograde trains which ran primarily along A-tubules. An additional, static fraction of IFT trains with a length of ~0.65 μm and 40-nm periodicity was also identified, consistent with the earlier study (Pigino

2009). The authors hypothesized that the selection of B- versus A-tubules by trains moving in opposite directions may avoid collisions between them, although we note that this could also be accomplished by the selective use of the 10–15pf tracks that are available per singlet or through the use of distinct A-tubules [83], and that collisions do not seem to pose a significant problem for IFT trains that move bidirectionally along the distal singlet A-tubules of *C. elegans* cilia [78]. The precise correlation between the morphologically-defined IFT-trains visible by EM and the biochemically-defined protein components of the IFT machinery (discussed next) is currently an outstanding unresolved problem in the field of cilium biology [81].

3. Biochemistry and Functions of components of the IFT Machinery

The overall molecular composition and structure-function analysis of the IFT machinery has recently been discussed in a scholarly review [84]. This machinery comprises kinesin-2 and IFT-dynein motors that move the IFT trains and associated cargo e.g. tubulin subunits back and forth between the base and tip of the cilium (Fig. 2). In this section we focus on those aspects relevant to motor-dependent transport and cargo delivery.

IFT trains

Composition—IFT trains are multimeric protein complexes that are related to the protein coats of intracellular transport vesicles [85, 86] and assemble from 16/17S IFT particles, which were first isolated in classic studies based on their temperature-dependent loss from cilia of conditional mutant *Chlamydomonas* cells lacking kinesin-2 function [10, 11]. Work done in a large number of laboratories supports the hypothesis of Cole *et al*, that IFT-particles comprise two subcomplexes, namely IFT-A, which contains six subunits (IFT144, 140, 139, 122, 121 and 43) and IFT-B, which contains sixteen subunits (IFT-172, 88, 81, 80, 74, 70, 57, 56, 54, 52, 46, 38, 27, 25, 22 and 20), with many of the subunits displaying characteristic protein-protein interaction motifs such as coiled-coil heptad repeats, tetratrico repeats and WD-40 repeats (Fig. 2A) [84]. The IFT-B complex is further organized into IFT-B1 (aka the IFT-B core comprising ten subunits IFT-88, 81, 74, 70, 56, 52, 46, 27, 25 and 22) and IFT-B2 (aka the peripheral IFT-B complex comprising six subunits, IFT-172, 80, 57, 54, 38 and 20), both of which are capable of assembly into stable complexes [87–91]. Available evidence discussed later suggests that the IFT particles transport axonemal precursors, notably tubulins, to their site of assembly at the axoneme tips.

Cilium membrane biogenesis requires the function of the BBSome, a complex of 8 of the known BBS proteins i.e. subunits BBS-1,-2,-4,-5,-7,-8,-9,-18 that is linked to the ciliary membrane by the small GTPase, ARL6/BBS-3 and is required for normal IFT (Fig. 2A) [92, 93]. Whether the BBSome represents a third integral component of the IFT particles, like IFT-A and IFT-B is debatable. In mammalian and nematode cilia it appears to be present in a 1:1 stoichiometry to the IFT particles and may be required for the structural integrity of IFT particles by holding together IFT-A and IFT-B as they are moved along the cilium by kinesin-2 motors [94, 95]. In *Chlamydomonas,* however, the BBSome appears to be highly substoichiometric (≈1:6) to IFT-A and IFT-B, and is proposed to serve as an adaptor involved in the export of signaling proteins from the cilium, rather than as an integral

component of IFT particles [96]. Loss of BBSome function leads to changes in the protein composition of ciliary membranes consistent with a role for the complex in ciliary membrane protein trafficking [33, 95, 97]. Taken together, the available evidence suggests that the BBSome can function as an IFT regulator, as a core IFT component, or as a cargo adaptor in manners that are apparently cell-type specific.

Cargo Binding to IFT trains—IFT-train subunits are proposed to bind a variety of cargo molecules, including ciliary tubulins, as well as motors such as the dynein arms of motile cilia and IFT motors themselves. For example, IFT dynein is moved base-to-tip along the cilium by anterograde IFT and, in some cilia, kinesin-2 motors are moved back to the cell body by retrograde IFT. IFT is proposed to transport tubulin subunits to their site of incorporation at the tips of the axoneme in low amounts when the assembling cilium has reached its steady-state length [63] but in substantially increased amounts during the elongation phase of ciliogenesis [98]. High-resolution structural studies showed that the $-NH_2$ domains of the IFT-B1 subunits, IFT-81 and IFT-74, form a tubulin-binding module in which the IFT-81 calponin homology (CH) domains serves as a recognition domain. This domain on IFT-81 binds tubulin relatively weakly, but binding is enhanced by the positively charged amino terminus of IFT74, which binds electrostatically to the acidic COOH-terminal tails of tubulin (E-hooks) [99]. In support of this model, disruption of either the CH-domain of IFT-81 or the IFT-74 amino terminal domain required for high affinity binding, led to a reduced rate of assembly of full-length cilia. The disruption of both domains, however, led to the assembly of only highly truncated steady-state cilia [100]. Interestingly, a second tubulin-binding CH-domain was also found in the N-terminal region of the IFT-B2 subunit, IFT-54, potentially allowing the transport of 2 tubulins per IFT-B complex, compatible with the kinetics of ciliogenesis [91]. The dynein arms of motile cilia were many years ago proposed to be delivered by IFT [101] and subsequent work suggests that the IFT-B1 subunit, IFT-46 is specifically involved in binding and transporting the outer, but not the inner, dynein arms as cargo along the cilium [102]. The delivery of inner dynein arms, in contrast, is thought to require the activity of IFT-56 [103].

While there is good evidence that some IFT-cargo molecules such as tubulins are ferried along the cilium via their binding to IFT-particle subunits, there are also indications that some cargo molecules may be delivered to the distal tips of cilia via their direct binding to the anterograde kinesin-2 motors instead, possibly circumventing the participation of IFT trains. These include tubulins [104], subunits of the retrograde IFT-dynein motor [105] and the GLI transcription factors that are involved in Hedgehog-related cilium-based signaling [106]. This interesting possibility merits further work.

Motor Binding to IFT-trains—In *C. elegans* amphid-channel cilia, heterotrimeric kinesin-2 (aka kinesin-II) appears to bind and transport IFT-A, whereas homodimeric kinesin-2 (OSM-3) binds IFT-B, because in BBSome mutants, the kinesin-II/IFT-A and OSM-3/IFT-B subcomplexes move separately along the axoneme at 0.5 and 1.2 μm/s, respectively [94]. The IFT-B1 subunit, IFT70 (DYF-1) was proposed to be involved in the IFT-B/OSM-3 interaction, which is supported by co-immunoprecipitation experiments in mouse, suggesting that IFT70 binds to multiple IFT-B subunits and to homodimeric

kinesin-2 (KIF17) [107]. In contrast to the findings from *C. elegans* however, co-immunoprecipitation experiments in *Chlamydomonas* (which lacks homodimeric kinesin-2) and mouse suggest that heterotrimeric kinesin-2 binds directly to IFT-B rather than IFT-A [108], but the significance of this difference is unclear. The retrograde motor, IFT dynein, is proposed to attach to IFT-B and is delivered to the distal tip of the cilium as a passive cargo of anterograde IFT and then docks onto IFT-A to drive retrograde IFT [109, 110]. The latter results, together with observations that loss-of-function mutations in IFT-B and IFT-A subunits often phenocopy mutations in heterotrimeric kinesin-2 and IFT-dynein, respectively, has led to the suggestion that IFT-B and IFT-A function separately in anterograde and retrograde transport along the axoneme [reviewed in [84]]. However, there are several reports that contradict this model, e.g. the requirement of IFT-A for import of some cargo molecules into the cilium and the participation of some IFT-B subunits in retrograde transport [111, 112]. For example an IFT-A subunit, IFT-121, is required for retrograde IFT [113] but also functions in the delivery of several membrane proteins to the cilium [114]. Clearly the biochemistry and functional consequences of the binding IFT-motors to subunits of the anterograde and retrograde IFT trains is a topic that requires additional research.

IFT motors

Heterotrimeric kinesin-2—Anterograde motors that drive the transport of IFT trains and associated ciliary precursors as cargo along the axoneme from the base to the distal tips of the cilium are members of the Kinesin-2 family, which are understood to exist in both heterotrimeric and homodimeric forms (Fig 2B) [reviewed by [25]]. Briefly, heterotrimeric kinesin-2 was first purified from sea urchin eggs as a MT-based motor consisting of a 1:1:1 stoichiometry of subunits 2α, 2β and KAP that moves towards the plus ends of MTs at ≈ 0.4μm/s and is required for ciliogenesis on the blastula stage embryo [115–118]. It is generally assumed to drive IFT along embryonic cilia, but this has not yet, to our knowledge, been confirmed. Similar complexes were subsequently found in several systems, including *Chlamydomonas* where it is clearly required for IFT and ciliogenesis [10, 119–121] and mouse where it is required for the assembly of nodal cilia and the establishment of left-right asymmetry, although here again, its assumed role in IFT along nodal cilia remains unconfirmed [122, 123]. Current mechanistic studies are aimed at elucidating the mechanical significance of this motor having two distinct motor subunits e.g. [124, 125] and the role of the unique KAP subunit, which contains multiple armadillo repeats and is an essential and specific subunit of heterotrimeric kinesin-2 [118, 126]. For example, in *C. elegans* cilia, in an *osm-3* mutant background, the loss of KAP subunit function results in the axoneme being completely absent, just like loss of motor subunit function [127, 128]. Available evidence suggests that KAP may stabilize the heterodimeric coiled-coil formed between the 2α and 2β motor subunits [129, 130], to target the motor to the base of the cilium and enhance its processive movement along the axoneme [119], and/or to bind IFT train/cargo complexes, although to our knowledge there is no evidence for the latter hypothesis in any cilia.

Homodimeric kinesin-2—Biochemical fractionation of *C. elegans* extracts suggested that their sensory cilia contain both a heterotrimeric and a homodimeric form of kinesin-2, the

latter consisting of two γ (aka OSM-3) motor subunits [131]. The formation of homodimers by purified recombinant mammalian kinesin-2 (aka KIF17) confirmed it oligomeric state and also permitted motility assays, which revealed that homodimeric kinesin-2 moves toward the plus end of MTs at ~1.0 μm/s [132]. Homodimeric forms of kinesin-2 have been found in cilia from a broad range of organisms but they appear to act differently in different cilia, even from the same organism [127]. In *C. elegans* amphid channel cilia, for example, homodimeric kinesin-2 clearly participates in anterograde IFT and it assembles the distal part of the axoneme, although in the absence of heterotrimeric kinesin-2 function, it is capable of building the full-length axoneme [128]. It also moves along the cilium in mammalian cells [95] and its activity is required for the localization of cyclic nucleotide gated channels and dopamine receptors to ciliary membranes [133, 134], but whether these signaling molecules are actually delivered to the cilium membrane by homodimeric kinesin-2-driven IFT remains unproven. Moreover, in some cilia the loss of homodimeric kinesin-2 function, unlike heterotrimeric kinesin-2, has no detectable effect on ciliary morphology and functions and consequently its ciliary function, if any, remains unclear [44, 135–137].

IFT-dynein—IFT-dynein (aka dynein-2) drives the retrograde transport of IFT-trains from the tip of the axoneme at rates of one to a few microns per second, thereby recycling components of the anterograde IFT machinery and turnover products back to the base of the cilium for export [reviewed by [24]]. The dynein-2 heavy chain (DYNC2 or DHC1b) was identified as a form of cytoplasmic dynein that is upregulated prior to ciliogenesis on the blastula stage sea urchin embryo, suggesting a role in cilium biogenesis [138]. A direct role for this dynein heavy chain isotype in driving retrograde IFT was subsequently supported by work done in *Chlamydomonas* and *C. elegans* cilia [139–141]. Earlier work, however, had implicated an accessory dynein light chain, LC8 in retrograde IFT, albeit one that has non-IFT-dynein-related functions as well e.g. stabilization of radial spokes, and inner and outer dynein arms [142]. In fact, IFT-dynein is a complex, multi-subunit motor, much more complex than the kinesin-2 motors, consisting of two DYNC2s and typically 2 each of three types of light chain (LC8, TcTex, roadblock), 2 light intermediate chains and 2 intermediate chains, whose specific functions are largely unclear and may be difficult to tease out [Fig. 2B] [105, 143–148]. For example, in a very rigorous recent study, it was shown that the loss of function of the *Chlamydomonas* light intermediate chain, D1bLIC, produces a range of phenotypes including decreased IFT-dynein stability, changes in protein expression level and a reduced frequency and velocity, but not a complete loss, of retrograde IFT [149]. This illustrates that much more work is required to understand the specific functions of IFT-dynein's multiple subunits in IFT and ciliogenesis. With respect to its mechanism, a significant advance was the determination of the crystal structure of the IFT-dynein triple A-containing motor domain in the ADP.Pi-bound pre-power stroke conformation [150]. However, unlike cytoplasmic dynein-1, the *in vitro* motility properties of purified IFT dynein have not been analyzed extensively, although in one study recombinant dynein-2 was observed to move towards the minus ends of MTs at 0.07 μm/s *in vitro,* i.e. with the predicted directionality but at a much slower speed than retrograde IFT [151]. Determining the molecular and biophysical mechanism of the IFT-dynein-driven transport of IFT trains is therefore also a topic that merits further attention [152].

Other motors—Several additional MT-based motors are involved in ciliogenesis, but are not thought to function primarily by transporting IFT particles and associated cargo along the axoneme [37, 153, 154]. Of these, a *C. elegans* kinesin-3 motor clearly modulates IFT and controls the localization of polycystins in the ciliary membrane, possibly via a non-IFT transport pathway [155, 156]. Kinesin-13 MT depolymerases are proposed to act at the axoneme tips or the mother centriole/BB to control ciliary length [157–159] and kinesin-4 controls the organization of the distal tip of the cilium to regulate transcription factors involved in Hedgehog signaling [160]. Because these and other fascinating ciliary motors are not thought to drive IFT, they are not a focus of the current review. Finally, it has been speculated that a novel dynein heavy chain, DHC-3, might represent a second form of IFT-dynein that drives retrograde transport along axonemes in certain *C. elegans* ciliated neurons, but this idea has not, to our knowledge, been tested [105].

4. Mechanism of Action of IFT-motors

The key drivers of IFT are IFT dynein for retrograde transport and heterotrimeric kinesin-2 for anterograde transport. In several cilia, an accessory homodimeric, kinesin-2 motor is deployed, but as noted above, its role may vary. Current work is aimed at understanding the biophysical mechanisms by which these kinesins and dyneins transduce the chemical energy contained in ATP into mechanical work. These IFT motors do not work on their own since they transport mechanically coupled trains containing multiple motors of either the same or different kinds. The resulting motor cooperation is one of the key areas of research in IFT, which we will address in the following section. First, we will focus on the properties of the individual motors, as obtained from *in vitro* and *in vivo* studies.

IFT dynein

As noted above, the slow minus end-directed motility of human recombinant dynein-2 has been documented *in vitro* [151] but more extensive studies of the mechanism by which IFT dynein generates force and motion are needed. It is known that IFT dynein is more closely related to the multifunctional transport motor, cytoplasmic dynein 1, than it is to axonemal dynein, which drives the beating of motile cilia [161]. Cytoplasmic dynein-1 has been extensively studied *in vitro* and shown to take sequential ~8nm steps to move processively towards the minus end of MTs [162], although the actual step size varies much more than for kinesins and the processivity strongly depends on the presence or absence of cofactors such as dynactin and cargo adaptors [163, 164]. It remains to be seen if IFT dynein displays similar *in vitro* motility properties to cytoplasmic dynein 1. Strikingly, the rate of retrograde IFT driven by IFT dynein *in vivo* varies, ranging between ~0.6 μm/s in mouse primary cilia [165], ~1.2 μm/s in *C. elegans* chemosensory cilia [128], ~3 μm/s in *Chlamydomonas* [96, 166], and ~5.6 μm/s in trypanosomes [167]. This remarkable difference in motor velocity between different cells and organisms warrants further study.

Heterotrimeric kinesin-2

As a preliminary step towards testing the role in anterograde IFT of the plus end-directed MT motility of heterotrimeric kinesin-2 observed in MT surface gliding assays [116], the rates of motility *in vitro* and *in vivo* have been compared [168]. For example, *C. elegans*

kinesin-II was observed to move at ~0.3 μm/s *in vitro* in "standard" PIPES buffer concentrations i.e. somewhat slower that *in vivo*, but faster velocities, very similar to the rates of kinesin-II-driven IFT train transport along sensory cilia (in mutants lacking homodimeric kinesin-2 function), could be obtained by simple variations of the PIPES concentration, supporting the hypothesis that kinesin-II serves as an anterograde IFT motor [168]. Later single-molecule optical tweezers experiments of KLP11/KLP20 motor heterodimers showed that the motor is processive (although substantially less so than kinesin-1), taking 8 nm steps along MTs and being capable of resisting opposing forces of ~5 pN [125]. Remarkably, chimeric constructs consisting of two KLP20 motor domains were processive as well, while constructs containing two KLP11 motor domains were not, indicating that the non-processive KLP11 motor domain is rendered processive by heterodimerization with its KLP20 partner. The authors also found evidence for an autoinhibition mechanism of heterotrimeric kinesin-2, which might be relieved by cargo (i.e. IFT train) binding.

More recently, full-length mouse KIF3A/B, has been studied extensively using single-motor bead tracking and optical tweezer assays [169, 170], yielding an unloaded run length of ~0.4 μm and a velocity of ~0.4 μm/s. Because the neck-linker regions of dimeric kinesin motors are proposed to transmit information about the mechanochemical state of one motor domain to the other, and *vice versa*, the shorter run length of kinesin-2 was proposed to be a consequence of its longer neck linker (17 amino acids) compared to that of the more processive kinesin-1 (14 aas [171, 172]. A recent study using truncated protein constructs attached to quantum dots, however, reported a very different run length of about 1.6 μm [173]. Further studies are needed to find the source of this apparent discrepancy, which might be due to the different constructs used, the use of quantum-dot versus polystyrene-beads, or other unknown differences in experimental conditions [169, 171–173]. Single-molecule fluorescence assays have further indicated that heterotrimeric kinesin-2 motors are less prone than other kinesins to detach from a MT upon encountering an obstacle e.g. a MAP on their MT track [174]. Stopped-flow and steady-state kinetics experiments on KIF3A/B indicate that, following dissociation, kinesin-2 rebinds to MTs very fast in the ADP-bound state, suggesting that its catalytic properties are optimized to restart a processive run after its progress has been impeded by such an obstacle [124]. A tentative mechanochemical cycle for heterotrimeric kinesin-2 based on these studies is shown in Fig. 3A.

Optical tweezers experiments have further demonstrated that opposing forces have relatively little effect on the velocity of heterotrimeric kinesin-2 compared to other kinesins but dramatically reduce its run length [169, 175]. Stopped-flow and steady-state kinetics experiments showed that the kinesin-2 construct, homodimeric KIF3A/A, spends a substantially longer fraction of its ATP hydrolysis cycle in a state with low MT affinity, but that dissociation from the MT in this state is slow under unloaded conditions [176]. This might be the underlying cause of the load dependency of heterotrimeric kinesin-2. In the optical tweezers study of Andreasson et al. [169], artificial homodimeric constructs were also studied, revealing that KIF3B/B is substantially less processive than KIF3A/A and that the properties of KIF3A/B are intermediate between the two homodimeric constructs.

The velocities measured *in vitro* match those observed for heterotrimeric kinesin-2 working on its own along cilia e.g. anterograde IFT occurs at ~0.5 μm/s in *osm-3* mutant *C. elegans* chemosensory cilia [128] and at ~0.4 μm/s in mouse primary cilia [165]. Remarkably, anterograde IFT in trypanosomes [167] and *Chlamydamonas* [10, 120, 121] is substantially faster (~2 μm/s). This faster transport might reflect the substantial phylogenic distance of the heterotrimeric kinesins in these organisms with those in mammals and nematodes [25]. Unfortunately, so far no *in vitro* assays have been performed on purified heterotrimeric kinesins from these latter organisms.

Homodimeric kinesin-2

The mechanism of action of homodimeric kinesin-2 has been less well-studied than that of heterotrimeric kinesin-2, perhaps because its homodimeric architecture resembles that of the extensively studied kinesin-1, suggesting that fundamental new principles are less likely to emerge. As noted above for heterotrimeric kinesin-2, the simple use of trial-and-error variations in the PIPES buffer concentration has revealed conditions in which the rate of plus end-directed MT gliding driven by *C. elegans* OSM-3 (~1.3 μm/s) matches the rate of transport driven along cilia under conditions when OSM-3 is the sole anterograde motor [128, 168]. Single-molecule fluorescence motility assays further revealed that full-length *C. elegans* OSM-3 motor constructs bind only very weakly to MTs and do not show processive motion *in vitro* [177]. This has been attributed to an autoinhibition mechanism that switches off motility in the absence of cargo. Deletion and point-mutation variants of OSM-3 motors devoid of this mechanism were shown to be relatively fast (~1 μm/s) and processive (run length ~1.6 μm). Optical-tweezers assays confirmed the autoinhibition mechanism and indicated that OSM-3's force-generation capability is comparable to that of kinesin-1.

5. Motor Cooperation and Regulation during IFT

Intracellular transport often depends on groups of motor proteins working together to transport a single cargo (Fig. 3B). During neuronal transport, for example, multiple kinesins with different motility properties and opposite-polarity dynein motors cooperate while transporting the same vesicles along MT tracks [178]. Their association with the same vesicle results in mechanical coupling between the different motors, which can give rise to frequent pausing and direction reversals [179]. In general, the motility properties of motor proteins such as run length and velocity are well known to be load dependent [169, 180]. In situations where multiple motor proteins are mechanically coupled, they can influence each other's behavior by applying assisting or resisting forces to each other. The cooperation of multiple, mechanically coupled motor proteins is a rich field of research, both theoretically [181–183] and experimentally using *in vitro* assays [184–188]. Several excellent reviews of this field have been published recently [189, 190]. Experiments and theoretical studies have shown that, depending on the properties of individual motor proteins and the way they are connected, cooperativity between motors can be positive or negative. Of particular note are situations where opposite-directed motors act on the same cargo, resulting in a 'tug-of-war', which can result in frequent directional reversals, pausing and even stalling as has also been observed in axonal transport [174, 178, 191].

In IFT, tens of motors act together on the same cargo in a very dense environment. In IFT, however, 'tug-of-war' kind of behavior involving reversals and pausing appears to be largely absent, with anterograde IFT trains moving steadily towards the ciliary tip before changing direction, and retrograde IFT trains moving steadily towards the base [94, 128, 192, 193]. This is remarkable since kinesins with different motility properties as well as opposite-polarity motors, the IFT dyneins, are coupled to the same IFT trains. In addition, the motor composition of IFT trains and the motor activity can vary depending on the track position. In *C. elegans* chemosensory cilia, for example, anterograde IFT-trains close to the base are mainly driven by kinesin-II motors, whereas after a gradual handover of the cargo, OSM-3 propels the trains solely towards the ciliary tip (Fig. 3C) [78]. Dynein-mediated direction reversals mostly occur at the ciliary tip [143], indicating that dynein is locally activated. Kymograph analysis of IFT in various organisms suggests that IFT is a highly orchestrated event with few aberrant movements [78, 94, 128, 192, 193]. Taking into account the number and variety of motor proteins involved in driving the smooth bidirectional movement of IFT-trains strongly suggests that motor activity is highly regulated. The regulation of the IFT motor proteins could occur at several different levels as described in this section.

At the base, the transition fibers serve as an assembly platform where IFT particles dock and form linear arrays of well-defined size [74, 81, 82]. The IFT-train assembly process sets the number of binding sites for IFT motors and also determines motor composition, a process that requires the activity of an additional protein complex, the BBSome [194]. How assembly takes place and how the IFT-train size is controlled is, however, not well understood. A recent study in *Chlamydomonas* has revealed that the docking of heterotrimeric kinesin-2 to IFT particles at the base is controlled by phosphorylation [108]. The authors showed that phosphorylated kinesin-2 did not bind IFT-particle-subcomplex B, whereas dephosphorylation of the FLA8/KIF3B motor subunit relieved this inhibition. In addition, the KAP subunit of heterotrimeric kinesin-2 could also play a role in IFT-particle docking and controlling motor activity. In *Chlamydomonas* it has been shown that the FLA3/KAP3 subunit is required for localization of heterotrimeric kinesin-2 to the ciliary base and for its motor activity [119]. Interestingly, in most organisms the KAP subunit is found to be slightly substoichiometric relative to the motor subunits, suggesting an additional form of regulation where the kinesin-2 motor might function as heterodimer [25], possibly having different motility properties. Targeting of the homodimeric kinesin-2, KIF17, to cilia involves a ciliary localization signal (CLS) that resides in the tail of the motor [71]. The same study showed that KIF17 entry into cilia is controlled by a RanGTP gradient and interaction of the motor with importin-β2 [71]. Interestingly, the authors identified the CLS by searching for sequences resembling a nuclear localization signal (NLS), and (as noted by the authors) that the KAP-subunit of heterotrimeric kinesin-2 motor harbors a NLS [195], indicating that IFT motor proteins might harbor (unidentified) NLS/CLS sites required for their cell-cycle dependent localization.

During IFT-train formation another form of motor regulation might come from the intrinsic properties of the motors themselves. In the absence of cargo interaction most kinesins are known to reside in an autoinhibited state, where the tail, which also contains the cargo-binding domain, folds back onto the motor domains, thereby preventing futile ATP hydrolysis, and inhibiting MT and cargo interactions [196]. Cargo binding can relieve this

autoinhibitory state upon which the motor extends and becomes active. Both heterotrimeric and homodimeric kinesin-2 can form a compact autoinhibited state [118, 177], which might be relieved upon their association with the IFT particles. In the case of KIF17 the autoinhibitory state is induced by a folded conformation where the tail domain and part of the coiled coil interact with the motor domains [197]. In the case of OSM-3 a single-point mutant (G444E), located in the hinge region of the coiled coil, was shown to relieve the autoinhibition in a series of ATPase and *in vitro* motility assays [177]. KIF17 is thought to have a similar hinge region, and a single point mutation (G754E) in this region together with mutations in a coiled-coil segment (762–772A) were shown to extend and activate the motor [197]. The hinge region of kinesin-2 motors might act as specific target site for regulatory proteins, which could stabilize the unfolded conformation, for example. In *C. elegans* the conserved ciliary protein DYF-1 (an IFT-particle B associated component) was shown to affect the autoinhibited state of OSM-3 [94, 198]. Using IFT assays the authors found that DYF-1 is required for targeting of the OSM-3 motor to IFT particles and for relieving its autoinhibition, DYF-1-OSM-3 protein-protein interactions were however not identified.

Once the IFT train is assembled and the kinesin-2 motors activated, the IFT-train has to navigate the structurally dense TZ. In *C. elegans*, kinesin-II is thought to function as a loader at the base and navigator in the TZ, whereas OSM-3 functions as a long-range transporter once inside the cilium [78]. This means that the localization and activity of both motors has to be precisely tuned via their docking and undocking to and from IFT trains. It is likely that the axonemal track and the IFT particles play important roles in this respect. Recent work in *Chlamydomonas* has shown that in anterograde direction IFT trains specifically move along the B-tubule of the axoneme, whereas retrograde IFT-trains move along the A-tubule [82]. In *C. elegans* the early termination of the B-tubule could hence act as a physical barrier to the kinesin-II motor, preventing the motor from entering the distal segment. The OSM-3 motor, however, is required to move along the A-tubule in order to enter the distal segment. It is therefore tempting to speculate that OSM-3 might preferably associate with the A-tubule and not the B-tubule. In this regard it is well known that tubulin PTMs and MAPs can affect motor protein activity [66, 196]. In *C. elegans*, for example, detyrosination of α-tubulin affects the activity of OSM-3, increasing its run length twofold without affecting its velocity [66], and tubulin deglutamylation has been shown to affect the ciliary localization of the kinesin-3, KLP-6, and the motility of OSM-3, but not that of kinesin-II [199]. Interestingly, the A- and B-tubule of the axonemal doublet can be modified in different ways. In *Chlamydomonas*, for example, the B-tubule is more detyrosinated in comparison to the A-tubule [67]. In zebrafish, the *C. elegans* DYF-1 homoloque *fleer* affects polyglutamylation of sensory cilia and its absence causes ultrastructural defects in the B-tubule [200]. [201]Although the axonemes of different organisms could be differently modified it is likely that tubulin PTMs play crucial roles in regulating the activity of IFT motor proteins.

While the IFT trains move along the axoneme, the IFT particles in concert with specific MAP kinases, regulate the docking of IFT motors to and from IFT trains thereby affecting motor activity and IFT dynamics. As mentioned above, the IFT-B associated DYF-1 can regulate OSM-3 motor activity by relieving its autoinhibition [94], whereas another IFT-B associated protein, DYF-2, is known to regulate turnaround of IFT particles at the ciliary tip [194]. In *C. elegans* chemosensory cilia the mitogen-activated protein (MAP) kinase DYF-5

is known to affect the undocking of the kinesin-II from IFT trains [202], and in *Chlamyodomonas* undocking of heterotrimeric kinesin-2 at the ciliary tip has been shown to require its phosphorylation [108]. Moreover, plus-end associated proteins such as EB1 localize to the tips of axonemal MTs and might interact with IFT machinery [63, 203, 204]. Regulation of IFT dynein in comparison to the regulation of the IFT kinesins is less well understood, but is likely to be controlled by its different subunits such as the light intermediate chain [149, 161]. It is clear that several layers of regulation orchestrate motor activity during IFT, and that further studies are required to unravel the intricate complexity of the different regulatory systems that control IFT in more detail.

In anterograde IFT in *C. elegans* chemosensory cilia, two different kinesin-2 motors, kinesin-II and OSM-3, cooperate [78, 128]. At the ciliary base, the IFT trains consist mainly of kinesin-II motors and move at a relatively slow velocity of 0.5 μm/s. This IFT-train motor composition might give rise to the dynamical properties required for loading IFT particles into the cilium and navigating the structurally dense TZ [78]. Along the MT doublets of the middle segment, the IFT trains, consisting of fewer kinesin-II and more OSM-3 motors, travel at an intermediate velocity of 0.7 μm/s, while along the distal segment IFT trains containing OSM-3 alone, travel at a terminal velocity of 1.3 μm/s [78, 128]. *In vitro* studies using well-defined ratios of kinesin-2 motors in MT gliding assays (performed in a pre-determined concentration of PIPES buffer as noted above for either type of kinesin-2 motor alone) initially showed that the rates of anterograde transport observed in cilia could be mimicked without the requirement for additional factors [168]. The intermediate velocity was explained by each motor influencing the other's motility parameters via alternating action or through mechanical competition but a recent *in vivo* study led to a refinement of this picture. We found that kinesin-II and OSM-3 motors gradually undock and dock to IFT trains, respectively, giving rise to a gradual increase in the velocity of anterograde IFT trains that travel along the middle segment [78]. This behavior can be explained by the introduction of a "bias parameter" which reflects additional factors that influence motor cooperation and cause an almost 10-fold increase in the contribution of OSM-3 relative to kinesin-II motors. It is not clear, however, what factors could create such a bias. It could involve regulatory processes (see above), but might also be the result of distinct load dependencies of the two motor proteins. This latter aspect could be studied using *in vitro* studies employing optical tweezers.

In *Chlamydomonas*, assemblies of heterotrimeric kinesin-2 motors move IFT trains at velocities well over 2.0 μm/s [10, 120, 121]. It has been shown that these IFT-motor assemblies can generate over 20 pN of force [193], well exceeding the 5 pN and 6 pN stall forces measured *in vitro* for the kinesin-2 family motors, kinesin-II and OSM-3, respectively [125, 177]. This indicates that kinesin-2 motor cooperation might be required to generate the forces for *Chlamydomonas* surface gliding. However, *in vitro* motor studies of kinesin-2 are required to obtain a deeper understanding of kinesin-2 cooperation during IFT in *Chlamydomonas*.

6. Cargo Delivery by IFT

Cargo transport by IFT is required for cilium assembly, maintenance and function [17] and it appears to fulfil two basic roles [23]. First, it is involved in selectively transporting proteins across the TZ, which controls the protein composition of the cilium and second it can move proteins against a concentration gradient, e.g. axonemal precursors that are produced in the cell body and are incorporated at the ciliary tip. Cargo molecules that are transported in cilia include the complex IFT machinery itself, axonemal building blocks, such as tubulins, signaling molecules and, in motile cilia, outer-arm dyneins [23]. In *Chlamydomonas*, IFT also drives gliding of the organism over surfaces that it adheres to. It is important to realize that, in contrast to many other intracellular transport mechanisms [205, 206], IFT cargoes are not contained in membranous vesicles, but in most cases comprise proteins that directly attach to the IFT machinery. Several cargo proteins are, however, membrane-bounded proteins, and in particular the BBSome appears to play a role in facilitating the transport of such proteins into and along the cilium by IFT [32]. For only a few cargoes, molecular details about their interaction with IFT trains are known. Above, we have discussed in detail the molecular basis of tubulin interacting with IFT components. For many other cargoes it is not yet understood how they interact with the IFT machinery. Interactions might be less tight, less specific and might involve unknown cargo adaptors [23]. The loading of cargo most likely happens at the ciliary base, where IFT proteins are enriched, apparently ‘waiting’ for assembly into trains and connection to cargo. Many aspects of train formation and cargo loading are unclear, but dephosphorylation of one of the heterotrimeric kinesin-2 subunits appears to be an important trigger [108]. IFT trains then depart in the anterograde direction and traverse the dense TZ, which has been shown to slow down transport [78]. Many cargoes are thought to be delivered in one go at the ciliary tip, where the trains disassemble and reassemble into retrograde trains [108, 167]. Certain components, however, including axonemal components in *Chlamydomonas* [98, 207] and kinesin-2 motors in *C. elegans* [78] have been shown to detach or attach to trains along the cilium. Below we discuss several specific cargoes in more detail.

IFT components

The IFT machinery consists of many proteins, including subunits of IFT trains as well as anterograde and retrograde motors. IFT trains are assembled at the base, remain intact as they move in the anterograde direction, disassemble at the distal tip then reassemble into retrograde trains that move all the way back to the base. Anterograde and retrograde trains appear different in EM images [82], but it is unclear whether this reflects a different conformation or composition. Anterograde motion is driven by kinesin-2 motors and IFT-dynein subunits have been identified as cargoes on anterograde moving trains in *C. elegans* [105, 143] and *Chlamydomonas* [149]. Remarkably, different subunits showed differences in their frequency of transport and location of turnaround [143]. In *C. elegans*, both homodimeric and heterotrimeric kinesin-2 motor proteins have been observed to be cargoes on retrograde trains [128], with homodimeric OSM-3 shuttling mostly between ciliary tip and end of the transition zone, and heterotrimeric kinesin-II from just beyond the TZ to the base [78]. Remarkably, in *Chlamydomomas* cilia, where heterotrimeric kinesin-II is the sole anterograde motor, no IFT-dynein-driven active retrograde movement of kinesin-2 has been

observed, suggesting that this motor diffuses back to the base [208]. This notable difference in retrograde transport of kinesin motors deserves further investigation.

Tubulin and other axoneme building blocks

The best characterized cargo of IFT is tubulin, the major building block of the axoneme that forms the core of the cilium. Based on studies of IFT mutants in *Chlamydomonas* it has long been thought that this IFT-based tubulin delivery for incorporation at the distal tip is required to build the cilium and maintain its length [17]. Indeed, careful studies in *Chlamydomonas* have resulted in the influential "balance point" model directly connecting IFT to ciliary length control [80]. More recent studies have revealed that length control might be more complex, involving upregulation of IFT during cilium growth [98] and kinase regulation of IFT speeds [209]. Direct evidence that tubulin is a cargo of IFT came from *in vivo* imaging of *Chlamydomonas* and *C. elegans* expressing fluorescent tubulin [63, 98] [although it has also been proposed that, in some cases, heterotrimeric kinesin-2 could directly bind and transport tubulin subunits along cilia independent of any IFT-particle subunits [104]]. In *Chlamydomonas*, additional components of the axoneme that have been shown to be IFT cargos include the radial spokes, the central-pair proteins, outer-arm dyneins and various regulatory complexes [23, 207].

Proteins involved in signalling

Cilia are important signaling hubs and IFT is thought to play a key role in delivering the signaling proteins involved in cilium-based signaling. Some signaling proteins, including TRP channels OSM-9 and OCR-2 in *C. elegans*, and PKD-2 in *Chlamydomonas* have been directly shown to be transported by the IFT machinery [210, 211]. In mouse olfactory cilia and human primary cilia, a more complex picture has been presented in which signaling proteins such as Arl, transmembrane olfactory signaling proteins [95], somatostatin receptor 3 [212] and smoothened [212, 213]) largely move by diffusion and appear to be only transiently connected to the IFT machinery. It could be that IFT is mostly involved in transporting these proteins across the TZ, while their distribution throughout the rest of cilium is mostly driven by diffusion. Further studies will be required to unravel what role IFT plays in localizing these signaling proteins.

IFT drives surface motility in Chlamydomonas

Chlamydomonas can use its flagella to adhere to surfaces and to glide along them [214]. Surface gliding is driven by IFT, mediated by flagellar membrane glycoproteins in a Ca^{2+}-dependent manner [193, 215]. The glycoproteins adhere to the surface transiently. Dynein motors then transport the glycoproteins to the ciliary base, driving the gliding of the algae over the surface in the opposite direction.

7. IFT in different Model Systems

The variety of model organisms that are being used to study cilia represent a fairly broad range of eukaryotes (Figs 1 and 4), as discussed in a comprehensive recent review [216]. These organisms range from protists such as the basal eukaryote *Giardia* [217] through plants where the IFT motor, kinesin-2 is thought to participate in ciliogenesis in mosses

[218, 219] to vertebrates such as *Xenopus* and mouse [123, 220]. Here we discuss only a few of the systems where direct assays of IFT have yielded mechanistic information about motor-dependent cargo transport in IFT.

Chlamydomonas

The green alga, *Chlamydomonas* uses its two motile cilia to swim through fluid and also as sensory organelles. Work in this system yielded a simple, and still very plausible, model for the mechanism of IFT in which heterotrimeric kinesin-2 moves IFT rafts and associated cargo from the base to the tip of the cilium and IFT dynein transports IFT subunits and turnover products back to the base of the cilium (Fig. 1B) [15, 17, 18]. The observation that the inactivation of the FLA10 subunit of heterotrimeric kinesin-2 using conditional mutants leads to a gradual cessation of IFT and defects in motile cilium assembly or maintenance, supports the hypothesis that it drives the anterograde transport of IFT trains [10, 120, 121]. However, it remains puzzling that the rate of anterograde IFT in this system occurs at 2 μm/s, much faster than the rate of anterograde motility driven by all purified heterotrimeric kinesin-2 motors so far studied [25]. Unfortunately the rate of motility driven by purified *Chlamydomonas* heterotrimeric kinesin-2, which may be unusually fast, has not to our knowledge been determined. Alternatively, although heterotrimeric kinesin-2 is clearly required for IFT in this system and can be observed moving along the cilium [119] it remains formally possible that this motor functions only to load the IFT machinery into the cilium (as in *C. elegans* [78]) and then an unidentified faster motor takes over and moves the IFT trains and heterotrimeric kinesin-2 as cargo the rest of the way along the cilium. Most researchers would be dismissive of this idea, but it should be noted that there does exist evidence for multiple ciliary kinesins in the cilia of this organism [221, 222]. If the former, arguably more likely alternative is correct, it may be that the presence of roadblocks such as dynein arms and radial spokes all along the axoneme may necessitate the use of only heterotrimeric kinesin-2 throughout, because of this motors' ability to circumnavigate roadblocks, thereby providing a rationale for the apparent difference with *C. elegans* (see next section).

In this system, the kinesin-2 motors are thought to transport inactive IFT dynein as cargo to the ciliary tip, but it is then returned to the base of the cilium via diffusion rather than as cargo of the very well-characterized retrograde IFT machinery [208] and it is thought that the switch from anterograde to retrograde IFT involves an unknown control mechanism that mediates reciprocal switching between ensembles of heterotrimeric kinesin-2 and IFT-dynein motors, thereby eliminating mechanical competition between them [223]. There is good evidence that IFT delivers both axonemal precursors such as tubulins and membrane proteins such as opsins to their site of assembly in the *Chlamydomonas* cilium [77, 98, 224].

C. elegans sensory cilia

This system is of interest because heterotrimeric and homodimeric kinesin-2 motors cooperate with one another and with IFT dynein to mediate the assembly of *C. elegans* amphid channel cilia on chemosensory neurons (Fig. 1C) [225]. The axonemes of *C. elegans* amphid channel cilia have a bipartite structure consisting of the axoneme core consisting of 9 doublet MTs from which 9 distal singlet MTs emanate and are required for certain forms

of chemosensory signaling. The assembly of these axonemes normally involves a subtle pattern of functional collaboration between the heterotrimeric kinesin-2, kinesin-II, and the homodimeric kinesin-2, OSM-3. Based on fluorescence microscopy assays of IFT in cilia of living worms of different mutant background, it was initially proposed that the assembly of the axoneme core involves both motors working together to transport IFT trains along the MT doublets whereas the assembly of the distal singlets depends only on OSM-3 transporting IFT trains along these singlets [94, 127, 128, 168]. Thus, in wild-type animals, kinesin-II and OSM-3 function redundantly to build the axoneme core, whereas OSM-3 alone builds the distal singlets and, moreover, in this system it was possible to obtain a good correlation between the rates of anterograde IFT and the rates of MT motility driven by purified kinesin-2 motors [25, 168]. The IFT trains are thought to deliver cargo consisting of axonemal tubulin subunits as well as subunits of the IFT-dynein complex to the tips of the axoneme [63, 105]. At the distal tip of the axoneme, there is a switch from anterograde to retrograde transport after which the kinesin-2 motors are recycled to the basal body as cargo of IFT dynein, rather than by diffusion [141]. It should also be noted that the pattern of motor collaboration seen in these cilia can be modulated to generate cilia with diverse sensory repertoires on neurons with different functions in the animal

Ever since the involvement of two anterograde motors in IFT was proposed [128], it was unclear why two different forms of kinesin-2 that move with the same polarity yet different speeds were deployed to move the same IFT-trains along the axoneme core. Recently, improved methods of genetic manipulation and fluorescence microscopic IFT assays led to a significant revision of the original model, with the recognition that there exists a functional differentiation between the two types of kinesin-2 motors [78]. Specifically it is now thought that the slower, less processive heterotrimeric kinesin-2, which is adapted to circumnavigate obstacles on the MT track, imports IFT trains and their associated cargo from the base of the cilium through the transition zone, where multiple roadblocks are encountered, into the cilium. There, homodimeric kinesin-2 gradually replaces heterotrimeric kinesin-2 as the IFT particles continue to move along the so-called “handover zone”, then it alone drives the long-range transport of these trains along the relatively obstacle-free axoneme to the distal tip of the cilium [78]. The new results are significant in explaining why the two distinct motors are used, because they are now seen to be functionally differentiated and it suggests why *Chlamydomonas* may use only heterotrimeric kinesin-2, because this motor is best suited to get around the roadblocks that exist along the full length of the axoneme in that system. In contrast, such obstacles are found mainly around the base of the cilium/transition zone in *C. elegans* and this is where this motor functions in these cilia. It is worth emphasizing that the new work supports the view that, along the handover zone, both heterotrimeric and homodimeric kinesin-2 attach to and move the same IFT particles, but their mole ratio varies with position. This gives rise to a range of intermediate velocities that could be due to either the alternating action and mechanical competition mechanisms proposed earlier [78, 168]. It is also notable that homodimeric kinesin-2 contributes to ciliary assembly and functions in other organisms and cell-types as well, although whether it does so by driving IFT is unclear.

Other invertebrates

In other invertebrates, the contribution of IFT to ciliogenesis varies, although direct assays of IFT are often lacking. As noted above, heterotrimeric kinesin-2 is thought to function alone to assemble the full-length axoneme in *Chlamydomonas* whereas in *C. elegans* amphid channel cilia, this motor can only build the axoneme core and in order to assemble the entire axoneme it must cooperate with the 'accessory' homodimeric kinesin-2. When heterotrimeric kinesin-2 is inhibited in another invertebrate model system where ciliogenesis and ciliary function have been extensively studied, the developing sea urchin embryo [226–228], the assembly of motile ciliary axonemes is impaired, but a short immotile 'procilium' is still assembled, plausibly by homodimeric kinesin-2 which is encoded by the sea urchin genome [117]. In contrast, the cilia on olfactory receptor neurons in *Drosophila* also have a bipartite organization and develop via a 2-step pathway, yet in this case heterotrimeric kinesin-2 alone appears to be sufficient to assemble the entire axoneme [229]. Thus this work shows that diverse means of deploying kinesin-2 motors seem to have evolved to build cilia, but working out the detailed mechanism of IFT in the sea urchin and fly systems would benefit from the development of IFT assays and to our knowledge, this has not yet been accomplished.

Protists

IFT can be assayed in several protists, including trypanosomes and ciliates (Fig. 4) [167, 230]. For example, the protist *Trypanosoma brucei* which causes African sleeping sickness contains a cilium that can be as long as ~40 μm and represents a powerful model system for studying IFT and ciliary function [231]. The trypanosome cilium contains a typical complement of IFT-A and B components, but is proposed to be unusual in containing IFT dynein with heterodimerized heavy chains and in lacking the KAP subunit of heterotrimeric kinesin-2 [148]. IFT has been directly visualized by GFP tagging IFT-particle and IFT-dynein subunits combined with RNA interference to knock-down specific proteins, with IFT-B knock-down usually blocking axoneme assembly and IFT-A knock-downs producing truncated cilia filled with IFT-particle subunits. In this system, once the cilium is assembled, IFT is required to maintain its molecular composition e.g. the presence of the regulatory subunit of PKA and the distribution of kinesin-9, but not ciliary length [232]. The IFT machinery displays complex dynamics along the cilia of trypanosomes and the availability of assays of IFT provide a powerful opportunity for dissecting how IFT-motors contribute to the process [167].

Vertebrates

A number of vertebrate model systems are available for studying IFT and ciliogenesis, including, for example, zebrafish, *Xenopus* and mouse. Malicki and others are systematically studying the functions of putative anterograde IFT motors of the kinesin-2 family and IFT-particle subunits in zebrafish with some interesting and surprising results [44, 233, 234]. For example, based on the mutant phenotype of the motor subunit of the heterotrimeric kinesin-2 motor, KIF3A, this motor is indispensable for ciliogenesis in all cells, but in some cells including photoreceptors, it seems to function primarily in basal body positioning prior to ciliogenesis *per se.* In some mechanosensory hair cell cilia, however, it is

dispensible for basal body positioning and following its loss, short procilia assemble [44], superficially similar to those seen following heterotrimeric kinesin-2 inhibition in sea urchin embryos [117]. In the zebrafish system, homodimeric kinesin-2 is apparently dispensible for ciliogenesis although subtle sensory functions cannot be ruled out [44]. The role of kinesin-2 motors and IFT in mouse photoreceptor morphogenesis is also an active area of investigation [35] and the heterotrimeric kinesin-2-driven transport of opsins and axoneme precursors, as well as the functions of homodimeric kinesin-2, if any, are being investigated intensively [135, 136, 235, 236]. It is worth commenting that the connecting cilium of vertebrate photoreceptors corresponds to the transition zone of more conventional cilia. The application of fluorescence microscopy IFT assays to these photoreceptors would be very useful for sorting out the roles of specific IFT motors in IFT and ciliogenesis, and the development of rigorous FRAP techniques is a significant step forward [235].

Light-microscopy assays allowing direct visualization of IFT have been developed for the cilia of *Xenopus* [220, 237] and of mouse olfactory sensory neurons (OSN) [95], where complex dynamics are observed. In the latter system, as in *C. elegans* amphid channel cilia, there is a 1:1 stoichiometry of the BBSome to IFT particles and both heterotrimeric (KIF3) and homodimeric (KIF17) kinesin-2 motors traffic along the OSN cilia, but unlike in *C. elegans* amphids, homodimeric kinesin-2 is not required to build the distal region of the axoneme [95]. Some evidence supports the hypothesis that KIF17 may be transported as passive cargo associated with IFT-train subcomplex B to the distal tip of the cilium, where its function is unknown [137]. Other studies, on the other hand, provide evidence that KIF17 may actively cooperate with IFT-B to deliver cyclic nucleotide gated channels and dopamine receptors to the membranes of primary cilia [133, 134]. This is consistent with a differential function model in which heterotrimeric kinesin-2 builds the ciliary axoneme and homodimeric kinesin-2 delivers signaling molecules to the ciliary membrane (Fig. 1D).

8. Concluding remarks

IFT is an outstanding system for studying the biophysical and molecular mechanisms of motor-dependent cargo transport. Much has already been learned from this system using a combination of *in vitro* motility assays and live-cell imaging in various IFT-protein mutant backgrounds, leading to plausible models for how motors move IFT trains along cilia in order to deliver cargo molecules to assemble functional cilia, but, as noted throughout, many questions remain. For example, there are outstanding questions concerning the exact timing of the initiation of IFT during ciliogenesis as well as the relationship between the biochemistry and EM ultrastructure of IFT trains. We would like to know if the elegant functional differentiation of cooperating kinesin-2 motors seen in *C. elegans* is a special case, or if it is deployed more broadly, not only in cilia but also in other intracellular transport systems where same-polarity motors cooperate [25, 78]. Related questions concern what are the functions of homodimeric kinesin-2 in various types of cilia and why is this motor present is some cilia where it has no apparent function [44]? What is the full spectrum of cargo molecules that are moved by the IFT machinery and how do they and the IFT motors bind to and dissociate from the IFT raft subunits? To answer some of these questions, an atomic level understanding of the structure of the IFT machinery, including the IFT-particles and motors, will be important, an area where good progress is being made [84]. In

addition, high-resolution single-molecule biophysical assays done at nanometer and millisecond resolution, combined with transient-state kinetic analysis of IFT-motor function, will be vital [173]. A major advance would be the reconstitution of the entire IFT machinery, including IFT trains, IFT motors and cargo molecules, from purified components, in a way that allows the high-resolution assay of its motility and a dissection of the role of individual components in the mechanism of IFT motor-dependent cargo transport.

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List of defined abbreviations

BB	Basal body
CLEM	Correlative light electron microscopy
CLS	Ciliary localization signal
CPC	Ciliary pore complex
DAVs	Distal appendage vesicles
DIC	Differential interference contrast
DS	Distal segment
EM	Electron microscopy
IFT	Intraflagellar transport
LECA	Last eukaryotic common ancestor
MAP	Mitogen-activated protein
MAPs	Microtubule associated proteins
MIPs	Microtubule inner proteins
MT	Microtubule
NLS	Nuclear localization signal
PFs	Protofilaments
PTMs	Post-translational modifications
TZ	Transition zone

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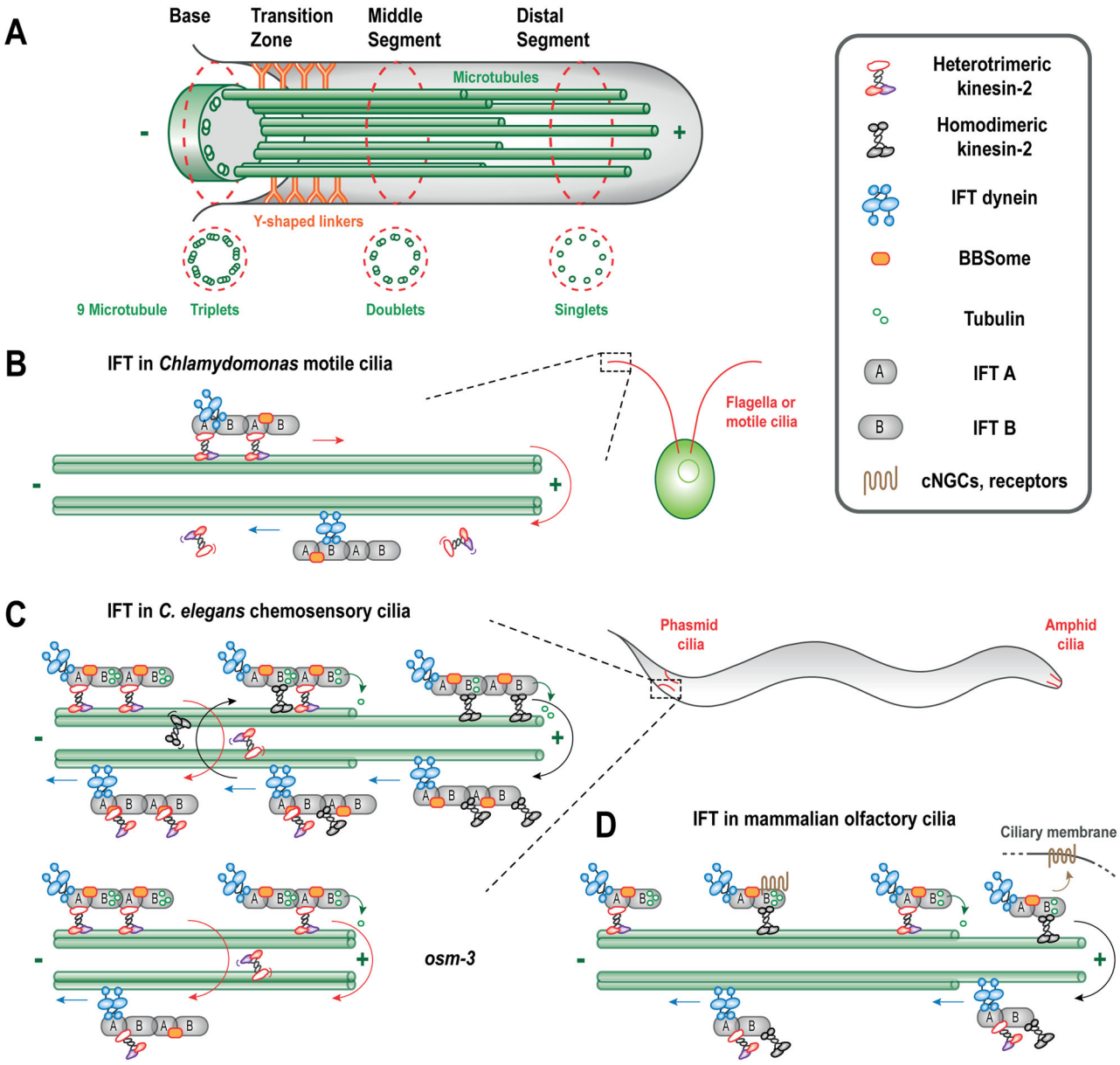

Figure 1.

Intraflagellar transport builds and maintains the cilium. (A) The axoneme is the core structural component of the eukaryotic cilium and it assembles by incorporation of precursors such as tubulin subunits onto MT plus ends at the distal tips of the axonemes (bacterial flagella, which rotate using a chemiosmotic mechanism add flagellin subunits at their distal tip, whereas the flagellum of archaea, the archaellum, rotates using ATP hydrolysis and adds subunits at its base). Shown is the chemosensory cilium of *C. elegans* neurons. It should be noted, however, that the basal body (BB, comprised of triplet MTs) degenerates in this organism. (B) Heterotrimeric kinesin-2 and IFT dynein are proposed to drive unitary rates of antero- and retrograde IFT, respectively in *Chlamydomonas*, whereas homodimeric kinesin-2 is absent. (C) IFT in *C. elegans* is driven by two distinct types of

kinesin-2 motors, heterotrimeric kinesin-II and homodimeric OSM-3, delivering cargoes such as tubulin to axoneme tips, while IFT dynein provides the retrograde transport, recycling the kinesin-2 motors. (D) Heterotrimeric kinesin-2 (kinesin-II aka KIF3) builds the axoneme whereas the homodimeric kinesin-2, KIF17 delivers signaling molecules to the ciliary membrane e.g. cNGCs and dopamine receptors in mammalian olfactory cilia, while IFT dynein drives transport towards the base. (A–D, simplified schematics, IFT-A and IFT-B stoichiometry, for example, can vary. See text for further details).

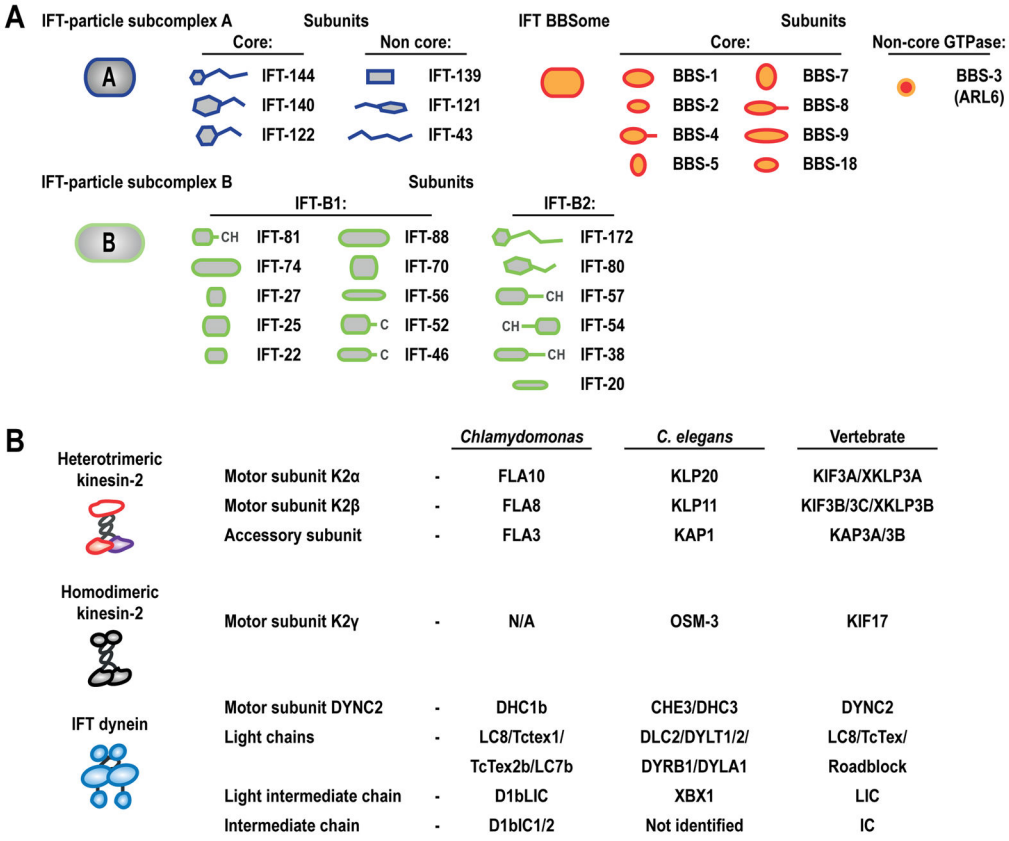

Figure 2.

The subunit composition of the IFT motors and the IFT trains/particles. (A) Detailed description of IFT particles based on a recent review [84]. (B) Description of motors involved in IFT and their subunits in different organisms. For more information on Kinesin-2 subunits see [25] and on IFT-dynein subunits see [24, 105, 143, 144]. See text for details.

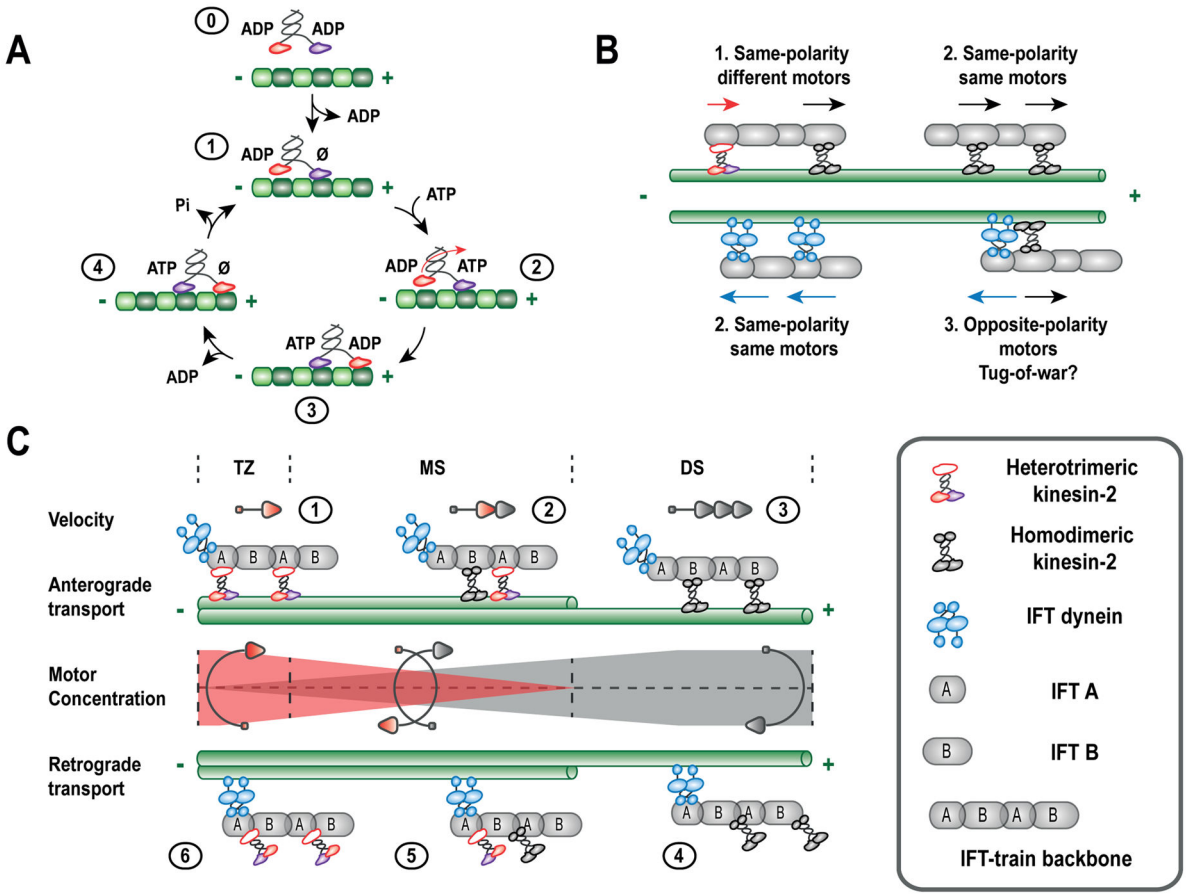

Figure 3.

Motor activity and cooperation of kinesin-2 motors. (A) A simplified mechanochemical cycle showing plus end-directed stepping of a truncated heterotrimeric kinesin-2 along a MT track (based on [124]). (B) Coupling of different molecular motors in a combinatorial fashion to the same cargo determines overall transport dynamics. (C) Cooperation of heterotrimeric and homodimeric kinesin-2 motors in *C. elegans* chemosensory cilia is determined by gradients of kinesin-2 motors. Numbered steps are; (1) A stable IFT-train backbone is loaded into the cilium and transported through the transition zone (TZ) at ~0.5 μm s^{-1} mainly by kinesin-II. (2) After navigating the TZ, kinesin-II gradually undocks while, at the same time, OSM-3 motors start docking, resulting in a gradually accelerating IFT train reaching ~1.3 μm s^{-1} at the end of the middle or proximal segment (PS), and ensuring reliable handover of the IFT-trains. (3) In the distal segment (DS), the train is occupied solely by OSM-3 reaching a terminal velocity of ~1.5 μm s^{-1}. (4) Following turnaround and remodeling at the ciliary tip, the backbone is returned to the base by IFT dynein, recycling OSM-3. (5) OSM-3 undocks from retrograde IFT trains along the PS, while kinesin-II gradually docks. (6) Close to the base, kinesin-II is the main kinesin cargo of retrograde IFT trains. Figure based on [78], see text for details.

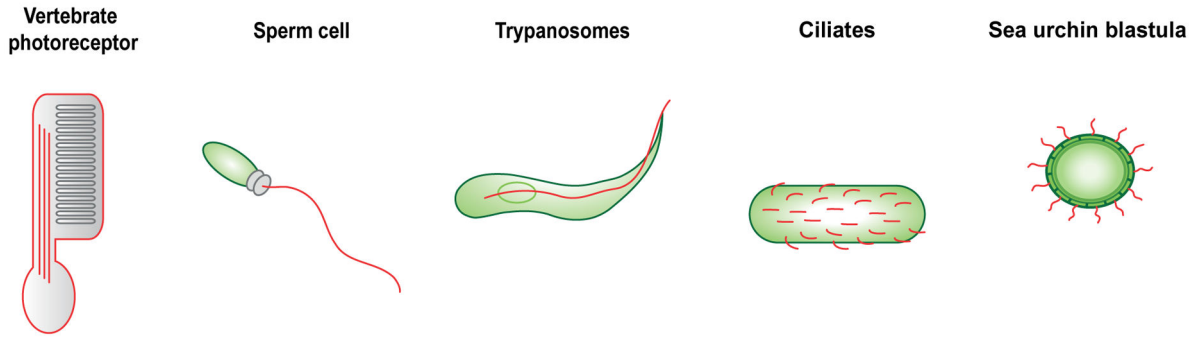

Figure 4.

Cilia of various cells and model systems. For motile cilia (aka flagella) of swimming *Chlamydomonas* cells and sensory cilia of *C. elegans* neurons, see Figure 1. Vertebrate photoreceptors are elaborate sensory cilia that detect photons of light. Spermatozoa, trypanosomes, ciliated protists and sea urchin blastula-stage embryos use motile cilia (aka flagella in sperm and trypanosomes) to swim through a fluid medium. Cilia or flagella are indicated in red. The three red lines inside the photoreceptor cilium represent the axonemal microtubules. See text for details.