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# INTRAFLAGELLAR TRANSPORT

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**Key Words** kinesin-II, IFT-dynein, cilia, flagella, motility, sensory signaling

■ **Abstract** It has been a decade since a novel form of microtubule (MT)-based motility, i.e., intraflagellar transport (IFT), was discovered in *Chlamydomonas* flagella. Subsequent research has supported the hypothesis that IFT is required for the assembly and maintenance of all cilia and flagella and that its underlying mechanism involves the transport of nonmembrane-bound macromolecular protein complexes (IFT particles) along axonemal MTs beneath the ciliary membrane. IFT requires the action of the anterograde kinesin-II motors and the retrograde IFT-dynein motors to transport IFT particles in opposite directions along the MT polymer lattice from the basal body to the tip of the axoneme and back again. A rich diversity of biological processes has been shown to depend upon IFT, including flagellar length control, cell swimming, mating and feeding, photoreception, animal development, sensory perception, chemosensory behavior, and lifespan control. These processes reflect the varied roles of cilia and flagella in motility and sensory signaling.

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## INTRODUCTION

Intracellular transport systems that utilize microtubule (MT)-based motor proteins to move and position subcellular components play critical roles in organizing the cytoplasm of eukaryotic cells (Goldstein & Yang 2000, Goldstein 2001, Hirokawa & Takemura 2003, Signor & Scholey 2000, Vale 2003). It is a well-established fact that many transported cargoes are moved by MT motors in association with membrane-bound transport vesicles, but work done during the past decade on

the assembly of eukaryotic cilia and flagella has revealed that some cargoes are moved along MTs in association with nonmembrane-bound oligomeric protein complexes in a process termed intraflagellar transport (IFT) (Kozminski et al. 1993, Rosenbaum et al. 1999, Rosenbaum & Witman 2002).

Cilia and flagella are ubiquitous organelles that project from the surfaces of many eukaryotic cell types, and they have evolved to carry out two very different functions, motility and sensory reception (Figure 1). There are abundant examples of motile cilia and flagella, whose rhythmic beating moves cells through fluid media or moves fluid media over stationary cell surfaces (Bray 2001, Johnson 1995, Porter & Sale 2000). In addition, many animal cells contain immotile primary cilia whose functions are less clear (Wheatley 1995, Wheatley & Bowser 2000), although the observation that the apical flow-induced deformation of primary cilia



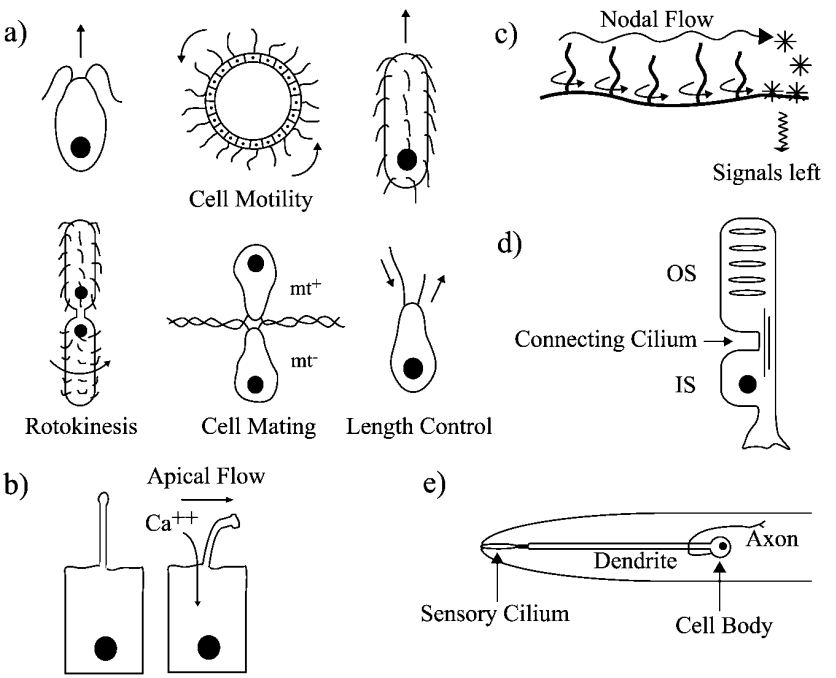


**Figure 1** Types of cilia and flagella. (*a*) IFT is required for the assembly and length control of motile cilia and flagella, which drive cell swimming, cytokinesis, and cell mating. (*b*) Renal epithelial kidney cells detect apical flow and respond by increasing intracellular $Ca^{2+}$. (*c*) The clockwise vortical beating of nodal cilia drives the nodal flow of perinodal fluid containing some factor (*) that signals left and initiates LR asymmetry. (*d*) Vertebrate photoreceptors use IFT along the connecting cilium to move proteins and membranes from the inner (IS) to the outer (OS) segment. (*e*) Sensory cilia form the dendritic endings of chemosensory neurons in the head of *Caenorhabditis elegans* and detect environmental chemicals.

on cultured kidney cells leads to an elevation of intracellular $Ca^{2+}$ suggests a sensory role (Praetorius & Spring 2001, 2003). Other immotile cilia, the sensory cilia, have obvious sensory functions and serve as specialized subcellular compartments that concentrate and organize the sensory signaling machinery. Such sensory cilia are found in vertebrate photoreceptors in the retina and on the endings of chemo-, thermo- and mechanosensory neurons in *C. elegans*, for example (Perkins et al. 1986, Marszalek et al. 2000). Certain primary cilia, the nodal cilia of vertebrate embryos, which play critical roles in development, are apparently motile (Nonaka et al. 1998), and, conversely, it is clear that motile cilia and flagella have sensory signaling functions (Pan & Snell 2002). Thus cilia and flagella participate in a variety of cellular and developmental processes, and defects in their function can lead to a range of diseases including respiratory distress, male sterility, polycystic kidney disease, retinal degeneration and situs inversus (Afzelius 1976, Pazour & Rosenbaum 2002).

All cilia and flagella have a similar design, consisting of a membrane-bound cylinder surrounding nine doublet MTs that make up the axoneme, which extends from a centriole called the basal body (Figure 2*a*). In motile 9+2 cilia or flagella, axonemal dynein motors cross-link and slide adjacent doublets, whereas accessory structures such as the central pair MTs, the nexin links, and the radial spokes convert MT-MT sliding to a coherent pattern of beating (Johnson 1995, Porter & Sale 2000). During the evolution of primary and sensory cilia, the structures required for motility were lost, which resulted in the formation of immotile sensory antennae on the cell surface. These sensory organelles have a relatively simple structure, sometimes referred to as 9+0, and lack dynein and nexin arms, radial spokes, and the central pair apparatus (e.g., Perkins et al. 1986). The membranes that surround both motile and sensory cilia play important roles in sensory perception and signaling, which has led to the interesting hypothesis that the sensory endings of neurons may have evolved from cilia and flagella (Clark & Grunstein 2000).

Elegant work suggests that ciliary and flagellar assembly involves the delivery of key components (e.g., tubulin subunits, dynein arms, radial spokes, membrane components) to the distal tip of the axoneme where assembly occurs (Johnson & Rosenbaum 1992, Johnson 1995, Stephens 1995). Because these components are synthesized in the cell body, they must traverse the length of the axoneme from the basal body to the distal tip, and they are now understood to be transported in association with the IFT particles by anterograde IFT (Kozminski et al. 1993, 1995; Piperno & Mead 1997; Cole et al. 1998) driven by the plus-end-directed heterotrimeric kinesin-II motor (Cole et al. 1993). Herein I review the mechanism and functions of IFT.

## THE MECHANISM OF IFT

IFT is a form of MT-based motility in which IFT particles move bidirectionally underneath the ciliary or flagellar membrane between the basal body and the distal tip of the axoneme (Figure 2). To illustrate current hypotheses of the basic

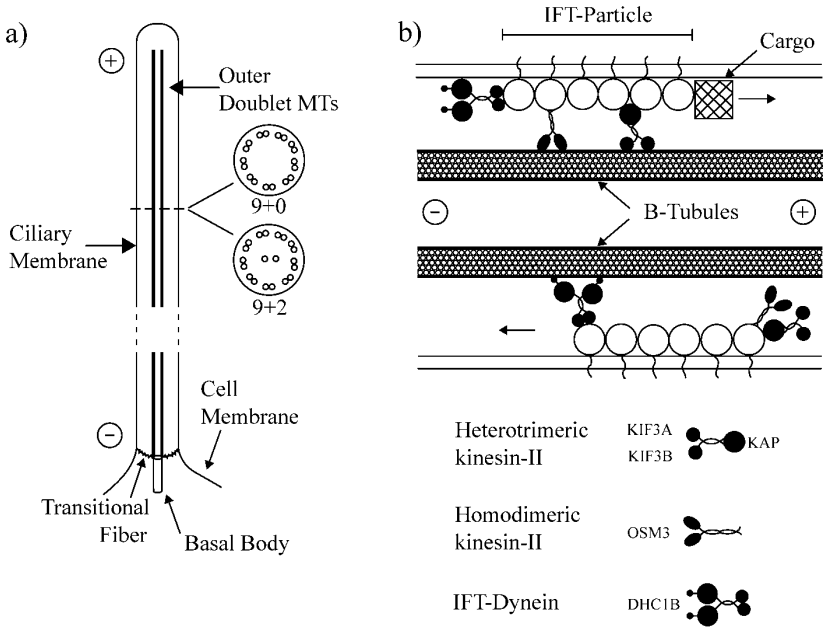


**Figure 2** Mechanism of IFT. (*a*) All axonemal MTs are oriented in parallel with their minus ends at the basal body and their plus ends at the distal tip. Motile axonemes are usually 9+2, whereas primary, nodal and sensory cilia are 9+0. (*b*) The anterograde transport of IFT particles, driven by heterotrimeric and homodimeric kinesin-II motors, is used to transport cargo, including the retrograde motor from the basal body to the distal tip. Retrograde IFT, driven by IFT-dyneins, is used to recycle the IFT particles and anterograde motors back to the cell body, forming a bidirectional shuttle system.

mechanism of IFT, it is useful to consider IFT particles undergoing a single cycle of IFT. First, IFT motors, IFT particles, and their associated cargo accumulate around the basal body prior to entering the axoneme (Johnson & Rosenbaum 1992, Piperno et al. 1996, Cole et al. 1998, Signor et al. 1999b, Orozco et al. 1999, Deane et al. 2001) where they appear to associate with the transitional fibers that extend from the distal portion of the basal body to the cell membrane and function as a loading dock for IFT (Deane et al. 2001). In this region, the IFT-motor-particle-cargo complex is assembled, sorted, and translocated to the base of the axoneme proper; the mechanism and sequence of these events are poorly understood (Deane et al. 2001).

Following assembly of the transport machinery, the anterograde kinesin-II motors move the IFT particles and associated cargo (e.g., axoneme and ciliary membrane precursors, retrograde motors, signaling molecules, etc.) along the length of the axoneme, using axonemal MTs as tracks (Figure 2*b*) (Huang et al. 1977, Lux & Dutcher 1991, Shakir et al. 1993, Walther et al. 1994, Kozminski et al.

1995, Tabish et al. 1995, Piperno et al. 1996, Morris & Scholey 1997, Cole et al. 1998, Nonaka et al. 1998, Marszalek et al. 2000, Iomini et al. 2001). At the distal tip, the IFT particles appear to be remodeled and become reduced in size (Piperno et al. 1998) presumably because axonemal precursors and other cargo are unloaded there; however, the mechanisms of cargo unloading are unknown. Finally, kinesin-II becomes cargo, and the retrograde IFT-dynein is activated, thus allowing it to transport the IFT particles and the kinesin-II motors back to the basal body (Pazour et al. 1999, Porter et al. 1999, Signor et al. 1999a).

It is notable that IFT particles move persistently along the axoneme from base to tip and backward from tip to base (Kozminski et al. 1993), switching direction only at the base and the tip. Thus it is plausible that a regulatory mechanism exists for differentially activating and inactivating the appropriate IFT motors at the base and tip to facilitate directional switching; however, the mechanism of this regulation is unknown.

## Microscopic Visualization of IFT

As in other areas of cell motility, light microscopic motility assays play critical roles in IFT research. Indeed, the discovery and initial characterization of IFT depended on the use of video-enhanced differential interference contrast (DIC) microscopy to visualize the movement of 500-nm particles along axonemes of paralyzed flagella mutants of *Chlamydomonas*, at rates of 2.0 $\mu$m/s from base to tip and 3.5 $\mu$m/s from tip to base (Kozminski et al. 1993, 1995). In these assays, IFT persisted under conditions of low free $Ca^{2+}$ ($<1$ $\mu$m) and was therefore distinct from other forms of nonbeating flagellar motility such as flagellar-mediated cell gliding and membrane glycoprotein motility. Using correlative DIC microscopy and thin-section electron microscopy (EM), the particles moving by IFT were visible by EM as electron-dense, linear arrays of subunits linking the B-tubules of the outer doublet to the flagellar membrane.

The trajectory of anterograde- and retrograde-moving IFT particles can be readily visualized under DIC optics by acquiring light intensity linescans along the flagellar long axis at time intervals (30 scans/s) and plotting intensity along the flagellum as a function of time (Piperno et al. 1998). Analysis of the resulting images reveals the velocity of motility (from the slopes), the frequency of motility events, and a decrease in contrast of the IFT particles as they switch from anterograde to retrograde motility, suggesting that IFT particle remodeling occurs at the tip of the axoneme.

Time-lapse fluorescence microscopy can be used to visualize specific proteins participating in IFT in contrast to the poorly defined particles visible by DIC (Orozco et al. 1999). Heritable transgenic lines of adult *C. elegans* that express GFP::IFT motor and GFP::IFT particles are immobilized by anaesthetization; IFT of the GFP::fusion proteins is assayed within their sensory cilia by standard or spinning disc confocal, time-lapse fluorescence microscopy (Orozco et al. 1999, Signor et al. 2000, Zhou et al. 2001, Dwyer et al. 2001). Although this technique

**TABLE 1** Protein machinery of IFT

| IFT component | Size of complex | Polypeptides | In vivo velocity ($\mu$m/s) | In vitro velocity ($\mu$m/s) |
|---|---|---|---|---|
| Anterograde motors | | | | |
| Heterotrimeric kinesin-II | 7.7 nm, 8S, 300 kDa | 1xKIF3A/KRP85/FLA10<br>1xKIF3B/KRP95<br>1xKAP | 0.7(A)<br>1.1(R) | 0.4 (+) |
| Homodimeric kinesin-II | 5.0 nm<br>6.8S<br>140 kDa | 2xOSM-3/KIF17 | 0.9(A)<br>0.9(R) | 0.8-1.2 (+) |
| Retrograde motors | | | | |
| IFT-dynein | 19S | 2xDHC1b/DHC2/CHE-3<br>D2LIC<br>LC8 | ND but likely<br>1.1 (R) | ND |
| IFT particle | | | | |
| Complex A | 16.3S<br>550 kDa | IFT-144<br>IFT-140/CHE-11<br>IFT-139<br>IFT-122/DAF-10<br>IFT-43 | 0.7(A)<br>1.1(R) | |
| Complex B | 15.5S<br>735 kDa | IFT-172/OSM-1<br>IFT-88/OSM-5/Tg737/Polaris<br>IFT-81<br>IFT-80<br>IFT-74/72<br>IFT-57/55<br>IFT-52/OSM-6/BLD1/NGD5<br>IFT-46/CHE-13<br>IFT-27<br>IFT-20<br>IFT-?/CHE-2 | 0.7(A)<br>1.1(R) | |

A, anterograde; R, retrograde; +, plus-end-directed; ND, not determined.



has not yet been sufficiently well explored to identify possible artifacts, it was demonstrated that GFP::IFT motor and GFP::IFT particle subunits display persistent, uninterrupted motility at similar rates along the length of the cilium (Orozco et al. 1999, Signor et al. 1999a, Qin et al. 2001) (Table 1) and that null mutations in IFT-dynein display a specific loss of retrograde, but not anterograde, IFT (Signor et al. 1999a). As genetic studies have identified dozens of genes that are essential for ciliary function in this system (Starich al 1995), the same approach can be used to dissect IFT pathways by visualizing IFT in multiple IFT-mutant organisms. Moreover, adapting these methods to fluorescence resonance energy transfer (FRET) microscopy could allow the direct observation of dynamic protein-protein

interactions in moving IFT-motor-particle-cargo complexes and the analysis of biophysical aspects of IFT within living animals (Periasamay & Day 1999).

## The Protein Machinery of IFT

Whereas light microscopy motility assays define the basic phenomenology of IFT, biochemistry and genetics are identifying and characterizing the molecular machinery of IFT, including IFT motors, IFT particle subunits, and cargo molecules (Figure 2*b*; Table 1).



IFT MOTORS Genomics reveals that organisms such as *C. elegans*, *Drosophila*, and mouse utilize scores of MT-based intracellular transport motors (Goldstein 2001, Hirokawa & Takemura 2003), but it is likely that many of these evolved from a limited number of prototypic "toolbox" motors emerging early in the evolution of primitive eukaryotes (Vale 2003). Two of these motors, the divergent cytoplasmic dynein, herein called IFT-dynein, and kinesin-II, play critical roles in IFT.

KINESIN-II Kinesin-II is found in single-cell flagellates and ciliates but not in fungi, consistent with the idea that kinesin-II evolved concomitant with the evolution of the axoneme for use in IFT, although in higher eukaryotes it is deployed for a variety of other transport functions as well (Marszalek & Goldstein 2000). Two forms of kinesin-II, which share a characteristic signature sequence (EDPKDALLRF/Y) in their neck domains, are thought to function as motors that drive anterograde IFT, namely heterotrimeric kinesin-II (also known as FLA-10-kinesin-II or KIF3A/3B/KAP3) and homodimeric kinesin-II (also known as Osm-3-kinesin or KIF 17) (Figure 2*b*).

Heterotrimeric kinesin-II was first purified from sea urchin embryonic cytosol via MT-affinity with the aid of pan-kinesin antibodies (Cole et al. 1992, 1993; Rashid et al. 1995; Wedaman et al. 1996), and it has subsequently been isolated from organisms ranging from algae to mammals (Yamazaki et al. 1996, Cole et al. 1998, Signor et al. 1999b, Scholey 1996, Marszalek & Goldstein 2000). Heterotrimeric kinesin-II holoenzymes contain two distinct motor subunits whose heterodimerization is directed by complementary charge interactions in a segment of the 35-nm long coiled-coil rod (De Marco et al. 2001). Associated with its tail domain is an accessory subunit, called KAP (Wedaman et al. 1996), that contains protein-binding armadillo repeats and is a candidate for binding IFT-cargo, and even forming the base of the IFT particles themselves (Table 1). In mouse, there are two heterotrimeric kinesin-II complexes, KIF3A/KIF3B/KAP and KIF3A/KIF3C/KAP, but the functional relationship between them is not yet known (Yang et al. 2001). Purified sea urchin kinesin-II moves toward the plus ends of MTs at 0.4 $\mu$m/s in an in vitro motility assay (Cole et al. 1993), and GFP::KAP fusion proteins move anterogradely at 0.7 $\mu$m/s in *C. elegans* sensory cilia and are returned as cargo by IFT-dynein at 1.1 $\mu$m/s (Orozco et al. 1999, Signor et al. 1999a) (Table 1).

The idea that heterotrimeric kinesin-II functions as an anterograde IFT motor gained strong support from studies of conditional mutants in the *Chlamydomonas FLA-10* gene, which encodes one of the motor subunits. Under restrictive conditions in the *fla-10* mutant, IFT particle particles are no longer transported along the flagellum, and consequently flagella cannot be assembled or maintained (Huang et al. 1977, Lux & Dutcher 1991, Walther et al. 1994, Kozminski et al. 1995, Piperno et al. 1996, Cole et al. 1998, Wittman & Rosenbaum 2002). The loss of heterotrimeric kinesin-II function leads to similar defects in cilia and flagella in a variety of systems, strongly suggesting that the function of heterotrimeric kinesin-II as an anterograde IFT motor is highly conserved (discussed below).

Evidence that homodimeric kinesin-II also participates in anterograde IFT (Figure 2*b*) has been obtained in only one system, sensory cilia on chemosensory neurons of *C. elegans*, where the OSM-3 kinesin-like protein is specifically concentrated (Signor et al. 1999b), based on observations that in *osm-3* loss-of-function mutants, sensory cilia are severely truncated (Perkins et al. 1986, Shakir et al. 1993, Starich et al. 1995, Tabish et al. 1995). Initially OSM-3 was thought to be a subunit of a heterotrimeric kinesin-II (Wedaman et al. 1996, Cole et al. 1998, Khan et al. 2000), but careful fractionation experiments showed that it is a subunit of a distinct homodimeric complex, whose two N-terminal motor domains are predicted to be linked by a 26-nm coiled-coil rod to a short tail (Signor et al. 1999b). Perhaps, homodimeric kinesin-II transports a cargo distinct from that transported by heterotrimeric kinesin-II, but the functional relationship between these motors is unknown. Homodimeric kinesin-II can be visualized moving along sensory cilia at 0.9 $\mu$m/s, but in vitro motility assays have not been performed. However, the OSM-3 homolog, KIF17, which transports NMDA receptor-containing vesicles in vertebrate neurons, moves to the plus ends of MTs at rates of 0.8–1.2 $\mu$m/s (Setou et al. 2000). Currently there is no evidence that homodimeric kinesin-II participates in IFT anywhere other than in *C. elegans*.

IFT-DYNEIN The retrograde motor for IFT, IFT-dynein (see below), was discovered in sea urchin embryos as a form of cytoplasmic dynein, which, similar to ciliary dyneins, is upregulated following de-ciliation, suggesting that it could be a cytoplasmic dynein involved in ciliary assembly (Gibbons et al. 1994). Definitive evidence for a role for IFT-dynein in retrograde IFT (Figure 2*b*) was obtained with the demonstration that mutations in genes encoding the IFT-dynein heavy chains (DHC-1b or CHE-3), light intermediate chains (D2LIC), or light chain (LC8) in *Chlamydomonas* or *C. elegans* produce short flagella or sensory cilia that are filled with IFT particle subunits (Pazour et al. 1998, 1999; Porter et al. 1999; Signor et al. 1999a; Wicks et al. 2000; Perrone et al. 2003; Schafer et al. 2003). Furthermore, retrograde, but not anterograde, IFT is disrupted in *CHE-3* null mutants (Signor et al. 1999a). These results are consistent with the hypothesis that following loss of IFT-dynein function, IFT particles are transported only anterogradely and thus accumulate at the tip of the axoneme because the retrograde transport system that normally returns them to the basal body is defective.

Little is known about the biochemistry and force-generating properties of IFT-dynein. Recent work suggests that it sediments as a 19S two-headed particle following detergent- or ATP-extraction from *Chlamydomonas* flagella and is dissociated to a 12S form upon high salt treatment (Perrone et al. 2003) (Table 1). Interestingly, DHC1b and D2LIC are components of this 19S complex but LC8 is not, suggesting that this multifunctional polypeptide plays an indirect role in retrograde IFT, perhaps loading axonemal precursors onto the IFT particles (Pazour et al. 1998, Perrone et al. 2003).



## IFT Particles

The purification of IFT particles from *Chlamydomonas* exploited the observation that the presence of IFT particles in flagella requires the activity of kinesin-II, and thus the particles are depleted from flagella of conditional *fla10* mutants grown under nonpermissive conditions (Kozminski et al. 1995). Consequently, matrix-containing fractions prepared from flagella of *fla10* cells grown under permissive conditions were found to contain a 16-17S complex of approximately 15 polypeptides that were absent from the flagella of cells grown under restrictive conditions (Piperno & Mead 1997, Cole et al. 1998). By varying the ionic strength during fractionation, the 16-17S complex was resolved into two complexes: IFT complex A (550 kDa), which sedimented at 16.2–16.4S, and complex B (710–760 kDa), which sedimented at 16S (Cole et al. 1998). Microsequence analysis showed that several of the polypeptides present in the IFT particle complexes were homologues of proteins required for sensory ciliary assembly in *C. elegans* (Perkins et al. 1986, Starich et al. 1995, Cole et al. 1998).

Detailed genetic and cytological studies of sensory cilia in *C. elegans* identified over 100 mutants with defects in sensory ciliary morphology and sensory function (Perkins et al. 1986, Starich et al. 1995). These mutants define 25 genes that fall into four classes: 6 chemotaxis (*che*) genes, 4 osmotic avoidance (*osm*) genes, 2 dauer larva formation (*daf*) genes, and 13 dye-filling (*dyf*) genes (Starich et al. 1995) (see below). Of these genes, several encode homologues of the IFT particle subunits that have been characterized from *Chlamydomonas*, including CHE-2, CHE-11, CHE-13, OSM-1, OSM-5, OSM-6, and DAF-10, supporting the hypothesis that the IFT particle subunits are essential for ciliogenesis (Perkins et al. 1986; Cole et al. 1998; Collet et al. 1998; Fujiwara et al. 1999; Haycraft et al. 2001, 2003; Qin et al. 2001) (Table 1).

Significantly, the homologue of *C. elegans* OSM-5, IFT-88, was shown to be required for motile flagellar assembly in *Chlamydomonas* and sensory ciliary assembly in *C. elegans* (Pazour et al. 2000, Haycraft et al. 2001). Moreover, its mammalian homologue, Tg737, is required for the assembly and maintenance of primary cilia in mouse kidney and of rod outer segments in mouse retinal photoreceptors (Pazour et al. 2000, 2002). This IFT particle subunit contains two TPR domains thought to participate in protein-protein interactions. Mutagenesis experiments have also revealed that IFT52 and IFT57 are required for the formation

of motile flagella on *Chlamydomonas* (Deane et al. 2001, Brazelton et al. 2001, Rosenbaum & Witman 2002).

The precise relationship between IFT particle complexes A and B is unclear. It is possible that the fragmentation of IFT particles into two subcomplexes is an in vitro artifact, but there are intriguing hints that they may be functionally distinct. For example, mutations in kinesin-II and complex B subunits result in more severely truncated cilia and flagella than do mutations in IFT-dynein and complex A subunits (Kozminski et al. 1995, Perkins et al. 1986). In the worm, the expression of all IFT complex B, but not complex A, genes are regulated by DAF-19, a RFX-type transcription factor (Swoboda et al. 2000, Schafer et al. 2003). Moreover, three *Chlamydomonas* mutants displaying defects in retrograde IFT, *FLA15*, *FLA16*, and *FLA17*, permit the assembly of flagella that are depleted of IFT particle complex A polypeptides (Piperno et al. 1998). These results suggest that IFT complex B might be associated with anterograde transport and flagellar assembly, whereas complex A might be involved specifically in retrograde IFT. It is also possible that complex A and B could be components of distinct anterograde IFT pathways, with heterotrimeric kinesin-II transporting one of the two complexes and homodimeric kinesin-II the other. Finally, in light of the distinct motility and sensory functions of cilia/flagella, it is possible that complex B functions in assembly by carrying axoneme precursors to the distal tip, whereas complex A functions in signaling by carrying sensory signals from the flagellar tip to the cell body.



## IFT Cargo

What is the cargo that associates with IFT particles? This cargo is apparently required for the assembly, motility, and sensing properties of cilia and flagella, and thus IFT particles are likely to deliver structural and force-generating components of the axoneme, as well as sensory ciliary membrane receptors and associated signaling molecules. Accordingly, direct experimental evidence supports the hypothesis that the IFT machinery transports axoneme components such as inner dynein arms (Piperno et al. 1996) and tubulin subunits (Marshall & Rosenbaum 2001) to their site of assembly in the flagellum. In support of the hypothesis that IFT particles deliver cargo associated with sensory signaling, *C. elegans* OSM-6, an IFT particle subunit, has been shown to be related to a 40-kDa mammalian protein, NGD5, which has been implicated in opioid receptor signaling (Wick et al. 1995, Collet et al. 1998). Similarily, an aurora-like protein kinase involved in signaling associated with mating requires kinesin-II for its transport into *Chlamydomonas* flagella (Pan & Snell 2000, 2003), and signaling molecules involved in photoreception are apparently delivered to rod outer segments in the vertebrate retina by IFT (Marszalek et al. 2000, Pazour et al. 2002). Finally, it is intriguing that the KAP subunit of kinesin-II associates with signaling molecules and may transport them as cargo (Nagata et al. 1998, Shimizu et al. 1996).

## Diverse Biological Functions of IFT

Experiments involving the disruption of components of the IFT machinery, particularly the IFT motor, kinesin-II, have provided abundant evidence that IFT plays important roles in a variety of cellular and developmental processes, some of which were quite unexpected.

MOTILITY The most obvious role of IFT is in the formation and maintenance of motile cilia and flagella whose rhythmic beating drives cell swimming (Johnson 1995, Porter & Sale 2000) (Figure 1*a*). A large body of work done in *Chlamydomonas* revealed that IFT drives motile flagellar assembly and thus is required for cell swimming (Kozminski et al. 1995; Piperno et al. 1996; Cole et al. 1998; Pazour et al. 1999, 2000; Porter et al. 1999; Rosenbaum & Witman 2002). In sea urchins, anti-kinesin-II mAb microinjection experiments reveal a role for kinesin-II (and presumably, therefore, IFT) in ciliogenesis on the blastula-stage embryo (Morris & Scholey 1997). Inhibiting kinesin-II function leads to the formation of short, 7-$\mu$m-long paralyzed "procilia," which are presumptive assembly intermediates that form by a kinesin-II-independent mechanism. Kinesin-II-dependent IFT is proposed to deliver components required for the elongation of these procilia to form the 18- to 30-$\mu$m-long motile cilia that produce the propulsive forces required for swimming and feeding (Morris & Scholey 1997). In the ciliate *Tetrahymena*, the deletion of genes encoding two kinesin-II motor subunits leads to an extreme shortening of locomotory and oral cilia and the concomitant disruption of cell swimming and phagocytosis (Brown et al. 1999b).

CYTOKINESIS Surprisingly, the loss of kinesin-II function in *Tetrahymena* led to a failure to complete cytokinesis (Brown et al. 1999b). In kinesin-II knockout cells, the initiation and ingression of the cleavage furrow was normal, but cell-cell abscission did not occur, suggesting that wild-type cells use cilia to generate the mechanical forces that pull the two daughter cells apart thus culminating in cytokinesis (Brown et al. 1999b). Light microscopy revealed that this cell-cell abscission requires multiple cilia-dependent rotations to sever the intracellular bridge, a process called rotokinesis (Brown et al. 1999a) (Figure 1*a*). Thus IFT is required for cell division in ciliates, albeit indirectly via its role in ciliogenesis. Little evidence exists for a direct role for IFT in cell division elsewhere (Morris & Scholey 1997, Matsuura et al. 2002).

CONTROL OF FLAGELLAR LENGTH IFT also contributes to the regulation of flagellar length (Marshall & Rosenbaum 2001) (Figure 1*a*). It is known that flagella are highly dynamic structures at steady state, exchanging more than 80 different polypeptides with a cytoplasmic protein pool (Song & Dentler 2001), with the addition and loss of tubulin subunits occurring at the distal tip of the axoneme. During steady-state turnover, the loss of tubulin subunits is balanced by IFT, which

delivers new tubulin subunits for distal tip assembly. This observation has led to a proposed model for flagellar length control in which the net rate of growth or shortening of the flagellum is equal to the difference between the IFT and length-dependent assembly rate and the length-independent disassembly rate (Marshall & Rosenbaum 2001). Such a simple mechanism for flagellar length determination may provide insights into the fundamental problem of how cells regulate the size of their organelles.

SENSORY TRANSDUCTION DURING MATING In addition to its role in ferrying molecules required for motile flagellar assembly and length maintenance, kinesin-II is also required for sensory transduction during mating in *Chlamydomonas* (Pan & Snell 2002, 2003). Fertilization in *Chlamydomonas* requires interactions between adhesion molecules on the flagella of cells of opposite mating types (mt+ and mt−) (Figure 1*a*), which initiates a signal cascade leading to an increase in intracellular cAMP concentration, gamete activation, and cell-cell fusion. However, loss-of-function kinesin-II mutants fail to increase their cAMP levels following flagella adhesion, and gamete activation and cell-cell fusion do not occur. Thus kinesin-II is proposed to function in sensory transduction, possibly by translocating and clustering transmembrane adhesion molecules to initiate the signal cascade, by transporting the signaling machinery into the flagellum, or by transporting IFT particles to the distal tip so that subsequent retrograde IFT can carry adhesion-activated signals from the flagellum into the cell body (Pan & Snell 2002).

PRIMARY CILIA IN THE KIDNEY Primary 9+0 cilia are present on renal epithelial cells lining the connecting ducts and tubules of the kidney (Figure 1*b*). The notion that IFT is required for the assembly of these primary cilia is supported by the observation that they are disrupted by mutations in the genes that encode the heterotrimeric kinesin-II subunit, KIF3A, and the IFT particle subunit, IFT-88 (Lin et al. 2003, Pazour et al. 2000). Specifically, in wild-type mice these monocilia are 3.1–3.5 $\mu$m long, but in mice homozygous for a hypomorphic mutation in the gene encoding IFT-88, the cilia are only 1–1.3 $\mu$m long. Thus it is proposed that IFT is required for the assembly and maintenance of full-length primary cilia, which normally perform a sensory function in the kidney. Significantly, two polycystic kidney disease proteins, PKD-2, a 6-span transmembrane $Ca^{2+}$channel, and PKD-1, an 11-span transmembrane protein, may require IFT to properly localize to mammalian renal cilia and to sensory cilia of *C. elegans* (Barr & Sternberg 1999, Yoder et al. 2002). It is possible that these two proteins form a stretch-activated $Ca^{2+}$ channel that allows renal cilia to sense apical flow and respond by elevating $Ca^{2+}$ levels (Praetorius & Spring 2001, 2003) (Figure 1*b*). The loss of renal ciliary function because of impaired IFT could be a cause of polycystic kidney disease (Lin et al. 2003, Pazour et al. 2000, Pazour & Rosenbaum 2002).

NODAL CILIA AND THE DEVELOPMENT OF LEFT-RIGHT ASYMMETRY During embryogenesis, vertebrates develop distinct and characteristic asymmetries along

the left-right (LR) axis, and consequently internal organs become asymmetrically positioned. For example, in all normal vertebrates, the rightward looping of the embryonic heart tube is an early event in the development of the LR axis and leads to the subsequent leftward tilting of the adult heart. Indeed, cardiac morphogenesis is particularly sensitive to LR positional cues (Supp et al. 2000). IFT plays a critical role in embryonic patterning by contributing to the establishment of the LR axis (Nonaka et al. 1998, Maszalek et al. 1999, Takeda et al. 1999).

In the mouse, gene targeting was used to generate mutants lacking either the KIF3A or KIF3B subunits of kinesin-II, yielding embryos that display randomization of cardiac looping. Moreover, LR-determining genes such as *lefty-2* and *Pitx2*, which are normally expressed only in the left lateral plate mesoderm of the embryo, were expressed bilaterally in the kinesin-II knockout mice. Significantly, primary cilia that are normally present on the embryonic node at the distal tip of the embryo (the mammalian equivalent of Spemann's organizer) were completely absent in the kinesin-II knockout mice, suggesting that kinesin-II-dependent IFT is required for both the assembly of nodal cilia and the development of the LR axis (Nonaka et al. 1998, Marszalek et al. 1999).

What is the connection between nodal cilia and LR asymmetry? In wild-type vertebrates, the embryonic node cells contain single 9+0 primary cilia, which undergo a vortical beating (Nonaka et al. 1998, Supp et al. 1999). Moreover, these beating cilia can drive the nodal flow of extracellular medium, which causes added fluorescent latex beads to move persistently leftward across the node. In kinesin-II knockout mice, there was no such flow, and added beads displayed only random Brownian motion.

The molecular mechanism underlying the curious vortical motion of nodal cilia is unknown, although it is significant that nodal cells express a form of axonemal dynein called LR dynein and that loss-of-function mutations in this motor protein give rise to rigid and immotile nodal cilia and a loss of LR asymmetry, suggesting that LR dynein drives nodal ciliary beating (Supp et al. 1997, 1999). This body of work has led to the provocative suggestion that kinesin-II-dependent IFT is required for the assembly of motile nodal cilia, whose vortical beating drives the nodal flow of perinodal fluid, serving to concentrate an unknown factor on the left side of the node, where it could bind a transmembrane receptor and initiate the expression of left-sided genes and the subsequent development of LR asymmetry (Figure 1*c*) (Nonaka et al. 1998). In support of this hypopthesis, it was recently demonstrated that artificial nodal flow can induce LR asymmetry (Nonaka et al. 2002).

IFT AND VISION Vertebrate photoreceptors are polarized sensory neurons consisting of a cell body, the inner segment (IS), and a sensory cilium, the outer segment (OS), which contains stacks of photosensitive membranous discs that concentrate phototransduction proteins such as opsin (Figure 1*d*). The photoreceptor OS turns over rapidly, and therefore it is necessary that large amounts of lipid and protein be continually transported from their site of synthesis in the IS to the OS. The IS and OS are connected by a short 9+0-connecting cilium, and it appears that

OS components are transported from the IS along the cilium by IFT (Marszalek et al. 2000, Pazour et al. 2002). Targeted mutagenesis of KIF3A was used to specifically knock out kinesin-II function in mouse photoreceptor cells, leading to defects in the targeting of phototransduction proteins to the OS (Marszalek et al. 2000). Similarly, mice lacking functional IFT-88, a subunit of the IFT particles, undergo abnormal OS development and retinal degeneration (Pazour et al. 2002). Thus IFT, presumably by transporting OS components along the connecting cilium, plays an important role in the assembly and maintenance of the vertebrate OS, and defects in this process may underlie certain human diseases that cause progressive blindness (Pazour & Rosenbaum 2002).



CHEMOSENSORY BEHAVIOR *C. elegans* senses environmental signals including chemicals, temperature, and mechanical pressure through ciliated sensory neurons (Figure 1*e*). For example, the animal undergoes chemotaxis in the presence of gradients of certain metabolites released by its bacterial food (Bargmann et al. 1990, Bargmann & Mori 1997, Chou et al. 1996, Culotti & Russell 1978, Ward 1973). The worm moves by lying on its side and using its body wall muscles to make long, sinusoidal runs interrupted by episodes of frequent turning, called pirouettes (analogous to runs and tumbles in bacterial chemotaxis) (Berg & Brown 1972). The animal responds to concentration gradients of attractants by increasing the frequency of pirouettes when moving down the gradient and decreasing the frequency of pirouettes when moving up the gradient (Pierce-Shimomura et al. 1999). This behavior depends upon the detection of the chemoattractant by ciliated chemosensory neurons in the nose of the animal.

The nervous system of adult hermaphrodite *C. elegans* is made up of 302 neurons, and of these, 26 are thought to be ciliated chemosensory neurons, including the amphid and inner labial neurons of the head (Figure 1*e*) and the phasmid neurons of the tail (White et al. 1986). The chemosensory cilia act as specialized compartments to concentrate receptors and other signaling molecules responsible for detecting environmental stimuli that control chemotaxis. Upon binding to a chemoattractant or chemorepellant, these cilia generate signals that are transmitted along the dendrite, through the cell body at the nerve ring, and out along the axon to the axon terminus. Here, synaptic neurotransmission sends information via networks of interneurons and motor neurons in the nerve cords to control the pattern of contraction of the body wall muscles, which results in movement toward or away from a chemical. It is likely that IFT delivers the appropriate chemosensory receptors and associated signaling molecules, as well as axoneme components, to the ciliary endings.

As noted above, two anterograde IFT motors, heterotrimeric and homodimeric kinesin-II, and one retrograde motor, IFT-dynein, are thought to drive bidirectional IFT in this system (Signor et al. 1999a,b), although direct functional evidence for a role of heterotrimeric kinesin-II is still being sought. Mutants in the IFT motor subunits, OSM-3, CHE-3, and D2LIC, and in several IFT particle subunits, OSM-1, OSM-5, OSM-6, CHE-2, CHE-11, CHE-13, and DAF-10, display severe

defects in sensory ciliary structure and chemosensory behavior, strongly supporting the notion that IFT plays critical roles in the formation and function of sensory cilia and the control of chemosensory behavior (Perkins et al. 1986, Starich et al. 1995) (Table 1). The precise roles and interrelationships between the multiple IFT components involved in IFT in this system remain unclear, but the ability to visualize specific proteins participating in IFT within living animals in various IFT mutant backgrounds (Orozco et al. 1999, Signor et al. 1999a) should improve our understanding of how IFT contributes to the formation and function of the chemosensory cilia and to chemosensory behavior.

CONTROL OF LIFESPAN Surprisingly, mutations in several components of the IFT machinery in *C. elegans* extend the animal's lifespan (Apfeld & Kenyon 1999). It is proposed that defects in IFT cause defects in sensory ciliary structure and corresponding defects in the sensory perception of environmental signals that cause *C. elegans* to age normally.

A DIRECT ROLE FOR IFT IN AN EXCITATION-ADAPTATION MECHANISM FOR SENSORY SIGNALING Abundant evidence, cited above, underscores the importance of IFT for sensory perception and signaling in both motile and sensory cilia/flagella. This raises the question: Is IFT involved in the sensory signaling mechanism itself, e.g., during chemosensation in *C. elegans*?

Similar to bacteria, adult *C. elegans* undergo chemotaxis in response to concentration gradients of chemoattractants rather than to their absolute concentrations (Berg & Brown 1972, Ward 1973, Pierce-Shimomura et al. 1999). This raises the possibility that *C. elegans* may utilize a fast-slow excitation-adaptation mechanism to adjust to the ambient concentration and sense their position in a gradient of chemoattractant, in a mechanism analogous to that deployed during bacterial chemotaxis (Bray 2001). Thus it is plausible that the ciliary-sensing mechanism utilized by *C. elegans* consists of two components operating at different timescales to store a past record of the chemical environment (the slow record) and compare it with a present (fast) record. For example, the initial event in chemosensation is presumably the binding of an attractive ligand to chemoreceptors in the ciliary membrane, leading to the activation of some fraction of the receptors within a submillisecond timescale, providing the fast record of the current concentration of attractant. A second slow record of the attractant concentration could depend on the bidirectional IFT of a record of the fraction of activated receptors from the ciliary tip to the transition zone and back again, possibly associated with IFT complex B. Because the sensory cilium is 10 $\mu$m long and IFT particles are moved at 0.7–1.1 $\mu$m/s, it would take the order of 10s for this slow record to develop as it traverses from tip to base and back again. It is plausible that the worm compares the fast submillisecond record of current fractional receptor occupancy with the slow record, which reports what the fractional occupancy was 10s previously. Thus the animal could determine if the fractional occupancy of the receptors and therefore the concentration of chemoattractant in the environment is increasing or decreasing

with time. If the two records differ by an amount less than some threshold amount, then the animal undergoes a random walk, but when this threshold is exceeded, a signal is generated that suppresses the frequency of pirouettes in a way that biases the random walk and causes the animal to move up a gradient of chemoattractant. This idea is speculative, particularly in the absence of any proposal for the biochemical mechanisms involved. However, it suggests that a fruitful experimental approach would involve testing if eukaryotic cilia and flagella, similar to bacteria, utilize a fast-slow excitation-adaptation mechanism to participate directly in sensory signaling.



## CONCLUDING REMARKS

In only a decade, remarkable progress has been made in IFT research. Much of the IFT machinery has been identified (including IFT motors and IFT particle components), the molecular mechanism of IFT is understood in outline, and there is an appreciation of the diversity of cellular and developmental processes that depend upon IFT and also on the diseases that can result from defects in IFT. However, many unresolved questions remain: (*a*) How does IFT machinery move through the cytoplasm to the transitional fiber loading dock; (*b*) what is the sequence of events during which the IFT machinery enters the axoneme and assembles for motility; (*c*) what is the relationship between IFT particle complex A and B or between heterotrimeric and homodimeric kinesin-II; (*d*) what are the important cargoes of IFT and how do they interact with the IFT particles; (*e*) how exactly does IFT contribute to sensory perception; and (*f*) how is IFT regulated? Future research is expected to answer such questions and to identify components of the IFT machinery that could be targets for the successful treatment of ciliary diseases.

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