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Intraflagellar Transport and the Flagellar Tip Complex

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Abstract Intraflagellar transport (IFT) is the term that refers to the microtubule dependent particle motility that is common to almost all flagella and cilia and is distinct from the mechanism of flagellar beating. IFT involves the rapid, bi-directional transport of molecular motors and their cargo proteins from the base to the tip of the flagellum and back again. While the basic mechanism of IFT is well established, the varied functions of this process are continually being elucidated. For example, although IFT plays a clear role in flagellar assembly, disassembly and stability, the exact sequence of events that take place when tubulin subunit addition and loss occur during flagellar assembly and disassembly, respectively, are unknown. Key to furthering our understanding of IFT is greater knowledge of the flagellar tip complex (FTC) because it is at the FTC that flagellar assembly and disassembly, cargo loading and unloading, and motor protein regulation occur. Yet these related processes may only represent one aspect of the importance of IFT in flagellar dynamics. IFT may also provide the basic elements of a signal transduction mechanism that functions to provide the nucleus with information about the outside environment and even about the state of the flagellum itself. Thus, IFT may function as the central component of a signal transduction system that controls flagellar gene transcription. J. Cell. Biochem. 94: 266–272, 2005.
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The terms cilia and flagella are for the most part interchangeable, as the structures of these organelles are highly conserved across species boundaries. Herein the term flagella will be used exclusively, although much of the data reviewed apply to cilia as well. Flagella are extensions of the cell cytoplasm and hence are enclosed by the plasma membrane. They are composed of an array of nine outer doublet microtubules surrounding a central pair of microtubules, all with their plus, or fast-growing, ends pointing toward the flagellar tip. This '9 + 2' arrangement of microtubules comprises the bulk of a structure called the axoneme. Completing the axoneme are radial spokes and dynein arms. The radial spokes are attached at precise intervals along the inside of each outer doublet and extend inward toward the central pair. Motive force-generating arms composed of the large, multimeric protein dynein [Gibbons, 1966] are bound at regular intervals along the length of the A-subfiber of each outer doublet. The dynein arms interact via an ATP-dependent crossbridge cycle with the B-subfiber of the adjacent outer doublet. The radial spoke/central pair complex together control the characteristic waveform produced by the organelle. Waveform is due to the varying activity of groups of dynein arms, which walk along the B-subfiber of an adjacent outer double [Satir, 1968]. This interaction of dynein with the microtubule of the adjacent outer doublet is controlled by the radial spokes and rotation of the central pair microtubules about their long axes (see [Smith and Yang, 2004] for a recent review); the spoke/central pair complex acts as a signal transducer that determines which group of dynein arms will be active at any given time. Thus, varying dynein activity occurs not only along one side or another of the axoneme but also along its length. The molecular events of outer doublet sliding, characterized by dynein-based motility under the control of the central pair/radial spoke complex, are observed microscopically as flagellar beating and macroscopically as cell motility.

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CHARACTERISTICS OF INTRAFLAGELLAR TRANSPORT (IFT)

The process of IFT, a flagella-specific phenomenon distinct from flagellar beating, was initially discovered in the bi-flagellate green alga *Chlamydomonas*. Using video-enhanced differential interference contrast microscopy and digital image processing, particles can be observed [Kozminski et al., 1993] moving in a directed, non-saltatory fashion from the flagellar base to the tip. At the tip of the flagellum, the particles pause briefly and then almost immediately begin the return journey to the base of the flagellum. Because the particles moved at different mean velocities outward (~2 μm/s) as compared to inward (~3.5 μm/s), and because microtubule based motility had been previously shown in other systems to be unidirectional [Gilbert et al., 1985] with respect to the motor involved [Vale et al., 1985; Vallee et al., 1988], it was assumed that two different molecular motors functioned to power IFT. Biochemical and genetic experiments have confirmed this initial assumption; kinesin II, a heterotrimeric kinesin composed of three non-identical subunits [Cole et al., 1993], moves IFT particles from the cell body to the flagellar tip [Kozminski et al., 1995]. When IFT particles reach the flagellar tip, they pause briefly and then return to the base, powered by a cytoplasmic form of dynein that is distinct from the axonemal dynein responsible for outer doublet sliding and flagellar beating. The cytoplasmic dynein involved in IFT is called dynein 1b [Gibbons et al., 1994; Pazour et al., 1999; Porter et al., 1999].

The particles that move during IFT can be resolved by transmission electron microscopy into electron dense patches of varying sizes, positioned between the flagellar membrane and the outer doublet microtubules. IFT occurs associated with the B-subfiber of each outer doublet, as it is with the B-subfiber that the IFT particles appear to interact, a conclusion based on analysis of EM thin sections [Kozminski et al., 1993, 1995]. IFT particles can be released from purified flagella by detergent extraction and resolved by sucrose gradient ultracentrifugation into two size classes called IFT particle complex A and complex B [Cole et al., 1998]. Five specific polypeptides are present in complex A, while complex B is composed of another 12 different polypeptides [Cole et al., 1998; Piperno et al., 1998]. These IFT polypeptides vary in relative molecular mass from 20 to 172 kDa.

Mutations in a number of the polypeptides of complexes A or B or mutations in kinesin-II or dynein 1b cause cells to have defective flagella or to lack flagella entirely. For example, *fla10* cells carry a temperature sensitive mutation in kinesin II [Walther et al., 1994], the motor that powers IFT during anterograde movement of the particles from the base to the tip of the flagellum. When *fla10* cells are shifted to the restrictive temperature, movement of IFT particles from the cell body into the flagellum ceases. Because dynein 1b is not affected and continues to move IFT particles back to the cell body, IFT particles are cleared from the flagella and the flagella shorten; during resorption, axonemal components are lost from the flagellar tip [Kozminski et al., 1995]. If the flagella of *fla10* cells are removed, the cells cannot regenerate new flagella at the restrictive temperature. Similarly, insertional mutants in dynein 1b, the retrograde (i.e., tip to base) IFT motor, can assemble only short, aberrant flagella [Pazour et al., 1999; Porter et al., 1999], while temperature sensitive mutations in dynein 1b cause the tips of the flagella to swell at the restrictive temperature. Swelling of the flagella tips occurs because anterograde IFT brings particles out to the tip where they accumulate, unable to return to the base due to a lack of dynein 1b activity. These observations indicate that IFT is required for flagellar growth as well as for maintenance of the flagella at the mature length [Marshall and Rosenbaum, 2001], which is about 12 μm in *Chlamydomonas*. The only exception to this rule noted thus far is that IFT is not required for the formation and function of sperm flagella in *Drosophila* [Han et al., 2003].

FLAGELLAR TIP COMPLEX (FTC) AND IFT

Structures at the flagellar tip were first reported about 25 years ago based on careful electron microscopic observations of flagella and cilia that had been gently extracted with detergent [Dentler and Rosenbaum, 1977; Sale and Satir, 1977; Dentler, 1980]. What will be referred to here as the FTC (Fig. 1) is composed of a central microtubule plate and ball that attaches the distal ends of the central pair microtubules to the membrane, while a short

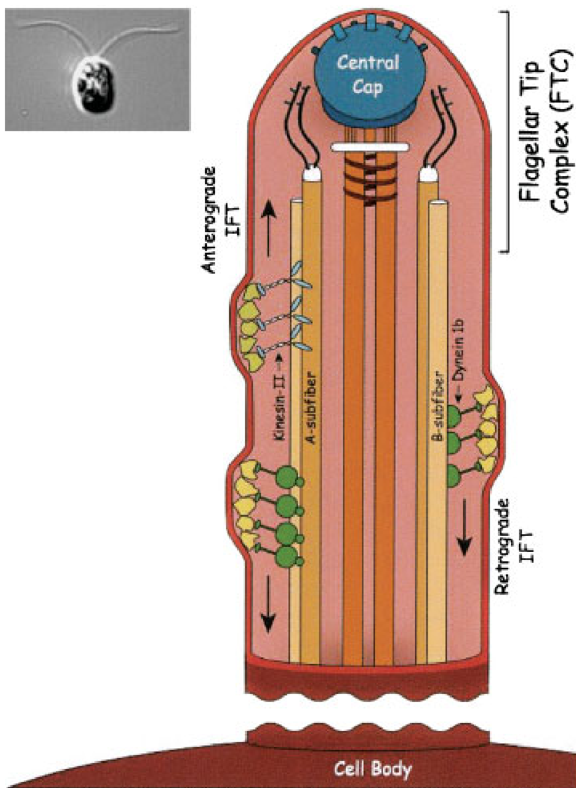

Fig. 1. Diagram of the components of IFT and the FTC, based on reports in the literature [cf. Dentler and Rosenbaum, 1977; Sale and Satir, 1977; Dentler, 1980; Cole et al., 1993]. In the upper left is a differential interference contrast image of a *Chlamydomonas* cell showing the size of the flagella (~12 μm) relative to the cell body. The artist's rendition on the right shows the major components of the flagellar axoneme, the IFT machinery, and the flagellar tip complex (FTC). See text for details.

filament wraps around the distal end of the central pair [Dentler, 1980]. Because the central pair rotates during flagellar beating [Omoto and Kung, 1979; Kamiya, 1982; Wargo and Smith, 2003], the plate and ball may play an essential role in this rotation, perhaps by acting as a bearing that mediates the interaction of the tips of the central pair microtubules with the flagellar membrane. Plugs insert into the A-subfiber of each outer doublet, and short filaments attach these plugs to the membrane [Dentler, 1980]. The plugs and filaments may be involved in microtubule assembly and disassembly, or they may serve to anchor the ends of the outer doublets to the membrane. In addition to these morphologically identifiable components, the FTC must also contain a set of unique proteins and enzymes related to IFT function because many IFT-related processes are restricted to the flagellar tip.

Three classes of proteins having specific IFT functions must be components of the FTC. The first class of proteins is involved in assembly and disassembly of the flagellum, because this activity occurs only at the flagellar tip [Johnson and Rosenbaum, 1992]. The microtubules of the flagellar axoneme add subunits at the tip during flagellar growth and lose subunits from the tip during flagellar resorption. In addition to tip growth and resorption, mature, full-length flagella undergo a continuous, steady state turnover of tubulin subunits, and this also occurs only at the tip [Marshall and Rosenbaum, 2001]. Therefore, proteins involved in the control of microtubule stability must be components of the FTC. Indeed, the microtubule plus end binding protein EB1 has recently been shown to reside at the FTC of the flagella of *Chlamydomonas* [Pedersen et al., 2003]. In non-flagellated eukaryotic cells, EB1 binds to the plus ends of cytoplasmic microtubules and, in the presence of APC (adematous polyposis of the colon protein), EB1 forms a complex with APC that promotes microtubule assembly [Nakamura et al., 2001]. However, the *Chlamydomonas* genome does not appear to encode an ortholog of APC. Moreover, although EB1 has microtubule binding activity, EB1 by itself does not promote microtubule assembly. Thus, the role of EB1 at the flagellar tip is yet to be determined.

A second class of proteins plays a role in cargo loading and unloading at the flagellar tip. IFT particles function, at least in part, like delivery trucks at a construction site, transporting materials from their site of synthesis in the cell body to their site of assembly at the FTC, and used components from the FTC back to the cell body for degradation or recycling. IFT cargo such as tubulin dimers, radial spokes, dynein arms, etc. [Qin et al., 2004] must be loaded onto the IFT particles in the region of the basal body and unloaded when the cargo arrives at the tip. In the case of the outer doublet and central pair microtubules, tubulin subunit assembly takes place at the growing tip, while in the case of radial spokes, assembly onto outer doublets can occur in a tip to base direction [Johnson and Rosenbaum, 1992]. For the return trip to the cell body, cargo (such as, 'old' radial spoke proteins, tubulin subunits, dynein arms, etc.) must be loaded at the tip to return to the base. Specific enzymes must be involved in the loading and

unloading of cargo in the region of the FTC, and these enzymes comprise a second class of FTC-specific components.

The third class of proteins are involved in control of the IFT motors kinesin II and dynein 1b. The motor proteins powering IFT must be regulated at the tip as well, because it is only at the flagellar tip that a change in the direction of IFT particle movement occurs. To accomplish this reversal in direction, kinesin-II must be downregulated or turned off, and dynein 1b must be upregulated or turned on. Thus, enzymes involved in IFT motor protein regulation must also be specific components of the FTC. The mechanisms used to control microtubule assembly/disassembly, cargo loading/unloading, and motor protein regulation at the flagellar tip are currently unknown. What is clear is that specific sets of FTC components must play a definitive role in these three key events that take place at the flagellar tip.

In contrast to these tip-specific events that define three classes of FTC components, note that other axonemal proteins, such as the radial spokes can, under certain conditions, assemble onto the microtubules of the axoneme along the entire length of the flagellum. This has been observed in dikaryon rescue experiments with mutant *pf14* cells. *pf14* cells have paralyzed flagella that do not beat because they lack radial spokes. When *pf14* cells are mated to wild-type cells, a dikaryon is produced having four flagella. Two flagella are derived from the mutant cell, and these are non-motile; the other two flagella are wild-type and motile. After a short time, radial spoke complexes from the wild-type cytoplasm of the dikaryon enter the mutant flagella via IFT and rescue the mutant phenotype. The originally spoke-less axonemes of the *pf14*-derived flagella of the dikaryon regain motility because they regain radial spokes, and analysis showed that attachment of complete radial spokes began at the flagellar tip and continued toward the base [Johnson and Rosenbaum, 1992] in the full-length, mutant flagella.

Identification and characterization of the protein components of the FTC, as well as understanding how the FTC components are involved in flagellar function in general and IFT in particular, are important goals. It is clear that the flagella of *Chlamydomonas* represent an ideal model system in which to study these FTC-specific aspects of the process of IFT. *Chlamydomonas* flagella are the only microtubule-based system in which *all* of the following have been established: (a) the motors powering movement are known for both directions along the length of the flagellum; (b) individual strains carrying temperature sensitive mutations in one or the other motors are available; (c) the cargo being moved can be isolated and the polypeptides have been cloned and sequenced; (d) mutations have been identified in many of the cargo polypeptides; (e) particle motility occurs in clearly defined directions (i.e., out and back along the flagellum, essentially in a single dimension); (f) the flagellum in which IFT occurs is a biochemically defined, easily isolated, membrane-bound compartment; (g) microtubule assembly/disassembly—indeed, assembly and disassembly of the entire organelle—occurs only at the flagellar tip; (h) regulation of motor activity occurs at a defined and highly localized morphological position (i.e., at the flagellar tip); and (i) the flagella can be easily removed experimentally and purified with little or no contamination by cytoplasmic components, whereupon the cells regenerate the missing flagella. Thus, detailed experiments aimed at understanding the molecular events of flagellar biogenesis using a synchronous population of cells can be readily conducted.

IFT AND FLAGELLAR GENE TRANSCRIPTION

When *Chlamydomonas* cells are deflagellated, they immediately begin to regenerate their missing flagella [Rosenbaum et al., 1969], a process which is complete in about 90 min and that has been extensively studied for the past three and a half decades. Cells begin to grow new flagella using a pre-existing pool of subunits, and this pool is sufficient to regrow the flagella to half-length. In order to generate full-length flagella, cells must turn on the transcription of flagellar genes and synthesize new flagellar proteins [Lefebvre et al., 1978, 1980]. How do the cells sense that their flagella are missing and thus upregulate flagellar gene transcription? A signal transduction mechanism must exist to inform the nucleus when the cell has been deflagellated, thus inducing the transcription of mRNAs encoding flagellar proteins. Is there a link between IFT and the control of transcription of the genes encoding flagellar proteins? Perhaps IFT provides a

central, as yet unrecognized, component in the signal transduction steps that are required to upregulate flagellar genes upon deflagellation.

In addition to providing precursor polypeptides for flagellar growth and maintenance, IFT may also function as a conveyor belt that moves sensor molecules from the cell body up to the flagellar tip via kinesin II and returns them to the cytoplasm via cytoplasmic dynein. The hypothesis, yet to be tested, is that the state of these sensor molecules when they leave the cell body and enter the flagellum via anterograde IFT as compared to when they return to the cell body after retrograde IFT could provide the nucleus with information about the state of the flagella or even about the environment outside the cell [Pazour and Rosenbaum, 2002b].

With respect to flagellar gene transcription, perhaps one or more IFT polypeptides are transcription factors or components of a signal transduction pathway controlling the transcription of flagellar genes. After these control polypeptides are synthesized in the cytoplasm, they would enter the flagellum via IFT. During their transit of the flagellum, they could become modified in some manner by one or more enzymes that are residents of the flagellum. For example, specific IFT cargo proteins could be activated by phosphorylation or by proteolysis while transiting the flagella. Phosphorylation is important in many protein kinase cascades that affect gene transcription, such as the MAP kinase and the Jak-STAT pathways. There is also precedent for proteolytic cleavage events initiating gene transcription, as this has been well documented in a process called regulated intramembrane proteolysis [Brown et al., 2000], which has been demonstrated to control various cell functions such as lipid metabolism and differentiation. IFT might play a role in flagellar gene transcription by providing the mechanism that moves an inactive signaling protein into a compartment (the flagellum) where it become activated by phosphorylation or proteolysis, and then returns the activated signaling protein back to the cell body. Upon exit from the flagella, the now activated IFT protein would initiate a signaling cascade that affects directly the transcription of nuclear genes encoding flagellar proteins.

One obvious place to localize enzymes, which would activate quiescent signaling molecules brought into the flagellum by IFT, would be at the FTC. How might such a process function? Specific IFT proteins could be post-translationally modified by interaction with FTC enzymes. When these activated IFT proteins return to the cell body, they would function as negative regulators of the transcription of flagellar genes. Transcriptional regulation could occur either directly, or through an amplification cascade; in either case, the result would be downregulation of flagellar gene transcription. Thus, as long as the cell has flagella and a functioning IFT system, active IFT cargo proteins that negatively regulate the flagellar genes are delivered to the cytoplasm, and these keep the flagellar genes quiescent. The cell thus 'knows' it has flagella. When the cells are deflagellated, the delivery of modified, active IFT cargo polypeptides back to the cell body ceases, the negative regulation of flagellar gene transcription is relieved, and the flagellar genes turn on.

Support for the idea that IFT proteins might serve as control elements in such a hypothesized negative feedback loop derives from what might appear to be two disparate lines of evidence: the first, aimed at understanding flagellar length control and the second on Hedgehog signaling in vertebrates. In the former example, a number genes that control flagellar length have been identified through mutational analyses. For example, cells having mutations in any one of four *LF* genes produce abnormally long flagella, while cells with mutations in any of three *SF* genes produce cells with shorter than normal flagella. The *LF4* gene encodes a MAP kinase that is localized to the flagella [Berman et al., 2003], indicating that a signal transduction cascade is involved in flagellar length control via a kinase that is resident in the flagella. The *LF3* gene, by contrast, encodes a novel protein that is present in the cell body but absent from flagella [Tam et al., 2003]. Of particular interest is the observation that *LF3* mutants accumulate IFT particles at the region of the FTC, suggesting a direct interplay between proteins involved in flagellar length control and the proper functioning of IFT.

In the second example, from the field of Hedgehog signaling, two genes have recently been identified in a screen for embryonic patterning mutants in the mouse [Huangfu et al., 2003]. Based on sequence analysis, the *wim* mutation is an ortholog of the *Chlamydomonas* protein IFT172, a complex B polypeptide; the *fxo* mutation is an ortholog of IFT88, another complex B polypeptide. The products of these genes

function downstream of Patched, the receptor for Hedgehog. Thus, elements of the IFT machinery are involved in a developmentally important signal transduction pathway that in flies controls segmentation and in vertebrates aids in the establishment of limb bud polarity as well as in determining the fate of the ventral cells of the somites and neural tube. The connection between IFT and these developmental events has yet to be determined.

It is clear that *Chlamydomonas* offers many advantages for the study of microtubule-dependent intracellular particle motility, including approaches employing techniques of cell biology, biochemistry, genetics, and molecular biology. Studies on such simple organisms often generate interesting connections relevant to our understanding of human health and disease. For example, knowledge gained from the study of IFT in the single-celled alga *Chlamydomonas* has already led to an enhanced understanding of the development of polycystic kidney disease, retinal degeneration, and *situs inversus* in humans [see Pazour and Rosenbaum, 2002a, 2002b for reviews]. Much more is sure to come.

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