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Protein transport in growing and steady-state cilia

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Abstract

Cilia and eukaryotic flagella are threadlike cell extensions with motile and sensory functions. Their assembly requires intraflagellar transport (IFT), a bidirectional motor-driven transport of protein carriers along the axonemal microtubules. IFT moves ample amounts of structural proteins including tubulin into growing cilia likely explaining its critical role for assembly. IFT continues in non-growing cilia contributing to a variety of processes ranging from axonemal maintenance and the export of non-ciliary proteins to cell locomotion and ciliary signaling. Here, we discuss recent data on cues regulating the type, amount, and timing of cargo transported by IFT. A regulation of IFT-cargo interactions is critical to establish, maintain, and adjust ciliary length, protein composition, and function.

Keywords

flagella; microtubule; intraflagellar transport; diffusion

Introduction

Compartmentalization is a key feature of eukaryotic cells. Besides membrane-enclosed entities such as the mitochondria or Golgi, regions of the cytoplasm itself are often specialized by possessing a particular complement of proteins (and lipids and nucleic acids) enabling them to perform exclusive tasks. Examples include the leading edge of crawling cells, the mitotic spindle, and various cellular extensions such as microvilli and axons. Such cytoplasmic domains are established and maintained by self-assembly or self-organization, intracellular transport locally concentrating components, and barriers limiting the free flux of proteins. Here, we will focus on cilia and flagella (interchangeable terms), thin projections with a diameter of 200 nm that extend for several microns from the cell surface (Fig. 1A). While not membrane-bound organelles, cilia are partitioned from the cell body by the transition zone, a region at the base of the cilium which functions as a diffusion barrier.[1] Proteomic studies indicate that of the ~20,000 nuclear-encoded proteins ~1,000 are present in cilia; many of them are highly enriched within the organelle.[2] Since ribosomes are absent from cilia, all proteins required in the organelle have to be imported from the cell body.[3] Most cilia are not assembled in the cell body and extruded, instead the axoneme, the

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microtubular scaffold of all cilia, grows by addition of subunits to its distal end.[4,5] Intraflagellar transport (IFT) plays a major role in this process by picking up ciliary precursors in the cell body and delivering them via molecular motors into cilia and to the ciliary tip.[6] Here, we will evaluate the role of IFT in establishing and maintaining the specialized protein content of cilia.

A briefing on cilia

Cilia are organized by basal bodies, barrel-shaped microtubule-based structures also termed centrioles (Fig. 1B). The A- and B-tubules of the centriolar triplets are continuous with the doublet microtubules of the axoneme (Fig. 1B-E). During ciliogenesis, basal bodies dock to the plasma membrane via the distal appendages or transitional fibers (TF) positioned at the distal end of the basal bodies (Fig. 1C).[7] Between the basal body and the axoneme proper resides the transition zone (TZ), an ultrastructurally and biochemically specialized segment of the flagellum that functions as a diffusion barrier between the cell body and the cilium (Fig. 1D).[8] However, large protein complexes such as the multimegadalton IFT trains move through the transition zone indicating that the TZ possesses a gating mechanism. In many IFT loss-of-function mutants, cilia terminate above the structurally intact transition zone indicating that the elongation of protruding cilia is IFT dependent.[9] IFT-independent assembly of flagella has been described as well: *Plasmodium* (and other apicomplexa), for example, rapidly (~10 min) assembles 12- 15 μm long axonemes within the cytoplasm which later will be surrounded by a membrane during exflagellation and remain active for ~1 hour once protruding from the cell.[10,11] Thus, IFT is not *per se* needed for cilia formation but standard cilia assembly in protrusions requires IFT.

Cilia have been long known as motile organelles which function in the locomotion of protists and spermatozoa or the transport of fluid across ciliated epithelia (Fig. 1A, E). The microtubules of motile cilia are densely decorated with protein complexes such as dynein arms and radial spokes (Fig. 1E). Many metazoans also possess non-motile cilia which function in sensing of the external and internal environment; sensing of light and odor, for example, involves receptors located inside ciliary membranes. Cilia are essential to mammalian development as the complete loss of cilia is embryonic lethal.[12,13] A plethora of diseases, termed ciliopathies, are associated with defects in ciliary motility and sensation.[14] Some of these conditions are caused by mutations impairing a cell- or tissue-specific function of cilia: The loss of an axonemal dynein, for example, will cause cilia paralysis but will not affect the sensory functions of non-motile cilia.[15] In contrast, defects in general cilia assembly, length control, composition, or maintenance often result in multiorgan phenotypes because cilia are widely distributed in the mammalian body. Genes encoding TZ proteins are hotspots for disease-causing mutations in humans: TZ defects affect ciliary protein entry and retention typically affecting cilia performance in many cell types, tissues, and organs.[16,17] Similarly, many IFT defects alter ciliary length and protein content affecting their sensory and signaling functions. In mammals, more or less subtle defects in IFT cause a wide range of diseases and developmental defects ranging from blindness and kidney anomalies to severe skeletal malformations and obesity.[14] After a brief introduction to the IFT pathway, we will review data on protein transport by IFT and its regulation.

IFT – the protein shuttle of the cilium

IFT is the bidirectional movement of large protein arrays (= IFT trains) along the axonemal microtubules (Fig. 2).[18] The trains are strings of IFT particles, each consisting of at least 22 distinct proteins organized into IFT-A, IFT-B1, and IFT-B2 subcomplexes (Fig. 2A, C).[19-22] IFT train assembly occurs near the TFs (Fig. 1C).[23-25] In the first part of the journey, anterograde trains move from the ciliary base to the tip along the B-tubule of the doublets using the molecular motor kinesin-2 and carrying inactive IFT dynein, the retrograde motor, as a cargo (Fig. 2B, D).[26,27] At the ciliary tip, anterograde IFT trains are remodeled for retrograde traffic and trains return to the cell body pulled by IFT dynein along the A-tubules.[26,28] IFT trains function as protein carriers allowing non-IFT proteins such as axonemal precursors to hitch a ride into the cilium. Cell fusion experiments using *Chlamydomonas* mutants with defective axonemes and wild-type cells provided initial evidence for protein delivery into cilia by IFT: During the repair of the mutant cilia, the missing axonemal proteins or substructures introduced by the wild-type cell are first added at the tip (instead of near the base where the proteins will enter cilia). Assembly then progresses toward the base of the cilium; a pattern indicative for transport of these components via IFT to the ciliary tip.[5,29,30] Direct imaging revealed that proteins of the ciliary matrix, membrane, and axoneme including tubulin, the major structural protein of cilia, move via IFT (Fig. 2E).[31] In selected cases, unloading of cargoes from IFT and subsequent incorporation into the axoneme have been observed directly.[32] The data confirm the role of IFT as the predominant protein transport pathway of cilia and flagella.

Post balance point: regulated loading of IFT trains contributes to ciliary length control

Ciliary length is typically tightly regulated in a cell type-specific manner and mutations that affect ciliary length reduce the swimming speed in protists and cause disease in mammals.[33,34] Numerous factors have been shown to participate in ciliary length control.[35] The supply of ciliary building blocks via the IFT pathway is likely to contribute to establishing and maintaining cilia of a defined length: Conceivably, reduced protein supply could result in shorter cilia; conversely, too much material might cause cilia to exceed their set length. A simple option to regulate the amount of protein transported into cilia would be an on demand system where IFT trains are only present or moving while cilia are assembled (Fig. 3A). While IFT is often abolished in mature sperm flagella, it continues in fully grown cilia in the vast majority of cells.[36] When IFT is switched-off using conditional mutants, cilia shorten and tubulin exchange at the ciliary tip is reduced.[37] These observations are the basis of the influential balance-point model which proposes that the capacity of IFT is restricting the length of cilia: As cilia elongate IFT trains will spend more and more time in transit and the frequency by which IFT trains reach the ciliary tip and drop off their cargoes will progressively decrease as the distance between the ciliary base and tip increases.[37-40] At one point, the balance point, assembly fueled by IFT-dependent cargo delivery and the assumed length-independent disassembly of cilia will balance each other establishing the steady-state length (Fig. 3B).[37] According to this model, cells could build cilia of a given length simply by limiting the number the IFT trains employed during assembly without

invoking complex mechanisms to measure cilia length or regulate the size of the precursor pool. A prerequisite of this model is that the cargo load of IFT trains is constant and length-independent. IFT-cargo complexes are transient in nature largely impeding their isolation and biochemical analysis, and, for the longest time, it was unclear whether the amount of protein transported by an IFT train was regulated or not. Recent advances in direct imaging of protein flux inside cilia revealed that IFT trains are highly loaded with tubulin and other axonemal precursors while cilia grow but are largely devoid of these cargoes once cilia reach their set length.[32,41] Thus, cells modulate the volume of structural proteins moved by IFT into cilia raising the question how IFT-cargo interactions are regulated.

The load on the IFT trains could simply reflect the availability of ciliary precursors in the cell body and ciliary elongation will cease once the precursor pool has been drained (Fig. 3C).[42] Classic experiments, however, revealed that even cells with full-length cilia still maintain a sizable precursor pool sufficient to rebuild half-length cilia in the absence of *de novo* protein synthesis.[43,44] Thus, IFT does not simply shuttle all available precursors into the cilium but other factors control how much of the axonemal proteins present in the cell body will be transported and used for ciliary assembly. The study of so called long-short cells provided crucial insights into the regulation of cargo loading: Mechanical shear can be used to remove just one of the two flagella from *Chlamydomonas*. The cells will regenerate the missing flagellum while shortening the remaining flagellum allowing for the analysis of cargo transport when growing and non-growing cilia are present on the same cell body.[44,45] While IFT traffic continues in both cilia, only those trains entering the growing cilium are highly loaded with tubulin.[41] This suggests that cells regulate IFT loading in a cilium-autonomous and length-dependent manner. Cells apparently possess a system to recognize cilia of insufficient length and respond by increasing the cargo load of just those IFT trains entering short cilia (Fig. 3D). Once cilia approach full length, loading of IFT trains with structural proteins tapers off and, in cilia exceeding their desired length, shortening is triggered.[46,47] These two feedback loops - increased cargo delivery with suppressed disassembly when cilia are too short, and suppressed IFT loading with increased disassembly when cilia are too long - could establish cilia of a defined length.

How to measure cilia length?

The molecular mechanisms by which cells measure the length of their cilia and respond to aberrant length by adjusting cargo influx into cilia via IFT remain essentially unknown. Mutations in several CDK-like and MAP kinases result in abnormally long cilia.[48-50] Some of these length-regulating protein kinases have been shown to be present inside cilia and to move by IFT.[51,52] Several such kinases phosphorylate the anterograde IFT motor kinesin-2 and phosphorylation of the Kif3b motor subunit by CAMK prevents the motor from associating with IFT particles and entry into the cilium.[52,53] Some of these length-regulating protein kinases including the aurora-like kinase CALK reside exclusively or predominately in the cell body.[50,54] The pattern of CALK phosphorylation changes in response to ciliary length and growth state (elongating, resorbing, or steady-state) providing evidence that cells register the condition of their cilia.[55,56] Tubulin transport is dysregulated in *lf2*, a *Chlamydomonas* flagellar length mutant defective in a CDK-like kinase.[41] These data establish a connection between length-regulating kinases, IFT, and cargo transport but how

precisely cilia length is sensed and how such a 'length signal' is transmitted to the cell body and the IFT machinery has not yet been established. In a speculative 'time-of-flight' model the activity of a length-regulating kinase could change as it transits via IFT through the cilium. Because IFT trains move with an essentially constant velocity, the time a kinase requires to cycle through a cilium would be proportional to ciliary length and the activity of the kinase as it returns to the ciliary base provides a biochemical read-out reflecting cilia length. If correct, changes in IFT velocity should affect ciliary length. A mechanistically distinct model assumes that protein import into cilia depends on the presence of an 'active' import factor allowing ciliary proteins and loaded IFT trains to pass through the TZ. If the activation of such a freely diffusible factor occurs at the ciliary tip and its inactivation is time-dependent, a tip-to-base gradient of active factor will be established which could adjust ciliary protein influx in response to changes in organelle length.[57] Several alternative models of how cells could sense ciliary length have been described.[58] Real-time measurements of ciliary length fluctuations in steady-state could help to pinpoint the length-regulating pathway. At the molecular level, the identification of the substrates of length-regulating kinases is required.

Understanding IFT loading and protein entry into cilia

How cells regulate the import of structural proteins via IFT in a ciliary length-dependent manner is currently unknown. Several mechanisms could be involved including the regulation of IFT-cargo interactions, of the passage of loaded IFT trains through the TZ, and of cargo availability in the area of IFT train assembly and loading.

Our knowledge of how cargoes and IFT proteins interact is still rudimentary and only a few such binding sites have been identified including those for tubulin and the outer dynein arms. Tubulin dimers, for example, are bound by the N-terminal domains of IFT81 and IFT74.[59-62] While many IFT proteins are phosphorylated, it is unknown whether posttranslational modifications or other structural changes in the trains modulate the binding capacity for tubulin and other cargoes.[63]

Protein entry into cilia is regulated at the TZ, which functions as a diffusion barrier and ciliary gate. Small soluble proteins (< 50kDa) and certain transmembrane proteins can move freely into cilia by diffusion[64-66]; such proteins could still require IFT to be enriched inside the ciliary compartment above cell body concentrations as we suggested for tubulin.[41] For proteins with larger diameters ciliary entry by diffusion is largely prevented.[65,67] In TZ mutants, some non-ciliary proteins are present in cilia while a subset of resident ciliary proteins are lost supporting its role as a bidirectional barrier.[16,17,68] How large protein complexes such as outer dynein arms (>1.5 MDa) or IFT trains pass through the TZ remains to be determined. Certain nuclear pore proteins (NUPs) have been localized in the TZ which has led to the controversial model that the TZ functions similarly to the nuclear pore complex.[69,70]

Passage through the TZ could depend directly on features of the ciliary proteins themselves such as targeting sequences which open up the TZ. Ciliary localization sequences (CLS) that are required and sufficient for ciliary import have been identified for certain transmembrane

and membrane-associated proteins.[71-73] CLS are variable in position and frequently encompass residues that are modified by acylation. Proteins predicted to be myristoylated and dual fatty acid modified are enriched in the *C. reinhardtii* flagellar proteome and evidence from various systems indicates that fatty acid modifications function in ciliary targeting.[2,74] Compared to the nuclear localization sequences (NLS), ciliary localization sequences are not conserved and more variable and many structural and soluble ciliary proteins lack recognizable import signals. Such proteins might bind to more universal import carriers. At least some cargoes require the activity of small G-proteins including Ran and Arl3 to enter cilia.[75-77] IFT trains pass with apparent ease through the TZ and binding of proteins to IFT trains could be the entry ticket for proteins, which are unable to enter the cilia on their own. Then, the cargo selectivity of IFT would decisively control the protein composition of cilia at the posttranslational level.

To regulate protein influx into cilia, cells could also regulate the space and time available for IFT trains and cargoes to interact in the cell: IFT trains exposed to cargoes for extended periods of time might pick-up more cargo than IFT trains passing rapidly by. IFT proteins and axonemal proteins have been detected on intracellular vesicles suggesting that they might already interact well before reaching the basal bodies.[78] In line with these observations, IFT20 is located at the Golgi in mammalian cells and aids in Golgi-to-cilium trafficking of certain membrane proteins.[79] Our work using long-short cells of *Chlamydomonas* showed that the loading of IFT trains can differ considerably between the cilia of a given cell even while the cilia emerge from adjacent basal bodies. This suggests that the mechanism that determines how much cargo enters cilia by IFT is likely to be locally confined to the basal body region.

Protein transport in full-length cilia

In nearly all cell types, IFT continues as long as cilia are present. After the initial rapid phase of assembly, many cilia continue to elongated somewhat which is likely to require IFT.[80] Further, IFT in steady-state cilia has diverse functions ranging from ciliary maintenance to cell locomotion and signaling. Most of these processes are likely to commence already during ciliary assembly and no principal difference between IFT in growing and full-length cilia is known, qualified by the observation that the transport of axonemal proteins is strongly reduced in the latter. In the following we will discuss IFT-based processes beyond cilia assembly.

Cilia maintenance

It has been reasoned that cilia and axonemes are intrinsically unstable and therefore require an ongoing supply of building materials to maintain their steady-state length.[37] Indeed, pulse labeling experiments suggest a continuous exchange of certain proteins in steady-state cilia.[81,82] Motile and primary cilia also lose material via the shedding of vesicles.[83,84] The outer segment (a structurally specialized sensory cilium) of rod cells in the eye continuously releases membranous discs at the distal end and new discs are formed at the proximal end.[85] This treadmilling of ciliary membranes requires a massive IFT-dependent transport of proteins such as opsin through the connecting cilium.[86,87] Treadmilling has not been

observed for axonemal microtubules in *Chlamydomonas* flagella[37,88] and the exchange of tubulin subunits at the tip of steady-state cilia is rather slow suggesting that the axonemes are relatively durable.[37,64]

The degree to which ciliary maintenance depends on IFT varies considerably between species and cell types. In *Chlamydomonas* and *Tetrahymena*, cilia and flagella shorten slowly when anterograde IFT is abolished indicating a continual demand of an essential component or a failure of ciliary length regulation, which could hinge on IFT (Fig. 3E).[89,90] *Trypanosoma* flagella maintain their length in the absence of IFT, but flagellar motility and protein distribution are increasingly affected.[91] At the other end of the spectrum are sperm flagella that once assembled lack IFT but nevertheless maintain their length and functionality for extended periods of time.[36] Apparently, the need for replacement proteins differs considerably between distinct types of cilia. More generally, IFT remains active in fully grown cilia of cycling cells, cells with sensory cilia etc., all of which require ongoing motor-based protein exchange between cilia and the cell-body.

Counteracting diffusional equilibration

Conceivably, the maintenance of cilia is unlikely to require the full-sized IFT system used for their assembly. Nevertheless, the size of the IFT machinery appears to be largely independent of the ciliary growth state.[92,93] Motor proteins are often autoinhibited in the absence of cargoes preventing wasteful ATP consumption.[94] However, the primary cargoes of the IFT motors are the IFT particles themselves. Thus, the IFT motors can be considered to be permanently engaged with “cargo” or “cargo adapters” while moving along cilia; it is unclear whether IFT trains also require a load of non-IFT proteins in order to enter and move along cilia.

What are the possible benefits offsetting the energy costs of continuously running IFT? Recent data link IFT-dependent protein transport in fully-assembled cilia to processes beyond structural maintenance. An example is the export of proteins from cilia. Just as certain proteins are highly concentrated inside cilia, many cytoplasmic proteins are efficiently excluded from the ciliary compartment. Small to midsize cell body proteins, however, are likely to diffuse across the transition zone continuously leaking into the cilium.[65] The membrane-associated protein phospholipase D can enter *C. reinhardtii* cilia in an IFT-independent manner but depends on IFT and the BBSome, an IFT-associated eight-subunit protein complex (Fig. 2), to be removed from cilia.[95,96] In mammals, loss of BBSome function causes an accumulation of cell body proteins in the outer segment of rod cells supporting the notion that the BBS/IFT system functions as a scavenger to export non-ciliary proteins from cilia.[97] Similarly, abundant soluble ciliary proteins could escape by diffusion. Thus, IFT counteracts diffusional equilibration between the cell body and the cilium (Fig. 3F).

Surface motility

IFT also drives flagellar surface and gliding motility, which are best studied in *Chlamydomonas* and other protists (Fig. 3G).[98,99] These on-and-off motilities are driven by transient interactions between IFT trains and ciliary transmembrane proteins. In contrast to

IFT itself, the binding of the involved transmembrane proteins to IFT depend on extracellular calcium.[100,101] In gliding motility, flagellar transmembrane proteins will adhere to a substrate immobilizing the associated IFT trains or capturing them after adhesion. Comparable to a filament gliding assay, the microtubule minus-end directed activity of IFT dynein will then pull on the axoneme dragging the entire cell in the direction of the cilium containing the adhesion.[101] In surface motility, extracellular particles move up and down the cilium. In hindsight, the back-and-forth movement of particles adhered to the flagellar membrane provided early evidence for the existence of a bidirectional transport system inside flagella.[99] Certain protists use their flagella to select and gather food probably involving this mechanism to move the pray to the ciliary base for endocytosis.[102,103] Cilia could function as receivers for extracellular vesicles.[104] In mammalian cilia, certain transmembrane proteins transiently associate with IFT while others appear to move essentially by diffusion once inside cilia.[105,106] While primary cilia are likely to display surface motility, it is not yet known whether it has any functional relevance, e.g., during cell migration.

IFT and ectosomes (… and the cell cycle)

Cilia shed vesicles or ectosomes (50-200 nm in diameter) containing IFT and ciliary membrane proteins. Components of the ESRCT complex are present in cilia-derived vesicles and the topology of ectosome formation from cilia recapitulates other ESCRT-based events.[107] Vesicle formation also involves the actin-based cytoskeleton.[108,109] The role of IFT in vesicle formation is unclear but it could delivery vesicle-specific proteins to the ciliary tip.[108] Due to their specific protein content, cilia-derived ectosomes possess biological functions in *Chlamydomonas* (during daughter cell release from sporangia and during mating) and likely elsewhere.[83,110] GPCRs and other proteins are shed in vesicles from the ciliary tip either as their natural route of exit or because protein export via the BBS/IFT system is impaired.[108,110] Further, IFT and other proteins are released from primary cilia prior to mitosis by shedding of the distal ciliary segment, a process termed decapitation.[109] Decapitation precedes cilia resorption and entry into the cell cycle emphasizing the tight coordination of cilia growth and disassembly with the cell cycle. In many cell types, ciliary disassembly promotes G1-S transition (mammals) or entry into mitosis (*Chlamydomonas*). Evidence implying IFT in the export of proteins liberated by axonemal disassembly during cilia shortening is weak.[111] IFT proteins reside at the mitotic spindle poles and loss of cilia or defects in IFT result in misoriented spindles and cell division planes.[12,112] In other systems, cilia loss is the only known phenotype of IFT mutants and mitosis and cell growth appear to be normal.[113] Clearly, the connections between IFT, cilia, ectosomes, and the cell cycle deserve further attention.

Adaptation and signaling

Cilia often undergo changes of composition or length in response to developmental or environmental cues (Fig. 3H). Examples include the import of cell adhesion molecules and the calcium channel PKD2 into *Chlamydomonas* flagella during gametic differentiation, the removal of certain GPCRs from cilia upon ligand binding, cyclic or pharmacologically induced changes in cilia length, light-dark adaptation of the outer segments, or the recruitment of Lis1-like into flagella to support axonemal dyneins under high-load

conditions.[114-120] Some of these changes are probably driven by diffusional entry and capture of proteins inside cilia.[64,121] However, IFT could provide the means to rapidly execute such changes.

IFT not only installs and maintains the ciliary signaling machinery but evidence suggests that it also participates directly in signaling cascades. In *Chlamydomonas*, cilium-to-cilium contacts between gametes initiate fertilization signals and active IFT is required for successful cell fusion.[122] The role of IFT is particularly well studied in the Hedgehog (Hh) pathway, which regulates a plethora of developmental processes.[123-125] In vertebrates, Hh signaling involves the controlled translocation of signaling proteins in and out of cilia. After binding of the Hh ligand, its receptor Patched exits the cilium and Smoothened translocates into the cilium; when IFT is defective, both proteins are mislocalized compromising Hh signaling.[123,126,127] Further down the cascade, Smoothened recruits β-arrestin in a process that depends on and might involve IFT.[128] Then, β-arrestin binds activated Gpr161, an orphan GPCR suppressing Hh signaling, and mediates its removal from cilia probably by facilitating loading onto retrograde IFT trains.[114,128] Evidence suggests further that Gli proteins, the transcription factors for Hh-responsive genes, are translocated from the cilium to the cell body in an IFT-dependent manner.[129-131] While IFT27 is expendable for cilia assembly and IFT itself in mice, *Ift27*$^{-/-}$ animals display features typical of defective Hh signaling emphasizing that IFT might transport Hh signaling proteins.[132] IFT-dependent transport of signaling proteins is likely to participate in other cilia-based signaling pathways.[133] It is intriguing to consider that signals received by the cilium could modify a receptor or effector enabling them to associate with IFT trains, translocate to the cell body, and transmit a signal (Fig. 3I). Then, a perpetually running IFT system would keep the cilia on high alert ensuring that signals are rapidly transmitted to the cell body and vice versa.

Summary

IFT trains are versatile with respect to the range of possible cargo proteins and the quantity of cargo bound to the carriers. While some aspects of the cellular circuits regulating the amount of structural proteins moved via IFT into cilia are emerging, it remains unclear how cells measure the length of their cilia and process this information to adjust the amount of cargo on the IFT trains. IFT also contributes in multiple ways to the maintenance and function of steady-state cilia. The ability of the cells to regulate when, which and how much protein is transported via IFT into cilia is likely to be a major determinant of cilia size, composition, and function and a major mechanism to adjust them in a controlled manner.

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Synopsis

Cilia and flagella are widely distributed cell organelles with motile and sensory functions. The intraflagellar transport (IFT) pathway moves proteins in and out of cilia and is required for ciliary assembly, maintenance, and signaling. Here, we discuss recent data revealing a complex regulation of IFT-cargo interactions. Continuously running IFT may prevent diffusional equilibration between the cell body and the cilium establishing and maintaining the specific protein content of cilia.

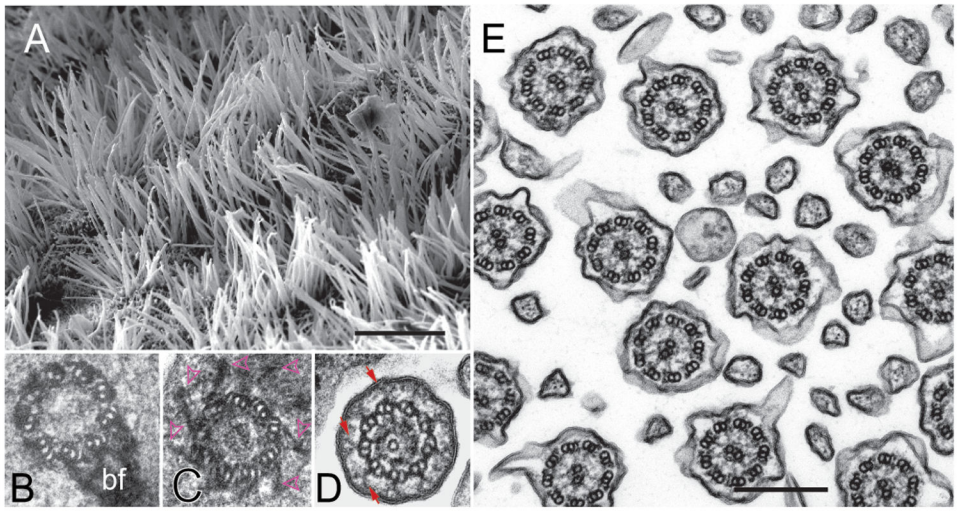

Figure 1. The structure of cilia and flagella

A) Scanning micrograph showing the ciliated epithelium lining the ventricular system of the brain in mouse.

B-D) Thin sections showing the proximal region of the basal body (B) with the attached basal foot (bf), a more distal section with the paddle-wheel like transitional fibers (C, arrowheads), and the transition zone (D) in cross-sections. In D, note the Y-shaped connectors (arrows) linking the doublet microtubules to the ciliary membrane.

E) Micrograph showing airway cilia with typical 9+2 axonemes in cross-section. Bars = 10 μm (A) and 250 nm (E).

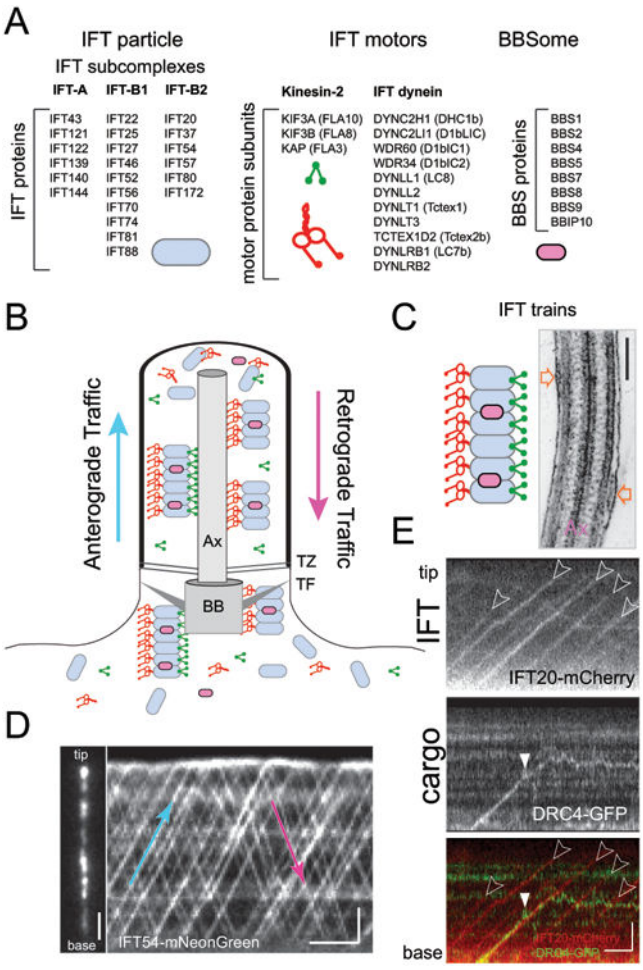

Figure 2. The intraflagellar transport machinery

A) Composition of IFT particles, IFT motors, and the BBSome. For the motors, the mammalian protein names are shown; the *Chlamydomonas* protein names are listed in the brackets.

B) Schematic presentation of IFT. Ax, axoneme, TZ, transition zone, TF, transition fibers, BB, basal body.

C) Schematic presentation and electron micrograph depicting IFT trains (open arrows). Bar = 200 nm.

D) Still image (left) and kymogram (right) showing IFT54-NG inside a *Chlamydomonas* flagellum. In the kymogram, anterograde trains are indicated by trajectories running from the bottom left to the top tight (blue arrow); trajectories running from the top left to the bottom right represent retrograde trains (red arrow). Bars = 2s 2 μm.

E) Kymograms depicting transport by IFT and unloading of the axonemal protein DRC4-GFP. IFT20-mCherry was expressed to visualize IFT. IFT trajectories are marked by open arrowheads. DRC4-GFP initially co-migrates with an IFT train but is then unloaded (white arrowhead) as indicated by the transition of the trajectory from a linear diagonal to a back-and-forth motion indicative for diffusion. Note that most cargoes are unloaded in the vicinity of the ciliary tip. Bar = 1s 2 μm.

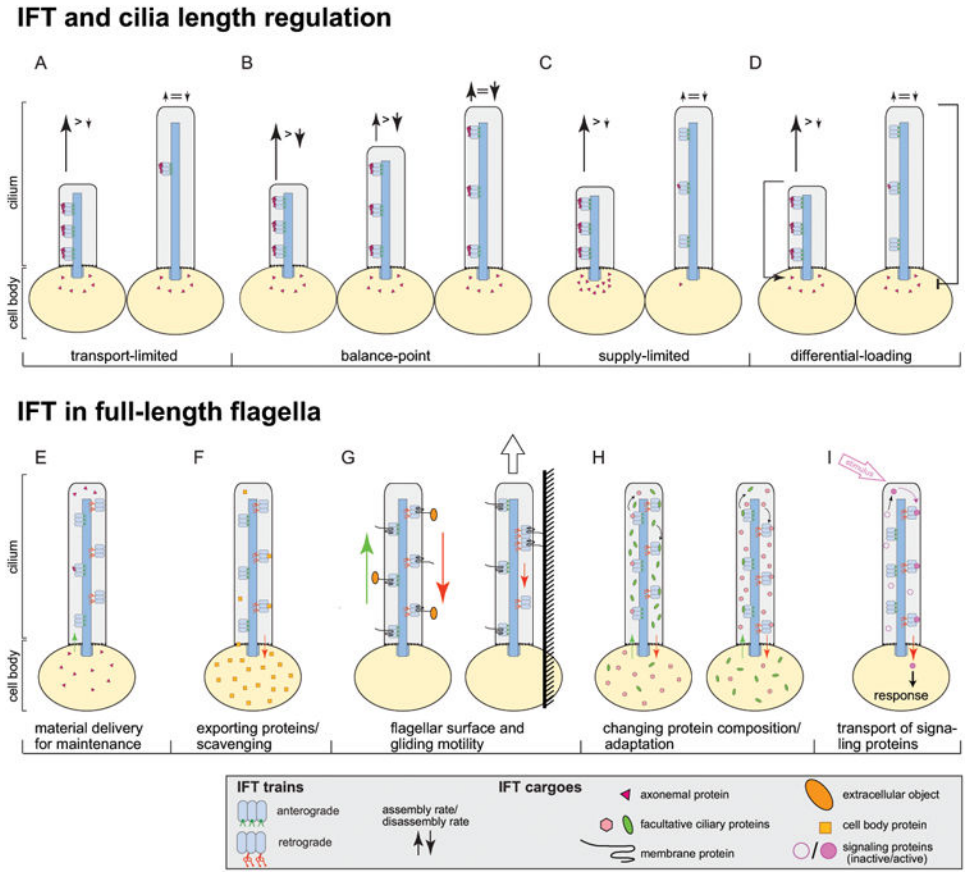

Figure 3. Cargo transport by IFT during ciliary assembly and maintenance

A-D) Models for ciliary length control.

A) The transport-limitation model suggests that cells will employ many IFT trains while cilia grow and reduce the number of trains in fully grown cilia. The cargo load per train is constant.

B) In the balance-point model, the ciliary assembly rate will decrease with increasing ciliary length because the time the trains spend in transit will increase. Neither the number of IFT trains nor the cargo load/train are regulated. *Chlamydomonas* flagella grow at a rate of up to 350 nm/min. In addition to delivering the building blocks accounting for this gain in length, IFT would have to provide those lost by ongoing ciliary disassembly. Our simulations showed that it is not possible to assemble cilia of 10 - 12 μm length in ~60 min when assuming that the continuous length-independent disassembly of cilia is large enough to balance the large amount of building blocks provided ceaselessly by anterograde IFT[32]; rather cilia would need hours of slow growth to reach steady-state length.

C) The supply-limitation model predicts that cilia will grow until the cell body pool of precursors is exhausted. IFT cargo load is regulated passively by the availability of cargoes.

D) The differential-loading model suggests that cells alter the amount of cargo per IFT train in response to changes in ciliary length. The length of the arrows near the flagellar tip indicate the rates of material delivery and cilia disassembly.

E-I) Models of IFT function in fully assembled cilia.

E) Material delivery for cilia maintenance via a low but steady influx of ciliary proteins.

F) Removal of non-ciliary proteins entering cilia via diffusion from the cell body.
G) Back-and-forth movement of extracellular objects on the ciliary surface (left). In gliding motility, IFT dynein, immobilized via IFT particles and transmembrane proteins to the substrate, pulls the cell by moving toward the minus-end of the axonemal microtubules.
H) Import and export of proteins to change ciliary protein composition; e.g. during adaptation.
I) Conditional transport of activated signaling proteins. Signals such as ligand binding change the properties of a protein allowing it to adhere to IFT trains.