Trends in Biochemical Sciences

CellPress

Review

IFT–Cargo Interactions and Protein Transport in Cilia

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The motile and sensory functions of cilia and flagella are indispensable for human health. Cilia assembly requires a dedicated protein shuttle, intraflagellar transport (IFT), a bidirectional motility of multi-megadalton protein arrays along ciliary microtubules. IFT functions as a protein carrier delivering hundreds of distinct proteins into growing cilia. IFT-based protein import and export continue in fully grown cilia and are required for ciliary maintenance and sensing. Large ciliary building blocks might depend on IFT to move through the transition zone, which functions as a ciliary gate. Smaller, freely diffusing proteins, such as tubulin, depend on IFT to be concentrated or removed from cilia. As I discuss here, recent work provides insights into how IFT interacts with its cargoes and how the transport is regulated.

Trends

Defects in intraflagellar transport (IFT), a process that moves proteins into cilia, cause disease in humans.

IFT functions in ciliary assembly, maintenance, protein export, and probably ciliary signaling.

Direct imaging of protein transport on IFT trains revealed that the amount of cargo attached to each train is regulated in a ciliary length-dependent manner.

Structural analysis of IFT proteins and complexes revealed how tubulin, the major protein of cilia and flagella, binds to IFT.

Cilia: Conserved Cell Organelles with Multiple Functions

Cilia (see Glossary) and flagella (interchangeable terms) are thread-like cell extensions of variable length with a diameter of approximately 200 nm (Figure 1A,B). The hallmark of all cilia is the **axoneme**, a microtubular scaffold typically containing nine doublet microtubules and often two central singlet microtubules (referred to as 9+2). The doublet microtubules are continuous with the microtubules of the basal bodies, which anchor and position the cilium within the cell (Figure 1C–F). Cilia are covered by the ciliary membrane, which is continuous with the plasma membrane but specialized in protein and lipid content [1,2].

Cilia are often structurally adapted to serve diverse functions. Motile cilia have **dynein** arms, which are large motor complexes that induce sliding of the axonemal microtubules and thereby bending of the cilium. Ciliary motility propels cells, such as protists and spermatozoa, or generates fluid flow above the ciliated epithelia lining, such as in the airways. A conserved 9 +2 axoneme is characteristic of most motile cilia (Figure 1C).

Many mammalian cells have a single nonmotile cilium, the primary cilium, which typically retains a 9+0 axoneme but mostly lacks the structures required for ciliary motility. Cilia are rich in signaling proteins [e.g., G-protein-coupled receptors (GPCRs), ion channels, protein kinases]. Some cilia are structurally modified for particular sensory functions. The outer segment of rods in the eye, for example, represents a structurally modified cilium functioning in light perception. Similarly, primary cilia in other organs and tissues sense chemical and mechanical cues. Cilia protrude from the cell surface into the environment and have a high surface:volume ratio, features likely fundamental for their role in sensing. Defects in ciliary function lead to a plethora of diseases referred to as 'ciliopathies' (Box 1). Many of these conditions are caused by cilia of incorrect size or composition, which has fostered a strong interest in understanding ciliary assembly.

IFT: The Protein Translocation Machinery of Cilia

Ribosomes are absent from cilia and approximately 600–1000 distinct polypeptides required to build the organelle need to be imported from the cell body; some of these proteins are

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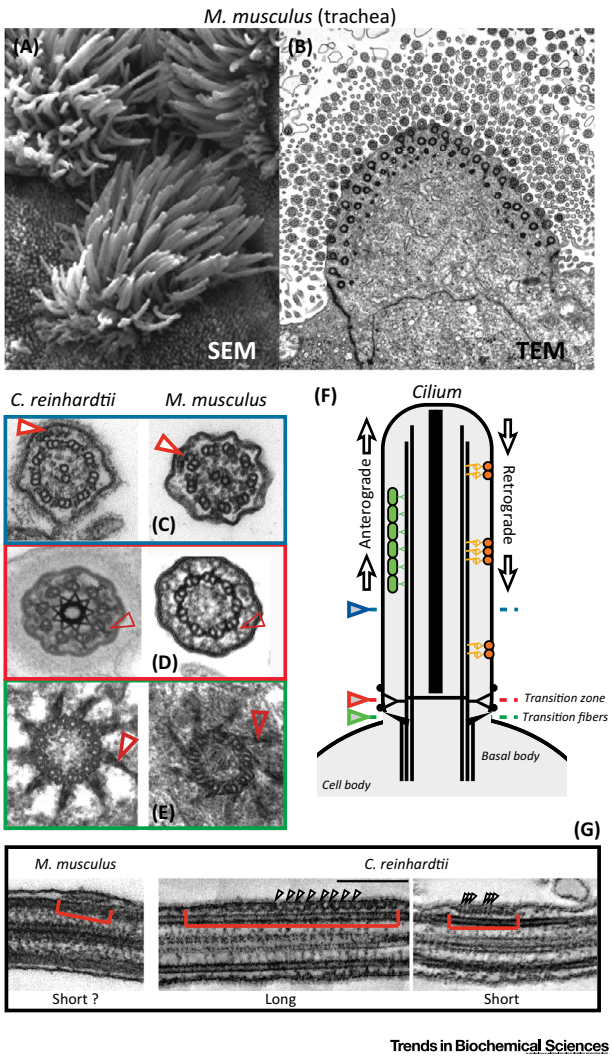

Figure 1. Cilia and Intraflagellar Transport (IFT). (A) Scanning electron micrograph and (B) transmission electron micrograph showing multiciliated epithelial cells from mouse *Mus musculus* airway. (C–E) Cross-sections through cilia of *Chlamydomonas reinhardtii* (left) and mouse ependymal cilia (right) showing 9 +2 axonemes (C); Y-shaped linkers (D); and a conserved structural element of the transition zone and the transitional fibers (E), which link the basal bodies to the plasma membrane and are the putative assembly sites of IFT trains [shown in (G)]. Arrowheads in (C) mark IFT trains. (F) Schematic presentation of a cilium and IFT trains. The colored arrowheads indicate the level of the cross-sections shown in (C–E). (G) Longitudinal sections through IFT trains in mouse ependymal cilia and *C. reinhardtii* flagella. Arrowheads mark the periodicities of long and short IFT trains. Scale bar = 100 nm. Images of *C. reinhardtii* IFT trains are reproduced, with permission, from Gaia Pagino.

concentrated several thousand-fold in cilia [2,3]. The axoneme elongates by addition of material to its tip, which points away from the cell body [4–6]. Thus, during cilia assembly, large amounts of building materials need to be transferred from the ciliary base to the distal end. These observations indicate the need for a ciliary protein translocation system and **IFT** is thought to be the predominant pathway to move proteins into and within cilia [7].

In brief, IFT is the bidirectional movement of supramolecular protein arrays inside cilia. These so-called '**IFT trains**' are appressed to the ciliary membrane and move via molecular motors on the axonemal microtubules [8] (Figure 1C,G). Although IFT trains are the primary cargo of the IFT motors, they also function as adaptors allowing other proteins (IFT cargoes) to be carried along. Cargoes bind to IFT near the basal body and move to the ciliary tip by anterograde IFT. At the tip, many cargoes are released from IFT; the trains reorganize and return to the cell body by retrograde IFT (Figure 1F). The IFT pathway is well conserved and required for the assembly of most cilia and eukaryotic flagella [9]. Here, I review recent progress in the structural analysis of IFT complexes and how they interact with various cargoes; I also discuss the regulation of transport frequency and capacity, and data obtained by direct imaging of protein transport in cilia.

Glossary

Axoneme: a ninefold symmetric microtubular scaffold in cilia and flagella. Primary cilia typically have a 9+0 axoneme. In motile cilia, the microtubules carry numerous protein complexes, such as the outer dynein arms (ODAs), radial spokes (RS), and the dynein regulatory complexes (DRC); most motile cilia have two additional central-pair microtubules, forming a 9+2 axoneme.
BBSome: a complex of at least eight BBS proteins that moves in association with IFT through cilia. Defects in this complex cause Bardet–Biedel syndrome (BBS), a rare disorder primarily characterized by blindness and obesity.
***Chlamydomonas reinhardtii*:** a unicellular green alga with two 9+2 flagella widely used as a model for studying flagella.
Cilia: thread-like cell protrusions surrounded by the plasma membrane and stabilized by a microtubular scaffold, the axoneme. The terms 'cilia' and 'flagella' both refer to this structure.
Ciliopathy: diseases caused by defects in ciliary assembly and function.
Dynein: a protein complex comprising heavy chains and associated regulatory, intermediate, light-intermediate, and light chains. Dyneins move toward the minus-end of microtubules; axonemal ODAs and IDAs power ciliary beating and IFT dynein transports trains to the ciliary base.
Kinesin-2: a class of plus-end directed molecular motors that includes the heterotrimeric kinesin-2 that moves IFT trains to the ciliary tip.
Intraflagellar transport (IFT): a bidirectional motility of IFT trains along cilia. Anterograde IFT moves toward the ciliary tip, whereas retrograde IFT moves toward the ciliary base.
IFT trains: array of multiple IFT particles, each comprising IFT-A and IFT-B subcomplexes.
Primary ciliary dyskinesia (PCD): a genetically heterogeneous condition caused by immotile cilia; features of PCD are reoccurring airway infections, male infertility, and often situs anomalies.
Polycystic kidney disease (PKD): an inherited disorder affecting approximately 1 in 1500 adults; most cases of PKD are caused by