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How Microtubules and Motor Proteins Enable the Maintenance and Functioning of Cilia

Introduction

Cilia are highly conserved, microtubule-based organelles present on the vast majority of human cells.[1] The two major types are primary cilia, also known as sensory or nonmotile cilia, and motile cilia (Figure 1a). Most cells have a single primary cilium[2] that serves sensory and signaling functions, whereas motile cilia are a particular feature of specialized multiciliated cells and beat in a coordinated fashion to drive extracellular fluid flow.[1,3] Ciliary beating enables mucus clearance by the respiratory epithelium, cerebrospinal fluid circulation via the ependyma, and egg transportation in fallopian tubes.[4]

Regardless of type, all cilia are anchored intracellularly by a modified centriolar structure known as the basal body.[4] The extracellular protrusion is referred to as the axoneme[5] and is supported by a cylindrical bundle of nine microtubule doublets (Figure 1b). In motile cilia, an additional central doublet is present and the combined axonemal microtubules act as a scaffold for other structural components,[3,5] most notably the axonemal dynein assemblies that power ciliary beating.[6]

In this newsletter, we will briefly review the unique roles of key ciliary motor proteins including kinesin-2, dynein-2, and axonemal dynein arms, and also consider the pathological consequences of mutations that affect their normal functioning.[7]

The Intraflagellar Transport System

Cilia are built and maintained through a dedicated intraflagellar transport (IFT) system that moves diverse molecular cargoes into and out of the axoneme.[6] Large protein assemblies referred to as IFT "trains" travel up and down the axonemal microtubules, powered by distinct molecular motors in each direction. The IFT-B complex is responsible for anterograde transport (i.e., toward the ciliary tip) and is driven by kinesin-2, a heterotrimer of KIF3A, KIF3B, and KAP3 proteins.[8] Retrograde transport relies on IFT-A trains carried by dynein-2, a larger motor complex consisting of at least eight subunits in mammals.[8]

Through mechanisms that remain largely uncharacterized,[9] anterograde to retrograde train conversion occurs in the ciliary tip region and may be triggered by IFT-B "derailing".[10] To facilitate the transition, dynein-2 is carried on IFT-B trains in an autoinhibited form that does not associate with microtubules, becoming active only when recruited to IFT-A assemblies.[6] Meanwhile, dissociation of kinesin-2 from the IFT-B complex induces autoinhibition[8] to enable switching to retrograde movement.

Remarkably, IFT-A and IFT-B trains avoid collision through selective binding to different sides of the microtubule doublet.[6,10] Anterograde trains track specifically along B-tubules (the truncated cylinder of each pair; Figure 1b), while retrograde trains associate only with A tubules. It is unclear exactly how this is regulated but tubulin posttranslational modifications may be involved.[6] Extensive polyglutamylation of the B tubule has long been established[11] and was recently demonstrated to inhibit dynein-2 binding.[12] Furthermore, kinesin-2 exhibits unique stepping behavior that seems to inherently restrict its motion to the B tubule of microtubule doublets.[13]

The main cargo for IFT is tubulin dimers, which are carried by two key proteins (IFT81 and IFT74) in the IFT-B complex.[14] In motile cilia, the inner and outer dynein "arms" required for ciliary beating are also moved into the axoneme via IFT, after initial preassembly in the cytoplasm.

Axonemal Dyneins and Ciliary Beating

Multiciliated cells possess tens to hundreds of motile cilia whose metachronal wave-like motion generates fluid flow along epithelial surfaces.[7] Ciliary beating is powered by a regular, repeating arrangement of axonemal dynein complexes and associated structures supported by the microtubule framework.[7,15] The inner and outer dynein arms are distinct multiprotein complexes named for their positioning relative to the cylindrical structure formed by the nine outer microtubule doublets of the axoneme.[16]

Each ciliary beat cycle consists of a power stroke followed by a recovery stroke to return to the starting position.[17] Motion is generated through microtubule sliding driven by the coordinated action of the axonemal dynein arms,[6,7] and there are two current models for how this is achieved. In the "switch point" model, half of the dynein arms are active (and the other half inactive) during the power stroke and the active/passive arms switch states during the recovery stroke. In the "switch inhibition" model, which has support from recent structural studies,[15] most or all of the axonemal dyneins are active at equilibrium and movement is generated by patterned spatiotemporal switching off of dynein arms.

Ciliary beating can be rapid, reaching a frequency of up to 30 Hz in the airways,[7] yet functions such as mucociliary clearance also demand coordinated movement of cilia over the scale of tissues as well as individual cells. Patterned, self-organized collective beating creates the metachronal waves that enable biological fluid propulsion, and the mechanisms underlying this exquisite

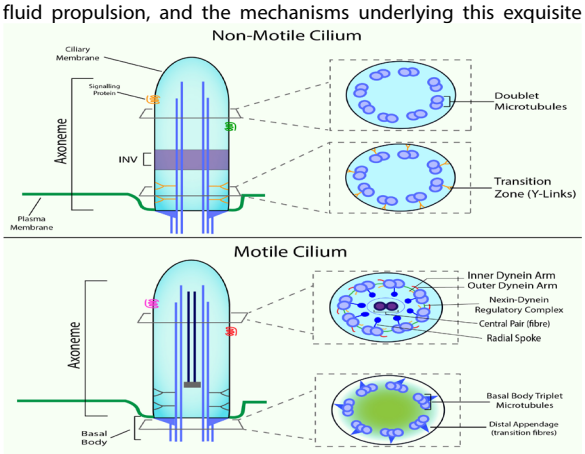

Figure 1. Schematic showing the structural differences in non-motile cilia and motile cilia. Adapted from Reiter and Leroux. 2018 (refs)

Continued from Page 1

coordination are an area of active research.[18,19] Notably, ciliary beating is tunable and responds to a range of physiological stimuli including Ca2+ levels and redox status.[7,15]

Ciliopathies

Mutations in genes affecting ciliary biogenesis and function cause a range of monogenic, mostly autosomal recessive, disorders that are collectively termed ciliopathies.[20] While no pathogenic mutations are known for kinesin-2, various dynein-related ciliopathies arise from dysfunction of either the IFT or axonemal motors.[7]

Dynein-2 mutations mostly affect the DYNC2H1 heavy chain and cause a group of skeletal disorders summarized as short-rib thoracic dysplasia (SRTD).[21] A severe example is Jeune syndrome or Jeune asphyxiating thoracic dystrophy (JATD), characterized by respiratory distress due to significantly shortened ribs.[20] With treatment, 40% of patients survive into adulthood but usually develop complications such as eye or kidney disease in middle age.[7]

In contrast, axonemal dynein mutations are associated with a group of chronic respiratory ciliopathies known as primary ciliary dyskinesia (PCD).[7] Impaired movement of motile cilia results in defective clearance of mucus and pathogens from the airways, causing recurrent infections such as bronchitis, pneumonia, and sinusitis5 and ultimately leading to respiratory tissue damage.[20] The first human gene associated with PCD was DNAI1 (dynein axonemal intermediate chain 1),[22] and more than 40 genes have now been implicated in PCD and related disorders. This includes cytoplasmic dynein assembly factors as well as a range of axonemal dynein arm subunits.[7]

KIF7: A Unique Ciliary Kinesin

Mutations in KIF7 cause multiple malformation disorders associated with disrupted Hedgehog pathway signaling and dysregulated GLI transcription factor (TF) activity.[23] Since the key Hedgehog pathway receptors Smoothened (SMO) and Patched (PTCH1) localize to primary cilia, KIF7 was proposed and confirmed as a ciliopathy gene,[23,24] but the relevant mechanisms have only recently been unraveled.

The KIF7 protein is an atypical kinesin-4 family member that has low affinity for microtubules and is immotile due to strong motor domain autoinhibition from a seemingly unique C-terminal inhibitory coiled-coil domain.[25] Removal of this domain resulted in tight microtubule binding but loss of normal KIF7 localization in cilia. A subsequent report from the same group confirmed low affinity of KIF7 for microtubules[26] and observed that the protein is transported to ciliary tips by IFT upon Hedgehog pathway activation.

In addition, KIF7 knockout abolished accumulation of GLI2/GLI3 in the tip region following Smoothened agonism, indicating that it carries these TFs into cilia.[26] It was independently shown that the coiled-coil dimerization domain of KIF7 binds GLI TFs through DNA molecular mimicry, with a second lower-affinity GLI binding site also identified in the motor domain.[27] Intriguingly, GLI2 knockout significantly reduced KIF7 transport to ciliary tips, suggesting that preformation of a KIF7–GLI2/3 complex may facilitate IFT loading for anterograde transport of both proteins to the cilium tip.

Current Developments and Outlook

One recent study highlights the potential of applying "omics" technologies in ciliary biology. Using the emerging technique of crosslinking mass spectrometry to measure in situ protein–protein interactions,[28] the authors discovered novel functional interactions in motile cilia and offered improved understanding of some human ciliopathy proteins.[29] For example, mutations in the microtubule inner protein ENKUR cause situs invertus, a laterality disorder in which the position of major abdominal organs is mirrored.[30] Knockdown of ENKUR in Xenopus multiciliated epithelial cells abolished recruitment of two inner dynein arm subunits, DNAI4 and DYNLT2B, to the axoneme,[29] consistent with pathogenic mutations disrupting the known requirement for ciliary motility in establishing the left–right axis during development.[30]

Elsewhere, advances in structural biology have enabled valuable new insights into ciliary ultrastructure. In contrast to the classical description, recent work unexpectedly revealed variable microtubule bundling arrangements and lengths in the distal portion of primary cilia, opening up exciting new avenues of research.[9] Undoubtedly, such ongoing investigations will continue to illuminate the fascinating structural and functional details of these "incredibly thin feet," as Anthony van Leeukwenhoek first described cilia upon observing them in 1674.[7,10]

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KIFC3 kinesin motor domain protein GST tagged: Homo sapiens recombinant	2 x 25 µg	KC01-A
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