Molecular Basis of Tubulin Transport During Cilium Formation

Rendicontazione

Informazioni relative al progetto

ciTTub

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Sito web del progetto

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Progetto chiuso

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Periodic Reporting for period 1 - ciTTub (Molecular Basis of Tubulin Transport During Cilium Formation)

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Sintesi del contesto e degli obiettivi generali del progetto

Cilia are organelles that protrude from the surface of most eukaryotic cells and are fulfilling important functions in motility and reproduction as well as sensory reception and signalling. In vertebrate organisms, cilia are essential for life. Motile cilia are usually present in multiple copies on the surface

of mammalian cells and beat in coordinated waves to create movement over apical surfaces in brains, lungs, Falopian tubes, and the embryonic node. Mammalian reproduction is also dependent on cilia which powers the movement of sperm cells towards the egg. Most animal cells contain a primary cilium (in general non-motile) that is normally present in one copy per cell. Immotile sensory cilia are found on photoreceptor cells in the eye and on olfactory neurons and are the sensory organelles of smell and sight. Both sensory reception and signalling in the cilium are a result of increased clustering of receptors in the ciliary membrane that are transmitting extracellular cues intracellularly.

The cilium is a semi-permeable organelle is surrounded by a ciliary membrane that is continuous with the plasma membrane and is partially closed by a transition zone. Cilia are supported by a central and microtubule-based structural scaffold called axoneme that is templated at the base by a modified centriole and built from multiple polymerized α- and β-tubulin subunits. Although more than 600 different proteins are residing in cilium, tubulin is by far the most abundant protein. As cilia does not contain ribosomes, the ciliary proteins require synthesis in the cytoplasm and transport inside the ciliary compartment through the transition zone by free diffusion or active transport along the axoneme.

The transition zone restricts the free diffusion inside of cilia to proteins smaller than 100kDa, therefore many proteins are trafficking cilium as cargoes on intraflagellar transport (IFT) trains that assemble into the cytoplasm and walks bi-directionally along the axoneme driven by kinesin or dynein molecular motors. The IFT is essential for cilium formation and was discovered in the green alga Chlamydomonas reinhardtii in 1993 as electron dense particulate material that moves between the ciliary membrane and the axoneme from base to the tip and back to the cytoplasm. These IFT trains turned out to be polymers of 22 different proteins organized into two biochemically stable IFT-A and IFT-B complexes.

Recent studies showed that IFT-B complex constitutes the backbone of polymeric IFT trains onto which IFT-A, dynein and finally the kinesin motor attaches. Moreover, the IFT-B complex contains the tubulin binding sites contributed by the calponin homology domain of IFT81 and the N-terminus of IFT74. So far it remained unclear how 16 different IFT-B proteins assembles into complexes to allow additional binding sites for cargoes such as tubulin. This was in part due to lack of high-resolution structures which were only available for smaller IFT-B sub-complexes leaving >50% of the IFT-B complex structurally uncharacterized.

In the ciTTub research project, I aimed at elucidating the molecular basis of tubulin transport during cilium formation by providing structural characterization of IFT-B complexes and tubulin. We approached this research project through three objectives: 1) Determination of the interaction interface between tubulin and the IFT-B complex (WP1); 2) Structural analysis of the IFT-B-tubulin complex (WP2); and 3) Elucidation of tubulin loading and unloading mechanism onto IFT-B (WP3). The first two objectives relied on the purification and reconstitution of various IFT-B complexes that contained the tubulin binding site of IFT81 and IFT74. This goal was achieved and allowed us to attempt the structural characterization of IFT-B complexes with or without tubulin by cryo-electron microscopy and X-ray crystallography. Because of several unexpected technical challenges, this strategy was unsuccessful.

However, we mitigated these setbacks by employing recent the developments in structure prediction by AlphaFold to obtain the structure of 15-subunit IFT-B complex. To verify the structural model, we have carried out a large body of work including chemical- and photo-crosslinking, mas-spectrometry, X-ray scattering as well as diffraction and mutagenesis in combination with protein-protein interaction assays. Currently. the results arising from the objectives 1) and 2) are under peer-review awaiting publication at EMBO Journal (Petriman et al., 2022; https://www.biorxiv.org/content/10.1101/2022.08.20.504624v1).

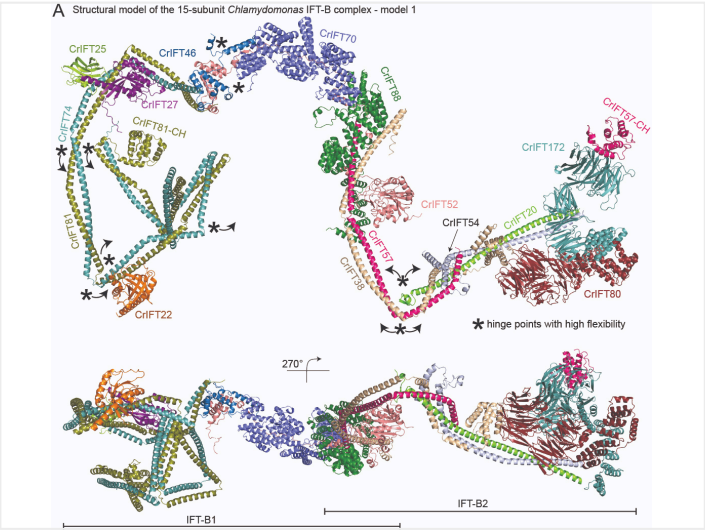
With the third objective I aimed at elucidating the role of small Rab-like GTPases, that are part of the IFT-B complex, in IFT-mediated tubulin transport. We have discovered that tubulin binds to IFT81 and IFT74 in vitro, independently of the three GTPases (Rabl2, IFT22 and IFT27). However, we have found that the tubulin binding proteins IFT81 and IFT74 enhances GTP hydrolysis to inactivate RabL2 during early steps of intraflagellar transport. The results arising from the third research objective are made available as pre-print through bioRxiv (Bøgholm et al., 2022; https://www.biorxiv.org/content/10.1101/2022.05.31.494111v1) and currently awaits per-review.

Lavoro eseguito dall'inizio del progetto fino alla fine del periodo coperto dalla relazione e principali risultati finora ottenuti

The work from the beginning to the end of the project was performed according to the Grant Agreement and is detailed in the final technical report. The main results of this research project are the solving of the 15-subunit IFT-B structure (Petriman et al., 2022) and the reveal of how RabL2 mediates the early stages of IFT (Bøgholm et al., 2022). Besides their open access publication, the results will be presented as posters at the International Cilia 2022 conference in Cologne, Germany.

Progressi oltre lo stato dell'arte e potenziale impatto previsto (incluso l'impatto socioeconomico e le implicazioni sociali più ampie del progetto fino ad ora)

The structure of the >1MDa IFT-B complex structure is highly elongated and flexible, is consistent with low-resolution cryo-ET reconstructions of IFT trains and provide us with a platform for understanding IFT function and pathological mutations (Figure 1). This represents a giant step forward in understanding IFT, cilium formation and allows us to rationalize decades of biochemical and cell biological work on IFT and cilium formation.



The model of 15-subunit Chlamydomonas IFT-B complex.

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