Ensemble and single-molecule dynamics of intraflagellar transport in C. elegans

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2018

document version
Publisher's PDF, also known as Version of record

Summary

Efficient and specific transport of proteins and other macromolecules inside cells is vital for cell function. This thesis investigates a type of intracellular transport, called intraflagellar transport (IFT) that is essential for building and maintaining cilia. Cilia are thin, membrane-enveloped protrusions from the cell surface that play an important role in signaling by acting as antennae to detect and respond to changes in the outside environment. Inside cilia, IFT trains, composed of molecular motors and IFT proteins, transport cargo along the microtubule track. Kinesin-2-driven trains drive transport from the ciliary base to the tip (anterograde transport), whereas IFT-dynein-driven trains return cargo from the tip back to the base (retrograde transport).

This thesis applies a combination of sensitive fluorescence microscopy, stochastic modeling and transgenesis to investigate IFT on three different levels: at the ensemble level, to characterize the functioning of groups of IFT components (**Chapter 2**); at the single-molecule level, to quantify the behavior of individual IFT components (**Chapters 2 and 3**); and at the systems-level to obtain insight into how motors, IFT particles and tracks collectively respond to stimuli (**Chapters 4-6**).

IFT at the ensemble and single-molecule level

Our results show that, at the ensemble level, IFT dynein traverses the cilium from tip to base as a group or 'train' of 30-40 motors. Retrograde trains are somewhat smaller and more frequent than anterograde IFT trains, resulting in equal flux in both transport directions (**Chapter 2**). Using dual-color fluorescence microscopy, we show that directionality of IFT trains is not modulated by the kinesin-2:dynein ratio, contrary to other tug-of-war, opposite-polarity motility systems. At the single-molecule level, we reveal dynamics that is not directly discernible at the ensemble level: diffusive behavior at the base and tip, pausing and directional switches (**Chapters 2 and**

3). We show, using stochastic simulations, that these directional switches are necessary to maintain the IFT-dynein distribution in the cilium.

It is evident from both the ensemble and single-molecule data that most directional switching occurs at the end of the ciliary distal tip, where all IFT trains need to reverse direction from anterograde to retrograde (**Chapter 2**). In **Chapter 3**, we focus only on this region of the cilium. We describe the experimental conditions necessary for single-molecule imaging of IFT at the *C. elegans* ciliary tip and directly visualize the turnaround of individual IFT components. Our results reveal that IFT components reverse direction in a ~1 μm region of the tip of the distal segment. In most cases, the components appear spatially confined without episodes of free diffusion. While most motor proteins and IFT-A proteins turn around almost immediately, IFT-B proteins, surprisingly, pause for several seconds. Our data is consistent with a scenario where the entire IFT-train machinery completely disassembles at the tip and IFT-B undergoes modifications (conformational change or chemical modification) before trains can reassemble and move in the retrograde direction.

In summary, we show that the underlying single-molecule dynamics of individual IFT components determines the ensemble behavior of IFT trains. During anterograde or retrograde transport, particle sub-complexes A and B form the backbone of the IFT train, with IFT dynein, kinesin-II and OSM-3 easily docking on and off to establish the required ciliary motor distribution. At the tip, the entire IFT machinery rapidly disassembles and then reassembles for retrograde transport.

IFT at the systems-level

In **Chapter 5**, we use ciliobrevin A to partially disrupt IFT dynein in *C. elegans* cilia. Acute, low-concentration ciliobrevin treatment results in ciliary shortening and a reduction of both anterograde and retrograde IFT velocities. In **Chapter 6**, we cut off the dendritic input of proteins into the cilium using femtosecond laser ablation and observe similar ciliary shortening and velocity reduction in both transport directions. The experiments described in these two chapters highlight several key aspects of the IFT system as a whole.

Firstly, and unsurprisingly, there appears to be interdependence between the two transport directions: disturbing retrograde transport also affects anterograde transport. Secondly, the ciliary axoneme shrinks in response to fewer (active) motors, indicating that ciliary length maintenance requires a minimum active number of dynein motors. Thirdly, the results from **Chapters 5 and 6** suggest that the system can rapidly respond to change by adapting IFT velocities and axoneme length. In other words, IFT is a relatively robust process and adjustments can be made to preserve functional transport for as long as possible.

In **Chapter 2**, we show that anterograde and retrograde flux are similar. This suggests that, at least to a certain extent, IFT is a closed system with no net loss of components during exchange at the tip or base. Dendritic femtosecond laser ablation experiments, described in **Chapter 6**, provide further support for this. In the first ~60-90 seconds following physical ablation of the dendrite, most motors and IFT particles remain in the cilium and IFT can continue for 7-10 cycles. It is tempting to speculate that there is a built-in window for restoration. That is to say, if influx of ATP and IFT components is restored within this time-window, IFT can also be rapidly restored, whereas permanent damage to the dendrite will result in loss of IFT components and loss of transport. We propose that such a multi-step response enables the cilium to rapidly respond to changes in the extracellular environment.

It is well-established that IFT motors build the axoneme that they walk on. This interplay between motors and track results in a coupling between motility and axonemal retraction: slowing down of motors results in axonemal retraction, as observed in **Chapter 5 and 6**. In **Chapter 4**, we probe the response of the IFT machinery to temperature using fluorescence microscopy of labeled IFT-dynein motors and tubulin. At temperatures above 30°C, the motors retract but axoneme length remains intact. This apparent uncoupling between motility and ciliary length maintenance is striking, but can be explained by the temperature-dependence of tubulin dynamics, where high temperatures favor polymerization, resulting in a very stable ciliary axoneme. This chapter shows that the interplay between motors and track is complex and can be modulated by extracellular factors such as temperature.

Outlook

Many intriguing questions about IFT remain. To date, very few ciliary cargoes and receptors have been identified. It will be interesting for future studies to explore the dynamics of cargo in the cilium and its interplay with the IFT machinery. Based on the current body of literature, the distal tip is postulated to be the main region for cargo exchange. Probing this region beyond the experiments in **Chapter 3** will likely yield interesting insights into IFT, signal transduction and the cilium's biological role as a sensory antenna. Important questions include: How does the cilium stage a signaling response? What are the events up- and downstream in such signal transduction? How does the ciliary signaling response differ in different model organisms and why? Providing answers to these questions will require approaches that extend beyond fluorescence microscopy, such as molecular biology tools to uncover novel ciliary receptors and ligands, or methods for sensitive detection of signaling molecules inside cilia. It will be particularly important for future work to regard cilia not as isolated closed systems, but rather as components of a complex, connected biological system that also includes the cell body, dendrite and extracellular environment.