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Introduction

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1.1 Molecular motors and the cellular highway

The correct localization of proteins and other macromolecules inside cells is crucial for function and survival. Prokaryotic organisms, such as bacteria, primarily rely on passive, thermal-energy driven diffusion to effectively distribute components to their required location within cells. Eukaryotic organisms, however, face a logistic challenge: their cells are too large for diffusion to effectively distribute organelles, vesicles, RNA and other components[1]. This problem is particularly severe in the long extensions of neurons, which can be meters long in larger mammals. To solve this problem, eukaryotic organisms have evolved intricate intracellular transport systems consisting of motor proteins, cargo and tracks[1]. The tracks are formed by structures called microtubules and actin filaments. These are part of ak cellular scaffold, the cytoskeleton, which gives the cell its structural integrity. Motor proteins are specialized proteins that are able to use these tracks as intracellular highways by converting chemical energy in the form of ATP to mechanical work. More specifically, ATP is converted to ADP, leading to a conformational change that enables force exertion and stepping along the track.

Three large superfamilies of motor proteins have evolved over time: dyneins,[2] myosins[3] and kinesins[4]. The myosins step along actin filaments, whereas dyneins and kinesins use microtubules as tracks. Both microtubules and actin filaments are polarized, enabling unidirectional transport: kinesins generally move in the plus-end direction along microtubules, whereas dyneins move in the opposite direction (Figure 1.1). The myosin superfamily contains motors that can move in either direction along actin (Figure 1.1). Kinesin and myosin motors are structurally similar and are thought to share the same evolutionary origin, both being related to G-proteins. Dyneins, on the other hand, belong to the AAA+ (ATPases Associated with various cellular Activities) family and are structurally distinct. They are larger (~1.4MDa) and, unlike the compact structure of myosins and kinesins, the ATPase domain is separated from the microtubule binding domain by a 10-15 nm stalk. In contrast to the regular 8

nm steps taken by kinesin motors, recent single-molecule studies have shown that cytoplasmic dynein 1 can take larger steps up to 32 nm, can step sideways and even backward [5-8]. Additionally, it can change its motility parameters by recruiting regulators such as NudE and Lis1 [9, 10].

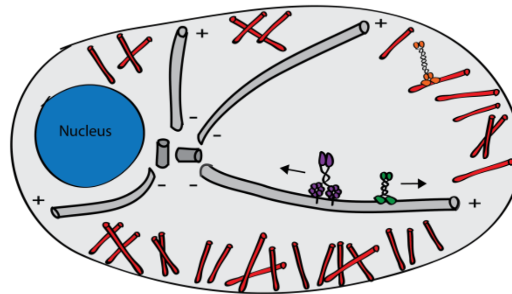

Figure 1.1: Schematic representation of intracellular transport in the cell. Myosins (orange) step along actin filaments (red). Microtubule filaments originate from the centrosome (grey cylinders). Kinesins (green) move in the plus-end direction, whereas dyneins (purple) move in the minus-end direction.

Over the past three decades, structural and functional studies of these motor proteins have taught us in great detail how they operate on the molecular level. *In vitro* single-molecule motility assays using optical tweezers or fluorescence microscopy have allowed detailed measurement of velocities, step sizes, processivity and force generation[11]. It has become clear, however, that understanding the behavior of a single motor protein is not sufficient to fully grasp intracellular transport. The main reason is that, in living organisms (*in vivo*), in many cases, multiple motors with distinct motility properties team up to move the same cargo[12]. This results in a complex interplay between motor proteins, that is also affected by cargo rigidity and by the properties of the tracks. Furthermore, the motors, tracks and cargo are under tight regulatory control, adding to the complexity. A complete understanding of

intracellular transport therefore requires a systems approach, such as the one presented in this thesis.

1.2 Intraflagellar transport (IFT) in *C. elegans*

In the work described here, we use intraflagellar transport (IFT) as a model for intracellular transport and motor-protein cooperation[13]. IFT is essential for the assembly, maintenance and function of cilia. These organelles are membrane-enveloped, hair-like protrusions from the cell body, that are composed of a circular bundle of 9 doublet microtubules called the axoneme. Motile cilia, such as the cilia in the human trachea that transport mucus, generally have an additional microtubule doublet in the middle (9+2 structure) which is used in ciliary or flagellar beating[14, 15]. Non-motile cilia (e.g. cilia that are responsible for olfaction or taste, or the connection between photoreceptor rod and cell body) lack this pair and have a 9+0 axonemal structure[16]. This thesis focuses on non-motile (or primary) cilia. Almost every eukaryotic cell has a non-motile cilium projecting from its surface. Non-motile cilia act as cellular antennae to detect and respond to chemical, mechanical and thermal changes in the extracellular environment[17]. Malfunctioning of these cilia is the underlying cause of many different human diseases, including Bardet-Biedl Syndrome and polycystic kidney disease[18-20]. Studying IFT therefore does not only contribute to a deeper understanding of intracellular transport, but could also increase our insight into ciliopathies.

The chemosensory cilia in the ~1 mm roundworm *C. elegans* have become important models to study IFT, for several reasons[21]. First, there are the general benefits of *C. elegans* as a model organism: fast reproduction, well-known genetics, well-developed transgenesis techniques, biobanks with numerous mutants and its transparency that renders it very suitable for fluorescence microscopy. Second, *C. elegans* chemosensory cilia show a particularly intriguing level of complexity: the ciliary axoneme has a bipartite structure with a middle segment composed of microtubule doublets from

which a distal segment, composed of nine microtubule singlets, protrudes[22]. In addition, there are human orthologs for most ciliary proteins.

In C. *elegans* chemosensory cilia, IFT is driven by the interplay between three different motor proteins: two motors of the kinesin-2 family, heterotrimeric kinesin-II (consisting of three subunits KLP-11, KLP-20 and KAP-1)[23, 24] and homodimeric OSM-3[25, 26], and IFT dynein, a cilium-specific cytoplasmic dynein[27]. Kinesin-II and OSM-3 transport cargo, as well as (presumably) inactive IFT-dynein motors, along microtubules from the ciliary base to the tip. Here, a directional switch occurs and IFT-dynein motors drive transport back from the tip to the ciliary base, carrying back kinesin-2 motors (Figure 1.2). The motors, together with protein complexes IFT-A and IFT-B, form transport units called IFT trains[28, 29].

Kinesin-II and OSM-3 do not work independently, but cooperate to transport cargo along microtubules from the ciliary base to the tip[30]. The key evidence for this was that both kinesin-2 motors and cargoes move at the same velocity of ~0.7 μm/s along the microtubule doublets of the middle segment, while IFT velocity increases to ~1.2 μm/s in the absence of kinesin-II and decreases to ~0.5 μm/s in the absence of OSM-3. An interesting redundancy in the IFT system was also uncovered: both kinesin-2 motors are capable of building the middle segment on their own, whereas OSM-3 is essential for the formation of the distal segment. These observations raised the question why the slower kinesin-II is employed when OSM-3 is capable of building the middle and distal segment by itself. The functional significance of this intriguing motor cooperation was recently addressed by Prevo *et al* in our group[31]. In brief, kinesin-II acts as the import motor to drive IFT trains close to the base and transition zone. It then slowly hands over transport to OSM-3, which facilitates fast, long-range transport[31].

1.3 Scope of this thesis

Compared to the kinesin-2 motors, IFT-dynein function is relatively underexplored, likely due to its large size and complex structure. In recent years this has started to rapidly change, with several groups probing IFT dynein structure[32, 33], composition of the motor complex[34, 35] and mutations implicated in human disease[36, 37]. IFT-dynein motility properties have also been investigated in different organisms at the ensemble (IFT train) level[38-43], but many open questions remain. This thesis uses improved imaging and analysis tools to address some of these unanswered questions about IFT dynein (**Chapter 2**) and the IFT transport system as a whole (**Chapters 3-6**), outlined in Figure 1.2.

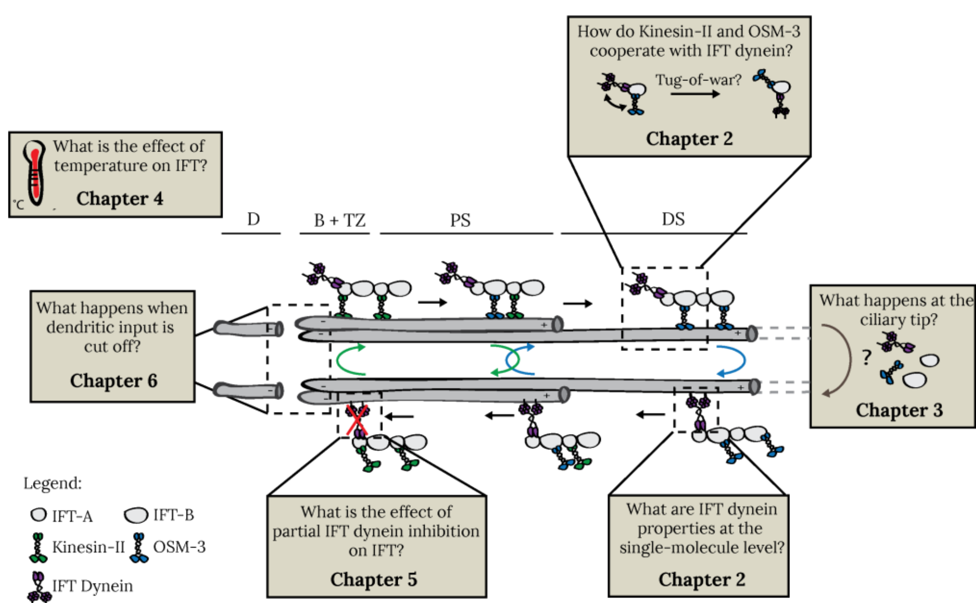

Figure 1.2: Visual outline of the topics addressed in this thesis.
Sketch of IFT in the C. *elegans* phasmid chemosensory cilium and the topics studied in each chapter. B, base; TZ, transition zone; PS, proximal segment; DS, distal segment; D, dendrite.

In **Chapter 2**, we investigate, for the first time, IFT-dynein motility at the single-molecule level in a living organism. We find unexpected dynamics, including diffusion at the base, pausing and directional switches along the cilium, and use stochastic simulations to connect single-molecule behavior (the probability of single-motor directional switches) to the ensemble IFT-dynein distribution.

Chapter 3 addresses the dynamics of dynein, kinesin and IFT particles at the ciliary tip: the region where IFT trains reverse direction from anterograde to retrograde. How this directional switch occurs is arguably one of the biggest questions in the field of IFT biology. We directly visualize and quantify the behavior of single IFT train components in this region and find that the entire IFT train first disassembles, and then reassembles for retrograde transport.

In subsequent chapters, we perturb the IFT system in different ways (**Chapter 4**, temperature; **Chapter 5**, partial dynein inhibition; **Chapter 6**, dendritic laser ablation) in order to study how the (connected) system of tracks, motors and IFT particles responds to such perturbations. At high temperatures, the axoneme remains intact in length while the motors retract (**Chapter 4**). Partial IFT-dynein inhibition results in a shortened axoneme and adapted, lower motor velocities in both transport directions (**Chapter 5**). In the final chapter, **Chapter 6**, we use pulsed femtosecond laser ablation to physically cut off the dendritic import of IFT proteins into the cilium. This triggers a signaling response resulting in rapid motor- and axoneme retraction.

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