Mini-Review

Kinesin-II, Coming and Going

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EUKARYOTIC cells are constantly challenged with a variety of transport problems. These are encountered during membrane trafficking, distribution of mitochondria and other membrane-bounded organelles, mRNA localization, and during special events such as mitosis and meiosis. Much of this transport is mediated by the concerted efforts of kinesins, dyneins, and myosins, the molecular motors that operate along the cytoskeletal network of microtubules and actin filaments. Differentiated cells generate special transport needs, such as the long distance axonal transport of materials in neuronal cells, the bidirectional intraflagellar transport of proteinaceous rafts in ciliated cells, and the dispersion and aggregation of pigment granules in melanophores. One motor protein that has been adapted to operate in these three specialized movements is kinesin-II, also known as the heterotrimeric kinesin, KIF3A/3B, and $KRP_{85/95}$. This paper briefly summarizes two independent studies that (a) identify a normally soluble enzyme as a cargo of kinesin-II during anterograde axonal transport (Ray et al., 1999), and (b) indicate that kinesin-II is carried as a cargo during retrograde intraflagellar transport within neuronal sensory cilia (Signor et al., 1999a).

Kinesin-II Is a Heteromeric Plus End–directed Microtubule-based Motor Adapted for Multiple Tasks

Kinesin-II is a heteromeric kinesin (reviewed in Cole, 1999). It is isolated from green algae, nematodes, echinoderms, and vertebrates as a heterotrimeric complex containing two unique, though related, motor subunits that are members of the KIF3A/3B or KRP85/95 kinesin subfamily (Cole et al., 1993, 1998; Yamazaki et al., 1996; Signor et al., 1999b). The two motor subunits are typically associated with a nonmotor subunit known as kinesin-associated polypeptide (KAP; Yamazaki et al., 1996). It appears that the heteromeric nature of kinesin-II allows the organism to treat it as a combinatorial protein. For example, at least three isoforms of the kinesin-II motor subunits (KIF3A, KIF3B, and KIF3C) are expressed in the mouse and rat. KIF3A is capable of forming heterodimers with either KIF3B or KIF3C, while KIF3B and KIF3C are unable to heterodimerize with each other (Muresan et al., 1998; Yang and Goldstein, 1998). In addition, several isoforms of the associated nonmotor subunit, KAP, can be generated from alternative splicing of KAP mRNA (Yamazaki et al., 1996). Kinesin-II, and thus far all NH_2-terminal kinesins, display only plus end–directed microtubule-based in vitro motor activity, suggesting that in vivo kinesin-II–driven transport will be limited to plus end–directed movement. Both kinesin-II motor and nonmotor subunit isoforms display differential tissue expression and even differential subcellular localization in the mouse, suggesting that different combinations of these subunits have been adapted for specialized transport needs (Muresan et al., 1998; Yang and Goldstein, 1998). Indeed, in frog melanophores, kinesin-II powers the dispersion of pigment granules (Tuma et al., 1998), while in the unicellular *Chlamydomonas* and *Tetrahymena*, kinesin-II drives anterograde intraflagellar transport (Kozminski et al., 1995; Brown et al., 1999). In higher animals, kinesin-II has also been adapted for anterograde axonal transport.

Drosophila Kinesin-II Is Responsible for the Axonal Transport of Choline Acetyltransferase

An axonal transport role for kinesin-II was hypothesized early in the studies of murine KIF3A/3B (Kondo et al., 1994). Reported to be associated with membrane-bounded vesicles, the true nature of the axonal kinesin-II cargo has eluded researchers. Now, a likely kinesin-II cargo has been identified in the axons of *Drosophila* neurons (Ray et al., 1999). In these studies, two *Drosophila* kinesin-II motor subunit homologues, KLP64D (KIF3A/KRP85) and KLP68D (KIF3B/KRP95), were found to coexpress in cholinergic neurons. Mutations in KLP64D cause uncoordinated, slow movement and result in death at the larval or early adult stages of development. Interestingly, heterologous expression of the mouse homologue, KIF3A, results in rescue of the KLP64D mutations, indicating that these two proteins are functional homologues. Transport of choline acetyltransferase, ChAT, from the cell body to the synapse is also disrupted in these mutants, suggesting that ChAT is normally transported out to the synapse by kinesin-II. To test this model, these researchers exploited a mutant (Klc^-) that is missing the light chains of conventional kinesin, a known anterograde axonal transport motor. The axons in segmental nerves of these mutant larvae contain periodic swellings caused by the accumulation of fast axonal transport cargoes (Hurd and Saxton, 1996; Gindhart et al., 1998). Some of the swellings contain both KLP64D and ChAT, while other swellings contain little of

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either protein, providing further evidence that kinesin-II may be transporting ChAT. These findings raise an interesting question. How does kinesin-II transport a normally soluble protein for long distances in the axon? Axonal forms of kinesin-II in the mouse appear to be associated with membranous vesicles (Kondo et al., 1994). If the same is true for insect axonal kinesin-II, then ChAT may associate with these vesicles during axonal transport. Alternatively, kinesin-II in the insect may be transporting a single protein (ChAT) or a protein complex which contains ChAT. A precedence for kinesin-II–mediated transport of protein complexes comes from studies of intraflagellar transport.

Intraflagellar Transport Is Powered by Kinesin-II and Cytoplasmic Dynein 1b

Initially identified in *Chlamydomonas*, intraflagellar transport (IFT) is the bidirectional movement of large proteinaceous rafts along the outer doublet microtubules of motile cilia and flagella and nonmotile sensory cilia (Kozminski et al., 1993; reviewed in Rosenbaum et al., 1999; Saxton, 1999). The anterograde movement of rafts out to the distal tip of these organelles is powered by kinesin-II, while the retrograde transport of the rafts back toward the cell body is mediated by cytoplasmic dynein 1b (Fig. 1). The IFT rafts are composed of repetitive subunits made from ~15 polypeptides that can be isolated as two large protein complexes known as IFT Complex A and IFT Complex B (Piperno and Mead, 1997; Cole et al., 1998). Functional roles of IFT include both the assembly and function of cilia and flagella. Disruptions of kinesin-II function in *Chlamydomonas*, *Tetrahymena*, *Caenorhabditis elegans*, echinoderms, and the mouse have all resulted in severe inhibition of the assembly of cilia and flagella (Kozminski et al., 1995; Morris and Scholey, 1997; Nonaka et al., 1998; Brown et al., 1999; Marszalek et al., 1999; Takeda et al., 1999). There is also compelling evidence for the role of cytoplasmic dynein 1b as the retrograde IFT motor. Disruption of this dynein in *Chlamydomonas* results in the formation of very short (~1 μm) flagellar stubs filled with kinesin-II and the IFT rafts (Pazour et al., 1999; Porter et al., 1999). Furthermore, disruption of an 8,000-D *Chlamydomonas* dynein light chain results in a less severe flagellar assembly phenotype which is accompanied by a loss of retrograde IFT but not anterograde IFT (Pazour et al., 1998). In the absence of retrograde IFT, the shorter-than-normal flagella are congested as they fill with both kinesin-II, and the IFT Complexes. This phenotype is similar to that observed in the *che-3* mutation in *C. elegans*; CHE-3 encodes the nematode cytoplasmic dynein 1b heavy chain. The hypothesis that CHE-3 dynein acts as the retrograde IFT motor in the nematode is strongly supported by Signor et al. (1999a).

As a Cargo, Kinesin-II Is Transported Out of Cilia and Flagella by Cytoplasmic Dynein 1b

Signor et al. (1999a) now show that kinesin-II can also be a cargo as well as a transporter. These researchers have examined intraflagellar transport in the sensory cilia found

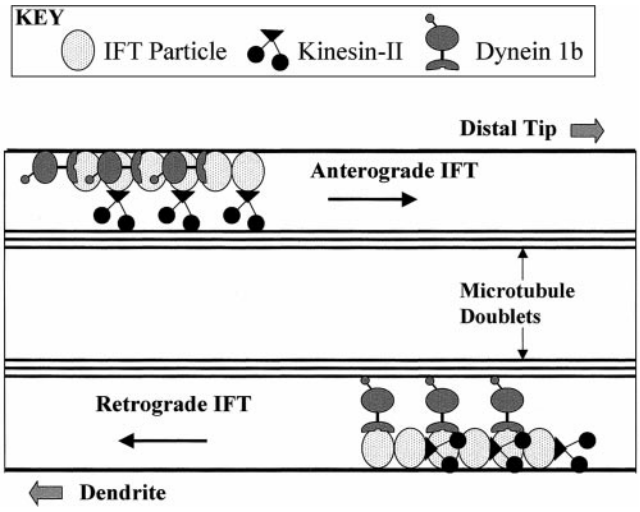

Figure 1. Intraflagellar transport within *C. elegans* sensory cilia. (Anterograde IFT) Kinesin-II transports IFT rafts and cytoplasmic dynein 1b along the microtubule doublets toward the sensory cilium tip (0.7 μm/s). (Retrograde IFT) Cytoplasmic dynein 1b transports IFT rafts and kinesin-II along the microtubule doublets back toward the dendrite (1.1 μm/s).

in the chemosensory neurons of *C. elegans*. They have used GFP fusions with the kinesin-II KAP subunit (Orozco et al., 1999) and two of the IFT raft subunits, OSM6 (Collet et al., 1998) and OSM1, to visualize anterograde and retrograde transport of these proteins in dendrites and the nonmotile sensory cilia that extend out from the distal end of the dendrites. Using a fluorescence-based assay with wild-type organisms, KAP::GFP, OSM6::GFP, and OSM1::GFP were seen moving bidirectionally in both the dendrites and the sensory cilia of chemosensory neurons; the cilia of these cells are nonmotile axonemal structures that extend out from the dendrites and are exposed to the environment. Since the microtubules of the axoneme are unidirectional, with their plus ends emanating away from the dendrite toward the distal tip of the cilium, the anterograde transport of 0.7 μm/s is very likely to be driven by the plus end–directed kinesin-II. The retrograde transport of 1.1 μm/s, however, requires a minus end–directed motor, indicating that kinesin-II is acting as a cargo during retrograde IFT. To identify the retrograde IFT motor in the nematode, Signor et al. (1999a) visualized IFT in a severe CHE-3 mutant that is effectively null for cytoplasmic dynein 1b. Severe mutations in the CHE-3 cytoplasmic dynein heavy chain completely abolish retrograde IFT along the cilium but do not interrupt anterograde IFT or the bidirectional dendritic transport of kinesin-II and IFT proteins. These results strongly suggest that CHE-3 cytoplasmic dynein serves as the retrograde IFT motor in the nematode and confirm earlier studies in *Chlamydomonas* that had implicated cytoplasmic dynein 1b as the retrograde IFT motor (Pazour et al., 1999; Porter et al., 1999). It also stands to reason that kinesin-II actively transports the cytoplasmic dynein 1b out to the tip as it seems unlikely that simple diffusion could keep up with the rapid pace of retrograde transport (~3.5 μm/s in *Chlamydomonas* and 1.1 μm/s in *C. elegans*).

In conclusion, the studies summarized above shed new light on the comings and goings of kinesin-II. Ray et al. (1999) provide evidence that kinesin-II is carrying the normally soluble ChAT through axons of cholinergic neurons while Signor et al. (1999a) have shown that kinesin-II becomes the cargo during retrograde IFT. Through the combined studies in *Chlamydomonas*, *C. elegans*, and other model organisms, it is now clear that kinesin-II and cytoplasmic dynein 1b are integral and essential parts of an ancient and conserved intraflagellar transport system designed to assemble and maintain ciliary and flagellar organelles. Indeed, due to the intrinsic polarity of the axonemal microtubules and tight space restrictions, retrograde IFT is required to prevent a serious congestion of IFT rafts at the distal tip. Thus, kinesin-II and cytoplasmic dynein 1b act in concert to keep intraflagellar traffic flowing. Likewise, in the neuronal axons and dendrites, where kinesin-II and other kinesins have been adapted for anterograde transport, retrograde transport may be required to remove anterograde motors and other material from the distal ends of these cells and return them back to the cell body.

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