Mini-Review

# Kinesin-II, a Membrane Traffic Motor in Axons, Axonemes, and Spindles

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THE kinesins constitute a superfamily of motor proteins that use energy liberated from ATP hydrolysis to move cargo along microtubules (MTs)[1] and thus play important roles in various intracellular transport events (4, 9). Kinesin-driven motility depends upon the action of kinesin-related polypeptides (KRPs), consisting of conserved motor domains sharing ~40% amino acid sequence identity that split ATP and move along MTs, linked to divergent tails that contain cargo-binding and oligomerization domains. Much has been learned about the activities of kinesin motors by studying these KRPs at the level of individual polypeptide chains, but inside cells, the specific functions of many KRPs may depend upon their self-assembly into multimeric complexes that also contain accessory subunits. Indeed, the founding member of the kinesin superfamily, named kinesin (24), was first purified as a heterotetrameric complex that contains such accessory subunits. Now a related complex, the heterotrimeric kinesin-II holoenzyme (26) has been described. Here I summarize the properties of the kinesin holoenzyme to provide a familiar context in which to review the structure, domain organization, and functions of the recently described kinesin-II motor protein. This comparison shows that kinesin-II has a similar overall structural organization to kinesin, having two motor domains lying at one end of the molecule linked by a rod to a tail where the accessory subunits are located. Such a structural arrangement appears to allow both kinesin and kinesin-II to serve as axonal vesicle transport motors that can be deployed as membrane traffic motors in other subcellular locations such as axonemes and spindles.

## *Kinesin, a Vesicular Transport Motor in Axons and Spindles*

Kinesin was first described in squid axons and shortly afterwards in sea urchin embryonic mitotic spindles (4). It is a heterotetrameric motor protein (Fig. 1) composed of two kinesin heavy chains (KHC) which contain the motor domains, and two accessory subunits, the kinesin light chains (KLCs). This motor protein moves toward the plus ends of MTs at about half a micron per second in a motility assay. Kinesin is a good candidate for being one of several fast anterograde axonal vesicle transport motors (4, 9) because when mouse peripheral nerves are ligated to block axonal transport, kinesin is found associated primarily with vesicles that accumulate on the side of the ligature proximal to the cell body (11) and some mutations in the *Drosophila* KHC appear to reduce the number of voltage-gated Na-channels in axons (12). These results suggest that kinesin transports vesicles containing Na-channels, and perhaps other membrane proteins and extracellular matrix components, along the axon for exocytosis at the neuronal cell surface (12).

Kinesin associates with vesicles in the sea urchin embryonic mitotic spindle asters (27), but kinesin-driven vesicular transport is apparently not essential for mitosis (28) and its significance has been unclear. However, new work suggests that the kinesin found in mitotic spindles may transport exocytic vesicles to the cell surface, just as it does in neurons (3, 21). This conclusion emerges from experiments in which the plasma membranes of sea urchin eggs and embryonic cells were damaged. The cells responded by increasing exocytotic activity, delivering new membrane to the wound site, and resealing the wounded membrane by a mechanism that resembles the vesicular trafficking events that underlie neurotransmission. The exocytic vesicles appear to be delivered to the cell surface by kinesin-driven transport along astral MTs because microinjection of a function-blocking antikinesin antibody specifically inhibits membrane resealing. This kinesin-dependent exocytic membrane resealing reaction may be a fundamental mechanism used by all cells to repair damaged membranes that can be deployed when needed for various tasks involving vesicular transport (4, 9), including neurotransmitter secretion by neurons.

## *Structure and Domain Organization of Kinesin-II in Comparison with Kinesin*

Kinesin-II is a more recently described membrane-translocating motor protein, whose structure is compared to that of kinesin in Fig. 1. The kinesin-II holoenzyme (formerly KRP[85/95]) was discovered and purified in a biochemical screen as a 300-kD heterotrimeric, nucleotide-sensitive MT-binding protein that reacts with pankinesin peptide antibodies (6, 7) and moves toward the plus ends of MTs at 0.4 μm/s (8). Sequence analysis (8, 17, 26) revealed that kinesin-II from eggs and embryos of the sea urchin *Strongylocentrotus purpuratus* (Sp) consists of heterodimer-

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1. *Abbrevations used in this paper*: KHC, kinesin heavy chain; KLC, kinesin light chain; KRP, kinesin-related polypeptide; MT, microtubule.

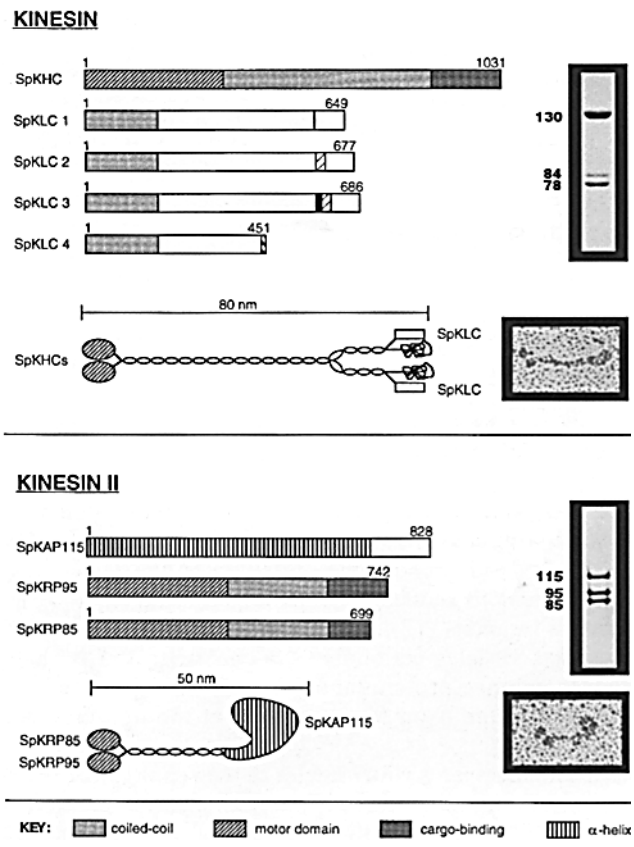


*Figure 1.* Structure and domain organization of two kinesin holoenzymes, kinesin (*upper panel*) and kinesin-II (*lower panel*). Each panel shows maps of subunits (*upper left*), SDS gels (*upper right*) and rotary shadow EMs (*lower right*) of purified proteins, and models of quaternary structure of kinesin or kinesin-II (*lower left*). The kinesin heterotetramer (see reference 20 for bibliography; *upper panel*) is assembled from two identical heavy chains (SpKHC; 1031 residues, 130 kD on gels) each having a globular $NH_2$-terminal motor domain, a central homodimerization domain, and a second globular COOH-terminal domain, together with two of four possible light chain isoforms (SpKLC 1-4; $M_r$ on gels, 78–84 kD) that differ due to alternative splicing. The EMs together with other data (4, 9) suggest that the assembled holoenzymes have two 10-nm motor domains on one end of the homodimeric rod, ending in a tail that binds vesicles and is juxtaposed to the associated light chains which may form heterodimeric coiled-coils with the heavy chains. The kinesin-II heterotrimer (see reference 26 for bibliography; *lower panel*) consists of 1 mol each of two different kinesin related polypeptides, Sp KRP85 and SpKRP95 ($M_r$ on gels 85 kD and 95 kD) with amino-terminal motor domains, central α-helical domains containing a 30–residue discontinuity that directs formation of heterodimeric coiled-coil rods, and globular presumptive cargo-binding domains at the COOH-terminal end, plus 1 mol of the accessory SpKAP115 polypeptide ($M_r$ on gels, 115 kD) that is predicted to be globular and highly α-helical. EMs suggest that the assembled holoenzyme consists of two 10-nm motor domains (*left*) linked to a heterodimeric rod, ending in a tail where SpKAP115 is localized. The figure compares kinesin and kinesin-II from sea urchin eggs because both holoenzymes have been purified from this system, but it also incorporates findings obtained using many other systems as well (4, 9). (The figure was kindly assembled by Karen Wedaman.)

ized 85 kD and 95 kD kinesin-related motor subunits, SpKRP85 and SpKRP95, together with a 115-kD "accessory" subunit, SpKAP115 of unknown function (where KAP = kinesin accessory protein).

The two motor subunits of kinesin-II are members of the Kif3 family (9), a family of motor polypeptides sharing >60% amino acid sequence identity within their motor domains (17). cDNAs that encode partial (22) and full-length (1, 13, 16) Kif3 family motor subunits were first identified using PCR screens in *Drosophila melanogaster* and mouse and subsequently the corresponding recombinant polypeptides were shown to move to the plus ends of MTs at 0.3–0.6 μm/s (13, 16, 29). Two additional members of the Kif3 family were discovered through the analysis of organisms carrying mutations in the *Fla10* and *Osm3* genes in *Chlamydomonas reinhardtii* and *Caenorhabditis elegans*, respectively (14, 19, 23, 25).

Sequence analysis suggests that polypeptides in the Kif3 family consist of an $NH_2$-terminal globular motor domain linked by an α-helical stalk to a second globular COOH-terminal domain, much like kinesin heavy chain (KHC). The motor domains of Kif3 family polypeptides display higher sequence identity to each other than to other members of the kinesin superfamily and, with the exception of Osm-3, there is significant sequence identity outside the motor domains (17). Sea urchin, mouse, and fly each contain at least two distinct Kif3 subunits with similar-sized stalks containing heptad repeats which are thought to form heterodimeric coiled-coils (8, 16, 17, 29), homodimerization being destabilized by unfavorable electrostatic interactions between residues of like charge lying within a discontinuity in the coiled-coils (17). This is in contrast to the motor subunits of kinesin (KHC), which readily form homodimers. The suppression of homodimer formation by the motor subunits of kinesin-II suggests that heterodimerization is functionally important, but if so it is unclear why.

The observation that sea urchin kinesin-II is a heterotrimer of 1mol SpKRP85:1mol SpKRP95: 1mol SpKAP115 (7, 8, 26) suggested that all Kif3 family members would be components of heterotrimeric complexes containing a nonkinesin-related accessory subunit (8) . In support of this hypothesis it was recently reported that a heterotrimeric complex of 1mol Kif3a:1mol Kif3b:1mol 100–105-kD polypeptide can be immunoprecipitated from mouse cytosol (29). Sequence analysis of sea urchin SpKAP115 predicts an 828–residue, 95-kD globular polypeptide rich in α-helical segments that are not predicted to form extensive coiled-coils, ending in a COOH-terminal, tyrosine-rich tail (26). EM analysis reveals that SpKAP115 is located at the COOH-terminal ends of the heterodimerized motor subunits (26), as are the accessory light chains of kinesin.

Thus, despite the lack of any sequence conservation outside the motor domains of the motor subunits or in the accessory subunits (e.g., SpKLCs vs SpKAP115), kinesin and kinesin-II display similarities in their structural organization, having two 10-nm $NH_2$-terminal motor domains linked by a protease-sensitive segment (8) to a dimeric rod that ends in a tail where the accessory subunits are located. These two kinesins also display similar functional properties in vitro, including ionic-strength–dependent conformational changes (4, 26), AMPPNP-enhanced, ATP-sensitive MT binding (4, 6–9, 24, 26), and plus-end–directed motion

along MTs at approximately half a micron per second (4, 8, 13, 16, 24, 29). Although kinesin and kinesin-II are both thought to move membranous cargoes to the plus ends of MTs in cells, the mechanism of cargo binding, the significance of motor subunit heterodimerization, the role of the conformational changes, and the function of the accessory subunits all remain to be determined.

### *Transport Functions of Kinesin-II in Axons, Axonemes, and Spindles*

Kinesin-II, like kinesin, may function as a neuronal vesicle transport motor that can also be deployed elsewhere. A neuronal function for kinesin-II in the fruitfly, for example, is suggested by the observation that KLP68D mRNA is maternally loaded, disappears at the time of cellularization, and is replaced by transcripts that are restricted to subsets of neurons in the peripheral and central nervous system in a manner consistent with a role in axonal outgrowth and synapse formation (16). Worms subjected to germline transformation with an Osm3:lacz construct specifically express the corresponding fusion protein in chemosensory neurons of larvae and adults (23). In mouse, Kif3a and Kif3b appear to be coordinately expressed, with the highest levels of Kif 3 transcripts and polypeptides being detected in brain and brain plus testis, respectively (1, 13, 29). Antibodies to Kif3a and Kif3b bind specifically to 90–160-nm neuronal vesicles that differ from synaptic vesicle precursors (29). The antibodies stain the cell bodies, axons, and dendrites of neurons (13, 29), sometimes in a "dotlike manner" (13). As with kinesin (11), in ligated peripheral mouse neurons, Kif3a and Kif3b both associate with vesicle-like structures that accumulate on the side of the ligature proximal to the cell body (13, 29). Together, these expression and localization data are consistent with a role for kinesin-II in the anterograde fast axonal transport of a subset of membrane-bounded vesicles.

A role in neuronal cell function is further supported by studies of the *osm-3* mutant in *C. elegans*, which displays defects in osmotic avoidance, chemotaxis, production of octopamine, and *dauer* larva formation. This suggests a role in chemosensation, consistent with the expression of Osm-3 protein in bundles of ciliated chemosensory neurons that are surrounded by an open channel formed by "sheath" and "socket" cells (2, 15, 19, 23). In wild-type worms, the MT-containing cilia forming the sensory endings of the neurons are immotile, and, distal to the channel opening, these cilia are surrounded by a matrix material. The surrounding sheath cells synthesize the matrix material, package it into vesicles, and then deliver these vesicles to the cell membrane for exocytosis and secretion of the matrix into the channel lumen (2, 15). In *osm-3* mutants, exocytosis of the matrix material is impaired and matrix-filled vesicles accumulate in the sheath cell cytoplasm, consistent with a role for Osm-3 protein in transporting matrix-containing vesicles to the sheath cell surface. *Osm-3* mutants also lack the distal segments of the neuronal cilia lying at the channel opening, suggesting that Osm-3 transports some essential ciliary stabilizing factor(s) out along MTs of the axons and axonemes of the chemosensory neurons (2, 15, 23).

Kinesin-II may function as a membrane traffic motor in motile axonemes as well, based on studies of the *fla-10* mutation in *C. reinhardtii*, which causes defects in flagellar assembly and stability. The mRNA that encodes FLA-10 in wild-type algae is upregulated at the end of mitosis when flagellar assembly normally occurs or after experimental deflagellation, and the corresponding protein is present in isolated axonemes (25). Immunoelectron microscopy has revealed that FLA-10 is localized between the outer doublet MTs and the flagellar membrane, where it is needed for the bidirectional movement of electron dense rafts beneath the flagellar membrane and of polystyrene beads on the flagellar membrane (14). Therefore FLA-10 may transport membrane-bound or raft-associated cargoes that include axoneme-stabilizing factors to their site of assembly at the axonemal tip (14, 25).

Kinesin-II is associated with punctate, detergent-sensitive vesicle-like structures in the anaphase spindle interzone of sea urchin embryonic cells (10). The aforementioned results with FLA-10 suggest that this protein may be stockpiled for use in ciliary assembly which occurs at the blastula stage, although we favor the idea that kinesin-II, like kinesin, delivers vesicles to the cell surface (10). For example, kinesin-II could deliver new cell membrane and extracellular matrix components to the cell surface at the developing cleavage furrow during cytokinesis. There is evidence from studies of *Xenopus* that new membrane for cytokinesis is derived from cytoplasmic precursor vesicles that are inserted into the membrane (5).

## *Conclusions*

Comparison of the structure and domain organization of kinesin and kinesin-II (Fig. 1) reveals that these two membrane traffic motors have similar overall structures with two motor domains at one end connected by a rod to a domain where the accessory subunits are localized. This overall structural similarity exists despite the lack of significant sequence conservation outside the motor domains or in the accessory subunits of these two motor complexes. The rods represent sites of motor subunit homodimerization in kinesin and heterodimerization in kinesin-II, but the functional significance of kinesin-II motor subunit heterodimerization remains to be determined. We hypothesize that the COOH-terminal tails of the motor subunits bind to receptors on the vesicular cargo of kinesin (20) and kinesin-II and that the accessory subunits target the motor subunits to specific classes of vesicles. For example, SpKLCs may target kinesin to a receptor on vesicles in the sea urchin spindle asters whereas SpKAP115 may target kinesin-II to a different receptor on vesicles in the spindle interzone. Obviously, understanding the molecular basis of the targeting and binding of kinesin and kinesin-II (as well as other motors) to their specific cargoes is an important area for future study. Finally, it would be interesting to know how many axonal vesicle transport motors are deployed for less well understood forms of vesicular transport such as those that occur in mitotic spindles. Interestingly enough, three of the five mouse neuronal Kifs (1) (or their homologues) can associate with spindle vesicles, namely Kif5 and Kif3 which are motor subunits of kinesin and kinesin-II, and also Kif4 (18), suggesting that the use of axonal transport motors for vesicle motility in spindles may be common.

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