CMLS, Cell. Mol. Life Sci. 56 (1999) 217–226
1420-682X/99/040217-10 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Kinesin-II, the heteromeric kinesin

D. G. Cole

Department of Microbiology, Molecular Biology and Biochemistry – LSS142, University of Idaho, Moscow (Idaho 83844-3052, USA), Fax + 1 208 885 6518, e-mail: dcole@uidaho.edu

Abstract. The kinesins constitute a large family of motor proteins which are responsible for the distribution of numerous organelles, vesicles and macromolecular complexes throughout the cell. One class of these molecular motors, kinesin-II, is unique in that these proteins are typically found as heterotrimeric complexes containing two different, though related, kinesin-like motor subunits, and a single nonmotor subunit. The heteromeric nature of these kinesins appears to have resulted in a class of combinatorial kinesins which can 'mix and match' different motor subunits. Another novel feature of these motors is that the activities of several kinesin-II representatives are essential in the assembly of motile and nonmotile cilia, a role not attributed to any other kinesin. This review presents a brief overview of the structure and biological functions of kinesin-II, the heteromeric kinesin.

Key words. Kinesin; motility; microtubule; cilia; flagella.

Introduction

The kinesins represent a diverse group of microtubule-based motor proteins that power a myriad of cellular transport events [1, 2]. The founding member of this large family, conventional kinesin, is the best characterized with regard to both molecular structure and activities. Originally purified from giant squid axon [3], sea urchin eggs [4] and bovine brain [5], kinesin was found to be a 350–400 kDa $\alpha_2\beta_2$ tetrameric complex that moves in vitro toward the plus ends of microtubules (MTs) at speeds of 0.6–0.8 μm/s. Each α heavy chain contains an amino terminal 350-amino acid force-generating motor domain that has become the defining feature of the kinesin family of proteins. Outside the motor domains, the two heavy chains homodimerize to form an α-helical coiled-coil stalk domain [6] and then diverge at their carboxyl-termini where the heavy chains associate with two globular β light chains [7].

Based on sequence homology with the kinesin motor domain, hundreds of kinesin-like proteins have been identified [1, 2]* with at least three dozen kinesins having been found in the mouse alone. Presently, many of these are grouped into 9 or 10 distinct classes based on motor domain sequence alignments [2]. Outside the motor domain, sequences of the various kinesins are richly diverse, likely reflecting the fact that different kinesins interact with different cargos [8]. Within most classes, some, but not all kinesins share sequence similarity outside the motor domain. However, despite the many genes sequenced, few kinesins have been isolated from native tissues, and thus we are only beginning to unveil a rich diversity in kinesin quaternary structure. Several kinesins, such as the Unc-104-related murine KIF1A and KIF1B, are believed to be monomeric when not bound to cargo [9, 10]. Most kinesin gene products, however, are believed to be subunits of multimeric complexes [1, 2, 11]. Conventional kinesin, for example, is generally purified as an $\alpha_2\beta_2$ heterotetrameric complex as described above [3–5], whereas the bimC-related *Drosophila* $DmKRP_{130}$/KLP61F is isolated as a bipolar homotetrameric complex [12–14]. Another example of a multimeric kinesin complex is that of the heterotrimeric or heteromeric kinesins, the subject of this review.

The heteromeric kinesins, also known as kinesin-II [15], KIF3 [16] or simply the heterokinesins [17], are plus end-directed motors ($\sim$0.4 μm/s) which have been purified as multimeric complexes from such diverse cell types as sea urchin egg [18–21], mouse neurons [22–25] and green algae [26]. The first of these to be purified, sea urchin $KRP_{85/95}$ [18], is now known as kinesin-II [15, 21], whereas the mouse complex is known as KIF3A/

* See the kinesin homepage at http://www.blocks.fhcrc.org/~kinesin/index.html

3B-KAP3 [25] and the *Chlamydomonas* complex is known as FLA10 kinesin-II [26]. In all three cases, these heteromeric complexes contain three subunits: two kinesin-like motor subunits encoded by separate, albeit related, kinesin-like genes termed KIF3 and one non-motor subunit termed KAP3 (kinesin associated polypeptide 3) or KAP115. This review summarizes our understanding of the molecular structure and in vivo functions of the heteromeric kinesins.

Structural organization

Our understanding of the molecular structure of the heteromeric kinesins has come primarily from biochemical, sequence and electron microscopic analyses of sea urchin egg kinesin-II [18–21, 27] and mouse neuronal KIF3A/3B-KAP3 [22–25]. Indeed, it was the characterization of these two holoenzymes that established the heteromeric nature of this class of kinesins. Kinesin-II is purified from sea urchin eggs as a 300-kDa heterotrimeric complex [18], whereas KIF3A/3B-KAP3 is immunoprecipitated in a molar ratio of approximately 1:1:1 from mouse neuronal tissue [24, 25]. In both systems, all three subunits of each heterotrimeric complex have been cloned and characterized, and expressed and native complexes have been carefully examined with electron microscopy. For these reasons, sea urchin kinesin-II and mouse KIF3A/3B-KAP3 serve as a structural template for the heteromeric kinesins.

The motor subunits found in these mouse and echinoderm heteromeric kinesins are encoded by two distinct KIF3-like kinesin genes, KIF3A(KRP85) and KIF3B-(KRP95). Interestingly, full-length KIF3A and KRP85 are much more similar to each other than they are to either KIF3B or KRP95. KRP85, for example, is 72.5% identical to KIF3A, but only 51.6% identical to KRP95. A similar relationship exists between KIF3B and KRP95, suggesting that these two mouse and echinoderm heteromeric kinesins are homologous complexes. KIF3A/KRP85 and KIF3B/KRP95 share very similar domain organization, as illustrated in figure 1.

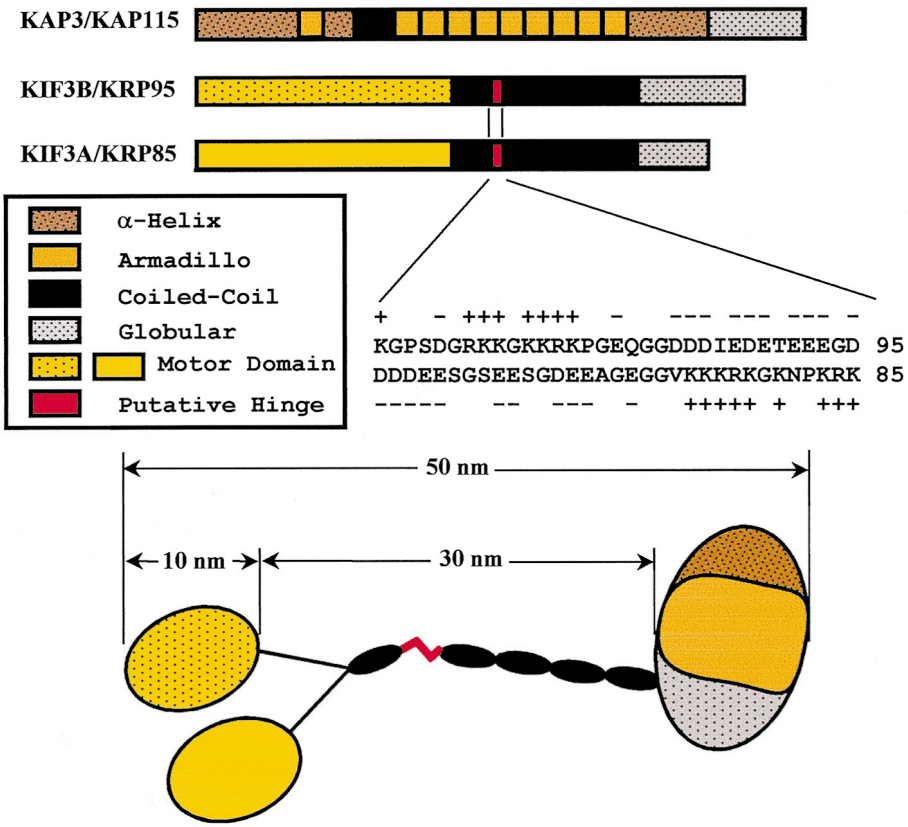

Figure 1. Domain organization of the heteromeric kinesins. The amino-terminal 350 amino acids of KIF3A/KRP85 and KIF3B/KRP95 form globular motor domains which contain both the ATP hydrolysis and microtubule binding sites. These subunits heterodimerize in an α-helical coiled-coil structure (aa 350–590) termed the stalk. KAP3/KAP115, mostly globular with 10 armadillo repeats (aa 158–199, aa 285–655), is found in association with the carboxyl-termini of the other two subunits (aa > 590). An asymmetric distribution of charged amino acids in KRP85 (aa 379–411) and KRP95 (aa 371–403) is shown.

The amino-terminal 350 amino acids form globular kinesin-like motor domains that are each approximately 10 nm across [21, 24]. The two motor subunits heterodimerize in an α-helical coiled-coil structure termed the stalk (amino acids 350–590), spanning 35–40 nm [21, 24, 27]. The structure of the carboxyl-terminal portion of the motor subunits is not well understood, but this region is thought to associate with KAP3/KAP115, the $\sim$100 kDa nonmotor subunit, to form a large globular region believed to function in cargo binding [21, 25].

The nature of the heterodimerization of the two motor subunits has been studied in both the mouse and echinoderm. Coexpression of KIF3A and KIF3B in a baculovirus expression system yielded a strong preference for the formation of the KIF3A/3B heterodimer over the formation of either homodimer [24]. Likewise, coexpression of KRP85 and KRP95 in a reticulocyte lysate system yielded a very strong preference for the formation of the KRP85/95 heterodimer over formation of either homodimer [27]. It has been suggested that a highly charged stretch of 33 amino acids in the stalk domain is responsible for the favoring of heterodimerization [27]. In KRP85, this sequence corresponds to amino acids 379–411, which contain 11 negatively charged residues in the first 18 positions, followed by 9 positively charged residues in the last 12 positions (fig. 1). Conversely, the corresponding KRP95 sequence spans amino acids 371–403 and contains 8 positively charged residues and 1 negatively charged residue in the first 14 positions, followed by 11 negatively charged residues in the final 17 positions. Alignment of these two sequences suggests a strong ionic interaction between the positively and negatively charged regions which should favor heterodimerization and disfavor homodimerization. Very similar asymmetric charge distributions are also present in the corresponding regions of the KIF3A and KIF3B sequences, suggesting a common mechanism for guiding heterodimerization of kinesin-II motor subunits. It should be noted, however, that sequence analysis reveals that certain members of the KIF3 family, such as *Chlamydomonas* FLA10 and *Caenorhabditis elegans* OSM3 do not share similar charge distributions and, therefore, might be capable of forming homodimers in vivo.

The third subunit of the heteromeric kinesins is a nonmotor, kinesin-associated polypeptide termed KAP3 or KAP115 [21, 25]; multiple isoforms of KAP3, believed to arise from alternative splicing of the carboxyl-terminus region, have been identified in the mouse [25]. Sequence analysis predicts that this protein forms primarily α-helical secondary structure which contains up to 10 armadillo repeats [28], degenerate 42-amino acid repeats that form α-helical coils [29]. In contrast, conventional kinesin nonmotor light chains contain tetratrico peptide repeats (TPRs) [30], degenerate 34-amino acid repeats that also form α-helical coils [31]. Interestingly, crystal structure analysis of representative armadillo [32] and TPR [33] domains reveals that both motifs generate similar superhelical structures. Since armadillo and TPR motifs are generally thought to mediate protein-protein binding [29, 31–33], it is tempting to hypothesize that these domains in kinesins function to transiently bind protein cargo. It should also be noted that the carboxyl-terminal 120 amino acids of KAP3/KAP115 contain numerous tyrosine residues, some of which are putative substrates for tyrosine kinases. Indeed, bacterially expressed SMAP, a human homologue of KAP3, can be phosphorylated by exogenous src kinase [34]. It is tempting, therefore, to speculate that the carboxyl-terminal region of the nonmotor subunit may serve as a phosphorylation-dependent cargo-binding regulatory domain.

There is evidence that kinesin-II holoenzymes are capable of undergoing a gross conformational change. Sea urchin kinesin-II, for example, undergoes a salt-dependent change in its sedimentation coefficient of 9.8 S to 8.0 S [21], consistent with the idea that kinesin-II is in a folded conformation in low salt and an extended conformation in high salt. Indeed, both folded and extended forms of sea urchin kinesin-II have been visualized by electron microscopy [21]. Since this conformational transition occurs at approximately physiological ionic strength, it is likely that this conformational shift can occur in vivo: Similar salt-dependent conformational changes (10 S to 6 S) are observed with both conventional kinesin [35] and smooth muscle and nonmuscle myosin-II [36, 37] and are thought to play a role in regulation of motor activity. Though the molecular nature of kinesin-II folding has not been determined, sequence analysis suggests the existence of a hinge site at the aforementioned putative heterodimerization site (amino acids $\sim$370–400); the corresponding sequences from KRP85 and KRP95 are shown in figure 1. Coiled-coil predictions [38] for KIF3A/KRP85 and KIF3B/KRP95 strongly suggest that the coiled-coil structure is disrupted in this short span by multiple proline and glycine residues [19, 23, 24, 27]. A similar break in the predicted α-helical coiled-coil is also found near the middle of the stalk domain of conventional kinesin heavy chain, which corresponds to a hinge identified by electron microscopy [39]. Alternatively, a conformational change may occur within the large, poorly understood, globular tail region of kinesin-II. Regardless of the mechanism, folding may serve an in vivo function; one possibility is that folding may render kinesin-II nonmotile unless bound to cargo. Binding of cargo to the tail region could induce unfolding which in turn could activate the kinesin-II complex, enabling it to move along MTs. Since *Chlamydomonas*

FLA10 kinesin-II also undergoes a similar conformational change [D. Cole, unpublished observations], it seems likely that other heteromeric kinesins will also be capable of similar salt-sensitive folding.

A combinatorial kinesin

Presently, the heteromeric kinesins are the only class of kinesins found to contain different motor subunits in the same complex. Until recently, the biological function of heterodimerization of the motor subunits had been a mystery. A credible solution has now been uncovered: kinesin-II is a combinatorial protein complex capable of existing in various combinations of motor and nonmotor subunits. Three different groups independently identified KIF3C, a third KIF3-like gene product identified in the rodent [40–42]. KIF3C is more similar to KIF3B (61.2% identical) than KIF3A (40.6% identity) [41]. Immunoprecipitation of neuronal extracts reveals that KIF3A and KIF3C, but not KIF3B, is immunoprecipitated with specific antibody against KIF3C and that KIF3A and KIF3B, but not KIF3C, is immunoprecipitated with specific antibody against KIF3B [40, 41]. This indicates that KIF3C and KIF3A are found in the same complex, but KIF3C and KIF3B are not. KIF3B and KIF3C also show differences in tissue distribution [40–42]. Sequence analysis of the putative heterodimerization domain reveals that an asymmetric charge distribution in KIF3C is quite similar to KIF3B (fig. 1), which should favor heterodimerization of KIF3A and KIF3C, but should disfavor formation of a KIF3B/KIF3C heterodimer. Interestingly, a species of neuronal KIF3C is partially purified in the absence of either KIF3A or KIF3B. KIF3C, therefore, may also exist as either a homodimer or a heterodimer with yet another, presently unidentified, KIF3-like kinesin [41]. In addition to multiple KIF3 genes, the kinesin-II accessory polypeptide, KAP3, is encoded by a single gene but expressed as multiple isoforms [25], further increasing the potential number of unique heterotrimeric complexes. Mixing and matching different kinesin-II motor and nonmotor subunits provides an organism with the ability to produce a variety of motors that can be specialized for diverse functions.

Biological functions

Heteromeric kinesins have been found in such diverse organisms as green algae, ciliated protozoa, nematodes, echinoderms and assorted vertebrates, where they appear to have been adapted for multiple tasks (table 1). In more complex organisms, such as nematodes, insects and vertebrates, these kinesins are clearly enriched in neuronal tissues and cells where they are thought to serve as a fast axonal transport motor. In mammals, however, kinesin-II is highly enriched in the testes and is found, to a lesser extent, in many other tissues, suggesting nonneuronal functions. In the frog, kinesin-II is found in melanophores and unfertilized eggs. In echinoderms, kinesin-II is found in both embryos and sperm, where it is localized to the mitotic spindle and the midpiece and flagellum, respectively. Kinesin-II in the biflagellate green algae *Chlamydomonas* is found both around the basal body region and along the flagellum in vegetative cells and associated with the mitotic spindle of dividing cells. This diversity of distribution suggests that these kinesins perform different tasks in different cell types, a hypothesis supported by an expanding wealth of evidence. In the face of this diversity, however, a common theme has arisen regarding the function of a number of kinesin-II motors; genetic phenotypes from *Caenorhabditis*, *Chlamydomonas*, mouse and *Tetrahymena* combined with antibody microinjection experiments in the sea urchin clearly demonstrate that kinesin-II plays an essential role in the assembly and function of motile and nonmotile ciliary structures.

With respect to in vivo function, one of the best-characterized heteromeric kinesins is *Chlamydomonas* FLA10 kinesin-II (reviewed in Rosenbaum et al., 1999) [74]. The *FLA10* gene encodes one of two KIF3-related motor subunits of the FLA10 kinesin-II holoenzyme [26, 52]. *Fla10-1*, which contains a motor domain point mutation at amino acid residue 329 [54], is one of a series of temperature-sensitive flagellar assembly mutants unable to form flagella when grown at the restrictive temperature (32 °C) [49, 50]. When *fla10-1* mutants are grown at the permissive temperature (22 °C), they possess full-length, motile flagella which are lost by resorption when the cells are shifted to the restrictive temperature [51]; prior to flagellar resorption, intraflagellar transport (IFT) ceases [53]. IFT, a bidirectional movement of particles beneath the flagellar membrane, consists of plus end-directed anterograde movement out to the flagellar tip (2 μm/s) and minus end-directed retrograde movement back to the cell body (3.5 μm/s) [75]. The IFT particles, identified in electron microscopy as long, narrow nonmembranous arrays termed rafts bridge the B-tubule of the outer doublet microtubules and the flagellar membrane [75]. With the disappearance of IFT in *fla10-1* flagella (shifted to 32 °C), there is a concomitant loss of both FLA10 kinesin-II [26] and the IFT particles as identified by electron microscopy [53]. These results argue that kinesin-II, a plus end-directed motor, is the anterograde IFT motor. Supporting evidence for this model has come from analysis of a retrograde IFT mutant, *fla14*, a null mutant for an 8-kDa light chain (LC8) [76] found associated with both axonemal and cytoplasmic dyneins [77, 78]. The *fla14*

Table 1. Table 1 Kinesin-II proteins.

Protein	Composition		In vitro motility (μm/sec)	Tissue/subcellular localization	Biological function	References
	Subunit	Size (aa, M_r)				
A. Heteromeric complexes						
C. elegans						
CeOSM3*	CeOSM3	671, 76k	nd	chemosensory neurons: cell bodies, dendritic cilia	ciliogenesis in select sensory neurons	43–47
CeKinesin-II	CeKRP85	~640, 74k	nd†	chemosensory neurons: cell bodies, dendritic cilia	anterograde IFT	47, 48
	CeKRP95	782, 89k				
	CeKAP	690, 78k				
C. reinhardtii						
FLA10 Kinesin-II	CrKRP85	unk, 85k	nd	basal body region; along flagella; mitotic spindle	assembly and maintenance of flagella; anterograde IFT motor	26, 49–57
	CrFLA10	786, 87k				
	CrKAP	unk, 100k				
M. musculus						
KIF3A/3B-KAP3	MmKIF3A	701, 80k	0.3–0.6	brain; testis; neuronal: cell bodies, axons, dendrites	fast axonal transport; ciliogenesis; left-right asymmetry	22–25, 58, 59
	MmKIF3B	747 85k				
	MmKAP3	772/793, 89/91k				
KIF3A/3C	MmKIF3A	701, 80k	nd	brain; retina: cell bodies, dendrites	nd	40
	MmKIF3C	796, 90k				
	KAP?					
R. norvegicus						
KIF3A/KIF3C	RnKIF3A	unk, 87k	nd	brain; retina; lung; muscle; neuronal: cell bodies, axons, dendrites	nd	41, 42, 60
	RnKIF3C	796, 90k				
	KAP?					
S. pupruratus						
Kinesin-II	SpKRP85	699, 79k	0.4	embryos: midzone of mitotic spindle	ciliogenesis	15, 18–21, 27, 61, 62
	SpKRP95	742, 84k				
	SpKAP115	828, 95k		sperm: midpiece and length of flagellum	nd	63
X. laevis						64–66
Kinesin-II	XlKRP85	unk, 85k	nd	melanophores: melanosomes	dispersion of melanosomes	
	KlKLP3	744, 85k				
	XlKAP	unk, 110k		ER/golgi interface	ER to golgi membrane transport	67
B. Other kinesin-II related proteins						
D. melanogaster						
	DmKLP68D	784, 88k	0.3	central nervous system; chordotonal organ	fast axonal transport? ciliogenesis?	68 68
	DmKLP64D		nd	nd	nd	69‡§
H. sapiens						
	HsKIF3A	702, 80k	nd	nd	nd	
	HsKIF3C	792, 89k	nd	brain	anterograde transport?	70, 71
	HsKIF3X	unk	nd	nd	interacts with MLK2 kinase	72
	HsKIF3B	747, 85k	nd	nuclei (COS-7 cells)	nd	73‖
	HsSMAP	792, 91k	nd	nuclei (COS-7 cells)	interacts with Smg GDS	34
				ER	interacts with MLK2 kinase	72
				nd	interacts with HCAP	73
T. thermophila						
	TtKIN1	735, 85k	nd	cilia	ciliogenesis	¶
	TtKIN2	697, 82k	nd	nd	ciliogenesis	¶

*The native CeOSM3 complex may be homodimeric. †The in vivo velocity of the GFP-KAP construct is 0.65 μm/s. ‡GenBank accession number: AAA28657. §GenBank accession number: AAC72294 ‖GenBank accession number: AB002357. ¶Brown et al., Mol. Biol. Cell **9S:** Abstract 173.

mutant retains apparently normal anterograde IFT in the absence of retrograde IFT [76]. In the absence of retrograde IFT, the flagella are only half-length, nonmotile and congested with large amounts of both IFT particles and FLA10 kinesin-II [76]. Recently identified mutants lacking the cytoplasmic dynein heavy chain 1b (DHC1b) display even more severe phenotypes with very short, bulbous flagellar stubs that contain nearly all the cell's pool of IFT particles [79, 80]. Taken together, the above findings are consistent with the model that kinesin-II transports IFT particles out to the flagellar tips, whereas cytoplasmic dynein 1b transports IFT particles back to the cell body.

What is the purpose of IFT? Since all mutations that adversely affect IFT also adversely affect flagellar assembly, it seems probable that IFT functions, at least in part, in the construction of the flagellum. Assembly of ciliary and flagellar organelles presents a transport problem to the cell because elongation occurs at the distal tip and not at the base [81]. Following synthesis in the cell body, sometimes into relatively large 'prefabricated' complexes [82] [D. R. Diener et al. (1996) ASCB Meeting, San Francisco, Abstract 273], axonemal precursors must then be delivered to the flagellar tip for assembly into the growing axoneme. The role of IFT in flagellar assembly may be to serve as a transport mechanism for axonemal precursors. This hypothesis is supported by elegant dikaryon rescue experiments in which *fla10* cells were mated with *ida4-fla10* double mutants, which are missing a subset of inner arm dynein complexes in the axoneme [55]. Mating of these two cell lines results in fused cells which share a common cytoplasm and have four flagella, two of which are lacking inner arm dynein complexes. Following mating at the permissive temperature, where IFT is normal, the *fla10* cytoplasm supplies inner arm dynein to the two *ida4-fla10* flagella; addition of the inner arm dyneins begins at the distal tips of the *ida4-fla10* flagella and progresses in toward the cell body. In contrast, following mating shortly after a shift to the restrictive temperature, where IFT disappears, no addition of inner arm dynein occurs in the *ida4-fla10* flagella. This is consistent with the idea that FLA10 kinesin-II-mediated IFT actively transports inner arm dynein complexes from the base of the flagellum out to the flagellar tips. Further evidence that IFT functions in axonemal assembly comes from analysis of the IFT particles.

The IFT particles in *Chlamydomonas* are composed primarily of two large, 16 S protein complexes: Complex A (550 kDa), consisting of 4–5 unique polypeptide subunits, and Complex B ($\sim$ 700 kDa), consisting of 11 unique polypeptide subunits [26, 56, 57]; the polypeptides range from 20 to 172 kDa. Partial protein and complementary DNA (cDNA) sequence of the individual *Chlamydomonas* IFT particle polypeptides, p52 and p172, were used to identify homologous proteins, OSM6 and OSM1, respectively, in *Caenorhabditis elegans* [26, 83]. Originally isolated due to an inability to sense changes in osmotic stress, the *osm6* and *osm1* mutants share specific and severe structural defects in the nonmotile $9 + 0$ sensory cilia found at the dendritic endings of chemosensory neurons [43, 44]. Expression of a GFP-OSM6 fusion protein showed that OSM6 is not only expressed in chemosensory neurons but is enriched at the ciliated dendrites of these cells [83]. Furthermore, the striking direct visualization of the anterograde intraflagellar transport of this GFP-OSM6 fusion protein within the sensory cilia of living *C. elegans* was recently reported [48]. These findings clearly indicate (i) IFT occurs in animal sensory cilia and (ii) that the p52/OSM6 protein is a conserved protein in the IFT rafts.

The role of nematode kinesin-II in ciliary assembly appears to be more complex than that seen in *Chlamydomonas*. Two separate kinesin-II complexes, CeKinesin-II and OSM3, are enriched in the ciliated dendrites of chemosensory neurons [47]. CeKinesin-II is a typical heterotrimeric complex consisting of two motor subunits, CeKRP85 and CeKRP95, and one nonmotor subunit, CeKAP [47]. OSM3 is either homodimeric or heterodimeric with no detectable kinesin-II-associated polypeptide or KAP [47]. The chemosensory ciliary defect in the *osm3* mutant is similar, though less severe, than that observed in the *osm1* and *osm6* mutants [43, 44], suggesting that OSM3 acts to transport the IFT proteins, OSM1 and OSM6, in the same way that FLA10 kinesin-II transports the analogous IFT proteins, p172 and p52, in the *Chlamydomonas* flagellum. However, a GFP-CeKAP fusion protein was shown to move to the tip of the sensory cilia at the same velocity observed for a GFP-OSM6 fusion protein (0.65 μm/s) [48], suggesting that CeKinesin-II may also function as an anterograde IFT motor in nematode. However, since these experiments were not done in an *osm3* background and CeKinesin-II and CeOSM3 are equally likely to be anterograde IFT motors, it is not clear which of these two kinesins actually transports GFP-OSM6 within cilia, though it is possible that both may share this task. It will be interesting to determine if these two kinesins transport unique cargos and perform separate functions in intraflagellar transport.

Multiple kinesin-II complexes may also be functioning in the cilia of *Tetrahymena thermophila* where two KIF3-related genes, KIN1 and KIN2, have been identified [J. Brown et al. (1998) ASCB Meeting, San Francisco, Abstract 173]. In this report, single deletion mutants in either KIN1 or KIN2 failed to result in a strong phenotype. A double-knockout mutant, however, missing both KIN1 and KIN2, displayed a very severe phenotype where ciliogenesis was completely blocked. These results are consistent with the idea that KIN1 and KIN2 are found in separate kinesin-II complexes and that both complexes perform overlapping functions regarding ciliary morphogenesis.

A role for kinesin-II in IFT and ciliary assembly also appears to be conserved in the echinoderms. Early work with sea urchin eggs revealed that kinesin-II is maternally loaded [18] and that following fertilization, kinesin-II levels stay fairly constant during the first few divisions until dropping off during the mid- and late blastula stages [61]. During mitosis, kinesin-II is localized to detergent-sensitive particles in the interzone of

the anaphase spindle [61]. These data originally suggested a possible role in mitosis or cell division of the early embryo. However, when K2.4, a monoclonal antibody raised against the KRP85 subunit, was microinjected into one- and two-cell embryos, the only detectable phenotype was observed several cell divisions later when the blastula were unable to complete ciliogenesis [62]. Short, nonmotile cilia of only 7 μm were present in the K2.4-microinjected cells. Ultrastructural analysis of these cilia revealed $9 + 0$ axonemes that were missing the central pair MTs and varying amounts of axonemal components such as dyneins and radial spoke complexes [62]. Frequently, the tips of these short cilia were bulbous, which was interpreted as a build-up of excess membrane, suggesting that delivery and incorporation of flagellar membrane occurs in the absence of active kinesin-II. The involvement of kinesin-II with ciliogenesis is particularly interesting, given that the only other echinoderm cell type known to contain kinesin-II is sperm [63]. Although it is not present in high concentrations, kinesin-II is found primarily around the midpiece and, to a lesser extent, along the length of the flagellum. The antibody-blocking experiments in the embryo suggest that kinesin-II may also be involved in the assembly of the flagellum during spermatogenesis, but its presence in fully mature and active sperm implies a functional role beyond axonemal assembly.

Studies in vertebrates have revealed that the heteromeric kinesins have been adapted to perform a variety of functions. Mouse neuronal KIF3A/3B-KAP3, for example, is highly enriched in the central nervous system where it is found associated with membrane-bounded vesicles that undergo fast axonal transport but are distinct from synaptic vesicles [22–25]; it is likely that KIF3A/3B-KAP3 is responsible for the axonal transport of these vesicles. KIF3A/3B-KAP3 is also abundant in the mouse testis where it may play a role in spermatogenesis [24]. This idea is supported by the recent finding that kinesin-II localization in rat testis is essentially limited to spermatid tails [84].

Kinesin-II-mediated IFT also appears to function in the vertebrate retina. Vertebrate photoreceptors are modified sensory neurons where the light-harvesting rod outer segment is a highly specialized nonmotile cilium. The proximal portion of the cilium, a $9 + 0$ axonemal structure known as the connecting cilium, connects the rod outer segment to the rod inner segment and is thought to act as a conduit through which material passes. Immunolocalization studies have shown that kinesin-II is strongly enriched at the base of the connecting cilium in the fish [85] and the rat [60]. Raftlike structures similar to the *Chlamydomonas* IFT rafts [53, 75] can also be seen in electron micrographs of the vertebrate connecting cilium [74, 86]. Lastly, targeted knockout of KIF3A in the mouse retina resulted in degeneration of the rod outer segment [J. R. Marszalek et al. (1998) ASCB Meeting, San Francisco, Abstract 75]. It seems likely that kinesin-II-mediated IFT is involved with both assembly and maintenance of the rod outer segments.

KIF3A and KIF3B are also essential in the early development of the mouse [58, 59]. Null mutants for murine KIF3B, generated via gene targeting, could not survive past 12 days postcoitum (dpc) and displayed slow growth and gross structural defects [58]. Interestingly, left-right asymmetry was randomized in these embryos [58]. This randomization may be due to a lack of nodal cilia which normally decorate nodal cells by 7.5 dpc; the lack of nodal cilia implies that KIF3B is part of a kinesin-II which functions via IFT in nodal ciliogenesis. It should be noted that the appearance of nodal cilia precedes the asymmetric expression of several genes (reviewed [87]); this asymmetric expression is disrupted in the KIF3B null mutant [58]. Contrary to the central dogma that nodal cilia are immotile, Nonaka et al. [58] showed that (i) nodal cilia generate a vortical beating pattern and (ii) that nodal cell beating causes a net leftward movement of exogenous fluorescent latex beads within the extraembryonic fluid; the authors suggest that nodal cilia might function to generate a left-biased gradient within the extraembryonic fluid of an unknown left-right signalling factor. In a similar study, Marszalek and co-workers [59] found that KIF3A null embryos also die very early (< 11 dpc), display multiple gross structural defects, randomization of left-right asymmetry and lack nodal cilia at 7.5 dpc. These findings strongly suggest that KIF3A and KIF3B, as a kinesin-II complex, are functioning together early in mammalian embryogenesis to generate nodal cilia and to establish left-right asymmetry.

Considerable evidence, presented above, has linked kinesin-II activity to the morphogenesis of eukaryotic motile and nonmotile cilia. There may be a secondary, nonassembly, nonmaintenance function for kinesin-II-mediated IFT: signal transduction. Robust IFT was recently visualized in fully grown, nonmotile *C. elegans* chemosensory cilia [48]. If these full-grown cilia are no longer undergoing active assembly, then perhaps IFT is involved with ciliary function. Identified functions of these sensory cilia are chemosensation and osmotic stress avoidance [43]. Kinesin-II might mediate these sensations via transport of MLK2, a MAP kinase kinase kinase [72]. A yeast two-hybrid screen using MLK2 identified a human KIF3 (HsKIF3X) and SMAP, the human KAP3 homologue, as MLK2-binding proteins [72]. Transfection of MLK2 in COS cells resulted in constitutive activation of three different MAP kinase cascades which are activated by extracellular stimuli: (i) the JNK (c-dun N-terminal kinase) MAP kinase cascade; (ii) ERK, the extracellular signal-

regulated kinase; and (iii) p38, [72]. These, or similar, kinase cascades could be operating in conjunction with sensory cilia. It should also be noted that JNK and p38 are both strongly activated in mammalian cells that are exposed to osmotic shock [88] and that the phenotype of the *C. elegans* kinesin-II mutant, *osm3*, includes the failure to avoid osmotic stress [43, 45]. The ultrastructural phenotype of *osm3* is considerably weaker than for *osm6* or *osm1*, indicating that some sensory cilium assembly proceeds in the absence of OSM3; perhaps CeKinesin-II-mediated IFT, rather than OSM3-mediated IFT, plays the primary role in ciliary assembly in *C. elegans*. The primary function of OSM3, however, may be to mediate extracellular stimuli via an MLK2-stimulated MAP kinase cascade.

Separate studies in *Xenopus* reveal two more adaptations of kinesin-II function. *Xenopus* kinesin-II, a heterotrimeric complex consisting of XlKRP85, Xklp3 (KIF3B homologue) and XlKAP [66], is found associated with membrane-bounded pigment organelles (melanosomes) in melanophore cells [65, 66]. In a microtubule-dependent process, melanosomes are transported from the cell center to the periphery (dispersion) and vice versa (aggregation). Since *Xenopus* kinesin-II is bound to melanosomes, it was thought to be the likely plus end-directed dispersion motor [65]. This hypothesis proved correct by the generation of a dominant-negative construct of GFP-headless Xklp3 which lacked the Xklp3 motor domain [66]. Overexpression of this fusion protein specifically inhibited pigment dispersion but had no effect on aggregation. *Xenopus* kinesin-II has also been implicated in membrane transport between the endoplasmic reticulum (ER) and Golgi [67]. Localization in frog cell lines revealed that Xklp3 is associated with the Golgi apparatus and is also found dispersed in the cytoplasm [67]. Expression of dominant-negative Xklp3 fusion proteins (headless Xklp3) in these cells blocked transport of specific proteins to the Golgi, implying that *Xenopus* kinesin-II functions in ER/Golgi membrane trafficking [67]. Additional clues to kinesin-II function in ER/Golgi trafficking have come from a two-hybrid study which identified an interaction between SMAP, the human kinesin-II-associated polypeptide, and Smg GDS, a small G protein regulator [34]. Smg GDS regulates the interaction of small G proteins, including Rho and Rap 1, with membranes. It is tempting, therefore, to speculate that vertebrate kinesin-II may be involved with G-protein-mediated ER/Golgi vesicle trafficking. It should also be noted that the approach of expressing a headless KIF3-related protein such as Xklp3 produces a one-headed heteromeric kinesin-II complex which can produce a dominant-negative phenotype; this strategy promises to be useful in future dissections of kinesin-II functions.

And now, for something completely different, kinesin-II may also be involved with chromosomal movement. In another yeast two-hybrid screen, now using SMAP as the bait, Shimizu et al. [73] identified a human SMAP-interacting protein which they termed HCAP due to a strong homology with *Xenopus* XCAP-E (*Xenopus* chromosome-associated polypeptide). Subcellular fractionation coupled with immunolocalization in COS-7 cells indicated that SMAP and KIF3B were present in both the cytoplasm and the nucleus. A specific interaction with HCAP, however, was confirmed when both SMAP and KIF3B coprecipitated with anti-HCAP [73]. Immunolocalization during mitosis revealed that HCAP associates with mitotic spindles. Combined with the observations that echinoderm [61] and green algae [54] kinesin-II is associated with mitotic spindles, we can speculate that kinesin-II plays a role in chromosome movement during mitosis.

Conclusions

The heteromeric kinesins represent an ancient class of kinesins that must have been established prior to the time plants and animals diverged. Indeed, heteromeric kinesins from diverse organisms are involved with a seemingly ancient process (IFT) that drives ciliary morphogenesis. Over time, other kinesin-II complexes, fueled by a combinatorial mechanism, have evolved to transport a variety of additional cargos.

Future challenges include identification of all KIF3 and KAP3 isoforms and the various combinations of those isoforms in model organisms. Second, cargos need to be identified, particularly at the molecular level, for each kinesin-II complex and each biological process. These cargos are likely to vary with cell type as well as with unique combinations of KIF3 and KAP3 isoforms. Once we understand the molecular nature of the specific cargos, we will be in a better position to address the mechanism of specific kinesin-II functions.

Acknowledgments. I thank J. C. Bulinski, D. R. Diener, K. Faire, J. Gaertig, L. S. B. Goldstein, R. L. Morris, V. Muresan, B. J. Schnapp, J. M. Scholey and Z. Yang for helpful discussions and sharing data prior to publication. I also thank J. L. Rosenbaum for helpful discussions and acknowledge support from the National Institute of Health grant GM14642 to J.L.R. and the University of Idaho Agriculture Experiment Station to D.G.C.

1 Bloom G. S. and Endow S. (1994) Kinesins. Proteins Profile **1:** 1059–1116
2 Moore J. D. and Endow S. A. (1996) Kinesin proteins: a phylum of motors for microtubule-based motility. Bioessays **18:** 207–219
3 Vale R. D., Reese T. S. and Sheetz M. P. (1985) Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. Cell **42:** 39–50
4 Scholey J. M., Porter M. E., Grissom P. M. and McIntosh J. R. (1985) Identification of kinesin in sea urchin eggs and evidence for its localization to the mitotic spindle. Nature **318:** 483–486

5 Brady S. T. (1985) A novel ATPase with properties expected for the fast axonal transport motor. Nature **317:** 73–75
6 de Cuevas M., Tao T. and Goldstein L. S. B. (1992) Evidence that the stalk of *Drosophila* kinesin heavy chain is an alpha-helical coiled coil. J. Cell Biol. **116:** 957–965
7 Hirokawa N., Pfister K. K., Yorifuji H., Wagner M. C., Brady S. T. and Bloom G. S. (1989) Submolecular domain of brain kinesin identified by electron microscopy and monoclonal antibody decoration. Cell **56:** 867–878
8 Goldstein L. S. B. (1993) With apologies to Sheherazade: tails of 1001 kinesin motors. Annu. Rev. Genet. **27:** 319–351
9 Nangaku M., Sato-Yoshitake R., Okada Y., Noda Y., Takemura R., Yamazaki H. et al. (1994) KIF1B, a novel microtubule plus-end directed monomeric motor protein for transport of mitochondria. Cell **79:** 1209–1220
10 Okada Y., Yamazaki H., Sekine-Aizawa Y. and Hirokawa N. (1995) The neuron specific kinesin superfamily protein KIF1A is a unique monomeric motor for anterograde axonal transport of synaptic vesicle precursors. Cell **81:** 769–780
11 Cole D. G. and Scholey J. M. (1995) Structural variations among the kinesins. Trends Cell Biol. **5:** 259–262
12 Cole D. G., Saxton W. M., Sheehan K. B. and Scholey J. M. (1994) A 'slow' homotetrameric kinesin-related motor protein purified from *Drosophila* embryos. J. Biol. Chem. **269:** 22913–22916
13 Kashina A. S., Baskin R. J., Cole D. G., Wedaman K. P., Saxton W. M. and Scholey J. M. (1996) A bipolar kinesin. Nature **379:** 270–272
14 Kashina A. S., Leszyk J. D., Saxton W. M. and Scholey J. M. (1996) An essential bipolar mitotic motor. Nature **384:** 225
15 Scholey J. M. (1996) Kinesin-II, a membrane traffic motor in axons, axonemes and spindles. J. Cell Biol. **133:** 1–4
16 Hirokawa N. (1998) Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science **279:** 519–526
17 Vale R. D. and Fletterick R. J. (1997) The design plan of kinesin motors. Annu. Rev. Cell Dev. Biol. **13:** 745–777
18 Cole D. G., Cande W. Z., Baskin R. J., Skoufias D. A., Hogan C. J. and Scholey J. M. (1992) Isolation of a sea urchin egg kinesin-related protein using peptide antibodies. J. Cell Sci. **101:** 291–301
19 Cole D. G., Chinn S. W., Wedaman K. P., Hall K., Vuong T. and Scholey J. M. (1993) Novel heterotrimeric kinesin-related protein purified from sea urchin eggs. Nature **366:** 268–270
20 Cole D. G. and Scholey J. M. (1995) Purification of kinesin-related protein complexes from eggs and embryos. Biophys. J. **68:** 158s–162s
21 Wedaman K. P., Meyer D. W., Rashid D. J., Cole D. G. and Scholey J. M. (1996) Sequence and submolecular localization of the 115-kD accessory subunit of the heterotrimeric kinesin-II (KRP85/95) complex. J. Cell Biol. **132:** 371–380
22 Aizawa H., Sekine Y., Tekamura R., Zhang Z., Nangaku M. and Hirokawa N. (1992) Kinesin family in murine central nervous system. J. Cell Biol. **119:** 1287–1296
23 Kondo S., Sato-Yoshitake R., Hodo Y., Aizawa H., Nakata T., Matsuura Y. et al. (1994) KIF3A is a novel microtubule-based anterograde motor in the nerve axon. J. Cell Biol. **125:** 1095–1107
24 Yamazaki H., Nakata T., Okada Y. and Hirokawa N. (1995) Kif3a/3b: a heterodimeric kinesin superfamily protein that works as a microtubule plus end-directed motor for membrane organelle transport. J. Cell Biol. **130:** 1387–1399
25 Yamazaki H., Nakata T., Okada Y. and Hirokawa N. (1996) Cloning and characterization of KAP3: a novel kinesin superfamily-associated protein of KIF3A/3B. Proc. Natl. Acad. Sci. USA **93:** 8443–8448
26 Cole D. G., Diener D. R., Himelblau A. L., Beech P. L., Fuster J. C. and Rosenbaum J. L. (1998) *Chlamydomonas* kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in *Caenorhabditis elegans* sensory neurons. J. Cell Biol. **141:** 993–1008
27 Rashid D. J., Wedaman K. P. and Scholey J. M. (1995) Heterodimerization of the two motor subunits of the heterotrimeric kinesin, KRP(85/95). J. Mol. Biol. **252:** 157–162
28 Gindhart J. G. and Goldstein L. B. S. (1996) Armadillo repeats in the SpKAP115 subunit of kinesin-II. Trends Cell Biol. **6:** 415–416
29 Neer E. J., Schmidt C. J., Manbudripad R. and Smith T. F. (1994) The ancient regulatory protein family of WD repeat proteins. Nature **371:** 297–300
30 Gindhart J. G. and Goldstein L. S. B. (1996) Tetratrico peptide repeats are present in the kinesin light chain. Trends Biochem. Sci. **21:** 52–53
31 Lamb J. R., Tugendreich S. and Heiter P. (1995) Tetratrico peptide repeat interactions: to TPR or not to TPR? Trends Biochem. Sci. **20:** 257–259
32 Huber A. H., Nelson W. J. and Weis W. I. (1997) Three-dimensional structure of the armadillo repeat region of beta-catenin. Cell **90:** 871–882
33 Das A. K., Cohen P. W. and Barford D. (1998) The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. EMBO J. **17:** 1192–1199
34 Shimizu K., Kawabe H., Minami S., Honda T., Takaishi K., Shirataki H. et al. (1996) SMAP, an Smg GDS-associating protein having arm repeats and phosphorylated by Src tyrosine kinase. 1996. J. Biol. Chem. **271:** 27013–27017
35 Hackney D. D., Levitt J. D. and Suhan J. (1992) Kinesin undergoes a 9 S to 6 S conformational transition. J. Biol. Chem. **267:** 8696–8701
36 Craig R., Smith R. and Kendrick-Jones J. (1983) Light chain phosphorylation controls the conformation of smooth muscle and nonmuscle myosin. Nature **302:** 436–439
37 Cross R. A. (1988) What is 10S myosin for? J. Muscle Res. Cell Motil. **9:** 108–110
38 Lupas A., Van Dyke M. and Stock J. (1991) Predicting coiled coils from protein sequences. Science **252:** 1162–1164
39 Hisanga S., Murofushi H., Okuhara K., Sato R., Masuda Y., Sakai H. et al. (1989) The molecular structure of adrenal medulla kinesin. Cell Motil. Cytoskeleton **12:** 264–272
40 Yang Z. and Goldstein L. S. B. (1998) Characterization of the KIF3C neural kinesin-like motor from mouse. Mol. Biol. Cell **9:** 249–261
41 Muresan V., Abramson T., Lyass A., Winter D., Porro E., Hong F. et al. (1998) KIF3C and KIF3A form a novel neuronal heteromeric kinesin that associates with membrane vesicles. Mol. Biol. Cell **9:** 637–652
42 Faire K., Gruber D. and Bulinski J. C. (1998) Identification of kinesin-like molecules in myogenic cells. Eur. J. Cell Biol. **77:** 27–34
43 Culotti J. G. and Russell R. L. (1978) Osmotic avoidance defective mutants of the nematode *Caenorhabditis elegans*. Genetics **90:** 243–256
44 Perkins L. A., Hedgecock E. M., Thomson J. N. and Culotti J. G. (1986) Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev. Biol. **117:** 456–487
45 Shakir M. F., Fukushige T., Yasuda H., Miwa J. and Siddiqui S. S. (1993) *C. elegans* osm-3 gene mediating osmotic avoidance behaviour encodes a kinesin-like protein. Mol. Neurosci. **4:** 891–894
46 Tabsih M., Siddiqui Z. K., Nishikawa K. and Siddiqui S. S. (1995) Exclusive expression of *C. elegans* Osm-3 kinesin gene in chemosensory neurons open to the external environment. J. Mol. Biol. **247:** 377–389
47 Signor D., Wedaman K. P., Rose L. S. and Scholey J. M. (1999) Two heteromeric kinesin complexes in chemosensory neurons and sensory cilia of *Caenorhabditis elegans*. Mol. Biol. Cell **10:** 345–360
48 Orozco J. T., Wedaman K. P., Signor D., Brown H., Rose L. and Scholey J. M. (1999) Movement of motors and cargo during intraflagellar transport. Nature **398:** 674
49 Huang B., Rifkin M. R. and Luck D. J. L. (1977) Temperature-sensitive mutations affecting, flagellar assembly and function of *Chlamydomonas reinhardtii*. J. Cell Biol. **72:** 67–85

50 Adams G. M. W., Huang B. and Luck D. J. L. (1982) Temperature-sensitive, assembly-defective flagella mutants of *Chlamydomonas reinhardtii*. Genetics **100:** 579–586
51 Lux F. G. III and Dutcher S. K. (1991) Genetic interactions at the FLA10 locus:suppressors and synthetic phenotypes that affect the cell cycle and flagellar function in *Chlamydomonas reinhardtii*. Genetics **128:** 549–561
52 Walther S., Vashishtha M. and Hall J. L. (1994) The *Chlamydomonas FLA10* gene encodes a novel kinesin-homologous protein. J. Cell Biol. **126:** 175–188
53 Kozminski K. G., Beech P. L. and Rosenbaum J. L. (1995) The *Chlamydomonas* kinesin-like protein FLA10 is involved in motility associated with the flagellar membrane. J. Cell Biol. **131:** 1517–1527
54 Vashishtha M., Walther Z. and Hall J. L. (1996) The kinesin-homologous protein encoded by the *Chlamydomonas fla10* gene is associated with basal bodies and centrioles. J. Cell Sci. **109:** 541–549
55 Piperno G., Mead K. and Henderson S. (1996) Inner dynein arms but not outer dynein arms require the activity of kinesin homologue protein KHP1^{FLA10} to reach the distal part of flagella in *Chlamydomonas*. J. Cell Biol. **133:** 371–379
56 Piperno G. and Mead K. (1997) Transport of a novel complex in the cytoplasmic matrix of *Chlamydomonas* flagella. Proc. Natl. Acad. USA **94:** 4457–4462
57 Piperno G., Siuda E., Henderson S., Segil M., Vaananen H. and Sassaroli M. (1998) Distinct mutants of retrograde intraflagellar transport (IFT) share similar morphological and molecular defects. J. Cell Biol. **143:** 1591–1601
58 Nonaka S., Tanaka Y., Okada Y., Takeda S., Harada A., Kanai Y. et al. (1998) Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. Cell **95:** 829–837
59 Marszalek J. R., Ruiz-Lozano P., Roberts E., Chien K. R. and Goldstein L. S. B. (1999) Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. Proc. Natl. Acad. Sci. USA **96:** 5043–5048
60 Muresan V., Lyass A. and Schnapp B. J. (1999) The kinesin motor KIF3A is a component of the presynaptic ribbon in vertebrate photoreceptors. J. Neurosci. **19:** 1027–1037
61 Henson J. H., Cole D. G., Terasaki M., Rashid D. J. and Scholey J. M. (1995) Immunolocalization of the heterotrimeric kinesin-related protein, KRP85/95, in the mitotic apparatus of sea urchin embryos. Dev. Biol. **171:** 182–194
62 Morris R. L. and Scholey J. M. (1997) Heterotrimeric Kinesin-II is required for the assembly of motile $9+2$ ciliary axonemes on sea urchin embryos. J. Cell Biol. **138:** 1009–1022
63 Henson J. H., Cole D. G., Roesener C. D., Capuano S., Mendola R. J. and Scholey J. M. (1997) The hetrotrimeric motor protein kinesin-II localizes to the midpiece and flagellum of sea urchin and sand dollar sperm. Cell Motil. Cytoskeleton **38:** 29–37
64 Vernos I., Heasman J. and Wylie C. (1993) Multiple kinesin-like transcripts in *Xenopus* oocytes. Dev. Biol. **157:** 232–239
65 Rogers S. L., Tint I. S., Fanapour P. C. and Gelfand V. I. (1997) Regulated bidirectional motility of melanophore pigment granules along microtubules in vitro. Proc. Natl. Acad. Sci USA **94:** 3720–3725
66 Tuma M. C., Zill A., Le Bot N., Vernos I. and Gelfand V. (1998) Heterotrimeric kinesin-II is the microtubule motor protein responsible for pigment dispersion in *Xenopus* melanophores. J. Cell Biol. **143:** 1547–1558
67 Le Bot N., Antony C., White J., Karsenti E. and Vernos I. (1998) Role of xklp3, a subunit of the *Xenopus* kinesin-II heterotrimeric complex, in membrane transport between the endoplasmic reticulum and the Golgi apparatus. J. Cell Biol. **143:** 1559–1573
68 Pesavento P. A., Stewart R. J. and Goldstein L. S. B. (1994) Characterization of the Klp68D kinesin-like protein in *Drosophila*: possible roles in axonal transport. J. Cell Biol. **127:** 1041–1048
69 Stewart R. J., Pesavento P. A., Woerpel D. N. and Goldstein L. S. B. (1991) Identification and partial characterization of six members of the kinesin superfamily in *Drosophila*. Proc. Natl. Acad. Sci. USA **88:** 8470–8474
70 Sardella M., Navone F., Rocchi M., Rubartelli A., Viggiano L., Vignali G. et al. (1998) KIF3C, a novel member of the kinesin superfamily: sequence, expression and mapping to human chromosome 2 at 2p23. Genomics **47:** 405–408
71 Telford E. A. R., Wightman P., Leek J., Markham A. F., Lench N. J. and Bonthron D. T. (1998) cDNA cloning, genomic organization and chromosomal localization of a novel human gene that encodes a kinesin-related protein highly similar to mouse Kif3C. Biochem. Biophys. Res Commun. **242:** 407–412
72 Nagata K., Puls A., Futter C., Aspenstrom P., Schaefer E., Nakata T. et al. (1998) The MAP kinase kinase kinase MLK2 co-localizes with activated JNK along microtubules and associates with kinesin superfamily motor KIF3. EMBO J. **17:** 149–158
73 Shimizu K., Shirataki H., Honda T., Minami S. and Takai Y. (1998) Complex formation of SMAP/KAP3, a KIF3A/B ATPase motor-associated protein, with a human chromosome-associated polypeptide. J. Biol. Chem. **273:** 6591–6594
74 Rosenbaum J. L., Cole D. G. and Diener D. R. (1999) Intraflagellar transport: the eyes have it. J. Cell Biol. **144:** 385–388
75 Kozminski K. G., Johnson K. A., Forscher P. and Rosenbaum J. L. (1993) A motility in the eukaryotic flagellum unrelated to flagellar beating. Proc. Natl. Acad. Sci. USA **90:** 5519–5523
76 Pazour G. J., Wilkerson C. G. and Witman G. B. (1998) A dynein light chain is essential for the retrograde particle movement of intraflagellar transport (IFT). J. Cell Biol. **141:** 979–992
77 Piperno G. and Luck D. J. (1979) Axonemal adenosine triphosphatases from flagella of *Chlamydomonas reinhardtii*. Purification of two dyneins. J. Biol. Chem. **254:** 3084–3090
78 King S. M., Barbarese E., Dillman J. F. III, Patel-King R. S., Carson J. H. and Pfister K. K. (1996) Brain cytoplasmic and flagellar outer arm dyneins share a highly conserved M_r 8,000 blight chain. J. Biol. Chem. **271:** 19358–19366
79 Pazour G. J., Dickert B. L. and Witman G. B. (1999) The DHC1b (DHC2) Isoform of cytoplasmic dynein is required for flagellar assembly. J. Cell Biol. **144:** 473–481
80 Porter M. E., Bower R., Knott J. A., Byrd P. and Dentler W. (1999) Cytoplasmic dynein heavy chain 1b is required for flagellar assembly in *Chlamydomonas*. Mol. Biol. Cell **10:** 693–712
81 Johnson K. A. and Rosenbaum J. L. (1992) Polarity of flagellar assembly in *Chlamydomonas*. J. Cell Biol. **119:** 1605–1611
82 Fowkes M. E. and Mitchell D. R. (1998) The role of preassembled cytoplasmic complexes in assembly of flagellar dynein subunits. Mol. Biol. Cell **9:** 2337–2347
83 Collet J., Spike C. A., Lundquist E. A., Shaw J. E. and Herman R. K. (1998) Analysis of osm-6, a gene that affects sensory cilium structure and sensory neuron function in *Caenorhabditis elegans*. Genetics **148:** 187–200
84 Miller M. G., Mulholland D. J. and Vogl A. W. (1999) Rat testis motor proteins associated with spermatid translocation (dynein) and spermatid flagella (kinesin-II). Biol. Reprod. **60:** 1047–1056
85 Beech P. L., Pagh-Roehl K., Noda Y., Hirokawa N., Burnside B. and Rosenbaum J. L. (1996) Localization of kinesin superfamily proteins to the connecting cilium of fish photoreceptors. J. Cell Sci. **109:** 889–897
86 Sanborn E. B. (1970) Cells and Tissues by Light and Electron Microscopy, vol. 1, Academic Press, New York
87 Harvey R. P. (1998) Links in the left/right axial pathway. Cell **94:** 273–276
88 Galcheva-Gargova Z., Derijard B., Wu I. H. and Davis R. J. (1994) An osmosensing signal transduction pathway in mammalian cells. Science **265:** 806–808