Moving on to the cargo problem of microtubule-dependent motors in neurons

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Vigorous investigation has finally begun to shed light on the cargo problem of the microtubule-dependent motors, kinesin and dynein superfamily proteins. Biochemical observations have suggested that the potential cargoes of certain populations of motor proteins seem to be in vesicle-form, each vesicle possessing specific functional marker molecules. In addition to the close relationship between microtubule-dependent motors and cargoes in vesicle-form, kinesin has also been highlighted as an apparent driving force for another cargo in non-vesicle-form, cytoplasmic protein. On the basis of new biophysical and cell-biological evidence, the controversy over the movement of cytoplasmic cargoes has entered a new phase.

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Abbreviations

CAM	cell adhesion molecule
ChAT	choline acetyltransferase
COP1	coatomer protein 1
DLG	discs large
GFP	green fluorescent protein
KAP3	kinesin superfamily associated protein of KIF3
KIF	kinesin superfamily protein
KIF5	kinesin superfamily protein 5/kinesin heavy chain
KLC	kinesin light chain
NMDA	*N*-methyl-D-aspartate
NMDAR2B	NMDA receptor 2B
PDZ domain	PSD-95, DLG, ZO-1 domain
PSD-95	postsynaptic density protein of 95 kDa
ZO-1	zona occludens-1

Introduction

The total sequencing of the *C. elegans* and *Drosophila* genomes has been completed and the human genome project is drawing to a close. From these data, we know that almost all of the motor proteins have either already been cloned, or are under investigation. Although the kinesin and dynein superfamily proteins are grouped into many subfamilies, at least one flagship constituent of each subfamily has already been studied intensively with few exceptions [1]. The period of identification and characterization of the new subfamilies seems to be continuing; nonetheless, we are now entering a new stage for the new millennium — the elucidation of the role of each player in the drama. We know the players by name, but only poorly by their characters or interrelations. This review focuses on some of the aspects known at the present stage that will boost the development of research into the exact function of the molecules.

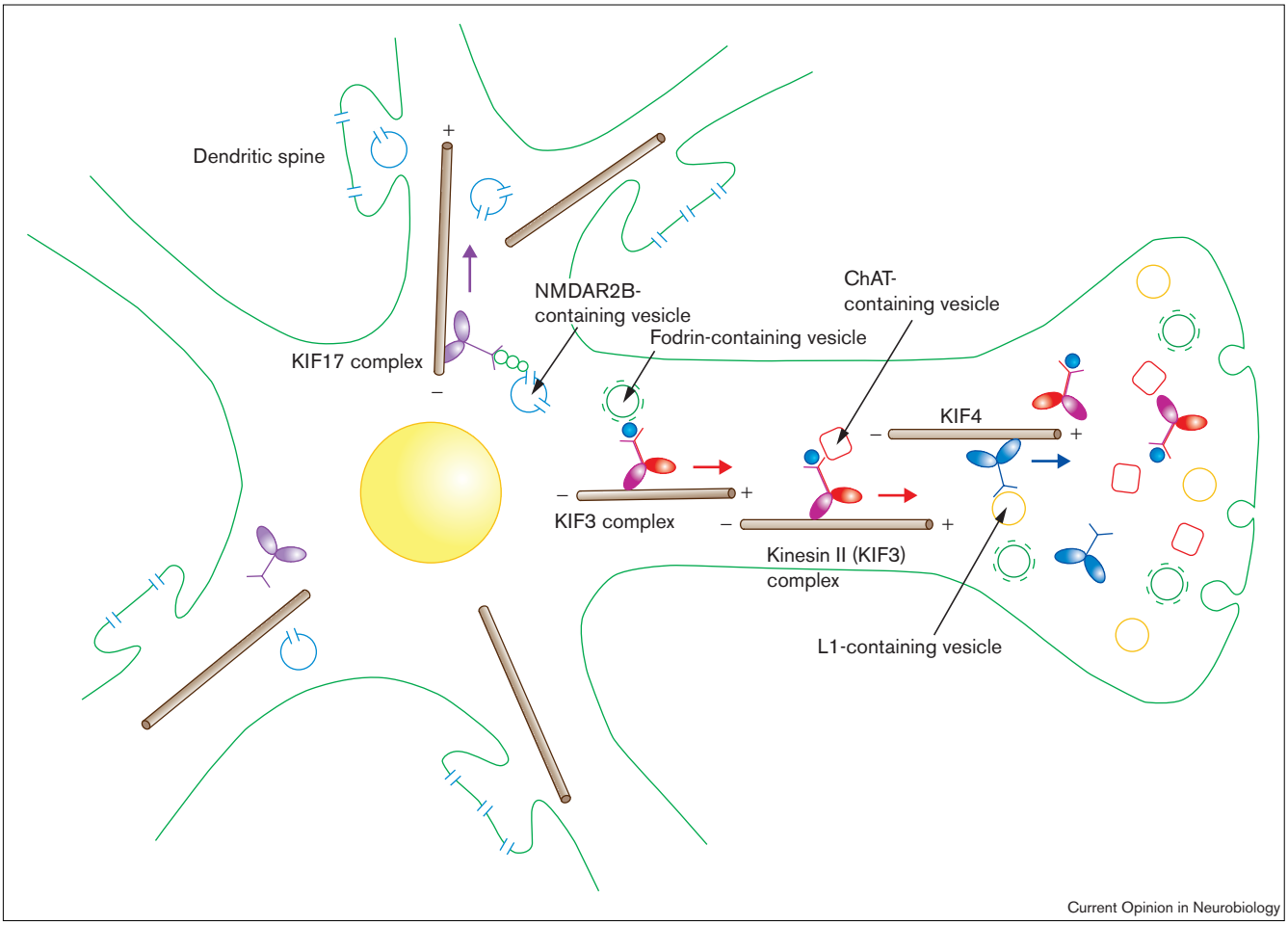
Conventional kinesin heavy chain, now designated as KIF5 or kinesin I, was first discovered 15 years ago [2,3], and cytoplasmic dynein was reported 13 years ago [4,5]. Recent advances in molecular biology revealed the existence of diverse superfamily proteins of both kinesin and dynein [6–8]. Since their discovery, many studies have been performed with the aim of answering the following three questions. First, concerning the mechanism of molecular motor movement: how do these proteins move? Second, concerning the cargo problem: what are their binding partners, or cargoes? Third, concerning the regulation problem: how is their movement regulated, and how is the specificity and the on–off nature of the cargo interaction regulated?

Although recent studies, mainly from the field of biophysics, have generated some clues for solving the first problem (see e.g. [9–11]), other long-awaited solutions to the second and the third questions are now arising, particularly in the field of neurobiology. We will pick out and describe some of the most important recent advances (mainly from May 1999) in this review.

Binding partners of kinesin superfamily proteins in neurons – their possible relationship with neurite formation and synaptic transmission

Rigorous screening of the binding partners of kinesin superfamily proteins has been carried out during the past several years, although few of the candidates had been identified. But finally, some of the putative cargoes have recently been elucidated. One example is KIF3A, which can form a KIF3 complex with KIF3B and KAP3 (kinesin superfamily associated protein of KIF3) [12–14]. Mice lacking the *kif3A* gene showed randomization of laterality, absence of motile monocilia on nodal pit cells and mesodermal hypoplasia [15••]. KIF3s are known to be expressed in the retina, and are presumed to play some role in eye development [16,17•,18]. But, contrary to expectations, *in situ* hybridization demonstrated that the distribution of *Pax6*, a developmental marker of eyes, was normal. These apparent phenotypes were similar to those of *kif3B* knockout mice [19], and implied that the KIF3 complex might function as an inducer of both nodal cilia and mesodermal cells. The direct binding partner of the KIF3 complex itself, however, remained unknown. Very recently, the first step in elucidating this problem was made, using yeast two-hybrid screening [20••]. Functional blocking of the KIF3 complex in cultured neurons inhibited both fast axonal transport and neurite extension, and subsequent yeast two-hybrid screening revealed that the KIF3 complex could bind fodrin-containing vesicles via KAP3. The KIF3 complex may be engaged in fast axonal transport conveying fodrin-associated vesicles for use in neurite extension. In combination with another report showing

Figure 1



The binding partners of kinesin superfamily proteins are now being elucidated. Biochemical observations suggest that the cargoes of the motor proteins seem to be in the form of vesicles that possess specific functional marker molecules. The KIF3 complex conveys fodrin-containing vesicles through the direct interaction of KAP3 with fodrin and plays a role in neurite elongation [20••], whereas the kinesin II (KIF3) complex associates with other vesicles containing ChAT [21•], although the factors linking the vesicle and the motor are unknown. KIF4 transports L1-containing vesicles and assists in axonal elongation [24•]; in this case, also, the exact players between L1 and KIF4 remain obscure. KIF17 conveys NMDAR2B-containing vesicles via a complex of mLin-10, mLin-2 and mLin-7, and may modulate the efficacy of synaptic transmission [22••].

that the *Drosophila* KIF3 complex, kinesin II, is required for axonal transport of choline acetyltransferase (ChAT) [21•], it seems that there are several different roles for the KIF3 complex *in vivo*. Various observations on putative KIF3 roles in ciliogenesis, mesodermal development, vesicular transport for neurite formation and transport of ChAT collectively suggest that the KIF3 complex is a multi-functional motor protein that can act in diverse conditions. Further studies on the specificity of each motor–cargo relationship will hopefully clarify the true nature of the molecule in the near future.

Another striking finding was recently made regarding a new member of the kinesin superfamily, KIF17 [22••]. This protein is predominantly expressed in neurons, and is localized in cell bodies and dendrites. Yeast two-hybrid screening revealed that the carboxy-terminal region of KIF17 could interact with the PDZ domain of mLin-10. These findings have enormous implications because mLin-10 is a well characterized linker protein that can form a protein complex with the NMDA receptor 2B (NMDAR2B), via mLin-2 and mLin-7. In fact, it was shown that KIF17 could bind to NMDAR2B through the interaction with this protein complex, and that KIF17 was able to transport vesicles containing NMDAR2B to the plus end of the microtubules *in vitro*. This ability of KIF to transport receptor proteins might be another regulatory step that could modulate the efficacy of synaptic transmission. As in the case of the KIF3 complex, further experiments regarding the functional significance and regulation of interactions are necessary.

The third example of successful cargo identification concerned KIF4, a developmentally regulated motor protein [23]

expressed in the growth cones of developing neurons [23,24•]. Vesicles containing the cell adhesion molecule (CAM) L1 were shown to associate with KIF4, and both KIF4 and L1 localized to the axon and growth cone. Interestingly, an antisense treatment against KIF4 expression induced suppression of L1-enhanced axonal elongation. As is the case with KIF3 and KIF17, these biochemical observations suggest that the cargo of the motor protein seems to be in the form of vesicles that contain specific functional marker molecules (Figure 1).

Kinesin heavy chain and light chain revisited: a new isoform of KIF5, its transport profile, and kinesin light chain knockout mice

The KIF5 proteins, conventional kinesin heavy chains, form homodimers with two kinesin light chains and thus constitute the classical kinesin complex. Though extensive studies have been carried out on these prototypic kinesin superfamily proteins, there remained some confusion over the data through lack of knowledge about KIF5 isoforms. We now know that KIF5s consist of three isoforms, KIF5A, KIF5B and KIF5C, and the first detailed analysis of one isoform has recently been published [25••]. This comprehensive study of the function of KIF5C, from molecular cloning to gene targeting, revealed a specific role for KIF5C in motor-neuron maintenance. The role of KIF5C differs from the roles of KIF5A or KIF5B, and this was further confirmed by the results of a sciatic-nerve-ligation study *in vivo*, using different anti-kinesin antibodies, H1 and H2 [26•]. Both of the structures stained by H1 and H2 accumulated proximal to the crush with a time-scale comparable to that of fast axonal transport, but H1-staining, which is presumed to be attributable to KIF5C [25••], showed more restricted distribution than H2-staining. We know that motor neurons sometimes act distinctively and that in diseases such as amyotrophic lateral sclerosis, selective motor neuron degeneration occurs. Together with these studies, we may now have a glimpse of the specific roles of each KIF5 isoform in particular neurons and their possible implications for pathogenesis.

Another report of *klc1* gene targeting, a predominant kinesin light chain (KLC) isoform in neuronal tissues, has shed light on the function of the classical kinesin complex [27•]. Mutant mice exhibit overt movement disabilities and small size and, in both the sensory and motor neurons of these mutants, KIF5A is found to be aberrantly accumulated and colocalized with the peripheral *cis*-Golgi marker giantin. (In sensory but not in motor neurons of mutants, KIF5B and COP1 (coatomer protein 1) were also aberrantly colocalized in a punctate distribution pattern, although this immunocytochemical finding should be re-evaluated. The authors used the anti-KIF5B antibody raised by Niclas *et al.* [28] but their staining pattern was totally different from that of Kanai *et al.* [25••]. We cannot evaluate the specificity of the antibody used by Niclas *et al.* here because of the lack of sufficient description and characterization of the antibody.) The expression level of KLC2, a more ubiquitously expressed kinesin light chain, in mutants seemed to be reduced in sensory neurons, but unaltered in motor neurons. At this stage, we have to say that the simple kinesin light chain inhibition model — that kinesin light chain negatively regulates the function of KIF5s — cannot explain these findings. If one postulates the model, KIF5A would accumulate in the cellular periphery in *klc1* mutants, but in fact it accumulates around the Golgi apparatus. This finding itself might suggest another idea — that without kinesin light chain, KIF5 could not bind to its putative cargo; thus, cargo binding to KIF5s is presumed to necessitate kinesin light chain. But to explain the whole story, it is apparent that the regulatory role of KIF5 function depends on another unknown factor, which is closely related to both KIF5 and kinesin light chain. In addition, although we do not know the exact behavior of KIF5C in *klc1* mutants, the reason for a clear difference in KIF5 and kinesin light chain function between motor and sensory neurons remains to be elucidated.

New molecular motors in neurons: remaining new faces

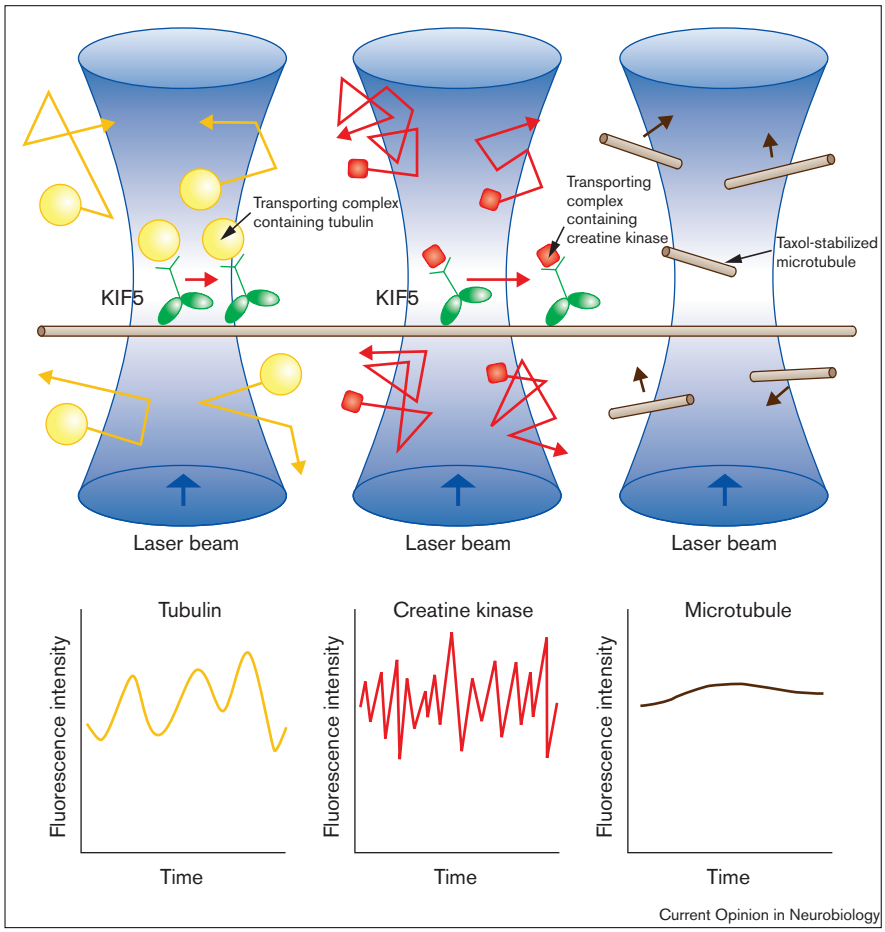
Recently, a few remaining new faces of neuronal kinesin superfamily proteins have been characterized. One example is KIF21 [29•]. One of the isoforms, KIF21A, is distributed pan-neuronally, whereas the other, KIF21B, is enriched in dendrites. In another recent study, the human homologue of KIFC3 was cloned, and the authors give a brief description of the related immunohistochemistry of the retina [30•]. Both of these superfamily proteins are in a preliminary state of characterization, thus further investigations to elucidate their biological functions are necessary.

Uncovering the molecular mechanism of slow axonal transport: in what way and what form?

Analysis of axonal transport led to the identification of fast axonal transport motor proteins in neurons, and subsequent cell-biological studies mainly pursued the cargo or regulation problems. The existence of slow axonal transport has been well recognized, but, in striking contrast to fast transport, the molecular mechanism of slow transport has remained obscure. Although slow axonal transport is presumed to support the movement of a large proportion of the cytoplasmic proteins, our knowledge of this system is limited to its cargo materials. We do not know what kind of motor proteins support the transport, to say nothing of its regulation mechanism. In other words, the enigma of the slow axonal transport mechanism, and its inherent combined problems of motor machinery and regulation, have escaped vigorous scientific investigation for the past twenty years. But now, at the end of this century, we have had some promising clues regarding the delineation of this molecular mechanism.

High-resolution *in vitro* observations of slow axonal transport are problematic because it is difficult to discriminate active transport from Brownian motion in the cytoplasm. The speed of slow axonal transport (around 1 mm per day)

Figure 2



Fluctuations in fluorescence intensity inform us about the transporting complex of cytosolic proteins in axons. Fluorescence correlation spectroscopy measures the fluctuation and signal strength of the fluorescently labeled molecules in a very restricted area (femto-liter scale; blue) *in vivo*. Both tubulin- and creatine-kinase-containing transport complexes (yellow and red, respectively) in the squid giant axon are conveyed by KIF5 (green) along microtubules (brown), but the signal from the latter fluctuates more rapidly than that from the former (compare the corresponding yellow and red graph traces); this indicates that the tubulin transporting complex is larger in both size and translational diffusion time than the creatine kinase complex. This might explain the difference between the transporting speed of tubulin and creatine kinase, observed in both mammalian and squid neurons (creatine kinase travels faster than tubulin). Polymerized tubulin (taxol-stabilized microtubules) shows very little fluctuation of fluorescence, thus may have the longest translational diffusion time; these did not travel far in squid giant axons, contradicting the classic polymer sliding hypothesis (see Terada *et al.* [31••] for details).

is at least one order of magnitude slower than the speed of simple diffusion. So, when we observe the movement of labeled cytoplasmic proteins at high magnifications, the substantial active transport phenomenon is easily obscured by background Brownian motion. To circumvent this problem, observations were performed at low magnification using thick giant axons from the squid [31••]. As Brownian motion is random, labeled molecules that are diffusing do not travel far under low magnifications, whereas one can observe slow but directional movement of proteins that are being transported actively. Using this *in vitro* experimental system, two important findings were made. First, injected labeled tubulin in excised squid giant axons was shown to be transported in a microtubule-dependent manner. Both pharmacological experiments and the functional blocking of motor proteins by an anti-kinesin antibody indicated that the directional movement of the fluorescence profile was dependent on kinesin motor function. Second, the translational diffusion time of injected tubulin by fluorescence correlation spectroscopy (Figure 2) revealed that the form of tubulin in the transporting complex is not a polymer. Although some researchers still maintain that polymer formation is a prerequisite for slow axonal transport (the polymer sliding theory; see e.g. [32,33] for reviews of the debates), these biophysical measurements proved that this classical idea is not plausible in *ex vivo* or *in vivo* conditions. This conclusion was confirmed by two more recent studies. One reports the observation of the movement of a single labeled microtubule in cultured neurons by speckle microscopy [34•]. The labeled single microtubule did not move as a polymer in cultured *Xenopus* neurons. The other study reports a transfection experiment of green fluorescent protein (GFP)-tagged neurofilament-M in a neuroblastoma cell line [35•]. The oligomeric structures of neurofilament subunits were transported in bright dot-like structures in a microtubule-dependent manner.

One of the remaining riddles is the slowness of this transport system. If both tubulin and neurofilament subunits are transported by kinesin superfamily proteins, why is their transporting speed so slow compared with that of membranous organelles? One of the clues for answering this question came from another observation of GFP-tagged neurofilament-M movement in cultured neurons [36•]. The observed velocity of the labeled protein was considerably faster than suggested by previous data using radioisotopic pulse labeling, but the movement was interrupted by prolonged pauses. From this result, one can

imagine that cytoplasmic proteins could be transported at the speed of fast axonal transport but, on average, as the proportion using active transport machinery is presumed to be small in quantity, the apparent speed will be slow. This assumption is supported by another observation using fluorescence correlation spectroscopy [31••]. The auto-correlation curve of fluorescent tubulin undergoing transport was successfully fitted by a simple one-component model, indicating that the population of proteins undergoing active transport might be small compared with the number of proteins diffusing freely in the axoplasm.

Dynein–dynactin complex and dynein light chains in neurons: multiple functionality and interrelationship among microtubule-dependent motor proteins

Moving from studies on kinesin to those on dynein motors, there have been a few reports on dynein–dynactin function in neurons this year. The cytoplasmic dynein complex consists of a homodimer of heavy chains, intermediate chains (IC74s) with WD repeats, four light intermediate chains, and several light chains (LCs), including two copies of the Tctex1/rp3 light chain and one dimer of the highly conserved LC8 protein. Although the cytoplasmic dynein intermediate chain IC74 seems to mediate the dynein–dynactin interaction through association with $p150^{glued}$, the role of this interaction remains to be elucidated. By dominant-negative expression of the truncated Glued protein (the *Glued[1]* mutation) in the *Drosophila* giant fiber system, synaptogenesis between the giant fiber axon and the tergotrochanteral motor neuron was impaired, the axon terminal being swollen with large vesicles [37•]. Analysis of path-finding and arborization of sensory axons during metamorphosis in *Glued[1]* and cytoplasmic dynein light chain mutants revealed that, in both mutants, proprioceptive and tactile axons reach the central nervous system on time but exhibit defects in terminal arborizations [38•]. So, as in the case of the KIF3 complex, we have to postulate multi-functionality of the dynein–dynactin complex, such as involvements in both synaptogenesis and the arborization of axons.

In addition, several genetic experiments have been published that suggest interrelationships between microtubule-based motors. For example, mutations in both cytoplasmic dynein (cDhc64C) and dynactin complex constituent (Glued) in *Drosophila* disrupt fast organelle transport in both directions and result in similar phenotypes as those caused by kinesin mutations, such as larval posterior paralysis and axonal swellings filled with organelles, with strong dominant genetic interactions between kinesin, dynein, and dynactin complex mutations in axonal transport [39•]. In *Caenorhabditis elegans*, kinesin-II (KAP–GFP) and its presumed cargo molecules move at around 1 μm/s in the retrograde direction along cilia and dendrites. This movement in cilia, but not in dendrites, is inhibited in a cytoplasmic dynein mutant background, whereas anterograde IFT (intraflagellar transport) movement is unaltered [40•]. Given these collective results, we are certain that the multi-functionality and interrelationship between motor proteins will be a popular topic for further study.

Whereas cytoplasmic dynein intermediate chain, IC74, seems to mediate the dynein–dynactin interaction and putative cargoes, the possibility has arisen that associated light chains may play various regulatory roles on dynein function. For example, genetic screening in *Drosophila melanogaster* based on the posterior-sluggish larval phenotype of motor mutants revealed the involvement of a gene called *roadblock*; the mutant exhibits intra-axonal accumulation of cargoes with a distal bias, axonal degeneration and aberrant chromosome segregation [41••]. The product of this gene turned out to be a homologue of *Chlamydomonas* outer-arm dynein-associated protein LC7, and could associate with both flagellar outer-arm dynein and cytoplasmic dynein. In addition, homologues of *roadblock/LC7* have been found in organisms lacking motile cilia/flagella such as *C. elegans*. Considering the facts that highly homologous dynein light chains are shared between cytoplasmic and flagellar dyneins, and that all cloned cytoplasmic dynein light chains including LC7 are thought to associate with IC74, we can imagine the possible functional linkage between cytoplasmic and flagellar dyneins. The dynein complex may have an interdependent functional relevance for intracellular transport, flagellar motility and mitosis, and this possibility has intriguing implications for the analysis of other motor proteins, including the kinesin superfamily proteins.

In an attempt to solve the motor, cargo, and regulation problems regarding cytoplasmic dynein, it is likely that the same kind of studies have been performed on the dynein proteins as have on the kinesin superfamily proteins. However, few dazzling results have appeared. This is a consequence of the complex subunit composition of cytoplasmic dynein itself. There was, however, a successful example last year [42••]. Using yeast two-hybrid screening, Tctex-1, a dynein light chain that was thought to be involved in the meiotic drive of mouse *t*-haplotypes, was isolated as a possible binding partner of the carboxyl terminus of rhodopsin. Rhodopsin-containing vesicles co-sedimented with microtubules in the presence of cytoplasmic dynein in a Tctex-1-dependent manner, and moved along microtubules via cytoplasmic dynein. From these results, we can infer a potential function of cytoplasmic dynein in the transport of rhodopsin-containing vesicles.

Conclusions

The binding partners of microtubule-based motors are now being elucidated. Biochemical observations have suggested that the cargoes of certain populations of motor proteins seem to be in the form of vesicles possessing specific functional marker molecules (Figure 1). This might be a coincidence, but we also have to test for the possibilities of other kinds of cargoes such as protein complexes, lipids, or

nucleotides, as well as investigating the functional and specific relevance of interactions between the molecules. Concerning the molecular mechanism of slow axonal transport, new biophysical techniques such as fluorescence correlation spectroscopy (Figure 2) have shed light on the enigmatic transporting complex. Without any doubt, the molecular dissection of this transporting complex is the next key step in solving the long-standing problem of slow axonal transport. We now have hints that link the function of motor proteins with physiological functions such as neurite elongation, synaptic transmission and cytosolic protein transport. The next millennium will surely witness the unfolding of the plot of this biological drama.

References and recommended reading

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Mice lacking the *kif3A* gene demonstrate severe developmental abnormalities, neural tube degeneration and mesodermal and caudal dysgenesis, and die before 10.5 days *post coitum*, probably as a result of the circulatory insufficiency with pericardial effusion. They also show randomization of laterality in heart looping and absence of motile monocilia on nodal pit cells, as do *kif3B* knockout mice [19]. Whole-mount *in situ* hybridization reveals that the distribution of *Pax6* is normal, but the staining for *sonic hedgehog* and *Brachyury*, both of which are midline mesodermal markers, is abnormal in the anterior–posterior direction at both mesencephalic and thoracic levels. This abnormal expression pattern might explain the mesodermal hypoplasia and degeneration with neural tube defects. In addition to revealing a relationship between KIF3 and nodal ciliogenesis, these studies implied the existence of a relationship between KIF3A and transport of the protein product of *sonic hedgehog*.

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The authors amplified a fragment of *HsKIF3A*, homologue of mouse *kif3A*, from a human retinal cDNA library. Using the Human Genome Project database, they determined its chromosomal localization (5q31) and genomic organization (17 exons interspersed by 16 introns, an open reading frame of 2106 bp). KIF3A is highly concentrated in photoreceptor inner segments, Muller cells and ganglion cells in human and monkey retina, whereas in *Xenopus* retina KIF3A and KIF3B are concentrated in the outer limiting membrane, photoreceptor inner segments and axonemes. Although there are some discrepancies between the observations made by these authors and the preceding data of Muresan and coworkers [18] (probably attributable to the technical differences in fixation), the authors surmised that KIF3 mediates transport of materials from the photoreceptor cell body to the outer segment.

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Microinjection of a functional blocking antibody against KIF3 into cultured superior ganglion neurons blocked fast axonal transport and inhibited neurite extension. Further yeast two-hybrid assays revealed a possible association of fodrin with the KIF3 motor via KAP3, and this binding was confirmed by immunoprecipitation and immunoelectron microscopy of fodrin-associated vesicles from cauda equina. Both fodrin and KIF3 co-migrated as fast transport components; thus, collectively, the authors concluded that the KIF3 motor is engaged in fast axonal transport conveying vesicles for neurite extension.

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In situ hybridization of *Klp64D* (the *Drosophila kif3A* homologue) demonstrated its preferential expression in cholinergic neurons. Mutations in the *Klp64D* gene cause uncoordinated sluggish movement and death, and impair the anterograde transport of ChAT. The inviability of this mutant was rescued by mammalian *kif3A*. Immunostaining for KLP68D (the KIF3B/C homologue) also revealed expression in cholinergic neurons; in kinesin light chain mutants, KLP68D accumulated in cholinergic neurons. (Comparing KIF3A and KIF3B, the overlap of expression between the two homologues in *Drosophila* was very limited, and could be determined only in the neurons of the chordotonal organs, bolwig organ cells, and in the antenno-maxillary complex. The authors insist that impairment of *Klp64D* mutants is a specific effect of KLP64D malfunction for two reasons: first, there was no significant change in axonal ChAT distribution in either kinesin heavy chain or light chain mutants; and second, no accumulation of SYT [synaptotagmin], CSP [cysteine string protein; both integral parts of synaptic vesicles], tubulin or actin was observed in *Klp64D* mutants. However, most of the data are not shown in their thesis.)

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A new member of the amino-terminal-type kinesin superfamily proteins, KIF17, which is brain-dominant and localized in cell body and dendrites, was cloned, and its possible binding protein was screened using the yeast two-hybrid system. The emergent results showed that one of the PDZ domains of mLin-10 is the binding partner of KIF17 carboxy tail. mLin-10 is known to interact with NMDAR2B via mLin-2 and mLin-7 complexes. The authors showed binding of KIF17 to NMDAR2B via this complex, and transport of NMDAR2B-containing vesicles on microtubules *in vitro* via KIF17, thus inferring its regulatory effect on synaptic transmission.

23. Sekine Y, Okada Y, Kondo S, Aizawa H, Takemura R, Hirokawa N: **A novel microtubule-based motor protein (KIF4) for organelle transports, whose expression is regulated developmentally.** *J Cell Biol* 1994, **127**:187-202.

24. • Peretti D, Peris L, Rosso S, Quiroga S, Caceres A: **Evidence for the involvement of KIF4 in the anterograde transport of L1-containing vesicles.** *J Cell Biol* 2000, **149**:141-152.
Using several biochemical techniques, the authors isolated vesicles associating with KIF4; these vesicles are concentrated in growth cones and contain L1, a CAM. Both KIF4 and L1 predominantly localize to the axonal shaft and growth cone of cultured hippocampal neurons. Antisense oligonucleotide treatment against KIF4 induced accumulation of L1 within cell bodies and suppressed L1-enhanced axonal elongation.

25. •• Kanai Y, Okada Y, Tanaka Y, Harada A, Terada S, Hirokawa N: **KIF5C, a novel neuronal kinesin enriched in motor neurons.** *J Neurosci* 2000, **20**:6374-6384.
The authors cloned KIF5C, another member of the conventional kinesin heavy chain KIF5 protein family, and generated mice lacking the *kif5C* gene. Although all three KIF5s share similar amino acid sequences and have been proven to be functionally redundant, specific antibodies against three KIF5s, KIF5A, KIF5B, and KIF5C, demonstrated distinct distribution patterns for each molecule. KIF5B is expressed mainly in glial cells, but is upregulated in neurons with elongating neurites, such as primary olfactory neurons and mossy fibers. Both KIF5C and KIF5A are expressed predominantly in neurons, but KIF5C is highly expressed in lower motor neurons in mice aged two weeks or older. Motor neurons of the abducens nucleus in *kif5C* knockout mice showed dominant loss of motor neurons (28%), suggesting a specific role for KIF5C in motor neuron maintenance. In addition, a detailed description and re-evaluation of the various anti-KIF5 antibodies, such as SUK4, H1 or H2, is provided, clarifying the conflicts of the previous data.

26. • Li J-Y, Pfister KK, Brady S, Dahlstrom A: **Axonal transport and distribution of immunologically distinct kinesin heavy chains in rat neurons.** *J Neurosci Res* 1999, **58**:226-241.
In this paper, the authors investigated immunoreactivity of anti-kinesin antibodies, H1 and H2, in rat peripheral nerve and spinal cord: both quantitative and qualitative differences between the two were detected. Both immunoreactivities were detected in axons proximal to the site of the crush as early as 1 hr after the crush operation – consistent with fast axonal transport – and virtually colocalized with synaptic vesicle proteins. On the other hand, the immunoreactivity of H1 demonstrated a more-limited distribution than that of H2, thus indicating that some specific kinesins were restricted to specific membrane organelles. As shown in the paper by Kanai *et al.* [25••], this limited distribution of H1 immunostaining is presumed to be representative of KIF5C, a novel neuronal kinesin heavy chain.

27. • Rahman A, Kamal A, Roberts EA, Goldstein LSB: **Defective kinesin heavy chain behavior in mouse kinesin light chain mutants.** *J Cell Biol* 1999, **146**:1277-1287.
Mice lacking the majority of the *klc1* (predominant KLC in neuronal tissues) gene, including the region encoding the TPR (tetratricopeptide repeat) domain, exhibit overt movement disabilities, small size, and their sensory and motor neurons have alterations in the intracellular localization of KIF5A, KIF5B and COP1 components. Both in sensory and motor neurons of mutants, KIF5A aberrantly accumulated and colocalized with the peripheral *cis*-Golgi marker giantin, but not with other endoplasmic reticulum, Golgi, mitochondrial or lysosomal markers. In sensory but not in motor neurons of mutants, KIF5B and COP1 were also aberrantly colocalized in a punctate distribution pattern (though this staining should be re-evaluated). The expression level of KLC2, a more ubiquitously expressed KLC, in mutants seemed reduced in sensory neurons, but unaltered in motor neurons.

28. Niclas J, Navone F, Hom-Booher N, Vale RD: **Cloning and localization of a conventional kinesin motor expressed exclusively in neurons.** *Neuron* 1994, **12**:1059-1072.

29. • Marszalek JR, Weiner JA, Farlow SJ, Chun J, Goldstein LSB: **Novel dendritic kinesin sorting identified by different process targeting of two related kinesins: KIF21A and KIF21B.** *J Cell Biol* 1999, **145**:469-479.
Neuronally enriched kinesin superfamily proteins, KIF21A and KIF21B, were cloned from a retinal mouse cDNA library using PCR, and proven to be anterograde motors, with amino-terminal head domains that could move along the microtubules to their plus end. Each protein contains a domain of seven WD-40 repeats, and shares a sequence identity of 61%, but their distributions are distinct. KIF21A protein was shown to be pan-neuronal, whereas KIF21B was highly enriched in dendrites, compared with the cell body and axon; however, its mRNA was almost exclusively confined to the cell body.

30. • Hoang E, Bost-Usinger L, Burnside B: **Characterization of a novel C-kinesin (KIFC3) abundantly expressed in vertebrate retina and RPE.** *Exp Eye Res* 1999, **69**:57-68.
Using RT–PCR screening, the authors cloned *HsKIFC3*, the human homologue of the kinesin superfamily proteins with carboxy-terminal head domains mouse *MmKifC3* and fish *FKIF2*, from a human retinal and retinal pigment epithelium (RPE) mRNA library. Immunolocalization with antibodies against the FKIF2 peptide show that FKIF2 is strongly concentrated in photoreceptor terminals in the outer plexiform layer in fish retinas. In human, this antibody labeled photoreceptor terminals, but labeled Muller cells and the ganglion cell and inner plexiform layers more strongly. *HsKIFC3* has been mapped to human chromosome 16q13-q21, which is within the critical region for the locus associated with Bardet-Biedl syndrome type II, a hereditary form of retinal degeneration.

31. •• Terada S, Kinjo M, Hirokawa N: **Oligomeric tubulin in large transporting complex is transported via kinesin in squid giant axons.** *Cell* 2000, in press.
To investigate the molecular mechanism of slow axonal transport, an *in vitro* experimental system for the observation of tubulin transport was constructed using squid giant axons. After injecting fluorescence-labeled tubulin into the axons, the movement of fluorescence was monitored using confocal laser-scanning microscopy and fluorescence correlation spectroscopy. Both pharmacological experiments and the functional blocking of motor proteins by an anti-kinesin antibody indicated that the directional movement of the fluorescence profile was dependent on kinesin motor function. The fluorescence correlation function was also used to monitor the directional movement of tubulin, and the estimated translational diffusion time revealed that each tubulin molecule was transported in the unique form of a large transporting complex. This transporting complex showed a diffusion time distinct from those of creatine kinase and taxol-stabilized microtubules.

32. Baas PW, Brown A: **Slow axonal transport: the polymer transport model.** *Trends Cell Biol* 1997, **7**:380-384.

33. Hirokawa N, Terada S, Funakoshi T, Takeda S: **Slow axonal transport: the subunit transport model.** *Trends Cell Biol* 1997, **7**:384-388.

34. • Chang S, Svitkina TM, Borisy GG, Popov SV: **Speckle microscopic evaluation of microtubule transport in growing nerve processes.** *Nat Cell Biol* 1999, **1**:399-403.
Under conditions in which the concentration of microinjected labeled tubulin is low, microtubules in the cytoplasm exhibit speckled variations in fluorescence intensity along their length. After microinjection of Cy3-labeled tubulin into *Xenopus* embryo neuronal cultures on ConA-coated substrates, the authors observed the movement of these fluorescent speckles in the microtubule lattice in axons. Despite robust axonal growth, the speckles were stationary, and the authors concluded that delivery of tubulin in the form of microtubules by slow axonal transport is negligible. They also found that microtubule-associated bright puncta, which are likely to represent clusters of labeled tubulin, moved to and fro along the axons. As these structures increased in number with the age of the culture, and as most of them resided in the cell bodies of the proximal axons, the authors prudently deduced that these tubulin-containing structures are associated with the endosomal/lysosomal pathway, and are thus unrelated to the slow axonal transport of tubulin.

35. • Yabe JT, Pimenta A, Shea TB: **Kinesin-mediated transport of neurofilament protein oligomers in growing axons.** *J Cell Sci* 1999, **112**:3799-3814.
The authors transfected the GFP-conjugated neurofilament-M gene into NB2a/d1, a neuroblastoma cell line, and observed translocation of brightly fluorescent dot-like structures, using real-time video analysis. These structures contained conventional kinesin immunoreactivity, and associated with microtubules. Their transport into axons was blocked by anti-kinesin antibodies and nocodazole. The authors proposed that the oligomeric structures of neurofilament subunits are transported in growing axons, using kinesin as a transport motor. The physiological significance of the bright dot-like structures of tagged neurofilament-M remains to be confirmed; it is still controversial whether the transporting form of neurofilament protein is a small oligomer, a polymer, or an aggregated protein complex. (See also the comments in [34•].)

36. • Wang L, Ho C-L, Sun D, Liem RKH, Brown A: **Rapid movement of axonal neurofilaments interrupted by prolonged pauses.** *Nat Cell Biol* 2000, **2**:137-141.
Using GFP-tagged neurofilament-M protein, the authors observed the movement of labeled neurofilament-M protein in rat superior cervical ganglion neurons in culture. In the naturally occurring gaps in the axonal neurofilament array, the authors observed intermittent, asynchronous, bidirectional but predominantly anterograde movement of the protein. The observed velocity was considerably faster than suggested by previous data using radioisotopic pulse labeling, but the movement was interrupted by prolonged pauses. Though the authors insist that they were observing the movement of neurofilament polymers, direct evidence for this assertion is missing.

37. • Allen MJ, Shan X, Caruccio P, Froggett SJ, Moffat KG, Murphey RK: **Targeted expression of truncated Glued disrupts giant fiber synapse formation in *Drosophila*.** *J Neurosci* 1999, **19**:9374-9384.
The protein product of the *Glued*^{1} mutation in *Drosophila* competes with the wild-type protein and forms complexes that can bind to dynein but are unable to bind to the cargo, thus disrupting retrograde transport. By

dominant-negative expression of this truncated *Glued* protein in the giant fiber system, the authors observed impairment of synaptogenesis between the giant fiber axon and the tergotrochanteral motor neuron, with swollen axon terminals with large vesicles. The transmission of this mixed electrochemical synapse in the mutant is weakened or absent and, interestingly, its chemical component seems to be compromised after repetitive stimulation. From these results, the authors concluded that both synaptogenesis and synaptic transmission necessitate proper dynein–dynactin function.

38. Murphey RK, Caruccio PC, Getzinger M, Westgate PJ, Phillis RW:
• **Dynein-dynactin function and sensory axon growth during *Drosophila* metamorphosis: a role for retrograde motors.** *Dev Biol* 1999, **209**:86-97.

Path-finding and arborization of sensory axons during metamorphosis in *Glued*^{1} and cytoplasmic dynein light chain mutants were analyzed. In both mutants, proprioceptive and tactile axons reach the central nervous system on time, but exhibit defects in terminal arborizations.

39. Martin M, Iyadurai SJ, Gassman A, Gindhart JG, Hays TS,
• Saxton WM: **Cytoplasmic dynein, the dynactin complex, and kinesin are interdependent and essential for fast axonal transport.** *Mol Biol Cell* 1999, **10**:3717-3728.

Mutations in both cytoplasmic dynein (*cDhc64C*) and dynactin complex constituent (*Glued*) disrupt fast organelle transport in both directions and result in similar phenotypes to those caused by kinesin mutations, such as larval posterior paralysis and axonal swellings filled with organelles. Though direct protein interactions of kinesin with dynein heavy chain and p150^{glued} were not detected, strong dominant genetic interactions between kinesin, dynein, and dynactin complex mutations in axonal transport were observed, suggesting their interdependent role in fast axonal transport.

40. Signor D, Wedaman KP, Orozco JT, Dwyer ND, Bargmann CI,
• Rose LS, Scholey JM: **Role of a class DHC1b dynein in retrograde transport of IFT motors and IFT raft particles along cilia, but not dendrites, in chemosensory neurons of living *Caenorhabditis elegans*.** *J Cell Biol* 1999, **147**:519-530.

The authors found that kinesin II (KAP–GFP) and two of its presumed cargo molecules, OSM-1 (OSM-1–GFP) and OSM-6 (OSM-6–GFP), move in the retrograde direction along cilia and dendrites in *Caenorhabditis elegans*. Inhibition of this retrograde transport in cilia, but not in dendrites, was observed in a *che-3* cytoplasmic dynein mutant background. In contrast, anterograde IFT movement proceeded normally in this mutant, implying the specific function of CHE-3 in the retrograde transport of kinesin II and its cargoes within sensory cilia.

41. Bowman AB, Patel-King RS, Benashski SE, McCaffery JM,
•• Goldstein LSB, King SM: ***Drosophila* roadblock and Chlamydomonas LC7: a conserved family of dynein-associated proteins involved in axonal transport, flagellar motility, and mitosis.** *J Cell Biol* 1999, **146**:165-179.

Based on posterior-sluggish larval phenotypic screening in *Drosophila melanogaster*, the authors identified *roadblock*; the mutant exhibits diverse defects in intracellular transport including axonal transport and mitosis. This gene encodes a homologue of *Chlamydomonas* outer-arm dynein-associated protein LC7, which could associate with both flagellar outer-arm dynein and cytoplasmic dynein.

42. Tai AW, Chuang J-Z, Bode C, Wolfrum U, Sung C-H: **Rhodopsin's**
•• **carboxy-terminal cytoplasmic tail acts as a membrane receptor for cytoplasmic dynein by binding to the dynein light chain Tctex-1.** *Cell* 1999, **97**:877-887.

In this paper, the authors used yeast two-hybrid screening to isolate Tctex-1, a dynein light chain, as a possible binding partner of the carboxy terminus of rhodopsin. Carboxy-terminal rhodopsin mutations responsible for retinitis pigmentosa inhibited this interaction. Rhodopsin-containing vesicles co-sedimented with microtubules in the presence of cytoplasmic dynein in a Tctex-1-dependent manner, and moved along microtubules via cytoplasmic dynein.