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Kinesins in the nervous system

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Abstract. Both the development and the maintenance of neurons require a great deal of active cytoplasmic transport. Much of this transport is driven by microtubule motor proteins. Membranous organelles and other macromolecular assemblies bind motor proteins that then use cycles of adenosine 5′-triphosphate hydrolysis to move these 'cargoes' along microtubules. Different sets of cargoes are transported to distinct locations in the cell. The resulting differential distribution of materials almost certainly plays an important part in generating polarized neuronal morphologies and in maintaining their vectorial signalling activities. A number of different microtubule motor proteins function in neurons; presumably they are specialized for accomplishing different transport tasks. Questions about specific motor functions and the functional relationships between different motors present a great challenge. The answers will provide a much deeper understanding of fundamental transport mechanisms, as well as how these mechanisms are used to generate and sustain cellular asymmetries.

Key words. Kinesin; kinesin-like proteins; microtubule-based motors; axonal transport; neurons.

Introduction

Many of the key signalling functions of neurons depend on their extended and polarized morphologies. A simple bipolar neuron can be modeled as: a dendritic tree that gathers external signals, an elongated axon that transmits signals to postsynaptic cells, and a cell body that helps support the dendrites and axon through synthesis of new cytoplasmic components (fig. 1). The development and function of the dendrites, cell body and axon require an asymmetric distribution of components. That distribution is achieved, at least in part, through selective cytoplasmic transport. A specific set of macromolecules and organelles moves from the cell body into the dendrites and a different but overlapping set moves into the axon. A number of cytoplasmic asymmetries in neurons depend on differential transport. For example, MAP2 (microtubule-associated protein 2) mRNA is present in cell bodies and dendrites but it is not seen in axons [1]. Conversely, other mRNA species are found in the axon [2]. Consistent with this, translational machinery is abundant in dendrites and has also been detected in axons [reviewed in 3–5]. Although the mechanisms that move mRNA and RNA protein complexes are not well understood, there is good evidence that microtubules and microtubule motors are used [reviewed in 4]. It is also evident that microtubules and motors are used for the selective transport of synaptic proteins from the cell body to the axon terminal [e.g. 6, 7–10]. There is little doubt that cytoplasmic transport in mature neurons is selective and well regulated [reviewed in 11].

Long-range transport in neurons involves two large families of microtubule motor proteins, the cytoplasmic dyneins and the kinesins. These force-generating enzymes bind to vesicles, mitochondria, or other cargoes and then pull them along microtubules, using the microtubules as polar tracks. The polarity of the tracks derives from the head-to-tail polymerization of α/β tubulin heterodimers [12–14]. A given motor binds to a microtubule and advances by stepping processively in one direction. Known members of the dynein family walk toward the minus (slow-growing) ends of microtubules [reviewed in 15, 16]. Some members of the kinesin family also walk toward the minus ends of microtubules, but many walk toward the plus (fast-growing) ends [reviewed in 17].

The organization of microtubules in neurons provides some insight into how differential transport is accomplished (fig. 1). In axons, the plus ends of microtubules are uniformly oriented away from the cell body [18]. Therefore, microtubule-based movement away from the cell body, or anterograde axonal transport, is thought to

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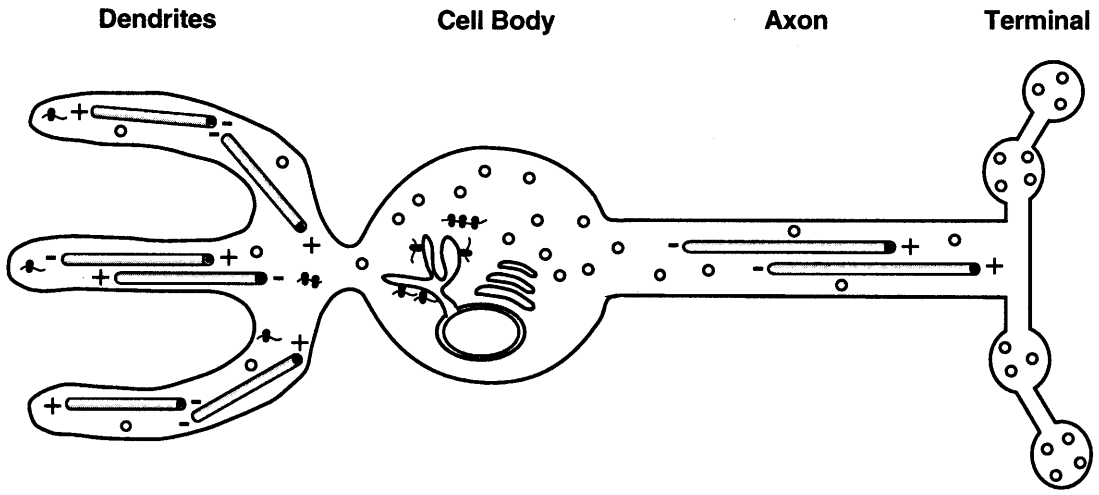

Figure 1. Cartoon of a bipolar neuron. The postsynaptic dendritic tree is to the left. Microtubules are oriented in both polarities; plus ends distal and minus ends distal. Dendrites contain mRNAs, polyribosomes, and other components of protein synthetic machinery. The cell body, in the center, contains extensive synthetic machinery and the nucleus. The axon extends to the right. It has less extensive synthetic machinery in comparison to dendrites. Axonal microtubules are uniformly oriented with their plus ends distal to the cell body.

be driven primarily by plus end-directed members of the kinesin family. Microtubule-based movement back toward the cell body, or retrograde axonal transport, is thought to be driven by one or more members of the dynein family and perhaps also by some minus end-directed kinesins [reviewed in 16, 19].

Microtubules in dendrites are organized differently than in axons (fig. 1). The plus ends of about half the microtubules are oriented away from the cell body, while the other half are oriented toward the cell body [20]. Organelle movement down an axon appears quite resolute, usually proceeding for long distances without changing direction [21], while organelle movement in dendrites appears more saltatory with frequent pauses and direction reversals [22]. Irresolute movement in dendrites might reflect alternating activities of plus- and minus end-directed motors on microtubules of a single polarity. Alternatively, it might reflect the activity of just one motor type that shifts between neighboring microtubules of opposite polarity. In either case, the fact that organelles and macromolecular assemblies get delivered to distant parts of dendrites despite the random polarity of the microtubule tracks presents an interesting puzzle.

Another puzzle has been revealed by the collective discovery of a large number of microtubule motor proteins. Why are there so many different motors? It is formally possible that the microtubule-based transport processes of cells could be accomplished using only two motors, one plus end-directed and one minus end-directed. However, this is not the case; even single-celled organisms have many microtubule motors. For instance the genome of the budding yeast *Saccharomyces cerevisiae* encodes one cytoplasmic dynein and kinesins [reviewed in Saunders, this volume and 23]. The genome of the nematode *Caenorhabditis elegans* contains two dyneins and at least 17 kinesins (J. A. Powers, personal communication). Genetic and molecular screens in the fruit fly *Drosophila melanogaster* have uncovered one cytoplasmic dynein [24, 25] and 14 kinesins so far [26–28]. Molecular and biochemical approaches in vertebrates have revealed as many as 4 cytoplasmic dyneins [29–31] and 30 kinesins [32–34].

Natural selection has presumably been the primary force for the diversification of microtubule motor proteins, so the advantages conferred by motor variations must be significant. Focusing on the kinesin family, the N-termi-

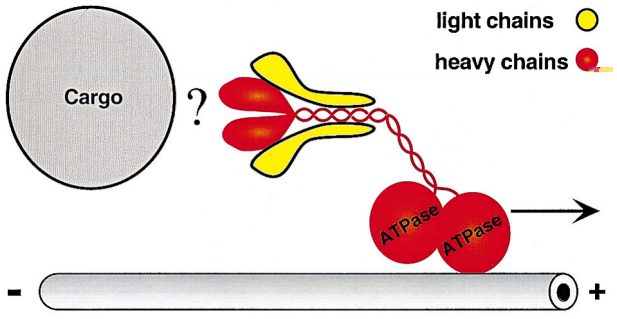

Figure 2. A cartoon of the kinesin holoenzyme. The heavy chains (orange) dimerize via coiled-coil interactions between the alpha-helical neck/stalk regions. The globular amino-termini hydroyze ATP to generate processive stepping movements along the microtubule toward the plus end. The light chains (yellow) may regulate cargo binding and modulate mechanochemical activity of the heavy chain. The question mark represents putative molecules that are involved in the linkage of kinesin to cargo.

nal ~350 amino acids that comprise the mechanochemical or motor domain are highly conserved (fig. 2). In many kinesins, the motor domain uses the hydrolysis of ATP (adenosine 5′-triphosphate) and a cycle of microtubule binding and release to generate progressive stepping movements. Amino acid differences in this motor domain separate the kinesin family into a number of subfamilies [28, 35]. (For a recent phylogenetic tree, see [36].) Such differences almost certainly alter the mechanisms by which different kinesins function. In fact, one subfamily of kinesins has members that instigate microtubule depolymerization [37]. Other kinesins do move at different velocities, ranging from 0.02 to 1.5 microns (μm) per second in vitro (table 1). They also probably vary in processivity; the relative number of steps made by a single motor protein before it diffuses away from the microtubule track. Differences in motor velocities may have evolved to tune rates of material delivery to match rates of consumption. Differences in processivity may provide a means to vary both rates of delivery and concentrations of free cargoes along the transport path.

Outside the motor domain, kinesins are not conserved except in the case of orthologs, which are homologous proteins that have equivalent functions in different species [35]. It is reasonable to expect that different nonmotor regions impart different functions to their motors. Although relatively little information has been gathered about the functions of nonmotor regions, they probably are important for cargo docking and regulation of motor domain activity. This suggests that the functions of different microtubule motors could be distinct and not overlap. However, some experimental results suggest that motors which normally have distinct functions may, under certain circumstances, be able to function in place of one another. For example, single disruptions of the *S. cerevisiae* kinesins CIN8p and KIP1p do not cause dramatic phenotypes while the disruption of both causes severe mitotic defects and lethality. These and other observations indicate that the functions of CIN8p and KIP1p overlap substantially despite the fact that their nonmotor domains have little or no sequence similarity [38, 39]. In contrast, a study of the single mutant phenotypes in real time with GFP labelled microtubules and chromosomes revealed that these motors normally do have separate functions in mitosis [40].

The large number of kinesins and the possibility of overlap under certain cellular conditions suggest that sorting out their functions will be challenging. At present, speculation on motor functions draws on a variety of information. First, expression patterns can provide clues about the sort of processes in which a motor participates. Obviously, the motors that drive mitotic movements must be present during mitosis and those that drive axonal or dendritic transport processes must be present in postmitotic neurons. Second, the polarity and velocity of movement of a motor on microtubules in vitro can be compared with the direction and velocity of transport processes in cells. For instance, a kinesin that is minus end-directed or moves very slowly is unlikely to participate in fast anterograde axonal transport. Third, phenotypic analyses of motor gene mutations and other functional inhibition strategies can reveal processes and movements that require a particular motor. Finally, an association of a motor with specific molecules or organelles revealed by localization in cells or biochemical fractionation can point to functional motor-cargo interactions. For example, if a biochemical approach identifies such an association, it can be pursued to reveal precise motor-cargo docking mechanisms.

In the remainder of this review we discuss the properties and potential functions of kinesins that are known or suspected of having functions in postmitotic neurons. To date, there is published evidence for the expression of 8 different kinesin subfamilies in neurons. Characterization of a few kinesins has been extensive, while characterization of a number of others, discovered more recently, is still in the early stages. Table 1 is a comparative chart that outlines information on the structure, motility, expression and potential roles of neuronal kinesins. While this review is necessarily abridged, it identifies some important features for motors from each subfamily and gives references to other literature that can provide more comprehensive information.

Conventional kinesin and the kinesin heavy chain subfamily

The driving force behind the discovery of cytoplasmic transport motors was curiosity about how organelles move in axons. The discovery of the first kinesin (often called 'conventional kinesin' but termed 'kinesin' for brevity in this section) stemmed from the development of video-enhanced differential interference contrast (VE-DIC) microscopy and the discovery that squid axoplasm can sustain organelle transport after being extruded from the axon [21, 41–43]. VE-DIC microscopy of extruded axoplasm allowed visualization of the movement of small organelles along individual microtubules and tests of the mechanism of movement with pharmacological agents. A key observation was that organelles moving along microtubules could be frozen in place by the nonhydrolyzable ATP analog, AMP-PNP [44]. This led to the isolation of microtubule/motor complexes, biochemical fractionation, and the reconstitution of microtubule-based motility in vitro [45, 46 and reviewed in 47]. The development of in vitro motility assays and the isolation of kinesin had a profound impact on the study of microtubule-based motility. Research on the mechanisms of microtubule-based movement has expanded and progressed tremen-

Table 1. Characteristics of the neuronal kinesins.

Kinesin subfamilies	Structure	In vitro motility and ligation analysis	Expression	Disruption phenotype in neurons	Proposed neuronal cargos
Conventional kinesin	Holoenzyme = tetramer: 2 identical motor subunits (~110 kDa), 2 assoc. proteins (~65 kDa). Motor subunit: N-term. motor domain, central coiled-coil-forming α-helices, C-term. globular. [17, 28]	Anterograde. 0.5–1.0 μm s^{-1}. Ligation: high conc. on proximal side, low conc. or absent on distal side. [17, 46, 103, 104]	Probably in most eukaryotes. Not in *S. cerevisiae*. In virtually all tissues. Relatively abundant. [56, 82, 105–109]	Inhibition of axonal transport. Organelle-filled axonal swellings. Reduced number of terminal boutons Weak action potentials and weak transmitter release. [6, 7, 9, 10, 107, 120–123]	In axons, anterograde transport vesicles and perhaps other membrane-bounded organelles. 17, 28]
Kinesin-II	Holoenzyme = trimer: 2 diff. motor subunits (1~85 kDa plus 1~95 kDa), 1 assoc. protein (~110 kDa). Motor subunits: N-term. motor domains, central coiled-coil-forming α-helices, C-terms. globular. [see Cole this volume and 126, 143]	Anterograde. ~0.3 μm s^{-1}. Ligation: high conc. on proximal side, low conc. or absent on distal side. [125, 128, 130–132]	Reported in sea urchin, mouse, fly, nematode and *Chlamydomonas*. Found in most tissues. Higher conc. in brain and testes. [see Cole this volume and 126, 143]	*osm-3* mutants in *C. elegans* fail to assemble dendritic axonemes in chemosensory neurons. [186]	In specialized dendrites, axoneme components. In axons, anterograde transport vesicles. [see Cole this volume and 126, 143]
UNC-104/KIF1's	UNC-104/KIF1A Holoenzyme = monomer: 1 motor subunit (~190 kDa). Motor subunit: N-term. motor domain, C-term. globular. [8, 144, 146]	Anterograde. 0.9–1.5 μm s^{-1}. Ligation: high conc. on proximal side, low conc. on distal side. [8]	Reported in human, mouse and *C. elegans*. Found in neuronal tissues. [8, 144, 146]	Reduced number of terminal boutons. Reduced number of synaptic vesicles in boutons. Increased number of transport vesicles in neuronal cell bodies. Neuronal degeneration. [145, 148]	In axons, anterograde vesicles bearing proteins required for synaptic vesicle formation at terminals. [8, 145, 148]
	KIF1B Holoenzyme = monomer: 1 motor subunit (~131 kDa). Motor subunit: N-term. motor domain, C-term. globular. [124, 150]	Anterograde. ~0.6 μm s^{-1}. Ligation: not reported. [150]	Reported in mouse. Found in all tissues assayed. [124, 150]	KIF1B antibodies block movement of mitochondria on microtubules in vitro. [150]	Mitochondria. [150]
	KIF1D Holoenzyme = monomer?: Motor subunit 122 kDa. Motor subunit: N-term. motor domain, C-term. globular. [187]	Hypothesized to be anterograde. [187]	Reported in rat. mRNA expressed in tissues throughout the brain and high levels of expression in epidermal secretory cells of the choroid plexus. [187]	Not reported.	In choroid plexus, possibly involved in secretion of cerebrospinal fluid. [187]
KIF2 and KIN I	KIF2 Holoenzyme = dimer?: 2 identical motor subunits (~80 kDa). Motor subunit: N-term. Globular, central motor domain, C-term. α-helical. [37, 124, 153, 155]	Murine KIF2: Anterograde. ~0.5 μm s^{-1}. Ligation: high conc. on proximal side, low conc. on distal side. Xenopus KIF2: Microtubule depolymerizing activity. [37, 153]	Reported in mouse. Found in axons of young neurons. Low expression in other tissues. [124, 153]	Antisense oligonucleotides inhibited neurite outgrowth and blocked accumulation of a specific protein in growth cones. [154]	In growing axons: 100–120 nm membrane-bounded organelles or other anterograde cargoes important for differentiation. [153, 154]

Table 1. (Continued).

Kinesin subfamilies	Structure	In vitro motility and ligation analysis	Expression	Disruption phenotype in neurons	Proposed neuronal cargos
KIF4 and Chromokinesin	KIF4 Holoenzyme = dimer?: 2 identical motor subunits (140 kDa). Motor subunit: N-term. motor domain, central α-helix, C-term. globular. [164]	Anterograde. 0.03 μm s^{-1}. Ligation: not reported. [164]	Reported in mouse. Found in young neurons and other neonatal mouse tissues and in adult spleen [164]	Not reported.	In mitosis, spindle associated membrane-bounded organelles. In axons, membrane-bounded anterograde organelles. [164]
MKLP1	MKLP1/CHO1 Holoenzyme = 362 kDa multimer: possibly 2 motor subunits (~110 kDa), undefined accessory proteins. Motor subunit: N-term. motor domain, central α-helical domain, C-term. globular. [167, 169–173]	Anterograde. ~0.06 μm s^{-1}. Ligation: not reported. [167]	Reported in human, hamster, flies and nematodes Found on centrosomes of interphase cells; on spindles and midbodies of mitotic cells. Found in cell bodies and dendrites of postmitotic cultured cells. [167–171, 174]	Antisense oligonucleotides inhibited formation of dendrites by cultured cells. [175, 176]	In cell body and dendrites, microtubules. [175, 176]
C-terminal motor	KIFC2 Holoenzyme = dimer: 2 identical motor subunits (~97 KDa). Motor subunit: C-terminal motor α-helical stalk. [184, 185]	Hypothesized to be retrograde. Velocity: none reported. Ligation: accumulates on both proximal and distal sides. [184, 185]	Reported in mouse. Found in neuron cell bodies, dendrites and axons. [184, 185]	Not reported.	In dendrites, novel class In axons, retrograde or of multivesicular bodies. ganelles. [184, 185]

dously. Now, a variety of novel mechanochemical tests and high resolution structural data for kinesin and NCD (non-claret disjunctional), a kinesin-related motor, provide a rich context for thinking about how motor proteins work [reviewed in 17, 48–50]

The physical composition of kinesin is well understood. Each holoenzyme is composed of two identical mechanochemical subunits, usually called heavy chains (KHCs), and two tightly bound nonmotor subunits, usually called light chains (KLCs) [51, 52]. Among the wide variety of eukaryotes in which kinesin has been identified, the heavy chains range in size from 92 to 117 kDa while the light chains range from 57 to 70 kDa [26, 28]. A cartoon of the organization of the heavy and light chains in the kinesin quaternary structure is depicted in figure 2.

As mentioned, KHC is responsible for the mechanochemical activity of kinesin. The gene encoding KHC was first cloned from *D. melanogaster* and subsequently from a number of other organisms, allowing good predictions of important secondary and tertiary structures [53–59]. KHC has a globular motor domain at the N-terminus, two central α-helices separated by a proline hinge and a globular region at the C-terminus [54, 60–62]. The motor domain is the site of ATP hydrolysis, microtubule binding, and is sufficient for anterograde movement on microtubules [52, 63]. The central α-helical 'stalks' of two heavy chains form coiled-coils to dimerize in parallel [64]. Thus, each kinesin holoenzyme has a pair of motor domains at one end.

The mechanochemical activity of kinesin is being studied in great detail. The use of VE-DIC microscopy, laser optical traps, and high-resolution tracking methods allows testing of the activities of individual motors on millisecond time scales. A single kinesin motor can step along microtubules for long distances following the path of a single protofilament [65, 66]. The dual-headed character of kinesin is likely to contribute to this highly processive behavior [66, 67]. It has been proposed that the two heads step alternately along a protofilament from one tubulin dimer to the next, at 8 nanometers (nm) per step [reviewed in 68, 69]. The step cycle is coupled to the ATP hydrolysis cycle of the motor domain with a ratio of one step per ATP [70–72].

The tail domain of kinesin is thought to be the site for the attachment of cargoes. At the C-termini of dimerized KHCs, the globular regions and the distal stalk,

form the binding sites for two KLCs. This complex is collectively referred to as the tail of kinesin (see fig. 2). KLC possesses coiled-coil-forming regions at its N-terminus that interact with KHC [73–80]. The C-terminus of KLC has 6 tetratrico repeats (TPRs) that may mediate further protein-protein interactions [81]. The respective contributions of the KHC C-termini and KLCs to kinesin function are not clear. There has been one report of a vertebrate KLC isoform that localizes to mitochondria perhaps to direct mitochondrial transport [82]. In addition to cargo binding, the tail may serve to regulate the KHC motor domains.

How might the tail of kinesin regulate the motor activity of the heads? In vitro, kinesin has been observed to exist in two distinct conformations, folded (at the proline hinge) and extended. The two forms are favored by low and high ionic strength, respectively. The ATPase activity of the motor appears to be much greater in the unfolded state. This leads to a model in which the tail of an active kinesin motor is extended and bound to cargo. When released from the cargo, the tail contacts and represses the mechanochemical activity of the head [83]. This is a particularly appealing model because it suggests coordinate regulation of cargo-binding and motor activities. Recently, studies comparing coexpression of KLC and KHC to KHC overexpression alone suggests that KLC and a portion of the KHC tail do indeed inhibit microtubule binding activity of the motor domain [84].

Mechanisms for kinesin regulation are likely to involve phosphorylation [reviewed in 85]. Both KHC and KLC are phosphoproteins [86–88] and the results of several experiments suggest that changes in kinesin activity correlate with differential phosphorylation although these experimental results are not wholly consistent [86, 88–92]. Additional evidence suggests that a slight shift to lower pH may trigger activation of kinesin motor activity by relieving the inhibition by the tail [84]. Further study into the influence of differential phosphorylation states and local pH levels on kinesin function will lead to a better understanding of the transitions between cargo-bound, active states and soluble, inactive states of kinesin [83, 84, 89].

How might motors select and bind to cargoes? It is known that membrane-binding sites for kinesin have protease-sensitive components and the binding of kinesin to membranes is saturable [92–94]. These data support the idea that the kinesin tail targets the holoenzyme to membrane-associated docking proteins [reviewed in 95, 96]. The vertebrate protein, kinectin, is a good candidate for a docking protein on endoplasmic reticulum (ER) [97–101]. Antibodies to kinectin produce punctate staining patterns in the cell bodies and proximal dendrites of motor neurons. Similar kinectin staining is not seen in axons despite the presence of strong kinesin staining [102]. This suggests that different proteins must be used to link kinesin to its axonal cargoes. Motor-cargo linkage mechanisms remain largely unexplored and constitute an important area for future research.

Which neuronal cargoes are transported by kinesin? When axonal transport is physically blocked by ligation, anterograde cargoes accumulate on the cell body-proximal side and retrograde cargoes accumulate on the distal side of the ligation. Immunolocalization data indicate that kinesin accumulates on the proximal sides of axon ligations, along with small vesicles, mitochondria, and smooth ER. In contrast, very little kinesin accumulates on the distal sides of ligations [103, 104]. This suggests that kinesin is an anterograde axonal transport motor. It also suggests that kinesin does not make the return trip to the cell body but is instead degraded at the axon terminal [103, 104]. Neuronal cargoes of kinesin then could include small vesicles, mitochondria, and smooth ER. Consistent with this, kinesin has been detected in association with vesicles, mitochondria, ER, and coated vesicles by various immunocytological approaches [56, 82, 105–109]. Biochemical fractionation of organelles suggests that kinesin can bind to the membranes of small vesicles [110, 111]. However, as with any data indicating a physical association of a motor and an organelle, the possibility that the motor may be a passive cargo in some instances needs to be kept in mind.

Function inhibition studies have also been used to search for kinesin cargoes. Both classical genetic and other inhibition approaches have been used to reduce or eliminate kinesin activity. A consistent conclusion is that kinesin indeed has a role in axonal transport. It also appears to function in a wide variety of cytoplasmic motility processes in nonneuronal cells [112–119]. We focus below on the disruption of kinesin function in neurons.

Mutations in metazoan kinesin genes and the resulting neuronal phenotypes have been reported only in *D. melanogaster*. The fly *Kinesin heavy chain* (*Khc*) and *light chain* (*Klc*) genes are essential. Animals bearing *Khc* or *Klc* null mutations exhibit distal paralysis then die during the larval stages of development [120, 121]. As distal paralysis develops, the posterior segments often curve up and over the back of the animal. This tail-flipping phenotype may be due to either strong tonic contraction of dorsal muscles or poor contraction of abdominal muscles [10, 121]. Eventually the entire animal becomes paralyzed and dies. The cause of the paralysis is a loss of neuron function [122]. The early distal paralysis probably occurs because the longest axons, which innervate the posterior muscles, are most sensitive to a loss of kinesin function. When compared to short axons, long axons in *Khc* mutants suffer more

severe reductions in both terminal size and neurotransmitter secretion [10, 122].

The underlying cause of the neuronal defects in *Khc* and *Klc* mutant larvae is a disruption of both anterograde and retrograde axonal transport. Electron microscopy of mutant nerves shows axonal swellings packed with vesicles, mitochondria, multivesicular bodies, and other membranous organelles [10]. It is thought that these organelle jams form when anterograde transport vesicles are stranded due to nonprocessive movement. While stalled, they hinder the passage of other cargoes, occasionally causing a massive pile-up of both anterograde and retrograde organelles. If this organelle jam hypothesis is correct, then the organelles that initiate the jams must be cargoes of kinesin. These initiator organelles have not yet been distinguished from innocent passers-by that are trapped by steric hindrance [10, 121].

Other inhibition studies also provide evidence that kinesin is important for fast axonal transport. For example, antisense KHC oligonucleotides injected into rabbit eyes were found to suppress the axonal transport of certain proteins destined for synaptic vesicles, secretory vesicles, and the plasma membrane [7, 9]. When applied to cultured hippocampal neurons, antisense KHC oligonucleotides caused abnormal accumulation of the terminal proteins GAP-43 and synapsin I in cell bodies [6]. These results and others support the hypothesis that kinesin acts as an anterograde motor for transport vesicles. However, the possibility remains that some of the antisense effects are due to global disruptions of axonal transport similar to those seen in *D. melanogaster Khc* mutants.

A cell-free axonal transport system has been used to search for specific cargoes of kinesin. Antibodies that bind squid KHC, when applied to extruded squid axoplasm, inhibited all vesicle movement in both anterograde and retrograde directions [107]. In a second study, antibodies against two different regions of KLC were used [123]. One was directed against the second tandem repeat of the TPR region, which is highly conserved and hypothesized to be involved in cargo binding. When applied to extruded squid axoplasm, it inhibited bidirectional organelle movement by apparently dissociating the organelles from microtubules. The second antibody, which was directed against a conserved region in the N-terminal region of KLC, did not interfere with organelle movements [123]. These data raise an interesting question. Since cytoplasmic dynein and probably other kinesins are present in the axoplasm, why does the inhibition of kinesin stop all movement? One possibility is that microtubule motors are highly interdependent and coordinately regulated which reveals further challenging questions to be answered in the study of motor regulation and cargo transport.

To study the role of kinesin in vertebrate neurons, it is possible to conduct gene disruption experiments in mice. It has been found that mice, like humans, have multiple KHC genes. Some of these KHC genes are expressed in all tissues while others are restricted to neuronal expression patterns [33, 57, 59, 124]. Disruption of the ubiquitously expressed mouse KHC gene, KIF5B, leads to an early embryonic lethal phase which is too early to assess phenotypes in the nervous system [119]. Tissue-specific KHC gene disruptions should provide an exciting way to determine functions of the ubiquitous KHC and neuronal KHC isoforms in the mouse nervous system. In addition, such approaches have the potential to provide important insights into the relationship between kinesin and neurodegenerative disorders in humans.

Kinesin II subfamily

Motors of the kinesin II subfamily have been found in a number of organisms [reviewed by Cole, this volume and 126]. The KRP85 and KRP95 motor subunits of sea urchin kinesin II were originally identified by biochemical purification of the holoenzyme [127, 128]. Independently, putative orthologs in vertebrates (KIF3A, KIF3B and KIF3C) and in *D. melanogaster* (KLP64D and KLP68D) have been identified based on their conserved kinesin motor domain or homology to KIF3s from other organisms [124, 125, 129–136]. Two other members of the kinesin II subfamily, encoded by the *fla10* gene of *Chlamydomonas rheinhardtii* [137] and the *osm-3* locus of *C. elegans* [138, 139], were discovered using classical genetic strategies followed by cloning and sequencing.

The structure of the kinesin II holoenzyme, a trimer, is distinct from conventional kinesin. The mechanochemical unit is two-headed, as is conventional kinesin, but it is a heterodimer [128, 132]. Although distinct, the kinesin II motor subunits are closely related. The motor domains are at the N-termini and are followed by long α-helices with coiled-coil-forming potential. It is likely that the specification for heterodimer formation resides in these α-helices [128, 132]. The tail of the heterodimer binds to a globular nonmotor subunit of approximately 110–115 kDa called the kinesin accessory protein or KAP [140, 141]. KAP may function in cargo binding and mechanochemical regulation of kinesin II motility [126, 140, 141]. Multiple isoforms of KIF3A and the mouse kinesin associated protein, KAP3, are expressed [124, 131, 132, 141]. Also, it has been shown that KIF3A is able to pair with either KIF3B or KIF3C [28, 35, 125, 128, 132–136, 142]. It is thought that the kinesin II structural diversity derived from mixing and

matching different KIF3 and KAP3 isoforms imparts functional diversity.

The heterodimeric nature of the kinesin II motor might produce mechanochemical characteristics that are distinct from those of conventional kinesin. However, tests with sufficient temporal resolution to uncover such differences have not been reported. Kinesin II monomer motor subunits, heterodimers, or the holoenzyme all drive plus end-directed microtubule motility at about 0.5 μm per second [128, 130, 132]. A shift from low to high ionic strength solution conditions causes a reduction in the sedimentation velocity of kinesin II. As with conventional kinesin, this suggests a conformational change from a folded to an extended state. It is not yet known if these changes alter mechanochemical activity as is observed with the conformational changes of conventional kinesin [140].

What are the neuronal cargoes of kinesin II? Extensive genetic, biochemical and cell biological studies of various kinesin II components have revealed that these motors transport protein assemblies required for construction and maintenance of nonmotile cilia in dendrites of sensory neurons. This rich area of research has recently been reviewed [in Cole, this volume and 143]. Are there axonal transport cargoes of kinesin II? KIF3A and B are expressed strongly in the nervous systems and testes of mice, frogs and flies but they are also expressed to a lower extent in other tissues [130–132]. KIF3C is expressed in the nervous systems of mice, rat, human and frog, with a particularly strong expression in eye tissues [125, 134, 135]. KIF3A, B and C accumulate by immunolocalization on the proximal side of an axon ligation and to a lesser extent on the distal side [125, 131, 132]. Yamazaki et al., have identified a class of 60 to 160 nm vesicles as possible neuronal cargoes of KIF3B [132] and KIF3C purifies with an anonymous class of vesicles as well [134]. These observations are intriguing and consistent with the hypothesis that kinesin II also transports vesicles in anterograde axonal transport.

UNC-104/KIF1 subfamily

UNC-104 was the first neuronal kinesin-related protein to be identified. It was discovered via screens for uncoordinated mutations in *C. elegans* followed by cloning and sequencing [144, 145]. Subsequently, KIF1A, the murine ortholog of UNC-104, was identified in a PCR-based screen for neuronally expressed genes with kinesin motor domain homology [8, 124]. The predicted amino acid sequences of KIF1A and UNC-104 are ~70% identical in their motor domains and 71% similar along their entire lengths, suggesting similar functions [8]. A putative human UNC-104 ortholog has also been identified [146]. Most of our knowledge of the structure and function of these motors comes from studies of the nematode and mouse proteins.

The UNC-104 holoenzyme is distinct from conventional kinesin and kinesin II. Results from rotary shadowing and sedimentation analyses showed that KIF1A exists as a globular 14 nm monomer [8]. Sequence analysis indicates that the motor domain is at the N-terminal end, followed by a region predicted to form α-helix. This putative helical region does not show the heptad repeat organization characteristic of a coiled-coil-forming structure. The C-terminal region is basic and contains pleckstrin homology domains, which highlights the possibility of protein-protein interactions [8, 144]. However, no proteins have been found to copurify with native KIF1A [8].

Mechanochemical tests with KIF1A purified from a heterologous expression system indicate that it is indeed a microtubule motor. It drives plus end-directed motility at 0.9–1.5 μm per second; faster than conventional kinesin or kinesin II [8]. These data pose an interesting question. How can a monomeric motor be processive? The ATP-driven stepping mechanism predicted for KHC requires alternation of microtubule-bound and unbound states. Two-headed holoenzymes like conventional kinesin can achieve processivity by coordinating their motors; one head is bound to the microtubule while the other seeks the next binding site. A single headed holoenzyme would need either a very different stepping mechanism or a nonmotor region that acts as a microtubule tether. Alternatively, processive movement could be achieved by cooperative operation of multiple monomers on a single cargo. Recently, high resolution tests of monomeric KIF1A fusion protein have shown that the motor domain can move processively and may employ electrostactic interactions to retain close contact with the microtubule after a step [147]. Further analyses into the differences and similarities between the mechanochemistry of different kinesin motors will be very exciting.

What are the neuronal cargoes of UNC-104? First, both UNC-104 and KIF1A appear to be expressed primarily in the nervous system [8, 145]. Immunolocalization data indicate that KIF1A accumulates strongly on the proximal sides of axon ligations and faintly on the distal sides. As with conventional kinesin and kinesin II, this is consistent with the activity of an anterograde axonal transport motor. KIF1A antibodies have been used to copurify KIF1A, small vesicles, and the synaptic terminal proteins synaptophysin, synaptotagmin and Rab3A. Other synaptic terminal proteins, SV2, syntaxin 1A, and SNAP-25, did not copurify with KIF1A [8]. These data support the notion that UNC-104 and KIF1A act as anterograde transport motors for vesicles that carry a subset of synaptic terminal proteins.

Hall and Hedgecock [145] provided the first indication that UNC-104 was responsible for the proper delivery of synaptic vesicle precursors to nerve terminals. Mutations in the *unc-104* gene cause behavioral defects that suggest impaired function of motor neurons, interneurons and possibly sensory neurons [145]. Ultrastructural analysis revealed axons with reduced numbers of synaptic swellings (boutons en passant) and reduced concentrations of synaptic vesicles within those boutons. Furthermore, neuron cell bodies contained higher than normal concentrations of small vesicles. Other membrane-bounded organelles, such as mitochondria, appeared to have relatively normal distributions in the mutant neurons [145]. Mutations in a vertebrate homolog give comparable phenotypes. Disruption of the KIF1A gene in mice leads to recessive lethality in newborn animals. Before death, these animals exhibited motor and sensory defects that were attributable to a retention of small vesicles and synaptic proteins in neuron cell bodies and neuronal degeneration [148]. Thus, UNC-104 and KIF1A appear to be motors for the anterograde transport of vesicles that are important for the formation of synaptic vesicles at axon terminals.

KIF1B belongs to the UNC-104 subfamily but is not an ortholog of UNC-104. The KIF1B motor has been identified by PCR based on its expression in mouse brain and a rat myogenic cell line [124, 149]. Its motor domain is at the N-terminal end and has a high degree of similarity to the motor domains of UNC-104 and KIF1A [124, 149, 150]. However, outside the motor domain the KIF1B sequence is unique. Rotary shadowing and sedimentation analysis of expressed KIF1B and the mobility of the native enzyme on gels suggest that it is a globular monomer, possibly with a short stalk. Mechanochemical tests indicate that it is a microtubule motor. It drives plus end-directed movement at approximately 0.7 μm per second [150]. These observations on a second member of the UNC-104 subfamily reinforce the hypothesis that these kinesins function as monomeric motors.

The neuronal cargo of KIF1B may be mitochondria. It is expressed in many tissues including brain, is enriched in mitochondrial fractions of cytoplasm, and it colocalizes with mitochondria in cultured cells stained with KIF1B antibodies [150]. Most impressive, Nangaku et al. have reconstituted mitochondrial movement on microtubules with purified KIF1B. Mitochondria alone did not move on microtubules. The addition of KIF1B stimulated some mitochondria to move and addition of an antibody to KIF1B blocked that movement [150]. The consequences of in vivo inhibition of KIF1B have not been reported. Other microtubule and microfilament-based motors may participate in mitochondrial movement [119, 151, 152]. If KIF1B inhibition proves to halt or hinder mitochondrial movement in vivo, the case for KIF1B as a mitochondrial motor will be very strong.

The list of proteins in this subfamily continues to expand. Another neuronal member, KIF1D, was identified by a PCR screen for kinesins expressed in rat oligodendrocytes and optic nerve [187]. Its motor domain is $\sim$70% identical to murine KIF1A and B. Outside the motor domain, KIF1D has more similarity to KIF1B and contains predicted structural features of a monomeric motor. The characterization of KIF1D is in the early stages. The rat gene is expressed in a variety of tissues throughout the brain. Additionally, high levels of expression are detected in epidermal secretory cells of the choroid plexus. This tissue secretes cerebrospinal fluid for the CNS [187]. Based on this expression pattern, it will be of interest to explore KIF1D's relationship to secretion from the choroid plexus and other possible functions in the CNS.

KIF2 and the KIN I (internal motor) subfamily

Differentiation of a neuron requires the assembly of massive amounts of new cytoplasm, expansion of the plasma membrane and reorganization of the peripheral cytoskeleton. This places heavy demands on transport and requires close regulation of cytoskeletal dynamics. The discovery and characterization of the mouse KIF2 protein suggest that vertebrates may use some specialized microtubule motors during neuron differentiation [124, 153, 154]. There is a *Xenopus* homolog, XKIF2, which is 90% identical overall with the murine KIF2 [155]. Other kinesin-related proteins grouped with KIF2 based on motor domain sequences include the mitotic motors XKCM1 and MCAK [155, 156]. Thus, members of this subfamily appear to participate in diverse cellular processes. We will focus on the murine KIF2 with some comparisons to what is known of other KIN I proteins.

KIF2 is clearly distinct from other neuronal kinesins. The motor domain of KIF2 and other KIN I proteins is located in the center of the polypeptide [37, 124, 155–158]. The KIF2 N-terminal nonmotor flank is predicted to be globular while the C-terminal flank is predicted to be α-helical [124]. There is evidence that KIF2 and other KIN I motors form homodimers [37, 153, 155]. Native KIF2 isolated from mouse brain extract with antibodies had no detectable associated proteins and electron micrographs of rotary shadowed, expressed KIF2 suggest a globular structure [153]. Murine KIF2 has been reported to drive plus end-directed microtubule movement at about 0.5 μm per second [153]. In contrast, no microtubule motility was observed in tests of the related *Xenopus* kinesins, XKIF2 and XKCM1 [37, 155]. Instead, these proteins have been shown to

exhibit microtubule depolymerizing activity [37, 155]. It will be of interest to see which motor properties are common to these proteins when direct comparisons of biochemical activity are available.

The expression pattern and subcellular location of KIF2 in rodents is consistent with a role in axonal transport during neuronal development. KIF2 is expressed at low levels in several tissues but strongly in the axons of young, postmitotic neurons [153]. Expression of KIF2 in most adult neurons is low with the exception of the olfactory bulbs which continue to grow throughout adulthood [153]. KIF2 staining accumulates very slowly at axon ligations in adult mice. Most is seen on the proximal side of the ligation but some is also on the distal side [153]. The majority of KIF2 in cells is membrane bound and it is especially enriched in growth cone particles purified from embryonic mouse brain [153]. Beads coated with murine KIF2 antibodies precipitated 100 to 120 nm membrane-bounded organelles from vesicle fractions of rat brain. These KIF2-associated organelles lacked synaptic terminal proteins, although such proteins were abundant in the starting membrane fractions. Some of the organelles had characteristics of multivesicular bodies [153]. This is a surprising association for a putative anterograde motor because multivesicular bodies are generally thought to be retrograde cargoes. It is possible that KIF2 is transported as a passive cargo on a class of multivesicular bodies.

One inhibition study supports the hypothesis that KIF2 is involved in neuronal differentiation. The β_{gc} subunit of the insulin growth factor-1 receptor protein, which is important for neuron differentiation, is enriched in the growth cones of developing neurites of PC12 cultured cells and in differentiating rat brain [159, 160]. Inhibition of KIF2 expression by antisense oligonucleotides in PC12 cells inhibited neurite outgrowth, prevented β_{gc} accumulation in growth cones, and caused abnormal accumulation of β_{gc} in the cell body [154]. Two additional observations imply that this effect is specific. First, other terminal proteins including synaptophysin, synapsin I, and GAP-43 accumulated normally in the growth cones of KIF2 antisense-treated cells. Second, antisense inhibition of KHC (conventional kinesin) did not prevent the accumulation of β_{gc} in growth cones [154]. These data support the model that KIF2 is a motor for the anterograde transport of organelles bearing β_{gc}. However, the conflict remains that other KIN I proteins, including XKIF2, exhibit control of microtubule dynamics in vitro. This suggests a novel function for these kinesins in developing neurons. Control of microtubule dynamics at the growth cone may be a key element of the a growing axon's response to guidance cues. If KIF2 were involved in such a process, then depletion of KIF2 could result in disruption of downstream events such as proper sorting of β_{gc} to the axon compartment. Further tests of murine KIF2 activities and examination of the expression of XKIF2 in the frog nervous system will eventually resolve the role of KIF2 in young neurons.

KIF4 and chromokinesin subfamily

KIF4 has been implicated in both neuronal differentiation and mitotic division. It was identified in the Aizawa et al. PCR screen for mouse neuronal kinesins [124]. As detailed below, KIF4 is expressed in young neurons and it may have neuronal transport functions. However, the amino acid sequence of KIF4 is about 60% similar to the other vertebrate motors, Xklp1 (*Xenopus laevis*) and chromokinesin (chicken), which appear to be involved in mitosis and germ plasm localization in *Xenopus* oocytes [161]. Since the similarity extends over both the motor and nonmotor regions, they are likely to be orthologs [28, 162–164]. Thus, KIF4 may have multiple cellular functions.

The predicted structures of KIF4 and the other chromokinesins are similar to the structure of KHC; an N-terminal motor domain followed by a long α-helical stalk and a globular C-terminus [162–165]. Recombinant KIF4 can dimerize. The presumed dimers, when examined by rotary shadowing and electron microscopy, appear to have an extended stalk with two heads at one end [164]. Associated proteins may bind to the tail of native KIF4 but none have been reported yet [164].

KIF4 could be a motor for anterograde transport in young postmitotic neurons. Mechanochemical tests using single microtubules have been complicated by a microtubule bundling activity. However, when purified flagellar axonemes were used for the tests, bundling was not a problem and plus end-directed movement occurred at approximately 0.2 μm per second [164]. Both KIF4 protein and mRNA have been localized in the neurons of neonatal mice, as well as in other tissues. The protein was not found in the neurons of adult mice. Subcellular immunolocalization of KIF4 in cultured cells suggest that it associates with microtubules and with some small organelles. This sort of fibrous plus punctate localization was seen in mitotic cells but also strongly in growth cones and interphase cell bodies. Thus, KIF4 was proposed as a motor for anterograde transport in differentiating neurons [164].

The hypothesis that KIF4 has mitotic function is based in part on its predicted structural similarity and partial coincidence of immunolocalization with the suspected mitotic motors, Xklp1 and chicken chromokinesin. The sequence similarities include a putative nuclear localization domain at a conserved position in the stalk region

and a weak zinc-finger domain in the C-terminal region [28]. Sekine et al., reported general KIF4 immunolocalization on mitotic microtubules and weak staining in interphase nuclei of mouse cells. In contrast, Xklp1 in *Xenopus* appears strongly concentrated in interphase nuclei, in various parts of the spindle, and on mitotic chromosomes [162]. Chromokinesin has also been localized to mitotic chromosomes in chicken neuronal cells [163]. The contrasts in the localization patterns may be due to differences in fixation protocols. Vernos et al., reported that they lost most Xklp1 localization from spindles and all staining of mitotic chromosomes when they used fixation conditions similar to those used by Sekine et al. [162, 164].

Multiple functions for Xklp1 in *Xenopus* also lead to the supposition that KIF4 participates in multiple cellular motility processes. Depletion of Xklp1 from frog egg extracts leads to defects in the maintenance of spindle structure and reduces chromatin-spindle associations [166]. Additionally, Xklp1 is necessary for the microtubule-dependent process of germ plasm aggregation in *Xenopus* oocytes [161]. An evaluation of possible Xklp1 expression and function in developing frog neurons would be of interest. Overall, it is difficult to distinguish between three possibilities: KIF4 could be primarily an axonal transport motor used in young postmitotic neurons; it could be primarily a mitotic motor with a residual presence in postmitotic neurons; or it could be both a mitotic and interphase neuronal motor. It is evident that further characterization of KIF4, including inhibition studies in neurons, will be required to distinguish between these possibilities. Due to the prediction of nucleic acid binding by members of this subfamily and the role in germ plasm aggregation, it would be interesting to test if KIF4 is involved in RNA trafficking in developing neurons.

MKLP1 subfamily

Recent studies of MKLP1 (CHO1) support the notion that some kinesins may indeed function as both mitotic and postmitotic neuronal motors. MKLP1 was discovered in an antibody-based search for novel spindle proteins [167, 168]. Monoclonal antibodies were generated against mitotic spindles isolated from Chinese hamster ovary cells [168]. Cloning and sequencing of the CHO1 antigen gene from human cells revealed a new kinesin which was named mitotic kinesin-like protein 1 or MKLP1 [167]. Beyond vertebrates, MKLPs have now been identified in *C. elegans* (CeMKLP1) and *D. melanogaster* (*pavarotti*) [169–172].

While the structure of MKLP1 appears similar to that of the heavy chain of conventional kinesin, its mechanochemical function is quite different. The predicted structure includes an N-terminal motor domain followed by an α-helical stalk and a C-terminal globular region [167, 169–173]. Kuriyama et al. report that the native hamster holoenzyme is 362 kDa, perhaps composed of two 110 kDa motor polypeptides and one or more accessory proteins [173]. Mechanochemical tests of expressed human MKLP1 indicate that it drives plus end-directed movement at 0.06 μm per second; one-tenth the rate of KHC driven movement. Furthermore, it can crosslink microtubules of opposite polarity and slide them past one another [167].

The CHO1 and other anti-MKLP1 antibodies have been used to localize MKLP1 in a variety of organisms and cell types. The hallmarks of MKLP1 localization often include centrosome staining in interphase and early mitosis, increased staining at the spindle equator and interzone during metaphase and anaphase, and midbody localization during telophase and cytokinesis [167–171, 174]. Mitotic mutant phenotypes for MKLP1 suggest a requirement of the motor for central spindle function and cytokinesis [169–171, 174]. There is also good evidence of MKLP1 expression in neurons. CHO1 antibody stains the dendrites and cell bodies of mouse neuroblastoma cells and rat cultured neurons [175, 176]. The staining is initially diffuse in the cell bodies of differentiating neurons that lack dendrites. The staining develops into elongate patches in the cell bodies and newly forming dendrites [175]. Experiments to define the subcellular localization of MKLP1 more precisely have not been successful. However, treatment of differentiated neurons with an antimicrotubule agent caused MKLP1 antibody reactivity to resume a diffuse staining pattern in dendrites and cell bodies [175]. Thus, MKLP1 localization in dendrites appears to be dependent on its association with microtubules [175].

The effects of inhibition of MKLP1 have been studied in postmitotic neurons and the results suggest a role for MKLP1 in organization of dendritic microtubules. Antisense oligonucleotides, when applied to cultured neuroblastoma cells and neurons, inhibited the development of dendrites [175, 176]. In addition, Sharp and colleagues observed that the expression of MKLP1 motor domain in cultured insect cells stimulated the formation of dendrite-like processes. These extensions contained microtubules with the bipolar organization characteristic of proximal dendrites; plus ends were both distal and proximal relative to the cell body [173, 177]. It has been proposed that such bipolar organization is generated by sliding transport of microtubules [178]. In this model, microtubules nucleated by the centrosome in the cell body are freed from the centrosome and some portion are then transported into dendrites with their minus ends leading to generate the bipolar array. The mechanochemical activity of MKLP1, its localization in developing neurons, and the

effects of inhibition and overexpression have led to the hypothesis that it is a motor for sliding microtubules into dendrites [173, 175–177]. However, the mechanisms that generate dendritic and axonal microtubule arrays are still under some debate [discussed in 11, 179–181]. Also, it should be kept in mind that MKLP1 may transport cargoes other than microtubules. Based on MKLP1's role in cytokinesis, such cargoes might be molecules important for cytoskeletal reorganization that would serve to direct the assembly of dendritic microtubule arrays.

MKLP1's neuronal function opens up the possibility that other mitotic motors might be involved in organization of neuronal microtubule arrays. The vertebrate mitotic plus end-directed motor, Eg5, has been localized to postmitotic neurons and is then available to be involved in dendritic microtubule organization [182]. Additionally, expression in Sf9 cells of a minus end-directed mitotic kinesin-like motor, CHO2, causes growth of axon-like processes [183]. This suggests that indeed, multiple mitotic motors may be available for further function in neuronal differentiation.

C-terminal motor subfamily

To date, minus end-directed kinesins share the characteristic of having C-terminal motor domains. The first C-terminal motors identified, NCD and KAR3, and most of the other members of this most divergent kinesin subfamily appear to function in mitosis and meiosis [19, 28]. However, other C-terminal kinesins have been identified in mouse. KIFC2 is expressed in mouse neurons and KIFC3 is ubiquitously expressed [34, 184, 185]. Thus, these neuronal kinesins have the potential to act as retrograde motors.

The structural and mechanochemical characterization of KIFC2 is still in progress. The amino acid sequence indicates clearly that it has a C-terminal motor domain. The nonmotor region is predicted to be α-helical with coiled-coil forming capability [184, 185]. Sedimentation and coimmunoprecipitation analyses of native KIFC2 purified from mouse brain suggest that it exists as a homodimer in vivo [185].

Because of its C-terminal motor domain, KIFC2 is expected to drive minus end-directed microtubule motility. However, when expressed in heterologous systems, it has failed to produce microtubule motility of any sort [184, 185]. KIFC2 may still be a microtubule motor; reconstitution of microtubule-based movement with some kinesins is quite difficult. Biochemical tests of truncated forms of the protein suggest that the motor domain can bind microtubules and hydrolyze ATP [185]. In addition, KIFC2 purified from mouse brain extract binds microtubules. However, the binding is not sensitive to ATP [184]. Thus, KIFC2 acts as a microtubule associated protein and an ATPase. Whether or not it acts as a microtubule motor remains to be seen. With the assumption that KIFC2 is a minus end-directed microtubule motor, it has been proposed to carry retrograde cargoes in axons and dendrites. Immunolocalization suggests that KIFC2 is present in axons, dendrites, and cell bodies of neurons but not in mitotic cells [184, 185]. Hanlon et al. reported that at axon ligations, KIFC2 accumulates on both proximal and distal sides. This is consistent with a motor that is synthesized in the cell body, transported as passive cargo down the axon, and then activated to transport material back toward the cell body [184]. Independently, Saito et al. focused on the possibility that KIFC2 functions in dendrites [185]. They reported that one-third of KIFC2 in mouse and rat brain homogenate cofractionates with a novel class of multivesicular body-like organelles; presumably components of an endocytic or some other prelysosomal pathway. KIFC2 also cofractionated with a complex of tubulin and various unidentified proteins [185]. Resolving the differences between these various studies will undoubtedly reveal more about this C-terminal kinesin and its potential role in axonal and dendritic transport.

Concluding remarks

Cytoplasmic transport is complex, even when one considers only transport by the kinesins in postmitotic neurons. The full complement of neuronal kinesins is yet to be defined in any one organism. As it currently stands, at least 8 are present in mice, 3 in flies, and three in nematodes. More certainly await discovery in these model systems. Furthermore, counting the number of different neuronally expressed kinesin-related genes underestimates motor diversity because multiple isoforms of motor subunits and associated proteins can be expressed from single genes. As various genome sequencing projects are completed, identification of the full complement of neuronal motors for each system should be relatively straightforward. The greatest challenges will be found in identifying the functions of each motor and learning how those functions are controlled.

Pioneering investigations of the last two decades have mapped out a set of information that should be gathered for each kinesin. This includes: subunit composition; structure; mechanochemical characteristics; expression pattern; association with cytoplasmic components; inhibition/overexpression phenotypes; modes of cargo-linkage; and regulatory mechanisms. Other neuronal motors, dyneins and myosins, have been and will continue to be discovered and characterized by similar approaches. An understanding of the mecha-

nisms that coordinate the activities of multiple motor types will be particularly important for a complete understanding of cytoplasmic transport.

Axonal transport provides a good context for considering questions of motor coordination. Different motor types must be associated with the same cargoes. For instance, cytoplasmic dynein and other retrograde motors must employ anterograde motors for transport to distal parts of the axon. During this delivery, their retrograde activity needs to be overcome. Perhaps anterograde motors like conventional kinesin simply overpower constitutively active retrograde motors. Alternatively, retrograde motors may be silenced during their delivery. After reaching the terminal, the anterograde motors are probably silenced by degradation or other regulatory mechanisms and the retrograde motors engage their specific cargoes for the return trip to the cell body.

Another question regarding motor coordination in axons is posed by the initial observation that transport organelles can move along both microtubules and actin filaments in axoplasm [188]. It has also been shown that mitochondria can move on both filament types in neurons [151, 189]. One interesting hypothesis is that the actin cytoskeleton facilitates short, local transport while microtubules direct long distance transport. Several excellent reviews discuss actin-based motility and the cytoplasmic myosins thought to be involved in this process [190–192]. It has been found recently that conventional kinesin heavy chain and a cytoplasmic myosin, MyoV, directly interact [193]. It will be of great interest to determine the underlying mechanisms that coordinate motility on microtubules and actin filaments.

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