Motor proteins for cytoplasmic microtubules

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It has been thought that motile structures within the cell are driven toward the plus and minus ends of microtubules by the ATPases, kinesin and dynein, respectively. Recently obtained data indicate that this model is far too simplistic. Kinesin is now understood to be one representative of a family of proteins. Another member of the kinesin family has been found to generate force toward the microtubule minus end. Evidence for either a bidirectional dynein, or closely related retrograde and anterograde forms of dynein has also received potent new support. The discovery of a third potential microtubule motor, the GTPase, 'dynamin', complicates matters further.

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Introduction

Microtubules serve as intracellular highways along which a rich variety of subcellular structures travel. During interphase the passengers are membrane-bounded organelles, such as mitochondria, lysosomes, endoplasmic reticulum, and assorted vesicles that participate in both the secretory and endocytic pathways. In dividing cells, chromosomes travel along microtubules from the metaphase plate to the spindle poles, and microtubules in one half spindle slide past anti-parallel microtubules of the other half spindle. Following fertilization, and conjugation in unicellular species, nuclei must travel toward one another as a prelude to fusion. To generate the forces for most, if not all, of these movements, the cell relies upon mechanochemical enzymes known as 'microtubule motor proteins' (Fig. 1).

In vitro motility assays have implicated two microtubule-stimulated ATPases, kinesin and dynein, as motors for microtubule-based transport. Movements toward the plus ends of microtubules have been thought to reflect the actions of kinesin [1], whereas motility in the opposite direction has been attributed to dynein [2]. Several predictions emerge from the kinesin/dynein model for bidirectional transport. For example, in the axon, where microtubules are oriented with their plus ends distal to the neuronal cell body, kinesin and dynein have been hypothesized to serve as the motors for anterograde and retrograde fast axonal transport, respectively. In the mitotic spindle, where kinetochores attach to microtubule plus ends, dynein might be a motor for anaphase A, the process by which chromosomes are transported toward the spindle poles.

Although experimental support for these predictions has been gained, the evidence must be weighed against an explosion of new data pointing to the existence of many additional motors, some of which already have been shown to possess novel or surprising properties. It is premature to formulate a detailed, refined model for microtubule-dependent transport on the basis of most current information, because most of the new apparent motors have been linked only in general terms to particular *in vivo* functions. Nevertheless, it is not too early to speculate that the cell makes use of numerous microtubule motors, each of which normally specializes in particular tasks, but may overlap functionally with other motor proteins. This review will focus on the work that has been carried out on microtubule motors in the past year.

Cousins of kinesin

A short while ago, kinesin was considered to be a unique, albeit widely distributed, protein comprising two ~124 kD heavy chains and a pair of ~64 kD light chains. The kinesin heavy chains are now known to be members of a rapidly expanding group of structurally related proteins. Predicted amino acid sequences have been published already for kinesin heavy chains from three species, and for seven proteins bearing extensive sequence homology to the heavy-chain motor domain (see Table 1).

This domain is located within a globular region situated at one end of kinesin heavy chain. It comprises ~340 amino acid residues beginning at the amino terminus, and contains binding sites for microtubules and ATP [3]. Each known member of the kinesin family includes a similar region with an average homology to the kinesin motor domain of about 40%.

Two general approaches led to the discovery of most new members of the kinesin family. The first of these involved the genetic mapping and subsequent sequencing of loci for known mutants in several species of organisms. In this manner, the *KAR3* gene in the budding yeast, *Saccharomyces cerevisiae* [4], and the *bimC* gene in the fungus, *Aspergillus nidulans* [5], were found to encode proteins

Abbreviations

MAP—microtubule-associated protein; **PCR**—polymerase chain reaction.

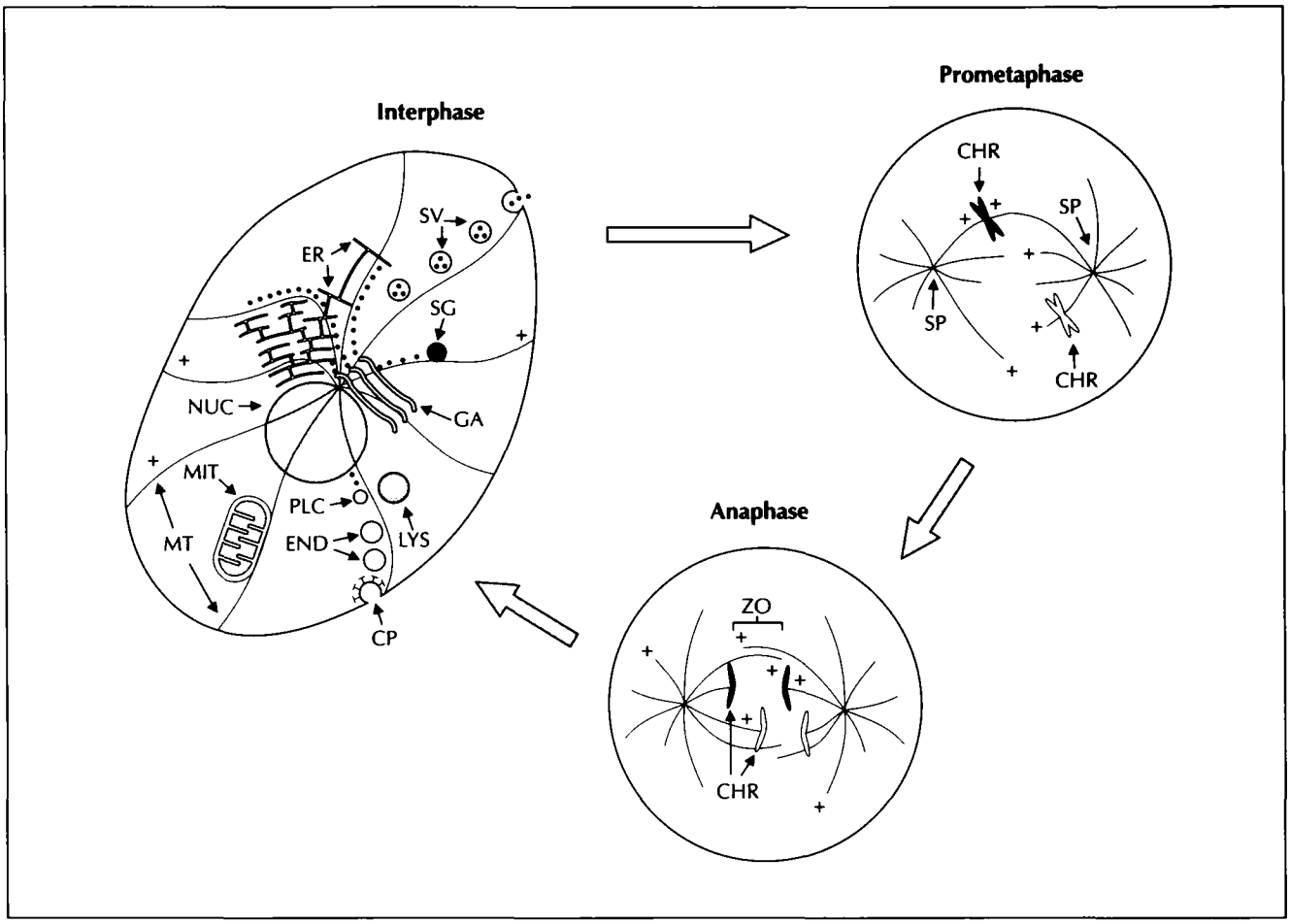

Fig. 1. Sites of action for microtubule motor proteins. A variety of motile events, several of which are illustrated here, are likely to reflect the actions of microtubule motor proteins in interphase and mitotic cells. To account for the numerous molecular species that probably serve as microtubule motors (see text), each may normally move a particular cargo in a unique direction relative to microtubule polarity, and may also be restricted to specific cell types or developmental stages. In interphase cells, where microtubules (MTs) often emanate from a centrally located organizing center with their + ends being distal to the nucleus (NUC), microtubule motors have been implicated in the following phenomena: elaboration of the endoplasmic reticulum (ER); transport of vesicles containing nascent secretory and membrane proteins fron the ER to the Golgi apparatus (GA, including *cis* and *trans* Golgi networks); a Golgi-to-ER recycling pathway; transport from the Golgi of secretory vesicles (SVs) to the plasma membrane, and of vesicles that provide larger secretory granules (SGs) and pre-lysosomal compartments (PLCs) with their characteristic contents; and motility of lysosomes (LYSs), mitochondria (MIT) and coated pit (CP)-derived endosomes (ENDs). During prometaphase, duplicated chromosomes (CHRs) move in both directions along microtubules that grow out of the spindle poles (SPs). These movements are apparently caused by microtubule motors that reside on kinetochores, and lead to the alignment of chromosomes in the center of the spindle at metaphase. During anaphase, chromosomal segregation is accomplished by two independent types of movement, each of which is likely to involve microtubule motors: first, chromosomes travel along microtubules toward the spindle poles (anaphase A), possibly with the aid of kinetochore-bound dynein [38,40,41]; and second, the two half-spindles move apart (anaphase B) as a result of inter-microtubule sliding that occurs within a zone of overlap (ZO).

with partial homology to the heavy chains of *Drosophila melanogaster* kinesin. Related proteins that were discovered using this strategy and were described subsequently include the *Drosophila* proteins, non-claret disjunctional, otherwise known as ncd or cand [6], and nod [7•], cut7$^+$ in the fission yeast, *Schizosaccharomyces pombe* [8•], and UNC-104 in the nematode, *Caenorhabdites elegans* [9•]. Another related protein, Eg5, which is encoded by a developmentally regulated mRNA found in oocytes and eggs of the toad, *Xenopus laevis*, was revealed by sequencing of the corresponding cDNA [10•]. The possible existence of more than 30 additional kinesin-like proteins has been indicated using the polymerase chain reaction (PCR) and PCR primers complementary to conserved sequences located within the known or suspected motor domains of kinesin heavy chain and related proteins, such as KAR3 and bimC [11•,12,13•]. The PCR approach has also led to an independent discovery of ncd [14].

The kinesin family members resemble each other physically in regions beyond those of primary sequence similarity. In the case of kinesin heavy chain, the remaining sequences form a long, largely α-helical structure that projects from the motor domain and extends to the carboxyl end of the polypeptide [15–18]. The available evidence suggests a similar organization for kinesin-like proteins, but in KAR3 [4] and ncd [6,14] the apparent motor domains are located at the carboxyl ends, and the tail regions of bimC [5], cut7$^+$ [8•] and nod [7•] evidently lack α-helical stretches. When present, the α-helical tail

Table 1. Properties of kinesin heavy chains and related proteins.

Protein	Species	Sequence similarity ATP*	Sequence similarity Mt†	*In vitro* MT binding	Transport direction	Location of motor domain	Likely or possible function	References
Kinesin heavy chain	*Drosophila melanogaster* (fly)	100	100	Yes	Plus end	Amino end	Organelle transport	[17]
	Loligo pealeii (squid)	95	83	Yes	Plus end	Amino end	Organelle transport	[20,51]
	Strongylocentrotus purpuratus (sea urchin)	90	80	Yes	Plus end	Amino end	Organelle transport	[25•]
UNC-104	*D. melanogaster*	71	43	Not determined	Not determined	Amino end	Synaptic vesicle transport	[9•,29••]
ncd (ca^{nd})	*D. melanogaster*	71	20	Yes	Minus end	Carboxyl end	Disjunction in female meiotic germ cells; mitosis	[6,14,30••,31••]
nod	*D. melanogaster*	43	55+	Not determined	Not determined	Carboxyl end	Like ncd, but for non-exchange chromosomes only	[7•,32••]
KAR3	*Saccharomyces cerevisiae* (budding yeast)	654••	27	Yes (*in vivo*)	Not determined (minus end suspected)	Carboxyl end	Nuclear fusion following karyogamy; mitosis	[4]
bimC††	*Aspergillus nidulans* (fungus)	62	47	Not determined	Not determined	Amino end	Separation of spindle pole bodies; spindle formation	[5]
cut7+††	*Schizosaccharomyces pombe* (fission yeast)	67	37	Not determined	Not determined	Amino end	Separation of spindle pole bodies; spindle formation	[8•]
Eg5††	*Xenopus laevis* (toad)	71	57	Not determined	Not determined	Amino end	Unknown; potentially pronuclear migration or mitosis	[10•]

*Per cent identity in optimally aligned sequences to amino acid residues 86–106 of *D. melanogaster* kinesin heavy chain, which includes the ATP-binding site and short flanking regions. †Per cent identity in optimally aligned sequences to amino acid residues 310–339 of *D. melanogaster* kinesin heavy chain, which includes most or all of the microtubule-binding site. +Per cent identity in optimally aligned sequences to amino acid residues 310–331 of *D. melanogaster* kinesin heavy chain. **Per cent identity in optimally aligned sequences to amino acid residues 86–105 of *D. melanogaster* kinesin heavy chain. ††May represent species-specific versions of the same protein. MT, microtubule.

domains may cause kinesin-like proteins to dimerize, as is known to occur for kinesin heavy chains. The tail regions are dissimilar to one another in primary sequence, except for those of Eg5, bimC and cut7+, which resemble each

other to a limited degree. The putative motor domains of these three proteins are also closely related to one another in primary sequence, raising the possibility that Eg5, bimC and cut7$^+$ represent species-specific versions of the same protein.

The (not quite forgotten) kinesin light chains

Although kinesin heavy chains and their homologues have attracted a lion's share of attention, the light chains have not been completely ignored. Recently, three distinct light chain cDNAs were cloned from rat brain and were completely sequenced [19••]. Assuming that translation begins at the first in-frame methionine codons, these kinesin light chains range in size from 542–560 amino acid residues and, as predicted from their electrophoretic mobilities, have molecular weights of about 62 700 ± 1050. The three isoforms are generated by alternative splicing of a single gene, are identical to one another except at their extreme carboxyl ends, and are unrelated to any other proteins whose sequences have been entered in the major databases. A series of 15 imperfect heptad repeats that begin at the amino termini of all three light chains probably forms an α-helical coiled-coil domain. This motif may enable pairs of kinesin light chains to dimerize, or may permit light chain binding to the α-helix-rich shaft of kinesin heavy chain. It is not yet known whether other members of the kinesin family contain subunits related to kinesin light chains.

The relation of structure to function in the kinesin family

Primary sequence data imply that a basic blueprint exists in the design of kinesin heavy chain and its relatives. Each contains a conserved, globular, ATP-binding domain attached to one of many rod-like appendages. It is tempting to speculate that, in all cases, the globular regions bind to microtubules and represent ATP-dependent motor domains, and that the tails specify the type of cargo that the protein transports. It must be noted however that, except for kinesin itself, none of the other family members has been purified, direct evidence for microtubule binding has been obtained only for KAR3 [4] and ncd [14] and, whereas all seven kinesin-like proteins contain a consensus ATP-binding sequence (Gly–X–X–X–X–Gly–Lys–Thr/Ser; where X is any amino acid), none have yet been reported to have ATPase activity. The ATP-binding site is evidently functional in ncd, however, as AMPPNP, a non-hydrolyzable ATP analog, stabilizes microtubule binding by ncd [14], just as it does for kinesin.

As well established as the kinesin family may be, questions regarding the *in vivo* functions of its members still abound. A steadily growing body of literature has implicated kinesin itself as a motor for organelle transport along microtubules. Antibodies to kinesin have inhibited vesicle motility in squid giant axons [20], the elongation of lysosomes in macrophages [21•] and pigment granule dispersion in fish melanophores [22•]. Isolated chromaffin granules were reported to move along microtubule-associated protein (MAP)-free microtubules in a kinesin-dependent manner [23]. Immunocytochemical studies demonstrated that kinesin co-accumulates with anterograde-moving organelles at nerve ligations [24•], and is associated with membranous organelles in mitotic spindles of embryonic sea urchin cells [25•]. In *Drosophila*, kinesin heavy chain is an essential gene product that probably transports axonal organelles toward the periphery of the organism [26•].

The collective studies cited above imply that the function of kinesin is to move many types of organelles, in most if not all cases toward microtubule plus ends. One recent report is difficult to reconcile with this model, however. Microinjection of anti-kinesin into fibroblasts was found to cause vimentin-containing intermediate filaments to retract from peripheral cytoplasm, and form dense, perinuclear aggregates [27].

Most classes of intermediate filaments behave as if they were cross-linked to microtubules. In light of the anti-kinesin microinjection results, the possibility that kinesin is a cross-bridging factor must be entertained. However, an alternative explanation that considers a broader body of evidence regarding the *in vivo* function of kinesin seems more plausible. Perhaps a membranous compartment connects intermediate filaments to microtubules, and continuously relies upon kinesin for its active transport toward the cell periphery. Such a membrane system could be disrupted by anti-kinesin, leading to collapse of the intermediate filament system.

In vivo functions of kinesin light chains have not been determined, but the available data raise two possibilities. Electron microscopy of purified kinesin indicated that at least two light chain epitopes are located at the end of the molecule opposite the globular motor domains, suggesting that the light chains are involved in binding to membrane-bounded organelles [15]. Consistent with this idea is the finding that a 10-residue amphipathic helix is located at the extreme carboxyl end of two of the three kinesin light chain isoforms [19••]. By analogy to other proteins with similar motifs, kinesin molecules bearing either of these light chain domains may be targeted to mitochondria.

The observation that kinesin heavy chain dimers have a more than fivefold higher microtubule-stimulated ATPase activity than tetrameric kinesin consisting of two each of the heavy and light chain subunits [28•], suggests another function for kinesin light chains. It is possible that they regulate the enzymatic, and by extension, mechanochemical properties of intact kinesin. The two potential light chain functions described here are not mutually exclusive, nor do they preclude other roles for these kinesin subunits.

Of the seven kinesin-like proteins highlighted here, only the *C. elegans* protein, UNC-104, appears to serve a function related to that of kinesin. Worms containing mutant UNC-104 have impaired mobility that ranges from slow and uncoordinated to nearly paralyzed. In UNC-104 mutants, synaptic vesicles are abnormally concentrated in neuronal cell bodies and are conspicuously rare in axons, whereas other membranous organelles, such as the Golgi apparatus, endoplasmic reticulum and mitochondria, have normal distributions [29••].

The UNC-104 data imply that different classes of organelles rely on distinct motors for anterograde transport along axonal microtubules. UNC-104 seems to be the motor for synaptic vesicle motility, whereas mitochondria and other types of organelles apparently rely on one or more other motors. The identities of the other nematode motors, whether a conventional kinesin is included among them, and the applicability of these findings to other organisms remain to be determined.

The other six kinesin-like proteins that have been fully sequenced are involved in events related to cell division, conjugation and fertilization. *Drosophila* ncd mediates disjunction of meiotic chromosomes in female, but not male germ cells, and of mitotic chromosomes in early embryonic cells [6,14]. To the amazement of many workers in the motor protein field, ncd, in contrast to kinesin, was shown to generate force toward the microtubule minus end [30••,31••]. The ncd protein also generates torque, causing microtubules to rotate [30••], and bundles microtubules in an ATP-independent manner [31••]. How these findings relate to the *in vivo* function(s) of ncd is not yet clear. They do raise the possibility, however, that ncd is a motor for the chromosome movements that mark the initiation of anaphase A.

Mutations of nod, another *Drosophila* protein, yield phenotypes that are nearly identical to those of ncd mutants, but are restricted to non-exchange chromosomes [7•]. Although mutants for either nod or ncd are recessive, double heterozygotes have a phenotype similar to that of homozygous nod mutants [32••]. This is the first well documented case for overlapping functions of multiple kinesin-like proteins in a single species. The direction of force production by nod has not been reported, but the protein appears to be important for maintaining chromosomes at the metaphase plate, and thus may be a plus-end-directed motor.

In yeast, the KAR3 gene product is required for nuclear fusion following mating, and also plays a non-essential role in mitosis. Nuclear fusion depends upon force production toward microtubule minus ends, raising the prospect that KAR3 is another minus-end motor. Further evidence for this possibility stems from the location of the KAR3 motor domain. KAR3 and ncd are distinct from kinesin and the other kinesin-like proteins in having motor domains located at their carboxyl, rather than amino ends. An appealing model which naturally follows is that the orientation of the motor domain determines the direction of force generation. Further design specifications are required, however, as a kinesin heavy chain motor domain engineered to the carboxyl end of an α-spectrin tail retains plus-end-directed motor activity (LSB Goldstein, personal communication).

The structural similarities of bimC [5], cut7$^+$ [8•] and Eg5 [10•] are echoed by apparent functional similarities. The separation of spindle pole bodies and mitotic spindle formation are controlled in part by bimC in *Aspergillus* and by cut7$^+$ in *S. pombe*. The *in vivo* function of Eg5 has not yet been investigated genetically or biochemically, but its presence in *Xenopus* oocytes and unfertilized eggs, and the rapid disappearance of its mRNA following fertilization suggest roles for Eg5 in pronuclear fusion or mitosis during early embryogenesis. Eg5 is the first, and so far only, kinesin-like protein from a vertebrate species to have been reported.

Dynein structure and function

Major achievements regarding the molecular biology of dynein were announced recently. A pair of technical *tours de force* resulted in the complete sequencing of axonemal dynein β heavy chains from two species of sea urchins. Each of these dynein subunits encompasses more than 4460 amino acid residues and is encoded by a nearly 15-kb mRNA! The two (mega)polypeptides are nearly identical in sequence, and each contains four apparent binding sites for ATP [33••,34••]. An extensive discussion of this work can be found in the review by Witman (this issue, pp 74–79).

A reversibly-associating subunit of rat brain cytoplasmic dynein has also been cloned and sequenced [35•]. This ~150 kD polypeptide is probably the mammalian equivalent of the Glued gene product of *Drosophila*. Consistent with the idea that Glued encodes an essential housekeeping protein, as dynein is presumed to be, mutations in Glued are lethal in homozygotes and lead to multiple developmental abnormalities in heterozygotes.

The role of dynein in organelle motility was the subject of several recent studies. Immunofluorescence and immunoelectron microscopy was used to examine ligated mammalian nerves, in which anterogradely and retrogradely moving organelles accumulated on the proximal and distal sides of the lesions, respectively. Immunoreactive dynein was associated principally with membranous organelles, and was present at high levels on both sides of the ligations [36•]. Co-localization of dynein with organelles on the distal sides was expected, in light of the evidence that brain dynein behaves as a retrograde, or minus-end-directed motor [2]. An attractive theory to explain the co-localization of dynein with organelles on the proximal sides of the lesions takes into account that dynein is synthesized in the neuronal cell body, but must be transported toward the end of the axon before it can begin to function as a retrograde motor. The suggestion was thus made that anterogradely moving organelles, perhaps driven by kinesin, include functionally inert dynein among their cargo. Once dynein reaches the axon terminal, it was hypothesized, the enzyme becomes activated to serve as a minus-end-directed motor [36•].

The dogma that dynein is solely a minus-end motor has been tested on a few occasions by studies of the giant amoeba, *Reticulomyxa*, and the firmest challenge yet was published recently. Detergent-lysed amoebae, which conduct vigorous organelle transport along microtubules at rates approaching 10 $\mu m\,sec^{-1}$, were challenged with a collection of 15 different ATP analogues and five conventional nucleoside triphosphates other than ATP. Only four of the nucleotides, all of which were ATP analogues, were able to promote organelle transport. Each of these supported motility in both anterograde and retrograde directions, and at a velocity that was independent of the direction of movement [37••]. The net effect of this study was the development of 'enzymatic fingerprints' for mo-

tor proteins in *Reticulomyxa*, and the demonstration that the anterograde and retrograde motors are virtually indistinguishable from one another. Earlier studies had implicated dynein as the only apparent organelle transport motor in *Reticulomyxa*. It appears, therefore, that this amoeba moves membranous organelles using either a bidirectional dynein, or two very closely related forms of dynein, one dedicated to plus-end movements and the other to transport towards the microtubule minus end.

In addition to moving membranous organelles along microtubules, dynein may be a mitotic motor. Both plus- and minus-end-directed ATP-dependent motors are located at the kinetochore [38,39], and the possibility that dynein serves as the latter was emphasized by a pair of recent immunofluorescence studies. A battery of polyclonal and monoclonal antibodies was used to demonstrate localization of dynein on or very near the kinetochores of mitotic chromosomes [40,41]. This places dynein at the location suspected for an anaphase A motor, and raises the possibility that dynein moves chromosomes poleward, toward microtubule minus ends.

Dynamin — a GTP-dependent motor?

Dynamin continues to yield surprises. Its classification as a microtubule motor has been based on its ability to bundle microtubules and cause microtubules within the bundles to slide apart from one another in the presence of ATP. The bundling activity was attributed to the principal protein found in the original preparations, an ~100 kD species, whereas the ATPase and motor activities required an additional fraction that comprised low levels of several polypeptides, and associated reversibly with the ~100 kD component [42].

It now appears that the natural substrate for dynamin is GTP. This conclusion is based on recent cloning and sequencing experiments demonstrating that the ~100 kD protein in rat brain contains a consensus GTP-binding site [43••], and on enzymological studies indicating that the equivalent bovine brain protein possesses a potent microtubule-stimulated GTPase activity in the absence of accessory factors (Shpetner HS, Vallee, RB: *J Cell Biol* [abstract] 1990, **111**: 290a). The activating fraction for ATP dependency has been analyzed and found to contain a nucleoside diphosphate kinase (RB Vallee, personal communication). This enzyme probably generates GTP from guanine nucleotides that are provided by the tubulin used for functional assays, and the ATP that is supplied to the system as an energy source. It is likely, therefore, that GTP is the natural substrate for the ~100 kD protein, which now must be regarded as the sole component of dynamin. In *Drosophila*, dynamin has been found to exist in at least two forms, which differ solely at their carboxyl ends and are encoded by a single gene [44•].

Analysis of the dynamin sequence has led to three additional conclusions of note: first, dynamin is unrelated to members of the kinesin family, except for a highly conserved 12-residue stretch that is important for nucleotide binding; second, dynamin does not contain any sequences related to microtubule-binding domains in conventional MAPs, such as tau, MAP-1B or MAP-2; and third, rat brain dynamin belongs to a family of novel GTP-binding proteins.

Presently, the other known members of this family include the mammalian Mx proteins, which are interferon-induced anti-viral factors [45], and a yeast (*S. cerevisiae*) protein, known as SPO15 or VPS1, that is involved in meiosis [46•] and vacuolar protein sorting [47]. These dynamin relatives range in size from ~74–100 kD, and exhibit the greatest degree of homology within their amino-terminal one-thirds, where the GTP-binding site of each is located.

The function of dynamin remains mysterious, but present evidence suggests that it plays a role in membrane trafficking, particularly in neurons. Dynamin is especially abundant in neuronal tissue [48•], and can be extracted from cells in a membrane-bound form [49,50•]. The *Drosophila* equivalent of dynamin is the product of the *shibire* gene, temperature-sensitive mutants of which have a paucity of synaptic vesicles at neuromuscular junctions when held at $\geq 29°$ [44•,50•]. The motor neuron-specific effects of *shibire* mutations probably reflect a defect in dynamin-dependent, endocytic recycling of synaptic vesicle membrane components following their fusion with the axollema at the synapse. Though microtubules have been postulated to be important for endocytosis, their precise roles in this process remain unknown.

Perspectives for the future

The state of knowledge about microtubule motor proteins has expanded enormously since early 1990, yet a profusion of fundamental questions remain unanswered. The *in vivo* functions of most putative motor proteins remain unclear, and genetic evidence alone clearly cannot remove all of the ambiguities. With the exception of Eg5 [10•], kinesin-like proteins are unknown in vertebrates, but the discovery of many others is widely anticipated. Now that a molecular handle has been gained for two of the dynein subunits [33••,34••,35•], it should be possible to determine whether a family of dynein-related proteins also exists. One other issue that is bound to attract attention in the future is regulation. None of the motor proteins are likely to function constitutively, and so determining how their activities are controlled by the cell represents another pressing challenge.

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