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Targeting of Motor Proteins

Richard B. Vallee and Michael P. Sheetz

Microtubules are responsible for chromosome segregation and the movement and reorganization of membranous organelles. Many aspects of microtubule-based motility can be attributed to the action of motor proteins, producing force directed toward either end of microtubules. How these proteins are targeted to the appropriate organellar sites within the cell, however, has remained a mystery. Recent work has begun to define the targeting mechanism for two well-studied motor proteins, kinesin and cytoplasmic dynein.

A role for microtubules in cell motility has been evident for nearly 50 years since these structures were identified as major components of cilia and flagella. That they function in the sorting of intracellular constituents was first suggested by the presence of these hollow polymers within the mitotic spindle. Depolymerization of microtubules was also found to block the segregation of chromosomes, and there is ample additional evidence that microtubules and their associated proteins are actively involved in many aspects of mitosis.

Evidence for a role for microtubules in sorting and transport of membranous organelles has been slower to emerge. The physiological importance of microtubules in some cases, such as axonal transport, is now evident, where the requirement for rapid, directed transport is obvious (*1*). However, the importance of microtubules and microtubule-based motor proteins in secretion and endocytosis, as well as in other aspects of membrane traffic, is still being elucidated. Certainly, microtubules are required for proper positioning of most, if not all, of the membranous components of the cytoplasm, presumably to facilitate their orderly interaction (*2*). However, depolymerization of microtubules allows most sorting activities to continue, albeit in some cases at slower rates. Such results may reflect a curious circularity inherent in experiments of this type: organelles that may normally be attached to microtubules and require microtubule motors for their transport become free to diffuse throughout the cytoplasm after microtubule depolymerization. Thus, microtubules may be required for aspects of subcellular sorting that have not been clearly revealed by microtubule disassembly experiments.

Additional roles for microtubules and motor proteins are beginning to emerge in concert with our view of membranous organelles as dynamic structures. For example, removal of microtubule motors from cell lysates has been found to inhibit fusion between early and late endosomes (*3*, *4*). There is also direct evidence for a role for microtubule motors in tubular network formation from endoplasmic reticulum membranes in vitro (*5*, *6*). Finally, elongation of membrane tubules of both Golgi and endocytic origin in cells treated with brefeldin A was found to be microtubule-dependent (*7*). Thus, microtubules may have both direct and indirect roles in the sorting of subcellular constituents.

The general principles of how microtubules and their associated motor proteins account for the distribution and redistribution of intracellular structures seem clear. Microtubules are polar polymers, the minus, or slowly polymerizing ends, of which tend to be located toward the cell center, whereas the plus, or rapidly polymerizing ends, tend to be located toward the cell periphery. Microtubules provide a directional track for organelle movement, whereas motor proteins provide the motive force. The first cytoplasmic motor proteins identified were kinesin and cytoplasmic dynein, which produce force toward the plus (*8*) and minus (*9*) ends of microtubules, respectively. Together with knowledge of the organization of microtubules within a given cell, it appeared that microtubule-based motility might be simply explained by the action of these two proteins. It has since become clear that both proteins are members of larger families and that microtubule-based movements within any given cell probably require a variety of different motor proteins. An additional factor critical to microtubule-dependent transport is the proper targeting and activation of motor proteins. Recent research has provided the first in-

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sights into these issues, particularly in regard to kinesin and cytoplasmic dynein, both of which are reviewed here.

Kinesins

Whether a protein is a kinesin or a kinesin-like protein is determined by a motor domain or head that is about 350 amino acids in length; this domain hydrolyzes adenosine triphosphate (ATP) and catalyzes movement. In the original kinesin, there are two heads that are linked by an α-helical coiled-coil tail that normally associates with two light chains. The kinesin tail links the motor domains to the structure to be carried and is responsible for regulating the motor activity. Intact kinesin has a very low microtubule-activated ATPase (adenosine triphosphatase) activity that is increased dramatically when the molecule is bound in a motile complex such as on the surface of anionic beads (*10*). Constructs of the kinesin molecule without its tail will support motility and have a high microtubule-activated ATPase activity because there is no inhibitory domain (*11, 12*). It may be expected that cellular conditions for activating kinesin should be tightly correlated with motility.

There has been a great proliferation of the identified kinesin-like motors [for reviews, see (*13–16*)]. On the basis of sequence similarities, the kinesin-like proteins have been grouped into eight major subfamilies (*13*). Three subfamilies have been exclusively associated with membranous organelle movements, and two others have been linked to movements of distinct vesicle populations (*17*). Spindle formation and chromosome movements in mitosis appear to be the major functions of three kinesin-like subfamilies. When the different subfamilies are compared, the greatest variation is found between the domains involved in linkage to the structure to be carried. An understanding of cellular function requires knowledge of the complete motility complex.

Because it is now possible to alter specific motors within cells, there is a rapidly expanding literature on the effects of motor depletion. Surprisingly, some of the phenotypes are quite mild, which raises the question of whether the motors are weak catalysts (increasing rates of transport only two- to threefold more than those of diffusive mechanisms) or whether there are overlapping functions of the many motors, such that other motors compensate for the loss of one. The loss of kinesin-like proteins is not instantly lethal in *Caenorhabditis elegans* (*18, 19*). In *Drosophila* mutations of kinesin, severe neurological phenotypes are observed but cells are viable and can proliferate (*20*). In *Saccharomyces cerevisiae*, the loss of a kinesin analog is not lethal until a myosin analog is also deleted (*21*). As a result of these studies, interest has been revived in a possible role for actin-based motors in vesicle transport (*22*). In some systems, such as squid axoplasm, there is direct evidence of an actin-based vesicle movement (*23*). The exact role that actin-based motility plays in organelle transport is obscure, although an obvious possibility is that it catalyzes the movement from the ends of microtubules through the actin cortex to the plasma membrane.

The NCD and Kar3 proteins are members of the kinesin family and move toward the minus end of microtubules, as does cytoplasmic dynein. These proteins are implicated in spindle organization and mitotic events (*15, 24*). With the identification of so many motors, there are increasing instances where multiple motors are expressed simultaneously in a single cell. A simple rationale for the multiple motors is that they will be under different control mechanisms that will be required for multiple transport phenomena to occur within the same cell.

At the heart of the function of the motors is attachment to the appropriate carrier and concomitant activation. In the case of kinesin, several of the important proteins in this process have been identified. As a largely soluble protein, kinesin can be recruited by diffusion to binding sites within the cytoplasm. Antibody labeling studies have localized subpopulations of kinesin to a variety of subcellular organelles, including the Golgi, the endoplasmic reticulum (ER)–Golgi intermediate compartment, and membranes in the sea urchin spindle (*25–27*). A problem with interpreting the antibody localization studies is that distribution does not necessarily correlate with activity (*28*). Binding studies have established that there are kinesin binding sites on the organelles that are saturable and protein-dependent (*26, 29*). The critical domain for binding to vesicles is in the kinesin tail, which maps to the coiled-coil regions of the tail.

Kinectin

A membrane protein thought to play a role in binding kinesin to intracellular vesicles, kinectin was purified with the use of a kinesin affinity column (*30*). Kinectin was originally localized to the endoplasmic reticulum, but preliminary studies find antigenically related forms on many intracellular compartments (*31*). Kinectin contains extensive α-helical coiled-coil regions in the COOH-terminal two-thirds of the molecule and has one hydrophobic domain starting seven amino acids in from the NH_2-terminus (amino acids 7 through 29). There is no obvious signal sequence, and thus the protein may associate with membranes post-translationally. In both size and sequence, the COOH-terminal domain of kinectin is similar to the two-headed myosin tail domain, which implies that the fully extended kinectin tail could reach over 100 nm.

Antibody inhibition studies demonstrate a role for kinectin in intracellular motility and glycoprotein processing. An antibody that binds a native epitope on kinectin inhibits kinesin binding and movement of vesicles in vitro (*32*). The antibody also inhibits cytoplasmic dynein binding to the same vesicles and dynein-based vesicle movement, but to a lesser extent. The kinectin antibody inhibits the movement of the major glycoprotein of vesicular stomatitis virus (VSV G protein) from the ER to the Golgi and subsequently to the plasma membrane when introduced into the cell (*33*). Whether kinectin alone is sufficient to activate kinesin motility has not been determined. In vitro vesicle motility, however, requires soluble components in addition to kinesin for motility (*34*), and there are a large number of proteins that bind to a kinesin affinity column (*30*).

Dyneins

The dyneins also represent a multigene family, though the relation between family members is of a quite different nature than that for the kinesins and myosins. Two general functional and structural classes of dynein have been identified: the axonemal dyneins, involved in ciliary and flagellar movement, and the cytoplasmic dyneins. In contrast to the kinesins and myosins, the force-producing heavy chains of the dyneins are all of similar size (471 to 538 kD) and primary structural organization, each polypeptide containing four equally spaced, centrally located P-loop consensus sequence elements corresponding to potential sites of nucleotidase activity (*35–43*). Ten to fifteen dynein heavy chains have been identified in each of several organisms, including rat (*44, 45*), sea urchin (*46*), *Drosophila* (*47*), *Chlamydomonas* (*48*), and humans (*45*). Many of these are likely to be components of cilia and flagella, which are known from biochemical and ultrastructural studies to contain multiple dynein isoforms. In contrast, only a single major cytoplasmic dynein has been identified, though additional minor cytoplasmic isoforms may exist (*44, 45, 49*). It is a curious feature of the dynein family that a diversity of dynein forms is required for the single function of ciliary and flagellar beating, whereas a limited number of cytoplasmic dyneins appear to serve in multiple roles.

Dynein Binding Sites

The axonemal and cytoplasmic dyneins differ with regard to their subcellular binding sites. From ultrastructural analysis of cilia

and flagella, the base of the axonemal dynein molecule can be seen to be stably anchored to the A microtubule of the fused microtubule pair, referred to as the outer doublet (*50*). The force-producing heads of the dynein molecule are positioned to interact with the adjacent outer doublet microtubules.

Cytoplasmic dyneins are thought to interact with a variety of structures, though the full range of interactions and functions has not been determined definitively. Cytoplasmic dynein has been localized to a number of discrete structures. These include late endosomes, lysosomes, and possibly elements of the Golgi apparatus (*51*), the distributions of which are consistent with control by a minus end–directed microtubule motor protein. Other studies have yielded a more general, fine punctate cytoplasmic staining pattern (*52, 53*), but it is uncertain whether these data reflect an association of cytoplasmic dynein with additional classes of membranous vesicles. In vitro analysis of the interaction of cytoplasmic dynein with membranes (*29, 54–56*) and studies of broken cell preparations (*4, 57, 58*) have supported an interaction with endosomes, apical exocytic vesicles in epithelial cells, and component membranes of the Golgi apparatus, and possible additional interactions with synaptic vesicles (*54*) and membranes of the endoplasmic reticulum (*6, 29*). Cytoplasmic dynein has also been implicated in nuclear migration in lower eukaryotes (*40, 41, 59, 60*) and humans (*61*).

Staining of kinetochores has also been observed during prometaphase in some studies (*52, 53, 62, 63*). Rapid minus end–directed chromosome movements have been observed in living cells during prometaphase, in support of a role for cytoplasmic dynein during this phase of mitosis (*64*), and a minus end–directed microtubule motor activity has been detected that is associated with kinetochores of isolated mitotic chromosomes (*65*). Cytoplasmic dynein also controls mitotic spindle positioning and elongation in *S. cerevisiae* (*40, 41, 66*) and could be involved in spindle assembly in vertebrates (*67*). It is not known how cytoplasmic dynein discriminates among different classes of membranous organelles nor how its interaction with kinetochores is temporally regulated during mitosis.

Dynein Accessory Subunits

Clues to resolving these questions and to understanding more general aspects of dynein regulation and force production have come from investigation of the component polypeptides of the dyneins and of functionally related proteins. In addition to the heavy chains, dyneins contain a variety of accessory subunits ranging in size from 8 to 150 kD [reviewed in (*68*)]. These polypeptides have been termed intermediate, light intermediate, and light chains, depending on their relative size. Subunit composition differs considerably between cytoplasmic and axonemal dyneins and even among axonemal forms of the motor protein. Because of the somewhat bewildering array of subunits, their functional interrelationship has been uncertain, but distinct accessory subunit classes have begun to emerge as their primary sequences have become known. Of particular interest have been the intermediate chains (ICs), involved in subcellular targeting. In addition, cytoplasmic dynein contains a class of as yet poorly understood 53- to 59-kD subunits (*69, 70*), known either as light intermediate or light chains, which, like the heavy chains, contain P-loop elements (*71, 72*) and could be involved either in force production or in regulation of dynein activity.

A number of distinct ICs (68 to 74 kD) have been cloned and sequenced from both axonemal (*73, 74*) and cytoplasmic dyneins (*75–77*). Conservation between axonemal and cytoplasmic ICs is limited to the COOH-terminal half of the sequences (*75*), which appears to be involved in the common function of heavy chain binding (*75, 77*). The NH_2-terminal portion may be involved in cytoplasmic- versus axonemal-specific functions. A variety of evidence suggests that the ICs are involved in targeting dynein molecules to appropriate binding sites within the cell. For example, the *Chlamydomonas* flagellar outer arm dynein ICs have been localized to the base of the dynein molecule by immunoelectron microscopy of purified protein (*78*), the appropriate location for a targeting role. One of the ICs could also be cross-linked to axonemal tubulin in situ (*79*) and was observed to bind to microtubules in vitro. Mutations in the IC genes result in loss of the entire dynein arm from the axoneme (*73, 74*). Together, these data are consistent with a role for the axonemal dynein ICs in anchoring dynein molecules to the axonemal microtubules.

To identify potential interaction targets for cytoplasmic dynein, binding partners for the cytoplasmic ICs were sought. A pair of polypeptides of 150 and 135 kD were identified in whole-cell lysates by blot overlay (*76*), immunoprecipitation, and immunoaffinity chromatography (*76, 80*). The polypeptides were identified as the $p150^{Glued}$ doublet. $p150^{Glued}$ exhibits partial co-purification with cytoplasmic dynein from some (*81–83*), though not all (*69*), tissues. It is a homolog of the product of the *Glued* gene in *Drosophila* (*82–84*), mutations in which lead to defects in eye morphology and neuronal development. $p150^{Glued}$ is a component of the ~20S dynactin complex (*83, 85*), which consists of a short F actin–like filament that is assembled from an actin-related protein, Arp1 (*85–88*), and associated proteins. $p150^{Glued}$ appears as a lateral projection emanating from the barbed end of the filament, with two globular domains at its end. Other components of the complex include the actin capping protein CapZ (*86*), a prominent 50-kD polypeptide (*63*), and at least three additional minor polypeptides. $p150^{Glued}$ appears to be an elongated protein, containing two regions of substantial coiled-coil sequence (*82, 83*). Distinct binding sites for Arp1 (*89*), microtubules (*82, 89, 90*), and the cytoplasmic dynein ICs (*76*) have been identified within $p150^{Glued}$, which suggests that it is a multifunctional "working arm" of the dynactin complex.

Function of Cytoplasmic Dynein-Dynactin Interactions

The specific role of dynactin in cytoplasmic dynein function has been uncertain. Dynactin was observed to stimulate the frequency of dynein-mediated organelle movements in an in vitro assay (*34*), suggesting a possible role as a dynein regulatory factor. Studies in *Saccharomyces* (*91, 92*), *Neurospora* (*60*), and *Drosophila* (*84*) also indicate that genes encoding subunits of cytoplasmic dynein and dynactin function in a common pathway. Dynein and dynactin are co-localized at the kinetochore of prometaphase chromosomes, which suggests that they can interact in vivo as well as in vitro (*63*).

That the interaction between cytoplasmic dynein and dynactin is mediated by the dynein ICs suggests that dynactin may serve to anchor the motor protein to its subcellular binding sites. Evidence in support of this possibility has come from analysis of the effects of overexpression of the p50 subunit of dynactin in cultured mammalian cells (*63, 93*). Transfected cells showed defects both in organelle distribution and in chromosome segregation (*63*). Late endosomes, lysosomes, and the Golgi apparatus were dispersed from their normal juxtanuclear or perinuclear positions (*63, 93*). Dividing cells were found to accumulate in a prometaphase-like state—that is, with condensed, unseparated chromosomes, further evidence for a defect in cytoplasmic dynein activity (*63*). The amounts of both dynactin and cytoplasmic dynein were clearly reduced at the kinetochore, which correlated with dissociation of $p150^{Glued}$ and other subunits from the Arp1 filament of the dynactin complex, as revealed by sucrose density gradient centrifugation. Together, these results suggest that dynactin serves to anchor cytoplasmic dynein to the kinetochore and to the surface of membranous organelles.

Comparison of Mechanisms

The properties of the kinesin- and dynein-binding proteins are quite different, suggesting that they function in distinct ways. Kinectin has a number of properties that are consistent with those of a membrane receptor. It has a putative transmembrane domain (*94*, *95*) and behaves as an integral membrane protein (*30*). Structurally, it is distinct from known cell surface receptors, especially with regard to its very extended coiled-coil region. Conceivably, this domain interacts with the coiled-coil regions within the kinesin heavy and light chains.

Dynactin behaves as a mostly soluble complex (*83*, *85*), though some dynactin is found in membrane fractions (*55*). Most of the subunits of dynactin have been cloned and sequenced, but so far, no evidence for a transmembrane domain or for lipid modification of the subunits has been obtained. Thus, whereas dynactin seems to play a role in linking cytoplasmic dynein to subcellular structures, it may be part of a more complex targeting mechanism.

The inhibitory effect of a kinectin antibody on the interaction of both kinesin and dynein with membranes (*32*) could indicate either that the binding sites for kinesin and cytoplasmic dynein on the membrane surface are physically close or that kinectin serves as a receptor for both motor proteins. Preliminary evidence has indicated an interaction between partially purified (20S) cytoplasmic dynein and a bacterially expressed kinectin fragment in support of the latter possibility (*31*), though it is not known whether the interaction involves dynein directly or other factors in the preparation. Kinectin was not detected among proteins that bound to cytoplasmic dynein ICs or to the p150^{Glued} component of the dynactin complex (*76*, *80*), but the possibility of an interaction with other dynein or dynactin subunits has not been explored extensively. In this context, analysis of "outward" and "inward" vesicle movements in cultured CV-1 cells (96) and fish scale chromatophores (*97*), which may represent kinesin- and cytoplasmic dynein–driven events, has suggested coordination between the two directions of membrane traffic. Such results would be consistent with a linked mechanism for motor recognition on the organelle surface. In one view, coordination might conceivably be regulated through kinectin, which would participate in membrane binding by both motors (98) (Fig. 1).

An alternative model for cytoplasmic dynein is suggested by the structure of dynactin (99) (Fig. 2). The short F actin–like filament of dynactin is reminiscent of the short actin filaments found at the vertices of the erythrocyte membrane skeleton, which is involved in linking spectrin molecules together. Perhaps dynactin serves in a similar capacity on the surface of minus end–directed organelles and in so doing serves to tether cytoplasmic dynein to the organelle surface (Fig. 2). It is noteworthy that Golgi-associated spectrin (*100*) and ankyrin (*101*) isoforms have been identified, which could serve in this capacity. By analogy with the plasma membrane skeleton, an organelle surface skeleton might be attached to the organelle by an "anchoring" molecule, akin to ankyrin.

An intriguing issue is how cytoplasmic dynein may be directed to sites as apparently distinct in structural organization as the surfaces of membranous organelles and mitotic kinetochores. So far, at least three proteins have been found in common between organelles and prometaphase kinetochores—cytoplasmic dynein, dynactin, and, recently, CLIP-170 (*102*)—and there may be a greater similarity between kinetochores and membranous organelles at the molecular level than is apparent from their gross morphological features.

The extended structure of kinectin may also reflect a skeletal role for this protein. Fully extended, the predicted coiled-coil region within kinectin (100 nm) is much greater than the coiled-coil region within kinesin and would be sufficient to circle a small vesicle completely. Conceivably, kinectin self-associates, and it is a kinectin network on the organelle surface with which kinesin, and possibly dynein or dynactin, associates rather than with individual kinectin molecules.

It is uncertain how the association of motor proteins with their target sites within the cell may be regulated. Although some studies have implicated protein phosphorylation in motor regulation [for example (*97*, *103*–*106*)], data demonstrating specific effects of motor or anchor phosphorylation on targeting have not yet been obtained. A number of considerations indicate that the

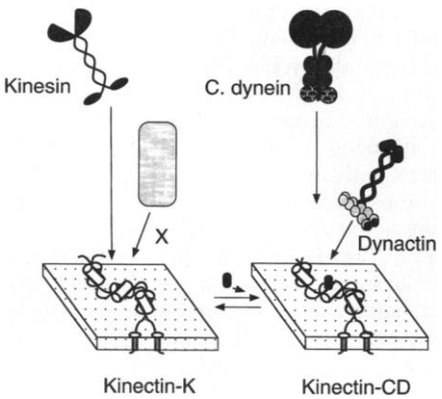

Fig. 1. Model of the motor binding to kinectin on the membrane vesicle surface. At left, kinesin is shown binding to kinectin (kinectin-K); an unknown accessory protein, X, is required for kinesin-dependent motility. Preliminary evidence suggests that dynein or dynactin may also interact with kinectin (right; kinectin-CD), though the nature of the interaction remains to be fully resolved. A modification of kinectin (circular symbol at bottom between kinectin-K and kinectin-CD) is postulated as the switch between kinesin and cytoplasmic dynein.

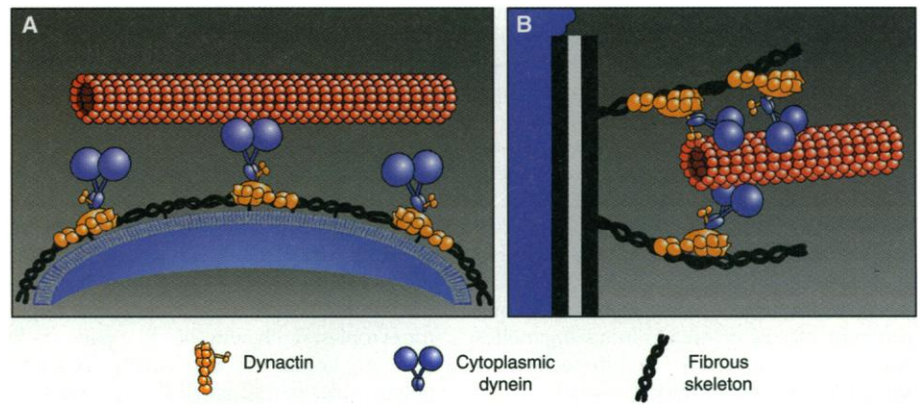

Fig. 2. Schematic representation of the possible organization of cytoplasmic dynein and dynactin on organelle membranes and kinetochores. Dynein is shown tethered to an organelle surface (**A**) or to a kinetochore (**B**) by its interaction with dynactin. The interaction between dynein and dynactin is mediated by the cytoplasmic dynein intermediate chains at the base of the motor molecule and by the p150^{Glued} component of dynactin, depicted as a projection emanating from one end of the dynactin complex. The actin-like filament of dynactin is speculated in (A) to be a component of an organelle surface skeleton, potentially analogous to the plasma membrane spectrin skeleton. Whether kinectin is a component of such a skeleton or forms an independent network remains to be determined. In (B), the cytoplasmic dynein and dynactin complexes are shown within the fibrous corona of the prometaphase kinetochore. The full extent to which kinetochore and organelle surfaces may be related in composition and organization remains to be determined [figure courtesy of C. Echeverri, modified from (*99*)].

interactions involved in targeting are likely to be complex and subject to regulation at several levels. For example, a curious feature of the dynein-dynactin interaction is the presence of microtubule-binding sites within both protein complexes. In addition to the force-producing interaction between the cytoplasmic dynein heavy chains and microtubules, the $p150^{Glued}$ component of dynactin contains what appears to be a static microtubule-binding site, as evidenced by both in vitro and in vivo expression assays (*82, 89, 90*). This region of ~80 amino acids near the NH_2-terminus (*82*) is homologous to similar-sized regions within the microtubule-endosome–linking protein CLIP-170 (*90*) and the yeast protein Bik1p, which is involved in mitosis (*107*). The site is distinct from the intermediate chain–binding site within $p150^{Glued}$ (*76*) and should be available for microtubule binding even when dynein and dynactin interact. Because such a stable interaction between $p150^{Glued}$ and microtubules should interfere with force production by cytoplasmic dynein, it is likely to be regulated in the cell. Perhaps the various interactions occur sequentially, with dynactin playing a role in docking cytoplasmic dynein to both organelles and microtubules before itself dissociating from the motor protein. In such a model, the cytoplasmic dynein light intermediate chains become of interest as possible regulatory nucleotidases (*71, 72*), which function in other aspects of membrane traffic. Alternatively, dynactin could serve as a means of maintaining the attachment of dynein to microtubules throughout the dynein cross-bridge cycle, serving to stabilize the association of either membranous organelles or chromosomes with microtubules.

Evidence that the interaction between kinesin and organelles may also be regulated has come from analysis of kinesin ATPase activity. States of low and high activity have been identified, which correspond, respectively, to the 9S and 6S conformational states (*12*). This behavior is reminiscent of that of smooth muscle and nonmuscle forms of myosin II (*108*). The association of the tail of the myosins with the head region has been found to inhibit ATPase activity, which suggests that nucleotide hydrolysis is coupled to assembly of the functional thick filament. It remains to be seen whether ATP hydrolysis by kinesin or cytoplasmic dynein are similarly coupled to organelle and kinetochore binding.

Conclusion

Evidence to date suggests that targeting of the two most extensively studied microtubule motors is likely to be complex. The binding of motors to subcellular structures may prove to be elaborately regulated, perhaps by means of mechanisms comparable to those for the recognition and docking of vesicles in the membrane traffic pathways, and the binding machinery may allow for regulation at multiple levels. Thus, future analysis of the targeting process promises to answer not only how organelles and chromosomes are sorted and redistributed both spatially and temporally during the cell cycle. In addition, it may identify new paradigms for the regulation of protein-protein interactions in general.

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