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Review

Molecular analysis of the microtubule motor dynein

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ABSTRACT **Dynein is a large enzyme complex that has been found in recent years to be responsible for a variety of forms of intracellular movement associated with microtubules. Molecular analysis of several of the polypeptide components of dynein and a related complex has provided important new insight into their structural organization and mechanism of action in the cell.**

Within the past decade cytoplasmic motor proteins have been identified that produce force along microtubules *in vitro*. These proteins are microtubule-activated ATPases, which convert chemical energy into mechanical energy. Kinesin was found to generate force toward the plus ends of microtubules and was proposed to account for anterograde axonal transport and other forms of plus-end-directed organelle movement along microtubules (1). Cytoplasmic dynein was identified as a minus-end-directed counterpart of kinesin and was proposed to account for retrograde, or minus-end-directed, movements along microtubules (2). It was subsequently shown to be structurally (3) and biochemically (4–6) related to the ciliary and flagellar ATPase dynein. In cilia and flagella, dynein is responsible for generating force between the array of 20 microtubules that make up the characteristic "axonemal" structure. Cytoplasmic dynein, in contrast, has been implicated in a variety of other forms of intracellular motility (reviewed in refs. 7–9), including retrograde axonal transport, protein sorting between the apical and basolateral surfaces of epithelial cells, and the distribution and redistribution of endosomes, lysosomes, and the elements of the Golgi apparatus within the cell. Cytoplasmic dynein has also been implicated in the poleward migration of chromosomes, at least during some stages of mitosis.

Substantial information is already available regarding the structural organization of kinesin based on primary sequence, physicochemical, and ultrastructural analysis. The molecule is a tetramer of two heavy and two light chains (10, 11). The N-terminal 35 kDa of the heavy chain contains a P-loop consensus sequence element indicative of nucleotide binding and hydrolysis and represents the force-producing "head" domain. The remainder of the heavy chain is predicted to form a coiled-coil α-helix (12–15). This arrangement is strikingly reminiscent of that of muscle myosin, though, other than the presence of the P-loop within the head domain, no evidence for primary sequence homology with myosin was detected. In further contrast to myosin, the kinesin light chains (16) are associated with the C-terminal tail portion of the molecule rather than the heads. As for myosin (reviewed in refs. 17–20), a family of kinesin-related genes has been identified, which exhibit clear sequence conservation within the head region and considerable variation within the remainder of the molecule and have roles in a variety of forms of intracellular movement (reviewed in refs. 21–23).

Dyneins are much larger and more complex molecules than the several forms of myosin or kinesin (molecular mass <600 kDa) that have been identified. Dyneins have a native mass between 1000 and 2000 kDa and contain either two or three force-producing heads, each of which is about as massive as the entire kinesin molecule (3, 24, 25). The heads are linked via stalks to a basal domain, which is as large as the heads but less well defined structurally. The heads are thought to be formed primarily or exclusively from the heavy chains, extremely large polypeptides responsible for ATP hydrolysis (see below). In addition, dyneins contain a highly variable number of accessory intermediate and light chains. Axonemal dyneins contain a variety of such subunits ranging in size from ≈14 to 120 kDa (26). Cytoplasmic dynein contains at least seven different accessory polypeptides ranging from 53 to 74 kDa (3, 4). In addition, a number of partially copurifying polypeptides have been identified that may be involved in regulating cytoplasmic dynein behavior (see below).

Because of the large size of the dyneins and their complex composition, defining their structure has been a daunting undertaking. Nonetheless, recent progress in the molecular characterization of their component polypeptides has provided new insight into their structural organization and mechanism of action. This work is reviewed here and in expanded form elsewhere (27).

Molecular Cloning of Dynein Heavy Chain

Heavy chains from sea urchin flagellar dynein (28, 29) and from *Dictyostelium* (30) and rat (microtubule-associated protein 1C; ref. 31) cytoplasmic dynein have been fully cloned and sequenced. Full-length sequences are near completion for two of the three different forms of *Chlamydomonas* flagellar dynein heavy chain (C. Wilkerson, S. King, and G. Witman, personal communication; D. R. Mitchell and K. Brown, personal communication) and for *Caenorhabditis elegans* (32) and *Saccharomyces cerevisiae* (33, 34) cytoplasmic dyneins. In addition, sequences from the catalytic domains of several other forms of dynein have been obtained by PCR from sea urchin (35), *Drosophila* (K. Rasmussen, J. Gepner, M. Serr, I. Gibbons, and T. Hays, personal communication), *Chlamydomonas* (36, 37), and *Paramecium* (38). The predicted sizes of the completed heavy chains are in the range of 510–540 kDa. Based on the pattern of expression of the different forms of heavy chain, genomic Southern blot analysis, and parsimony analysis of the evolutionary relationship between the different forms of heavy chain, it appears that organisms generally have a single cytoplasmic dynein heavy chain gene and numerous axonemal dynein heavy chain genes (30, 31, 35). The latter are thought to reflect the multiplicity of heavy chain polypeptides found within individual axonemal dynein molecules (the *Chlamydomonas* outer arm dynein molecule is three-headed and contains three distinct heavy chain gene products; refs. 39 and 40) and among the several forms of dynein observed within a given axoneme (one outer arm dynein and at least three different inner dynein arms; refs. 41–43). Overall sequence identity between cytoplasmic and axonemal dyneins can be as low as 27% (rat cytoplasmic dynein vs. sea urchin axonemal dynein), whereas the degree of conservation between species for the same functional form of dynein tends to be higher (for example, 54% between rat cytoplasmic and *Dictyostelium* cytoplasmic dynein; see ref. 31). Little evidence for significant homology with the kinesins and myosins was detected (though see ref. 44).

The most remarkable common features of the several dynein heavy chain sequences are four P-loop consensus sequence elements spaced at 35- to 40-kDa intervals (Fig. 1). The region surrounding the first P-loop represents the most highly conserved part of the molecule. UV-induced cleavage of dynein heavy chains in the presence of vanadate, which results in the inactivation of the ATPase activity, is estimated to occur in the vicinity of this site (28), suggesting an involvement in ATP hydrolysis. The third P-loop region is also relatively well conserved among dyneins and most similar to the first P-loop sequence. This suggests that the four domains may have arisen during evolution by successive duplications of the first sequence and then of the first and second sequence. Such duplications would presumably be very ancient, appearing as they do in all forms of dynein heavy chain. Whether the second, third, and fourth P-loop elements are vestigial or serve a regulatory role remains to be investigated. Kinetic evidence has been interpreted to support binding of only one ATP molecule per heavy chain (45). However, because of the complexity of the dynein holoenzyme (which contained three heavy chains in the study cited), detailed kinetic analysis of recombinant heavy chain or other simpler forms of the molecule seems warranted.

Clear sequence conservation between axonemal and cytoplasmic dynein heavy chains extends over the C-terminal two-thirds of the polypeptides (30, 31). However, the N-terminal 1300 amino acids appear to be completely unrelated between the flagellar and cytoplasmic sequences. In contrast, the rat and *Dictyostelium* sequences show 43% sequence identity over this region (31), suggesting a role in cytoplasmic- and axonemal-specific functions. Because the predicted masses of the heavy chains are much greater than the observed masses of the force-producing head domains [327 kDa for cytoplasmic dynein (3) and 375–400 kDa for axonemal dyneins (24, 46)], either or both the N- and C-terminal portions of the heavy chain may lie outside of the head domain and contribute to the stalk and basal portions of the dynein molecule. Conceivably, the nonconserved N-terminal domain specifies cytoplasmic vs. axonemal function, playing a role either in subunit interactions or in targeting the enzyme to distinct subcellular sites (Fig. 2).

While molecular cloning of the dynein heavy chains has provided a basis for further exploration of the relationship between dynein structure and function, it has also raised many questions. There is still no indication as to why the dynein heavy chain is so large (the head alone is much larger than the 100-kDa tubulin dimer with which it interacts), and the primary sequence has offered relatively little in the way of insight into the tertiary structural organization of the molecule. The large head domain probably reflects the multiplicity of ATP-binding regions (Fig. 2*A*), but the requirement for this level of structural redundancy remains obscure.

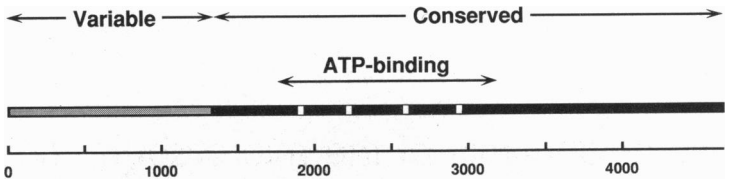

FIG. 1. Diagram of dynein heavy chain. White rectangles represent phosphate-binding P-loop sequence elements; the shaded region differs between known complete axonemal (sea urchin flagellar) and cytoplasmic dynein heavy chain sequences (rat and *Dictyostelium*).

Molecular Cloning of Dynein Accessory Subunits

Progress has also been made in the molecular cloning of the dynein accessory subunits, which has provided some insight into their functional relationship.

The *Chlamydomonas* flagellar outer arm dynein, which has been one of the most extensively studied forms of the enzyme, contains three distinct heavy chains, two intermediate chains of 70 and 78 kDa, and a series of light chains in the 10- to 30-kDa range (39). Purified rat cytoplasmic dynein contains a prominent polypeptide of 74 kDa, which has been observed to split into as many as three electrophoretic bands, and additional polypeptides of 59, 57, 55, and 53 kDa (3, 4). The differences in subunit composition between the two dynein forms have made it difficult to judge to what extent individual polypeptides are structurally or functionally related.

Molecular cloning of the 70-kDa *Chlamydomonas* flagellar dynein outer arm intermediate chain (IC70) was accomplished in conjunction with the analysis of outer dynein arm (*oda*) mutants (47). Several of these mutants, including *oda6*, lack outer arms completely as judged morphologically and biochemically, and show abnormal flagellar motility (48). cDNA clones encoding the 70-kDa intermediate chain (49) were shown to map to the *ODA6* locus and were used to deduce the primary sequence of its polypeptide product (47).

Cloning and sequencing of overlapping cDNAs encoding the rat cytoplasmic dynein 74-kDa species revealed clear, albeit relatively distant, homology with the *Chlamydomonas* IC70 (50). Sequence conservation was detected primarily within the C-terminal portion of the two sequences. Heterogeneity among 74-kDa transcripts indicated by PCR analysis and amino acid sequencing of proteolytic fragments revealed at least three alternative sequences near the 5′ end, suggesting that the multiple 74-kDa electrophoretic species were produced by an alternative splicing mechanism. More recently, cDNAs encoding the 78-kDa *Chlamydomonas* dynein subunit (IC78) were cloned and sequenced revealing a similar pattern of homology with both the *Chlamydomonas* flagellar and the 74-kDa rat cytoplasmic dynein subunits (51). These results provide further evidence for the common ancestry of axonemal and cytoplasmic dyneins and identify a novel intermediate chain gene family.

These findings, in conjunction with structural and functional studies of the axonemal dynein intermediate chains, have suggested a role for the 74-kDa cytoplasmic dynein subunit. Axonemal and cytoplasmic forms of dynein are presumed to produce force via a common mechanism involving their conserved head domains. However, they differ in the nature of their additional interactions within the cell, which are thought to be mediated by the basal portion of the molecule (Fig. 2*B*). In the case of axonemal dynein, this part of the molecule forms a fixed attachment to a second microtubule within the axoneme, which results in sliding between the axonemal microtubules and bending of the entire cilium or flagellum. The base of the cytoplasmic dynein molecule is predicted to form a comparable attachment to the surface of membranous organelles and, possibly, kinetochores.

Both IC70 and IC78 of *Chlamydomonas* flagellar outer arm dynein have been deduced to reside in the basal portion of the molecule, as judged by immunoelectron microscopic analysis of purified dynein particles using an anti-IC70 antibody (52) and biochemical evidence showing a direct interaction between IC70 and IC78 (53). Cross-linking studies revealed a direct interaction of the 78-kDa species with tubulin (54), as did binding of the *in vitro*-translated polypeptide to purified microtubules (51). These data suggest that the role of IC78, and possibly of IC70, is in producing the noncatalytic link to microtubules (Fig. 2).

An analogous role for the 74-kDa cytoplasmic dynein subunit would be in binding to organelles and kinetochores (Fig. 2*B*), but the details of this interaction are poorly understood. Cytoplasmic dynein,

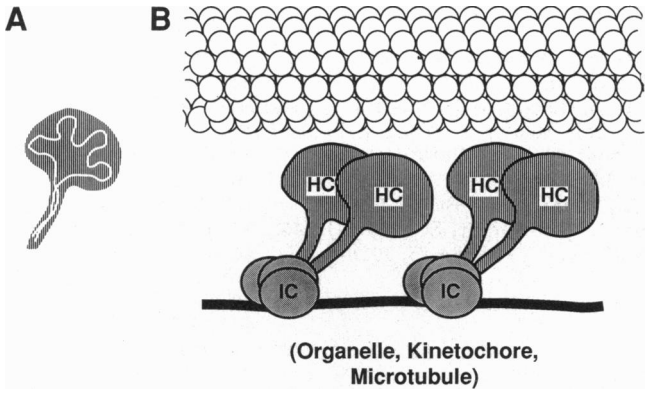

FIG. 2. (*A*) Sketch of heavy chain, indicating hypothetical folding pattern. The P loops are speculated to demarcate repeated structural domains of 35–40 kDa. While the first such domain is thought to be involved in catalytic activity, the role of the other domains is uncertain. The C- and N-terminal portions of the heavy chain are speculated to make up the stalk of the molecule. (*B*) Interaction of dynein with microtubules and other structures. Dynein heads, representing ATPase region of heavy chains (HC), interact with a microtubule at top to produce force. Intermediate chains (ICs) are shown attached to the basal region of the dynein molecule, where they interact with a second microtubule in cilia and flagella or with organelles and possibly kinetochores in the cytoplasm. (Adapted from ref. 27.)

like kinesin, is found at a substantial concentration in the soluble phase of tissue homogenates, with little evidence for residual membrane-associated enzyme. Whether this indicates a weak affinity for membrane binding sites or a regulated interaction is not known. No evidence for a membrane-spanning domain in the sequence of the 74-kDa subunits was found, and sequence motifs clearly indicative of lipid modification were not identified. It seems likely, therefore, that the cytoplasmic dynein subunit will prove to interact with other polypeptides on the surface of organelles and kinetochores.

Immunocytochemical analysis has revealed a clear association of cytoplasmic dynein with two classes of membranous organelles, lysosomes and late endosomes (55). These organelles often exhibit a perinuclear distribution, consistent with a role for a minus-end-directed microtubule motor in controlling their subcellular distribution. While anti-dynein antibodies have not shown comparable Golgi staining so far, dynein may be associated with this organelle as well. Golgi membranes introduced into broken cell preparations have been reported to become localized to the centrosomal region, and this behavior was abolished by immunodepletion of cytoplasmic dynein (56).

While direct binding of cytoplasmic dynein to purified minus-end-directed organelles *in vitro* has not been assessed, binding to synaptic vesicles (57) and microsomes (58) has been reported. It will be of interest to determine whether cytoplasmic dynein will discriminate between different classes of organelles in *in vitro* binding assays, and it should be of value to identify organelle and kinetochore surface proteins with which the 74-kDa cytoplasmic dynein intermediate chain interacts.

Role of the Glued (Dynactin) Complex

In addition to the biochemically well-behaved components of cytoplasmic dynein described above, a number of partially copurifying polypeptides have been seen, including species of 150, 135, 50, and 45 kDa (59–61). These polypeptides cosediment with microtubules, though less efficiently than the heavy chain and 74-kDa intermediate chains of cytoplasmic dynein; they dissociate from microtubules in the presence of ATP along with cytoplasmic dynein; and they cosediment with cytoplasmic dynein at 20 S (61, 62). However, the four polypeptides, along with additional minor species of 62, 34, and 32 kDa, can be separated from cytoplasmic dynein by FPLC (61). Antibody to the 50-kDa polypeptide has also been found to immunoprecipitate the same components in a comparable ratio from total brain cytosol (45 kDa > 50 kDa > 150 kDa > 135 kDa >> 62 kDa > 32 kDa > 34 kDa). Together, these data reveal these polypeptides to be components of a discrete complex (62).

Molecular cloning of the 150-kDa species in rat brain revealed it to be homologous throughout its length to the similar-sized product of the Glued gene in *Drosophila* (60). A cDNA encoding a polypeptide corresponding to the C-terminal 117 kDa of the rat and fly polypeptides was subsequently reported under the name dynactin (61). (The complex has come to be referred to by the names Glued or dynactin.) The original Glued mutant is dominant and produces defects in the development of the eye and nervous system. Homozygotes have a cell lethal phenotype, indicating a role in an essential cell function, but no information is available identifying a specific cellular defect.

The 45-kDa species, which is the major component of the complex, also proved to have an interesting identity. Peptide sequence from both the chicken (63) and rat (62) polypeptides revealed them to represent a member of a family of actin-related proteins, referred to as centractin (64) or actinRPV (63). The 34- and 32-kDa polypeptides have been identified immunologically as the actin capping protein CapZ (65). Immunoblotting of sucrose gradients of brain cytosol indicated the three major components of the complex, p150^{Glued}, p50, and centractin to exist exclusively in a 20S form (62), a surprising result considering the polymorphic nature of actin.

While the complex is incompletely separated from cytoplasmic dynein by FPLC (61), complete separation was seen by immunoprecipitation (62), raising the issue of whether the two structures interact at all *in vitro* or *in vivo*.

The evidence in favor of such an interaction is intriguing but incomplete. Cytoplasmic dynein alone was found to have no effect on microtubule-associated organelle movements in an *in vitro* assay using organelles stripped of peripheral membrane proteins (66). However, addition of the complex stimulated the number of organelle movements per unit time.

Immunocytochemical analysis using antibodies to the components of the complex has revealed a punctate cytoplasmic distribution, which may correspond to vesicular structures, with particularly bright staining at the centrosome [refs. 61, 62, and 64; and hence the name centractin for the actin-related component (64)]. The latter distribution can be disrupted using microtubule depolymerizing drugs (62). This suggests that the complex is associated with the centrosome peripherally via the minus ends of microtubules, which are anchored there. The immunocytochemical behavior of the components of the complex is consistent with that expected for a cytoplasmic structure under the spatial control of cytoplasmic dynein. Curiously, however, while lysosomes, endosomes, and the Golgi apparatus are often found in the pericentrosomal region, they are not sharply focused at the centrosome itself. Thus, the complex may be directly associated with the microtubule minus ends, or it may serve as a marker for an, as yet, poorly characterized vesicular compartment that resides in this region.

Evidence for a specific interaction of the complex with microtubules has also come from work on two other proteins, CLIP170 and BIK1. CLIP170 copurifies with and colocalizes with both microtubules and endosomes, implicating the protein in cross-linking the two structures (67). An N-terminal repeated sequence of ≈100 amino acids was found to be responsible for microtubule binding *in vitro*. BIK1 (68, 69) is a yeast gene, the product

of which partially colocalizes with microtubules. Mutations in BIK1 result in altered levels of microtubule assembly. BIK1, Glued, and rat p150^{Glued} each contains a single N-terminal copy of the CLIP170 microtubule-binding motif (67), suggesting that all members of the family are capable of direct interaction with microtubules. Results of transfection of p150^{Glued} into cultured mammalian cells have, in fact, revealed colocalization with microtubules (C. Waterman and E. Holzbaur, personal communication).

At present it is difficult to make complete sense out of the disparate but fascinating properties of the components of the Glued or dynactin complex. However, the ability of a component of the complex to bind microtubules directly might be expected to hinder rather than stimulate cytoplasmic dynein-mediated motility. Conceivably, therefore, microtubule binding is a transient stage in a multistep mechanism by which the complex functions. It should be of considerable interest to continue to probe the role of the other components of the complex, the ability of the complex to interact with cytoplasmic dynein, and, specifically, with the 74-kDa intermediate chain, and the effects of mutations in the Glued gene to address this issue.

Note Added in Proof. Since submission of this article, cloning of the rat cytoplasmic dynein heavy chain has also been completed by another laboratory (70).

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