Mechanism of Force Production for Microtubule-dependent Movements

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Our current thinking about the driving force for the movements of intracellular particles or chromosomes has been dominated by our knowledge of the two most common contractile systems, those based upon actin-myosin interactions or upon microtubule-dynein interactions. Abundant quantities of material in well-ordered arrays have allowed considerable progress to be made in the investigation of the mechanisms responsible for movement in these systems. There are many examples of the involvement of cytoplasmic actin and myosin in nonmuscle cell motility (1, 2). Evidence in favor of a cytoplasmic dynein in microtubule-dependent motility is beginning to accumulate, and recent work from several laboratories has provided preliminary evidence that a dyneinlike ATPase is present in the mitotic spindle of sea urchin eggs (3–5). In addressing the question of a cytoplasmic dyneinlike ATPase, it is primarily important to establish a general definition of dynein and to characterize those features that distinguish it from myosin and other ATPases. Dynein has been loosely defined as an ATPase that associates with microtubules and has high molecular weight polypeptides in excess of 300 kdaltons (6). A more meaningful definition includes its direct role in generating a force for movement coupled to the hydrolysis of ATP, which must be approached in terms of the biology as well as the biochemistry of the system. In this review we will summarize the important biochemical properties of ciliary dynein in terms of a comparison with myosin and relate the results to the potentially distinguishing features of a cytoplasmic dynein.

Biochemistry of Dynein

Dynein has been isolated and extensively studied from only three sources, *Tetrahymena* cilia, *Chlamydomonas* flagella, and sea urchin sperm flagella. Each system provides the investigator with unique advantages in studying dynein and has led to unique contributions to the field. Dynein from mammalian sperm has been isolated but has not been extensively studied (7). Dynein was first isolated from the cilia of *Tetrahymena* by Gibbons (8). Subsequent work with sea urchin sperm flagella established that the ATPase activity was coupled to movement (9, 10) in the intact axoneme. Gibbons and Fronk (11) and Gibbons and Gibbons (12) established an important criterion for biological activity based upon the ability of dynein to rebind to partially extracted axonemes and restore motility and showed, furthermore, that dynein could be artificially activated 10-fold, leading to an enzyme that had lost this important biological activity. This work defining a "latent activity dynein" was significant in that it emphasized that a good preparation of dynein was one with a low rather than a high specific activity. Activation by a number of treatments, such as gentle heating or the addition of a low concentration of detergent, was useful in distinguishing latent activity dynein from dead dynein. However, there are problems with this as a general criterion for dynein, because the ability of a given dynein to be activated cannot be predicted on the basis of any important biological activity of the protein. Consequently, activation by a sublethal treatment has not proven to be a universal property of dynein. Nonetheless, the work on latent activity dynein importantly points to the need for caution in isolating a protein with a low specific activity, typically in the range of 0.1–0.2 μmol/mg·min^{-1} (11).

The ability of dynein to associate with microtubules provides a specific biochemical criterion for dynein that most clearly distinguishes it from myosin or other ATPases. Dynein obtained from either *Chlamydomonas* or *Tetrahymena* will rebind to microtubules from a variety of sources ranging from axonemal tubules to bovine brain microtubules to surf clam mitotic spindles (12–16). In each case thus far examined, the dynein bound to the microtubule with a 24-nm linear repeat and saturated the surface lattice with six to seven rows of dynein arms (14, 16). In some cases, the dynein bound by both its structural attachment site and its ATP-sensitive site, leading to the net cross-linking of microtubules (14). In other cases, the dynein bound only by the ATP-sensitive end (16). Although the basis for the binding to one end or the other has not been established, in each case, the addition of ATP led to the rapid dissociation of the dynein attached by the ATP-sensitive end.

One can argue that the microtubule-dynein interaction is

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specific if the binding to the microtubule surface lattice shows a 24-nm periodic repeat and if the complex is rapidly dissociated with moderately low concentrations of ATP (10–100 μM) (17). This concern for specificity is important inasmuch as tubulin has an acidic isoelectric point and microtubules can act as cation-exchangers to bind proteins nonspecifically. Since the ATP-induced dissociation of one end of the dynein from the microtubules is part of the cycle by which the hydrolysis of ATP is linked to the production of force (see below), it is likely that the ATP-sensitive binding of dynein to a microtubule will represent a general property of dynein. Thus, a general criterion for dynein can be based upon periodic, ATP-sensitive binding to microtubules. We are limited only by our ability to find conditions that will allow the reactions to occur in vitro.

Dynein Polypeptide Composition

Work on *Chlamydomonas* has taken advantage of mutants lacking a single component of the axoneme. By correlating the loss of the outer arm with the loss of a particular set of polypeptides, it was established that the outer arm is composed of two separable ATPases, sedimenting at 18S and 12S (18, 19). The 18S species was found to be composed of two heavy chains (>320 kdaltons), two intermediate chains (73 and 86 kdaltons), and eight light chains (<20 kdaltons), whereas the 12S species consisted of a single heavy chain (315 kdaltons) and a single light chain (19 kdaltons) (19, 20). In addition, a 13S ATPase was missing in mutants lacking the inner arm (21). The 13S inner arm dynein contains a distinct set of polypeptides and can be separated from the 12S outer arm component by chromatography (20, 21).

The sea urchin outer arm dynein is a 1.25-million-dalton particle sedimenting at 21S and composed of two heavy chains (>300 kdaltons), three intermediate chains (76, 90, and 122 kdaltons), and four light chains (14–24 kdaltons) (22). Furthermore, the outer arm can be biochemically dissected to yield two ATPases, each containing a single heavy chain (23, 24). The major dynein isolated from *Tetrahymena* cilia is a 22S particle (formerly known as 30S dynein) and consists of three heavy chains (~400 kdaltons), three intermediate chains (70, 85, and 100 kdaltons), and an undetermined number of light chains in the range of 20 kdaltons (16).

The molecular weights of the dynein polypeptides were estimated by their mobility on SDS polyacrylamide gels, but there was concern that extrapolation of gel standards had led to gross underestimation of the molecular weight of the dynein heavy chains. Bell (25) isolated the heavy chains from sea urchin and estimated their molecular weight by sedimentation equilibrium studies, obtaining a molecular weight of 450,000–500,000. It therefore seems most reasonable to assume that all previous estimates of the molecular weights of dynein heavy chains based upon gel electrophoresis are underestimates and that the actual molecular weights are in the neighborhood of 450,000.

To a first approximation, the polypeptide compositions of the dyneins isolated from the three sources are nearly identical, the most important difference being that sea urchin dynein has only two heavy chains, whereas both *Chlamydomonas* and *Tetrahymena* outer arm dyneins contain three heavy chains. Interestingly, dynein isolated from bull sperm can be fractionated to yield a 19S species containing two heavy chains and a 12S species containing a single heavy chain.[1]

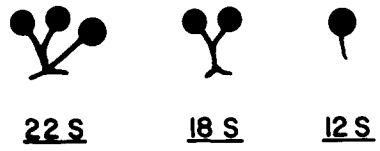

FIGURE 1 Structural comparison of *Tetrahymena* and *Chlamydomonas* dyneins. The structures of *Tetrahymena* 22S dynein (26) and the *Chlamydomonas* 18S and 12S dyneins (27), as revealed by scanning transmission electron microscopy, are diagrammatically represented. See text for details.

Structure of Dynein: A Unified View

Until recently, the similarities as well as the differences in the dyneins isolated from the various sources have not been fully appreciated. The similarity in polypeptide composition suggests that the results obtained independently with each dynein species should be applicable to the other systems. However, the significance of this view has not been fully appreciated; for example, the relationship of the separable ATPase fragments of sea urchin 21S dynein to the 18S and 12S dyneins observed in *Chlamydomonas* dynein was rather puzzling. Analysis of dynein structure and molecular weight by scanning transmission electron microscopy has done much to reconcile the conflicting views of dynein isolated from different sources. The electron microscope at Brookhaven National Laboratory has provided a unique opportunity for the examination of unstained biological specimens and reliable measurement of the mass and structure of a particle from the intensity of its electron scattering. Scanning transmission electron microscope analysis of *Tetrahymena* 22S dynein established that the protein consists of a bouquet with three globular heads connected by slender threads to a rootlike base (Fig. 1). Two of the heads have a diameter of ~120 Å and a mass of 400 kdaltons, whereas the third has a diameter of ~140 Å and a mass of nearly 500 kdaltons. The mass of the entire particle is 1.95 million daltons (26).

Subsequent analysis of *Chlamydomonas* dyneins showed that the 18S dynein contains two 400-kdalton globular heads connected to a fibrous base by two slender threads and has a net mass of 1.25 million daltons (27). The 12S particle is a single globular unit with a mass of 470 kdaltons. It is clear that the two *Chlamydomonas* dyneins taken together equal the single *Tetrahymena* 22S dynein in terms of structure, mass, and polypeptide composition (Fig. 1). Preliminary scanning transmission electron microscope analysis of the sea urchin 21S particle, which had been previously shown to have a molecular weight of 1.25 million (11), confirmed the molecular weight and indicated a two-headed structure similar to that of the *Chlamydomonas* 18S dynein.[2] Recent work by Pfister and Witman (28) has shown that the 18S dynein can be broken apart into two ATPases analogous to the sea urchin subfragments (23).

These analyses have led to a unified view of the dynein outer arm as consisting of two or three globular units con-

[1] Gagon, C., Centre Hôpital de Université Laval, Quebec, Canada, personal communication.

[2] Gibbons, I. R., K. A. Johnson, and J. S. Wall, unpublished results.

nected by slender strands to a rootlike base. *Chlamydomonas* and *Tetrahymena* outer arm dyneins are essentially identical, whereas the sea urchin outer arm dynein resembles the *Chlamydomonas* 18S dynein and lacks the head corresponding to the *Chlamydomonas* 12S species. Although the reason for only two globular heads in the sea urchin dynein is not understood, the current data provide a framework with which to understand the mechanism of force production by dynein. In the ATP-sensitive complex formed between *Tetrahymena* 22S dynein and bovine brain microtubules (26), the heads are in close proximity with the microtubule. These observations suggest strongly that the roots of the bouquet anchor the dynein to the A subfiber and that the globular heads are connected to the A subfiber by flexible strands and interact with the adjacent B subfiber in an ATP-dependent reaction to produce the force for sliding (Fig. 2). Because three molecules of ATP are required to induce the dissociation of each molecule of dynein (29), it is likely that each head contains one ATP-binding site and one microtubule-binding site. Although this model remains to be definitively established, it provides a rational basis for understanding the mechanism of force production for sliding.

The bouquet model can account for the images of dynein observed in situ by a variety of techniques. The length of each strand connecting the dynein heads to the base is ~20–25 nm, such that the overall length of the particle from the base to the top of the head is 35 nm. However, the span of the dynein arm seen in cross sections of fixed and embedded axonemes is only 25 nm. This suggests that if the roots of the bouquet are connected to the A subfiber, then the strands are at an oblique angle tilted toward the base of the cilium, as shown in Fig. 2. Because the length of the strand is equal to the distance between two dynein molecules along the microtubule, the strands connecting the roots to the heads appear as a connection between successive groups of globular units. Such connections were first described as a spur by Witman and Minervini (30) in negatively stained preparations of axonemes and subsequently as an interdynein linker by Goodenough and Heuser (31) in replicas of shadowed, rapidly frozen axonemes.

This arrangement of the globular heads connected to the microtubule by the strands (Fig. 2) makes sense in terms of the direction of force production for active sliding in the axoneme. Sale and Satir (32) established that the dynein arms on each outer doublet cause the adjacent outer doublet to slide outward, away from the cell body. Thus, if each head were connected to the A subfiber by a somewhat flexible strand, the involvement of the head in force production with the B subfiber would pull on the strand until it was angled downward, as shown in Fig. 2.

The differences in structural detail and polypeptide composition of myosin and dynein are remarkable. Striated skeletal myosin is well known as a two-headed molecule with a molecular weight of 500,000 consisting of two heavy chains (200,000 daltons) and four light chains (17,000–20,000 daltons) (for reviews, see references 33 and 34). Each head is pear shaped, measures ~120 × 40 Å, has a mass of ~120,000 daltons, and contains one ATPase site and one actin-binding site. The heads are connected to the rod portion of the molecule by flexible random coil segments, allowing the heads to rotate independently in solution. As illustrated in Fig. 2, the similarities in the principles that appear to govern the design of myosin and dynein are profound. In both actin-myosin and microtubule-dynein systems, globular heads are anchored to one filament by flexible strands and reach out to interact with the other filament to form an ATP-sensitive cross-bridge.

The macroscopic movements of muscle result from relatively small changes in protein conformation, probably involving the rotation of the ATPase head against the actin to produce a force for sliding. The force-producing mechanism for dynein may be similar, employing the rotation of the dynein head against the microtubule. In the ATP-sensitive complex between *Tetrahymena* dynein and bovine brain microtubules, dynein saturates the microtubule surface lattice at a ratio of one dynein to six tubulin dimers (16). Each head

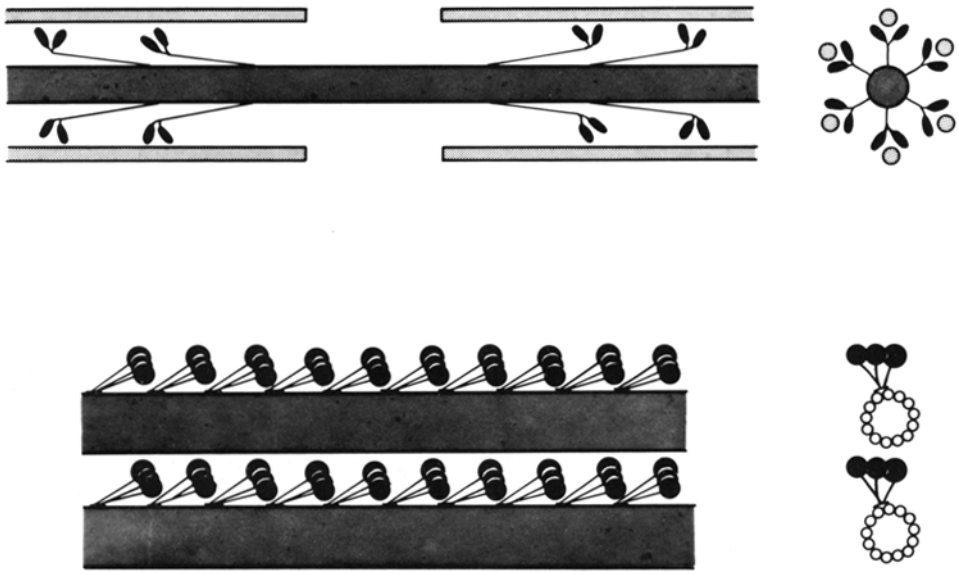

FIGURE 2 Sliding-filament models for actomyosin and microtubule-dynein. Schematic representations of the structure of the muscle actomyosin (*top*) and the microtubule-dynein system (*bottom*) are drawn to the same scale. Longitudinal (*left*) and cross sections (*right*) are shown for each system. For simplicity, a singlet microtubule is shown, although the figure is intended to represent the arrangement of dynein on a doublet microtubule in the axoneme as well. The outside diameter of the microtubule (25 nm) provides a magnification factor for the drawing.

occupies an area equal to two tubulin dimers on the microtubule surface; certainly the size of each head is consistent with this assignment.

The major difference between the two systems, aside from the size of the heads, is that myosin has two nearly identical heads and ciliary dynein has two similar heads and a third, larger head. The necessity for at least two heads for muscle contraction has been the subject of much speculation. For example, it has been suggested that the two heads cooperate to go hand over hand up the actin filament, although such a model has been difficult to experimentally test. The function of the third dynein head is even more uncertain. Inasmuch as the third head is not seen in sea urchin dynein, it may be that the third head is somehow involved in the regulation or generation of a ciliary waveform.

One notable exception to the rule that all myosins have two heads is *Acanthamoeba* myosin I, which is a single-headed species (35). Thus, in looking for a cytoplasmic dynein, it must be considered that the species may have one, two, or three heads. In view of the relatively small power requirements to move a chromosome (36), a single-headed species may provide sufficient force. Only one thing is certain. The size and density of dynein cross-bridges seen in cilia and flagella is not found in the mitotic spindle. Rather, as evidenced by the difficulty in finding cross-bridges in thin section, either the size or the number of dynein molecules must be smaller than in the axoneme.

A Common Kinetic Pathway

Dynein and myosin both couple the binding and hydrolysis of ATP and the release of ADP and P_i to the association and dissociation of a cross-bridge to produce a net sliding movement. We have recently shown that, in spite of rather gross structural differences, dynein and myosin couple the hydrolysis of ATP to force production by the common pathway shown in Fig. 3 (17, 37, 38). The first two steps of the dynein reaction pathway parallel that observed for actomyosin (33, 35, 39); ATP binding induces a very rapid dissociation of the microtubule-dynein complex, which is followed by a slower hydrolysis step on the free dynein molecule. Further work is required to establish the sequence of events following the hydrolysis of ATP in the dynein pathway, and only indirect evidence suggests that the binding of the ADP-phosphate intermediate to the microtubule completes the cycle, with the subsequent release of products coupled to the production of force for sliding. In the case of actomyosin, it is well established that the force-producing step occurs with the release of products from the ATPase site (40).

It is significant that the first step of the pathway for both ATPases involves the ATP binding-induced dissociation of the cross-bridge from the filament. It is precisely this step in which the substrate binding energy is utilized to couple the hydrolysis reaction to the vectoral process of cross-bridge motion. Moreover, the kinetic constants for the steps involving the binding and hydrolysis of ATP are surprisingly similar, within a factor of two or three when measured under identical conditions.[3] The only large difference is in the rate of product release, which is about 100-fold faster for dynein; this may reflect the greater importance of the resting state in muscle and the stronger selective pressure to maintain the products (ADP and phosphate) bound to the myosin for long periods of time.

[3] Chilcote, T. J., and K. A. Johnson, unpublished results.

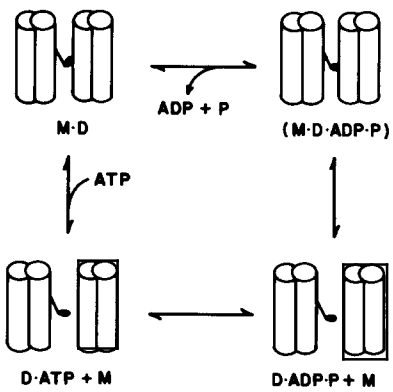

FIGURE 3 Mechanism of dynein cross-bridge motion. The dynein ATPase reaction sequence showing the pathway by which ATP binding, hydrolysis, and product release are coupled to the interaction of the dynein head, *D*, with a microtubule, *M*. The cycle starts in the upper left hand corner and proceeds counterclockwise with the addition of ATP. For clarity, only one of the three dynein heads is shown. Reproduced with permission from reference 37.

The faster rate of product release by dynein explains our failure to observe an activation of the steady-state ATPase activity upon the addition of microtubules, analogous to the actin-activated ATPase of myosin, that one would expect. The binding of microtubules to dynein, at concentrations of tubulin accessible in solution, is too slow to affect the lifetime of the dynein-ADP-P_i intermediate (37, 38). When dynein rebinds to the axoneme, the high local concentration of microtubules is sufficient to lead to an approximately sixfold activation of the ATPase, which is coupled to the propagation of waves (9, 10). Theoretically, one should see an activation of dynein by microtubules in solution, but the concentration required may be beyond that readily attainable.

The mechanism of inhibition of dynein by vanadate has also been shown to be identical to the mechanism observed for myosin (41). However, several studies have shown that vanadate can be used as a selective probe to inhibit dynein but not myosin, and several investigators have attempted to use vanadate to determine whether a given movement was driven by myosin or dynein (42, 43). We can understand the selective inhibition of dynein as due solely to a kinetic difference rather than a mechanistic or thermodynamic difference between the two ATPases. Vanadate binds to a dynein-ADP intermediate to form the inhibitory dynein-ADP-vanadate complex at a rate that exceeds by a factor of at least one million the rate of the corresponding reaction with myosin (41). Thus, although the equilibrium constants for the formation of the ADP-vanadate complex at the active site of the enzyme may be the same for both myosin and dynein, the large difference in rates of formation of the complex allow one to selectively inhibit dynein at concentrations of vanadate and over periods of time at which myosin is not affected. Because we know little about the kinetic properties of cytoplasmic myosin and nothing about the kinetic properties of a putative cytoplasmic dynein, we must exercise caution in interpreting the results of vanadate inhibition of complex cellular events in terms of distinguishing the two ATPases.

Unidirectional Force Production

Both systems are capable of producing force in only one direction relative to the structural polarity of the filaments. The direction of force production is established at the interface

between the working head of the ATPase and the surface of the filament. In the actomyosin systems, actin filaments are organized in antiparallel arrays and are pulled together by bipolar myosin filaments (Fig. 2). In contrast, microtubules are organized in parallel arrays in cilia, flagella, and the chromosome-to-pole region of the mitotic spindle (6, 15, 44). Unidirectional force production in microtubule-dynein systems in general can be understood with respect to the ciliary axoneme. The notable feature of the microtubule array in the axoneme is that lateral asymmetry of the microtubules of the axoneme converts the parallel arrangement of microtubules into a system capable of producing force in only one direction. That is, the structural attachment site (base) of the dynein binds on only one side of a microtubule, whereas the heads of the dynein molecule can interact in an ATP-dependent reaction only on the opposite side of that microtubule. This prevents the formation of counterproductive cross-bridges between two parallel microtubules. In the axoneme, this lateral asymmetry is achieved by the doublet microtubule. Cytoplasmic microtubules may assemble in a B-type lattice containing a seam along one edge involving different tubulin bonding contacts (45) and therefore may possess an intrinsic lateral asymmetry. The significant point is that the observed parallel array of microtubules in the mitotic spindle is consistent with a dyneinlike force-producing system, in terms of our understanding of the axoneme, and supports, rather than contradicts, a sliding-filament model of chromosome movement.

Summary and Prospectus for a Cytoplasmic Dynein

Over the past two years, we have gained new insights into the structure and biochemistry of dyneins isolated from cilia and flagella that can serve as a basis for new approaches to the question of microtubule-dependent motility. In reviewing the work on dynein, it is reasonable to propose that many of its similarities with myosin reflect the principles that govern the conversion of chemical energy to the mechanical force required for sliding. The differences, in turn, may reflect adaptation to the unique constraints of each system. With regard to the latter, it is essential to carefully scrutinize the many predictions of the biochemical properties of a cytoplasmic dynein that are based upon analysis of ciliary and flagellar dyneins. One should expect that cytoplasmic dynein will have at least one globular head and should be structurally attached to one microtubule by a flexible strand and that the head will bind to an adjacent microtubule ATP in an ATP-sensitive reaction. These predictions are based upon what appear to be fundamental properties of a cross-bridge involved in sliding microtubule motility. However, further predictions concerning the activation of ATPase activity or inhibition by vanadate, for example, cannot be made, because these properties are a function of subtle kinetic differences in the protein and are likely to change due to the requirements of adaptation to a particular system.

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