Eur Biophys J (1998) 27: 466–473

ARTICLE

Melissa Gee · Richard Vallee

The role of the dynein stalk in cytoplasmic and flagellar motility

Received: 13 March 1998 / Revised version: 17 April 1998 / Accepted: 17 April 1998

Abstract We have recently identified a microtubule binding domain within the motor protein cytoplasmic dynein. This domain is situated at the end of a slender 10–12 nm projection which corresponds to the stalks previously observed extending from the heads of both axonemal and cytoplasmic dyneins. The stalks also correspond to the B-links observed to connect outer arm axonemal dyneins to the B-microtubules in flagella and constitute the microtubule attachment sites during dynein motility. The stalks contrast strikingly with the polymer attachment domains of the kinesins and myosins which are found on the surface of the motor head. The difference in dynein's structural design raises intriguing questions as to how the stalk functions in force production along microtubules. In this article, we attempt to integrate the myriad of biochemical and EM structural data that has been previously collected regarding dynein with recent molecular findings, in an effort to begin to understand the mechanism of dynein motility.

Key words Microtube · Molecular motor

I Introduction

The dyneins comprise a class of minus-end directed microtubule motor proteins which include cytoplasmic and axonemal isoforms. Over 11 forms of axonemal dyneins have been identified that function in sliding events which give rise to flagellar and ciliary beating (Holzbaur and Vallee 1994; Kamiya 1995). The cytoplasmic dyneins function in minus-end directed organelle movements along microtubules (Paschal and Vallee 1987; Paschal et al. 1987) and have been shown to function during nuclear migration in fungi as well as in movements of other organelles, including lysosomes, endosomes, and Golgi, in mammalian cells (Sweeney and Holzbaur 1996; Corthesy-Theulaz et al. 1992; Burkhardt et al. 1997). In addition, cytoplasmic dynein also plays a role in chromosome capture, bipolar spindle formation and spindle orientation during mitosis (Vaisberg et al. 1993; Heald et al. 1996; Echeverri et al. 1996).

The dynein complex ranges in size from 1 to 2×10^6 Da and is composed of 2–3 different heavy chains ≥500 kDa in axonemal dyneins and 2 identical heavy chains of about 530 kDa in cytoplasmic dyneins. The heavy chains each contain a globular head domain and are tethered together via a stem emerging from a globular base (Johnson and Wall 1983; Witman et al. 1983; Goodenough and Heuser 1984; Vallee et al. 1988; Amos 1989). The entire complex resembles a fist holding 2–3 balloons. The heavy chains contain the catalytic ATPase and microtubule-binding domains required for motor function. The dynein complex also contains an extensive array of lower molecular weight subunits ranging from 8–122 kDa, some of which have been localized to the base of the complex (King and Witman 1990; Steffen et al. 1997).

Sequences for approximately 15 cytoplasmic or axonemal dynein heavy chain genes or cDNAs have been reported (Gibbons et al. 1994; Tanaka et al. 1995; Asai and Lee 1995; Vaughan et al. 1996). Sequence analysis indicates the presence of four conserved nucleotide-binding P-loop motifs located within the central ~115 kDa of the protein. Only the first P-loop has been shown to have ATPase activity (Tang and Gibbons 1987; Mocz and Gibbons 1996; Gee et al. 1997). Recent evidence indicates that the remaining three P-loops may also be active in nucleotide binding (Mocz and Gibbons 1996), perhaps serving some regulatory function.

M. Gee · R. Vallee (✉)
Worcester Foundation for Biomedical Research,
University of Massachusetts Medical School,
222 Maple Avenue, Shrewsbury, MA 01545, USA
e-mail: gee@sci.wfbr.edu,
e-mail: vallee@sci.wfbr.edu

II Comparison of dynein to myosin and kinesin

The overall structure of dynein differs from that of the other motor proteins, kinesin and myosin. The catalytic head domain of dynein is approximately 350 kDa in size (Johnson and Wall 1983; Vallee et al. 1988) and appears to correspond to ~3300 a.a. of the heavy chain (Koonce and Samso 1996; Gee et al. 1997). This is quite large compared to the motor domains of kinesin and myosin which consist of ~340 a.a. and ~850 a.a. respectively (Rayment et al. 1993; Kull et al. 1996; Sablin et al. 1996). The two or three dynein heavy chains appear to be stably linked only near the base of the complex (Goodenough and Heuser 1984; Amos 1989). The dyneins also differ from kinesin and myosin in having many more non-catalytic subunits. Given the size and complexity of dynein compared to the other motors, it is no wonder that progress in understanding dynein has been slower.

The kinetics for the cross-bridge cycle of axonemal dynein have been analyzed in detail by Johnson and coworkers (1985). ATP binding to dynein causes the protein to release rapidly from the microtubule, similar to ATP-stimulated release of myosin from the actin filament. ATP is hydrolyzed to ADP and phosphate, followed by release of phosphate. Microtubule binding to the resulting dynein-ADP complex stimulates the release of ADP, which is considered to be the rate limiting step in the reaction (Johnson 1985; Omoto and Johnson 1986). The power stroke for dynein is likely to occur during product release (Johnson 1985; Omoto and Johnson 1986). Basal ATPase levels for axonemal dyneins are quite high (0.15–0.38 μmol ATP/mg · min), as is the K_m for microtubule stimulation (Omoto and Johnson 1986). Cytoplasmic dynein, on the other hand, has a lower basal ATPase activity (32 nmol/mg · min), and microtubules stimulate ATP hydrolysis with a K_m of only 0.16 mg/ml (Shpetner et al. 1988). The two classes of dynein also vary in rates of motility in in vitro gliding assays. Axonemal dyneins typically have a motor velocity of 4.5 μm/s, whereas the cytoplasmic dyneins tend to be somewhat slower (~1.2 μm/s) (Paschal et al. 1987 a, b; Vale and Toyoshima 1988; Sale and Fox 1988).

Much has been learned about the mechanism of motility of kinesin and myosin by use of single-molecule experiments and structural analysis (reviewed by Block 1996; Vale 1996; Cope et al. 1996; Howard 1997; Amos and Cross 1997). Such experiments have provided information as to step size, processivity, and cooperativity between heads. Single molecule systems for studying dyneins have yet to be fully developed. As a result, mechanistic details of dynein motility are still lacking. The density of protein required to translocate microtubules in in vitro motility assays performed on glass coverslips suggests that more than one axonemal dynein may be required for microtubule gliding (Vale et al. 1989). In that study, 50 μg/ml or more of the *Tetrahymena β*/IC complex was required for motility with an estimated surface density of ~1000 dyneins per μm^2. Dilution studies of cytoplasmic dynein have been performed using dynein-coated latex beads in in vitro motility assays (Wang et al. 1995). In these experiments, dynein was adsorbed to beads at concentrations calculated to yield one dynein molecule per bead and was found to produce continuous movement along microtubules. However, bead motility included a high percentage of sideways and backwards movements suggesting that the dynein wobbled between protofilaments. This movement was postulated to result from random diffusion of one of the dynein heads while the other head was engaged in force production.

Axonemal dyneins are likely to work coordinately in the flagellum or cilium in which case that any one dynein would be expected to spend little time actually applying force to the microtubule, reminiscent of the muscle actinomyosin system (Johnson 1985). In contrast, the functions of cytoplasmic dynein are more similar to that of conventional kinesin in transporting organelles within the cell; thus, it seems reasonable to expect that cytoplasmic dynein might be processive, since it would be inefficient for the organelle to dissociate from the microtubule repeatedly en route. In order to achieve processivity, one head could remain attached to the microtubule during the power stroke of the second head, similar to the hand-over-hand mechanism proposed for kinesin (Howard 1996; Howard 1997). Another possibility is that multiple dyneins on the same organelle act coordinately to propel it along a microtubule. Finally, the dynactin complex which has been shown to both bind microtubules and be required for dynein-directed organelle motility in vitro (Vallee and Sheetz 1996), could function in keeping dynein and the organelle attached to the microtubule between power strokes. Such a functional role for dynactin would explain why this complex is required for dynein-directed organelle motility in vitro, but not for microtubule gliding (Schroer and Sheetz 1991).

III Microtubule binding domain of dynein

Until recently, very little had been done to correlate dynein sequence information with functional domains of the protein, largely owing to the immense size of the heavy chain subunit which has made standard recombinant and molecular genetic experiments difficult. We have generated a series of 36 mutant and truncated forms of the rat cytoplasmic dynein heavy chain for expression and subsequent analysis in cultured mammalian cells (Gee et al. 1997). These constructs were used to define the motor domain and to identify the microtubule-binding region.

Analysis of recombinant dynein containing a point mutation showed the first P-loop to be necessary for vanadate-mediated photocleavage (Gee et al. 1997). (In this reaction, which blocks further ATPase activity and is characteristic of all dyneins, vanadate is thought to exchange with the phosphate in the dynein-ADP-phosphate complex subsequent to ATP hydrolysis and, once bound, to prevent release of ADP blocking further dynein function (Shimuzu and Johnson 1983). The bound vanadate acts as a chromophore and causes cleavage of the polypeptide chain when

irradiated with UV light (Gibbons et al. 1991)). Work by Koonce and Samso (1996) showed that the C-terminal two thirds of the protein forms a globular mass which likely includes the globular head domain observed in EM and STEM. When we truncated further into this region of the heavy chain from either the N- or C-terminus, the resulting fragments were no longer susceptible to vanadate photocleavage and co-localized with microtubules as judged by immunofluorescence microscopy. These results indicated that nearly the entire C-terminal two-thirds of the protein is required for normal ATPase activity and proper regulation of microtubule binding. As these properties are required for dynein motor function, this region likely corresponds to the minimal motor unit.

We mapped the microtubule binding site to a 131 a.a. region using primarily a microtubule-co-localization assay, combined with in vitro microtubule binding. Interestingly, this region is located quite far downstream (~1,400 a.a.) from the first functional P-loop and ~340 a.a. downstream from the fourth P-loop. We note that studies using in vitro microtubule binding assays alone have identified other potential sites. For example, Lee and Asai (1995) identified three different microtubule binding regions within the P-loop containing segment of the rat cytoplasmic dynein heavy chain using co-sedimentation analysis of bacterially expressed fragments with microtubules. Koonce (1997) in vitro translated a series of dynein heavy chain segments located in the vicinity of the site identified by our analysis. Several of the translation products corresponding to distinct regions were observed to cosediment with microtubules. The basis for the disparity in the results from the several studies is not entirely clear. However, the functional importance of the site identified in our study is supported by a significant body of ultrastructural and physiological evidence (see below).

The microtubule-binding site defined by our analysis is flanked by two relatively long regions that are predicted to form coiled coils and are structurally conserved among all dyneins sequenced to date (Mitchell and Brown 1994; Gee et al. 1997). We reasoned that these two regions might interact in anti-parallel to form a hairpin resulting in a 10–12 nm long structure, with the microtubule-binding region occurring as a globular mass at the tip. Goodenough and Heuser (1984) had reported the presence of a "stalk" with similar proportions emanating from the globular head of purified axonemal dyneins when viewed using quick-freeze deep-etch EM techniques. A similar structure was also reported by Amos (1989) using negative stain EM to view purified porcine cytoplasmic dynein. Goodenough and Heuser (1982) showed that dynein in the axoneme contacts the B microtubule via a thin filament, referred to as the B-link, similar in length to the stalk observed on purified dyneins. As other investigators had failed to see the stalk, however, its presence had remained controversial in the dynein field (Satir 1989).

In order to examine the morphology of the microtubule-binding region identified by deletion analysis, we expressed a 45 kDa polypeptide encompassing the microtubule-binding region and flanking coiled-coils in insect cells infected with recombinant baculovirus. When viewed by EM, two types of particle were observed; rods measuring ~23 nm×6 nm and astral arrays composed of 12–18 spokes radiating from a central mass (Fig. 1). The individual spokes of the astral arrays were each composed of ~12 nm long filaments containing a globule measuring 6 nm in diameter at the tip. The size and morphology of the individual spokes approximated that of the stalks observed on purified dyneins using the same EM technique (Goodenough and Heuser 1984). These data strongly imply that the microtubule-binding region that we identified using deletion analysis corresponds to the stalk previously observed on purified dyneins and axonemal dyneins in situ. The individual rods that we observed in our recombinant protein preparations could represent single stalks thickened by the metal shadow, or dimers or trimers.

We have not yet determined the specific amino acids involved in microtubule contact. Polymer binding regions of myosin and kinesin have been mapped more precisely (Rayment et al. 1993b; Woehlke et al. 1997; reviewed by Cope et al. 1996). The actin filament binding region of myosin lies opposite to the nucleotide-binding face and consists of at least two large loops that are non-contiguous at the sequence level (Rayment et al. 1993b). Filament binding is controlled by nucleotide binding on the "backside" of the head domain, and vice versa. The actin-binding face separates into two separate surfaces upon ATP binding, resulting in release from the filament. Upon hydrolysis and phosphate release, the two surfaces move into close proximity to one another, likely resulting in the 10,000 fold increase in actin-binding affinity displayed by the myosin-ADP complex (Taylor 1992; Rayment et al. 1993b; reviewed by Vale 1996). A low-affinity filament binding state mediates the events between ATP binding to myosin and high-affinity binding of myosin to the filament.

The microtubule-binding region of kinesin was recently defined at the residue level using the mutagenic method of alanine scanning to replace individual amino acids with the neutral amino acid alanine (Woehlke et al. 1997). The microtubule contact residues of kinesin are found in an approximately 2.5 nm wide strip on the back side of the protein opposite the nucleotide-binding pocket (Woehlke et al. 1997). The regions that provide polymer contact correspond spatially to the peptide loops of myosin that interact with the actin filament. Two classes of solvent-exposed amino acids were found to affect microtubule-binding. These included primarily basic amino acids, including K and R, which when mutated individually caused a decrease in microtubule binding as determined by microtubule-stimulated ATPase activity. The other class of residues were primarily acidic amino acids, including D and E, which when mutated caused an increase in microtubule-binding affinity. Thus, in kinesin, microtubule-binding affinity appears to be regulated by a balance of strategically placed acidic and basic residues.

Work from several laboratories suggests that both kinesin and dynein interact with the acidic C-terminus of tubulin. Each motor has been reported to bind the glutamic acid-

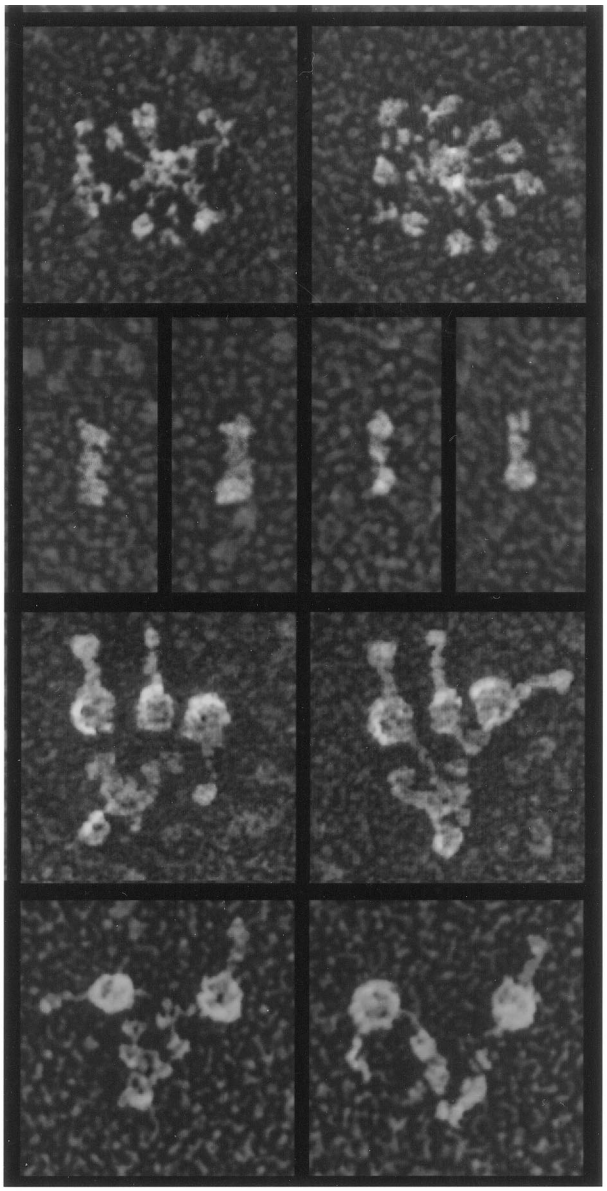

Fig. 1 Electron micrographs of dynein stalks and purified dyneins (from Gee, Heuser and Vallee 1997). Top Row, astral arrays of recombinant dynein microtubule binding domain. These structures are aggregates of 12–18 spokes emanating from a globular mass. The individual spokes correspond in size and dimension to the stalks on axonemal and cytoplasmic dyneins observed at the same magnification in rows three and four. Second Row, rod-shaped structures observed in preparations of recombinant dynein microtubule binding domain. These structures are larger in dimension than the individual stalks in the astral arrays and may be dimers or trimers of stalks. Third Row, purified axonemal dynein corresponding to outer arms of *Tetrahymena* flagella. Fourth Row, purified cytoplasmic dynein

rich C-terminal tails of both α and β tubulin (Paschal et al. 1989; Goldsmith et al. 1995; Larcher et al. 1996); however, only peptides corresponding to the C-terminus of β tubulin were found to interfere with microtubule stimulated ATPase activity of kinesin (Tucker and Goldstein 1997). Since dynein and kinesin have both been proposed to bind the same region of tubulin, the microtubule-binding contacts of dynein with microtubules are likely to involve electrostatic forces similar to those of kinesin. Several conserved basic residues in the globular region of the dynein stalk are likely candidates for polymer interaction (Fig. 2). Several of these charged residues are more highly conserved with in cytoplasmic dyneins than between cytoplasmic and axonemal dyneins. Such differences could be important in imparting different microtubule-binding affinities to the different dynein complexes to facilitate their independent functions. Microtubule stimulation of cytoplasmic dynein ATPase activity has been estimated to involve a K_m for microtubules that is at least 100 fold lower than that for axonemal dynein (Shpetner et al. 1988), and likely reflects differences in microtubule-binding affinity.

Why dynein should contact the microtubule via such an unusual structure is uncertain. One reasonable possibility relates to the large size of the dynein head relative to that of the tubulin subunits which make up the microtubule surface lattice. The diameter of the dynein head (13–14 nm) is about three times that of the tubulin monomer (4–5 nm). Direct microtubule contact by multiple dynein heads within a single molecule, or by adjacent dyneins within the axoneme or on the surface of a membranous organelle, may well be sterically impossible; thus, the stalk may have evolved to alleviate this problem.

The length of the stalk as observed by freeze-etch electron microscopy appears to be comparable among a wide range of dyneins (Goodenough and Heuser 1982, 1984, 1989). Some variability in the predicted length of the two coiled coil regions is observed (Mitchell and Brown 1995; Gee et al. 1997). However, we note that the position of prolines that flank these regions is highly conserved, suggesting that the actual length of the α-helices is constant. Perhaps ten to twelve nanometers is a sufficient length to overcome steric hindrance between dynein heads, but short enough to function efficiently in force transduction (see below).

IV Ultrastructural and biochemical analysis of dynein head behavior

Evidence for dynein conformational changes comes from direct observations of dynein morphology in the axoneme in which the dynein head changes from a squat "corn kernel" conformation in the rigor state to a ellipsoidal shape in the presence of ATP (Goodenough and Heuser 1982, 1984; Burgess 1995). The individual heads within each dynein molecule cannot be discerned in these images and are likely to be intimately associated with one another, as is observed for purified axonemal dynein complexes fixed with glutaraldehyde (Goodenough and Heuser 1984) and for the so called "phi particles" of cytoplasmic dynein (Amos 1989). Nonetheless, the dynein head, or head conglomerate, appears to change position in relationship to the base of the molecule depending on ATP availability.

Burgess (1995) has used computer image averaging of quick-freeze deep-etch EM images of outer arm dyneins

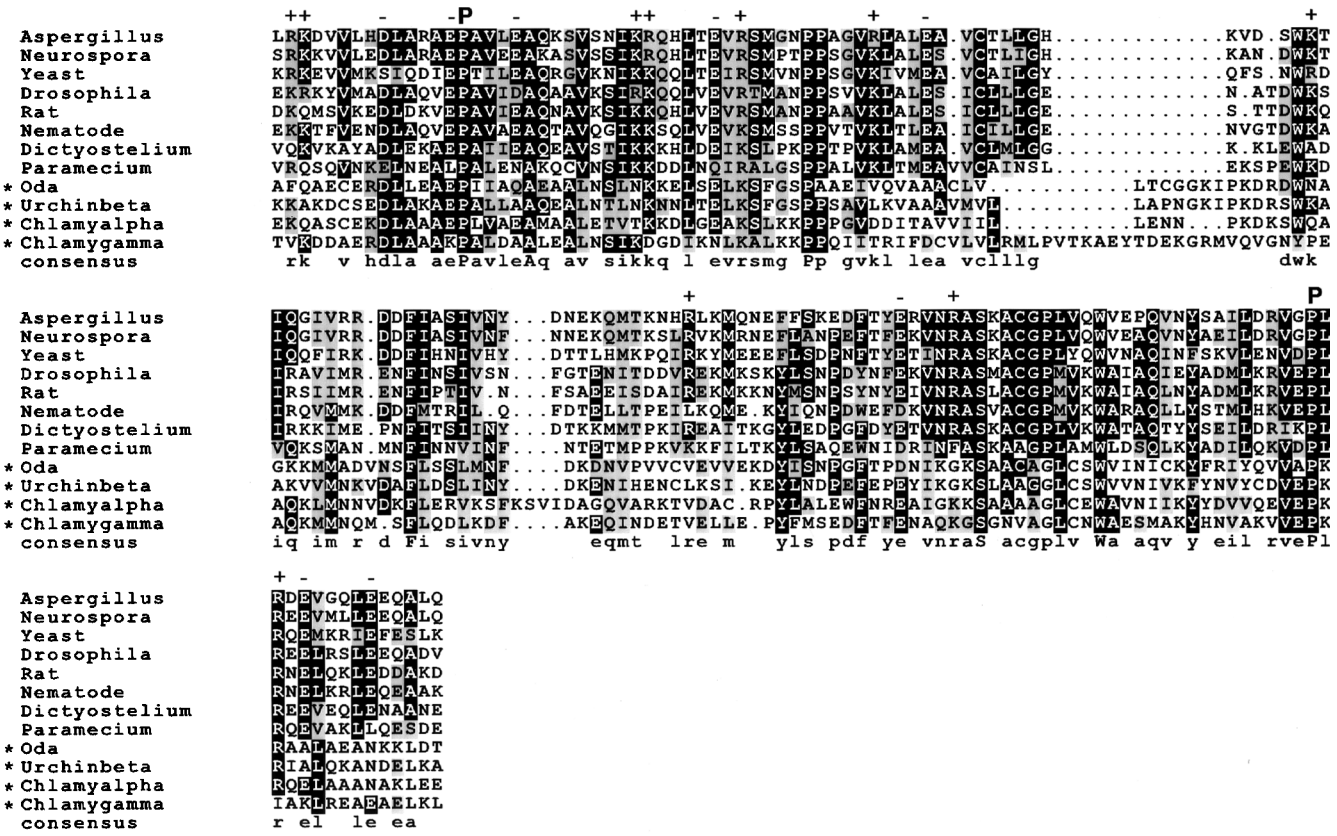

Fig. 2 Sequence comparison of the microtubule binding region of cytoplasmic and axonemal dyneins. Sequences from eight cytoplasmic and four axonemal (*) dyneins were compared within the region of the microtubule binding domain using the pileup function of the GCG program, and were analyzed for conservation using the Boxshade 3.21 software from the Swiss Institute for Experimental Cancer Research. Sequences were obtained from Genbank and contain 14 amino acids upstream and downstream of the conserved prolines (**P**) that we propose define the beginning of the flanking predicted coiled coil regions. Sequences showing fifty per cent or higher identity or conservation are boxed in black or gray, respectively. In the consensus line, capitol letters indicate complete identity and small case letters indicate conserved residues. Conserved basic amino acids are indicated by +'s and conserved acidic amino acids by –'s. Note that some of these residues are more highly conserved in the cytoplasmic versus axonemal dynein heavy chains

observed in the rigor, relaxed, or active states in order to assign ATP-related states to dynein in active axonemes. This analysis corroborated the conformational changes previously described by Goodenough and Heuser (1982, 1984), but also provided evidence that, in rigor, the dynein head is displaced ~12 nm-roughly its own diameter-towards the distal (plus) end of the axoneme in relationship to the A microtubule. These data suggest that the dynein molecule may become more extended, or that the head may shift relative to the base (stem) of the dynein complex, during the relaxed state. These data also imply that the dynein step size could be relatively large (i.e. as much as 12 nm).

There is also biochemical evidence that the dynein complex undergoes fairly dramatic conformational changes as a result of ATP binding and hydrolysis. Experiments using limited protease treatment indicate that different regions of the dynein heavy chain are solvent-exposed depending on whether the protein is incubated with ATP or ADP plus vanadate (Ianaba and Mohri 1989).

In the relaxed (ADP and VO_4 bound) and active (unlimited ATP) states, the stalk emanates from a proximal position on the dynein head(s) and is nearly perpendicular to the B microtubule. The position of the stalk appears to be uniform amongst dyneins along the axoneme. In the rigor state, the stalk emanates from the top of the dynein head and appears to tilt at a variety of angles (Goodenough and Heuser 1982; Burgess 1995).

Contact of the stalk with microtubules in the axoneme has been observed using freeze-etch EM (Goodenough and Heuser 1982). The globular tip that is observed on stalks on purified dynein, however, has not been observed on the B-links. Several possibilities could account for this phenomenon. The globular tip could intercalate into the microtubule lattice making it invisible in these preparations. Alternatively, the tip might assume another conformation when in contact with a microtubule such that it lies flush against the polymer. Interestingly, in one study the globular tip of purified cytoplasmic dynein appeared to have multiple conformations which result in variable stalk lengths (Amos 1989). In the extended examples, the tip seems to have "uncoiled" as an extension of the filamentous region. In addition, longer stalks have been observed in outer arm dyneins in the axoneme when stretched during rigor (Goodenough and Heuser 1984). Thus, this region could act as a spring to push or pull along the microtubule.

Another curious feature of the B-links is that the stalk was not observed to break contact with the B microtubule in either the relaxed or rigor state, leaving open the possibility that the stalk contains both a low-affinity and higher-affinity microtubule-binding surface or that the stalk resides close to the B microtubule when not engaged in the power stroke. It is unlikely that these contacts form strong attachments since the outer doublet microtubules must be allowed to slide distances of up to 100 nm in relation to one another during flagellar beating (Satir 1989).

A low affinity contact by the stalk could act to tether the dynein to the microtubule between strokes, while a higher affinity interaction might be engaged to grip the microtubule during the power stroke. A low affinity interaction with microtubules has been reported by Vale et al. (1989) in which microtubules were observed to undergo bidirectional, one dimensional diffusion in in vitro motility assays in the presence of vanadate and either ADP or limiting ATP. This interaction with microtubules may very well constitute an intermediate state during motility since the dynein molecule is presumably locked into an intermediate state of ATP hydrolysis under the former conditions.

Another question that arises from in situ analysis of axonemal dynein is why only one filamentous contact with the B microtubule is observed per multi-headed dynein molecule. This observation suggests that only one dynein head is in contact with the microtubule at a time. Alternatively, the stalk from the additional heads could be hidden, or the stalks from multiple heads might closely associate while making contact with the microtubule in situ forming a compound coiled coil. Observation of "phi" particles in preparations of purified porcine cytoplasmic dynein and glutaraldehyde-fixed outer arm dyneins supports the latter possibility (Amos 1989; Goodenough and Heuser 1984, 1989). In these samples, the two or three dynein heads appeared to interact to form one globular mass with the individual stalks fused into a single filament. As noted above, the dimensions of the rod shaped structures that we observed in preparations of baculovirus-expressed stalks suggests that these particles could represent dimers or trimers (Gee et al. 1997). Whether stalks do, indeed, interact under physiological conditions, and whether this interaction might function in modulating microtubule binding, remain fascinating questions for future research.

V The role of the dynein stalk in motility

A major question remaining to be answered is how the stalk functions during motility. Two issues must be resolved. First, how is force transmitted through the stalk? Second, how is conformational information transmitted? That the latter form of signal transduction is at issue is evident from consideration of the dynein cross-bridge cycle. In order to function in force production, microtubule binding must cycle between "on" and "off" states in response to nucleotide binding states within the head region. Since the ATPase domain is presumed to reside within the head region, a distance of at least 10 nm from the site of microtubule binding, some form of feedback between these important functional regions must occur via the stalk which links the two. Thus, the stalk must function to process conformational changes that arise at or near the nucleotide-binding pocket in the head and transmit this information to the site of microtubule contact. Furthermore, microtubule binding at the tip of the stalk must stimulate product release within the head domain.

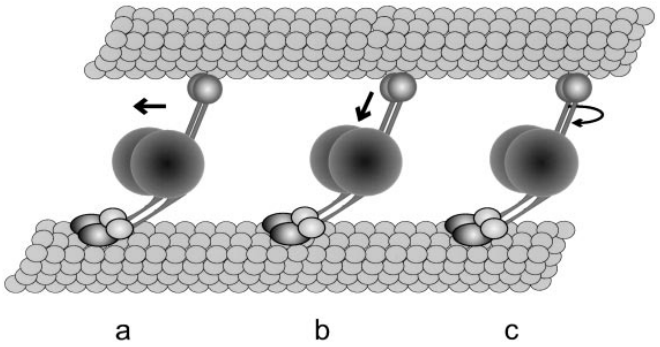

Fig. 3a–c Three models portraying possible activities of the dynein stalk during force production. In diagram **a**, the stalk acts as a directional lever arm to amplify conformational changes within the head domain. In diagram **b**, the head domain tugs on the stalk which acts as an intermediary between the dynein head and the microtubule. In diagram **c**, conformational changes within the dynein head during the cross-bridge cycle cause the stalk to twist and dissociate from the microtubule

Based on current knowledge of the changes in morphology of dynein during its cross-bridge cycle (see above), the stalk may be envisioned to participate in dynein motility in a number of ways (Fig. 3). An obvious possibility is that the stalk acts as a "lever arm" similar to the regulatory region of conventional myosin (reviewed in Block 1996) (Fig. 3a). In such a model, the stalk would act to amplify subtle conformational changes that occur within the head to produce larger-scale movements along the microtubule. The anti-parallel coiled coil structure we propose for the filamentous region of the stalk seems likely to provide adequate rigidity to serve as a lever arm similar to the rigidity imparted by binding of light chains to the myosin regulatory region (reviewed in Block 1996 and Howard 1997). Rigidity of the dynein stalks could be further increased by interaction of multiple stalks (see above).

We note that an extended intramolecular coiled-coil structure similar to the dynein stalk has also been reported projecting from the catalytic domain of seryl t-RNA synthetase (Cusack et al. 1990). This structure has been shown to rotate ~30° relative to the catalytic domain upon substrate binding (Biou et al. 1994). A 30° angular rotation of the ~12 nm long dynein stalk would accommodate a movement of roughly 6 nm by the stalk tip along the microtubule.

Observations that the dynein head itself may move during the cross-bridge cycle, however, suggests that rotation of the stalk(s) does not constitute the only force-producing motion (Tsukita et al. 1983; Burgess 1995). Thus, combined movement of the head and stalk may contribute to the length of the physical lever arm, and thus the dynein

step, in this mechanism. The head and stalk may not act as a rigid unit at all times, however, since the position of the stalk in relationship to the head was reported to be variable in the rigor state and to differ between the rigor and relaxed states (Burgess 1995).

Whether the stalk plays an active or a passive role in force production remains an open question. It is unclear whether the stalk changes position only as a consequence of head movement, or if it, indeed, acts as an independent lever arm. Rather than causing an angular rotation of the stalk, the dynein head may simply tug on this structure to cause movement along the microtubule (Fig. 3b). This mechanism is easy to imagine for dynein in the axoneme where the dynein base is firmly attached to a fixed position, i.e. the A microtubule; but it is less compelling for cytoplasmic dynein whose cargo, i.e. organelles, are not fixed in position.

In neither scenario described above is it clear how conformational information is transmitted through the stalk between the dynein head and the microtubule binding site. For a coiled-coil structure, only very small movements between the interacting α-helices can be accommodated without their dissociation; thus, it is possible that local or general melting of the coiled-coil is involved in transducing conformational information between the head and the microtubule. However, such changes should lead to loss of stalk rigidity.

Rigidity and signal transduction can both be accommodated by models involving twisting of the stalk (Fig. 3c). Such a motion could supply enough force to break the microtubule-dynein interface or cause the microtubule-binding face to tilt into a lower-affinity position. In addition, strain within the twisted stalk could be transmitted to the head to enhance product release as a result of microtubule-binding at the tip of the stalk.

Compared to myosin and kinesin, our understanding of dynein-directed motility at the mechanistic level is still in its infancy. Much will be gained by further mutational and structural analysis of the dynein complex. Likewise, high resolution motility assays using single and multiple dynein heads should contribute greatly to our understanding of dynein force production. It is already clear that dynein differs significantly from the other motor proteins in the structural relationship between its polymer-binding and ATPase sites. It will be interesting to see just how closely dynein follows in the footsteps of its fellow motor proteins in mechanistic terms.

Acknowledgements The authors would like to thank Jorge Garces, Sharon Hughes Tynan, Patricia Okamoto, and Atsushi Mikami for critical reading of the manuscript and Katy Sherlock and Jorge Garces for help in preparing the manuscript.

References

Amos LA (1989) Brain dynein crossbridges microtubles into bundles. J Cell Sci 93:19–28

Amos LA, Cross RA (1997) Structure and dynamics of molecular motors. Curr Opin Struct Biol 7:239–246

Asai DJ, Lee S (1995) The structure and function of dynein heavy chains. Mol Cell Biol 5:299–305

Biou V, Yaremchuk A, Tukalo M, Cusack S (1994) The 2.9 A crystal structure of *T. thermophilus* seryl-tRNA synthetase complexed with $tRNA^{Ser}$. Science 263:1404–1410

Block SM (1996) Fifty ways to love your lever: myosin motors. Cell 87:151–157

Burgess SA (1995) Rigor and relaxed outer dynein arms in replicas of cryofixed motile flagella. J Mol Biol 250:52–63

Burkhardt JK, Echeverri CJ, Nilsson T, Vallee RB (1997) Overexpression of the dynamitin (p50) subunit of the dynactin complex disrupts dynein-dependent maintenance of membrane organelle distribution. J Cell Biol 139:469–484

Cope MJT, Whisstock J, Rayment I, Kendrick-Jones J (1996) Conservation within the myosin motor domain: implications for structure and function. Curr Biol 4:969–987

Corthésy-Theulaz I, Pauloin a, Pfeffer SR (1992) Cytoplasmic dynein participates in the centrosomal localization of the golgi complex. J Cell Biol 118:1333–1345

Cusack S, Berthet-Colominas C, Hartlein M, Nassar N, Leberman R (1990) A second class of synthetase structure revealed by X-ray analysis of *Escherichia coli* seryl-tRNA synthetase at 2.5 A. Nature 347:249–255

Echeverri CJ, Paschal BM, Vaughan KT, Vallee RB (1996) Molecular characterization of the 50 kD subunit of dynactin reveals function for the complex in chromosome alignment and spindle organization during mitosis. J Cell Biol 132:617–633

Gee MA, Heuser JE, Vallee RB (1997) An extended microtubule-binding structure within the dynein motor domain. Nature 390:636–639

Gibbons IR, Gibbons BH, Mocz G, Asai DJ (1991) Multiple nucleotide-binding sites in the sequence of dynein β heavy chain. Nature 352:640–643

Gibbons BH, Asai DJ, Tang W-JY, Hays TS, Gibbons IR (1994) Phylogeny and expression of axonemal and cytoplasmic dynein genes in sea urchins. Mol Biol Cell 5:57–70

Goldsmith M, Yarbrough L, van der Kooy D (1995) Mechanics of motility: distinct dynein binding domains on α- and β-tubulin. Biochem Cell Biol 73:665–671

Goodenough UW, Heuser JE (1982) Substructure of the outer dynein arm. J Cell Biol 95:798–815

Goodenough UW, Heuser JE (1984) Structural comparison of purified dynein proteins with in situ dynein arms. J Mol Biol 180:1083–1118

Goodenough UW, Heuser JE (1989) Structure of the soluble and in situ ciliary dyneins visualized by quick-freeze deep-etch microscopy cell movement, vol 1: The dynein ATPases, vol 1. Liss, New York, pp 121–140

Heald R, Tournebize R, Blank T, Sandaltzopoulos R, Becker P, Hyman A, Karsenti E (1996) Self-organization of microtubules into bipolar spindles around artificial bipolar spindles around artificial chromosomes in *Xenopus* egg extracts. Nature 382:420–425

Holzbaur ELF, Vallee RB (1994) Dyneins: molecular structure and cellular function. Annu Rev Cell Biol. 10:339–372

Howard J (1996) The movement of kinesin along microtubules. Annu Rev Physiol 58:703–729

Howard J (1997) Molecular motors: structural adaptations to cellular functions. Nature 389:561–567

Johnson KA (1985) Pathway of the microtubule-dynein ATPase and the structure of dynein: a comparison with actomyosin. Annu Rev Biophys Chem 14:161–188

Johnson KA, Wall JS (1983) Structure and molecular weight of the dynein ATPase. J Cell Biol 96:669–678

Kamiya R (1995) Exploring the function of inner and outer dynein arms with *Chlamydomonas* mutants. Cell Motil Cytoskeleton 32:98–102

King SM, Witman GB (1990) Localization of an intermediate chain of outer arm dynein by immunoelectron microscopy. J Biol Chem 265:19807–19811

Koonce MP (1997) Identification of a microtubule-binding domain in a cytoplasmic dynein heavy chain. J Biol Chem 272:19714–19718

Koonce MP, Samso M (1996) Overexpression of cytoplasmic dynein's globular head causes a collapse of the interphase microtubule network in *Dictyostelium*. Mol Biol Cell 7:935–948

Kull FJ, Sablin EP, Lau R, Fletterick RJ, Vale RD (1996) Crystal structure of the kinesin motor domain reveals a structural similarity to myosin. Nature 380:550–555

Larcher J-C, Boucher D, Lazereg S, Gros F, Denoulet P (1996) Interaction of kinesin motor domains with α- and β-tubuline subunits at a Tau-independent binding site. J Biol Chem 271:22117–22124

Lee S-W, Asai DJ (1995) Identification of microtubule-binding sites on dynein heavy chain by using bacterially expressed MAP1 C fusion proteins. Mol Biol Cell 6:35a

Mitchell DR, Brown KS (1994) Sequence analysis of the *Chlamydomonas* alpha and beta dynein heavy chain genes. J Cell Sci 107:635–644

Mocz G, Gibbons IR (1996) Phase partition analysis of nucleotide binding to axonemal dynein. Biochemistry 35:9204–9211

Omoto CK, Johnson KA (1986) Activation of the dynein adenosinetriphosphatase by microtubules. Biochemistry 25:419–427

Paschal BM, Vallee RB (1987) Retrograde transport by the microtubule associated protein MAP 1 C. Nature 330:181–183

Paschal BM, King SA, Moss AG, Collins CA, Vallee RB, Witman GB (1987) Isolated flagellar outer arm dynein translocates brain microtubules in vitro. Nature 330:672–674

Paschal BM, Shpetner HS, Vallee RB (1987) MAP 1 C is a microtubule-activated ATPase which translocates microtubules in vitro and has dynein-like properties. J Cell Biol 105:1273–1282

Paschal BM, Obar RA, Vallee RB (1989) Interaction of brain cytoplasmic dynein and MAP 2 with a common sequence at the C terminus of tubulin. Nature 342:569–572

Rayment I, Rypniewski WR, Schmidt-Base K, Smith RJH, Tomchick DR, Benning MM, Winkelman DA, Wesenberg G, Holden HM (1993) The three-dimensional structure of myosin subfragment 1: a molecular motor. Science 261:50–58

Rayment I, Holden HM, Whittaker M, Yohn CB, Lorenz M, Holmes KC, Milligan RA (1993) Structure of the actin-myosin complex and its implications for muscle contraction. Science 261:58–65

Sablin EP, Kull FJ, Cooke R, Vale RD, Fletterick RJ (1996) Crystal structure of the motor domain of the kinesin-related motor ncd. Nature 380:555–559

Sale WS, Fox L (1988) Isolated β-heavy chain subunit of dynein translocates microtubules in vitro. J Cell Biol 107:1793–1797

Satir P (1989) Structural analysis of the dynein cross-bridge cycle. Cell Movement 1:219–234

Schroer TA, Sheetz MP (1991) Two activators of microtubule-based vesicle transport. J Cell Biol 115:1309–1318

Shimizu T, Johnson KA (1983) Kinetic Evidence for Multiple Dynein ATPase Sites. J Biol Chem 258:13841–13846

Shpetner HS, Paschal BM, Vallee RB (1988) Characterization of the microtubule-activated ATPase of brain cytoplasmic dynein (MAP 1 C). J Cell Biol 107:1001–1009

Steffen W, Karki S, Vaughan KT, Vallee RB, Holzbaur ELF, Weiss DG, Kuznetsov SA (1997) The involvement of the intermediate chain of cytoplasmic dynein in binding the motor complex to membranous organelles of Xenopus Oocytes. Mol Biol Cell 8:2077–2088

Sweeney HL, Holzbaur ELF (1996) Mutational analysis of motor proteins. Annual Review of Physiology, vol 58. Annual Reviews, Inc., pp 751–792

Tanaka Y, Zhang Z, Hirokawa N (1995) Identification and molecular evolution of new dynein-like protein sequences in rat brain. J Cell Sci 108:1883–1893

Tang W-JY, Gibbons IR (1987) Photosensitized cleavage of dynein heavy chains. J Biol Chem 263:17728–17734

Taylor EW (1992) Mechanism and energetics of actomyosin ATPase. In: Fozzard HA (ed) The heart and cardiovascular system, vol 2. Raven Press, New York, pp 1281–1293

Tsukita S, Tsukita S, Usukura J, Ishikawa H (1983) ATP-dependent structural changes of the outer dynein arm in *Tetrahymena* cilia: a freeze-etch replica study. J Cell Biol 96:1480–1485

Tucker C, Goldstein LSB (1997) Probing the kinesin-microtubule interaction. J Biol Chem 272:9481–9488

Vaisberg EA, Koonce MP, McIntosh JR (1993) Cytoplasmic dynein plays a role in mammalian mitotic spindle formation. J Cell Biol 123:849–858

Vale RD (1996) Switches, latches, and amplifiers: common themes of G proteins and molecular motors. J Cell Biol 135:291–302

Vale RD, Toyoshima YY (1988) Rotation and translocation of microtubules in vitro induced by dyneins from *Tetrahymena* cilia. Cell 52:459–469

Vale RD, Toyoshima YY (1989) Microtubule translocation properties of intact and proteolytically digested dyneins from *Tetrahymena* cilia. J Cell Biol 108:2327–2334

Vale RD, Soll DR, Gibbons IR (1989) One-dimensional diffusion of microtubles in vitro bound to flagellar dynein. Cell 59:915–925

Vallee RB, Sheetz MP (1996) Targeting of motor proteins. Science 271:1539–1544

Vallee RB, Wall JS, Paschal BM, Shpetner HS (1988) Microtubule associated protein 1C from Brain is a two-headed cytosolic dynein. Nature 332:561–563

Vaughan KT, Mikami A, Paschal BM, Holzbaur ELF, Hughes SM, Echeverri CJ, Moore K, Gilbert DJ, Copeland NG, Jenkins NA, Vallee RB (1996) Identification of multiple genomic loci involved in dynein-based motility. Genomics 36:29–38

Wang Z, Khan S, Sheetz MP (1995) Single cytoplasmic dynein molecule movements: Characterization and comparison with kinesin. Biophys J 69:2011–2023

Witman GB, Johnson KA, Pfister KK, Wall JS (1983) Fine structure and molecular weight of the outer arms of dyneins of *Chlamydomonas*. J Submicrosc Cytol 15:193–197

Woehlke G, Ruby AK, Hart CL, Ly B, Hom-Booher N, Vale RD (1997) Microtubule site of the kinesin motor. Cell 90:207–216