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COMMENTARY

AAA domains and organization of the dynein motor unit

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SUMMARY

Dyneins contain one-three microtubule motor units that are each derived from the C-terminal globular head of a heavy chain. The N-terminal regions of the heavy chains form stems that are required for intra-dynein associations. The microtubule-binding sites are located at the terminus of a short stalk that emanates from each globular head. Recent electron microscopic analysis indicates that the dynein head has a heptameric toroidal organization. This finding is echoed by the identification of six AAA (*A*TPases *a*ssociated with cellular *a*ctivities) domains and a seventh unrelated unit within this heavy chain region. At least two of these AAA domains can bind nucleotide, although only one appears able to hydrolyze ATP. Several other AAA domain proteins exhibit a similar annular organization of six AAA units. Detailed structural information is available for several AAA proteins, including *N*-ethylmaleimide-sensitive vesicle-fusion protein and the RuvB motor involved in DNA migration and resolution of Holliday junctions. The resulting structural parallels allow intriguing predictions to be made concerning dynein organization and motor function.

Key words: AAA domain, Dynein, Flagellum, Microtubule, Molecular motor

INTRODUCTION

Dyneins, kinesins and myosins represent the three major classes of molecular motor that translocate along cytoskeletal elements. The kinesins and myosins have a motor domain that contains both ATPase and filament-binding sites attached to an α-helical lever arm and neck region. Structural analysis of the kinesin and myosin heads identified a striking similarity in the motor domains of these enzymes (Kull et al., 1996; Rayment et al., 1993). This in turn implies that the general mechanisms by which these two motor classes generate force along their respective filament systems, although obviously differing in detail, are related at a fundamental level (Rayment, 1996; Kull et al., 1998). Much recent work has fleshed out many of the molecular parameters of myosin- and kinesin-based motility. For example, the lever arm and neck regions determine the polarity of movement (Case et al., 1997; Henningsen and Schliwa, 1997), whereas coordination between heads from a single particle sets the processivity of the motor along the filament (Kozielski et al., 1997; Romberg et al., 1998). These observations have raised the possibility that all cytoskeletal motors function similarly. However, there has remained one thorn in the side of this rather comforting picture of molecular motor conformity – dynein.

Dyneins exhibit much greater molecular complexity than the other cytoskeletal motors: compare, for example, the 360-kDa kinesin tetramer with the 1.9-MDa outer dynein arm, which contains 13 different polypeptide components. Unlike in myosin and kinesin, in dynein the microtubule-binding domain is located far from the ATP-hydrolysis site (Gee et al., 1997; Koonce, 1997); the in vitro microtubule-translocation parameters of dynein also are quite different from those of kinesin (Wang et al., 1995). Recent studies indicate that the dynein motor unit is constructed around a series of AAA (*A*TPases *a*ssociated with cellular *a*ctivities) domains (Neuwald et al., 1999), which suggests that this enzyme class is fundamentally distinct from kinesin and myosin at both the structural and mechanistic levels.

The AAA domain is found in a very wide variety of proteins ranging from bacterial protease regulators and metal chelatases to transcriptional regulators and the microtubule-severing protein katanin (Hartman and Vale, 1999; Patel and Latterich, 1998). This domain consists of an ATP-binding motif and P-loop combined with a core α/β structure on which protein-specific modules are added. High-resolution structures have been reported for two members of this protein class: the D2 oligomerization domain of *N*-ethylmaleimide-sensitive vesicle fusion protein (NSF; Lenzen et al., 1998; Yu et al., 1998) and the clamploader (δ′ subunit) of DNA polymerase III (Guenther et al., 1997). Electron microscopic (EM) and image analysis has also revealed many structural features of RuvB, which is thought to act as a DNA pump during branch migration and resolution of Holliday junctions (Yu et al., 1997). In combination with the recent sequence comparisons revealing dynein as a member of this group (Neuwald et al., 1999), the new structural data allow us to draw conclusions about the

organization of this motor and the mechanisms of dynein function.

DYNEIN DOMAIN ORGANIZATION

Dyneins consist of a series of globular motor domains (1-3, depending on the number of heavy chains (HCs) in each specific enzyme) attached via stems to a basal cargo-binding unit (Fig. 1A). These enzymes are built around 1-3 approx. 520-kDa HCs that each contribute an N-terminal, apparently flexible, stem and a C-terminal globular head domain of approx. 12 nm diameter that contains the ATP-binding and -hydrolysis sites (for review see King, 2000; Witman et al., 1994). The microtubule-binding region is located in a separate small globular domain located at the tip of a stalk that protrudes from the main head (Gee et al., 1997; Koonce, 1997). The N-terminal stem and microtubule-binding domain emanate from opposite sides of the globular head unit (Fig. 1A; Goodenough and Heuser, 1984). Mass analysis indicates that these heads account for approx. 350 kDa of the HC (Johnson and Wall, 1983; Witman et al., 1983), and negative-stain EM suggests they each contain a central, apparently hollow, core (Marchese-Ragona et al., 1988; Samso et al., 1998). More recent averaging of individual motor domain images has revealed additional features of this intriguing construction, defining seven distinct globular subdomains surrounding the central unfilled cavity (Samso et al., 1998).

How might the primary sequence of the dynein motor domain conform to the heptameric construction suggested by EM? In NSF, the toroidal hexameric organization revealed by EM (Hanson et al., 1997) is echoed by the sequential arrangement of six AAA domains within the ring (Lenzen et al., 1998; Yu et al., 1998). The original sequence analyses of dynein revealed only four putative nucleotide-binding motifs (P1-P4) within the central region of the HC (Gibbons et al., 1991; Ogawa, 1991), with ATPase activity being ascribed to the first, most highly conserved, motif. However, more recent studies clearly indicate that the dynein motor also contains six AAA domains plus an unrelated seventh unit at the C terminus (Fig. 1B; Neuwald et al., 1999). The first four AAA units correspond to the previously identified motifs. The fifth and sixth AAA domains had not been noted originally, because they lack intact consensus P-loop motifs. The coiled coil and

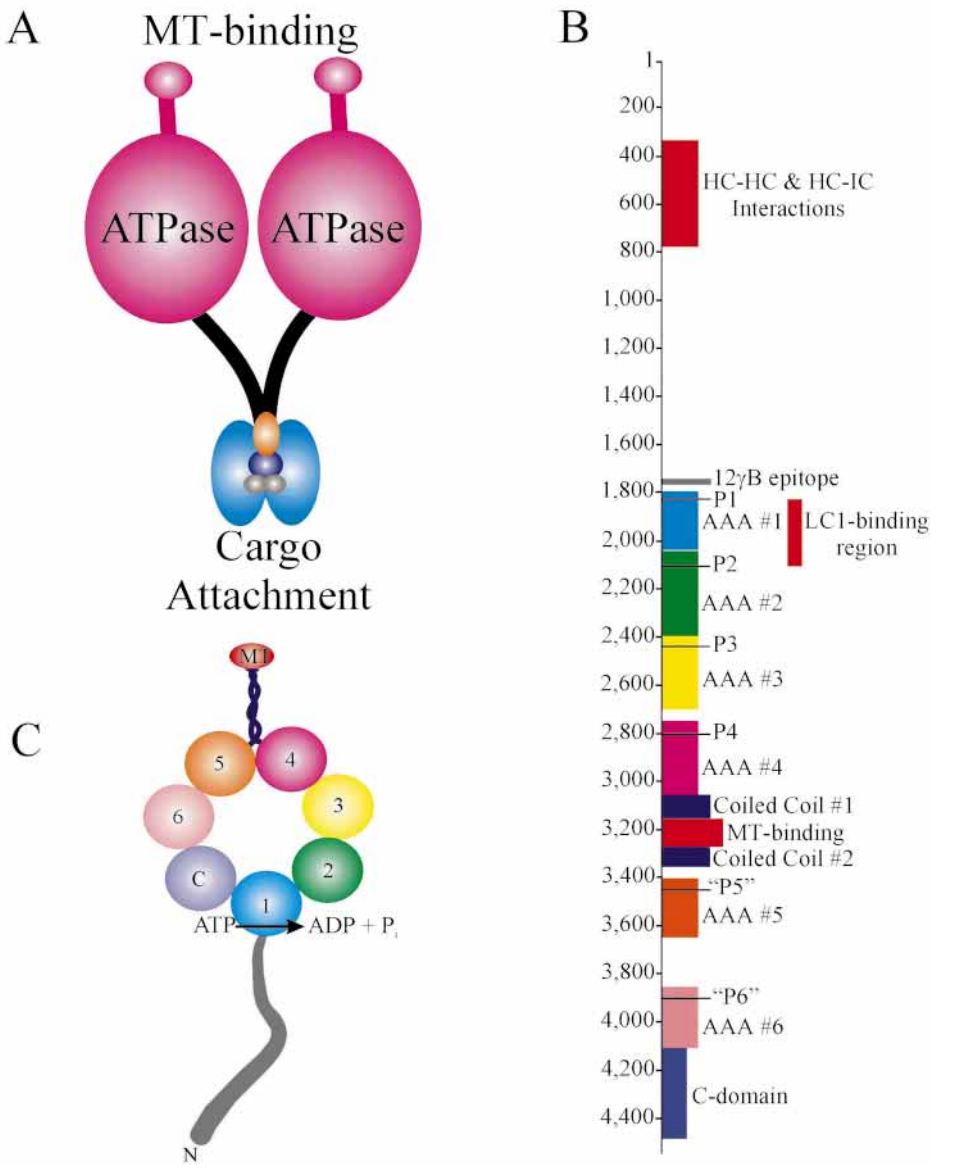

Fig. 1. Organization of the dynein motor domain. (A) Generic model of a dynein particle containing two heavy chains (HCs). The C-terminal portion of each HC forms a globular head containing the ATPase sites and has a small stalk-like structure that terminates in a microtubule-binding globular unit. The base of the dynein particle consists of the N-terminal regions of the HCs and a series of accessory proteins that function in cargo binding and might also have regulatory roles. (B) Map of the γ HC from *Chlamydomonas* outer arm dynein indicating the six AAA domains (AAA#1-AAA#6), both evident (P1-P4) and cryptic (P5-P6) P-loops (Neuwald et al., 1999), the microtubule-binding region and coiled-coil segments (Gee et al., 1997; Koonce, 1997). Also illustrated is the section that in cytoplasmic dynein is involved in HC-HC and HC-IC interactions (Habura et al., 1999), the LC1-binding domain (Benashski et al., 1999) and the epitope (Q1735-Q1758) recognized by monoclonal antibody 12γB (Wilkerson et al., 1994). Note that almost the entire globular motor unit consists of the six AAA domains, an unrelated C-terminal subdomain and the coiled-coil microtubule-binding region. (C) Model for the organization of an individual HC illustrating the heptameric structure of the head. Each subdomain is color-coded to illustrate the predicted concordance with individual regions shown on the map (B) on the basis of the structural parallels with NSF.

microtubule-binding region that protrudes from the main head (Gee et al., 1997; Koonce, 1997) is located between the fourth and fifth AAA domains.

Sequence analysis combined with the recent EM reconstructions and the resulting structural parallels with the hexameric NSF complex (Hanson et al., 1997; Neuwald et al., 1999; Yu et al., 1998) suggests a plausible model for the arrangement of these various domains within the globular motor head of dynein (Fig. 1C). The six AAA units and C-terminal segment are proposed each to correspond to one of the dynein subdomains visualized by EM (Samso et al., 1998) in a manner analogous to that observed for NSF. In the model shown in Fig. 1C, the AAA domains are arrayed sequentially in a toroidal structure; the C-terminal unit contacts the first AAA domain (AAA#1) and completes the ring. This arrangement would place the N-terminal stem and the microtubule-binding structure on opposite sides of the head – as is observed by EM (Goodenough and Heuser, 1984).

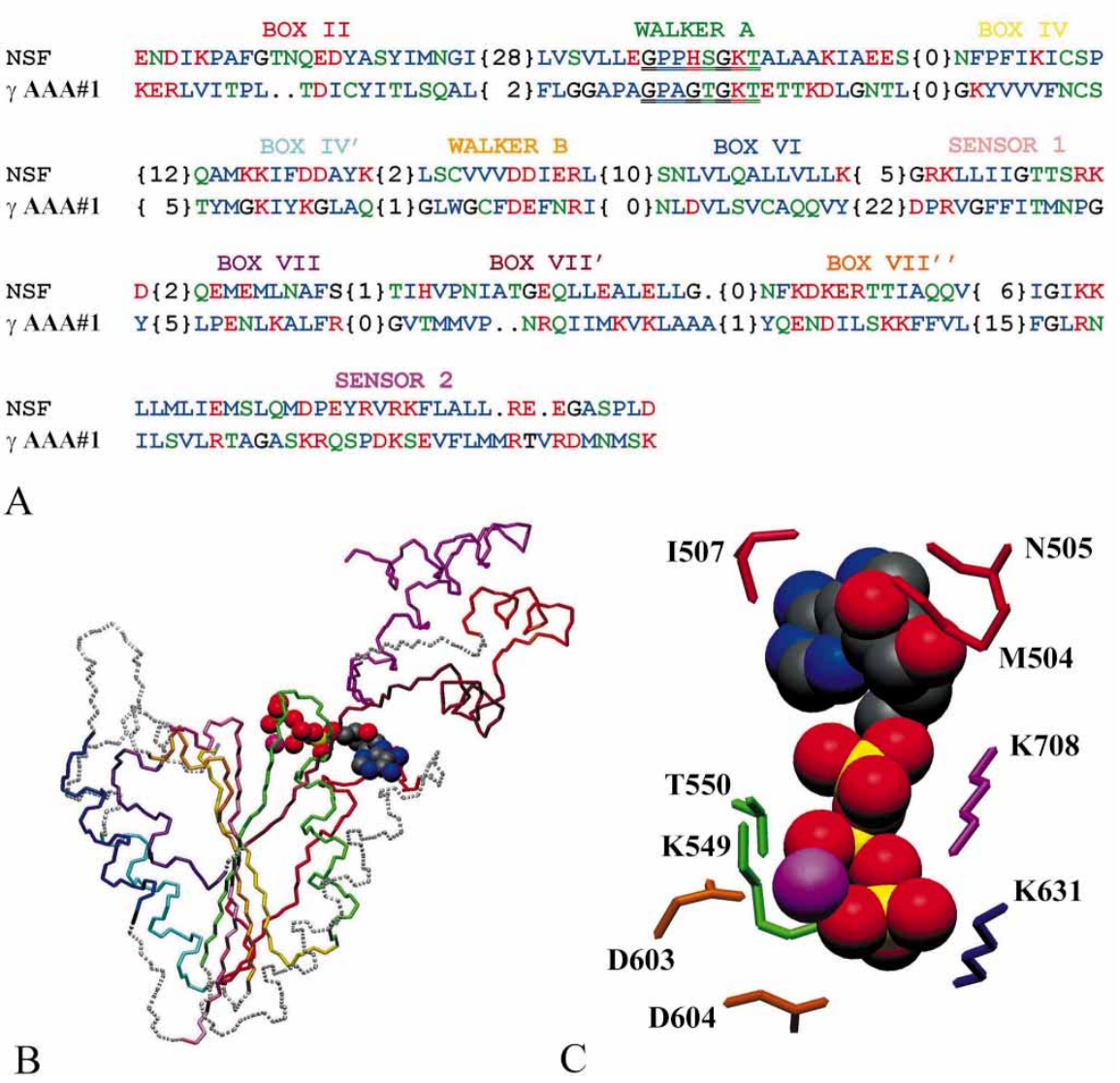

Fig. 2. Structure of the AAA domain. (A)Sequence alignment of the D2 hexamerization domain of NSF (residues 485-742; accession # P18708) and the first AAA domain of the γ-HC from *Chlamydomonas* outer-arm dynein (residues 1789-2030: accession # Q35975). The alignment is based on that described by Neuwald et al. (1999). Residues in the alignment are color coded as follows: red, charged; green, polar; blue, hydrophobic; black, glycine. Numbers in parentheses indicate the number of residues that join adjacent conserved elements. The different segments of the AAA domain (Box II, Walker A, etc.) are indicated in the same color on both the alignment and the C_α backbone structure of NSF shown in B. The double underscore indicates the P-loop motif within the Walker A box. (B) C_α backbone of one AAA domain with bound $Mg.ATP^{2-}$ from NSF (accession #1D2N). The color code for conserved elements follows: Box II, red; Walker A, green; Box IV, yellow; Box IV′, cyan; Walker B, orange; Box VI, blue; Sensor 1, pink; Box VII, purple; Box VII′, brown; Box VII″, dark orange; Sensor 2, magenta. Interconnecting regions between these elements are shown in grey. (C) Details of NSF side-chain interactions with $Mg.ATP^{2-}$. The adenine base has few specific contacts and sits in a hydrophobic pocket within the binding site. Two highly conserved lysine residues (K549 and K708) contact the β and γ phosphates. A third interacting lysine residue (K631) derives from the adjacent AAA domain and could allow communication and/or coordination between domains. The metal ion (magenta) also interacts with the β and γ phosphates, with T550 and with a water molecule hydrogen-bonded to D603. All of the residues (except K631) that make specific contacts are conserved in the first AAA domain from the γ HC.

Alternative assignments or arrangements of the various subdomains are of course possible, although they do not appear to account for the available data so readily.

AAA DOMAIN STRUCTURE

An alignment of the conserved elements within one NSF D2 AAA domain and AAA#1 (containing P-loop 1) of the γ HC from *Chlamydomonas* outer arm dynein is shown in Fig. 2A (this is the only dynein region known to hydrolyze ATP). Each element corresponds to a unit of the NSF AAA domain tertiary structure depicted in Fig. 2B. For example, the Walker A segment contributes a β strand to the twisted sheet that forms the core of the structure, a loop that interacts with the β and γ phosphates of ATP, and a helix that connects to Box IV.

In NSF, several residues coordinate the nucleotide and Mg^{2+} ion. Two lysine residues (K549 and K708) interact with the β and γ phosphates. Both basic residues are conserved in dynein AAA#1. Most interestingly, in the NSF hexamer, K631 from one AAA subunit interacts with the γ phosphate of ATP within an adjacent AAA subunit in the ring. This side chain occupies the site normally filled by the water molecule required for γ-phosphate hydrolysis and appears to stabilize the ATP-bound form of NSF. These observations lead to the hypothesis that ATP-dependent structural alterations are propagated through the protein. In both flagellar and cytoplasmic dyneins, a basic residue occurs at this position in AAA#5 and potentially could interact with and stabilize nucleotide bound to AAA#4. This hypothesis predicts that mutation of K3515 would alter the motor properties of the *Chlamydomonas* γ-HC. Analysis of a dynein required for setting up left-right asymmetry during vertebrate development (Supp et al., 1997) provides further support for the hypothesis that conformational changes in AAA domains other than AAA#1 are required for motor function. In murine left-right dynein, a single base change causes an E→K transition and results in motor dysfunction, leading to immotile nodal cilia and the consequent randomization of organ placement in the developing embryo (Supp et al., 1999). This highly conserved glutamate residue (E2216 in the γ-HC sequence) is located in the region of AAA#2 that connects the Sensor 1 motif with Box VII.

Both NSF and dynein require Mg^{2+} for catalysis. In NSF, the metal interacts with six ligands, including the side chain of T550 (within the P-loop), the β and γ phosphates and three water molecules, one of which is hydrogen bonded to D603. The latter residue is important for the correct orientation of $Mg.ATP^{2-}$ and forms part of an acidic pair located in the Walker B region that also is conserved in dynein. Within NSF, ATP is bound in the *syn* conformation – that is, with the base stacked above the ribose sugar. Whether the nucleotide adopts the same structure when bound to dynein is, however, unclear because this enzyme most readily hydrolyzes nucleotide analogues that favor the alternate (*anti*) conformation, in which the base is oriented away from the sugar (King et al., 1989; Omoto and Nakamaye, 1989). Given that the base-binding region of NSF is principally hydrophobic and engages in few specific interactions, there might be no necessity for conserving the base orientation of the bound nucleotide within an AAA domain.

HOW MANY NUCLEOTIDE-BINDING SITES ARE PRESENT IN DYNEIN?

Although each dynein HC contains six AAA domains, at least four of which could interact with ATP, the actual number of nucleotide-binding sites has proven difficult to determine. Phase-partition analysis of sea urchin sperm dynein suggested that each HC indeed can bind four ATP molecules in a slightly cooperative fashion (Mocz and Gibbons, 1996). Subsequent fluorescence anisotropy measurements using a methylanthraniloyl nucleotide analogue provided evidence for only two high-affinity sites that have association constants in the 10^{-4}-10^{-5} M^{-1} range (Mocz et al., 1998). This latter result agrees with data from in vitro microtubule-translocation assays, which suggest that the *Chlamydomonas* γ-HC has both high- and low-affinity nucleotide-binding sites, which have K_ms of 8 μM and 80 μM, respectively (Wilkerson and Witman, 1995). The results from mapping studies locating sites of azidoATP photoinsertion within dynein HCs also can be interpreted in terms of a two-site model (King et al., 1989).

THE MICROTUBULE-BINDING SITE

Quick-freeze/deep-etch microscopy of isolated dynein particles has clearly demonstrated that a short stalk-like structure terminating in a small globular domain emanates from the head unit (Goodenough and Heuser, 1984). Analysis of various HC subfragments indicates that this protruding structure forms the microtubule-binding site (Gee et al., 1997; Koonce, 1997). It is derived from two regions that have high coiled-coil structure probabilities and an intervening segment of approx. 125 residues, all of which are located between AAA#4 and AAA#5 (Fig. 1B). The coiled-coil regions presumably are arranged in an anti-parallel fashion. However, the segment between the coiled-coil domains that apparently contacts tubulin is rather enigmatic, because it is not highly conserved between flagellar and cytoplasmic dyneins – a high degree of conservation might be expected for an essential functional domain. For example, the *Dictyostelium* cytoplasmic dynein HC and the γ-HC from *Chlamydomonas* flagellar dynein share only 24.6% sequence identity in this region, even though both proteins functionally interact with microtubules. This implies that only a few highly conserved residues within this region actually form the dynein-microtubule interface. Furthermore, on its own, this domain is insufficient for high-affinity microtubule binding in vitro, which suggests that the adjacent coiled-coil regions are required to form the active folded structure (Koonce and Tikhonenko, 2000).

THE N-TERMINAL REGION

The N-terminal portion of the HC (approx. 150 kDa) is required for assembly of the dynein particle: it both mediates HC-HC dimerization and interacts with the IC-LC complex to form the basal cargo-binding unit. The *Chlamydomonas* mutant *oda4-s7* expresses a truncated form of the outer-arm β-HC that allows dynein assembly but lacks the C-terminal β-HC motor unit (Sakakibara et al., 1993). Deletion analysis of cytoplasmic dynein has defined a relatively small subregion required for HC-HC and HC-IC interactions (Habura et al., 1999; Fig. 1B). This N-

terminal domain appears flexible and apparently does not adopt a rigid conformation as seen in the α-helical tails of myosin and kinesin (Goodenough and Heuser, 1984; Johnson and Wall, 1983). Circular dichroism spectroscopy of this region from the β-HC of sea urchin outer-arm dynein suggests that it has relatively low (approx. 26%) α-helical content, which would support a non-helical flexible arrangement. Intriguingly, however, sequence analysis predicts a large α-helical content (>60%) with high probability. A possible solution to this apparent anomaly would be for this domain to adopt a partially helical structure in situ, but for structural changes to occur following its removal from the axoneme and/or during preparation for EM. Certainly the dimensions of isolated flagellar outer arm dynein particles are such that rearrangement and/or intermolecular association of the N-terminal regions must occur for the splayed particle seen by EM to correspond with the compact structure observed in situ (Goodenough and Heuser, 1984).

DYNEIN-BASED MICROTUBULE TRANSLOCATION

Both flagellar outer arm and cytoplasmic dyneins are able to translocate microtubules in vitro when attached to a substrate at high surface density (Paschal et al., 1987; Paschal and Vallee, 1987; Vale et al., 1992). The velocity of movement can reach approx. 6 $\mu m.second^{-1}$, depending on the particular dynein being assayed. Intriguingly, the two dynein classes behave somewhat differently at lower motor densities; they might therefore have distinct duty ratios (i.e. time in strongly-bound state [τ_s]/total cycle time [τ_c]) (Vale et al., 1992). However, a recent report indicates that *Chlamydomonas* inner dynein arm c is a single-headed processive motor (i.e. a single motor molecule can go through multiple force generating events and traverse long distances along a microtubule without dissociating), even though it exhibits a low duty ratio (Sakakibara et al., 1999). Thus, in this dynein, mechanisms that keep the motor loosely attached to the microtubule throughout most or all of the mechanochemical cycle, and therefore avoid the separation of motor and filament that would otherwise be enforced by thermal diffusion, have evolved. Intriguingly, this motor slips backwards along the microtubule when placed under high load, which supports the idea that it is only loosely tethered for most of the cycle (Sakakibara et al., 1999).

Kinesin exhibits precise tracking along the microtubule in that it will move down a single protofilament for a considerable length (Gelles et al., 1988). Dynein, by contrast, does not have this property but, rather, wanders across the microtubule surface as it goes through multiple force-generating cycles (Wang et al., 1995). This meandering over the surface lattice might be related to the observation that several dynein species are capable of generating torque and thus cause the microtubule to rotate as it is translocated in the in vitro assay (Kagami and Kamiya, 1992; Vale and Toyoshima, 1988) – i.e. microtubule rotation might be due to a directional bias in the off-axis motion of certain dynein motors.

MECHANISTIC PARALLELS WITH OTHER AAA PROTEINS

The structural homology in AAA domain proteins implied by sequence analysis raises the possibility that dynein-based motility shares more mechanistic similarity with the hexameric AAA domain-based motor RuvB than with kinesin or myosin (Neuwald et al., 1999). RuvB is involved in migration of the RuvABC complex along DNA at Holliday junctions. The DNA strand is passed through the RuvB ring of AAA domains in an ATP-dependent manner.

The structural parallels between dynein, NSF and RuvB suggest a potential mechanism by which dynein ATPase activity is coupled to force generation and the location at which the power stroke occurs. In both myosin and kinesin, ATP- and filament-binding sites are located close to each other, which allows direct communication between the two. However, in dynein, the microtubule-binding site is likely to be located at the end of a stalk far from the ATP-hydrolysis domain (Fig. 1C). How then might ATPase activity in AAA#1 affect the microtubule-binding properties at this distant site? One solution to this dilemma is that adjacent AAA domains might sense the presence of nucleotide in the binding sites (as apparently occurs in NSF) and pass this information around the ring through conformational changes.

Another possibility, first suggested by Samso et al. (1998), is that the power stroke actually occurs between the globular head and N-terminal stem (not the microtubule-binding region). These two ideas are not necessarily mutually exclusive. The second scenario is attractive because the known ATP-hydrolysis site would then be located close to this junction. However, it does raise the questions of whether the N-terminal stem has sufficient mechanical rigidity to act as a lever arm and of how microtubule affinity at a distant site might be altered. As argued above, the apparent flexibility of the N-terminal region observed by EM might be artifactual; i.e. in the native state, additional interactions between heavy chains might enhance the stability of this domain. Indeed, Neuwald et al. (1999) propose that in RuvB, repetitive cycles of structural rearrangements drive DNA migration through the junction. How might alterations in microtubule-binding affinity be achieved if the power stroke occurs at a point distant from the binding interface? This is a clear problem if all movement is directed along the filament axis – i.e. if no change in orientation of the dynein-microtubule interface occurs. However, within the axoneme, the globular heads of flagellar dyneins appear to rotate during the mechanochemical cycle (Goodenough and Heuser, 1984). Thus, if the power stroke contains a rotary as well as a linear component, the rigid microtubule-binding stalk would dramatically change its orientation with respect to the microtubule axis. This in turn could disrupt the interface and lead to a significant alteration in the affinity of this dynein segment for the microtubule.

CONCLUSIONS

The recent identification of dynein as a member of the AAA domain class of ATPases has provided intriguing insight into the organization of these motors. Combined with EM studies indicating that the dynein head has a heptameric arrangement, these data suggest a model for how the heavy chain is organized. Clearly, we must now demonstrate the proposed concordance of AAA units and subdomains within the head, using, for example, high-resolution immunoEM. The parallels that can be drawn between dynein and other AAA domain

proteins provide a tantalizing glimpse into the structural organization of the dynein motor and make intriguing predictions that can be tested by site-directed mutagenesis.

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