Molecular mechanism of force generation by dynein, a molecular motor belonging to the AAA+ family

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Abstract

Dynein is an AAA+ (ATPase associated with various cellular activities)-type motor complex that utilizes ATP hydrolysis to actively drive microtubule sliding. The dynein heavy chain (molecular mass > 500 kDa) contains six tandemly linked AAA+ modules and exhibits full motor activities. Detailed molecular dissection of this motor with unique architecture was hampered by the lack of an expression system for the recombinant heavy chain, as a result of its large size. However, the recent success of recombinant protein expression with full motor activities has provided a method for advances in structure–function studies in order to elucidate the molecular mechanism of force generation.

Introduction

Dynein is one of the most distant members of the AAA+ (ATPase associated with various cellular activities) family [1], and more than ten different types of dynein are found in a eukaryotic cell [2]. It is a molecular motor that utilizes energy released from ATP hydrolysis to move toward the minus end of the microtubule network in a cell, performing essential cellular functions such as intracellular transport, mitosis and cell motility. Our understanding of the molecular mechanism of dynein force generation is very limited compared with that of other AAA+ machines or cytoskeletal motors. This is partly because the development of an expression system for recombinant dynein was hampered as a result of the huge size of the dynein heavy chain that contains the motor domain (molecular mass > 500 kDa). However, expression of recombinant cytoplasmic dynein heavy chain with full motor activities has now been successfully developed, using either *Dictyostelium discoideum* [3] or *Saccharomyces cerevisiae* [4]. Together with the expression of recombinant dynein heavy chain, advances in electron microscopy studies [5] and single-molecule force measurement [6–8] have provided insights into the structure of dynein and the mechanism of its force generation.

Structural model of dynein heavy chain

Dynein is a huge protein complex which contains one to three heavy chains [9]. Among the proteins that form this complex, the heavy chain is responsible for all of the motor activities: ATP hydrolysis, ATP-sensitive microtubule binding and microtubule sliding [3,10]. Electron microscopy studies have shown that the dynein heavy chain is composed of three structurally and functionally distinct structures: the 'tail', the 'head' and the 'stalk' (Figures 1a and 1b) [5]. The N-terminal one-third of the dynein heavy chain constitutes a slender tail, which is responsible for dimerization of the heavy chain, as well as for binding of associated polypeptides and cargo [11,12]. The remaining C-terminal portion of the heavy chain contains six tandemly linked AAA+ modules [13–16] and folds to form a ring-like head with a diameter of ~13 nm [17]. The ATP hydrolysis sites of dynein are within this ring-like head. Finally, there is an elongated structure called the stalk that has a long coiled coil (~12 nm), with a small globular tip which is a microtubule-binding site [18–20]. Thus, among the AAA+ family members, the dynein heavy chain has a very unusual structure. First, the dynein heavy chain has six AAA+ modules within a single heavy chain, among which four modules (AAA1–AAA4) contain nucleotide-binding/hydrolysing sites, whereas the sequences of two modules (AAA5 and AAA6) are too degenerate to contain a nucleotide-binding/hydrolysing site. Secondly, from the ring-shaped head of the six AAA+ modules, the long tail and stalk, which are essential for force generation, protrude. In the heavy chain, the C-terminal 380 kDa segment has been identified as the motor domain capable of driving the active translocation of microtubules (Figures 1a and 1b) [3].

The power-stroke model of force generation by dynein

It is generally assumed that sliding of microtubules is driven by a cross-bridge cycle: a co-operative cycle involving association/dissociation of dynein to and from a microtubule track as well as its directional translocation, which is driven by ATP hydrolysis by dynein. From single-particle analyses

Key words: ATP associated with various cellular activities (AAA+), ATP hydrolysis, dynein, microtubule, motility, motor protein.

Abbreviations used: AAA+, ATPase associated with various cellular activities; BFP, blue fluorescent protein; FRET, fluorescence resonance energy transfer; GFP, green fluorescent protein; V_i, orthovanadate.

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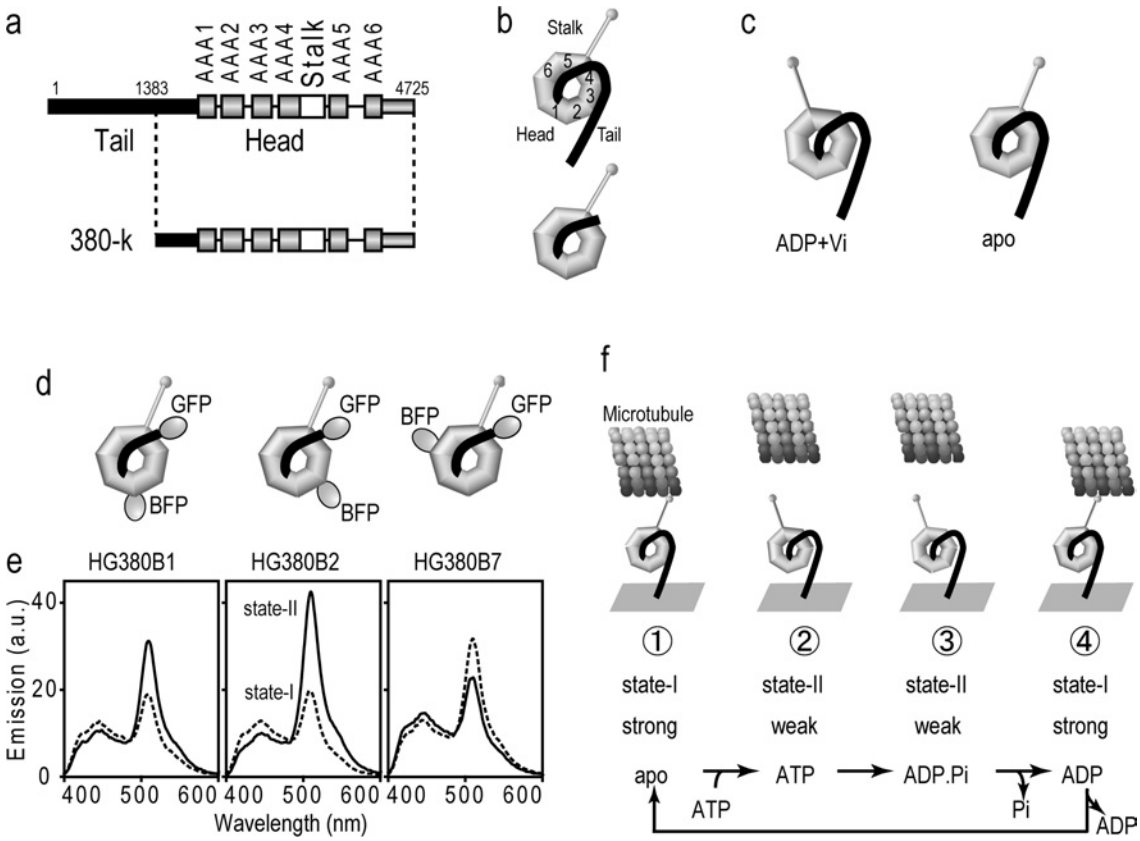
Figure 1 | Structural organization and ATPase-dependent tail swing of the dynein heavy chain

(**a**) The domain structure and features of a dynein heavy chain. The heavy chain is composed of the tail, head containing six AAA+ modules (AAA1–AAA6) and stalk. The 380 kDa (380-k) C-terminal recombinant protein is shown below. (**b**) A structural model of the heavy chain illustrating the heptameric ring-like structure of the head, and the elongated stalk and tail; 1–6, AAA1–AAA6 modules. (**c**) A scheme illustrating two structures of dynein in the apo and ADP + V_i-bound states, based on electron microscopy images. (**d**) Design of GFP- and BFP-labelled dyneins for detection of the tail swing. In these constructs, GFP and BFP were genetically inserted into the tail and head respectively. (**e**) Emission spectra of the GFP/BFP-labelled dyneins excited at 380 nm in the presence of 200 μM ADP (broken lines) or ATP (solid lines). The emission measurements (a.u., arbitrary units) detected two FRET states: state I and state II. Reproduced from [21] with permission. (**f**) Model of the cross-bridge cycle of dynein.



on negative-staining electron microscopy images of an axonemal dynein, Burgess et al. [5] have shown that dynein adopts two distinct conformations depending on nucleotide states (Figure 1c). The most significant structural difference between the ADP + V_i (orthovanadate) state, mimicking the pre-stroke state, and apo (no nucleotide) state, mimicking the post-stroke state, was the location of the tail on the head. On the basis of those observations, the power-stroke model has been proposed, in which the swinging motion of the tail relative to the head (and the stalk) generates force and directional movement of a microtubule [5].

Since this power-stroke model is the first mechanistic model of dynein force generation on a microtubule, it was necessary to examine whether the tail really swings against the ring-like head during the ATP hydrolysis cycle, as assumed in the model. To detect this swing motion of the tail, double-labelled motor domains were constructed using recombinant 380 kDa dynein, in which GFP (green fluorescent protein) was fused to the tail and BFP (blue fluorescent protein) was inserted into the head (Figure 1d), and ATPase-dependent changes in FRET (fluorescence resonance energy transfer) between GFP and BFP were measured (Figure 1e) [21]. The results revealed that dynein has at least two distinct FRET states (state I and state II), corresponding to two distinct orientations of the tail against the head, which depends on the ATP hydrolysis steps. The FRET change seems to reflect the tail swing against the head, which will result in translocation of a microtubule bound on the stalk, as suggested by the power-stroke model.

By using this GFP-based FRET technique and the co-sedimentation assay of motor domains and microtubules, the coupling of an intermediate state of the ATP hydrolysis cycle of dynein, the tail orientation and the microtubule-binding states were all examined. In the apo and ADP states, dynein binds tightly to microtubules and the tail takes the post-stroke orientation (state I). In the ATP and

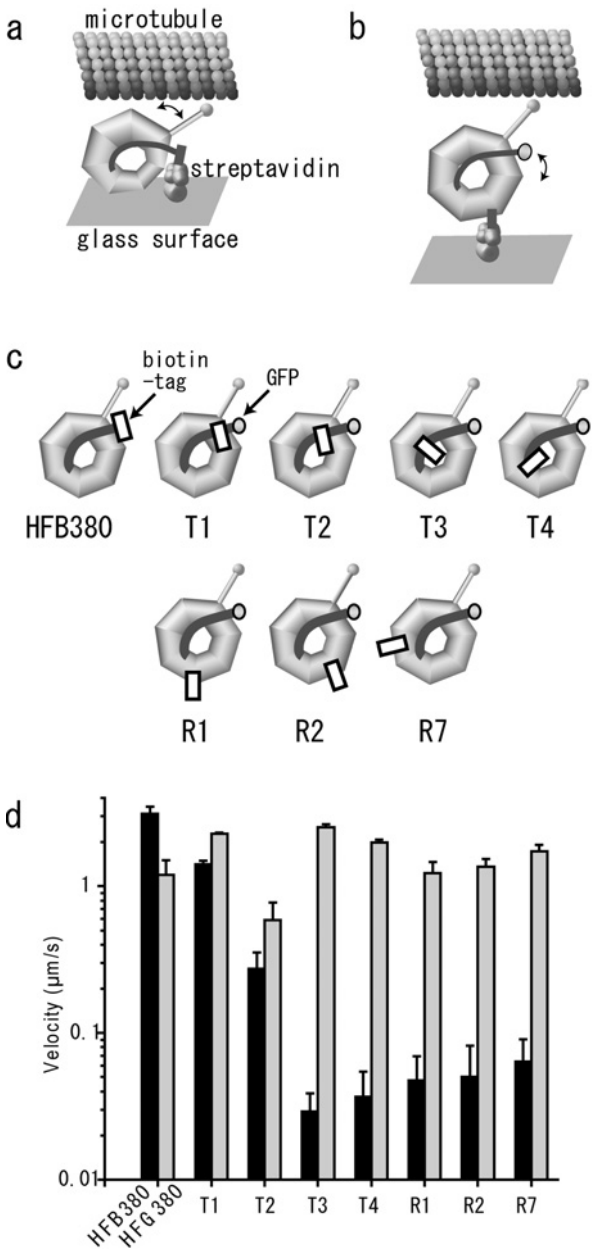
$ADP + V_i$ states, dynein binds weakly to microtubules and the tail takes the pre-stroke orientation (state II). On the basis of these findings, the cross-bridge cycle of dynein has been proposed (Figure 1f). In the apo state, dynein binds tightly to microtubules and the tail is in the post-stroke orientation (1). On binding to ATP before ATP hydrolysis, dynein dissociates from microtubules and the tail takes the pre-stroke orientation (2). When dynein adopts the $ADP + P_i$ state after ATP hydrolysis, it still remains dissociated from microtubules and the tail remains in the pre-stroke orientation (3). In the ADP state after phosphate release, dynein binds tightly to microtubules and the tail swings from the pre-stroke to the post-stroke position, resulting in the power stroke (4). After ADP release, dynein goes back to step (1).

Coupling of the tail swing and microtubule translocation

The FRET studies have established that the tail swings against the head during the ATP hydrolysis cycle. However, it remains to be clarified whether this tail swing is coupled directly with the translocation of microtubules. To address this issue, novel experiments were designed to assess the relationship between the tail swing and the ATPase-dependent translocation of microtubules [22]. In conventional microtubule-sliding assays, the end of the tail is anchored on to a glass surface and microtubule sliding over immobilized dynein was observed (Figure 2a). In order to uncouple the tail motion and the microtubule translocation, the dynein motor domain was anchored on to the glass surface at different points through streptavidin–biotin linkage (Figures 2a and 2b). The biotin tag was inserted genetically at eight locations along the tail and on AAA modules in the head of the dynein motor domain without much effect on its motor activities (Figure 2c). After linking the biotinylated motor domains on a glass surface by biotin–streptavidin linkage, *in vitro* motility assays were carried out. It should be noted that the tail motion did not contribute to microtubule-sliding activity when the anchoring point was within the head (Figure 2d). The observation that dynein moved microtubules at a high speed (1–3 μm/s) only when dynein was anchored near the end of the tail indicated that the robust microtubule-sliding activity requires the tail motion (Figure 2d). All three constructs that had a biotin tag within the head moved microtubules at a low speed ($\sim$0.05 μm/s). This result suggests the presence of a second mode of sliding other than the power stroke of the tail. Although the detailed mechanism is still not clear, this second mode of microtubule sliding may be the result of directional Brownian motion, as observed for both myosins and kinesins [23].

Figure 2 | Gliding assay with genetically engineered dyneins containing site-specific biotin tags

(**a**, **b**) Schematic diagram of the gliding assay. The tail (**a**) or head (**b**) of the dynein construct was anchored on to the glass surface through biotin–streptavidin linkage. (**c**) Design of biotinylated dyneins. In these constructs, a biotin tag was inserted into the tail (HFB380, T1, T2, T3 and T4) or the head (R1, R2 and R7). (**d**) Microtubule-sliding velocities are plotted on a logarithmic scale. The dynein motors were anchored on to the glass surface via the biotin moiety (black bars) or the N-terminal GFP moiety (grey bars). Reproduced from [22] with permission. © 2006 National Academy of Sciences, U.S.A.

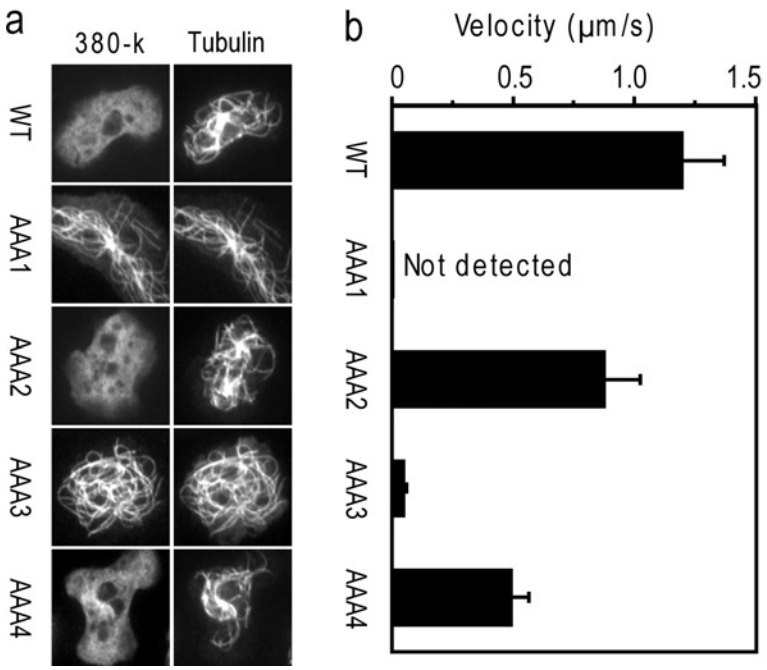


Functions of multiple ATP-binding/hydrolysis sites in the dynein motor domain

Sequence analyses have shown that the dynein heavy chain has multiple ATP-binding/hydrolysis sites in the six tandemly linked AAA+ modules [13–16]. The AAA+ module has a series of conserved motifs that are important for ATP binding/hydrolysis. These include the Walker A (or P loop) motif, which is essential for ATP binding, and the Walker B motif, which is necessary for ATP hydrolysis.

Figure 3 | Functional significance of the four ATP binding/hydrolysis sites in dynein

(**a**) Representative images of cells expressing wild-type or Walker A mutants (AAA1–AAA4 module mutant constructs) of 380 kDa recombinant dynein. (**b**) Sliding velocity of microtubules driven by the wild-type or Walker A mutants (AAA1–AAA4 module mutant constructs) of 380 kDa recombinant dynein. Reproduced with permission from [26]. © 2004 American Chemical Society.



These motifs are present in the N-terminal four AAA+ modules (AAA1–AAA4) and absent from the other two AAA+ modules (AAA5 and AAA6), suggesting that a dynein heavy chain has four ATP-binding/hydrolysis sites. In fact, it was shown that four ATP/ADP nucleotides can bind to an axonemal dynein heavy chain [24], and it has been suggested that at least the AAA1 and AAA3 modules of cytoplasmic dynein have ATPase sites, and ATP hydrolysis cycles in these two sites are tightly coupled [21].

In order to clarify further the functional roles of the four AAA+ modules, *in vivo* mutational analyses of the Walker A motif have been carried out using cytoplasmic dyneins of *Drosophila melanogaster* [25], *S. cerevisiae* [4] and *D. discoideum* [26]. All of these studies showed that the ATP hydrolysis cycles at the AAA1 and AAA3 modules were essential for dynein, as revealed by abnormal subcellular localizations of these Walker A mutants. Figure 3(a) shows the subcellular localization of the wild-type and the four Walker A mutants of the 380 kDa *Dictyostelium* cytoplasmic dynein fragment. The wild-type recombinant dynein fragment did not co-localize with microtubules, and showed a diffuse distribution throughout the cytoplasm. However, when ATP binding to either AAA1 or AAA3 modules was inhibited by Walker A mutation, dynein exhibited clear co-localization with microtubules, suggesting that the mutant dynein fragments were trapped in a state where they bound tightly to microtubules and were unable to function normally. Thus the ATPase cycles at the AAA1 and AAA3 modules are critical for *in vivo* dynein functions.

The motor activities of these mutants evaluated *in vitro* has revealed that the functions of the AAA1 and AAA3 modules were actually not identical. As shown in Figure 3(b), the AAA1 mutant completely lost microtubule-sliding activity. In contrast, the AAA3 mutant could drive microtubule sliding, although the velocity was 20-fold lower than that of wild-type dynein [26]. It was also found that the AAA1 mutant was unable to operate the cross-bridge cycle at all, as judged from microtubule-binding assays and FRET analyses. On the other hand, the AAA3 mutant was able to perform steps 1 and 2 of the ATP hydrolysis cycle (ATP-dependent dissociation from microtubules and post-stroke to pre-stroke transition), but was unable to proceed further. From these results, it seems likely that the ATP hydrolysis cycle at AAA1 is essential, and drives microtubule sliding directly. The ATP binding and hydrolysis at AAA3 plays a critical role in microtubule sliding and participates indirectly in the process through tight coupling with the ATPase cycle at the AAA1 module. Furthermore, since a mutation in AAA2 or AAA4 affected microtubule sliding, these two ATP-binding/hydrolysis sites may also be coupled with the ATP hydrolysis cycle at the AAA1 module. In fact, it has been reported that, in axonemal dyneins, ATP and ADP regulate motor activity [27–29]. ATP/ADP binding to AAA2 and/or AAA4 may play a role in this regulation.

Conclusions and perspectives

Dynein is an AAA+-type molecular motor with sequence similarity to other AAA+ family proteins, even though the architecture of its AAA+ ring, with two long structures (tail and stalk), is very unusual in this family, whereas its mechanical output of force generation (step size, maximum force, processivity etc.) is very similar to that of kinesin, a molecular motor belonging to the G-protein family. The similarity of mechanical output of dynein and kinesin suggests that the underlying mechanism of force generation, and therefore structural parts required for the process, would be very similar between these two distinct motors, even though these two classes of motors have evolved independently. The unique structures in dynein, the stalk and the tail, would be the mechanical parts that may have been acquired during evolution for the power stroke to amplify structural changes in the AAA+ modules, which many other AAA+ machines use for actively pulling polypeptides and polynucleotides into the central hole for unfolding [30,31]. Further molecular dissection of this unique molecular motor may reveal common features which will help to explain how the AAA+ proteins have acquired diverse cellular functions.

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