

Special Issue: 40 Years of TIBS

## Review

## How Dynein Moves Along Microtubules

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**Cytoplasmic dynein, a member of the AAA (ATPases Associated with diverse cellular Activities) family of proteins, drives the processive movement of numerous intracellular cargos towards the minus end of microtubules. Here, we summarize the structural and motile properties of dynein and highlight features that distinguish this motor from kinesin-1 and myosin V, two well-studied transport motors. Integrating information from recent crystal and cryoelectron microscopy structures, as well as high-resolution single-molecule studies, we also discuss models for how dynein biases its movement in one direction along a microtubule track, and present a movie that illustrates these principles.**

## Overview of the Dynein Motor Protein

Eukaryotic cells use motor proteins to transport a variety of cargos, which include membrane-bounded organelles, mRNAs and proteins, along cellular highways of actin filaments and microtubules [1,2]. The molecular motor myosin V moves cargos unidirectionally towards the barbed end of actin filaments. On microtubules, cytoplasmic dynein and kinesins are the two main classes of cargo-transporting motors. Microtubules have an intrinsic polarity, with a plus end and a minus end; in most cells, the plus ends are directed primarily towards the cell cortex, whereas most minus ends localize towards the cell center. Most kinesin family members transport cargoes towards the plus end, or cell cortex, whereas dynein transports cargo towards the minus end, or cell center. Kinesin-1 and myosin V are the best understood cargo-transporting molecular motors; their mechanisms of motility have been reviewed elsewhere [3–5] and are summarized in Box 1. In this review, we focus on how the dynein motor steps along a microtubule. Broadly, dyneins can be divided into two classes: axonemal and cytoplasmic. Axonemal dyneins regulate microtubule sliding in the axonemes of cilia and flagella, whereas cytoplasmic dynein facilitates movement of organelles and other cargos necessary for cellular function.

Cytoplasmic dynein, a homodimer of two identical motor domains and several additional subunits attached to the non-motor tail domain, has numerous cargos and is involved in many cellular functions [1,6,7]. Cytoplasmic dynein 1 transports mRNA–protein complexes, viruses, membranous organelles, and certain proteins toward the cell center, whereas cytoplasmic dynein 2 transports cargo exclusively in the cilium. In metazoan cells, dynein 1 plays a critical role in mitosis, where it creates forces that slide microtubules to help build and maintain a bipolar spindle [8–10]. Dynein 1 also transports mitotic checkpoint proteins from the kinetochore towards the poles, which helps to regulate the timing of the metaphase–anaphase transition [11,12]. During development, dynein 1 localized at the cell cortex positions the spindle during asymmetric cell division and transports critical mRNA complexes involved in developmental patterning [13,14]. The nucleus is also positioned by dynein 1, and errors in this process cause

## Trends

The architecture and nucleotide-dependent conformational changes of the dynein motor domain were recently resolved in several recent structural studies.

Dynein displays conspicuous differences from kinesin and myosin, including the independent stepping behavior of the two motor domains in the homodimer, its much weaker directional bias, and the long separation between the polymer-binding domain and the catalytic body of the enzyme.

The dynein linker domain plays an important role in the mechanics of movement but also regulates specific transitions in the ATPase cycle.

Dynein may use several mechanisms to bias its movement towards the minus end, including conformational changes of its linker domain, Brownian search, and an asymmetric binding mechanism of its microtubule-binding domain.

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### Box 1. Principles of How Kinesin-1 and Myosin V Move Processively along Tracks

Work on kinesin-1 and myosin V have revealed general principles of how these homodimeric motors move processively along tracks; schematics depicting the kinesin-1 (Figure 1A) and myosin V (Figure 1B) cycles are shown. In both cases, the two motor domains move in a hand-over-hand fashion and one ATP is consumed per step [49,60]. Energy from the ATP hydrolysis cycle is transferred into structural changes in the motor that are needed for the following two critical events in the motility cycle.

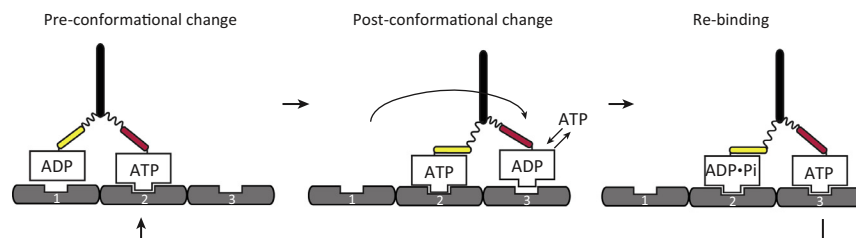
#### 1. Changing the Strength of Binding between the Motor and Its Track

Just as G proteins use a GTPase cycle to change their affinity for effector proteins, motor proteins use ATP energy to change their strength of binding to their polymeric track. A strong binding state allows the motor to resist an opposing load. A weak binding state is needed to release the motor so that it can be displaced (see below) and then rebound to a forward binding site on the track and lock a 'step' in place. Breaking a strong interaction between the motor and its track requires energy from the nucleotide hydrolysis cycle. For kinesin-1, the weak binding state occurs with ADP binding, whereas for myosin V, the weak binding state is ATP bound. The binding energy from the motor-polymer interaction also can weaken the binding of ADP in the active site, thus advancing the chemical cycle of the motor by allowing ATP/ADP exchange.

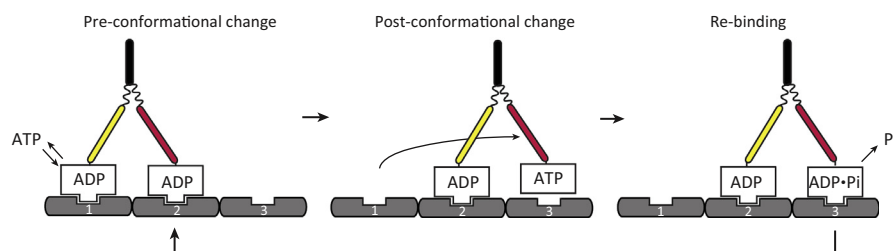
#### 2. Biasing the Stepping of the Motor in One Direction along the Polymer

If the motor dissociates and rebinds to the same position on the track, then no net motion will occur. For kinesin-1 and myosin V, at least two mechanisms favor the rebinding of the dissociated motor domain to a forward binding site and help to coordinate the alternating actions of the two motor domains. First, a structural change in a mechanical element (two conformational states shown in red and yellow) in the polymer-bound motor displaces the detached partner motor domain and biases its Brownian search for a forward binding site [61,62]. In myosin V, this conformational change is the swing of its long lever arm, whereas in kinesin-1, the docking of the neck linker to the catalytic core serves a similar role. A second mechanism for promoting directionality is through an asymmetry in ADP release kinetics of the two motor domains, which is regulated by the weak-to-strong binding transition of the motor to the polymer. In the case of kinesin, the transition to the strong binding state is disfavored if the detached motor domain attempts to bind at the rearward binding site [63,64]. This asymmetry is likely mediated by the different neck linker position in the front and rear head. In the case of myosin V, the fully strongly bound actin state and concomitant ADP release are repressed in the front head of the myosin V motor domain; once the rear head releases, then the lever arm swing and ADP release can occur in the front head [5,60,65].

#### (A) Kinesin



#### (B) Myosin



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**Figure 1. Principles of How Kinesin (A) and Myosin (B) Move Along Their Tracks, Microtubules, and Actin, respectively.** Nucleotide-dependent conformational changes are coordinated between the two heads to generate alternating, hand-over-hand stepping along the tracks. See details in Box 1.

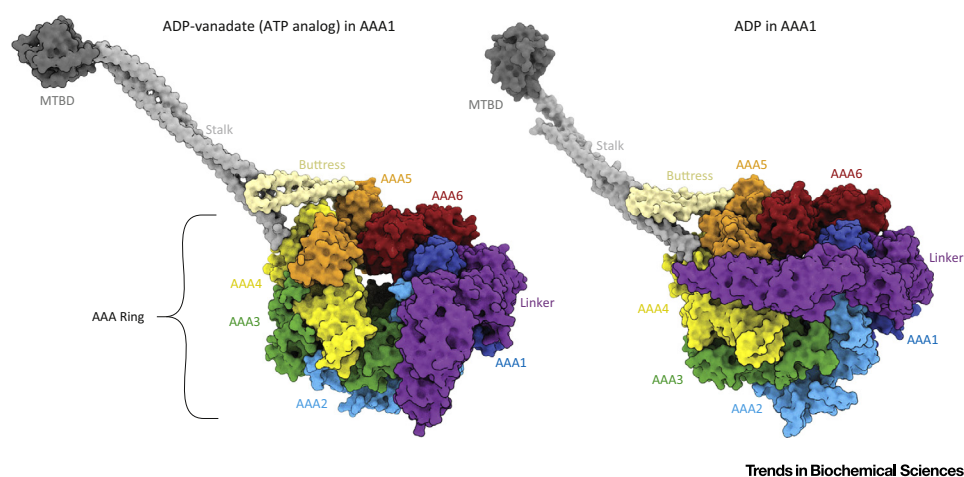
defects in brain development [15–17]. Many of the activities described above are carried out by dynein 1 working in conjunction with dynactin, an  $\sim 1.2$ -MDa multisubunit protein complex, and adaptor proteins that link dynein–dynactin to specific cargos [18]. In fact, mammalian cytoplasmic dynein 1 appears to exhibit poor motility in the absence of dynactin and an adapter [19,20]. In contrast, budding yeast *Saccharomyces cerevisiae* dynein exhibits robust processive motility in the absence of dynactin [21]. In *S. cerevisiae*, dynein only appears to serve a nonessential function of pulling on microtubules from the cell cortex in order to position the mitotic spindle at the juncture of the mother–daughter bud neck; perhaps because of its limited functions, the regulation of yeast dynein appears to be less complex. Thus, yeast dynein has served as a good model system for studying the motility properties of dynein *in vitro*.

In the past few years, our understanding of dynein has advanced considerably; several X-ray and cryoelectron microscopy (cryo-EM) structures of various dyneins from different species have been reported, and the single molecule motility properties of *S. cerevisiae* dynein have been studied in exquisite detail. In this article, we review this recent progress and highlight similarities and differences between dynein and the other well-studied cargo transport motors kinesin-1 and myosin V. We integrate structural data on cytoplasmic and axonemal dyneins from different species and single-molecule studies on yeast dynein into a model for the biased stepping of dynein towards the minus end of the microtubule. This review does not cover the regulation of dynein motility by associated factors (e.g., Lis1 and dynactin), for which considerable recent progress has also been made [19,22,23].

## The Dynein Motor Domain and Its Conformational Changes

### Architecture of the Dynein Motor Domain

The first crystal structures of the dynein motor domain were obtained in 2011 [24,25], shedding light on the architecture of the motor domain; subsequent crystal structures revealed details at higher resolution and in different nucleotide states [26–29]. The cytoplasmic dynein heavy chain is a homodimer comprising two identical motor domains. Each motor domain contains a hexameric AAA ring, with three appendages that protrude from the ring: the stalk, buttress (in some studies also called the strut), and linker (Figure 1). The building blocks for each AAA



**Figure 1. Architecture of the Dynein Motor Domain and the Linker Conformational Change.** Two crystal structures are shown: (left panel) human cytoplasmic dynein 2 with ADP-vanadate, which is thought to mimic ATP or ADP-Pi in AAA1 (PDB code: 4RH7) and (right panel) *Dictyostelium discoideum* cytoplasmic dynein with ADP bound in AAA1 (PDB code: 3VKH). AAA1 is the main hydrolytic site that drives the motility cycle. Transitions of the linker between bent and straight conformations may be involved in driving dynein motility (see Figure 2). This figure was generated using the software ePMV (Embedded Python Molecular Viewer) [66].

domain in the AAA ring are a large  $\alpha\beta$  domain (AAAL) and a small  $\alpha$ -helical domain (AAAs). Together, AAAL and AAAs form one AAA domain. Most AAA proteins are homo-oligomeric rings, comprising multiple copies of an identical protomer [30]. Dynein is unusual in that its six AAA domains are synthesized as a single polypeptide, which folds to form an asymmetric AAA ring. Of the six AAA domains in dynein, AAA1–4 bind ATP [26,27]; domains AAA1, AAA3, and AAA4 also are capable of hydrolyzing ATP. Hydrolysis at AAA1 is critical for motility, and most of this review focuses on how AAA1 drives dynein motility. Hydrolysis at AAA3, and perhaps AAA4, plays a regulatory role, which will be briefly discussed.

In addition to the six AAA domains, dynein contains three appendages that protrude from the ring (Figure 1). The stalk and buttress are two coiled coils that extend from the small domains of AAA4 and AAA5, respectively, and interact with one another. The stalk is a long ( $\sim 140$  Å) coiled coil that contains a small,  $\sim 10$ -kDa microtubule binding domain (MTBD) at its distal tip. The buttress is a smaller coiled coil that interacts with the stalk approximately one-third of the way along the stalk from the ring. The stalk coiled coil also provides a pathway of communication between the AAA ring and the MTBD. The two helices of the coiled coil can interact with one another in two possible ways, which results in two different registrations of interacting amino acids. Experiments by Gibbons *et al.* [31] first suggested that the  $\alpha$  registry is associated with a strong binding state of the MTBD and a four-residue shifted  $\beta^+$  registry is associated with a weak binding state. Several subsequent experiments have provided additional support for this hypothesis [29,32–35]. The sliding of one of the helices of the stalk between the  $\alpha$  and  $\beta$  registries is thought to provide a mechanism for communicating between the AAA ring and the MTBD.

The third appendage of the ring is a predominantly helical linker domain that arches over one face of the ring. The N terminus of the linker domain extends into the cargo-binding tail region, while its C-terminal region continues into AAA1, the main ATPase site of the dynein ring. The linker is hypothesized to serve as a mechanical element. This hypothesis originally arose from 2D negative stain EM data of axonemal dynein [36] and later EM studies of cytoplasmic dynein [37]. In the ADP-vanadate trapped state, the linker is bent, and its N terminus is positioned at AAA2/3; in the ADP state, the linker extends straight across the ring towards AAA4/5 (Figure 1). The bent-to-straight ‘linker swing’ is proposed to act as a power stroke, although its roles might be more complex, as discussed later.

#### Nucleotide-Induced Conformational Changes

The conformational changes in different nucleotide states are described in detail in a recent review that compares crystal structures of the dynein motor domain bound to ADP, AMPPNP, ADP-vanadate or no nucleotide (‘apo’, in which all nucleotide-binding sites are empty except AAA2, which constitutively binds nucleotide) [29]. In brief, structural studies suggest that ATP binding at AAA1 triggers a series of conformational changes that propagate around the entire ring and reposition all of the AAA domains into a closed ring conformation (Figure 1). Additionally, the linker detaches from its docking site at AAA5, where it resides in the apo state. In the ATP-bound state, the linker can adopt a bent conformation, in which it contacts AAA3/2 (referred to in the dynein literature as the pre-power stroke state) [38]. In addition, it can explore various other conformations [28]. One important consequence of the AAA ring adopting a closed conformation is that AAA4 and AAA5 move relative to one another. This movement results in the buttress pulling on one of the helices of the stalk to which it is attached, thus shifting the coiled coil to its  $\beta^+$  registry and inducing a weak binding state of the MTBD [29]. ATP hydrolysis and/or release of phosphate at AAA1 then allows the MTBD to return to a strong binding state ( $\alpha$  registry of the stalk), the linker to return to its straight conformation (post-power stroke state), and the AAA ring to adopt a more open conformation.

### Allosteric Roles for AAA3 and the Linker

The nucleotide state of AAA3 plays a regulatory role in modulating the allosteric changes in the motor domain. When ADP is bound in AAA3, large conformational changes in the entire ring (AAA1–6) and linker occur, as described above. However, when ATP (mimicked by AMPPNP) is bound in AAA3, only conformational changes in AAA1–4 are observed. In this case, the linker is straight and the MTBD remains locked in a tight binding state [28,39,40]. Consistent with the idea that the linker bending occurs when AAA3 is bound to ADP, a crystal structure of human dynein 2 in the presence of ADP-vanadate captures a bent conformation of the linker and reveals electron density for ADP-vanadate for AAA1, but only ADP in AAA3 [29]. Single-molecule studies agree with this structural data, showing that ATP binding at AAA3 results in strong microtubule binding, whereas ADP binding at AAA3 weakens the binding to microtubules [39]. However, when force is applied at the N terminus of a linker, under high load, the binding and release from microtubules is independent of the nucleotide bound at AAA3 [40]. A more detailed understanding of how this AAA3-based regulatory mechanism is used to control dynein motility under low and high load in a cell remains to be elucidated.

The linker conformational change from straight to bent is likely key for promoting ATP hydrolysis in AAA1. In its straight conformation, the linker sterically inhibits the catalytic arginine finger in AAA2 from reaching its optimal position to promote hydrolysis of ATP in AAA1 [27,28]. Linker bending removes this steric block to AAA1–AAA2 closure [29], allowing positioning of the arginine finger optimally for catalysis, and thus may facilitate ATP hydrolysis [28]. Conversely, docking of the N-terminal end of the straight linker onto AAA5, as seen in the apo state, may facilitate the opening of AAA1–AAA2 and the release of ADP to complete the ATPase cycle (Figure 1) [26]. Thus, the nucleotide-dependent allosteric changes of both the linker and AAA3 are important in regulating the chemomechanical cycle of dynein. The linker conformational change facilitates ATP hydrolysis at AAA1, the main site of hydrolysis, and AAA3 gates conformational changes around the ring and thus impacts binding of dynein to microtubules.

### Comparison of Dynein with Kinesin and Myosin

In order to understand the mechanism of dynein, it is useful to compare and contrast dynein with the two best-studied processive motors: kinesin-1 and myosin V. Although they operate on different tracks (microtubules for kinesin-1 and actin for myosin V), kinesin-1 and myosin V use a similar mechanism for converting ATP energy into the hand-over-hand movement of their two identical motor domains along a filament (Box 1). First, while bound to the polymer, both motors use a large conformational change of a mechanical element (lever arm for myosin V and neck linker for kinesin-1) to displace a detached, partner motor domain, thereby biasing its reattachment to a forward binding site after a Brownian search. Second, as a result of having different orientations in the mechanical elements in the two-head-bound intermediate, the transition to the strong-binding state is favored in only one of the two motor domains (Box 1). This asymmetry helps to coordinate the chemical cycles of the two motor domains and helps to ensure that the motor domain, once detached, steps forward rather than revisits its last binding site. As discussed later, dynein may exploit similar principles in how it moves towards the microtubule minus end. However, as discussed next, dynein also differs from kinesin-1 and myosin V in several important ways, and these differences provide clues to its mechanism. We focus our discussion on results from yeast dynein, which is the most extensively studied dynein motor to date and where there appears to be an emerging consensus with regard to its single-molecule behavior. We note that mammalian dynein has not been studied in as much detail as yeast dynein, and may exhibit a different stepping behavior [41–43].

### The Two Motor Domains of Dynein Operate Largely Independently during Motility

Single-molecule fluorescence imaging of the individual motor domains of kinesin-1 and myosin V reveals an invariant hand-over-hand movement in which the rear head always passes the front

head (Box 1). By contrast, imaging studies of the two dynein heads reveal that they step largely independently of one another, which gives rise to diverse types of steps [44,45]. Like kinesin-1 and myosin V, the rear head of dynein can step past the front head, but these hand-over-hand steps account for <20% of the total steps [45]. In addition, dynein displays an inch-worm type of motion, which is never observed for kinesin-1 or myosin V. Here, the front head can detach and then step forward, while the rear head remains fixed in position. The rear head also can take a step without necessarily passing the front head. Furthermore, the order of stepping of the two dynein heads appears to be largely stochastic; in some cases, one motor domain can step two or three times in a row before its partner head steps. This stepping pattern is very different from kinesin-1 and myosin V, which display a strict alternating pattern in which one motor domain steps and then the other. An exception to this stochastic stepping occurs when the two dynein motor domains become widely separated on the microtubule lattice. In this scenario, the probability of forward head stepping decreases, and either the rear head steps forward or the front head steps backward, both of which reduce the tension between the two motor domains.

Experiments by the Yildiz Laboratory with recombinant dynein heterodimers further underscore the conclusion that an individual dynein motor domain acts as an autonomous stepper. In these experiments, a wild-type dynein motor domain was heterodimerized either with a 'dead' motor domain bearing a crippling mutation in the AAA1 active site [44] or with only a 'crutch' consisting of the dynein coiled-coil stalk and MTBD without the ring [46]. Remarkably, both types of active-inactive heterodimeric motors step at close to wild-type velocities along the microtubule. The active motor is usually (~75%) in the lead, and its movement forces its dysfunctional partner also to step along the microtubule. This finding contrasts results obtained from a comparable kinesin wild-type plus hydrolysis-dead heterodimer, which moves 10–20-fold slower than native homodimeric kinesin [47]. These experiments indicate that one dynein head can step along the microtubule effectively, provided that it has a tether (a partner motor domain or even a microtubule-binding crutch) that prevents it from diffusing away from the microtubule surface when it is searching for a new tubulin-binding site after releasing from the microtubule. Thus, the walking mechanism of dynein does not require communication between two actively cycling ATPase motor domains, as is true of kinesin-1 and myosin V.

#### Dynein Exhibits a Weak Directional Bias and Variable Step Sizes

Kinesin-1, myosin V, and yeast cytoplasmic dynein display comparable processivity, moving at ~1–2 microns per encounter with the track. During such an excursion under zero load, kinesin-1 and myosin V rarely take backwards steps. By contrast, dynein frequently (20% of the time) takes backward steps [21,44,45]. This finding indicates that the mechanisms that dynein uses to produce directional bias are less efficient than those of kinesin-1 or myosin V.

Single-molecule data show that the step sizes of yeast dynein (measured by marking the position of the motor domain ring and not the MTBD) are highly variable: 8–32 nm in both the forward and backward directions, with an average of 10–12 nm [21,44,45,48]. Sideways stepping to adjacent protofilaments is also common. These results suggest that the detached dynein motor domain can access many possible tubulin-binding sites on the lattice. By contrast, kinesin only takes 8.2-nm steps [49] (the size of the tubulin dimer), and the step sizes of myosin V are close to 36 nm [50], which is the helical pitch of the actin filament.

#### The Main Catalytic Site of Dynein Is Spatially Distant from the Microtubule-Binding Domain

In kinesin and myosin, the polymer-binding and catalytic sites are integrated within the same domain. Thus, during a step, the polymer-binding and catalytic sites advance by the same distance. By contrast, the microtubule-binding and catalytic AAA ring of dynein are separated by a long (~140 Å) coiled coil. The main role of the large AAA ring is to displace the small (~15 kDa) MTBD along the microtubule towards the minus end. The MTBD of dynein must move by a



unitary distance corresponding to the spacing of tubulin-binding sites; however, because the stalk is flexible and can potentially bend or alter its angle with respect to the ring, the ring itself may not undergo the same net displacement as the MTBD. The length of the stalk coiled coil is highly conserved throughout the dynein superfamily. Thus, the separation between the microtubule and catalytic sites is likely to be an important feature in the dynein mechanism.

### How Dynein Steps towards the Microtubule Minus End

How is dynein stepping biased towards the minus end of a microtubule? The answer to this central question is not entirely clear, but here we synthesize data and models from the literature, and discuss how several mechanisms might synergize with one another to promote the weak directional preference of dynein movement along the microtubule.

#### How Does the Leading Head Step Towards The Minus End?

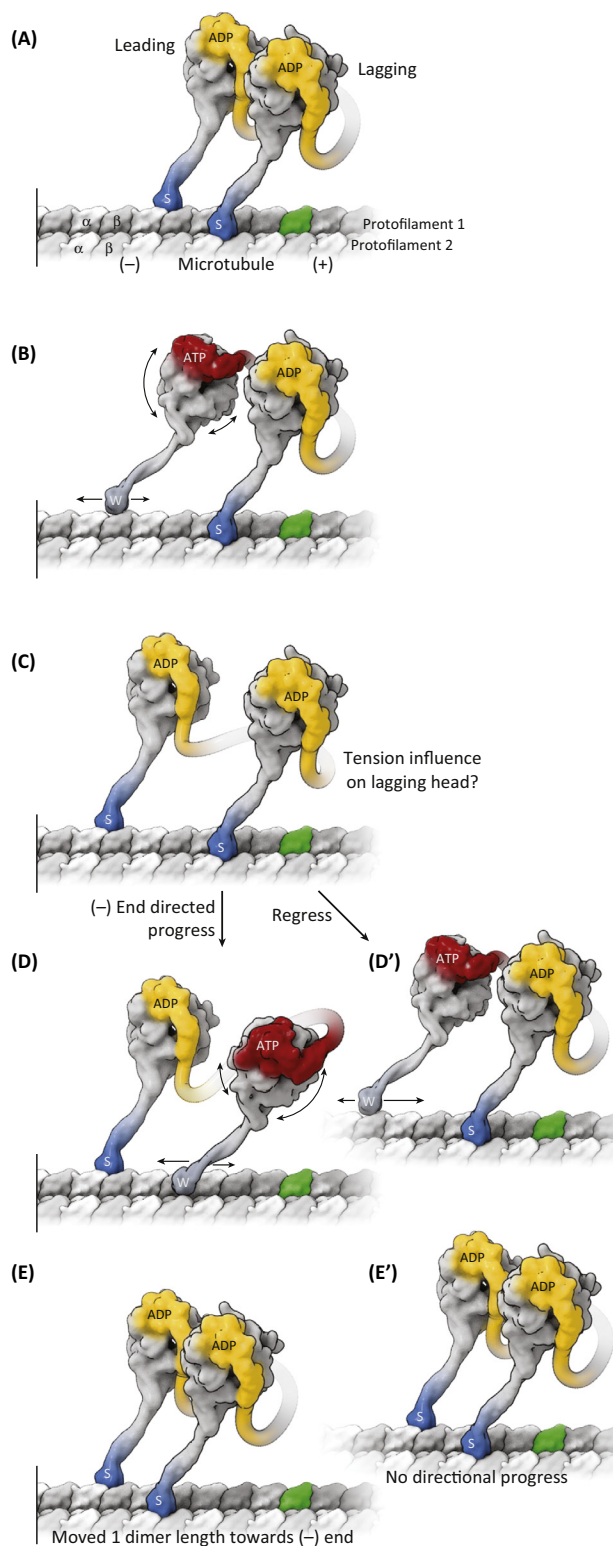
Figure 2 shows a model in which the leading motor domain of dynein takes a step forward to a new tubulin-binding site, while the lagging head remains bound in place. As described earlier, neither kinesin-1 nor myosin V can execute this type of step. Two potential mechanisms might enable the front motor domain of dynein to step without any active assistance from the lagging head. It is also important to keep in mind that these biasing mechanisms are relatively weak, because the leading head frequently takes backward as well as forward steps [44,45] (Figure 2).

#### Repositioning of the Stalk/MTBD by Rotation of the AAA Ring

Several studies [25–27,38,36] have shown that the N terminus of the linker is positioned primarily over AAA4/AAA5 in ADP/apo states and over AAA2/3 in ATP/ADP-Pi states of AAA1 (note: other positions of the linker between AAA2–4 are also observed with ATP or ATP analogs [28,38]). How does this conformational change impact movement on the microtubule? The answer will likely depend upon whether dynein is strongly bound or detached from the microtubule. If strongly bound, then the ring will be held in place by the docked MTBD/stalk and the linker will swing relative to the microtubule, analogous to the power stroke of myosin (Box 1). However, if detached from the microtubule in the ATP state, then the ring can potentially rotate relative to the microtubule to allow AAA2/3 to make contact with the N-terminal domains of the linker [37] (Figure 2). Indeed, ring rotation has been recently observed in EM reconstructions for *in situ* axonemal dynein in an ATP-like state, while the linker was more or less stationary perhaps due to its connections with the tail domain or with the partner head [51]. Importantly, the angular change of the stalk associated with ring rotation shifts the position of the MTBD further towards the microtubule minus end, and thereby biases its rebinding to a new tubulin site towards the minus end (Figure 2). Because of the 140-Å length of the stalk, a small rotation of the ring can lead to a larger (>8 nm) displacement of the MTBD. This type of biasing mechanism could explain how a dynein motor domain can step without a functional partner motor domain [46].

#### Brownian Motion and Rebinding of the MTBD

To further bias the directionality of dynein, the ring rotation described above may be augmented by an inherent asymmetry in microtubule rebinding during a Brownian search. A mechanism of a random diffusional search combined with the asymmetric binding of a motor to a polymer was first suggested by Huxley in 1957 as a model for muscle contraction (Figure 3). Similar to this model, dynein has been shown to undergo 1D diffusion along the microtubule surface in its weakly bound (ATP) state. This result suggests that the MTBD can hold on to the microtubule weakly and that thermal energy can displace the MTBD from one tubulin subunit to the next along the microtubule [35,52,53]. Furthermore, optical trap studies have uncovered a directional asymmetry in dynein binding to microtubules. Specifically, dynein holds on more tightly to the microtubule when pulled backward compared to when it is pulled forward [40,46,54]. The angle between the MTBD and the stalk, where there appears to be a flexible joint [55,56], may be involved in governing this binding asymmetry. Based upon these results and others [32], we

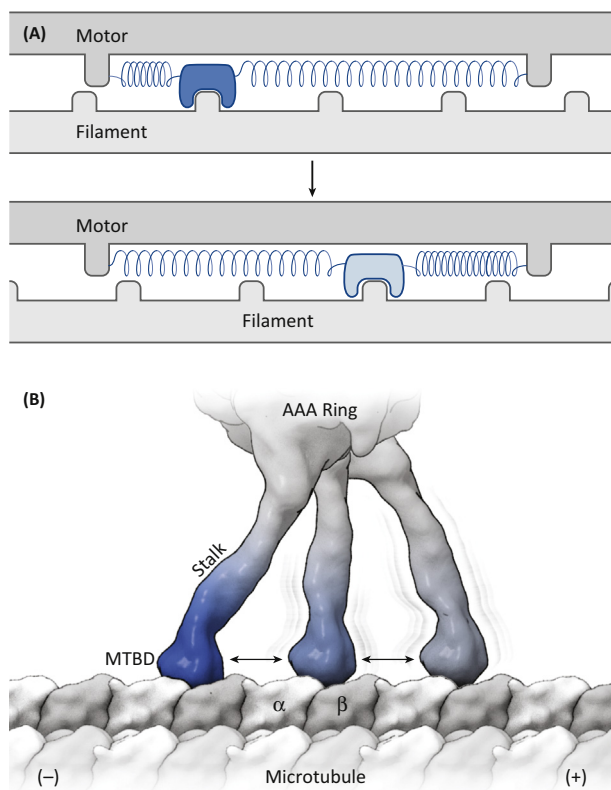


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**Figure 2. A Model for Dynein Stepping along a Microtubule.** See also

Movie S1 in the supplemental information online. (A) The sequence starts with a cytoplasmic dynein dimer with ADP bound in both motor domains (yellow) and both MTBDs tightly bound to the microtubule in the strong-binding state (indicated by 's' and darker blue color). One tubulin dimer in the microtubule is highlighted in green to allow easier tracking of dynein's relative progression towards the minus end. (B) When ADP is exchanged for ATP in the leading head (red), conformational changes occur in the ring, which are communicated to the MTBD via a change in registry of the coiled-coil stalk, thus releasing the MTBD from the microtubule. This sequence triggers the repositioning of the N terminus of the linker over AAA2/3 and a resultant rotation of the ring/MTBD and a slight forward re-positioning of the MTBD. The weakly bound motor domain (indicated by 'w' and lighter blue color) is also subject to Brownian motion, which results in a 2D search of the MTBD on top of the microtubule lattice. The weak-to-strong binding transition is favored when the stalk is angled backwards, which tends to lock a forward step in place when the front head MTBD happens upon an accessible MT-binding site (see Figure 3). This asymmetry in the binding properties of the MTBD provides an additional mechanism that favors a forward versus a backward step. (C) This strong rebinding favors the movement of the linker from AAA2/3 to AAA4/5 and ATP hydrolysis/Pi release. This minus-end directed shift of the N terminus of the linker reduces the slack in the connection between the motor domains and applies tension to the lagging motor domain (or at least constrains motion to favor positions towards the leading head). At this point, two potential subsequent steps are shown (D, E and D', E'). (D, E) The rear head now detaches after ADP/ATP exchange and undergoes the same sequence of events described for the front head, again resulting in a step forward towards the front head and relieving the tension between the two motor domains. (D', E') The leading motor domain undergoes ADP/ATP exchange and detaches, but tension between the motor domains overrides the forward biasing mechanisms and the leading MTBD steps backward. In this instance, the homodimer does not undergo a net displacement from A to E'. Abbreviation: MTBD, microtubule-binding domain. Movie S1 shows several other stepping possibilities.





**Figure 3. Asymmetry in Polymer Binding Can Create a Directional Bias.** (A) A depiction of a model by Andrew Huxley for muscle contraction [67]. In this model, which was developed before the myosin structure was known, the motor contains a small actin-binding element linked to a core by a spring-like element, which moves back-and-forth by thermal motion relative to the actin filament. The binding of this fluctuating element to the actin filament increases when displaced by thermal fluctuations in one direction (to the left) (top panel, dark blue reveals a state with a high association rate); once bound, the restoring force of the spring slides the filament from left to right. If stretched in the opposite direction, the motor tends to dissociate (bottom panel, light blue) when stretched in the opposite direction. (B) A model for the binding of the MTBD to the microtubule. The stalk/MTBD can explore different binding sites by Brownian motion; the weak-to-strong transition of the MTBD (dark blue) is enhanced when displaced towards the minus end (left in this figure) with the stalk tilting backward but less likely when interacting with other binding sites (lighter blue, to the right).

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speculate that the detached MTBD, exploring space by thermal motion, will more likely undergo a weak-to-strong binding transition if it reaches a forward tubulin subunit and has a preferred geometry of its stalk pointing backward (Figure 3).

#### How Does the Lagging Head Step towards the Minus End?

Each dynein motor domain can step in an independent and autonomous manner. Thus, the lagging motor domain can detach and bias its forward motion using the same mechanism described for the leading motor domain. However, the forward movement of the lagging motor domain might also be aided through tension created by the leading head. How is this tension generated? If the leading head succeeds in taking several successive forward steps, then the inter-head distance increases and the connection between the two heads becomes increasingly taut. This tension appears to increase the probability of the lagging head taking a forward step [44,48]. In addition, the bent-to-straight conformational change of the linker in the strongly bound leading head also would be expected to increase tension and thus potentially pull the lagging motor domain towards the microtubule minus end [6] (Figure 2). However, the degree to which the lagging head ‘feels’ the tension from the linker conformational change and shifts its position towards the minus end depends upon the compliance of the elements holding the two heads together, which is still poorly understood for native dynein [23,57].

#### Dynein Stepping: an Animation

To illustrate how the structural properties of dynein and conformational changes described above might lead to the complex stepping behavior of yeast dynein, we have generated a 3D animated model based upon the crystal structures of dynein in different nucleotide states and its

single-molecule stepping behavior (Movie S1). Specifically, this animation highlights the following features of dynein stepping, most of which have been described earlier in this review: (i) The inch-worm step (the leading head takes a step followed by the lagging head; (ii) an occasional hand-over-hand step (20% of the time according to Qiu *et al.* [45]); (iii) both alternating and consecutive steps of the two dynein motor domains; (iv) backwards steps (~20% of the steps for yeast dynein; (v) different-sized steps (8–32 nm for the AAA ring); (vi) switching of a motor domain to an adjacent protofilament track [21]; and (vii) an inherent asymmetry in positioning of the motor domains, such that the leading motor domain is most often positioned to the right of the lagging motor domain when viewed down the axis of the microtubule towards the minus end, as oriented in Figure 2 [44,45]. The animation also illustrates conformational changes in the linker, Brownian motion of the entire motor protein, and how such dynamics might enable the motor to step along the microtubule.

In generating this animation, gaps in information or understanding also become more apparent; indeed, many aspects of this animated model are speculative. First, the structure and compliance of the element connecting the two motor domains is not known, and its modeled role in the animation is somewhat arbitrary. These details are important for understanding how the two motor domains might communicate with one another through tension. Interestingly, and unlike kinesin-1, the dynein dimer is able to move processively with diverse structural elements connecting the two motor domains, including a short connection to an antiparallel GST (glutathione S-transferase) dimer [21,24], DNA-based dimerization [58], or the longer flexible linker that exists in native dynein [57]. We currently do not understand how dynein can accommodate such diverse connections between its two motor domains and the role of this region in the native dynein molecule. Second, defining hinge points that allow relative motions of dynein subdomains is important in generating the animation; these frames of reference and relative motions impact the degree of ring rotation (leading head stepping), how the MTBD can undergo a Brownian search along the microtubule lattice, and how one motor domain can pass its partner. While some information is known, many of these details are hypothetical at this time. Third, the relative contributions of linker conformational changes, asymmetric binding of the MTBD, and perhaps other biasing mechanisms are not well defined at present. Fourth, while we have ‘snapshots’ of dynein in different nucleotide states, the temporal sequence of conformational changes within this large motor is not known. For example, the exact order of events of ATP binding to the motor, microtubule release, and the linker bending remains speculative. Fifth, the conformational states of the AAA ring are derived from crystal structures, and we do not know how microtubule binding might impact these conformational states. Sixth, current information on stepping is based upon high-resolution tracking data from a fluorescent dye in the AAA ring, not in the MTBD. As discussed earlier, the positions of the ring and the MTBD may differ substantially due to the flexibility of the stalk and rotations of the ring.

### Concluding Remarks

Recent crystal and EM structures have focused on the dynein motor domain and have illuminated how transitions in the nucleotide state of AAA1 and AAA3 in the asymmetric, hexameric AAA ring trigger allosteric changes over long distances in the motor domain (reviewed in [59]). Single-molecule and mutagenesis studies also have provided details on the stepping behavior of yeast dynein and how it differs from kinesin-1 and myosin V. Despite this recent deluge of information (including new information on the regulation of dynein, reviewed in [68]), the structural basis of unidirectional motion of dynein remains incompletely understood. Thus, the challenge remains to develop more direct evidence for or against the models discussed here. Part of this challenge will be in understanding the dynamics of the dynein molecule. We have snapshots of dynein from X-ray crystallography, but dynein is undoubtedly a dynamic protein machine that explores many conformations. To help understand such dynamics, conformation-sensitive probes will be needed to observe changes in the structure of dynein in real time and

### Outstanding Questions

How does dynein move toward the microtubule minus end? We discuss several ideas in this review, but are they correct? What are the relative contributions of the linker versus the MTBD in directional motion?

How are the two dynein motor domains connected to one another in a homodimer? Can a linker conformation in one dynein motor domain increase tension and bias the position of the partner motor domain?

Why does dynein use a long coiled coil to separate the polymer-binding domain from the ATPase ring? Why is its length so highly conserved?

Does dynein use dynactin or other associated proteins to improve the coupling between the two motor domains or change the efficiency of forward stepping?

ascertain how these changes relate to specific steps that dynein takes on a microtubule. In addition, new advances in cryo-EM allow identification of different conformational states even within a single nucleotide-bound chemical state; such conformational heterogeneity will provide information on the dynamics of the motor domain. It also will be important to obtain higher-resolution structures of the entire motor domain when it is in microtubule-bound states, particularly in the dynein dimer. Finally, future single-molecule studies of mammalian dynein–dynactin and axonemal dynein will provide a broader understanding of dynein stepping and extend the current extensive work on yeast dynein. Collectively, such experimental information will help to provide a clearer understanding of the dynein mechanism.

### Acknowledgments

We are grateful to members of the Vale laboratory for their comments on this review, and to our referees for helpful suggestions. Our work on dynein is funded by the NIH (R01GM09731 to R.D.V. and K99GM112982 to G.B.), a QB3 Fellowship grant from the California Institute for Quantitative Biosciences, qb3@UCSF, to G.T.J. and a UCSF School of Pharmacy, 2013 Mary Anne Koda-Kimble Seed Award for Innovation to G.T.J.

### Supplemental Information

Supplemental information associated with this article can be found, in the online version, at [doi:10.1016/j.tibs.2015.11.004](https://doi.org/10.1016/j.tibs.2015.11.004).

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