Directionality and processivity of molecular motors

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Analysis of a mutant with altered directionality has led to new insights into motor directionality. The prediction from current models for processivity of a two-heads-bound state has been confirmed by electron microscopy for myosin V and by unbinding experiments for kinesin. Evidence is emerging that non-processive motors bind their filament with one head, hydrolyze ATP and then release, requiring binding by a second motor to complete a step.

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Current Opinion in Cell Biology 2002, 14:50–57

0955-0674/02/$ – see front matter

Introduction

Molecular motors are protein machines that use the energy from ATP hydrolysis to perform work [1••]. Motor proteins move directionally along a cytoskeletal filament, either actin filaments or microtubules, to transport vesicles, organelles or possibly even chromosomes, or to slide actin filaments or microtubules past one another. The three known classes of cytoskeletal motors, the actin-based myosins and microtubule-based kinesins and dyneins, each consists of an extended family of related proteins that function in muscle contraction, organelle transport, spindle and chromosome motility in dividing cells, and cell motility. Mutations in the cytoskeletal motors can cause profound cellular defects, including paralysis, aneuploidy and developmental defects, for example, a lack of bilateral asymmetry needed to form organs such as the heart [2,3].

Motor directionality

A property intrinsic to molecular motors is their ability to move unidirectionally along a cytoskeletal filament. To understand the molecular basis of motor directionality, it is important to determine the structural domains that are essential for directionality. Insights have come from the discovery of 'backwards' myosin and kinesin motors that move with the opposite directionality to the other members of their families, towards the slow-growing or minus ends of actin filaments or microtubules. Comparisons of the structures of these motors and the effects of mutations on directionality will give clues as to the mechanism of directionality.

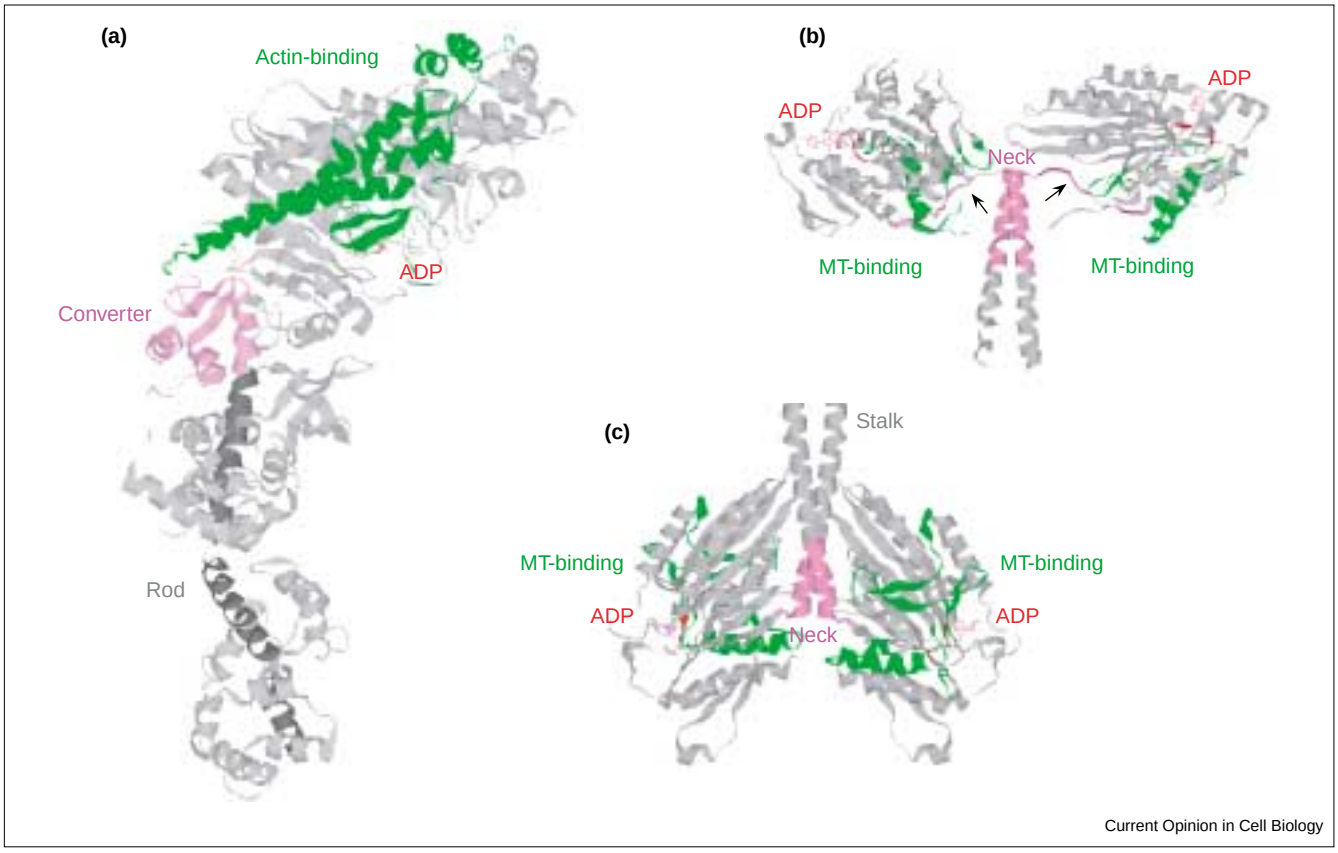
The minus-end kinesin motors, such as Ncd, are distinctive in that they have a reversed domain organization compared with the plus-end kinesins; the conserved motor domain is at the carboxyl terminus of the coiled-coil stalk instead of the amino terminus. In contrast, the minus-end myosin VI motors have an N-terminal motor domain like the plus-end myosins (Figure 1a). The myosin VI motors differ from other myosins, however, in that they have a 53-residue insertion in the 'converter' at the base of the rod-like lever arm [4] (Figure 1a). Remarkably, the myosin VI lever arm appears to rotate in the opposite direction to smooth muscle myosin II, a plus-end motor, when ADP is released from the motor or 'head', as analyzed by cryoelectron microscopy [4]. This has been interpreted to mean that movement of myosin VI towards actin minus ends is due to a molecular cog in the converter region that reverses the direction of movement of the lever arm. The converter domain could thus modulate interactions between the motor and the lever arm to determine motor directionality.

Questions have been raised regarding this proposed mechanism of directionality by a recent report that directionality of myosin VI is determined by the motor core and that the insertion in the converter is not necessary for minus-end directionality [5]. Further studies are needed to determine the structural elements of the myosin motor domain involved in directionality determination and the interactions of these elements with the converter and lever arm, which are essential for myosin motility.

The region of the kinesin motors implicated in motor directionality is structurally analogous to the myosin converter, in that it lies at the base of the coiled-coil stalk, adjacent to the conserved catalytic core (Figure 1b,c). This region in plus-end conventional kinesin, the neck, consists of the proximal end of the coiled-coil stalk, a region of less stable coiled coil, together with 15–18 residues that join the stalk to the motor core, the so-called 'neck linker' (Figure 1b). The neck linker is found in two different conformations in crystal structures, either docked against the motor core, forming β strands that interact with the β sheet of the catalytic core, or not visible in the model, presumably in a mobile or disordered random coil.

Mutating the neck linker by randomizing the residues results in an extremely slow plus-end motor with ~460-fold reduced velocity compared to the wild type [6]. Although the mutations dramatically reduce motor velocity, the maximum microtubule-activated ATPase rate per head is reduced by only ~2.6-fold and the K_m (MT) by ~6.6-fold compared to wild type, much less than the reduction in velocity. Thus, the neck linker may be needed for high-efficiency transfer of the energy of ATP hydrolysis into plus-end-directed movement. One possibility is that the neck linker amplifies the conformational changes of the catalytic core that are induced by ATP hydrolysis.

Figure 1

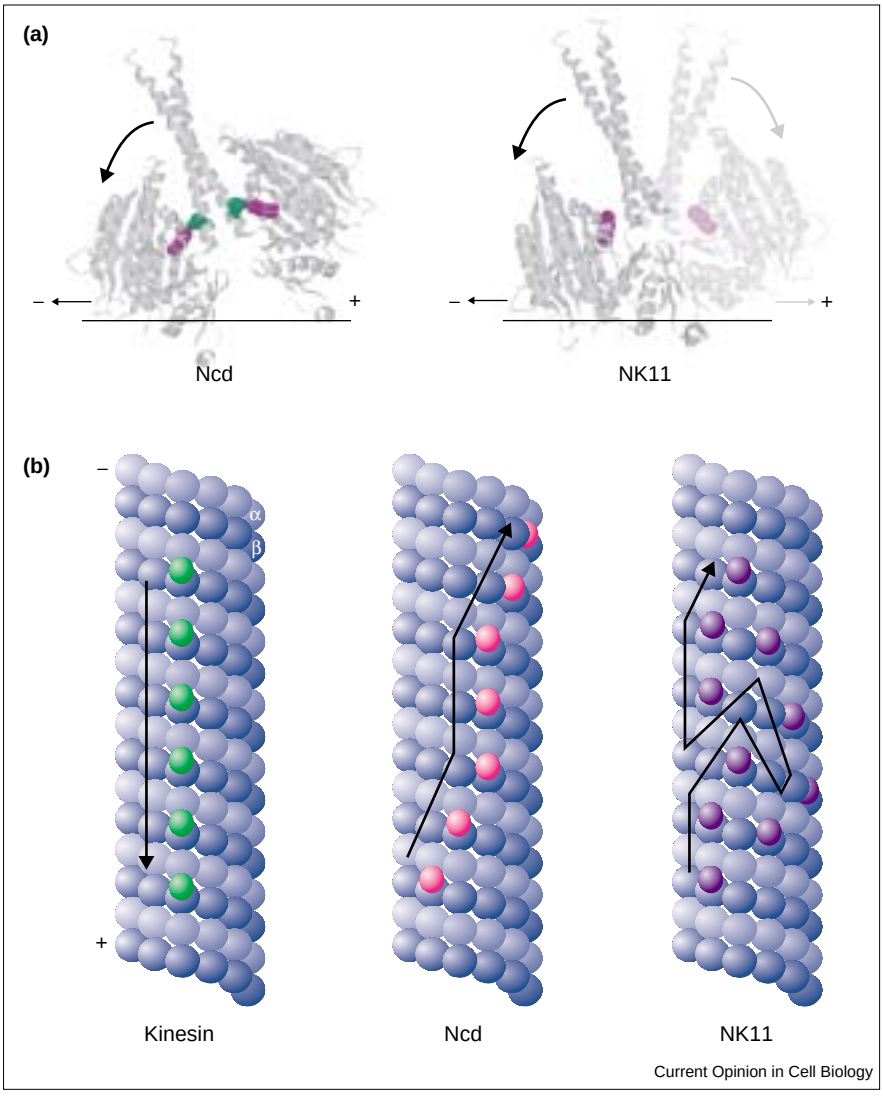


Structures of myosin and kinesin motors. (a) Scallop smooth muscle myosin S1 (PDB 1B7T [46]). For myosin, only the crystal structure of the monomeric motor has been solved. The essential and regulatory light chains (light gray, bottom) are bound to the rod (dark gray), which is thought to act like a lever arm to amplify small movements of the motor. The converter element (dark pink) consists of short β strands and α helices together with the proximal end of the helical rod, and is structurally analogous to the neck of the kinesin motors. The actin-binding region of the motor is shown in green and the bound ADP in red. (b) Dimeric rat conventional kinesin (PDB 3KIN [47]). The two β strands of the neck linker (purple, arrows) join the helical neck (dark pink) to each head. (c) Dimeric Ncd (PDB 2NCD [48]). The neck linker is missing from minus-end-directed Ncd. The neck (dark pink) consists of the distal end of the coiled-coil stalk joined directly to the two heads. The microtubule-binding region of kinesin and Ncd is colored green and the bound ADP is red.

The neck of minus-end-directed Ncd, a kinesin-related motor of *Drosophila*, differs from conventional kinesin in that the neck linker is missing; the Ncd neck consists only of the distal end of the coiled-coil stalk joined directly to the conserved catalytic core (Figure 1c). Despite the differences in neck structure, analysis of chimeric motors implicates both the kinesin and Ncd stalk/necks in the determination of motor directionality. Fusing the kinesin stalk/neck, including the neck linker, to the motor core (strand β1 to helix α6) of minus-end Ncd resulted in a plus-end-directed chimeric motor [7,8], whereas fusing the Ncd stalk/neck to the motor core of plus-end kinesin resulted in a minus-end-directed chimeric motor [9]. Unexpectedly, mutation of two neck residues in the reversed kinesin motor reverted its minus-end movement to slow plus-end motility [9]. The slow plus-end movement could be due to directionally biased conformational changes of the motor core that occur during ATP hydrolysis. The kinesin neck and neck linker could act to amplify these movements and increase motor velocity. But the Ncd neck is required to direct movement of the motor to microtubule minus ends and, to do this, must overcome the slow plus-end movement intrinsic to the catalytic core.

How does the Ncd neck function in motor directionality? Remarkably, a point mutation in the Ncd neck causes the motor to move either towards the microtubule plus or minus end [10••]. The mutation changes a neck residue from asparagine to lysine, weakening an interaction of the neck asparagine with a lysine of the motor core [10••]. Gliding assays of microtubules moving on motor-coated glass coverslips showed random movement of the mutant motor, Ncd NK11, towards the microtubule plus or minus end. The gliding excursions were long, some >20 μm, and microtubules infrequently but abruptly reversed direction. A few microtubules broke as they glided on the motor-coated surface; this was attributed to differing forces on the microtubule due to motors moving with different velocities

Figure 2



Stalk/neck function in Ncd directionality and paths of the kinesin motors along the microtubule. (a) The stalk/neck of wild-type Ncd may undergo a change in angle to bias motor movement towards microtubule minus ends. A conformational or angle change detected by single motor analysis occurs upon binding of the motor to the microtubule [10••] and may be associated with release of ADP from the bound head. The interacting neck residue (green) abd motor core residue (purple) are shown as space-filled models. The stalk/neck of the Ncd NK11 neck mutant contains a point mutation that allows the movement to occur in either direction. Analysis of Ncd and the Ncd NK11 mutant demonstrates that the directional bias of wild-type Ncd toward the microtubule minus end is dependent on neck-motor core interactions [10••]. (b) The paths of Ncd and kinesin along the microtubule differ from one another. Kinesin moves parallel to the protofilament axis along one (as shown) or two protofilaments as the motor moves towards the microtubule plus end [11]. Ncd tends to step off-axis to the right as it moves to the microtubule minus end [10••,12]. The Ncd NK11 neck mutant steps to the left or right as it moves toward the plus or minus end [10••].

or in opposite directions. The mean velocities of plus-end and minus-end motor movement were similar to one another and overlapped with the mean minus-end gliding velocity of wild-type Ncd [10••], indicating that the mutant motor functioned normally but directionality was defective. Single-motor laser-trap assays showed that a conformational change or angle change, for example, a movement of the stalk relative to the motor core, occurs upon binding of the wild-type or mutant motor to a microtubule. This movement is directional and is biased towards the minus end in wild-type Ncd, but it occurs in either the plus or minus direction in the NK11 neck mutant [10••]. The interpretation of the nature of this movement, based on the mutant analysis, is that it involves a change in angle of the stalk/neck relative to the motor core and is the basis of the minus-end directionality of wild-type Ncd (Figure 2a). Directionality of Ncd is thus dependent on interactions of the neck with the motor core.

A simple mechanical model for directional force generation involves a lever arm-like movement by the stalk/neck (Figure 2a). A conserved glycine at the base of the stalk/neck that inactivates the Ncd neck when mutated together with an adjacent residue [9] may act as the pivot point for the proposed lever arm. Alternatively, the position or angle of the Ncd neck region may regulate an asymmetrical interaction between the Ncd head and tubulin that results in directional movement.

The single-motor assays of the NK11 mutant and wild-type Ncd also showed that wild-type Ncd tends to step obliquely to the right as it moves to the microtubule minus end, rather than following the protofilament axis like kinesin [11] (Figure 2b). This off-axis stepping generates torque, causing microtubules in gliding assays to rotate as they move on the motor bound to the coverslip [12] and may be important for motor function in the spindle [13,14]. The NK11 neck

mutant steps obliquely to either the left or the right as it moves towards the microtubule plus or minus end (Figure 2b), indicating that the off-axis stepping is intimately tied to motor directionality.

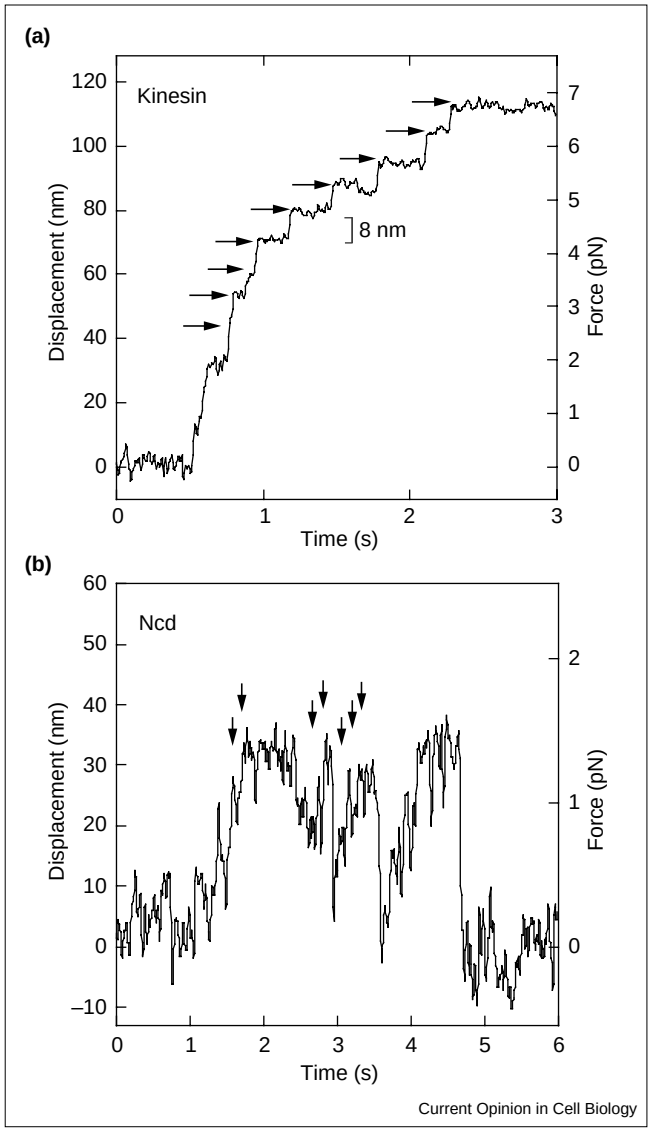
The finding that mutation of a single residue causes Ncd to move either towards the microtubule plus or minus end indicates that a local structural change can alter directionality. This raises the question of whether motors that are not unidirectional occur naturally in the cell. One candidate is KIF1A, a single-headed kinesin motor that moves with random bidirectional movement in single-motor assays but shows long excursions, indicating processive movement, and net displacement towards the microtubule plus end [15,16]. Although the KIF1A motor that has been demonstrated to show biased diffusional processive movement is a chimera with a neck originally derived from conventional kinesin [15,16], a truncated native KIF1A motor protein has also been observed to move in a processive manner (see on-line Supplementary Material in [15]). The neck of the KIF1A motor may thus be as flexible as that of the Ncd NK11 neck mutant, permitting it to move towards either the microtubule plus or minus end.

Other candidates for a naturally occurring bidirectional motor are dyneins: the first report of bidirectional movement by a motor, although as yet unconfirmed by identification of the motor, was in extracts of the giant amoeba *Reticulomyxa* [17]. The bidirectional movement could be converted to unidirectional minus-end movement by phosphorylation and was attributed to a variant form of cytoplasmic dynein. More recently, axonemal dyneins have been reported to move on microtubules in an oscillating fashion with alternating forward and backward processive stepping [18]. This oscillating movement, which may underlie the beating of cilia and flagellae, has been simulated by computer modeling as a strain-dependent switching between forward and backward stepping by a bidirectional motor [19]. The basic mechanism of the bidirectional movement of dynein may be similar to the Ncd NK11 neck mutant in which a local structural change affects motor directionality.

Motor processivity

Like directionality, processivity is a property that is intrinsic to motor function. Processivity refers to the ability of a motor to bind to a filament and take successive steps before detaching. Processive movement by a molecular motor was first demonstrated for single molecules of conventional kinesin [20], which steps by 8 nm increments along the microtubule [21], corresponding to the spacing of tubulin dimers in a protofilament, reaching a maximum force of 7–8 pN ([22]; Figure 3a). Myosin V [23] and certain dyneins [18,24,25] have also recently been shown to move processively. Interestingly, processivity of 22S outer arm dynein is sensitive to ATP concentration [25]. The increased processivity of the motor at low ATP concentration is presumably due to an increase in its duty ratio or filament-bound state. The low processivity of cytoplasmic dynein [26] increases in the presence of its receptor protein, dynactin [27], which may increase the binding affinity of the motor for microtubules. The ability of a dimeric motor to move processively is thought to involve alternating filament binding and ATP hydrolysis by the two motor domains with one of the heads always bound to the filament; otherwise the motor would diffuse away from the microtubule within a few microseconds. Such an alternating head catalysis model for processivity requires the existence, at least transiently, of a 'two-heads-bound' state to permit the

Figure 3



Processive movement by single kinesin motors or multiple Ncd motors. Traces from laser-trap assays show movement along a microtubule of motors attached to beads. (a) Single kinesin motors show successive displacements of 8 nm (arrows), the distance between adjacent tubulin dimers in a protofilament, generating a maximum force of ~7 pN. (b) Multiple (~3–4) Ncd motors show sequential but irregular steps (arrows), interpreted to be due to the stochastic binding of a second head from a different motor to complete each step.

heads to switch in binding to the filament without the motor releasing from the filament.

A two-heads-bound state has recently been observed in negatively stained electron micrographs of dimeric myosin V bound to actin [28]. Two-headed binding to actin was increased by adding ADP at low concentration, consistent with the idea that myosin V complexed to ADP binds tightly to actin. The average distance between the two heads bound to the actin filament was 36 nm, which corresponds to the helical repeat of the filament. Remarkably, a myosin V molecule can walk linearly along an actin filament, stepping over the helical turns by taking 'strides' or physical steps of 36 nm. The electron microscopy analysis has allowed workers to distinguish between physical steps and working strokes of the motor (often referred to as 'steps' in laser trap assays). Working strokes correspond to changes in conformation or angle of the motor and differ from steps of the motor along its filament; for example, the angle change at the head–tail junction of myosin V results in a working stroke of ~26 nm as measured by electron microscopy, significantly smaller than its 36 nm stride or physical step [28].

How is a physical step of the motor achieved? An emerging idea is that one or more conformational changes produce a working stroke of the motor [29]; several working strokes occur during the hydrolysis cycle, resulting in a step of the motor along its filament. Considerable evidence now indicates that kinesin moves on microtubules with regular steps of 8 nm [21,22]. But, recently, fast and slow 4 nm substeps within the 8 nm step of kinesin were detected at higher time resolution using smaller beads [30•]. These substeps could represent the conformational changes or working strokes predicted to occur during the kinesin–microtubule cycle by recent models [31,32], for example, docking of the neck linker, binding of ATP to the attached head, or binding of the unbound head to the microtubule. The substeps of myosin V should also be detectable at higher time resolution. The detection of putative substeps for kinesin in single-molecule assays is a technical *tour de force* that should enable workers to detect other changes in conformation or angle of the motor and correlate them with chemical steps of the ATPase cycle.

Despite the recent evidence from electron microscopy for two-headed binding by myosin, evidence for a two-heads-bound state of kinesin has been elusive. Cryoelectron micrograph reconstructions showing both heads of dimeric kinesin bound to the microtubule have been obtained by some kinesin workers, but not others (see [33]). Because kinesin binds to microtubules without force generation (zero load) under conditions used for electron microscope decoration, it is important to determine the binding state of kinesin to the microtubule during force generation, that is, under physiological conditions. The most convincing evidence so far for a two-heads-bound state for kinesin has been obtained using an optical trap to measure the unbinding force for single kinesin molecules attached to a microtubule in the presence or absence of added nucleotide [34••]. The detachments showed nucleotide dependence, indicating that it was release of the motor from the microtubule (rather than, for example, detachment of the motor from the bead) that was being monitored. The unbinding force was approximately twice as large with AMP•PNP [a slowly hydrolyzable nucleotide analog, adenosine 5′-(beta,gamma-imino)triphosphate] (~14 pN) as with no nucleotide (~7 pN). The elastic modulus (the constant defining the force per area required to cause a relative length change) of the motor–microtubule complex was also twice as high in AMP•PNP as with no nucleotide [34••]. The elastic modulus is influenced by several factors, however, for example, the compliance between the bead and the motor, and between the coiled-coil region and the heads. The elastic modulus can also increase with force due to nonlinearity of elastic components [22]. Thus, the increase in the elastic modulus can be attributed to two-heads-binding and an increase in the modulus of other elastic components. The increase in unbinding force, caused by an increase in binding constant of kinesin to tubulin due to the second bound head, together with the increase in the elastic modulus, is compelling for a two-heads-bound state with AMP•PNP. The motor with no nucleotide was interpreted as a one-head-bound state. Adding ADP + AMP•PNP to the motor resulted in an unbinding force of ~7 pN, consistent with the interpretation that ADP binds to one head causing it to release from the microtubule, converting the motor plus AMP•PNP to a one-head-bound state.

The negatively stained electron micrographs of myosin V and unbinding experiments for kinesin provide convincing evidence that myosin and kinesin can bind to their filament with both heads at the same time. These observations have been interpreted as evidence for the alternating head catalysis model of motor stepping, which requires both heads to be bound at least momentarily as they switch in binding to the filament. But evidence for a two-heads-bound state does not exclude other models, such as the 'inchworm' model in which the leading head always precedes the lagging head to the next binding site along the filament, requiring both heads to be bound during a part of the hydrolysis cycle. Models for processivity should also take into account the monomeric motors, for example, KIF1A [15] and inner arm dynein c [24], and a three-headed outer arm dynein [25] that have been reported to be processive motors. Monomeric motors require a different mechanism for processivity than alternating head binding for the single head of the motor to remain bound to the filament and, at the same time, to move along the filament. KIF1A appears to have solved the problem of a one-headed motor remaining attached to its filament as it moves along the filament by having an extra microtubule-binding site built into its motor domain, the lysine-rich 'K-loop' [16]. The K-loop is thought to maintain weak binding by

the motor to the microtubule, substituting for the binding by the second head of a dimeric motor and permitting processive diffusional movement by the motor. Processive motors with more than two heads, for example, the three-headed outer arm dynein reported to be a processive motor [25], could bind with each of the heads in turn, or use only one or two of the heads to remain attached to the filament while moving along it. These motors could use variations of the currently proposed mechanisms for dimeric or monomeric motors, or new, as yet undescribed modes of processivity.

Nonprocessive motors

Evidence from several types of experiments has led to the idea that, in contrast to processive motors, nonprocessive motors bind to their filament and undergo conformational or angle changes, then detach without taking successive steps along the filament. First, biochemical rate constants are consistent with the interpretation that Ncd, which considerable evidence now indicates is a nonprocessive motor, releases its bound head before binding with its second head [35]. Thus, only one head of the dimeric motor may be capable of binding to its filament at a time. Unbinding experiments have not yet been carried out for Ncd, but a one-head attachment/release model is predicted to show only one-headed binding even in the presence of AMP•PNP.

Second, several recent studies [10••,36•] have not only provided compelling evidence that Ncd is a nonprocessive motor, but have also detected working strokes of the motor bound to the microtubule. One of these conformational or angle changes is directional and biases movement of the motor to the microtubule minus end [10••], as discussed above. This displacement may be associated with ADP release, since it occurs upon binding of the motor to the microtubule. A second recently detected conformational or angle change occurs just prior to release of the motor from the microtubule [36•] and has been suggested to be the large conformational change predicted to occur when ATP binds to the empty head [32]. Although the nature of these conformational or angle changes is still speculative, they are likely to involve movements of various structural elements, for example, tilting or rotation of the so-called 'relay' helix of the motor catalytic core [37••] or movement of the coiled-coil stalk relative to the motor core. The binding and displacements occurred as single events, consistent with the interpretation that a single head binds, hydrolyzes ATP, then releases from the microtubule.

The two Ncd single-motor studies discussed above [10••,36•] each provided new information about the movements of the Ncd motor as it interacts with a microtubule, but each failed to detect the movement reported by the other, possibly due to the different geometries of the laser trap assays. The single-bead assay used to detect the directional movement of Ncd [10••] may be more sensitive to the angle change of the head or neck, which is amplified by the large bead. On the other hand, the three-bead assay used to detect the movement late in the binding phase may be advantageous in detecting translational movement by the motor along the microtubule [36•]. The interpretation of results from the two analyses, which are complementary to one another, is that the head or neck of Ncd changes in angle upon binding to microtubule and releases ADP. Upon binding of ATP or hydrolysis of ATP to ADP•P_i, Ncd may move translationally along the microtubule with a slight backward angle change. The movements occur in the bound head, followed by release of the motor from the microtubule.

Myosin II is also a nonprocessive motor, like Ncd, in contrast to myosin V and conventional kinesin, which are processive motors. The step size of myosin II is still controversial. At least part of the reason for this is that it is technically difficult to measure the step size precisely at low force in laser trap assays because of the large compliance and large Brownian noise. Although both the step size and the motor displacement per ATP hydrolysis have been extremely controversial over the past ten years or so, there is now substantial agreement that an elemental step, or substep, of myosin II is ~5 nm (reviewed in [1••]).

The interactions of a nonprocessive motor, for example, skeletal muscle myosin II or Ncd, with actin or microtubules thus do not result in successive steps of the motor along its cytoskeletal filament. Instead, the changes in conformation or angle of the bound motor can translocate the filament past its initial position, the motor then unbinds and another motor binds to continue the translocation of the filament. In the case of myosin II, a single-headed myosin S1 motor may remain weakly bound to the actin filament and diffuse along the filament to the next binding site [38], resulting in a large displacement of 11–30 nm [39]. The large displacements would appear to consist of successive 5.3 nm steps if the motor paused at binding sites along the filament. The necessity for a second motor to complete a step has led to the idea that nonprocessive motors work in large arrays in the cell [40]; for example, the thick filament of skeletal muscle myosin or the arrays of Ncd, a spindle motor, along the spindle fibers [13]. These large arrays of motors, with each motor capable of attaching to an actin filament or microtubule, also have the potential to hold on to the cytoskeletal filament without translocating it if a number of available heads bind. This may be important in maintaining the integrity of the mitotic spindle, for example, attaching centrosomes to spindle poles, spindle microtubules to one another, and maintaining attachment of chromosomes to kinetochore fibers.

Although single nonprocessive motors do not take successive steps along their filament, multiple nonprocessive motors can show processive movement along a filament. What is a step for a nonprocessive motor? A nonprocessive motor is capable of taking successive steps along a filament if a

second motor binds before the first motor releases. This means that processive stepping of a nonprocessive motor requires more than one motor and, unless the binding of the second motor is somehow coordinated with release of the first motor, the steps will be stochastic. These characteristics contrast with a highly processive motor like kinesin, whose traces from single-motor laser-trap assays show highly regular stepping (Figure 3a). Similar records for multiple Ncd motors show irregular steps (Figure 3b), supporting the idea that the attachment of the second motor is stochastic rather than coordinated with detachment of the first motor.

Nonprocessive motors that are present in arrays in the cell will be constrained in stepping along their filament both by the orientation of the motors in the array [41] and the availability of the binding sites along the filament. For example, the binding of a second myosin II motor to an actin filament after detachment of a first motor may occur only when a binding site is present on the same face of the filament, ~36 nm from the original binding site, giving a disproportionately long stride or physical step of 36 nm for myosin II (see [1••] for further discussion).

How can a nonprocessive motor be converted into a processive motor? The neck linker of kinesin is thought not only to amplify the conformational changes of the catalytic core, as noted above, but also to regulate movement of the second head (see [42] for a recent model). This results in a coordinated alternative binding and catalysis of the two heads that enables the motor to move processively along the microtubule. Fusing the neck linker and neck/stalk of kinesin to the Ncd motor core reverses the direction of Ncd movement and converts it into a plus-end motor [7,8], but it does not convert Ncd into a processive motor [8]. Processive movement involving the neck linker probably requires interactions with amino acids of the kinesin motor core that are not conserved in Ncd. Further analysis of these residues could provide important information regarding the molecular basis of motor processivity and regulation of the kinesin neck linker.

Conclusions

Despite recent progress, the conformational changes that underlie directionality, processivity and other aspects of motor function are still relatively poorly defined. Detecting these structural changes and correlating them with the chemical steps of the nucleotide hydrolysis cycle is the immediate challenge for workers in the field. The use of sensitive fluorescence assays should prove informative [43,44], together with high-resolution laser-trap assays [30•] and X-ray crystallographic structures of motors in different states of the hydrolysis cycle [45].

Acknowledgements

We thank HP Erickson for comments on the manuscript. Work in our laboratories is supported by grants from the Japan Ministry of Education, Science, Sport & Culture (HH), National Institutes of Health (SAE) and Human Frontier Science Program (HH and SAE).

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