Conformational changes during kinesin motility
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Nucleotide-dependent movements of the head and neck of kinesin have been visualized by cryo-electron microscopy and have been inferred from single-molecule studies. Key predictions of the hand-over-hand model for dimeric kinesin have been confirmed, and a novel processivity mechanism for the one-headed, kinesin-related motor KIF1A has been discovered.

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**Current Opinion in Cell Biology 2001, 13:**19–28

**0955-0674/01/$—see front matter
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**Abbreviations**
cryo-EM  cryoelectron microscopy
FRET  fluorescence resonance energy transfer

**Introduction**
Some of the most intriguing questions about molecular motors focus on the conformational changes of these proteins as they move and do work. These are largely structural questions, such as: what is the sequence of structural states that produces a net displacement? And, how are the transitions between those states linked with the chemical steps of ATP hydrolysis? But more subtle and general issues arise when the load is considered: how do protein conformational changes do mechanical work? Do proteins employ a stereotyped ‘power-stroke’ to generate force? What is the role of thermal fluctuations in force generation?

Atomic structures of the motor proteins and their filaments in all the nucleotide states of the motor’s ATP hydrolysis cycle would help to understand their action. For myosin II, atomic structures in different nucleotide states have provided compelling evidence that the protein generates movement through a ‘swinging lever arm’ mechanism. For kinesin, the atomic structure has been solved in only one nucleotide state (ADP bound). Nevertheless, clues to the conformational changes that take place during kinesin motility have been obtained from recent cryoelectron microscopy (cryo-EM), biochemical, and single-molecule experiments. This review focuses on kinesin results reported during the past year and touches on parallel results from myosin.

**Anatomy of motor proteins**
Kinesin and myosin motor proteins appear to operate using the same basic design. A conformational change in the motor domain, which is associated with a change in nucleotide state, is transmitted via a converter domain to a lever domain. In this way, small conformational changes (on the order of a few Angstroms) in the nucleotide-binding pocket (which are associated with binding or hydrolysis of ATP or release of products) are amplified into the large displacements (which can be several nanometers) associated with motility.

Kinesin and myosin use a conserved core motor domain, which is structurally similar to a core region of G proteins, to produce the initial nucleotide-dependent conformational change [1]. However, the converter and lever domains of kinesin and myosin are quite different. Myosin’s lever is an 8 nm light-chain-binding domain that undergoes a large rigid-body rotation, as seen when myosin is crystallized in different nucleotide states [2,3,4*]. Evidence that the myosin lever arm rotates during motility is reviewed by Geeves and Holmes [5*]. Recent fluorescence resonance energy transfer (FRET) measurements report a large, nucleotide-dependent angle change in myosin’s lever arm [6*] and thereby provide additional support for the swinging lever arm model for myosin.

Kinesin (Figure 1) has no single domain corresponding to myosin’s lever, and the inability of one-headed kinesin to step suggests that movement of dimeric kinesin relies partly on mechanical amplification by the second (tethered) head [7,8*]. However, for the free head to reach the next binding site (8 nm away) while the other head remains bound, a large conformational change is required to alter the relative positions of the two heads: either the coiled-coil dimerization domain (red and blue helices in Figure 1) opens up [9,10] or the ‘neck linker’ (a stretch of ~15 amino acids that links the conserved motor domain to the coiled-coil stalk [red and blue spheres in Figure 1]) peels away from the surface of the motor domain [11*], or both [12]. Thus a number of domains — the motor, the neck linker, and the dimerization domain — probably contribute to kinesin’s lever.

**Directionality and the converter domain**
What determines the direction of a motor protein along its filament? The concept of a converter domain that acts as a sort of gear box is useful for understanding the directionality of both myosin- and kinesin-related motors. For myosin, the ‘converter’ domain forms the junction between the motor domain and the light-chain-binding domain (the lever): a change in the nucleotide bound in the active site alters the structure of the converter domain [13], and this, in turn, causes rotation of the lever [4*]. The connection between the active site and the converter domain is made by the relay helix (also called the switch II helix), which may couple the opening of the nucleotide-binding pocket to rotation of the converter and the attached lever [4*,14*]. Myosin VI has a large insert in the converter domain, and this motor protein moves towards the minus end of actin filaments, in the opposite direction from all other characterized myosin motors [15**].
For kinesin-related proteins, the ‘neck’ region that forms the junction between the head and dimerization domain (Figure 1) probably plays the same role as the converter domain of myosin [11*,16]. Support for this idea first came from studies of chimeras of kinesin and the kinesin-related motor protein ncd (reviewed in references [17–19]). When the conserved motor domain of ncd (i.e. the region of ncd that is highly conserved among all kinesin-related proteins) is put into the kinesin ‘body’, the resulting chimera is plus-end directed, like kinesin. Conversely, when the conserved motor domain of kinesin is put into the ncd body, the chimera is minus-end directed, like ncd. Truncation experiments with kinesin [20] place the direction-determining region in the neck linker domain (Figure 1). Although ncd has no neck linker, the direction-determining sequences of ncd are also located outside of the conserved motor domain, in the coiled-coil domain just amino terminal to the motor domain. Mutations in this domain, called the ncd neck, can reverse the direction of movement. Remarkably, change of a single residue in the ncd neck creates a motor that can alternate between plus-end- and minus-end-directed states [21*].

In summary, considerable evidence supports the idea that a ‘converter’ domain can modulate directionality in both kinesin- and myosin-related motors. More research is needed, however, to reveal the structural mechanisms that underlie this directional control and to answer questions such as how does a molecular gear box really work.

**Processivity and the hand-over-hand model**

The processivity of molecular motors, which is the ability to make multiple steps along a filament before dissociation, varies widely. Kinesin is a highly processive motor that can take hundreds of steps before falling off the microtubule (reviewed in [16]), whereas the kinesin-related protein ncd is non-processive and cannot manage even two steps in succession [21*,22**,23**]. Myosin V moves processively along actin filaments [24 •,25•,26••,27•,28•], whereas muscle myosin II is non-processive [29]. These differences are thought to reflect different biological functions: motors that operate in large ensembles to drive rapid movements (e.g. muscle myosin) should spend only a small fraction of their mechanical cycle attached to the filament and thus be non-processive, whereas motors that operate alone or in small numbers to transport organelles (e.g. kinesin) should be processive [29].

What are the properties of a motor protein that allow it to be processive? In the case of kinesin, recent work from several groups supports a model [30] in which the two heads move in a coordinated, hand-over-hand fashion. A crucial aspect of this coordination was recently demonstrated, namely that the detachment of one head is contingent on the attachment of the other [7,8•,31•]. For conventional kinesin and *Neurospora* kinesin, binding of the detached, tethered head to the microtubule accelerates the ATPase and detachment rates of the bound head by a factor of 10 or more [8•,31•]. The physical basis of this coordination between the heads is thought to be the intramolecular strain generated upon binding of the forward head, although direct evidence for this is still lacking.
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Figure 2

The proposed mechanochemical cycle of kinesin, with possible branches indicated. An animation of this cycle can be found at www.current-opinion.com/subjects/jcel/mov1.mov. The neck linker and dimerization domain are red and blue, as in Figure 1. Black denotes one side of the motor domain (the side view of the attached head in Figure 1b), and gray denotes the other side. Motor domains are labelled with their nucleotide state: D represents ADP, T represents ATP, DP represents ADP·Pi, and φ represents no nucleotide. Nucleotides are shown as colored spheres: ADP, yellow; ATP, green; ADP·Pi, blue. The microtubule protofilament is schematized as light gray (α subunits) and dark gray (β subunits) hemispheres. State 0 represents the crystal structure with ADP bound [9]; in this structure the neck-linker clings to the motor core. The heads are related by a rotation of 120° about an approximately vertical axis, so if one imagines the black head to lie in the plane of the page, the gray head is actually rotated into the page by approximately 60°. In state 1, one head attaches and rapidly releases its ADP; the tethered head does not attach in the absence of added nucleotide [31•,40•,55]. The dimerization domain is depicted as if it were attached to a small load, so it points backward rather than out of the page as in Figure 1b. In state 2, the attached head binds to ATP, causing a conformational change that moves the tethered head from pointing into the page to pointing out of the page [33•,43,44] and advances the load by 1–2 nm (see ‘load-dependent steps’). Here the conformational change is depicted as a movement of the neck-linker on the surface of the attached motor (red linker moves from one position to another), which causes an opening in the lower ~2.5 nm (two heptads) of the coiled-coil dimerization domain [56] and thereby allows the tethered head to rotate and fluctuate about its neck. In state 3, ATP hydrolysis in the attached head usually precedes and accelerates attachment of the tethered head (see text), but no information is available on the specific conformational changes due to hydrolysis (here represented by the average position of the tethered head moving closer to the forward microtubule-binding site). Also, two-headed binding is possible without hydrolysis [31•,55] (left branch, states 3–4). In state 4, a conformational fluctuation of the neck linker has allowed the tethered head to attach to the microtubule and become the forward head. In state 5, the forward head releases its ADP rapidly following attachment, thus binding more tightly to the microtubule [8•,31•,40•,50], trapping its neck-linker in a peeled-away configuration, and causing intramolecular strain. In state 6, sustained strain induces detachment of the rear head, owing to the weaker attachment in ADP·Pi compared to no nucleotide [31•,40•,50]. ATP binding in the forward head might cause additional strain and hence further accelerate rear-head release [11•] (left branch, states 6→7). The rear head probably detaches before releasing its phosphate (see text), but earlier phosphate release is possible [31•,40•] (right branch). States 6→7: the newly detached head swings forward, and the load may move forward, as the strain of the two-heads-bound state is relieved. We hypothesize that phosphate release causes the neck linker of the detached head to shift back to the ADP position, allowing the re-coiling of the dimerization domain. Kinesin has now advanced by 8 nm with respect to state 1, and the coiled-coil has gained a half twist (red α-helix and neck linker are now in front of blue). Whether the next step will reliably relieve this half-twist is a question that remains to be answered.
A key prediction of the hand-over-hand model is that the two heads of kinesin can bind simultaneously to the microtubule. Two-headed binding of kinesin to microtubules has been demonstrated by cryo-EM [10,32], although other laboratories see predominantly one-headed binding under similar conditions [35••]. This apparent discrepancy might be accounted for by differences in motor concentration (higher motor concentrations favor one-headed binding) and/or ADP concentration (ADP, even in the micromolar range, is likely to displace one head from the microtubule, owing to its very high affinity for a free head). Direct mechanical evidence has now been obtained for a two-heads-bound configuration [34]. Single dimeric kinesin molecules attached to beads and an optical trap were used to measure the unbinding force and mechanical stiffness of the kinesin–microtubule complex. In the presence of saturating concentrations of the nonhydrolyzable ATP analog AMP-PNP both the unbinding force and the stiffness were twice that measured in the absence of nucleotide when the trap pulled the bead toward the plus end of the microtubule. This strongly suggests that two-headed binding predominates in the presence of AMP-PNP, whereas one-headed binding predominates in the absence of nucleotide.

**Processivity of a one-headed motor**

Although evidence indicates that the two heads of kinesin alternate in a hand-over-hand stepping fashion, a novel processivity mechanism appears to operate in the one-headed, kinesin-related motor protein KIF1A. Recent work surprisingly indicates that KIF1A is processive: on average, each encounter of KIF1A with a microtubule results in a net displacement of about 1 μm toward the plus end [35••]. Like dimeric kinesin, monomeric KIF1A uses two tubulin-binding motifs to maintain its association with the microtubule, but unlike kinesin, which has only one motif per head, KIF1A has two different motifs in the one head. One of these motifs is conserved among all kinesin-related proteins but the second motif is not. The second motif is a lysine-rich loop (the ‘K loop’) that interacts with the glutamate-rich carboxyl terminus of tubulin [35••,36••,37••], and deletion or mutation of this loop results in loss or reduction of processivity [36••,37••]. In addition, a non-processive motor could be converted into a processive one by extending the K-loop of a conventional kinesin monomer with six lysines [37••]. Experiments with microtubules whose tubulin subunits were enzymatically digested at the carboxyl terminus identified the carboxy-terminal region of tubulin as the binding partner of the K loop [37••].

The movement of KIF1A differs from kinesin in that its velocity fluctuates wildly. This suggests that diffusion along the microtubule plays an important role in motility [35••,37••]. Consistent with a diffusional mechanism, the coupling between nucleotide hydrolysis and stepping is loose: several ATP molecules are hydrolyzed per 8 nm step even in the absence of load. This contrasts with conventional kinesin, which hydrolyzes one ATP per 8 nm step at low load (see below).

Although the evidence presented on the processivity of KIF1A is rather compelling, these results are not without controversy, as the findings have been made in only one laboratory, and other groups have found evidence that the monomeric motor unc104, the KIF1A homologue in Caenorhabditis elegans, is not processive [38]. One possible source for the discrepancy is the particular motor constructs used: the ‘processive’ KIF1A was actually a truncated form of KIF1A (351 amino acids) fused to 23 amino acids of the neck region of kinesin, and the ‘non-processive’ unc104 was a longer construct with 651 amino acids from unc104 fused to GFP (green fluorescent protein). It is important to confirm the processivity of the KIF1A constructs in other laboratories and to test other constructs. Furthermore, although experiments with KIF1A knock-out mice implicated this monomeric motor in the transport of vesicle precursors [39], it remains to be determined whether KIF1A’s diffusion-based mechanism can still work against the high loads expected when the motor is challenged to move organelles through the crowded cytoplasm (for further discussion of KIF1/UNC104 see the review by Bloom, pp 36–40 of this issue).

**Kinesin’s mechanochemical cycle**

Whether a motor protein is processive or not, the motor’s filament-binding sites must alternate between at least two mechanical states (attached to filament and detached), and the nucleotide-binding pockets must cycle through four chemical states (empty, ATP, ADP-Pi and ADP). To understand the mechanism by which a motor protein moves, one must define the dominant pathway(s) that the motor follows through all possible mechanochemical states. All pathways occur with some non-zero probability, and the most frequently followed path may change depending on the external load, nucleotide concentration, temperature, pH, and salt concentration. Here we discuss the dominant path of kinesin under conditions typical of a laboratory motility or biochemical assay: low load (<1 pN), saturating ATP (~1 mM) and negligible ADP and Pi (~nM), room temperature, and a pH of 7.

Several models for kinesin’s dominant mechanochemical cycle have been proposed recently [8••,11••,14••,31••,40••,41]. Although some aspects of the proposed pathways are agreed upon, there are several important differences among the models. First we will review those aspects that are widely accepted, then in a separate section below we will delve into the controversial issues. To frame the discussion, we propose a mechanochemical cycle for kinesin in Figure 2 (central panel) that extends the model in reference [8••], and we highlight possible branches from the main path (side branches in Figure 2).

A key early finding was that microtubules accelerate the release of ADP, which binds tightly to both heads of kinesin in solution. But when microtubules are mixed with dimeric kinesin, ADP dissociates from only one head unless ATP is present in the solution (Figure 2, states 0→1) [42]. It is
therefore widely believed that binding of ATP to the attached head precedes a conformational change that allows the other (tethered head) to attach and release its ADP (Figure 2, states 2→5), although the details of this conformational change are a matter of debate. The presence of the non-hydrolyzable ATP analogue AMP-PNP also accelerates release of the second ADP, showing that hydrolysis is not necessary for ADP release from the second head (Figure 2, left branch, states 3→4). However, as argued below, hydrolysis usually takes place before attachment of the second head (main path of Figure 2, states 3→4).

Cryo-EM of kinesin-decorated microtubules has also provided hints about the movements of kinesin. Comparison of microtubules decorated with kinesin dimers in the absence of nucleotide and in the presence of AMP-PNP shows that the binding of AMP-PNP is associated with a large conformational change that moves the tethered head from the left of the attached head to the right of the attached head, where the plus end of the microtubule is up ([33•,43,44]; see Update, but see also [10] for another interpretation). This conformational change appears to take place within the neck-linker of the attached head, because a similar structural change is seen when AMP-PNP binds to a kinesin monomer whose neck-linker has been labeled near its carboxyl terminus with a gold particle [11•]. These electron microscopy studies thus support the notion that the tethered head acts like a lever and the neck acts like a converter domain (and possibly also part of the lever).

Several other important aspects of kinesin’s pathway in Figure 2 have been agreed upon. One is the fuel efficiency: kinesin is a ‘tightly coupled’ motor, it hydrolyzes one ATP molecule for each 8 nm step under low load [45•,46•,47,48,49•]. Optical trapping data suggest that the coupling remains tight even under loads as large as 5 pN [49•]. A second feature is the maximum motor speed: conventional kinesin takes ~100 steps per second at saturating ATP and low load (so moving 8 nm from state 1 to state 7 in Figure 2 takes ~0.01 s). Another widely agreed feature of kinesin motility is that the attachment between the motor domain and the microtubule is nucleotide dependent: the motor dissociates slowly (‘binds strongly’) with ADP or ADP-Pi bound [8•,31•,40•,50]. These differences in binding strength ensure that when both heads of kinesin are attached to the microtubule the rear head with ADP-Pi is more likely to be released than the forward head with no nucleotide bound (Figure 2, states 5→6). Finally, it has recently become clear that kinesin possesses an internal conformational switch that prevents motility unless cargo is bound to its carboxyl terminus, termed ‘tail domain’. Without cargo (which can be an organelle, head, or glass surface, for example) bound to the carboxyl terminus, kinesin folds up so that the tail binds to the motor domain and inhibits ATP hydrolysis [51•,52]. So, the pathway in Figure 2 only applies to kinesin with cargo bound, or with the tail domain deleted.

**Uncertainties regarding kinesin’s mechanochemical cycle**

Many important aspects of the conformational changes during kinesin motility are not understood. Given that ATP binding to the attached head of kinesin induces a positional change in the tethered head, one important question is: how is the initial conformational change in the nucleotide-binding pocket of the attached head transmitted and amplified into a larger motion of the tethered head?

As the neck-linker and dimerization domain provide the physical connection between the heads, recent work [11•] has focused on changes within kinesin’s neck-linker upon ATP binding. Electron paramagnetic resonance (EPR) spectroscopy of probes attached to the neck-linker suggests that this region is highly mobile in the presence of ADP and in the absence of nucleotides when the motor is in solution or attached to a microtubule. However, the neck-linker is immobile in the presence of AMP-PNP when the motor is attached to a microtubule [11•]. These observations have led to a model in which ATP binding induces a disordered-to-ordered conformational change of the neck-linker [11•,14•,53]. However, this model appears to be at odds with a battery of existing data that support a rather different conclusion. A highly disordered neck in the ADP state is inconsistent with the ordered conformation in the dimer crystal structure [9] (Figure 1), the lack of two-fold symmetry in the dimer solution structure ([54]; see also Update) (the coiled-coil is symmetric, so a disordered neck should lead to two-fold symmetry of the heads), and the reasonably well ordered conformation seen in the cryo-EM studies of dimeric kinesin mentioned above ([33•,44] and Update). Indeed, the observation using cryo-EM of a reduced mass of the tethered head in the presence of AMP-PNP [33•,43,44] is consistent with the tethered head having greater conformational freedom when ATP is bound to the attached head compared to when no nucleotide is bound. The ADP-release experiments mentioned above [42] are also more consistent with an ordered neck in the ADP state: if the ADP state were highly disordered, one would expect that the tethered head would be able to attach to the microtubule and release its ADP even in the absence of ATP. The EPR studies of Rice et al. [11•] were performed on severely truncated monomeric motors whose ATPase cycles are poorly coupled to motility (many ATPs hydrolyzed per mechanical step). Thus, it is possible that the high mobility of the neck-linker in these constructs arises from this truncation.

The results from cryo-EM [11•,33•,43,44] and ADP-release experiments [31•,42,55] are consistent with a model in which ATP binding to the attached head results in greater mobility of the tethered head, in contrast to the conclusion of Rice et al. The proposal by Schnitzer et al. [41] of an isomerization following ATP binding, in which kinesin undergoes rapid fluctuations between two conformational substates in equilibrium, is also consistent with ATP-induced conformational freedom of the tethered head.
head. Finally, a disordered conformation of the ATP state may explain the lack of a kinesin–ATP crystal structure.

How could ATP binding to the attached head increase the mobility of the tethered head? ATP binding to the attached head might result in a partial opening of the coiled-coil dimerization domain, as depicted in Figure 2 (state 2). In this scenario, ATP binding causes the neck-linker of the attached head to shift from one ordered conformation to a second that is incompatible with a fully closed coiled-coil (Figure 2, states 1→2). In fact, the first segment (~2.5 nm) of kinesin’s coiled-coil departs from the classical leucine zipper pattern and has the potential to uncoil [56]. In summary, although there is considerable evidence that ATP binding causes a forward-directed motion of the tethered head, the details of this conformational change remain to be elucidated. Single molecule studies have provided additional insight into changes associated with ATP binding and these are discussed in the section on load-dependent steps.

Currently, we have no direct information on the conformational changes associated with ATP hydrolysis, but biochemical data allow us to address the important question of whether ATP hydrolysis in the attached head takes place before or after attachment of the tethered head (Figure 2). Several lines of evidence indicate that hydrolysis precedes attachment (Figure 2, states 2→4): first, ATP binding to the attached head accelerates the release of the fluorescent ADP-analog mantADP from the detached head several-fold (a factor of 3–12) more effectively than AMP-PNP [31•,55]. Second, the rate constant for ATP hydrolysis by the kinesin monomer attached to a microtubule (which may be approximately the same as the rate constant for hydrolysis by the attached head of the dimer) is more than six-fold higher than the rate constant for release of mantADP from the tethered head in the presence of AMP-PNP (200 s⁻¹ [57] versus 30 s⁻¹ [55]). Third, the rate constant for ATP hydrolysis is likely to be even greater in the attached head of the dimer, in order to reconcile the low affinity of the attached head for AMP-PNP (Kₐ ~1 mM [50,55]) with the high apparent second order association rate (or ‘on-rate’) for ATP binding (1–2 μM⁻¹ s⁻¹ [55]). (The low affinity and high apparent on-rate indicate that binding of ATP to the attached head is followed by a very rapid step, with a rate constant of k = K_{on-apparent} × K_{d} =1000–2000 s⁻¹, that does not occur after binding of AMP-PNP and is therefore probably hydrolysis.)

The above data suggest that hydrolysis in the attached head usually precedes and accelerates the binding of the tethered head, as shown in Figure 2 (states 2→4), implying that the hydrolysis step is associated with a productive conformational change. On the contrary, Rice et al. [11•] find that a kinesin mutant defective in ATP hydrolysis (E236A) is nevertheless able to accelerate tethered head ADP release to the same rate as wild-type (Figure 2, left branch, states 3→4), and they conclude on that basis that ATP binding alone drives forward stepping. Evidently the E236A motor with bound ATP has different properties than the wild-type motor with bound AMP-PNP. Because other kinetic properties of this mutant are aberrant (ADP release is 100 times faster than in wild-type [11•]), we do not think it provides a good model for the ATP state.

The next chemical step following the hydrolysis of ATP, the release of phosphate from the rear head, is another important area of debate. Hancock and Howard [8•] argue that detachment of the rear head is a prerequisite for phosphate release, on the basis of their findings with a one-headed heterodimer (Figure 2, states 5→7). Rice et al. [11•] propose, without evidence, that a one-headed monomer detaches after phosphate release so that attachment and detachment take place in the same nucleotide state (i.e. with ADP bound). However, this model is probably incorrect because it fails to account for the single-motor motility observed with monomers in the assays of Young et al. [58]. In these assays, single monomeric motors anchored to glass coverslips could propel microtubules, even though the motors could not remain continuously attached to the microtubules, because methylcellulose prevented microtubule escape from the surface. In the model of Rice et al. [11•], the monomers would attach and detach in the same conformation, so a single monomer could not move a microtubule. This problem does not exist if detachment takes place before phosphate release, as argued by Hancock and Howard [8•]. In this case, the bound phosphate prevents premature release of ADP and subsequent futile hydrolysis cycles in the attached rear head (Figure 2, right branch), helping to ensure that more than 90% of the ATP molecules hydrolyzed result in a mechanical step [45•]. Cross and colleagues [40•] postulate that phosphate might be released just before rear-head detachment (Figure 2, right branch). Direct measurements of the rear-head phosphate release kinetics are necessary to resolve this issue with certainty.

A final area of uncertainty concerns the release of the rear head from the microtubule. It is clear that detachment of the rear head is contingent upon attachment of the forward head (Figure 2, states 3→6) [8•,31•] but it is not known whether the intramolecular strain generated by the simultaneous binding of both heads is sufficient to pull the rear head off the microtubule [8•,31•], or whether subsequent ATP-binding to the forward head is required as well [11•,14•]. Interestingly, if ATP binding is strictly required (Figure 2, left branch, states 6→7) then the first 8 nm step of kinesin on a microtubule will require two ATPs.

Rationale for kinesin’s cycle

While debates on the exact mechanochemical pathway (or pathways) of kinesin continue, is there a simple way to rationalize the mechanochemical coordination between the two heads? An intriguing possibility is that the symmetry of kinesin may provide a structural basis for the communication between the heads [16]. Most homodimeric proteins have an (approximate) axis of two-fold
symmetry due to the isologous interaction of the monomers [16]. This means that the two heads are related by a 180° rotation. Interestingly, the rat kinesin homodimer (with ADP bound to both heads; Figure 1) deviates more from symmetry than any other homodimeric protein, to our knowledge. Nevertheless, the heads are still related by rotation of ~120°, so binding both heads to a translationally symmetrical microtubule will require very considerable intramolecular strain when the heads are in the same nucleotide state [9]. It is therefore reasonable to hypothesize that, whenever both heads of kinesin are in the same nucleotide state, simultaneous binding to a microtubule will be difficult (i.e. a fluctuation large enough to reach such a highly-strained state will be rare).

It follows that the two heads of kinesin might only bind simultaneously to the microtubule if they are in different nucleotide states; conformational differences between the heads, presumably in the neck regions, might then facilitate the achievement of the required translational symmetry by a directed conformational change or a thermally induced fluctuation (e.g. Figure 2, transition from state 3→4). We suggest a simple symmetry rule that helps account for the mecanochemical path of the kinesin dimer shown in Figure 2: there are two classes of states, those with phosphate (ATP, ADP-Pi) and those without phosphate (nucleotide-free and ADP), and two-headed binding is only allowed when the heads are in different classes.

**Load-dependent steps**

To understand how motor proteins do mechanical work we need to resolve which of the motor’s chemical steps are associated with mechanical displacements, and determine the size of each displacement. Optical trapping experiments have revealed that kinesin makes 8 nm displacements (e.g. [49*]). But is the entire 8 nm displacement of kinesin accomplished within a single chemical step such as ATP binding? Or do several chemical steps each contribute incremental advances (substeps) that add up to 8 nm? The chemical steps associated with displacements will be slowed down by opposing external loads; these are the load-dependent steps. From a functional point of view, the important question is which steps are rate limiting (i.e. limit the speed) when the motor is challenged by a load?

What is known so far about the load-dependent steps of kinesin? Mechanical experiments are consistent with ATP binding being load dependent. The key observation is that at very low ATP concentrations, when ATP binding is rate limiting, kinesin slows down under even a small load. If ATP binding is irreversible (or if it is reversible, but followed immediately by an irreversible step such as hydrolysis) then this observation implies that the on-rate for ATP binding is load dependent. Alternatively, if ATP binding is reversible and is followed by rapidly reversible steps, then the load dependence could be distributed over any of these steps.

For example, in the scheme of Schnitzer *et al.* [41], the load dependence is attributed to a rapidly reversible isomerization of the ATP-bound state.

An estimate for the displacement associated with ATP binding can be made using Boltzmann’s law as follows. If the transition state for the ATP-binding step corresponds to moving the load through a distance $d$ relative to the nucleotide-free state, then application of an opposing force of magnitude $F$ will reduce the rate constant for the ATP-binding step by the factor $\exp(-Fd/kT)$, where $k$ is the Boltzmann constant and $T$ the absolute temperature. If this step is irreversable, then at low ATP concentration kinesin’s velocity can be modeled as $v(F) = v_0 \exp(-Fd/kT)$, where $v(F)$ and $v_0$ are the velocities of loaded and unloaded kinesin, respectively. In the low-load limit, the slope of the velocity–load curve is $-c_0 F/d kT$. This expression for the slope can be compared to measured velocity–load curves to extract a value for $d$. From velocity–load curves of kinesin measured at low levels of ATP using optical traps [49*,59,60] or glass microneedles [61] the slope at low load falls in the range $-c_0 F/d (2.5 \text{ pN})$ to $-c_0 F/d (7 \text{ pN})$, so we find that $d = 0.6–1.6 \text{ nm}$. Thus ATP binding (or steps that are in rapid equilibrium with the ATP-binding step) is associated with a conformational change of some 1–2 nm.

If ATP binding is associated with an advance of only 1–2 nm, is there evidence for additional load-dependent steps that are rate limiting? First, the shape of the velocity–load curves measured for kinesin are inconsistent with a single load-dependent step. All the velocity–load curves measured for kinesin measured at low levels of ATP using optical traps [49*,59–63] or glass microneedles [61] the slope at low load falls in the range $-c_0 F/d (2.5 \text{ pN})$ to $-c_0 F/d (7 \text{ pN})$, so we find that $d = 0.6–1.6 \text{ nm}$. Thus ATP binding (or steps that are in rapid equilibrium with the ATP-binding step) is associated with a conformational change of some 1–2 nm.

‘Substeps’ of kinesin’s 8 nm step might be detectable experimentally. By lowering the ATP concentration and raising the load, it might be possible to slow down the two different steps: an ATP-binding step and a second step with stronger load dependence. A plausible candidate for the second step is the release of the rear head from the microtubule (Figure 2, states 6→7). When the rates of the two steps are close, they will both be rate limiting, and in principle the displacements associated with both steps should be detectable with the typical time resolution of ~1 ms for an optical trap. Alternatively, the absence of substeps at low ATP levels and high load will indicate that ATP binding has the strongest load dependence.
Conclusions
Over the last few years, a general picture of how motor proteins move and generate force has emerged: small conformational changes in the nucleotide-binding pocket of the motor domain are amplified into large structural changes in the protein. For kinesin, the details of these conformational changes are becoming clearer. Biochemical, cryo-EM, and single-molecule experiments all indicate that a major conformational change takes place upon ATP binding to the attached head. This change permits the attachment of the tethered head to the microtubule and is associated with a movement of the load towards the plus end of the microtubule. Hydrolysis probably accelerates attachment of the tethered head in the course of kinesin motility. The attachment of the tethered head is a prerequisite for the release of products: first of ADP from the forward head, and then of phosphate from the rear head. The coupling between stepping and nucleotide hydrolysis is tight because the chemical steps are prerequisite for mechanical steps and mechanical steps are necessary for chemical steps. Further clarification of the conformational changes associated with kinesin motility may come from several directions, including a crystal structure of kinesin in the ATP state (comparison with the ADP structure may reveal kinesin’s ‘swinging lever arm’), direct measurement of the relative motions of the two heads while stepping, recording substeps, and measuring the elasticity of kinesin. Careful modeling that incorporates the available biochemical, biophysical, and structural data will also be important to further elucidate kinesin’s mechanism of motion.

Update
Single-molecule recordings on myosin II constructs with genetically engineered levers shows that the working-stroke scales with the length of the lever [66], providing strong support for the swinging lever-arm model. A recent study employing small-angle X-ray and neutron scattering provides strong evidence that the structure of dimeric kinesin in solution is consistent with the asymmetric conformation in the ADP crystal structure [67].

Cryo-EM of the microtubules decorated with dimeric kinesin-related proteins confirms that these proteins, like kinesin, attach by one head only. In the absence of nucleotides, the tethered head of Neurospora kinesin can occupy either one or two positions, leading the authors to suggest that either head of the asymmetric dimer can bind to the microtubule [68].

Acknowledgements
We appreciate comments on this manuscript from the members of the Howard Laboratory. We thank Linda Amos for a suggestion on Figure 1.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as: • of special interest ** of outstanding interest


FRET experiments report a nucleotide-dependent ~70° rotation of the myosin lever arm that is complementary to and consistent with previous crystallographic data. The authors provide evidence for two distinct ‘pre-stroke’ angles of the lever.


By comparing the biochemical cycles of one-headed and two-headed kinesin, the authors show that, in dimeric kinesin, release of the rear head is contingent upon attachment of the forward head. Evidence is provided that the rear head contains ADP·Pi when it is released. A mechanochanical pathway for kinesin is proposed that is consistent with a wide body of data.

12. Cryo-EM studies reveal nucleotide-dependent conformational changes in the neck linker of kinesin monomers bound to microtubules, consistent with earlier cryo-EM data on kinesin dimers. Spectroscopic analysis of kinesin monomers is inconsistent with existing data.
15. Vale RD, Milligan RA: The way things move: looking under the hood of molecular motor proteins. Science 2000, 288:88-95. A provocative comparison of potential motility mechanisms in kinesin and myosin. The authors make the astonishing hypothesis that the crystal structure of dimeric kinesin containing ADP in each head actually represents the conformation of the molecule in the ATP-bound state.
17. Myosin VI, which has a large insert in its converter region, moves in the opposite direction to all other characterized myosin motors.
19. Generally kinesin and dynein move in the opposite direction along microtubules.


The authors describe the development of a new surface-immobilization method for histidine-tagged proteins (the surfactant F108-NTA). This paper provides strong evidence that ncd is not processive.


Optical tweezers and a three-bead, suspended microtubule assay are used to confirm that ncd is not processive. ATP-triggered working strokes of single ncd motors at the end of ncd-microtubule binding events are resolved.


This is the first direct evidence that myosin V moves processively on actin filaments.


A detailed kinetic analysis of a monomeric construct of myosin V indicates that the rate-limiting step of the actin-activated ATPase is the release of ADP.


This study describes the detection of two-headed binding of Myosin V to actin filaments by electron microscopy.


The authors provide definitive proof that Myosin V is processive.


Single fluorescently labelled myosin V molecules move several micrometres along actin filaments before dissociating. Solution ATPase measurements in this study may be affected by tail inactivation of the motors.


This is a comprehensive biochemical and biophysical analysis of the nucleotide-dependent interaction between Neurospora kinesin and microtubules, illustrating essential consistency with conventional kinesin. It confirms a key aspect of the hand-over-hand model of processivity—that release of the rear head is contingent on attachment of the forward head.


The authors present cryo-EM maps of kinesin bound to microtubules in the presence of ADP. They propose a docking of kinesin-ADP onto the microtubule that is consistent with proteolysis and mutagenesis studies and that avoids steric clash between kinesin’s dimerization domain and the microtubule. They show that the same orientation is appropriate for the docking of ncdADP into a cryo-EM map of microtubules decorated with ncd in the presence of ADP.


The first report that a single-headed motor protein—a construct of KIF1A—can move processively. The motion is accounted for by a biased-Brownian movement model. The K-loop is proposed as the domain that maintains the motor’s association with the microtubule.


High resolution EM density map of kinesin-related KIF1A bound to a microtubule in the presence of AMP-PNP. Docking of atomic models and gold labelling suggest that the lysine-rich loop of KIF1A (which is absent from kinesin) forms a novel microtubule-binding site.


The authors characterize the nucleotide-dependent binding of the KIF1A K-loop mutants to microtubules. Proteolysis indicates that the binding partner of the positively charged K-loop is the negatively charged carboxyl tail of tubulin. Processivity of K-loop mutants increased with the number of positive charges in the K-loop.


The authors showed directly that each ATP hydrolyzed by kinesin results in a displacement of 8 nm, with a high probability in an 8 nm step.


This study provides strong confirmation of kinesin’s tight coupling using cysteine-modified motors: as the number of modified cysteines increased, the step size remains fixed whereas the motor velocities and maximum ATPase rates decline to the same extent.


An optical trap with a force-clamp provides evidence for two load-dependent transitions, and for tight coupling under loads up to ∼5 pN.

Inhibition of kinesin’s ATPase activity is relieved when artificial cargo is bound to the tail domain. Deletion of the tail activates the ATPase without the need for cargo binding. Inhibition is re-established by addition of exogenous tail peptide. 


Low angle X-ray and neutron scattering are employed to investigate the solution structures of several kinesin constructs of different lengths. For dimeric constructs similar in length to the dimeric construct that has been crystalized [9], the scattering patterns and the radius of gyration were in good agreement with the crystal structure.


