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Kinesin: world's tiniest biped

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Kinesin, an essential motor protein that moves intracellular cargo along microtubules, walks like a person. When we walk, our feet exchange roles with each step, one moving and one remaining stationary. The moving foot travels twice as far as our torso during a single step, and our body alternates between two configurations (left vs. right leg leading). Recent work shows that kinesin shares all three of these hallmarks of bipedal walking. The challenge now is to determine how the gait of this lilliputian biped is coordinated.

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Introduction: two models for kinesin movement

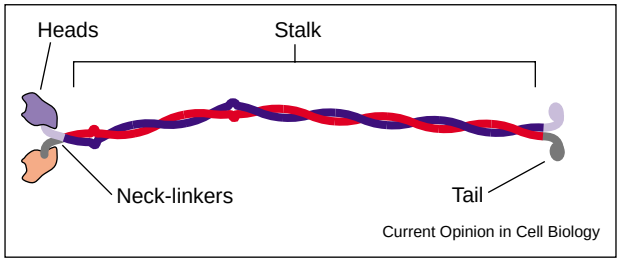
Kinesin is a motor protein that transports cargo across cells by moving along microtubule filaments. Establishing how kinesin moves is critical for understanding how it performs essential tasks in living cells. More generally, kinesin serves as an important model for how the structure of a protein determines its dynamics and function. The molecule consists of two identical polypeptide subunits that dimerize to form a rod-shaped, coiled-coil stalk, with a cargo-binding tail at one end and twin globular domains, usually called heads, at the other (Figure 1) [1]. Each head is a catalytically active ATPase that attaches to microtubules with nucleotide-dependent affinity. Once attached, kinesin advances stepwise over the microtubule surface lattice in 8-nm increments [2], hydrolyzing one ATP per step [3–5]. One molecule can generate hundreds of steps during a single encounter with a microtubule [6], even under load [7]. This remarkably high processivity indicates that at least one head stays attached to the microtubule at all times. To explain how kinesin moves without detaching, two broad classes of models have been invoked: hand-over-hand, where the two heads exchange roles with each step, and inchworm, where one head always leads. Here, I review the debate over which mechanism applies to kinesin, and summarize recent experiments providing strong evidence for a hand-over-hand mechanism. Important questions and discrepancies remain, and these are also discussed.

Coordination is required for highly processive motion

The movement of kinesin implies that its two heads do not act independently. To move along a microtubule without falling off [6,7], the motor requires at least two sites for attachment, so that one can maintain a grip while the other advances. Each kinesin head has one site for microtubule attachment [8], and both heads are required for highly processive motion [9,10] (but see also [11•]). Moreover, the motor steps unidirectionally [12,13] and follows a path parallel to the protofilaments in the microtubule lattice [14,15]. To achieve such a regular motion, the first head cannot release its grip until the second head has attached to a particular location on the microtubule. Thus, the heads must somehow communicate their states to one another to coordinate their attachment and detachment.

A popular hypothesis, consistent with a host of experimental observations, is that the two heads of kinesin work hand-over-hand, alternating in microtubule attachment and in ATP hydrolysis. In hand-over-hand models, the heads exchange leading and trailing roles with each step. The rear head detaches from the microtubule, moves into the front, and then re-attaches. Along with this mechanical alternation, the heads are also proposed to exchange catalytic roles. Strict catalytic and mechanical alternation gives hand-over-hand models a key feature, called 'head equivalence', that accords well with the homodimeric structure of kinesin. The structurally identical heads are functionally equivalent in the sense that they follow the same cycle of biochemical events, but out of phase from one another. That is, the heads do the same things, but not at the same time. The exact order of events is a matter of debate, but a plausible cycle for an individual head is as follows: stage one, ATP binding; stage two, hydrolysis; stage three, phosphate release; stage four, microtubule detachment; stage five, forward movement (by 16 nm); stage six, microtubule attachment; and stage seven, ADP release (followed by stage one again). (For discussions of the kinetic cycle of kinesin, see [16,17,18•].) Coordination would be lost if the heads traversed this cycle independently of one another, so additional constraints, discussed below, are necessary to ensure that they alternate in attaching to the microtubule, moving and hydrolyzing ATP. With these additional

Figure 1



The structure of a kinesin molecule. Two identical polypeptides (red and blue) dimerize to form a coiled-coil stalk, with a cargo-binding tail at one end, and twin globular heads at the other. Each head is a catalytically active ATPase that attaches to microtubules with nucleotide-dependent affinity. Short (~15 amino acid) polypeptide segments, called neck-linkers, join each head to the stalk.

constraints, the models correctly predict that each ATP hydrolysis corresponds to one 8-nm advance of the stalk [3–5]. The moving head travels 16 nm for each ATP hydrolyzed, while the other head remains attached. The stalk adopts the average position of the two, traveling only 8 nm.

Step equivalence implies rotation in hand-over-hand models

In addition to the functional equivalence of the heads, a subset of hand-over-hand models also include 'step equivalence', where all steps are generated in precisely the same manner. Put differently, step equivalence means the kinesin molecule goes through an identical sequence of conformations during every 8-nm advance. In hand-over-hand models with step equivalence, the moving head always passes on the same side of the attached head (Figure 2a, top view), and the whole molecule rotates during each step by 180° [1]. The rotation is in the same direction every time, either clockwise or counterclockwise. After each step, the three dimensional structure of the molecule is identical, except that the polypeptide subunits, and hence the two heads, have swapped places (Figure 2a). This subset of hand-over-hand models has been called 'symmetric', to emphasize the symmetry of head movements [19•]. The prediction that kinesin rotates as it moves may seem surprising, but there is precedence for it. Rotary motion is generated by other mechanoenzymes, such as the bacterial flagellar motor [20], and F_1F_o-ATP synthase [21]. Like these motors, kinesin's structure has rotational symmetry [22].

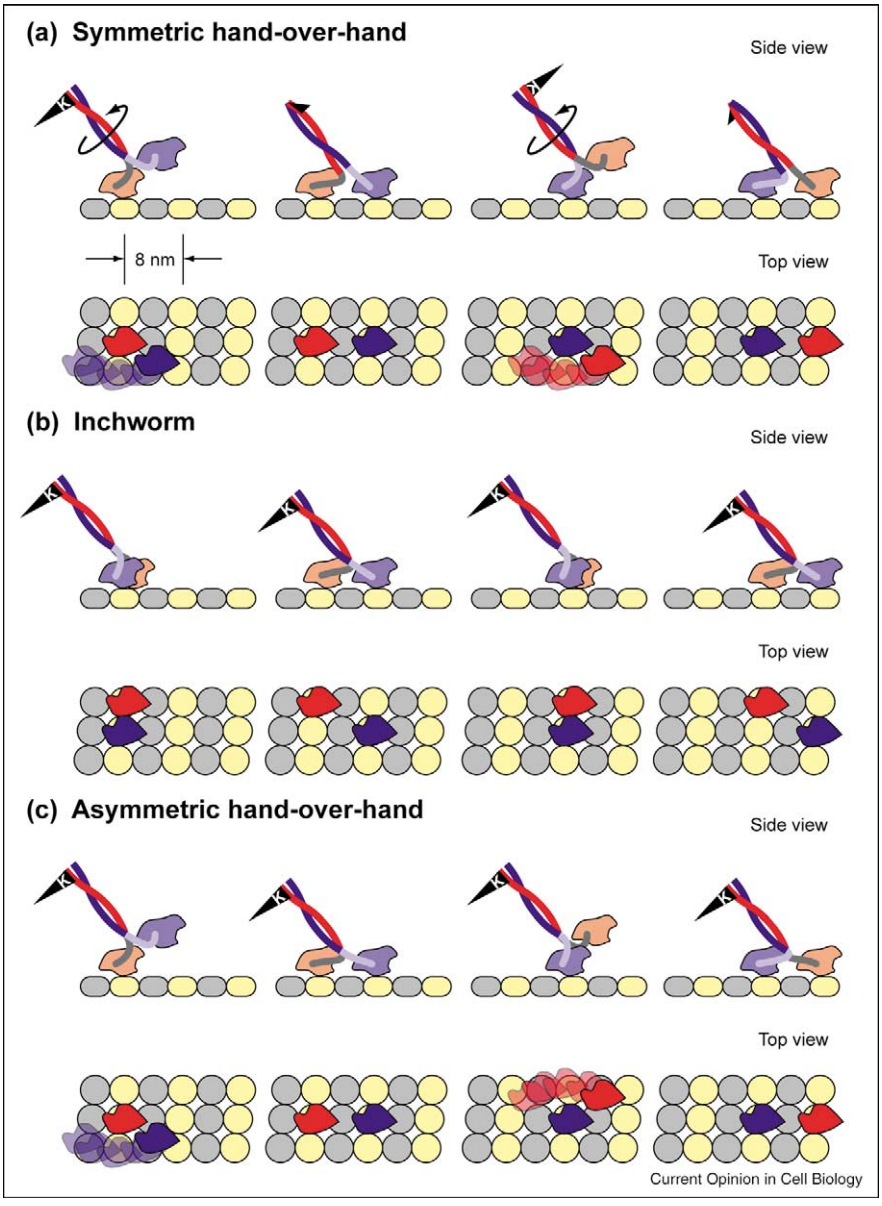
When gliding filament or bead assays are used to observe the motion of single kinesin molecules, the filaments or beads do not rotate, but this does not preclude symmetric hand-over-hand mechanisms. In gliding filament assays, microtubules in solution are pushed along by motors bound through their stalks or tails to a coverslip [6]. In bead assays, the motors are instead bound to micron-sized beads, which they carry along coverslip-bound microtubules [7]. Despite the absence of rotation in these single-molecule assays, symmetric hand-over-hand mechanisms were considered plausible, until recently [19•], because the motor-to-coverslip or motor-to-bead attachments allowed swiveling [1,19•]. With a swiveling linkage, kinesin could execute a symmetric hand-over-hand motion without transmitting torque to its cargo. In other words, kinesin could spin, and the bead or filament would not have to. Given uncertainties about the motor-to-cargo linkages in living cells, such a swivel could occur *in vivo* as well.

Rigid linkages show that kinesin does not rotate

A simple modification to the gliding filament assay forced kinesin researchers to consider the issue of symmetry, and caused a re-evaluation of the hand-over-hand dogma. Hua *et al* showed that a biotin–avidin linkage between the kinesin stalk and the coverslip creates a rigid motor-to-surface attachment that does not allow swiveling [19•]. Then they measured the orientation of gliding filaments moved by single, rigidly bound motors. The rigid attachment ensured that if kinesin executed a symmetric hand-over-hand movement, the spinning stalk would transmit torque to the filament and cause it to rotate 180° with each step. They worked at low nucleotide concentration so that stepping was very slow, leaving plenty of time between steps for the filament to rotate in response to the torque. No rotations were seen. Filament orientation never changed in any systematic way; it only fluctuated randomly over ~30°. On the basis of this result, the authors ruled out symmetric hand-over-hand models, and instead proposed an inchworm model [19•]. (Asymmetric hand-over-hand models were not ruled out, as discussed below.)

Inchworm models make the unorthodox prediction that the heads behave very differently from one another, but retain step equivalence, one of the attractive features of symmetric hand-over-hand models. In inchworm models, one head always leads, and each 8-nm advance of the motor consists of two rapid substeps. The leading head moves 8 nm and binds the microtubule, and then the trailing head moves the same distance (Figure 2b, top view). Only one head is proposed to be an active ATPase, to account for the finding that one ATP is consumed per 8-nm advance. The other head is a non-catalytic 'passenger' head. The heads do not swap roles after each 8-nm advance, so inchworm models predict no reorientations of the stalk. Even though the heads are decidedly not equivalent, the mechanism still has step equivalence. The whole kinesin molecule goes through an identical sequence of transitions during each 8-nm advance, and the three-dimensional structure of the molecule is identical after each advance (Figure 2b).

Figure 2



Candidate models for kinesin. Hand-over-hand models (a and c) predict that the heads are functionally equivalent, alternating in microtubule attachment, movement and hydrolysis of ATP. **(a)** In symmetric hand-over-hand models, the steps are also equivalent. The molecule goes through the same sequence of structural and biochemical transitions for every 8-nm step, and its configuration is identical after each step, except that the two polypeptide subunits, and hence the two heads, have swapped places. The moving head always passes on the same side of the attached head (a, top view), implying that the whole molecule rotates 180° with each step (a, side view, *arrows*) [1]. A fiducial mark on the red subunit (black pennant) emphasizes the predicted stalk rotation. **(b)** Inchworm models predict that the heads behave very differently, but that the equivalence of the steps is retained. One head always leads, and each 8-nm step is composed of two rapid substeps: the leading (blue) head detaches and moves 8 nm to its next attachment site, and then the trailing head (red) moves the same distance (b, top view). One head is predicted to be catalytically inactive, to account for the 1:1 coupling between ATP hydrolysis and stepping [3–5]. Even though the heads behave very differently, the steps are all generated in the same way. The molecule goes through the same sequence of transitions for every step, and its conformation is identical after each step [19•]. Because the heads do not swap places, the stalk does not reorient with each step. **(c)** The lack of stalk reorientation [19•] can also be accommodated by asymmetric hand-over-hand models, where the heads are functionally equivalent, but the steps are different. The heads follow the same sequence of biochemical transitions and gross-scale movements, but the moving head passes on alternate sides of the attached head (c, top view), and structures joining the heads to the stalk make compensatory movements to suppress the 180° reorientation that would otherwise occur when the heads swap places (c, side view). Recent work provides strong evidence that kinesin moves using an asymmetric hand-over-hand mechanism [23••–25••].

The experiment of Hua *et al* [19•] did not formally exclude all hand-over-hand mechanisms, but it did force a choice between head equivalence and step equivalence. The lack of stalk rotation can be accommodated by a class of hand-over-hand mechanisms termed 'asymmetric', with functionally equivalent heads but non-equivalent steps. Both heads can still undergo the same basic sequence of biochemical transitions and gross-scale movements (e.g. the hypothetical sequence above). But the moving head must pass on alternate sides of the attached head in successive steps (Figure 2c, top view), and structural elements joining the heads to the stalk must make compensatory movements to suppress the 180° reorientation that would otherwise occur when the two heads swap places (Figure 2c, side view). These are familiar features of our own bipedal walk, so it might seem natural to ascribe them to kinesin. Our feet swap places, but our hips articulate so that our bodies do not reorient with each step. Unlike our bodies, however, the structure of kinesin does not have mirror (bilateral) symmetry [22]. Kinesin does not have one left and one right foot; it has two left feet. Therefore, for kinesin to walk like we do, it must take two different types of steps, and the molecule must switch between two fundamentally different configurations after each step (Figure 2c). So, with the demonstration that kinesin does not rotate, researchers were faced with a choice. Some favored the head equivalence postulated by asymmetric hand-over-hand models, while others favored the step equivalence postulated by inchworm mechanisms.

New evidence for an asymmetric hand-over-hand mechanism

Recent experiments, performed in three independent labs and published nearly simultaneously, provide strong evidence that kinesin moves by an asymmetric hand-over-hand mechanism. Several hallmarks of this class of mechanisms were confirmed. First, the heads alternate in ATP hydrolysis [23••]. Second, the molecule switches between two different conformations after each step [24••]. Third, the heads advance in 16-nm increments, twice the distance moved by the stalk during each step [25••]. All three experiments relied on high-resolution tracking of single kinesin molecules, either with optical traps [23••,24••] or with single-molecule fluorescence techniques [25••].

An optical trap is essentially a very small, very bright light spot that traps micron-sized objects, such as glass or polystyrene beads, and exerts a restoring force whenever the object moves away from the center of focus. In the kinesin bead assay, a trap can be used to grab and place beads near immobilized microtubules [7]. When kinesin attaches to a microtubule it begins to move, pulling the bead. Tension supplied by the trap reduces thermally-driven (Brownian) motion to the nanometer level, so the bead follows the stepping motion of the motor stalk, advancing in discrete increments and dwelling at well-defined positions in between advancements [2].

While using optical traps to record the motion of native and recombinant kinesins, two groups independently found that some molecules limp along the microtubule, exhibiting a difference in the timing of every other step. The most severe limpers were heterodimers with one mutant head that hydrolyzed ATP more slowly than the other [23••]. The strict alternation in the timing of steps for these heterodimers implies that the heads alternate in ATP hydrolysis, which rules out inchworm mechanisms, where one head is catalytically inactive. Significant limping was also seen for homodimers, despite their identical heads [24••,26•]. Limping of these homodimers cannot be accommodated by any mechanism with equivalent steps, because identical conformations would produce identical timing. To generate a timing difference, the molecules must switch between two different conformations after each step, as predicted by asymmetric hand-over-hand models.

Using single molecule fluorescence techniques, a third team showed that kinesin's moving head travels twice as far as its stalk during each step [25••]. To track this motion, a fluorescent dye molecule was attached to an exposed cysteine on the surface of one head, but not to the other head. The position of the dye was then monitored as a labeled motor moved along a microtubule immobilized on a coverslip. With low background fluorescence and sufficient light collection efficiency, dye position can be located with nanometer accuracy in a fraction of a second [27]. With this method, kinesin head motions were tracked at low ATP concentrations, when the steps occurred about once per second. The individual heads advanced in increments of 16 nm, not 8 nm like the stalk, a finding that is inconsistent with inchworm mechanisms. Also, the timing of head movements confirmed that the labeled head paused after each advance, presumably to wait for the unlabeled head to advance.

Each of these experiments makes significant and separate contributions to the overall conclusion that kinesin moves using an asymmetric hand-over-hand mechanism. The trapping experiments were performed at high ATP concentrations, and with external load applied to the motor, while the fluorescence work addressed the low ATP, unloaded regime. The mechanism therefore seems to apply regardless of whether the motor speed is limited by the rate of nucleotide binding or by other mechanochemical events in the cycle. Also, the experiments used a variety of protein constructs from several species, so the conclusions are likely to apply to all highly processive, dimeric kinesins.

Puzzling results to consider

There is substantial agreement now that kinesin walks hand-over-hand. Still, there are some puzzling results to

consider, and fundamental questions about the mechanism remain unanswered. First, what causes limping of homodimeric kinesins? Limping is expected for the heterodimers that have been studied [23••,28], because these molecules were deliberately engineered to have one slow and one normal head. Homodimers, however, have no obvious structural asymmetry, so these limp for more subtle reasons. The available evidence suggests that mechanical stiffness of the coiled-coil stalk affects the severity of limping. A structural transition involving stalk movement is clearly implicated, because the degree of limping varies with the amount of force applied to the stalk [26•], and with the length of the stalk [24••]. Both effects can be explained by a dependence on stalk stiffness. Shorter filaments are more stiff than longer ones, all else being equal, and applied load also increases the stiffness of the molecule, as shown by the decrease in thermal fluctuations with increasing load in optical trap assays [2,29].

Properties of the stalk could influence step timing in various ways. First, in bead assays, a stiff stalk would efficiently transmit any asymmetry in the motor-to-bead linkage at one end to the heads at the other end. With an asymmetric linkage, for example, one head might tend to project away from the surface of the bead, while the other projects toward it. The head projecting toward the bead could require more time to reach its microtubule attachment site during a diffusional search, thereby causing a difference in the timing of every other step in situations where this search is rate-limiting. More compliant stalks will allow larger thermal fluctuations of the heads relative to the bead, reducing any asymmetric effect of the motor-to-bead linkage and lowering the severity of limping.

In a second scenario, limping is caused by over- and under-winding of the stalk during hand-over-hand motion [24••]. Coiled-coils are thought to have asymmetric torsional compliance because of their twisted (i.e. chiral) structure. Hence, the energetic barrier for over- and under-winding of the stalk should differ with the handedness of the winding. Compliant stalks will present lower torsional barriers. If such barriers affect the rates of head advance, they could cause a kinetic alternation that is less severe for compliant stalks.

In a third model, limping is induced by axial misregistration between the α-helices of the coiled-coil stalk [24••]. The two helices interact through periodic heptad repeats of hydrophobic amino acids that pack together in a snug 'knobs-in-holes' arrangement. They are usually assumed to dimerize in register, but this assumption has not been tested. Other coiled-coil proteins can adopt various packing arrangements [30], raising the possibility that misregistration occurs in the kinesin stalk as well. Misregistration by one heptad repeat would shift the heads relative to one another, effectively increasing the maximum neck-linker length for one head by ~1 nm (i.e. from 3 to 4 nm). The shift could cause one head to require more time to reach its microtubule attachment site in a diffusional search, as compared to the other head. In this case, the reduction of limping with increased stalk length can be explained if longer stalks are less likely to be misregistered. More experiments will be required to determine if any of these models supply the correct explanation for limping of homodimeric kinesins.

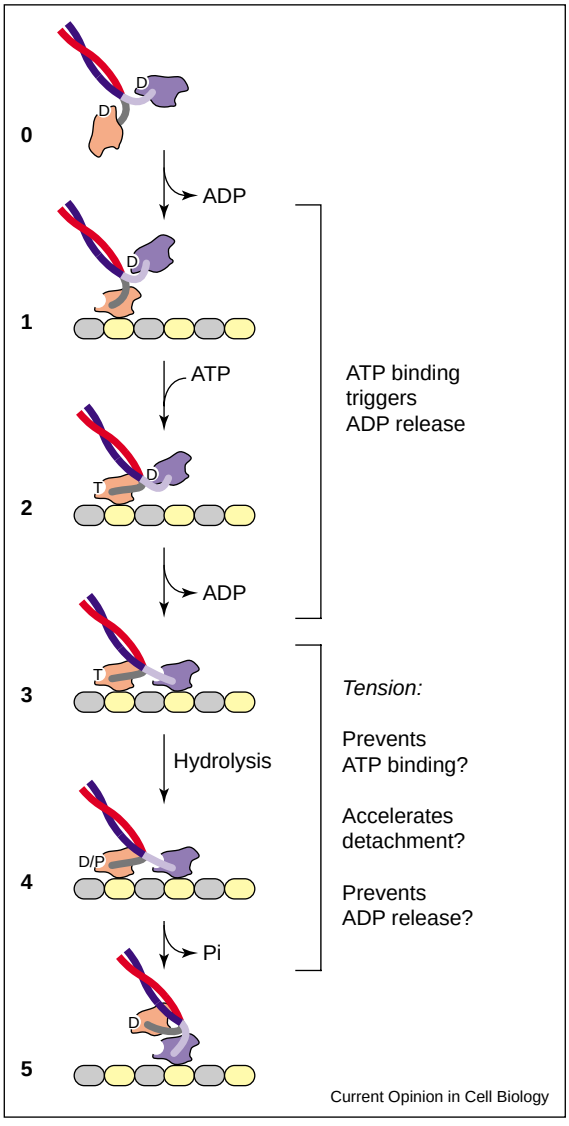
Another interesting observation may challenge the hypothesis of head equivalence. Kinesin constructs with a point mutation in both heads that reduces ATPase activity 700-fold or more do not produce movement in bead assays. Surprisingly, heterodimers with this mutation in only one of the two heads are capable of long-distance movement [31•]. This result seems paradoxical, and it could mean that highly processive motion is possible without alternating catalysis. However, an alternative hypothesis is that the defective ATPase activity of the mutant head is rescued by dimerization with a wild-type head. Some defect must be rescued in order to explain the motility of the heterodimers. ATPase rescue seems plausible, at least, because the structural changes caused by this mutation are very subtle, and the functional defect is very limited in scope. The mutation does not disrupt microtubule association, ADP release or coordination between the heads [32]. Whether or not the ATPase activity is rescued, this result raises fascinating questions about how one head can influence the activities of the other.

Fundamental questions remain unanswered

The question of how the two heads of kinesin coordinate with one another is arguably the most fundamental gap in our understanding of the mechanism of kinesin. It is clear enough that one head can influence several aspects of the other's behavior. The challenge now is to decipher how these influences are communicated between the heads, and how they lead to hand-over-hand motion.

The clearest demonstration of one head affecting the other is an example of negative cooperativity, where one head delays ADP release from the other. In the absence of microtubules, ADP binds tightly to both heads (Figure 3, state 0). Upon first encountering a microtubule, one head attaches to the filament and immediately releases ADP (Figure 3, state 1). The second head retains ADP, either staying detached from the filament or attaching only weakly, until the first head binds ATP [33–35]. This ATP-dependent ADP release may be driven by a conformational change in the neck-linker. Experiments with single-headed constructs show that the neck-linker undergoes a nucleotide-dependent, disorder-to-order transition [36]. In the presence of ADP, or when no nucleotide is present, the neck-linker is a disordered,

Figure 3



Possible forms of cooperativity between the two heads of kinesin. One clear example of negative cooperativity is ATP-dependent ADP release. In the absence of microtubules, ADP binds tightly to both heads of kinesin (state 0). Upon first encountering a microtubule, one head attaches and releases ADP, but the other head retains ADP (state 1) until ATP binds the first head. This ATP-triggered ADP release is thought to depend on a sequence of three events. First, ATP binding to the attached head causes a structural transition in the neck-linker, which brings the stalk, and hence the second head, close to the next attachment site on the microtubule (state 2). The close proximity promotes attachment of the second head, which then causes ADP release (state 3). Other forms of cooperativity, both negative and positive, may also be important. For example, in states where both heads are attached (states 3 and 4) the molecule may be strained. The resulting rearward tension on the front head (blue) could delay ATP binding until the rear head (red) detaches (state 5). Also, forward tension on the rear head may accelerate its detachment. In principle, these forms of cooperativity could provide the coordination required for hand-over-hand motion, and states 1–5 could represent the kinesin cycle during processive stepping. However, the roles of these forms of cooperativity during stepping are not well understood, and the order of events shown here is speculative. (Pi denotes inorganic phosphate. D, T, and D/P indicate ADP, ATP, and ADP+Pi in the catalytic site, respectively.)

flexible tether emanating from a point on the back of the head. When a head attaches to a microtubule in the presence of ATP analogs (AMPPNP, ADP-AlF_x), its linker becomes immobilized on the surface of the head, and the distal end of the linker points toward the front of the head. On the basis of this finding, a model for the full two-headed motor has been proposed where immobilization of the neck-linker on one head moves the stalk, and therefore the second head, forward and closer to the next attachment site on the microtubule lattice [36] (Figure 3, state 2). This in turn promotes microtubule attachment of the second head, which triggers ADP release (Figure 3, state 3). Consistent with the model, point mutations that disrupt neck-linker dynamics also prevent ATP-dependent ADP release [36].

Neck-linker dynamics may drive ATP-dependent ADP release when kinesin first encounters a microtubule, but the role of the neck-linker during processive stepping is uncertain. Its dynamics have only been directly observed using constructs that lack the stalk and the second head, which are incapable of processive movement. In the full, two-headed motor, the position of the neck-linker and its relationship to the particular nucleotide in the active site may be different from what is found in one-headed constructs [16]. Also, the free-energy change associated with neck-linker immobilization is small [37], so even moderate load applied to the stalk should prevent its immobilization. Kinesin can still generate steps under such loads, suggesting that neck-linker docking may not be an essential part of its mechanism. On the other hand, cross-linking the neck-linker to the head has a dramatic effect on motility [38], suggesting that neck-linker movements are somehow essential.

Additional forms of cooperativity must occur

By itself, ATP-dependent ADP release is not sufficient to coordinate the heads. As an example, consider the situation after ATP-induced ADP release from the second head, which is presumably in front (Figure 3, state 3). Without further constraints, the front head could bind ATP, so that both heads contain ATP. If hydrolysis and phosphate release then occurs in both heads, both could simultaneously detach, without completing a single 8-nm step. Some additional constraint is necessary to ensure that detachment of the rear head is contingent on attachment of the front head.

At least two other forms of cooperativity have been proposed, and these could provide the additional constraint necessary to fully coordinate the heads. After ATP-induced ADP release, the nucleotide-free front head is delayed in binding ATP [39•], suggesting another type of negative cooperativity. The delay in ATP binding persists, at least in one mutant, even after hydrolysis and phosphate release have occurred in the other head [18•]. On the basis of this result, a model has been

proposed where ATP binding to the front head is contingent on detachment of the rear head from the microtubule [18•,39•]. This hypothesis, in combination with the hypothesis of ATP-triggered attachment of the front head described earlier, could fully coordinate the motor, as follows. ATP binds to the attached head only when the other head is detached, causing the detached head to move into the front position, whereupon it attaches to the microtubule and releases ADP. With the motor in this two-heads-attached state, the front head cannot bind ATP, even though its active site is empty. Once hydrolysis and phosphate release have occurred in the rear head, it detaches, which relieves the block on the front head and completes the cycle. If complete detachment of the rear head is required to relieve the block, then under sufficiently low ATP concentrations the motor will dwell in a state with only one head attached to the microtubule. This prediction is apparently at odds with evidence suggesting a two-heads-attached waiting state [25••], but the discrepancy can be resolved with one change to the model: complete detachment of the rear head is not required, but rather a transition from a strongly bound to a weakly bound state is sufficient to allow ATP binding to the front head, which must then trigger complete detachment of the rear head, and movement of the rear head into the front. ATP-triggered detachment of the rear head is an example of positive cooperativity, which has also been suggested by experiments with one-headed kinesins. For some one-headed kinesin derivatives, spontaneous detachment from the microtubule is too slow to account for the rate of detachment during processive stepping of the two-headed motor [40]. If the same spontaneous rate applies to the rear head during processive stepping, then detachment of this head must be somehow accelerated by the front head [10,16,40,41•].

Mechanical tension could provide a signal through which the heads influence one another, but evidence for this is so far indirect. The heads can be detached from microtubules with externally applied force, and the unbinding force is lower for forward load (i.e. toward the plus-end of the microtubule) than for backward load (toward the minus-end) [42]. Also, ADP binds more tightly to the heads when forward load is applied [43•]. These two findings lend support to the hypothesis that the front head causes detachment of the rear head, and prevents the rear head from releasing ADP, by pulling forward on it. Furthermore, the affinity of the heads for ADP can be reduced by applying backward load [43•]. Assuming the affinity for ATP is similarly affected, this suggests that the rear head prevents ATP binding to the front head by pulling backward on it. Inter-head tension is expected when the molecule adopts a two-heads-attached state, because significant distortion of the crystal structures is required to orient the two heads for simultaneous attachment to the microtubule [44]. Unfortunately, the amount of tension, and hence the plausibility of this model, is difficult to predict. Coordination through stereo-specific interactions between the heads, as occurs in other allosteric enzymes (e.g. hemoglobin), is unlikely for kinesin, because the heads share few points of contact in crystal structures [22], and the neck-linkers that join them are often disordered.

Conclusions

Our understanding of kinesin continues to advance very rapidly. To some extent, progress is driven by technological improvements. The motion of single kinesin molecules and sub-domains within these molecules can now be followed in real time using advanced microscopy techniques. Specific amino acid substitutions and fluorescent tags are also easily added using modern methods of molecular biology and protein engineering. These methods have allowed us to determine many details about the mechanism of kinesin, including the recent confirmation that its two heads probably work hand-over-hand. We do not understand how the heads are coordinated, but new techniques may help uncover the salient details. Techniques allowing simultaneous observation of mechanical and biochemical events at the single molecule level [21] will be particularly useful.

Apart from technological improvements, the study of kinesin and other motor proteins has advanced because of the widespread interest these molecules inspire. Motor proteins are interesting not only to cell biologists and biophysicists, but also to engineers, nanotechnologists and even televangelists (!) [45•]. Kinesin is especially fascinating because of its small size, its very high processivity, and now, because its motion bears a resemblance to the way we walk. More importantly, kinesin and kinesin-like proteins are involved in fundamental cell processes, including mitotic spindle formation, chromosome dynamics, and vesicle and organelle movement. By elucidating how kinesin moves, we gain insight into the exquisite dynamics of living cells. Taking a broader view, the study of kinesin offers rare, direct insight into fundamental questions about how a protein's structure determines its dynamics and function.

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