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Kinesin: walking, crawling or sliding along?

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Kinesins are microtubule-based motor proteins that are involved in cargo transport and mitosis. They are called 'motors' because they convert chemical energy to mechanical energy (i.e. force and motion). They use the energy of ATP hydrolysis for their enzymatic processes by walking on microtubules. However, the mechanism underlying their motion has been unclear. Recently, conventional kinesin, which was the first-identified member of the family, has been shown to walk by swapping its two heads in a 'hand-over-hand' mechanism. There is also experimental evidence supporting an asymmetric walking of kinesin in which two identical heads of the motor take alternate slow and fast steps. Other cargo-carrier and mitotic kinesins remain uninvestigated and are of great interest to biophysicists.

Kinesins and organelle transport

Kinesin constitutes a large motor-protein superfamily (referred to as the KIF family) [1] (Box 1) that transports cargoes within a cell by moving on microtubule filaments. The genome-sequencing project has revealed that there are 45 different kinesins in humans [1]. The catalytic core of the protein (often referred to as the motor domain) is the only conserved region (~50%) [2]. It is used to identify which genes belong to which kinesins. Diversity of the genes corresponds to different working mechanisms, structure and cargo-binding affinities. KIFs are separated into three major classes according to where the motor domain is on the peptide sequence: N terminus, C terminus or middle of the amino acid sequence (M kinesin) [3]. Of the 45 kinesins, there are only three C-terminal and three M kinesins, the rest being N-terminal [1]. N-terminal kinesins move towards the microtubule plus ends, whereas C-terminal kinesins move towards the minus ends [3]. (The plus ends of microtubules are oriented towards the cell periphery; the minus ends are oriented towards the nucleus.)

Figure 1a shows the structure of conventional kinesin. Each monomer consists of an N-terminal motor head, a neck linker, a long coiled-coil dimerization region and a globular tail domain [4]. The active form of conventional kinesin is a dimer in which the coiled-coil regions of two monomers are wound around each other to form a common stalk [5]. The motor regions are responsible for binding to the microtubule and to nucleotide [6,7], and each head is connected to a neck linker, which is a mechanical element that undergoes nucleotide-dependent conformational changes [8]. Neck-linker docking and undocking creates the powerstroke [9] and determines the directionality of the motor movement [8,10–12]. The neck linker is, in-turn, connected to a common stalk that leads to the globular tail domain [13]. The kinesin light chain (KLC), localized at the tail region, modulates the cargo-binding affinity of the motor [7]. Inhibition of KLC function results in impaired axonal transport and release of kinesin from

Box 1. Kinesins in action

The prototypical member of the KIF superfamily, conventional kinesin [55,56], is abundant in nerve cells [3]. It has a motor region at the N terminus and, hence, moves cargo towards the cell membrane [2]. Humans have three genes for conventional kinesin [1]. *KIF5B* exists in many tissues and it transports cargoes associated with endoplasmic reticulum, mitochondria, Golgi complex, lysosomes and endosomes [3,13,57,58]. *KIF5A* and *KIF5C* are expressed only in nervous tissue, in which they transport membranous organelles towards axons [59,60]. Mutations in neuron-specific kinesins have been linked to neurological diseases in humans [2].

KIF1, KIF3 and KIF4 families are other N-terminal kinesins involved in cargo transport [1]. KIF1A and its *Caenorhabditis elegans* homolog Unc104 are the fastest kinesins ($\sim 2\ \mu m\ sec^{-1}$) that transport synaptic vesicles towards axons in neurons [34,61]. Interestingly, it exists as a monomer in solution [34] and, presumably, dimerizes *in vivo* to transport the cargo over long distances [42]. In *KIF1A*-knockout mice, a decreased number of synapses and synaptic vesicles were observed [62]. KIF1B, another member of the KIF1 family, functions as a monomer and transports mitochondria from the cell body to axons [63]. KIF1C is dimeric and is involved in endoplasmic reticulum and Golgi transport [64]. KIF3A/B (referred to as kinesin II) has two nonidentical motor heads [65]. It is involved in embryonic development, melanosome transport and Golgi trafficking [66–70]. *KIF3A/B*-knockout mice could not develop cilia and they displayed diseases linked to impaired intraflagellar transport and embryo development. KIFC2 is the only C-terminal kinesin that transports vesicles and membranous organelles towards the minus end in dendrites [71].

Other kinesins have a major role in cell division. Formation of the bipolar structure of the mitotic spindle, transport of chromosomes towards the equator of the spindle, chromosome segregation and poleward transport of chromosomes after segregation are among the many duties of various kinesins in mitosis [44]. For example, chromokinesin, a member of the KIF4 family, binds to DNA as its cargo and carries segregated chromosomes towards the opposite poles [72,73]. KIF2, which is a family of M kinesins, can bind to the end of microtubules and destabilize them [43]. By shrinking the length of microtubules, the KIF2 MCAK is thought to mediate the poleward motion of separated chromosomes [74]. Ncd is a C-terminal kinesin that forms a pole in centrosome-free spindles (i.e. female meiosis) by binding to one microtubule from the tail region and walking towards the minus end on the other microtubule [75].

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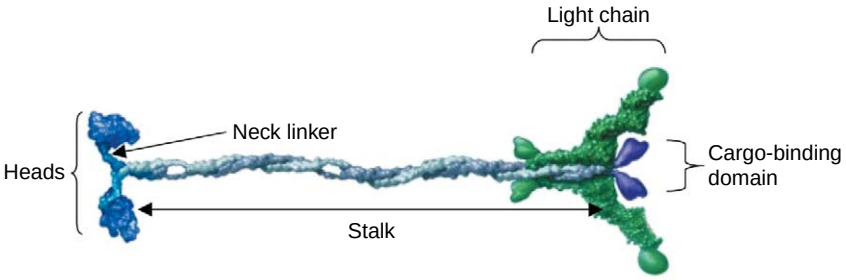

Figure 1. Structure of conventional kinesin. Conventional kinesin is a homodimeric motor protein that walks processively along microtubules. The motor heads bind to microtubules and ATP. Each head is connected to a flexible neck linker that enables motor stepping. The neck linker is connected to a 70-nm long coiled-coil stalk (body) that holds two heads together. At the end of the stalk, kinesin has a cargo-binding domain that recognizes membranous organelles and vesicles. The distance between the heads is 8.3 nm – approximately the distance between adjacent tubulins. Reproduced, with permission, from Ref. [2].

membrane vesicles [14]. Moreover, in the absence of cargo, the kinesin stalk is bent in such a way that KLC blocks the movement of the head region. Presumably, this prevents futile hydrolysis of ATP [15,16].

Cargo transport and processivity

Kinesins have an important role in intracellular cargo transport (Box 1), and loss of function of these proteins causes several diseases, including deafness, pigmentation, left–right asymmetry defects, sensory problems and neurological disorders, in humans and other organisms [17]. The most striking example of specific and fast cargo transport, which is essential for survival, is axonal transport. Neurons are extremely thin and can be up to 1 m in length. Because the axon does not include a ribosome, all of the proteins required for the axon and the synapses must be transported from the cell body. Transport of a membranous vesicle to the axon would take several years by diffusion [2], which is obviously too long for a cell. Kinesins haul these cargoes and travel these long distances in the order of minutes by moving along the microtubule. Transport of cargo requires a highly processive motor that can undergo many catalytic ATP cycles before fully detaching from the track.

From biophysicists' perspective, the central issue is to determine how the chemical and mechanical steps are coupled so that the motor moves in a coordinated manner. The conformational change in the motor region caused by ATP hydrolysis is converted to a net displacement. Most of the processive kinesins are two headed, one of which is required for detaching from the microtubule, moving forward and attaching to the next binding site for a net movement. At the same time, the other head should be bound to the track to prevent complete dissociation of the motor. To remain on the track, each of the heads should spend at least 50% of its ATP turnover bound to its respective track. The working stroke of the motor should also be long enough to reach the next binding site [18].

In the past decade, single-molecule studies have revealed that kinesin takes 8.3-nm steps per ATP hydrolyzed, which is equal to the distance between adjacent tubulins [19–22]. The motor can complete ~100 ATP turnovers and walk 800 nm sec^{-1} [20]. A single powerstroke of the motor can generate a force of ~6 pN [20].

The ATP cycle has also been analyzed; biochemical studies show that the two heads are strongly coordinated so that binding of the second head accelerates dissociation of the already bound head [23]. The kinesin head binds to a microtubule in its ATP-bound state and detaches in the ADP-bound state. ATP binding causes the neck linker to be docked pointing towards the plus end of microtubules. This conformation mediates plus-end-directed movement of kinesin by pulling the other head forward [8]. Authors of earlier studies combined these findings to fit into a simple walking mechanism called the 'hand-over-hand' model [9,24].

Hand-over-hand model

In the hand-over-hand model, kinesin moves in much the same way as humans walk. The rear head (head 1) takes a step while the front head (head 2) remains stationary on the microtubule (Figure 2b). Head 1 attaches to the next tubulin-binding site and becomes the leading head. Thus, kinesin moves along the microtubule by alternating the positions of the heads. This means that, if the centroid position of the motor moves 8.3 nm, the rear head must move 16.6 nm and the front head must not move at all (in humans, when the left foot takes a step, the right foot is stationary). In the next step, head 2 moves forward and becomes the leading head again while head 1 stays fixed to the track. Therefore, each of the heads alternately takes 0-nm (i.e. it is stationary) and 16.6-nm steps, and the cargo is moved 8.3 nm in each case.

The simplest example of the hand-over-hand model is a 'symmetric' model, meaning that the motor reverts to the exactly the same 3D conformation after each step [25]. This would enable each head to repeat the ATPase cycle starting from the same physical condition and to create identical steps. This assumption requires the stalk region to undergo half a revolution when kinesin swings the rear head. Figure 2b shows two possible ways for kinesin to walk in a symmetric hand-over-hand fashion. In the first, the rear head always passes the stalk from the same side, presuming that the stalk rotates 180° along the same direction every step. Because the tail region is fixed when kinesin is attached to the cargo, rotation along one direction would overwind the stalk. After several steps, the torsional barrier would prevent kinesin from walking [26]. In the second, the rear head alternately passes the stalk from the right side and then the left side, presuming that the stalk is rotated 180° back and forth every other step. First, kinesin winds its stalk by half a revolution and then the rear head passes the stalk from the other side to

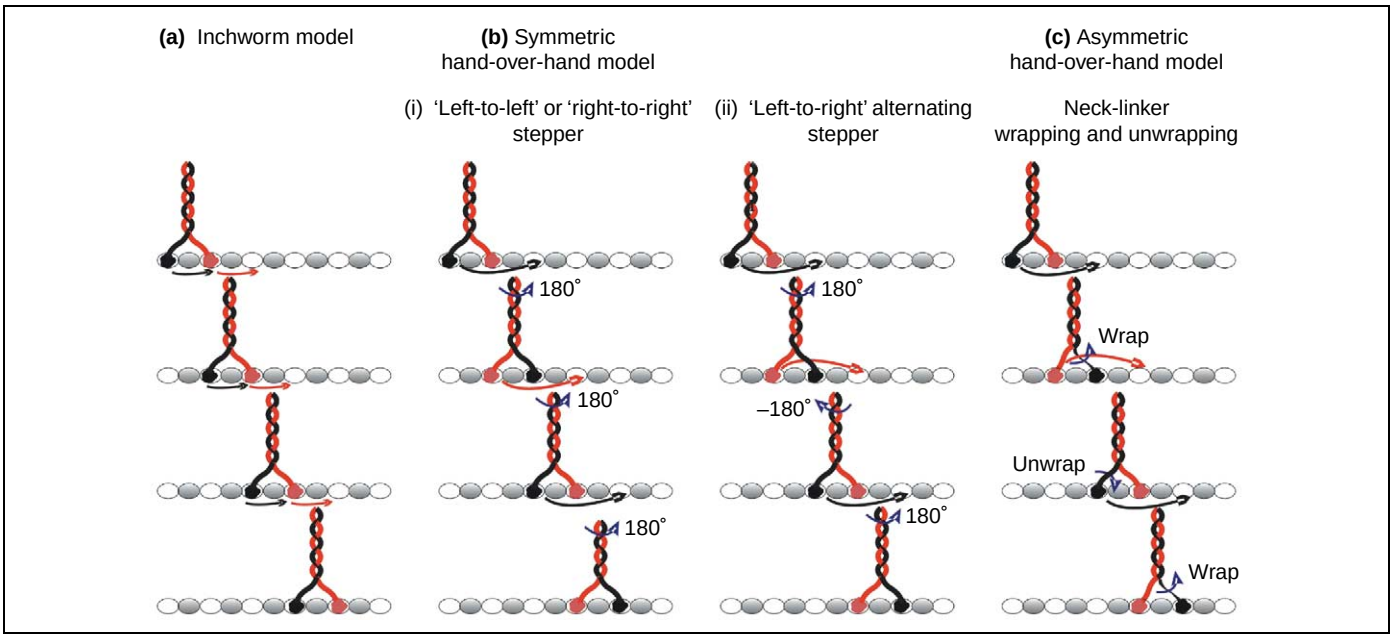

Figure 2. Stepping models of conventional kinesin along microtubules. **(a)** The inchworm model states that both of the heads move 8.3 nm per ATP hydrolyzed. The motor reverts to the same conformation without requiring the stalk to rotate. **(b)** In the symmetric hand-over-hand model, the rear head moves forward while the front head stays bound to the microtubule. To revert to same physical state, kinesin should rotate its stalk 180° every step. Two possible ways of symmetric hand-over-hand motion. (i) The rear head always steps from the left or right side (called 'left-to-left' or 'right-to-right' stepper, respectively). This requires the stalk to rotate constantly along one direction. (ii) The two heads alternately pass the stalk from the right and left side (called 'left-to-right' stepper). In this case, kinesin should swivel its stalk 180° clockwise and counterclockwise every other step to walk symmetrically. **(c)** Asymmetric hand-over-hand motion is a 'left-to-right' stepper that alternates between two physical states. To avoid rotating the stalk (or twisting, in the case of cargo transport) kinesin might alternately wrap and unwrap one of its two neck linkers (black) every other step. In this case, the two heads take a step that starts from different physical conformations and that would cause kinesin to move asymmetrically.

release the tension. Therefore, kinesin can take alternating right and left steps, and carry the cargo without being prevented from moving after several steps.

To test rotation of the stalk, Gelles and colleagues performed a microtubule-gliding assay [27] (Figure 3a). In this experiment, the tail regions of kinesin motors were fixed on a glass surface. When a single kinesin translates the microtubule, the torsion would rotate the microtubule instead of twisting the stalk. If more than one kinesin were attached, the microtubule would not be able to rotate. The assay was performed at low motor concentration and some microtubules displayed a pivotal movement, indicating that a single kinesin was attached. Importantly, these microtubules did not show 180° rotation either along the same direction or back and forth (Figure 3b). To explain this result, the authors proposed a new mechanism that was referred to as the 'inchworm model'.

The inchworm mechanism suggests that only one head is catalytically active. In this model, one head always leads and the other head follows (Figure 2a). It is a symmetric model in which the motor can revert to the same state after each step without rotating the stalk. Hence, it is a symmetric model. It is, however, not clear how the motor moves both of its heads forward by hydrolyzing a single ATP. It should be noted that the authors did not rule out an asymmetric hand-over-hand model in which the stalk undergoes less than half a revolution per cycle or does not show rotation at all (Figure 2c).

Hand-over-hand versus inchworm

Following the work by Gelles and colleagues, several groups presented evidence almost simultaneously that supported hand-over-hand motion. These experiments clearly excluded the possibility of an inchworm-type movement.

(i) Kaseda *et al.* [28] performed a motility experiment with a heterodimeric construct in which one head catalyzed ATP 18 times slower than the other head did. The experiment was designed to test whether both of the heads catalyze ATP, as in the hand-over-hand model, or whether only a single head does so, as in the inchworm model. If kinesin walks hand-over-hand, the construct would be expected to move by alternating between slow and fast dwell periods because each head would move every other step. The speed of the construct, which is limited by the slow head, would be 9 times (18 ÷ 2) slower than wild type. However, the inchworm model suggests a slow-walking kinesin and a fast-walking kinesin, depending on the position of the mutant head, but no alternating dwell times. In a single-molecule optical-trapping assay (Box 2), the authors observed alternating short and long dwell periods during which the kinesin stalk took 8.3-nm steps. The overall speed of the motor decreased nine times compared with the speed of wild-type kinesin. The data are completely consistent with the hand-over-hand model and they disprove the inchworm model.

(ii) Asbury *et al.* performed an optical-trap assay with homodimeric kinesins that had stalks truncated at different positions [29]. Under high load (~4.5 pN), kinesins truncated near the motor domain limped; the motor alternated between fast and slow motion, much like the observations that Kaseda *et al.* made. The limping behavior shows that kinesin uses its heads alternately, which is in agreement with the hand-over-hand model. Remarkably, the authors observed limping by using non-mutated (except for the short stalk) homodimeric kinesin. This

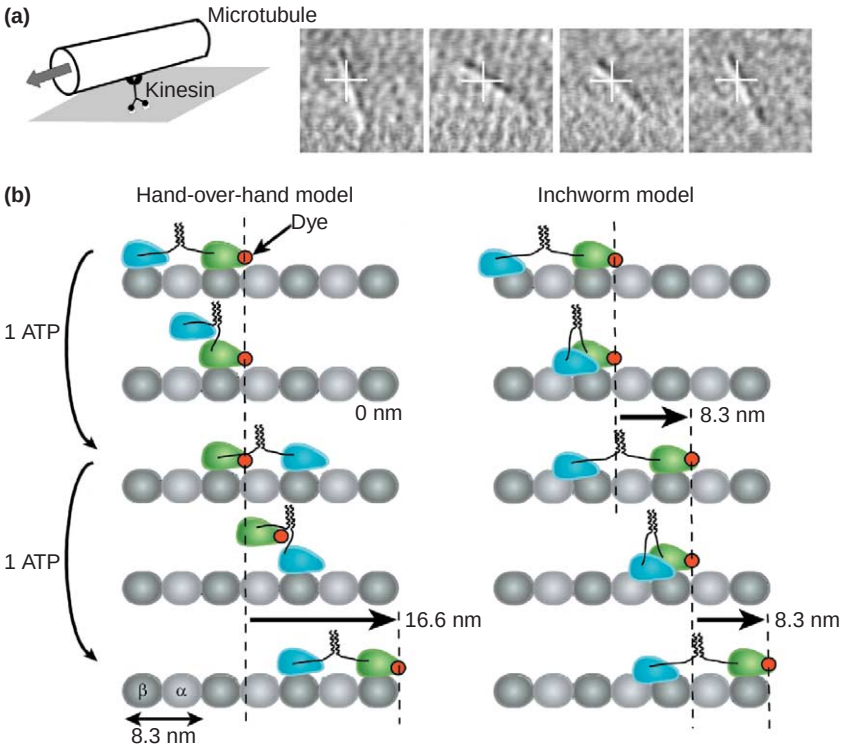

Figure 3. Neck-rotation measurements of kinesin proposed an inchworm model. **(a)** Microtubule-gliding assay. The assay was performed to determine whether the stalk is rotated half a revolution every step, as in a symmetric model. When a single kinesin translates the microtubule (left), the symmetric model predicts that the microtubule rotates 180°. However, the microtubules were moved without any rotation (right). Based on these results, the inchworm model was proposed to explain the motion of kinesin. Reproduced, with permission, from Ref. [27]. © (2002) AAAS (www.sciencemag.org). **(b)** Stepping patterns of an individual head in hand-over-hand versus inchworm models. In the hand-over-hand model of kinesin motility, the rear head moves forward 16.6 nm while the front head stays stationary. Therefore, kinesin walks by swapping its heads, resulting in alternating steps of 16.6 nm and 0 nm for each head. In the inchworm model, only the front head is catalytically active: the rear head follows. Both heads move 8.3 nm in each step. Reproduced, with permission, from Ref. [31]. © (2004) AAAS (www.sciencemag.org).

result differs from that of Kaseda *et al.* because, in the latter, the kinesin construct was designed to limp. The experiment has additional implications for the processive motion of conventional kinesin that will be discussed later.

(iii) The most direct way to understand how kinesin moves is to visualize the motion of an individual kinesin head. However, this requires the nanometer localization of a small probe placed on the head to distinguish whether the head takes an 8.3-nm or a 16.6-nm step as kinesin walks. Recently, it has been shown that organic dyes can be localized to within a few nanometers while enabling continuous monitoring of the molecular motor for minutes [30]. The technique has been named fluorescence imaging with one-nanometer accuracy (FIONA) (Box 2). Originally, it was used to show that myosin V walked hand over hand [30].

In this experiment, the hand-over-hand model predicts that an individual head would take alternating 16.6-nm and 0-nm steps, whereas the inchworm model predicts uniform 8.3-nm steps (Figure 3b). The head of a 'cysteine-light' kinesin was labeled with a single Cy3 dye. Kinesin was then made to walk on surface-immobilized microtubules. Because the temporal resolution of this method is several-hundred milliseconds, limiting amounts of ATP were used to measure the individual steps taken by the kinesin head. The head moved 17.4 ± 3.3 nm, which is two times the centroid step (8.3 nm). Furthermore, a kinetic analysis showed that kinesin moves its heads with alternating steps of 0 nm and 16.6 nm. The results directly show that kinesin walks in a hand-over-hand manner rather than using an inchworm mechanism [31].

Symmetric versus asymmetric hand-over-hand models

The experiments discussed previously prove that kinesin walks in a hand-over-hand, not an inchworm, motion. The combination of the work by Gelles' group with the other results indicates that an asymmetric hand-over-hand model is an attractive mechanism for explaining the processive motion of kinesin. In the asymmetric model, kinesin alternates between two distinct conformations as it moves. This occurs if the motor does not twist its stalk 180° every step. The model is called asymmetric because the beginnings of two successive 8.3-nm steps are physically different.

Hoenger *et al.* proposed an asymmetric model to avoid twisting the stalk or rotating the cargo every step [26]. They suggested that the trailing head can move forward alternately from the right and the left side of the stalk. The left step should follow a distinct pathway from the right step if there is no stalk rotation. The authors proposed a model in which the neck linker alternates between two physical structures to minimize the build up of torsional strain in the stalk region. According to the Hoenger model, the neck linker first wraps around the stalk and then unwraps during the second step (Figure 2c). When the neck linker wraps around the stalk, it is harder for the trailing head to move forward because of the torsional

Box 2. Nanometer-precision, single-molecule techniques

Optical traps

In optical traps [76], dielectric beads are decorated by motor proteins and trapped by focused a laser beam. The trap functions as a spring and, after several steps, the force applied to the bead stalls the motor because it exceeds the force generated by the powerstroke. 1-μm-sized beads can be trapped with piconewton levels of force, providing sub-nanometer precision within 1 ms. Such traps were used to detect the stepwise motion of motor molecules [20,77,78]. Applying a constant force to a motor by force-feedback control [79] provided tracking of motor movement over longer distances.

Cantilever probes

The attachment of a glass microneedle or atomic-force microscope (AFM) tip to protein and DNA complexes can also achieve nanometer-range spatial resolution, and manipulates single molecules by applying forces [76,80]. However, they are less compliant than optical traps, and only the most compliant probes can achieve sufficient sensitivity to observe the forces exerted by single molecules.

Single-pair fluorescence resonance energy transfer (spFRET)

Closely spaced fluorophores can transfer their energy to each other through dipole–dipole interaction. Excitation of the donor molecule whose emission is in resonance with the absorption of the acceptor yields the emission of an acceptor. The energy transfer depends on the distance (R) between the donor and the acceptor molecule with R^6, which makes energy transfer highly sensitive to distance changes between the donor and the acceptor. Labeling of two sites of a protein with a FRET pair yields its conformational dynamics, including open and closed states or docking and undocking mechanisms, with several Angstroms of resolution for the distances between 2 nm and 10 nm [81].

FIONA

In light microscopy, a point-like fluorescent object cannot be observed any clearer than at ~250 nm in the visible region of light because of the diffraction limit. The position, however, can be localized with arbitrarily high precision by determining the center of the diffraction-limited spot [30]. This precision can be achieved by collecting a sufficient number of emitted photons. It has been shown experimentally that single organic dyes can be localized to within a nanometer. This technique was used to determine the translational motion of the individual heads of processive motors [30,31,82].

barrier. As the neck linker unwraps, this barrier is removed and the motor can take a faster step. Consequently, switching between wrapped and unwrapped states implies that kinesin, despite having two identical heads, moves by alternating slow and fast steps.

As mentioned, Asbury *et al.* [29] performed an optical-trap assay under constant load and observed that homodimeric kinesin walks asymmetrically. Because the ATP concentration was saturating, the speed of the motor was determined solely by the catalytic rate of the heads. Because the two heads are identical, each head would be expected to move with the same speed if kinesin walked symmetrically. However, Asbury *et al.* observed that kinesins with a truncated stalk limped – one kinesin head took a step faster than the other did, which is indicative of an asymmetric type of walking. The limping factor (the ratio of even and odd dwell periods) was 6:1 for the constructs in which the stalk was truncated near the motor domain. The experiment indicates that the limping is caused by the difference in geometry between the even and odd steps. Basically, the motor does not necessarily revert to exactly the same state after each step; instead, it does so every other step.

Surprisingly, kinesins with a full-length tail did not limp to a significant degree. The authors truncated the stalk region in many positions and observed that the limping factor was inversely correlated to the length of the stalk. This was counterintuitive; the general idea is that the motor head and neck linker, not the tail, are responsible for motor stepping. The conclusion is that the observed asymmetry cannot be directly explained solely by switching between two physical states. The function of the length of the tail remains to be explored.

The authors proposed several explanations for how the asymmetry depends on the stalk. One possibility is that kinesin twists its stalk 180° back and forth every step – a symmetric model – but the optical-trap bead limits this motion. Rotating the stalk would cause winding and unwinding of the stalk because the tail region is fixed (the optical-trap bead restricts the rotation at the end of the stalk because the bead cannot respond to the instantaneous rotation). It is more difficult and, hence, slower to take a step when the stalk is overwound. For this reason, the bead can bias the motor asymmetrically, particularly when the tail is too short. In full-length kinesin, however, the rotation of the stalk can average out on the long coiled-coil region. Therefore, no limping should be observed. Based on this explanation, although it contradicts Gelles' result [27], one could speculate that kinesin indeed walks symmetrically and that the observed limping is due to the experimental conditions. Nevertheless, the experiment is important because it shows that kinesin is more likely to move in an asymmetric hand-over-hand manner.

A collaboration between the Higuchi and Endow laboratories has recently carried out an experiment similar to that performed by Asbury *et al.* [32]. They reported that wild-type kinesins did not limp to a significant degree, consistent with the observations of the Block group [29]. Although the limping factor increased slightly in correlation with the applied load, under a high load (7 pN) it was less than a statistically significant value. To study the limping of a homodimeric construct further, Higuchi *et al.* made a point mutation that was expected to open the nucleotide-binding cleft for rapid nucleotide binding and release. They observed that mutant homodimers presented a severe limping that correlated with the applied load. Under no load, the construct moved like a wild-type kinesin (no limping) [32]. The presence of limping in the mutant homodimers, but not in wild type, suggests that the mutation enhanced a load-sensitive step in the ATPase cycle of kinesin. Again, the results are in agreement with asymmetric-type walking.

How do unconventional kinesins walk?

Kinesins are diverse in structure so that they can function in cells as monomers, dimers and tetramers. They might use different mechanisms from the hand-over-hand motion. However, other kinesins have not been studied as well as conventional kinesin. The next section is a summary of recent findings about four unconventional kinesin motors.

KIF3A/B

KIF3A/B (kinesin II) is a heterodimeric kinesin involved in intraflagellar transport and Golgi trafficking. It has two distinctive heads (head A and head B). To characterize the motion of these non-identical heads, Zhang *et al.* created chimeric constructs with two identical heads (KIF3A/A and KIF3B/B) [33]. The speeds of these constructs were compared with those of wild type by performing a microtubule-gliding assay. KIF3A/B was observed to be a processive motor. It moves more slowly ($200\ nm\ sec^{-1}$) than conventional kinesin but travels longer distances (3.6 μm) without dissociating. KIF3B/B moved at two times the speed of wild type, whereas KIF3A/A moved five times slower than KIF3A/B. The results indicate that both of the heads (head A and head B) affect the speed of KIF3A/B, which is in agreement with the hand-over-hand motion. The relationship between the speeds of the mutants and those of wild type, however, cannot be directly accounted for by alternating steps of the two heads. If head A catalyzed ATP ten times slower than head B did, the speed of KIF3A/B would be limited by the slow head. Although KIF3A/B was expected to move two times faster than KIF3A/A (similar to the relationship observed by Kaseda *et al.*), it moved five times faster. The results indicate that there is coordination between the two heads so that the fast head (B) speeds up the rate-limiting step of the slow head (A).

Unc104/KIF1A

Unc104/KIF1A is a monomeric cargo-carrier kinesin [34]. The Hirokawa laboratory reported that KIF1A, a mouse ortholog of Unc104, moves processively along surface-immobilized microtubule filaments *in vitro* [35]. Although the motion of wild-type KIF1A was highly variable, a chimeric construct in which the KIF1A head was followed the neck linker of conventional kinesin moved constantly to the microtubule plus end. The observed movement of the construct was not unidirectional; instead, it was relatively oscillatory. The motor took forward and backward steps but the overall movement was towards the plus end. An optical-trapping assay with a wild-type motor also showed that a single KIF1A motor was sufficient for net movement of a bead under a load of 0.15 pN [36].

To explain the oscillatory motion of KIF1A, the authors suggested that it undergoes biased Brownian diffusion along microtubules [37]. In this model (Figure 4a), KIF1A is weakly anchored to the microtubule by electrostatic attraction, providing free movement along the microtubule. Brownian motion is biased so that the motor takes more steps towards the plus end than towards the minus end. The Hirokawa laboratory then presented structural evidence to support their model [38,39]. By obtaining crystal structures of the KIF1A-head–microtubule complex under different nucleotide conditions, the authors showed that kinesin uses the energy of ATP hydrolysis to switch alternately between the two microtubule-binding sites (L11 and L12). The conformation of the head on the first binding site (L11) that is pointed towards the plus end provides the bias. ATP binding detaches L11 and brings the second loop (L12) into contact with the microtubule. L12 provides a flexible tether that enables the motor to undergo 1D Brownian motion along microtubules without dissociation. Therefore, ATP hydrolysis is not used for the mechanical step; instead, it modulates active detachment and attachment of the head to the microtubule. The mechanical step is achieved through the microtubule-binding energy of the head.

Arguably, this mechanism cannot explain how KIF1A rapidly transports the cargo within a cell. The observed net motion was eight times slower than speeds reported previously *in vivo* and *in vitro* [34,40]. Increasing the number of attached Unc104/KIF1A motors to the cargo achieved smooth processive motion in both optical-trap and *in vitro* cargo-transport assays [36,41]. The continuous movement of cargo (or bead), however, was not observed where attached motors were dispersed. These observations raise the possibility that the motor can dimerize *in vivo* to transport the cargo processively. The dimerization would be reversible depending upon the motor concentration and it would have a regulatory role in the biological function of the motor (Figure 4b).

To test whether Unc104/KIF1A dimerizes for smooth and fast movement, the Vale laboratory artificially created dimeric forms of Unc104 [42]. The authors showed that the constructs displayed smooth processive motion that was as fast as the *in vivo* speed of Unc104. The reversible dimerization of Unc104 was also tested by increasing the motor concentration in solution [42]. In previous single-molecule fluorescence measurements, an extremely low concentration of motor was used to separate the fluorescent spots from each other. The addition of an excess amount of non-labeled motor mixed with a small amount of fluorescently labeled motors resulted in fast and smooth Unc104 motion. This was probably due to dimerization of Unc104 at high motor concentration. Consequently, all of these measurements present a counterexample to the biased-diffusion model and present strong evidence in favor of *in vivo* dimerization of Unc104. It is possible that many monomers can carry a cargo simply by cooperation. However, a single dimerized motor is more efficient and can achieve maximal speed of transport. The mechanism underlying the motion of Unc104/KIF1A is still controversial, and which model accounts for the biological activity of the motor needs to be elucidated.

Mitotic centromere-associated kinesin

Mitotic centromere-associated kinesin (MCAK) is a dimer that has a motor domain located in the middle of the polypeptide chain. It binds to the end of a microtubule and depolymerizes the filament [1]. During cell division, MCAK depolymerizes the microtubules that are attached to chromosomes [43]. This activity is thought to help the poleward movement of chromosomes after segregation [44]. Recently, it was shown that MCAK searches for the ends of the microtubule by diffusing along the microtubule lattice [45]. It attaches to the end of the filament with high affinity and processively depolymerizes tubulins in an ATP-dependent manner at a rate of $20\ s^{-1}$ [45]. The issues of how the motor removes tubulin subunits from the filament and remains on the track are unclear.

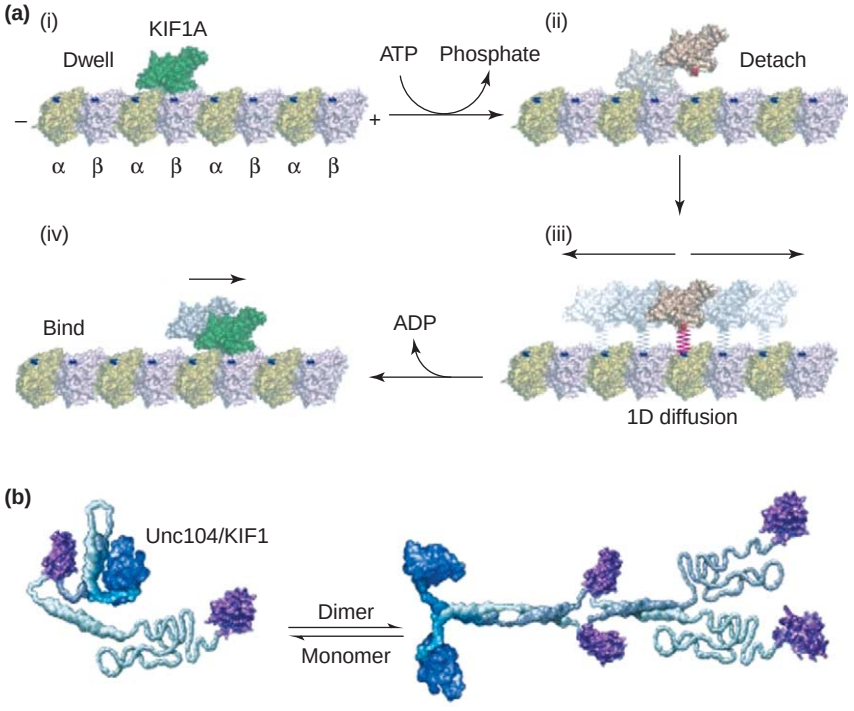

Figure 4. Alternative mechanisms of Unc104/KIF1A movement. **(a)** Biased Brownian motion. (i) The KIF1A head stays bound to the microtubule in the rigor state, which is pointed towards the plus end. (ii) ATP binding and hydrolysis releases the strong microtubule-binding site of the KIF1A head (loop 11) and (iii) the head immediately releases the phosphate, which brings the second binding site (loop 12) close to the microtubule. The weakly anchored state of KIF1A prevents the motor from detaching while the head is free to diffuse along the microtubule lattice. The plus-end-directed conformation in (i) provides the bias to the motor, and the KIF1A head is more likely to take a step towards the plus end. (iv) The head releases ADP and binds strongly to the microtubule. Reproduced, with permission, from Ref. [36]. **(b)** Reversible dimerization. Increasing the concentration of the motor results in unidirectional processive movement of Unc104 motors towards the plus end of the microtubule. This indicates that the motor might dimerize reversibly *in vivo* in a concentration-dependent manner. Transport of cargo by a dimerized motor is faster and more efficient than cooperative transport by many monomers. Reproduced, with permission, from Ref. [42]. © (2002) AAAS (www.sciencemag.org).

BimC

BimC is a bipolar homotetrameric kinesin that has two heads on each side [46,47]. In mitosis, it grabs the antiparallel microtubules in the equator of the cell. BimC then slides the microtubules apart by walking on both microtubules towards the opposite directions [48]. This mechanism determines the distance between the poles, and mutations produce exceptionally long mitotic spindles [49,50]. Whether the four heads work in a coordinated manner or whether the two heads on each side walk independently on microtubules is an unresolved issue.

Concluding remarks

Single-molecule experiments have recently shown that conventional kinesin walks hand over hand. Optical-trapping assays showed that several kinesin constructs walked asymmetrically. However, neither full-length wild-type kinesin nor kinesin constructs under no load presented limping behavior. It is not clear whether the observed asymmetry is inherent to motor stepping or due to experimental conditions. The bead might limit the rotation of the stalk and force the molecule to walk asymmetrically. To exclude this possibility, rotation of the stalk when kinesin is not attached to a cargo should be studied. Such asymmetric models are attractive because the motor does not need to rotate its cargo within the cytoplasm, and many motors can work together more easily using this mechanism. This is important because a single kinesin can travel only several microns, whereas the cargo can be transported more than 1 m. Therefore, many kinesins must carry the cargo cooperatively and prevent its detachment from the microtubule. However, many motors could also work together using the symmetric mechanism if the 180° rotation were averaged out by twisting the long stalk instead of rotating the cargo.

Also, little is known about how unconventional kinesins function in a cell. A recent study of kinesin II showed that this motor probably walks hand over hand [33]. Unc104/KIF1A was shown to be a single-headed processive motor [35] that is reminiscent of myosin VI and IX [51,52]. However, it probably dimerizes *in vivo* and walks using head-to-head coordination, in the same way as conventional kinesin [42]. Whether many monomeric motors transport the cargo or whether the motor dimerizes *in vivo* to transport the cargo efficiently at high speeds remains controversial. Elucidating the mechanisms of mitotic kinesins can help to determine how the cell divides properly into two. For example, information about how much force is produced by each mitotic motor will help the understanding of how the mitotic spindle forms and how chromosomes are aligned at the center of the spindle.

Loss of function of each motor is linked to neurological, sensory or genetic disorders. Mechanistic information about the motors will help to unravel complex biological systems such as mitosis and vesicle trafficking. This knowledge could also be used to recover disfunctional motors in clinical trials. Moreover, knocking out the activity of a motor can also be used to cure some diseases [2].

For example, inhibition of mitotic kinesins can be used to slow down the uncontrolled division of carcinogenic cells or tumor activity. Inactivation of a cargo-transporting motor might be used as a tool against viruses that hijack the cytoskeletal transport system [53,54]. Clinical applications, however, require a deeper understanding of the mechanism and regulation of the motor proteins.

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