Kinesin motors as molecular machines

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Summary
Molecular motor proteins, fueled by energy from ATP hydrolysis, move along actin filaments or microtubules, performing work in the cell. The kinesin microtubule motors transport vesicles or organelles, assemble bipolar spindles or depolymerize microtubules, functioning in basic cellular processes. The mechanism by which motor proteins convert energy from ATP hydrolysis into work is likely to differ in basic ways from man-made machines. Several mechanical elements of the kinesin motors have now been tentatively identified, permitting researchers to begin to decipher the mechanism of motor function. The force-producing conformational changes of the motor and the means by which they are amplified are probably different for the plus- and minus-end kinesin motors.
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Introduction

The microtubule motor protein kinesin, also known as conventional kinesin, was identified in 1985 [1,2] as the motile force underlying movement of particles along the microtubules of the giant axon of the squid.[3–5] Kinesin was shown to be capable of binding to microtubules and, in the presence of ATP, of moving towards the fast polymerizing/depolymerizing plus ends of microtubules,[6] representing the first cytoplasmic microtubule motor protein to be discovered. Conventional kinesin is a tetramer of two heavy chains, each consisting of a motor domain, coiled-coil dimerization domain and tail,[7] and two light chains, which are thought to bind to membranes associated with cellular vesicles or organelles (Fig. 1).[8] Essential features of the motor domain include highly conserved nucleotide- and microtubule-binding motifs, which enable the motor to generate force and move along microtubules.[9] Conventional kinesin is the founding member of the kinesin family—together with related proteins that display a high degree of sequence identity to its motor domain, these proteins constitute the kinesin family of microtubule motor proteins. Kinesin motor proteins have been found in all eukaryotes examined to date, including the protista, fungi, invertebrates, animals and higher plants.[10,11] The number of kinesins identified to date in genomes that have been fully sequenced and at least partially annotated varies from 6 in budding yeast to 19 in *C. elegans*, 24 in *Drosophila*, 45 in humans and 61 in *Arabidopsis*.[12,13]

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Cellular function

Conventional kinesin and other members of the kinesin family bind ATP and microtubules at specific sites in their conserved motor domain, and use the energy from ATP hydrolysis to produce force and move along microtubules. The nonmotor region of the motor protein is believed to interact with other proteins or cellular components, enabling the motors to perform essential roles in vesicle and organelle transport, spindle function and chromosome motility, and regulation of microtubule dynamics (Fig. 2).

The large number of kinesin proteins in many organisms has given rise to the idea that different kinesin proteins could bind to specific vesicles or organelles and transport them between cellular compartments. The adaptor or receptor proteins that couple kinesin motors to proteins associated with membrane-bounded cargo have recently begun to be identified using genetics, yeast two-hybrid screens, and co-precipitation by antibodies. Several of the adaptor or receptor proteins identified so far are components of large complexes that may include other receptor and signaling proteins, e.g., the NR2B subunit of the NMDA neurotransmitter receptor,[14] the AP-1 adaptor complex,[15] amyloid precursor protein[16] and JNK signaling pathway interacting proteins.[17] Transport of specific signaling proteins to particular targets within the cell may be important in regulating their activity. It is furthermore possible that the signaling proteins themselves could be important in regulating the motors that transport them. The identification of these proteins represents a breakthrough in understanding the cellular function and regulation of the kinesin motors.

Besides their roles in vesicle and organelle transport, a large number of kinesin proteins have been implicated in

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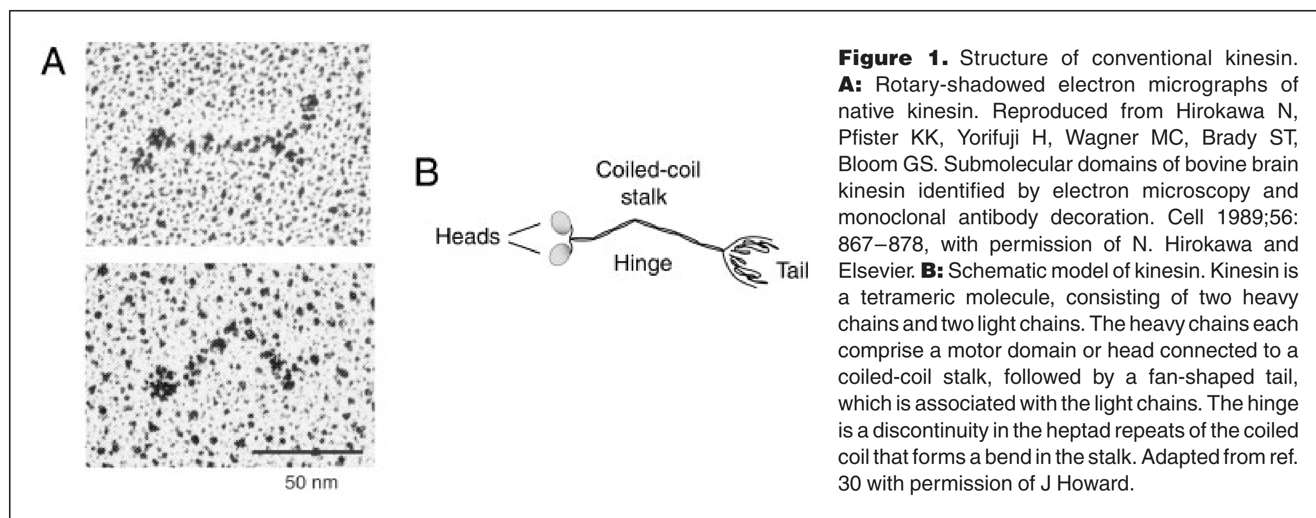

Figure 1. Structure of conventional kinesin. **A:** Rotary-shadowed electron micrographs of native kinesin. Reproduced from Hirokawa N, Pfister KK, Yorifuji H, Wagner MC, Brady ST, Bloom GS. Submolecular domains of bovine brain kinesin identified by electron microscopy and monoclonal antibody decoration. Cell 1989;56: 867–878, with permission of N. Hirokawa and Elsevier. **B:** Schematic model of kinesin. Kinesin is a tetrameric molecule, consisting of two heavy chains and two light chains. The heavy chains each comprise a motor domain or head connected to a coiled-coil stalk, followed by a fan-shaped tail, which is associated with the light chains. The hinge is a discontinuity in the heptad repeats of the coiled coil that forms a bend in the stalk. Adapted from ref. 30 with permission of J Howard.

chromosome distribution by their localization to the meiotic/mitotic apparatus or mutant effects on spindles or chromosomes (Fig. 2).[18–20] The motors can bind to and crosslink spindle fibers and use energy from ATP hydrolysis to move directionally along microtubules, performing essential roles in spindle assembly and maintenance, centrosome duplication, and attachment of centrosomes to poles. Several of the kinesin motors are associated with chromosomes and may play a role in mediating chromosome attachment to the spindle[21] or congression to the metaphase plate.[22] The identification of microtubule motors as components of the mitotic apparatus represents a major advance in understanding the forces that underlie chromosome and spindle dynamics during mitosis and meiosis.

Unexpectedly, some kinesin microtubule motor proteins have been found to destabilize or depolymerize microtubules, providing a link between regulation of microtubule depolymerization and assembly, and force-producing proteins associated with the spindle and chromosomes.[23–25] Kar3, a motor required for karyogamy, or nuclear fusion following mating in yeast, has been shown to destabilize microtubules in vitro, causing them to shorten in motility assays, presumably by depolymerizing.[23] *kar3* mutants cause microtubules to become very long in vivo, evidence that its microtubule-destabilizing activity is an essential part of Kar3 function in the cell.[26] MCAK, a chromosome-associated kinesin protein required for mitosis, has been shown to depolymerize microtubules in vitro and is needed to regulate microtubule length in live cells.[24,27] The mechanism by which MCAK and related proteins depolymerize spindle fibers is not certain, although a model has been proposed that involves bending of protofilaments at microtubule ends by the motor, causing them to circularize and be released as tubulin rings (Fig. 2).[28] This mechanism of microtubule disassembly does not require ATP hydrolysis by the motor and can be observed in the presence of the nonhydrolyzable ATP analogue, AMP · PNP. Strikingly, MCAK also possesses an ATP-dependent depolymerase activity that acts catalytically and has been proposed to remove tubulin dimers processively from both ends of the microtubule.[29]

Molecular motor function

A major unanswered question in the motors field is the mechanism by which motors convert energy from ATP hydrolysis into work, enabling the proteins to bind to and move along microtubules or actin filaments. Answering this question will be essential to understand how the motors generate force to transport vesicles and organelles along cytoskeletal fibers and contribute to forces in the spindle during mitosis and meiosis. Remarkably, motor proteins hydrolyze nucleotides and translocate along a filament, converting chemical energy from ATP hydrolysis *directly* into work without undergoing an intermediate heat or electrical conversion step, as do man-made machines.[30,31]

How do motors capture the energy released by nucleotide hydrolysis and turn it into work? Motor proteins are thought to couple steps of ATP hydrolysis to force-producing conformational changes and changes in binding affinity for their filament to move along microtubules or actin filaments. A prevailing idea is that ATP binding, hydrolysis, or product release induces conformational changes in the protein that, under load, create strain.[30] The strain drives movement of the load, and the movement, which is referred to as a working stroke of the motor, relieves the strain. Several working strokes, corresponding to different chemical steps, can occur within one ATPase cycle.[32] The coupling between nucleotide hydrolysis and steps by the motor may differ for different motors—conventional kinesin takes one step along its filament for each

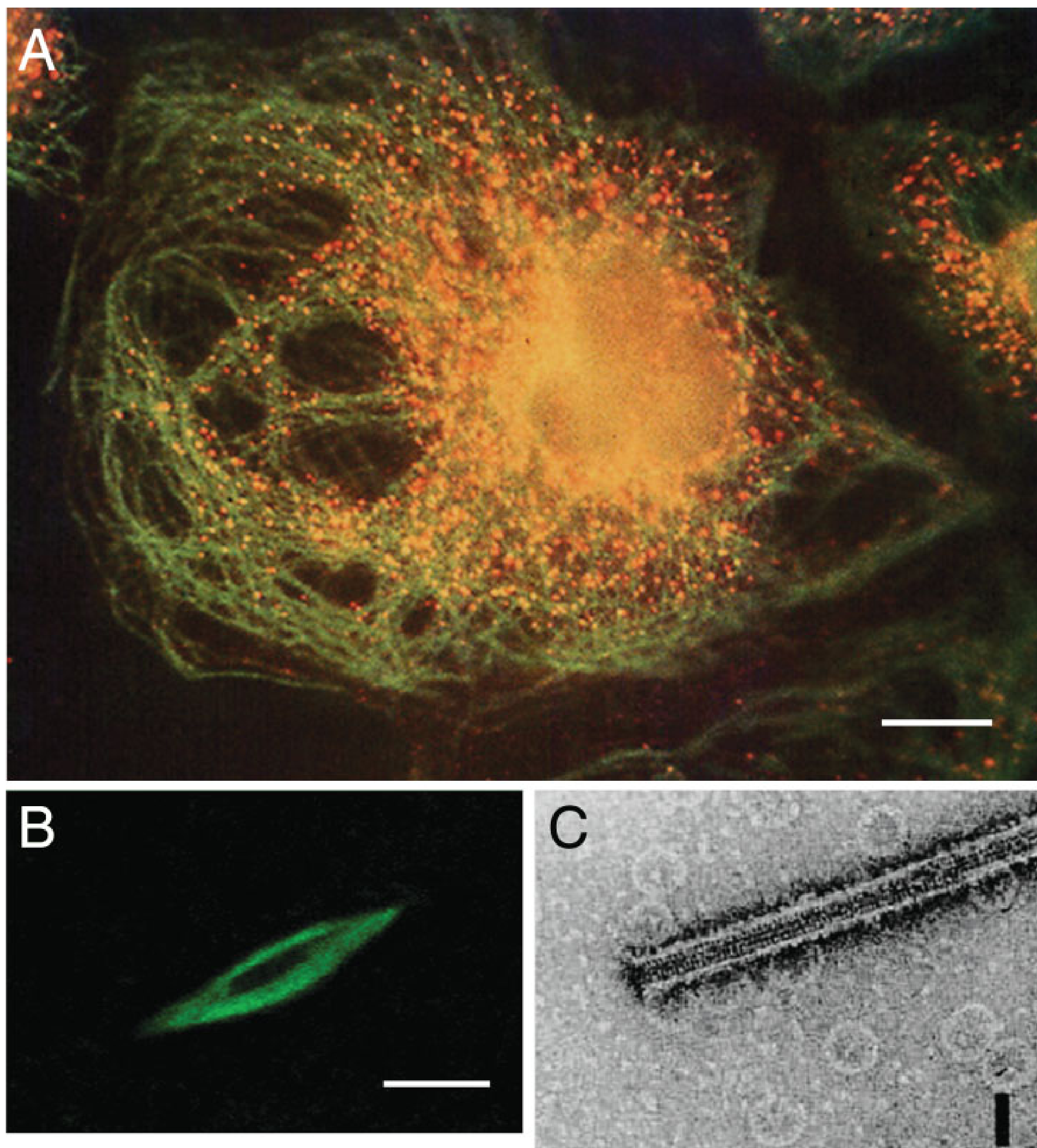

Figure 2. Kinesin motors at work. **A:** Moving membranes or vesicles. PtK_1 vertebrate cultured cells stained with a monoclonal antibody to kinesin heavy chain (red) show staining of a post-ER, pre-Golgi system of membranes known as the intermediate compartment (microtubules, green). Kinesin is thought to be the motor for moving these membranes from the Golgi to the ER. Image provided by G Bloom. Bar, $\sim$5 μm. **B:** Spindle assembly. Ncd, the minus-end kinesin motor of *Drosophila*, is required for normal assembly of meiotic spindles in oocytes (Ncd-GFP, green). The Ncd motor is needed to form bipolar spindles in the acentriolar oocytes, presumably by crosslinking and bundling microtubules and moving to microtubule minus ends. The dark region in the center of the spindle corresponds to the metaphase-arrested meiosis I chromosomes. The Ncd motor also functions in mitotic spindles of early embryos. Bar, 5 μm. **C:** Depolymerizing microtubules. pKinI, a kinesin motor found in *Plasmodium falciparum*, disassembles microtubules at their ends, forming rings from single protofilaments in the presence of the nonhydrolyzable ATP analogue, AMP · PNP.[28] By contrast, the KinI motor, MCAK, catalytically depolymerizes microtubules in the presence of ATP, which has been proposed to occur by release of tubulin dimers from microtubule ends.[29] Bar, 40 nm. Reproduced from Moores CA, Yu M, Guo J, Beraud C, Sakowicz R, Milligan RA. Molec Cell 2002;9:903–909 with permission of R.A. Milligan and Elsevier.

ATP hydrolyzed.[33,34] Thermal fluctuations are expected to play an important role in the motor mechanism, either by driving diffusional movements of the motor to its next binding position[35] or by promoting diffusive structural changes that drive force-producing conformational changes.[30]

The structural elements that undergo strain have not been identified with certainty for any molecular motor, but are likely to have spring-like or elastic properties that allow them to extend or rotate, then recoil back into their original conformation.[30] A schematic model of a molecular motor as a machine portrays the protein as consisting of binding sites for its filament and nucleotide with a spring to enable the protein to change in conformation and to produce force upon recoil to its original position, a lever to amplify the force-producing conformational changes, a hinge to allow the lever to move, and a latch to regulate nucleotide binding or release of hydrolysis products (Fig. 3A). Important goals of motors researchers are to identify the spring-like or elastic elements of the motor, the force-producing conformational changes, and the steps in the hydrolysis cycle at which they occur.

Motor mechanical elements

Work largely over the past two years has led to the tentative identification of several mechanical elements of the kinesin motors. A potential spring-like element of the motor may be the so-called "relay" helix, α4, a structural element of the motor at the motor–tubulin interface. When crystal structures of the kinesin motor, KIF1A, bound to ADP or an ATP analogue are compared to one another, they show a difference in position of helix α4 that consists of a rotation coupled to a translation in position.[36] This difference in position of helix α4 has been interpreted to be a movement of the helix that occurs upon ATP binding. The movement could correspond to a winding up motion of a spring, which releases at a subsequent step of the nucleotide hydrolysis cycle.

The question of whether this movement produces force has not yet been answered, as the force-producing steps of the kinesin motors have not yet been identified. For the F_1 ATPase, a rotary motor for which somewhat more information is available, the force-producing steps are thought likely to correspond to ATP binding or release of ADP or P_i.[37] Contrary to intuition, ATP hydrolysis or cleavage of the gamma phosphate bond is believed to be a step of the F_1 ATPase hydrolysis cycle that exists in equilibrium with ATP synthesis or bond formation and is therefore unlikely to cause a major structural change in the motor that produces force.[38] By contrast, binding of nucleotide in a conformation that enables it to be hydrolyzed, or release of hydrolysis products may cause large changes in

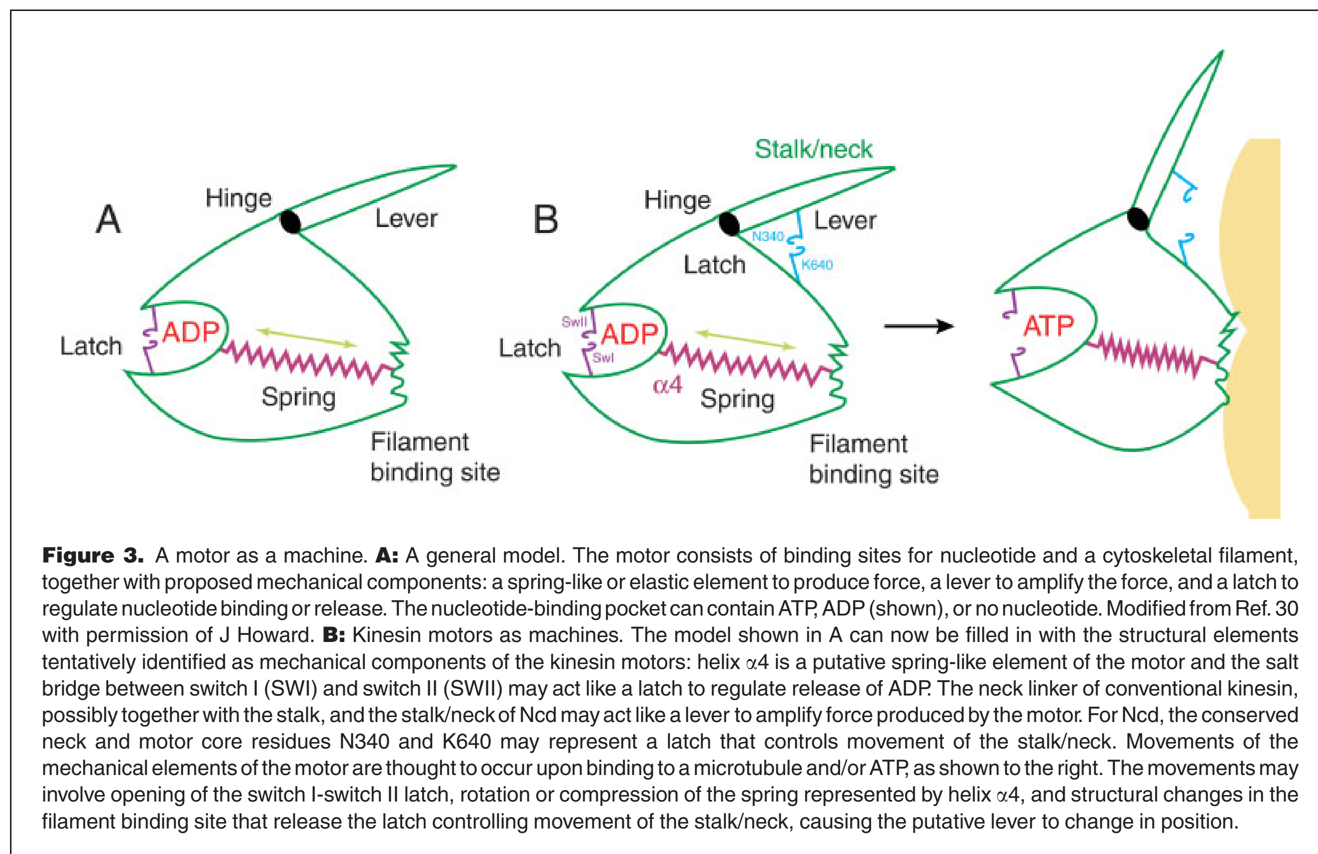

Figure 3. A motor as a machine. **A:** A general model. The motor consists of binding sites for nucleotide and a cytoskeletal filament, together with proposed mechanical components: a spring-like or elastic element to produce force, a lever to amplify the force, and a latch to regulate nucleotide binding or release. The nucleotide-binding pocket can contain ATP, ADP (shown), or no nucleotide. Modified from Ref. 30 with permission of J Howard. **B:** Kinesin motors as machines. The model shown in A can now be filled in with the structural elements tentatively identified as mechanical components of the kinesin motors: helix α4 is a putative spring-like element of the motor and the salt bridge between switch I (SWI) and switch II (SWII) may act like a latch to regulate release of ADP. The neck linker of conventional kinesin, possibly together with the stalk, and the stalk/neck of Ncd may act like a lever to amplify force produced by the motor. For Ncd, the conserved neck and motor core residues N340 and K640 may represent a latch that controls movement of the stalk/neck. Movements of the mechanical elements of the motor are thought to occur upon binding to a microtubule and/or ATP, as shown to the right. The movements may involve opening of the switch I-switch II latch, rotation or compression of the spring represented by helix α4, and structural changes in the filament binding site that release the latch controlling movement of the stalk/neck, causing the putative lever to change in position.

the motor that could result in a power stroke when amplified by other structural changes. For kinesin, release of P_i is faster than the reversal of ATP hydrolysis,[39] thus the hydrolysis step itself may be essentially irreversible, raising the possibility that it might also produce force.

Amplification of conformational changes that occur in the catalytic domain of conventional kinesin has been proposed to occur by structural changes in the neck linker,[40] an element that joins the conserved motor domain to the end of the coiled-coil stalk.[41] Although the neck linker was disordered in the first crystal structure of a kinesin motor,[42] presumably due to its mobility in the crystal lattice, subsequent crystal structures show a visible neck linker consisting of two β-strands that interact with other β-strands of the motor domain.[41,43] The mobile or undocked conformation of the neck linker is thought to alternate with docking against the motor core. Undocking of the neck linker would not only potentially enable the two heads of dimeric conventional kinesin to reach between two adjacent binding sites along the microtubule, but could also amplify a small movement of the motor core, producing a power stroke. A model has been proposed in which docking of the neck linker occurs upon binding of ATP and involves a force-producing transition from a disordered to an ordered state, causing the neck linker to move towards the microtubule plus end.[44] This model is based on evidence that the neck linker of kinesin assumes different conformations when the motor is bound to nucleotides that represent different steps of the hydrolysis cycle,[44] however, several critical steps of the model lack direct evidence. Models that propose alternating docking and undocking of the neck linker in essence rectify a disordered element or Brownian ratchet to drive movement of the motor along a microtubule, and have been referred to as "molecular ratcheting" mechanisms.[45]

Kinesin is expected to undergo several small conformational changes that may comprise several working strokes, culminating in 8 nm steps along the microtubule;[30] thus understanding the mechanism by which the motor walks along the microtubule will be essential to understand how the motor works. The nucleotide state of the two heads at each substep of the motor along the microtubule must be established, together with the conformational changes that occur and the changes that result in the force-generating strokes of the motor. The stepping mechanism of conventional kinesin is currently controversial: most workers favor a hand-over-hand mechanism in which the two heads of the motor bind alternatively to the microtubule and hydrolyze ATP.[30,46] However, some researchers have proposed a model in which only one of the two heads hydrolyzes ATP and advances in an "inchworm" fashion along the microtubule, dragging the second head along.[47] This model was proposed because of the failure to detect the alternating rotational movement predicted for a hand-over-hand model as the two heads step around one another to bind to the microtubule. The present data rule out a hand-over-hand model in which the two heads alternate and step around one another symmetrically with each step, but do not exclude a asymmetric model. Further work, currently in progress in several laboratories, is needed to resolve the kinesin stepping mechanism before we can understand how the motor generates force and moves along the microtubule.

Other conformational changes detected in crystal structures of the kinesin motors have not yet been correlated with events of the hydrolysis cycle. These conformational changes have been observed in mutants of Kar3, a minus-end kinesin motor of budding yeast, *S. cerevisiae*, but are likely to occur in all kinesin motors, based on the conservation of the residues that they involve. The mutants alter highly conserved or invariant residues of switch I and switch II,[48] elements of the kinesin motors that are structurally homologous to G protein regions that move or change in conformation upon nucleotide binding or exchange.[49] The mutants show basal ATPase activity, but activation of the motor ATPase by microtubules, which is essential for movement of the motors along microtubules, is completely blocked.[50] The two mutated residues interact with one another in wild-type motors, forming a salt bridge between the switch I and switch II regions of the motor. The mutational changes destabilize the motor-ADP, the most stable form of the kinesin motors in solution and the conformation observed in almost all the crystal structures of kinesin motors. The mutants are interpreted to trap the motor in transition states that proceed away from the ADP state.[50]

The structural changes of the Kar3 mutants include melting of the switch I loop and the two helices flanking the loop, and stabilization of the switch II loop, which is disordered in wild-type Kar3 crystal structures.[50] The structural changes perturb water-mediated interactions with the magnesium ion, which could weaken binding of the Mg^{2+} by the motor, destabilizing the bound ADP. The conformational changes have been observed in Kar3 motors complexed with ADP and therefore probably represent post-hydrolysis changes that occur prior to ADP release by the motor. The mutant motors show a pathway of structural changes that extend from helix $\alpha 4$ at the motor interface with tubulin to the active site. This pathway is likely to be important in signaling changes from one part of the motor to another. The observation that changes in single residues of switch I and switch II cause structural changes in the motor that differ markedly from the ADP state indicates that the residues act to stabilize the motor-ADP conformation. The salt-bridge between switch I and switch II could thus act like a latch to regulate ADP release from the motor.

Further crystal structures are needed to determine the nature of the conformational changes that have been detected in the kinesin motors by fluorescent probes attached to specific motor residues[51] or fluorescence polarization microscopy,[52] and determine the steps of the hydrolysis cycle at which they occur.

Minus-end kinesin motors

Although each 8 nm step by conventional kinesin may be due to cumulative small conformational changes of the motor, current evidence for the kinesin-related motor, Ncd, indicates that the motor undergoes only one or possibly two large displacements during each interaction with a microtubule.[53,54] Ncd is a minus-end motor that moves with the opposite directionality along microtubules as conventional kinesin, towards the more stable microtubule minus ends. Ncd has now been demonstrated to be a nonprocessive motor—single motors bind to the microtubule with one head, hydrolyze ATP and then release without completing a step along the microtubule, which requires binding of the second head of the motor to the microtubule.[53,54]

A large conformational or angle change of the Ncd motor has been detected in single molecule laser trap assays upon binding of the motor to the microtubule.[53] Strikingly, the displacement is directionally biased towards the microtubule minus end, the direction of Ncd movement along the microtubule. Its occurrence upon motor binding to the microtubule suggests that it may be caused by release of ADP from the motor as it binds to the microtubule. Mutant analysis indicates that the displacement probably involves movement of the coiled-coil stalk/neck relative to the motor domain because it depends on interactions between a key neck residue, N340, and motor core residue, K640.[53] The movement might represent a lever arm-like angle change of the coiled-coil stalk/neck of Ncd, similar to the large rotation of the coiled-coil rod believed to occur in myosin.[55–57]

A second displacement has been detected just before release of Ncd from the microtubule and has been postulated to be the large conformational change predicted to occur when ATP binds to the motor.[54] The structural basis of this movement has not yet been determined, although it may correspond to the rotation coupled to a translation of the relay helix, $\alpha4$, detected in the kinesin motor KIF1A, which is thought to be triggered by binding of ATP to the motor.[36] The only two single-molecule studies of Ncd reported so far each detected only a single displacement of the motor, which differ in their time of occurrence during the binding phase of the motor to the microtubule.[53,54] This might be due to the configuration of the laser trap assays used in the two studies, which differed in geometry and may have been less sensitive to the displacement detected by the other group. Further work is needed to characterize the displacements that occur when the Ncd motor interacts with a microtubule and the steps of the hydrolysis cycle at which they occur.

Kinesins as machines

Basic mechanical elements of the kinesin motors have now been tentatively identified (Fig. 3B): helix $\alpha4$ is a putative spring-like element of the motor and the salt-bridge between switch I and switch II may act like a latch to regulate release of ADP from the motor. The neck linker of conventional kinesin and the stalk/neck of Ncd are possible amplifiers of motor conformational changes. An interaction between neck and motor core residues, N340 and K640, of Ncd may act like a latch to control movement of the stalk/neck. The stalk/neck of other minus-end kinesin motors may also act to amplify motor structural changes and contain a latch that controls its movement, judging from the conservation of residues in the stalk/neck region of the C-terminal motor kinesins, which are probably all minus-end motors.[58]

The mechanism by which the plus- and minus-end kinesin motors produce their powerstroke could differ—conventional kinesin and other plus-end kinesin motors may undergo several small conformational changes, which are amplified by movements of the neck linker, culminating in a step along the microtubule, while Ncd and other minus-end kinesin motors may undergo only one or possibly two large conformational changes to produce a single powerstroke prior to releasing from the microtubule. Thus, the working strokes of the motor and the mechanism by which they are amplified may differ markedly for the plus- and minus-end kinesin motors.

Conclusions

Distinguishing among models to establish the mechanism by which the motors work will require new crystal structures, together with motility assays and biochemical tests to determine the force-producing conformational changes of the motor and the steps of the nucleotide hydrolysis cycle at which they occur. Crystal structures corresponding to major events of the hydrolysis cycle are still missing and are needed to understand the changes that the motors undergo. Informative new states of the kinesin motors are likely to be metastable or transient, making them difficult or impossible to study in wild-type motors under normal conditions, requiring researchers to use innovative strategies to crystallize the motors in the missing states. These states correspond largely to the strongly bound states of the motor, when the motor interacts with the microtubule. Conditions are needed to crystallize motor–tubulin complexes or increase the resolution of available structural techniques, such as cryoelectron microscopy. Alternatively, certain mutants might mimic the motor bound to a microtubule. The structures of the mutants are expected to show changes at the active site coupled to changes at the microtubule-binding region of the motor, enabling workers to understand the changes that the motor undergoes as it binds to the microtubule and hydrolyzes ATP.

Although crystal structures are needed to define the conformational or structural changes that the motors undergo and identify the predicted spring-like elements within the motor, they will not identify the force-producing conformational changes of the motor. This will rely on techniques such as laser trap assays to apply load at specific steps and measure the effects on the working strokes of single motor molecules.

Determining the dependence of specific conformational changes on ATP or ADP concentration should help to establish the step of nucleotide hydrolysis, while the load dependence of the conformational change should reveal whether it is force-producing. Mutants will help by confirming the structural elements involved in specific conformation changes. The crystal structures, together with laser trap assays of single motors, will elucidate the motor mechanism and reveal whether it is the same or different for kinesin motors of different directionality. Only with this information will researchers fully understand how the kinesin motors function as molecular machines.

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