

The Way Things Move: Looking Under the Hood of Molecular Motor Proteins

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The microtubule-based kinesin motors and actin-based myosin motors generate motions associated with intracellular trafficking, cell division, and muscle contraction. Early studies suggested that these molecular motors work by very different mechanisms. Recently, however, it has become clear that kinesin and myosin share a common core structure and convert energy from adenosine triphosphate into protein motion using a similar conformational change strategy. Many different types of mechanical amplifiers have evolved that operate in conjunction with the conserved core. This modular design has given rise to a remarkable diversity of kinesin and myosin motors whose motile properties are optimized for performing distinct biological functions.

Movement is one of the defining attributes of life. Not surprisingly, the study of motility has a long history, as theories for muscle contraction span three millennia (1). In recent years, interest in biological motion has blossomed because of the realization that much of cell behavior and architecture depends on the directed transport of macromolecules, membranes, or chromosomes within the cytoplasm. Indeed, modern microscopy has transformed our view of the cell interior from a relatively static environment to one that is churning with moving components, not unlike the bustling traffic in a metropolitan city. Just as disruption of commercial traffic impairs the welfare of a city, defective molecular transport can result in developmental defects as well as cardiovascular and neuronal diseases.

The motions of muscles as well as much smaller intracellular cargoes are driven by molecular motors that move unidirectionally along protein polymers (actin or microtubules). In this review, we primarily discuss muscle myosin, an actin-based motor, and conventional kinesin, which transports membrane organelles along microtubules. Muscle myosin, whose study dates back to 1864, has served as a model system for understanding motility for decades. Kinesin, discovered using *in vitro* motility assays in 1985, is a relative newcomer by comparison.

Five years ago, it appeared as though kinesin and myosin had little in common. In addition to operating on different polymers, kinesin's motor domain is less than one-half the size of myosin's, and initial sequence comparisons failed to reveal any important

similarities between these two motors. Their motile properties also appeared to be quite different. Conventional kinesin was found to be a highly processive motor that could take several hundred steps on a microtubule without detaching (2, 3), whereas muscle myosin was shown to execute a single "stroke" and then dissociate (4, 5) (Fig. 1).

The last few years of research, however, have cast a different light on the relationship between kinesin and myosin. The crystal structures of kinesin revealed a striking structural similarity to myosin (Fig. 2), and the structural overlap pointed to short stretches of sequence conservation (6, 7). This suggested that myosin and kinesin originated from a common ancestor, which in turn may be linked even further back in evolution to a protein predecessor that also gave rise to the G protein superfamily (Fig. 3) (8).

Further insight into the evolution and function of molecular motors has come from recent sequencing efforts, which have uncovered large numbers of kinesin and myosin genes (most likely >50 of each in the mammalian genome) (Fig. 3). The numerous myosin motors arose to execute a variety of distinct biological activities, including muscle contraction, cytokinesis, cell movement, membrane transport, cell architecture, and certain signal transduction pathways (9). Kinesins are involved in membrane transport, mitosis and meiosis, messenger RNA and protein transport, ciliary and flagellar genesis, signal transduction, and microtubule polymer dynamics (10). Analyses of these newly discovered kinesins and myosins have revealed as much diversity in the motile properties among motors within a superfamily as between conventional kinesin and muscle myosin themselves (Fig. 3).

The opportunity to study and compare numerous kinesin and myosin motors provides a valuable resource for understanding the mechanism of motility. Because kinesin and myosin

share a similar core structure and evolutionary ancestry, comparison of these motors has the potential to reveal common principles by which they convert chemical energy into motion. In this article, we review recent models for how muscle myosin and conventional kinesin produce movement. Although these models superficially appear very different, a similar core mechanism becomes apparent when the working parts of kinesin and myosin motors are examined closely. This common core has proven very versatile, as it has provided a scaffold for the evolution of a large number of functionally diverse motor proteins.

Motility Models for Muscle Myosin and Conventional Kinesin

In the 1960s, electron microscopy of muscle tissue revealed myosin cross-bridges reaching out from the thick filaments to contact the actin thin filaments. Like many out-of-time rowers in a boat, the myosin cross-bridges were envisaged to attach asynchronously to actin, tilt their angles like oars stroking through the water, and then detach and re-cock, similar to pulling the oar out of the water at the end of the stroke (11, 12). This conceptual idea was coupled to the enzymatic cycle in 1971 by Lynn and Taylor (13), who showed that the tight actomyosin complex was dissociated by adenosine triphosphate (ATP) binding and that the power stroke was most likely associated with phosphate release after the hydrolytic step. Although muscle myosin is a dimer, the two heads appear to act independently, and only one head attaches to actin at a given time (Fig. 1). In the last few years, this model has received increasing experimental support. The crystal structure of myosin (Fig. 2) revealed a large "catalytic core" that binds nucleotide and actin, and an adjacent "converter domain" that links the core to an extension ~80 Å in length (termed the lever arm) composed of a helix enveloped by calmodulin-like light chains (14–17). A battery of biochemical, biophysical, and structural methods have shown that the converter and lever arm domains rotate relative to the catalytic core in a nucleotide-dependent manner (Figs. 1 and 4) (16, 18–22). Thus, muscle myosin moves the actin filament by the angular rotation of its long, rigid lever arm [see animation (23)]. Because the motor is strongly attached to actin only during this brief motion-producing phase of its enzymatic cycle, a single muscle myosin molecule cannot move continuously along its track.

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In contrast to muscle myosin, conventional kinesin walks methodically along a microtubule protofilament, stepping from one tubulin subunit to the next (distance of 80 Å), similar to a person walking across a pond along a row of stepping stones (24, 25). How kinesin, which lacks an obvious long lever arm, could take a step larger than the dimension of its motor domain remained a mystery for several years. However, Rice *et al.* (26) have shown that unidirectional motion is produced by a pronounced conformational change in kinesin's "neck linker," a 15-amino acid region that is COOH-terminal to the catalytic core (Figs. 1 and 4) (17). The neck linker is mobile when kinesin is bound to microtubules in its nucleotide-free and adenosine diphosphate (ADP)-bound states. However, when the microtubule-bound kinesin binds an ATP analog, the neck linker becomes docked on the catalytic core with its COOH-terminus pointing toward the microtubule plus end (Fig. 4). Thus, the energy associated with ATP binding drives

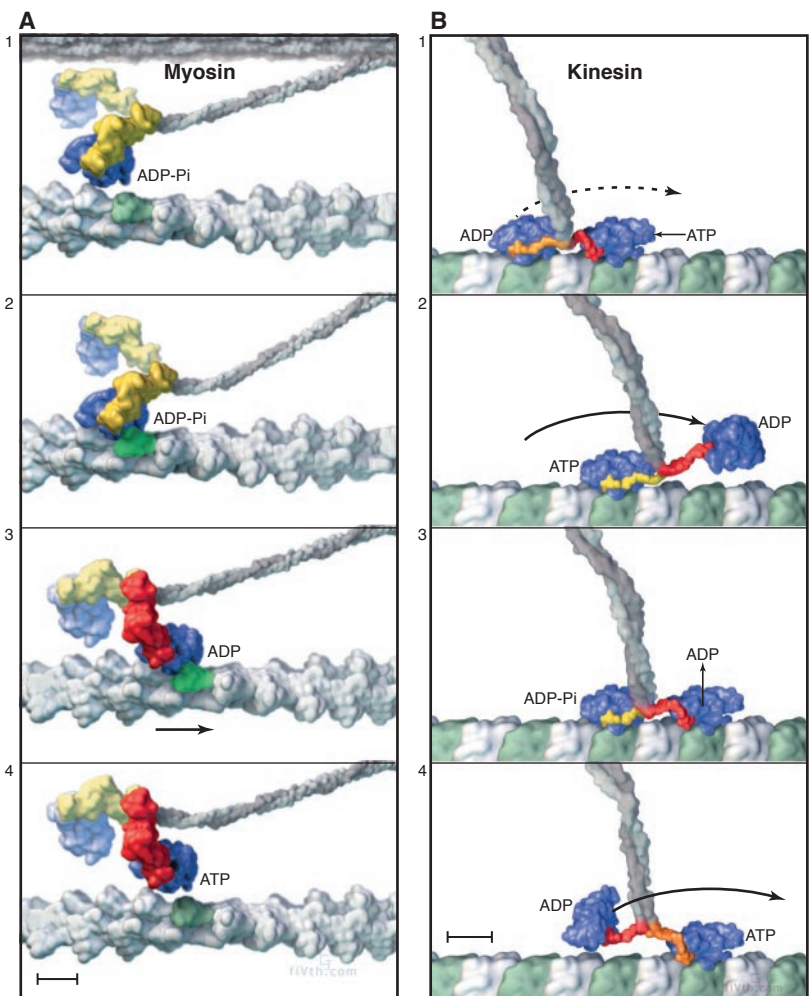
a forward motion of the neck linker and any object attached to its COOH-terminus (27).

In a truncated kinesin monomer whose neck linker is attached directly to a bead or slide surface, the docking of the neck linker on the core will deliver a plus-end-directed pull on its cargo. However, such monomer-based motility is nonprocessive and slow relative to the kinesin dimer (28–30). In the native kinesin dimer, the neck linker is connected to a coiled-coil dimerization domain, and neck linker motion in one head is conveyed to its partner to enable processive motion. Specifically, when ATP binding "zippers" the neck linker of the forward head into the docked position, the trailing head detaches from its binding site and is thrust forward to the next tubulin binding site, akin to a judo expert throwing an opponent with a rearward-to-forward swing of the arm (Fig. 1). Thus, the kinesin step is initiated by the ATP-driven swing of the neck linker, which positions the partner head over the forward subunit. The

tight binding of the partner head to its new tubulin site then locks the step in place and produces a force that pulls kinesin's cargo forward by 80 Å [see animation (23)].

The above models highlight several differences in how muscle myosin and conventional kinesin produce motion. Muscle myosin undergoes a large angular rotation of a long and relatively rigid mechanical element within the myosin head to produce a displacement of ~100 Å per ATP hydrolyzed. For conventional kinesin, a smaller conformational change is produced by the neck linker peptide, which is rigid (docked) only in the ATP/ADP-Pi-bound state (Pi, phosphate). This small change becomes amplified into an 80 Å net movement as a consequence of the partner head binding to the next tubulin binding site. The different mechanical strategies of conventional kinesin and muscle myosin reflect their distinct biological roles (31). Conventional kinesin transports small membrane organelles or protein complexes, and

Fig. 1. Models for the motility cycles of muscle myosin and conventional kinesin [see animation (23)]. **(A)** Muscle myosin. Frame 1: Muscle myosin is a dimer of two identical motor heads (catalytic cores are blue; lever arms in the prestroke ADP-Pi state are yellow), which are anchored to the thick filament (top) by a coiled coil (gray rod extending to the upper right). In the ADP-Pi-bound state, the catalytic core binds weakly to actin. Frame 2: One head docks properly onto an actin binding site (green). The two myosin heads act independently, and only one attaches to actin at a time. Frame 3: Actin docking causes phosphate release from the active site. The lever arm then swings to the poststroke, ADP-bound state (red), which moves the actin filament by ~100 Å. Frame 4: After completing the stroke, ADP dissociates and ATP binds to the active site, which rapidly reverts the catalytic core to its weak-binding actin state. The lever arm will then recock back to its prestroke state (i.e., back to frame 1). **(B)** Conventional kinesin. Unlike myosin, the two heads of the kinesin dimer work in a coordinated manner to move processively along the track. The coiled coil (gray) extends toward the top and leads up to the kinesin cargo. Frame 1: Each catalytic core (blue) is bound to a tubulin heterodimer (green, β subunit; white, α subunit) along a microtubule protofilament (the cylindrical microtubule is composed of 13 protofilament tracks). To adopt this position, the neck linker points forward on the trailing head (orange; neck linker next to but not tightly docked to the core) and rearward on the leading head (red). ATP binding to the leading head will initiate neck linker docking. Frame 2: Neck linker docking is completed by the leading head (yellow), which throws the partner head forward by 160 Å (arrow) toward the next tubulin binding site. Frame 3: After a random diffusional search, the new leading head docks tightly onto the binding site, which completes the 80 Å motion of the attached cargo. Polymer binding also accelerates ADP release, and during this time, the trailing head hydrolyzes ATP to ADP-Pi. Frame 4: After ADP dissociates, an ATP binds to the leading head and the neck linker begins to zipper onto the core (partially docked neck indicated by the orange color). The trailing head, which has released its Pi and detached its neck linker (red) from the core, is in the process of being thrown forward. The surface features of the motors and filaments were rendered by G. Johnson (fiVth media: www.fiVth.com) using the programs MolView, Strata Studio Pro, and Cinema 4D (also for Figs. 4 and 5). Protein Data Bank (PDB) files used throughout the figures are as follows: ADP-AIF₄⁻ smooth muscle myosin [prestroke, yellow: 1BR2 (76)], nucleotide-free chicken skeletal myosin [poststroke, red: 2MYS (74)], human conventional kinesin [prestroke, red: 1BG2 (6)], and rat conventional kinesin [poststroke, yellow: 2KIN (40)]. Scale bars, 60 Å (A) and 40 Å (B).



processive motion along the polymer enables efficient long-range transport using one or a few motor proteins. In contrast, muscle myosin operates in the context of a large array of motors, where it is essential for each one to attach, produce motion, and then detach quickly so as not to impede the actions of other motors producing force on the same filament.

The conformational changes that drive forward motion in myosin and kinesin also occur at different steps in the ATPase cycles (Figs. 1 and 4). ATP binding causes the forward motion of kinesin's neck linker (power stroke) but causes myosin to dissociate from actin and recock its lever arm (recovery stroke). Conversely, release of phosphate after ATP hydrolysis causes myosin to bind tightly to actin and swing its lever arm forward while it weakens kinesin's grip on the microtubule and detaches the neck linker. However, these distinct mechanochemical cycles reflect different mechanical "readouts" of a similar underlying process that takes place in the cores of kinesin and myosin, as discussed below.

The Molecular Motor Parts List

For motors to produce forward motion, the ATP hydrolysis cycle must be linked to a conformational change cycle. Therefore, some element(s) must sense key enzymatic transi-

tions and then relay this information to the polymer binding interface and the mechanical element. This pathway operates in reverse as well, because polymer binding (32) or strain on the mechanical element (4, 25) can affect enzymatic rates. Interestingly, the nucleotide site is far removed from both the polymer and mechanical regions of these proteins. Therefore, just as in an automobile, the site that processes the chemical fuel must be linked through intermediate components to the site that ultimately generates the motion. In the automobile, the breakdown of the chemical fuel is coupled to the stroking of a piston, which in turn is linked through the crankshaft and transmission to the turning of the wheels. A somewhat analogous situation for translating chemical changes into mechanical motions exists in molecular motors (33).

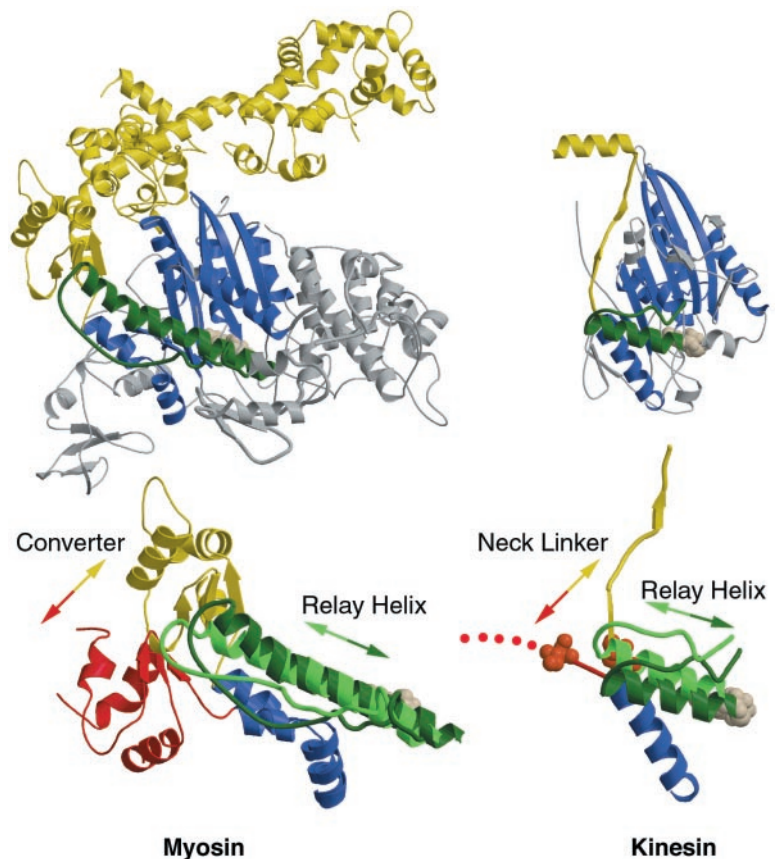
The nucleotide site: Swinging loops act as triggers. To change conformation between ATP- and ADP-bound states, motor proteins must sense the presence or absence of a single phosphate group. The identity of the "γ-phosphate sensor" became evident when myosin structures with and without bound ATP analogs were compared (34, 35). The sensor consists of two loops, called switch I and switch II, which form hydrogen bonds with the γ-phosphate and also position a cat-

alytic water and important side chains for cleavage of the β- to γ-phosphate bond. To accomplish these actions, the switch II loop operates like a spring-loaded gate that swings in by several angstroms to interact with the γ-phosphate and swings out when the γ-phosphate is released. The "ATP"-bound state of the sensor is also stabilized by a salt bridge that forms between the switch I and II loops.

Kinesins contain switch I and II loops that are almost identical to those in myosin (36). Moreover, a very similar "γ-phosphate sensor" operates in the G protein superfamily as well (7, 8), indicating that the switch loops are ancient and predate the appearance of molecular motors (Fig. 3). The strong conservation of the switch loops most likely reflects evolutionary pressures to maintain the chemistries involved in γ-phosphate bonding and nucleotide hydrolysis.

Inspection of the switch regions of myosin, kinesin, and G proteins suggests that ATP binding (switch regions engaged with the γ-phosphate) and phosphate release (switch regions disengaged) trigger the most critical structural changes in the nucleotide cycle. Although the nucleotide hydrolysis and ADP release steps probably induce conformational changes as well, the ATPase cycle can be simplified by proposing that transitions between ATP/ADP-Pi-bound structures and

Fig. 2. Atomic structures of the myosin and kinesin motor domains and conformational changes triggered by the relay helix. The motor domains of smooth muscle myosin (ADP- AlF_4^-) and rat conventional kinesin are shown in the upper panels (both structures are proposed in this article to represent an "ATP/ADP-Pi" conformation). The common structural elements in the catalytic cores are highlighted in blue, the relay helices and polymer loops are dark green, and the mechanical elements [neck linker for kinesin; the converter and lever arm domains for myosin (17)] are yellow, and nucleotide is shown as an off-white space-filling model. The complete lever arm was not obtained in the smooth muscle myosin crystal structure, but was added here by atomic modeling for purposes of illustration. The kinesin and myosin structures are shown in the same orientation (by superimposing their P-loops) and are displayed as viewed from the polymer surface (~90° clockwise view of the motors shown in Fig. 4). The similar positions of the relay helices and the mechanical elements in kinesin and myosin can be seen in relation to their common cores. The details and similarity of the conformational changes elicited by the relay helix are shown in the lower panels. For both myosin and kinesin, the relay helix undergoes similar motions during the transition from the "ATP/ADP-Pi-bound" state (dark green; upstroke) to the "ADP/nucleotide-free state" (light green; downstroke). A loop following the relay helix (in greens, which likely controls polymer affinity, and the mechanical elements ["ATP/ADP-Pi" (yellow) and "ADP/nucleotide-free" states (red)]) both shift their positions in response to the relay helix motion. With the relay helix in an upstroke position, kinesin Ile³²⁵ (orange space-filling residue) in the neck linker inserts into a pocket on the catalytic core [see (48)]. This event is proposed to trigger the docking of the rest of the neck linker onto the catalytic core. In the downstroke position, the relay helix (light green) occludes the pocket, which pushes Ile³²⁵ out, and the neck linker becomes disordered (red dots). Nucleotide-free chicken skeletal myosin (red, light green) and ADP- AlF_4^- smooth muscle myosin (yellow, dark green) are shown on the left, and human conventional kinesin (red, light green) and rat conventional kinesin (yellow, dark green) are shown on the right.



ADP/nucleotide-free structures drive the primary events in the motility cycles of motor proteins, as outlined below.

Long-Range Communication: Piston-Like Motions of a Relay Helix

Comparison of the myosin and kinesin structures reveals that small movements of the γ -phosphate sensor are transmitted to distant regions of the protein using a similar element: a long helix that is connected to the switch II loop at its NH₂-terminus, and that interacts along its length and at its COOH-terminus with the polymer binding sites and the mechanical elements, respectively. This highly conserved helix has been called the switch II helix (kinesin literature) or relay helix (myosin literature); for uniformity, the term relay helix is used in this review. The relay helix is the key structural element in the communication pathway linking the catalytic site, the polymer binding site, and the mechanical element in both kinesin and myosin. Myosin's relay helix undergoes a nucleotide-dependent conformational change that approximates the motion of a piston (Fig. 2) (16, 34, 35, 37). In crystal structures with several ATP/ADP-Pi analogs, the motion of the switch II loop toward the γ -phosphate tilts and translates the relay helix along its axis toward the nucleotide ("upstroke" position). Conversely, without a nucleotide in the active site, switch II swings away and the relay helix moves to a "downstroke" position (38). Interestingly, myosins from chicken muscle (14, 16) and the cytoplasm of the slime mold *Dictyostelium* (34, 35) show nearly superimposable upstroke and downstroke positions, indicating a strong conservation of these two protein con-

formations. However, the structural state of the relay helix need not always be tightly coupled to a particular nucleotide state. For example, with ADP-BeF_x (BeF_x is a phosphate analog) in the active site, the relay helix is in a downstroke position in a *Dictyostelium* myosin crystal structure (34) but is in an upstroke position in a chicken smooth muscle myosin structure (16). Rather than reflecting a mechanistic difference between these two myosins, the relay helix is most likely in a conformational equilibrium in this nucleotide analog state, and distinct crystallization conditions shifted it into an upstroke position (ATP/ADP-Pi-like state) in one case and a downstroke position (ADP/nucleotide-free-like state) in the other.

Despite efforts to obtain kinesin crystal structures with bound ATP analogs, the only structures to date contain ADP (or ADP analogs) in the active site. However, a comparison of crystal structures of two almost identical kinesins (rat and human conventional kinesin) reveals two distinct positions of the relay helix (39). In the rat conventional kinesin structures (40), the relay helix is translated along its axis toward the nucleotide and away from the β sheet when compared to human conventional kinesin. This structural difference is similar in nature and magnitude to that observed in myosin's relay helix in different nucleotide states (Fig. 2). This difference in the two kinesin structures cannot be explained by sequence variation, because switch II and the relay helix (residues 229 to 271) are 100% identical in these two motors. Furthermore, the relay helices in the human conventional kinesin, *Drosophila* Ncd (41), and yeast Kar3 (42) structures all closely

superimpose, even though these motors are from different kinesin subfamilies and distantly related species. Hence, it is likely that the relay helices in rat and human kinesin structures became trapped (possibly by different crystallization conditions) in upstroke (ATP/ADP-Pi-like) and downstroke (ADP/nucleotide free-like) states, respectively, analogous to the two states of the relay helix observed in the two ADP-BeF_x myosin structures.

These crystal structure findings suggest that the relay helices in kinesin and myosin can be dynamic structures that can access different conformations in a single nucleotide state. Other studies also suggest that the mechanical elements of myosin (lever arm) (20, 43, 44) and kinesin (neck linker) (26) also are not rigidly fixed in nucleotide-dependent positions when these motors are in solution or very weakly bound to their tracks. The motor-polymer interaction, however, markedly affects this conformational equilibrium by "cementing" the relay helix and the mechanical element in well-defined nucleotide-dependent conformations. For example, distinct ATP/ADP-Pi and ADP/nucleotide-free conformations of kinesin's neck linker are only observed when the motor is complexed with microtubules (26), and a well-defined position of myosin's lever arm is observed when the motor docks tightly onto actin under ADP/nucleotide-free conditions (11, 19, 20).

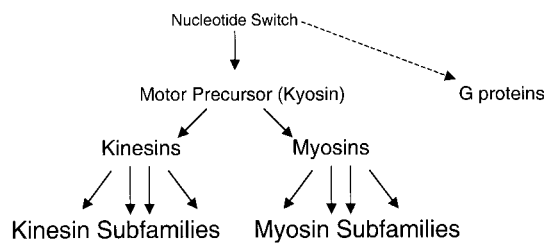
In summary, the relay helices in both myosins and kinesins undergo similar conformational changes that resemble the motions of a piston (37). During the normal enzymatic cycle, the upstroke motion of the relay helix is a consequence of the inward motion of switch II toward the γ -phosphate, whereas the downstroke is initiated by phosphate release. Because the relay helix is long but incompressible, it is a perfect device for transmitting information from the nucleotide site to distant polymer binding and mechanical elements.

Controlling Polymer Binding Affinity and Generating Unidirectional Motion

Although myosin and kinesin have similar switch loops and relay helices, they have evolved very different polymer binding sites and mechanical elements. In kinesin, a 12-amino acid loop serves as the main microtubule binding element, whereas in myosin this loop is attached to a ~140-amino acid actin binding domain (45). As discussed previously, the mechanical elements of kinesin (neck linker/second head) and myosin (converter/lever arm helix) also differ considerably in architecture. Here, we describe how the actions of the polymer binding and mechanical elements in kinesin and myosin are coordinated with the movements of the relay helix.

The polymer binding site. Although the polymer binding interfaces of kinesin and

Evolution of Motor Proteins



Examples of Kinesin Subfamilies		Direction	Processivity	Biological Activities
Conventional	(Dimer)	Plus End	Yes	Membrane Transport
Ncd	(Dimer)	Minus End	No	Meiotic/Mitotic Spindle Function
Examples of Myosin Subfamilies				
Myosin I	(Monomer)	Barbed End	No	Cell Motility/Membrane Functions
Myosin II	(Dimer)	Barbed End	No	Muscle Contraction/Cytokinesis
Myosin V	(Dimer)	Barbed End	Yes	Membrane/mRNA Transport
Myosin VI	(Dimer)	Pointed End	Unknown	Membrane Transport

Fig. 3. Evolution and function of kinesin and myosin motors. On the basis of structural similarities, it is proposed that kinesin, myosin, and most likely G proteins evolved from a common ancestor (7, 8). Various classes of motors then evolved within the kinesin and myosin superfamilies. Motor classes discussed in this review are shown here. For detailed information on the biological roles served by kinesin and myosin motors, see (9, 10).

myosin are different, they appear to communicate with the relay helix in a similar way. In kinesin, the microtubule binding loop begins COOH-terminal to the relay helix, doubles back, and makes extensive contacts with the relay helix (green loop in Fig. 2). Myosin has a comparable loop that follows a similar path, but, unlike kinesin, it does not contact actin directly. Instead, this loop plays an intermediate role by linking the relay helix to the actin binding elements. The polymer loop of kinesin and the analogous loop in myosin are both pulled toward the nucleotide during the upstroke of the relay helix and are pushed away during the downstroke (Fig. 2) (46). These loop motions very likely affect polymer binding affinity in both motor proteins, but in opposite ways. Myosin in its ATP/ADP-Pi-bound state binds polymer more weakly than in its ADP-bound state, whereas in kinesin the opposite is true.

The mechanical element. The mechanical elements of kinesin and myosin also are linked to the motions of the relay helix (Fig. 2). In myosin, the converter makes extensive contacts with the end of the relay helix and the beginning of the following loop (16, 18). During the upstroke, the converter adopts a final position above the relay helix (toward the "pointed" end of the actin filament) (Figs. 2 and 4). During the downstroke, the converter is pushed below the relay helix (toward the "barbed" end of the actin filament) (16). These swings of the converter require hinge-

like movements about two glycine residues that lie near the boundary between the catalytic core and the converter domains (16). Because of the rigid embrace between the converter and the base of the lever arm helix, the motion of the converter is translated into the angular motion of the lever arm. Interestingly, the converter motion may occur in two steps in several myosins. For example, in myosin I, two mechanical steps occur per ATP cycle (47) and an extra motion of the lever arm after ADP release has been observed (19). This behavior may be elicited by a two-stroke action of the relay helix, the first downstroke occurring with phosphate release and the second occurring with the release of ADP.

In kinesin, the neck linker emerges from the catalytic core in a position analogous to the converter region of myosin and undergoes a similar type of swing (Figs. 2 and 4). In response to the relay helix upstroke, a pocket is created that provides a docking site for conserved residues in the neck linker [e.g., Ile³²⁵ in Fig. 2; for a detailed view of the pocket and the proposed docking mechanism, see (48)]. This interaction most likely initiates the "zippering" of the remainder of the neck linker onto the catalytic core. In the relay helix downstroke, this critical binding pocket is occluded, forcing the detachment of the neck linker from the core. The hinge-like action of kinesin's neck linker probably occurs around a conserved glycine/alanine (human kinesin Gly³¹⁹) at the boundary between

the catalytic core and neck linker, which lies in a similar position to a glycine at the catalytic core-converter boundary in myosin.

In summary, despite their different structures, kinesin's and myosin's polymer binding and mechanical elements display similar movements in response to the upstroke (ATP/ADP-Pi-bound state) and downstroke (ADP/nucleotide-free state) actions of the relay helix. Thus, these two motor superfamilies appear to use a similar overall strategy for converting small motions at the nucleotide binding site into a power stroke that drives forward motion.

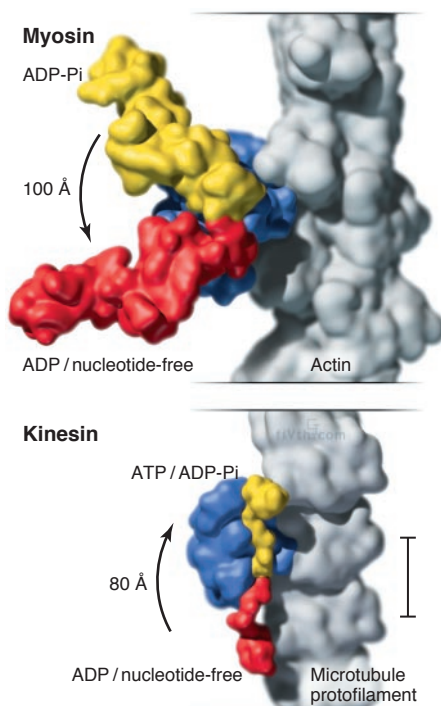
Engineering Motors with Different Properties

Despite their similar core architectures, kinesin and myosin motors have evolved a remarkable diversity of motile activities. Within each superfamily, there are "forward" and "reverse" motors as well as motors that move processively or nonprocessively along the polymer track. Myosins and kinesins independently arrived at similar solutions for creating motors with these intriguing properties. These engineering efforts have been focused on the specific architecture of the motor's mechanical element.

Throwing the gearshift into reverse. One class of kinesins (typified by *Drosophila* Ncd) (49, 50) and one class of myosins (myosin VI) (51) travel in the opposite direction to most kinesins and myosins. One way to create an opposite-polarity motor is to reverse the orientation of the motor on its track. However, such a solution necessitates the evolution of a completely new polymer binding interface, and electron microscopy and sequence comparisons of the polymer binding regions indicate that neither Ncd (52) nor myosin VI (51) have adopted such a strategy. Alterations in the switch II and relay helix mechanism are also unlikely, given that these regions are highly conserved between opposite-polarity motors. Therefore, direction reversal must involve a new way in which the nucleotide-dependent motions of the relay helix are coupled either to changes in polymer affinity or to the swing of a mechanical element.

An example of how changing polymer affinity can reverse the direction of motion is illustrated by comparing the structural actions and enzymatic cycles of muscle myosin and conventional kinesin. Although kinesin and myosin bind in a similar orientation along their polymer axes and undergo similar nucleotide-dependent swings of their mechanical elements, they nonetheless move in opposite directions relative to their common catalytic cores (Fig. 4). This is because the order of the tight and weak polymer binding states is reversed in the kinesin and myosin enzymatic cycles. In the ATP-bound state, for example, kinesin is strongly bound to the

Fig. 4. A model for the "power strokes" of myosin and kinesin motors complexed with their polymer tracks. In myosin, a ~100 Å motion of the lever arm domain is generated when the motor undergoes a transition from an ADP-Pi-bound state to an ADP/nucleotide-free conformation (78). This figure was generated by superimposing the structures of smooth muscle myosin (ADP- AlF_4^-) and the nucleotide-free chicken skeletal myosin. Shown are the converter/lever arm positions in ADP-Pi (yellow) and nucleotide-free (red) states, the similar catalytic cores (blue), and the actin filament (gray; "pointed end" toward the top). In the motility cycle of a kinesin dimer (only one head shown here) along a microtubule (a single protofilament is shown in gray; "plus end" toward the top), the neck linker swings from a rearward-pointing position (ADP/nucleotide-free; red) to a forward-pointing position (ATP/ADP-Pi; yellow). The "ATP/ADP-Pi" state is rat conventional kinesin, whereas the "ADP/nucleotide-free" position of the neck linker was modeled on the basis of cryo-electron microscopy from Rice *et al.* (26). Myosin and kinesin structures were superimposed using their P-loops, showing that they bind in similar orientations to their tracks. (Note: The actin filament runs parallel to the plane of the image, but the microtubule is tilted ~20° with respect to the plane of the image.) Although the mechanical elements are similarly positioned in kinesin and myosin, the power strokes occur in opposite directions (arrows) because of the different polymer binding cycles of the two motors (see text for details). Scale bar, 80 Å.



microtubule but myosin is detached from actin. As a result, the ATP-induced docking of the neck linker generates forward motion in kinesin, whereas the analogous swing of the myosin converter/lever arm generates the recovery stroke (Figs. 1 and 4). This example illustrates how reversing the order of tight and weak binding states can lead to direction reversal. However, current enzymatic and binding data indicate that neither Ncd nor myosin VI reverse direction by such a mechanism (53).

“Reverse” motion could also be achieved by developing a new mechanical element that swings in the opposite direction in response to similar cues from the catalytic core. Both sequence and structural comparisons of opposite-directed motors tend to support this model. The lever arm of myosin VI, for example, rotates in the opposite direction to other myosins (toward the pointed end of the actin filament) when ADP is released from the catalytic core (51). The structural basis of this different conformational change most likely resides within a ~50-amino acid insertion in the converter region, which is unique to the myosin VI class. One possibility is that this insertion repositions the lever arm domain so that it projects from the opposite side of the converter domain. In this way, the same rotational motion of the converter around the end of the relay helix would swing the myosin VI lever arm in the opposite direction to other myosins.

The minus-end-directed kinesin motors (e.g., Ncd) have undergone an even more radical change, dispensing with the COOH-terminal neck linker entirely and using a structurally different mechanical element (also called the neck) that precedes the NH₂-terminus of the catalytic core (54). A clue to the functional consequence of this change is found by comparing the ADP crystal structures of Ncd and human conventional kinesin. In both structures, the relay helices are in similar downstroke positions. However, Ncd’s neck is docked onto the catalytic core, whereas kinesin’s neck linker is detached. Therefore, the upstroke of the relay helix may elicit an opposite response in Ncd relative to kinesin, detaching the Ncd neck from the catalytic core and displacing it toward the microtubule minus end.

In conclusion, classes of “reverse” kinesin and myosin motors appear to have been created through the evolution of new mechanical elements. These findings highlight how structurally distinct mechanical elements can be hooked up to the same core allosteric mechanism with remarkably different outcomes. From sequence gazing, the number of distinct mechanical amplifiers in the kinesin and myosin superfamilies may be rather large (55). Another example of how the evolution of a specific architecture for a mechanical element can give rise to a particular motor property is

illustrated by comparing processive myosin and kinesin motors, as described below.

Learning to walk. For many years, processive motion was thought to be a hallmark of kinesins but not of myosins. Recently, however, myosin V, which transports small membrane organelles along actin filaments, was found to be highly processive (56). Conversely, several classes of kinesin motors display limited or possibly no processivity (57–59). Therefore, like directionality, processivity appears to involve specific adaptations that have evolved in only some classes of motor proteins.

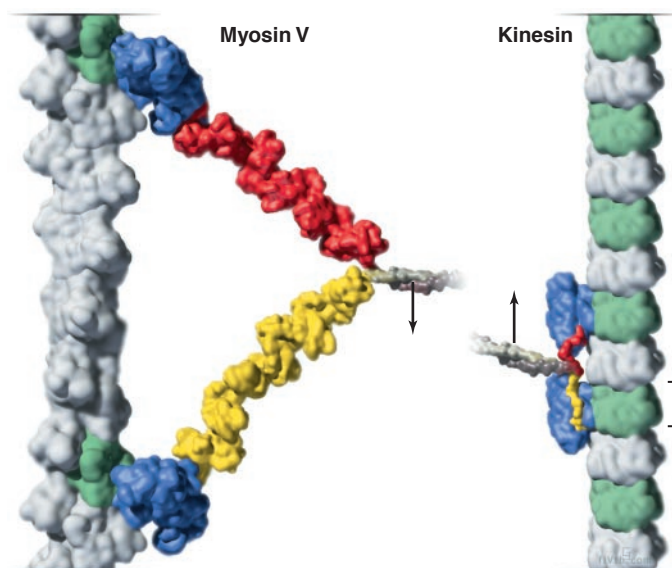
Studies of conventional kinesin have uncovered features that allow this motor to move processively. Coordination between the two motor domains of the kinesin dimer appears to be required, because single kinesin heads produced by truncation do not move processively (29, 30, 60). A coordinated enzymatic mechanism, termed alternating-site catalysis, has also been described in which an enzymatic transition in one head triggers a chemical transition in its partner head (61–63). As a result, the two heads are always in different nucleotide states as the motor dimer moves along the microtubule. This coordinated kinetic mechanism requires the microtubule as a participant, because the two kinesin heads behave independently and do not influence each other when the motor dimer is in solution.

How do the two identical heads of the kinesin dimer “talk” to one another? One

head could relay a conformational change directly to the active site of its partner head through an allosteric mechanism. However, the solution that appears to have evolved relies largely on the geometry of the microtubule as well as the length and physical motion of the mechanical element, the neck linker. This “mechanically controlled access” model works as follows. When the kinesin dimer makes an initial interaction with the microtubule, only one kinesin head can readily make contact with the microtubule, because of restraints imposed by the coiled-coil and prestroke conformation of the neck linker in the bound head (64). As a result, the detached head must wait patiently in an ADP-bound state, because microtubule binding is required to bypass this kinetic bottleneck. Then, in the critical transition, ATP binding to the attached head induces neck linker docking, which swings the detached head forward and allows it to reach the next tubulin binding site. This creates a two-head-bound intermediate in which the neck linkers in the trailing and leading heads are pointing forward (poststroke) and backward (prestroke), respectively (Fig. 5) (65). The newly bound leading head can then proceed through its kinetic checkpoint, bind ATP, and produce a power stroke that throws the trailing head forward by 160 Å to a new binding site (Fig. 1) (23).

The match between the length of the myosin V lever arm and the geometry of the

Fig. 5. A critical two-head-bound intermediate for processive movement requires mechanical elements of very different sizes in myosin V and kinesin. Because of the helical arrangement of subunits in an actin filament and the linear arrangement of subunits in a microtubule protofilament, equivalent motor binding sites (green subunits) occur every 360 Å along actin (“pointed end” toward the top) and every 80 Å along the microtubule protofilament (“plus end” toward the top). The direction of motion is shown by the arrows



next to the coiled-coil domains (gray). The two heads of myosin V and conventional kinesin can span the distance between these binding sites only if the trailing head is in a poststroke state [“ADP/nucleotide-free” for myosin V (red); “ATP/ADP-Pi” for kinesin (yellow)] and the forward head is in a prestroke state [“ATP/ADP-Pi” for myosin V (yellow); “ADP/nucleotide-free” for kinesin (red)]. Myosin V is modeled here by extending the lever arm crystal structures of chicken skeletal muscle (upper head; red lever arm) and chicken smooth muscle myosin (ADP-AlF₄⁻; lower head, yellow lever arm) to include six light chains. The two myosin catalytic cores (blue) are docked in same orientation (by aligning their P-loops), but appear slightly different because of structural changes in the core associated with these two nucleotide states. Scale bar, 40 Å.

actin filament (31) suggests that this motor also may operate by a mechanically controlled access mechanism. Accessible tubulin binding sites are spaced every 80 Å along a microtubule protofilament, but subunits with identical orientations in the helical actin filament are separated by 360 Å (Fig. 5). To operate processively on this geometrically challenging substrate, myosin V has developed a lever arm domain three times the length of that of muscle myosin. In solution, both myosin V heads are kinetically trapped in a pre-power stroke state (ADP-Pi). We predict that, like kinesin, geometrical constraints allow only one head of the myosin V dimer to dock initially onto the actin filament. This docked head can then bypass its kinetic checkpoint (Pi release) and swing its long lever arm forward, which would position the detached head near the next binding site 360 Å away. The new leading head can then bind to actin (Fig. 5) and release its Pi. The motor will remain in this two-head intermediate until the trailing head detaches after its bound ADP is exchanged for ATP (66). This allows the strained leading head to complete its power stroke, which swings the trailing head 720 Å forward to the next accessible binding site. Evidence for this model comes from optical trapping data, which reveal that the myosin V dimer moves in discrete steps of ~360 Å (67). The 360 Å steps generally occur very rapidly and are separated by long dwell times (67), suggesting that myosin V spends most of the time with its two heads bound to the track, as also appears to be true for conventional kinesin. Thus, myosin V and conventional kinesin appear to have convergently arrived at mechanistically similar solutions for generating processive motion by evolving mechanical elements and power strokes that match the spacing between binding sites along their respective cytoskeletal tracks.

The mechanically controlled access model provides a simple physical explanation for alternating-site catalysis. The formation of the critical two-head-bound species for conventional kinesin and myosin V is designed to occur only when the mechanical elements in the trailing and leading heads are in post-stroke and prestroke conformations, respectively. Because the bound nucleotide governs the position of the mechanical element and vice versa, this geometry constrains the two identical motor domains to have out-of-phase mechanical and chemical cycles. This proposed mechanism has interesting similarities and differences with respect to the well-known alternating-site catalytic mechanism of F_1 ATPase (68). In the F_1 ATPase, the enzymes (β subunits) are fixed in position in a ring, and they mechanically push the allosteric activator, the γ subunit, around in a circle. In the kinesin and myosin systems, the polymer subunits (the allosteric activators) are

fixed along the track, and the enzyme subunits must move to reach their allosteric activators.

Perspective

When watching motion under a microscope, one cannot help but feel that the molecular machines generating such activities must be endowed with unique properties. However, as we learn more about these molecular motors, they have become more familiar and less magical. At the heart of kinesin and myosin motors is an allosteric core whose actions are not so different from those of many well-studied enzymes. The partnership between the motor and its track in generating conformational changes is also recapitulated in many enzymes that interact with protein substrates. Furthermore, the large movements of motor mechanical elements are similar in scale to domain motions in other proteins (e.g., the ribosomal G protein EFG or viral fusion proteins). Remarkable, however, is the way in which these familiar themes have come together in a molecule that has evolved to move along a track. And still appearing magical is the way in which protein motors can work with such high efficiency and be carefully tuned to have so many different motile properties. These aspects of motor function will no doubt become better understood as the field continues to explore evolution's treasure trove of cytoskeletal motors.

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38. A recent scallop myosin structure (with bound ADP) has been obtained that shows a third positioning of the relay helix, rigid relay loop, and converter/lever arm (18). How this structure fits into the mechanochemical cycle is unclear. The authors suggest that it may represent an ATP- rather than an ADP-bound state, whereas Cooke (71) has suggested that it represents a mechanically strained ADP-bound state of the motor.
39. Although the positions of the relay helix and the polymer loop (loop 12) differ in the rat and human kinesin crystal structures [root mean square (rms) deviation of 4.0 Å], the overall catalytic core structures are much more similar (rms deviation of 1.8 Å). The reason for the different relay helix positions in rat kinesin may be traced to the formation of a salt bridge between switch I and II (Arg²⁰⁴ and Glu²³⁷), which is not seen in other kinesin structures. An analogous salt bridge is observed in myosin structures with ATP analogs.
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 55. Other examples of motors with different amplifiers include *Toxoplasma* myosin XIV, which lacks a long lever helix (73) and may operate using only a "converter-based" amplifier. Many types of kinesin necks also have evolved, some of which may stimulate mechanical disassembly of microtubules (74).
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 79. Because of space constraints, we can cite relatively few articles; we regret not being able to acknowledge many of the important contributions in this field. We thank G. Johnson, A. Lin, E. Sablin, and B. Sheehan for figure preparation. We are also grateful to C. Cohen, R. Cooke, R. Fletterick, S. Rice, L. Sweeney, E. Taylor, and K. Thorn for many stimulating discussions and for providing comments on the manuscript.

REVIEW

Motility Powered by Supramolecular Springs and Ratchets

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Not all biological movements are caused by molecular motors sliding along filaments or tubules. Just as springs and ratchets can store or release energy and rectify motion in physical systems, their analogs can perform similar functions in biological systems. The energy of biological springs is derived from hydrolysis of a nucleotide or the binding of a ligand, whereas biological ratchets are powered by Brownian movements of polymerizing filaments. However, the viscous and fluctuating cellular environment and the mechanochemistry of soft biological systems constrain the modes of motion generated and the mechanisms for energy storage, control, and release.

In his famous letter of 1676 to the Royal Society, the 17th-century microscopist Leeuwenhoek (*1*) described how the body of a

simple unicellular organism, probably a vorticellid, was connected by a slender stalk to a fragment of leaf and wrote this about its movement: ". . . their whole body then leapt towards the globul of the tayl. . . and unwound again. This motion of extension and contraction continued a while . . ."

This example of motility, the retraction by the stalk of peritrich ciliates, is caused not by the sliding action of a motor protein but by a spring that operates according to a simple

mechanism: the entropic collapse of polymeric filaments. Although they are regarded as unusual engines for motility, springs and ratchets composed of filaments and tubules power many of the largest, fastest, and strongest cellular and molecular movements. Just as muscles magnify forces and movements by a clever geometrical hierarchy, these unusual mechanochemical engines (2) use a similar principle: Small changes in a protein subunit are amplified by their linear arrangement in filaments and bundles. From the biochemical and physical characteristics of several molecular springs and ratchets we will argue that they represent ancient and commonplace eukaryotic molecular engines.

Supramolecular Springs—Conformation Changes Driven by Ion Binding

Biological springs are active mechanochemical devices that store the energy of conformation in certain chemical bonds that act as

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