Kinesin: switch I & II and the motor mechanism

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Summary
New crystal structures of the kinesin motors differ from previously described motor-ADP atomic models, showing striking changes both in the switch I region near the nucleotide-binding cleft and in the switch II or ‘relay’ helix at the filament-binding face of the motor. The switch I region, present as a short helix flanked by two loops in previous motor-ADP structures, rearranges into a pseudo-β-hairpin or is completely disordered with melted helices to either side of the disordered switch I loop. The relay helix undergoes a rotational movement coupled to a translation that differs from the piston-like movement of the relay helix observed in myosin. The changes observed in the crystal structures are interpreted to represent structural transitions that occur in the kinesin motors during the ATP hydrolysis cycle. The movements of switch I residues disrupt the water-mediated coordination of the bound Mg2+, which could result in loss of Mg2+ and ADP, raising the intriguing possibility that disruption of the switch I region leads to release of nucleotide by the kinesins. None of the new structures is a true motor-ATP state, however, probably because conformational changes at the active site of the kinesins require interactions with microtubules to stabilize the movements.

Key words: Molecular motor, Structure/function, Conformational changes, Kinesin, Myosin

Introduction
Life inside the cell is an incessant frenzy of rapid back-and-forth movements of myriad cellular components, including proteins, vesicles and organelles, driven by collisions of water and other molecules (see Howard, 2001). This Brownian motion allows molecules such as newly synthesized RNAs to diffuse randomly from their site of entry into the cytoplasm but is far too slow to move molecules the relatively large distances required in eukaryotic cells and cannot localize them to specific regions of the cell (Wilkie and Davis, 2001). Localization to a site within the cell requires facilitated movement, usually aided by one of a battery of cytoskeletal motor proteins - the myosins, kinesins or dyneins.

Cytoskeletal motors
Cytoskeletal motor proteins are specially built to perform their transport function in the cell: one region of the protein binds to an actin filament or microtubule, hydrolyzes ATP and moves along the filament, while another region attaches to cargo for transport to a specific site within the cell. Cytoskeletal motors are thus specialized ATPases that transport cargo by coordinating the hydrolysis of ATP with binding to and movement along a filament. Remarkably, these molecular motors convert the chemical energy from ATP hydrolysis directly into mechanical energy. How do motors capture the energy released by nucleotide hydrolysis and turn it into work? Motor proteins are apparently capable of sensing and responding to the presence or absence of a γ-phosphate, and then transmitting this information along a pathway of increasingly larger conformational changes that culminates in a force-generating event. A prevailing idea is that one or more steps of ATP binding or hydrolysis induces small conformational changes in the protein that, under load, create strain (Howard, 2001). The strain is relieved by further changes in the motor that produce force and then amplify the force, resulting in movement of the motor along its filament. The structural elements that undergo strain are likely to have spring-like or elastic properties that allow them to extend or rotate, and then recoil back into their original conformation (Howard, 2001). Movements of the motor catalytic core are further expected to involve the highly conserved switch regions (Kull et al., 1996; Sablin et al., 1996), switch I and switch II, so-named because of their structural homology to regions of G proteins that move upon nucleotide hydrolysis and exchange (Sprang, 1997). Thus, an understanding of the motor mechanism is likely to come only after workers have identified the spring-like or elastic elements within the motor, together with the force-producing structural changes in the motor and the steps in the hydrolysis cycle at which they occur.

The two best-studied cytoskeletal motors, myosin and kinesin, are dimeric proteins that have two catalytic domains joined by a coiled-coil rod or stalk. These two motor proteins and other highly related proteins in their respective families contain a central core of structural elements that are remarkably similar to one another (Kull et al., 1996) (Fig. 1). Despite this structural homology, however, there are indications that the kinesin motors differ substantially from the myosins in their mechanism of function. A fundamental difference is the nucleotide-dependent interactions of the motors with their filament: myosin bound to ATP is weakly bound to or detached from actin, whereas kinesin-ATP is strongly bound to microtubules. Conversely, myosin-ADP is strongly bound to actin, whereas kinesin-ADP is weakly bound to or detached
from microtubules. A further basic difference between the kinesins and myosins is that myosin hydrolyzes ATP while detached from actin, whereas kinesin hydrolyses ATP while attached to the microtubule. But, for both motors, the rate-limiting step in the hydrolysis cycle is accelerated by binding of the motor to its filament, which results in a characteristic actin- or microtubule-activated ATPase activity that underlies the ability of the motor to move along its filament.

**Myosin**

Work on myosin has identified three distinct conformations of the motor that are thought to correspond to different nucleotide states (Rayment et al., 1993a; Fisher et al., 1995; Dominguez et al., 1998; Houdusse et al., 1999; Houdusse et al., 2000). These have been classified as detached, near rigor, and transition forms (Houdusse et al., 2000) (Fig. 2), all of which bind weakly to actin. The nucleotide bound to the motor is not always an indication of the motor state, since the detached form can be crystallized with either ADP or ATP bound to the active site (Houdusse et al., 1999; Houdusse et al., 2000). The three forms show major differences in the position of the rod-like lever arm, as well as a striking change in the ‘converter’ region at the base of the lever arm. The converter, a rigid α/β subdomain of 67 residues that includes the first three turns of the helical rod (Fig. 1), is thought to convert movements at the nucleotide-binding cleft of the motor, which are transmitted by the adjacent switch II or ‘relay’ helix and the SH1 helix, into the swinging of the lever arm. The SH1 helix, which is also adjacent to the converter, is intact in the near-rigor and transition structures but disordered in the detached form, which has been interpreted to be a motor-ATP state (Houdusse et al., 1999). This mobility of the SH1 helix reflects its role in transmitting and directing movements of the relay helix to the converter domain and lever arm. Structural changes at the nucleotide-binding cleft of myosin are thus coupled to movements of the relay helix, SH1 helix, converter domain and the lever arm (Fig. 1). The power stroke is thought to correspond to the swinging of the lever arm (Rayment et al., 1993a; Rayment et al., 1993b); however, the structural elements that undergo strain and the conformational changes in myosin that produce force have not yet been identified.

**Kinesin motors**

By comparison with myosin, the structural states of the kinesin motors have been elusive. Almost all of the crystal structures solved so far are complexed to ADP and are interpreted to represent weak-binding or detached ADP states (reviewed by Sack et al., 1999). The kinesin-ADP models do differ from one another, indicating that they may represent structural transitions within the ADP state, which could help identify the mechanical elements of the motor that undergo conformational changes during the hydrolysis cycle. For example, human kinesin (Kull et al., 1996) and rat kinesin (Kozielski et al.,...
myosin) forms a hydrogen bond with an oxygen from the \(\gamma\)-phosphate; (2) a conserved serine (or threonine) residue from switch I (SSRFG in myosin) forms a strong hydrogen bond from its \(\gamma\)-oxygen to the Mg\(^{2+}\) ion and a weaker one to the \(\gamma\)-phosphate of the nucleotide; and (3) the same serine or threonine forms a hydrogen bond from its amide nitrogen to an oxygen on the \(\gamma\)-phosphate. Given that the KIF1A-AMP-PCP structure does not show any of these features, it probably represents a collision ATP complex - a first step in ATP binding that does not produce major conformational changes (Cooke, 1986) - rather than a catalytically active state similar to Ras-GMP-PNP (PDB 5P21) (Pai et al., 1990) or myosin-VO\(_4\) (PDB 1VOM) (Smith and Rayment, 1996).

Crystal structures of kinesin mutants designed to destabilize the ADP state, stabilizing conformations that may normally be unstable or transient, reveal extensive changes in the conformation of switch I and switch II (Yun et al., 2001). The kinesin mutants affect highly conserved or invariant arginine and glutamic acid residues of switch I (SSRSH) and switch II (DLAGSE), respectively, and disrupt a salt bridge between the two residues that is observed in some, but not all, kinesin-ADP structures (Sack et al., 1999; Yun et al., 2001). The R-E salt bridge is also observed in myosin (Fig. 4D), where it has been

![Image](https://example.com/image.png)  
**Fig. 2.** Comparison of the three observed structural conformations of myosin. The structures are weak-binding states, but are modeled as complexed with actin for illustration. Motor domains (gray) and converter domains/lever arms (colored) for the myosin detached state (red) (scallop S1-ADP, PDB 1B7T) (Houdusse et al., 1999), near-rigor state (green) (nucleotide-free scallop S1, PDB 1DFK) (Houdusse et al., 2000) and transition state (cyan) (scallop S1-ADP-VO\(_4\), PDB 1DFL) (Houdusse et al., 2000) are shown bound to a short actin filament of five molecules (yellow). In the detached state, the lever arm and associated light chains (not pictured) would collide with an extended actin filament. Motor domains were aligned using a least-squares alignment of 19 \(\alpha\)-carbon atoms, including residues 168-186.
proposed to stabilize the CLOSED form of the motor, in which the switch I and switch II regions close in around the bound nucleotide, a conformation thought to be essential for ATP hydrolysis (Geeves and Holmes, 1999). In contrast, the OPEN conformation of myosin, in which the R-E salt bridge is not formed (Geeves and Holmes, 1999; Kliche et al., 2001), is catalytically incompetent (Furch et al., 1999). The kinesin salt-bridge mutants show striking changes in the switch I and switch II regions that include transitions from a disordered to a visible switch II loop and from ordered to disordered switch I loop and two flanking helices (Yun et al., 2001) (Fig. 5). The mutants thus stabilize the switch II region, resulting in a longer switch II helix as well as a visible switch II loop, and destabilize the switch I region, causing the switch I loop to become mobile and the helices on either side to partially melt (Fig. 5). The mutant conformations have been interpreted to represent new ADP crystal forms that differ from those previously reported (Sack et al., 1999) or intermediates between the kinesin-ADP and kinesin-ATP states (Yun et al., 2001).

The structural transitions between KIF1A-ADP and KIF1A-AMP-PCP, together with those observed in the two Kar3 salt-bridge mutants, shed important new light on the mechanism of the kinesin motors. They show that the switch I region can dramatically alter its conformation, changing from a small helix or helices [as in human KHC (Kull et al., 1996), rat KHC (Kozielski et al., 1997) and KIF1A-ADP (Kikkawa et al., 2001)] to a pseudo-β-hairpin [as in KIF1A-AMP-PCP]

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**Fig. 3.** Structural changes at the active site and the filament-binding region in kinesin and myosin. (A,B) Comparison of human kinesin (PDB 1BG2) (Kull et al., 1996) and rat kinesin (PDB 2KIN, monomer structure) (Sack et al., 1997) showing the nucleotide-binding site with bound ADP and movements of helices α4 and α5 (human, orange; rat, red). Helix α4 can be seen in (A) and is the upper red/orange helix pair in (B). The lower helix pair is α5. The superposed structures show little difference at the active site in the P-loop (green), switch I (purple), or switch II (cyan), but helices α4 and α5 are displaced relative to one another by a simple translation of 4.5 Å. The kinesin neck linker, observed in the rat structure, is shown in magenta. (C,D) Superposed KIF1A-ADP (PDB 1I5S) and KIF1A-AMP-PCP (PDB 1I6I) (Kikkawa et al., 2001). The movement of helices α4 and α5 (KIF1A-ADP, orange; KIF1A-AMP-PCP, red) is more complex than in (B), and consists of a translation coupled with a rotation. There is also a substantial structural rearrangement of the switch I region (C) (KIF1A-ADP, pink-purple; KIF1A-AMP-PCP, yellow), which changes the short loop-helix-loop-helix (KIF1A-ADP) to a short pseudo-β-hairpin (KIF1A-AMP-PCP). (E,F) Myosin-ADP·BeF3 CLOSED (F.J.K. & K.C. Holmes, unpublished) compared with myosin-ADP OPEN (PDB 1G8X) (Kliche et al., 2001). The relay helix (analogous to kinesin helix α4) in the CLOSED form (red) is translated along its axis ~4.5 Å toward the nucleotide-binding site with little rotational movement at its N-terminus. However, the pronounced bend in the middle of the relay helix in the CLOSED structure causes a ~100° rotation of its C-terminal end, causing the position of the helix end in the OPEN (orange) and CLOSED (red) forms to differ by 15 Å. This movement is further amplified by the myosin converter domain, and is thought to drive the myosin power stroke. All of the comparisons and distances described in this paper are based on a least-squares alignment of 19 α-carbon atoms in the structurally conserved P-loop, the preceding β-strand, and the N-terminal end of the following α-helix (residues 77-95 of human kinesin, 78-96 of rat kinesin, 89-107 of KIF1A, 466-484 of Kar3 and 171-189 of Dictyostelium myosin II).
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To a completely disordered structure [as in the Kar3 salt-bridge mutants (Yun et al., 2001)], the switch I region can almost certainly adopt a conformation not yet observed in the kinesin motors in which conserved serine residues (SSRSRSH) interact with the Mg·ATP at the active site. The intriguing possibility that arises from structural analysis of the Kar3 mutants (Yun et al., 2001) is that disruption of the switch I region may be responsible for the mechanism of nucleotide release in the kinesins. A disruption of the switch I region following nucleotide hydrolysis could interfere with the coordination of Mg²⁺, causing Mg²⁺ to be released.

Motor-microtubule interactions

What do the new structures tell us about interactions between the kinesin motors and microtubules? Cryoelectron microscopy (cryoEM) density maps of KIF1A bound to microtubules in the presence of ADP or the ATP analogue AMP-PNP (Kikkawa et al., 2001) have been interpreted to show that the long axis of the motor is offset by ~20° in the AMP-PNP state compared with the ADP state, suggesting that the motor undergoes a directional rotation between these two states. However, the difference in motor orientation between the two states is slight, and this, together with the low resolution of the ADP density map (22 Å) and the absence of structural features in the central region of the motor that could be used as landmarks to orient the motor, makes it difficult to demonstrate unequivocally that such a rotation occurs. The KIF1A-ADP and KIF1A-AMP-PCP crystal structures have also been fitted into the cryoEM density maps - but the crystal structures do not fit precisely into the density maps, and structural elements can be seen to protrude from the electron density even though the fittings have been optimized to...
minimize these effects. This indicates that the positions or conformations of at least some of the structural elements in the microtubule-bound motors differ from the atomic models. This is not unexpected, since the crystal structures of the motor are not complexed with tubulin and are highly unlikely to be in the same states as the motor bound to microtubules and imaged by cryoEM. The authors interpret their fittings of the atomic structures into the cryoEM maps to show that the rotational movement they observe is centered around helix \( \alpha_4 \), the relay helix, which rotates \( 20^\circ \) between the ADP and AMP·PCP atomic models. They propose that, when the motor is bound to the microtubule, the motor itself rotates around the helix, which remains fixed in place (Kikkawa et al., 2001).

Kikkawa et al. propose that the rotational movement of the catalytic core drives docking of the neck linker onto the motor core and is accompanied by a displacement of the motor towards the plus end of the microtubule, thus accounting for the plus-end-directed movement of KIF1A and other kinesin motors (Kikkawa et al., 2001). They further propose that a similar rotation of the catalytic core of Ncd, a kinesin-related motor that has the opposite directionality, disrupts an interaction between the neck and the motor core that drives movement of Ncd towards the minus end. These interpretations of the static crystal structures provide models for motor function that require confirmation by further experimental work. Several aspects of the proposed mechanisms lend themselves to testing—for example, by biochemical analysis of mutants or spectrophotometric analysis of motors containing fluorescent probes at specific sites (Xing et al., 2000) to determine whether the proposed rotation occurs during force production by the motor and is therefore relevant to motor function. It is also of interest to determine whether the rotation, if it occurs, is coupled to directional movement of the motor. Such a rotation could represent a basic force-producing movement of the motor with the relay helix acting like a spring or elastic element that undergoes strain and enables the motor to produce force. If so, the movement would differ from the piston-like movement of the relay helix thought to occur in myosin (Dominguez et al., 1998) (Fig. 3E,F) and proposed to drive the swinging of the lever arm (Vale and Milligan, 2000). Although the relay helix could serve as the spring or elastic element involved in force generation in both kinesin and myosin, the mechanistic details of force generation would differ. The rotational movement thought to occur in KIF1A could represent an ancient conformational movement inherited from the G proteins, which evolved into the kinesin and myosin motor mechanisms. This could explain the non-processive, plus-end-directed motility of neck-mutated Ncd-kinesin and Ncd constructs (Endow and Waligora, 1998; Case et al., 2000) that appears to be intrinsic to the kinesin motor domain. Amplification by the neck and/or neck linker of such an initial strain-generating movement could direct motor movement towards the microtubule plus or minus end.

The salt-bridge mutants provide further information about the changes that occur in the kinesin motors in states subsequent to the ADP state. Biochemical analysis of the mutant motors demonstrates that they can hydrolyze ATP and bind to microtubules but show no microtubule-activated ATPase activity (Yun et al., 2001), which is essential for movement of the motor along the microtubule. Activation of the motor ATPase is thought to occur in the kinesin motors by accelerating the rate-limiting step, under nonsaturating microtubule concentrations, of ADP release (Hackney, 1988). Because the mutants can bind to microtubules but binding to microtubules fails to activate their ATPase, the mutants ‘decouple’ (Ruppel and Spudich, 1996) microtubule- and nucleotide-binding by the motor (Song and Endow, 1998). The decoupling of these two essential motor activities is interpreted to be due to a block in communication between the microtubule- and nucleotide-binding regions of the motor (Yun et al., 2001). Together with a previous mutant in which microtubule-activated ATPase activity is blocked (Song and Endow, 1998), the salt-bridge mutants define a structural

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**Fig. 5.** Comparison of wild-type and mutant R-E salt-bridge Kar3 motor domains. Wild-type Kar3 (PDB 1F9T) (Yun et al., 2001) superposed with (A) the E631A (EA) mutant structure (PDB 1F9W) (Yun et al., 2001) or (B) the R598A (RA) mutant structure (PDB 1F9V) (Yun et al., 2001). Blue regions represent areas of least change with <0.5 Å difference between main-chain atoms. Gray areas show differences of 0.5-1.0 Å, after which the color changes to orange (for wild type) or red (for the mutants), becoming solid at 2.0 Å structural difference. Yellow elements are present in the wild-type structure, but not the EA mutant. Green elements are observed in the RA mutant, but not wild-type Kar3. The ADP and Mg\(^{2+}\) from wild-type Kar3 are also shown. The arrow in (A) indicates the position of the switch I loop, which becomes disordered in the EA and RA mutants. The arrow in (B) indicates loop 11 and the N-terminus of helix \( \alpha_4 \), the relay helix, which become ordered upon mutation of R598. The \( \alpha \)-helix preceding switch I also undergoes a substantial movement in the RA mutant.
signaling pathway within the motor for ATPase activation by microtubules. This pathway extends from helix α4, the relay helix, at the microtubule-binding region of the motor to the R-E salt bridge between switch I and switch II to the active site. The mutants further show that direct interactions between switch I and switch II are required for activation of the motor ATPase by microtubules.

The salt-bridge mutants are trapped in states that alter the ability of the motor to bind to microtubules: one of the mutants (RA) binds weakly to microtubules compared with the wild type, whereas the other (EA) binds tightly (Yun et al., 2001). The regions of the motors that are altered in the crystal structures identify structural elements that are likely to be involved in weak and strong binding of the motor to microtubules. The structures of the switch II loop and helix differ between the RA and EA atomic models, and this may be responsible for differences in the interactions of the motors with microtubules - the switch II helix is longer by one turn in the RA mutant compared with one of the two available wild-type structures, and the switch II loop is visible in the RA mutant rather than disordered, as in the EA mutant. Stabilization of the switch II loop and the N-terminus of the switch II helix might interfere sterically with microtubule binding, causing the weak binding of the RA mutant to microtubules. Loop L8 is displaced slightly in the EA mutant relative to its position in the wild-type motor. This difference, although small, may reflect an inherent difference in loop mobility that causes the mutant motor to bind more tightly to microtubules than the wild type. Previous studies implicate the switch II loop/helix and loop L8 in binding to tubulin (Woehlke et al., 1997; Alonso et al., 1998; Hirose et al., 1999; Kikkawa et al., 2000), supporting these interpretations.

The atomic structures of the salt-bridge mutants further show that changes in the water-mediated coordination of the nucleotide at the active site occur when the salt bridge between switch I and switch II is disrupted (Yun et al., 2001), as noted above. The movements of switch I residues toward the nucleotide, although <1 Å, disrupt coordination of the Mg²⁺ ion by water molecules. This could result in loss of Mg²⁺, which would be followed immediately by ADP release. Thus, the rate-limiting step in nucleotide hydrolysis would be overcome (Hackney, 1988), explaining the activation of the motor ATPase by microtubules.

Kinesin and myosin motor mechanisms – a comparison

Although motor-filament interactions of myosin and the kinesin motors during the hydrolysis cycle differ, the structural homology of their catalytic cores suggests that movements of conserved structural elements parallel one another. For example, analogous movements of the switch I and switch II regions, which are highly conserved in myosin and the kinesin motors, are expected to occur during nucleotide hydrolysis and exchange in the two classes of motors. Such movements of the switch regions have now been observed in crystal structures of both motors. In myosin, the movements change the structure of the nucleotide-binding cleft, as in the G proteins (Sprang, 1997): conversion of the OPEN form of myosin to the CLOSED form is accompanied by movement of switch II into the active site (Fisher et al., 1995; Geeves and Holmes, 1999) (see Fig. 3E,F and Fig. 4C,D). In the new kinesin structures, switch I moves towards the bound nucleotide when the motor is complexed with AMP-PCP (Kikkawa et al., 2001) or when the salt bridge between switch I and switch II is disrupted (Yun et al., 2001), whereas switch II remains unchanged in position. But the movement of the invariant switch I serine residues (SSRSR) in the KIF1A-AMP-PCP model and the salt-bridge mutants is small (<1.0 Å) and does not result in additional interactions between switch I and the nucleotide. This means that we have not yet seen the critical transition in kinesin corresponding to the CLOSED conformation of myosin in which the switch II glycine flips in to hydrogen bond with the γ-phosphate. When kinesin does this, there is likely to be a movement of switch II toward the nucleotide, although perhaps not so large as that in myosin, since switch II of kinesin is already intermediate in position between that of myosin in its OPEN and CLOSED forms. It also seems likely that this conformation will be seen only when the motor is complexed with microtubules (as noted above) or in a mutant that somehow mimics the binding of the motor to microtubules. The requirement for stabilization of motor movements by the microtubule is a crucial point for the kinesin motors - it not only explains why a true ATP state has not yet been seen in the crystal structures but is important for the interpretation of available structures.

Mutants that disrupt the salt bridge between switch I and switch II inhibit or block the filament-activated ATPase activity of both myosin and the kinesin motors (Onishi et al., 1998; Furch et al., 1999; Yun et al., 2001). This indicates that direct interactions between switch I and switch II are needed for activation of the myosin and kinesin motor ATPase. Disruption of the salt bridge in myosin appears to interfere with the formation of the catalytically active CLOSED conformation (Kliche et al., 2001), shifting the mutants toward the switch II OPEN conformation, whereas it causes the kinesins to move towards a switch I OPEN conformation. The disrupted salt bridge of both myosin and the kinesins thus appears to permit movements of the switch I and switch II regions that are prevented when the salt bridge is formed (Geeves and Holmes, 1999; Yun et al., 2001). This could prevent the mutants from populating a state essential for ATPase activation in the wild-type motors.

Although it is unclear how far the mechanistic similarities between kinesin and myosin extend, the following movements are expected to occur during the kinesin ATPase cycle on the basis of the structural changes observed in the myosins and G proteins. As noted above, the new kinesin structures show a conformation predicted to occur in myosin that we refer to as switch I OPEN, switch II OPEN (F.J.K. and K.C. Holmes, unpublished). Nucleotide hydrolysis in both myosin and G proteins requires both switch elements to be CLOSED. When kinesin is not bound to microtubules, the microtubule-binding region and switch II appear to be uncoupled from one another (see below), and it seems likely that the link between these regions is established upon microtubule binding, perhaps through the ordering of loop 11 by interactions with the microtubule. If binding to the microtubule causes switch II to become even more OPEN, this, in combination with the already OPEN switch I, could result in destabilization and release of the bound Mg-ADP. Subsequent binding of ATP might then pull switch II into a CLOSED position, in which a hydrogen bond forms between the invariant switch II glycine
(DLAGSE) and the γ-phosphate, thereby causing the switch II helix at the microtubule-binding site to change in conformation and the neck linker to dock against (or undock from) the motor core (Schief and Howard, 2001). In myosin, the conserved salt bridge between switch I and switch II stabilizes the CLOSED conformation, which is necessary for ATP hydrolysis. The role of the salt bridge in the kinesins is less certain - the salt bridge is observed in some, but not all, motor-ADP crystal structures and, in cases in which it forms, its geometry is imperfect. It is not clear whether a perfect salt bridge forms when the motor is in a hydrolysis-competent state, since the salt bridge is not formed in the closest structure yet to an ATP state, KIF1A-AMP-PCP, or in the Kar3 salt-bridge mutants, which can be interpreted to be transitions towards an ATP-bound state. If the switch I and switch II elements in the hydrolysis-competent conformation of kinesin assume the same conformation as they do in myosin, the salt bridge would form with perfect geometry, leading to the formation of a hydrolysis-competent CLOSED conformation similar to that of myosin and the G proteins. However, the switch I region of kinesin has never been observed in a conformation that resembles switch I CLOSED of myosin and could function in a different manner, reflecting differences that exist between myosin and the kinesins in the motor-filament state during the hydrolysis step. Formation of the salt bridge, even with imperfect geometry, when the motor is detached from the filament may stabilize the unbound motor, whereas the absence of the salt bridge may be needed to permit changes in the active site when the motor is bound to its filament.

**Further uncertainties**

In addition to the uncertainties regarding the rotation of the relay helix that has been inferred from the KIF1A atomic structures and cryoEM analysis (Kikkawa et al., 2001), questions regarding other aspects of the kinesin mechanism remain. One of these concerns the docking of the neck linker onto the motor catalytic core. Several crystal structures now show a visible neck linker docked onto the motor core forming β-sheet structure with other β-strands of the catalytic core, rather than disordered and not visible in the model. But it is still uncertain whether the docking occurs in the ADP or ATP state, since the neck linker has been observed docked onto the motor core in motors bound either to ADP (Kozieliski et al., 1997; Sack et al., 1997) or the ATP analogue, AMP-PCP (Kikkawa et al., 2001). Kikkawa and co-workers interpret the previously reported kinesin-ADP structures with a docked neck linker (Kozieliski et al., 1997; Sack et al., 1997) to be motor-ATP structures. But the distinction between the two states is not clear if the ADP and ATP conformations differ only slightly and if the states do not depend on the bound nucleotide because the energy transition between them is low, as is seen in myosin (Urbanke and Wray, 2001).

Another way of phrasing this question is what is the structure of the neck linker of kinesin in a conformationally distinct ATP state? A recent model proposes that the neck linker docks against the catalytic core when the motor binds to ATP and microtubules, directing the motor towards the plus end (Rice et al., 1999). However, evidence supporting this model is controversial (see Schief and Howard, 2001), and more work is needed to determine its validity. It should also be noted that the KIF1A structures solved were those of a chimera consisting of the neck linker from conventional kinesin fused to the KIF1A catalytic core (Kikkawa et al., 2001). It is therefore still uncertain exactly how the native KIF1A protein behaves, especially given its unusual nature. KIF1A has been reported to move in a biased diffusional manner along the microtubule (Okada and Hirokawa, 1999), rather than by alternative binding of two heads like conventional kinesin (Howard, 2001), and it is the alternative binding of the kinesin heads that the neck linker docking and undocking are thought to regulate. Whether KIF1A is regulated by nucleotide binding in the same way as conventional kinesin is unclear, given its atypical mechanism of motility.

Moreover, the structural changes observed in helices α4 and α5 of KIF1A-AMP-PCP are uncoupled to changes in the nucleotide-binding site. In myosin, the C-terminal end of the relay helix is linked to the converter domain (Fig. 1) by strong hydrophobic interactions and the two move as a rigid body - when the relay helix moves, so does the converter, and a tightly coupled pathway of conformational change connects the relay helix/converter to the nucleotide-binding site. If γ-phosphate is present at the active site, the relay helix is pulled up and in, the helix bends, the converter rotates, and the lever arm swings. These movements occur even in detached motors not bound to actin, in which they function to reprimed the myosin head to position it for binding to the next actin site. In the kinesins, there is a short loop (L12) between helix α4, the relay helix, and helix α5 that differs from the large actin-binding domain inserted at this site in myosin. The movement of the two helices appears to be coupled in the KIF1A-AMP-PCP structure (Kikkawa et al., 2001), but the link between the nucleotide-binding site and the two helices, which form part of the motor interface with the microtubule (Woehlke et al., 1997; Alonso et al., 1998; Hirose et al., 1999; Kikkawa et al., 2000), is weak in motors not bound to microtubules. That is, the changes in helices α4 and α5 at the microtubule-binding site of KIF1A appear to be unlinked to structural changes at the active site, at least in the absence of microtubules. The Kar3 uncoupling and salt-bridge mutants (Yun et al., 2001) show that a pathway of structural changes from helix α4 to the active site does exist in the kinesins, but the structural details of this pathway are not evident from the KIF1A-AMP-PCP crystal structure. The conformational changes described by Kikkawa et al. could therefore be a result of crystal contacts affecting the position of the helices, rather than the nucleotide state inducing a conformational change (Kikkawa et al., 2001). This is also a likely explanation for the differences in the positions of helix α4 and α5 observed in the rat and human kinesin structures (Sack et al., 1999). Variable regions in different crystal structures can be indicative of movements that occur in vivo, however. The observed movements of helices α4 and α5 could therefore approximate actual conformational changes that occur during the kinesin cycle, although they may also differ significantly in detail and magnitude. The true ATP state of kinesin remains to be visualized.

**Future directions**

A major question that has not yet been answered either for myosin or the kinesins is what are the changes in conformation that occur in the strongly bound states of the motor? High-
resolution atomic structures that reveal the changes in position and conformation of structural elements both at the motor filament-binding interface and the nucleotide-binding cleft are needed to understand the motor mechanism. The fitting of available atomic models into the cryoEM density maps is an initial step forward, but crystal structures of the motors complexed with different nucleotides in strongly bound states are needed to reveal the changes in conformation of the motor at higher resolution. Finally, further work is needed to identify the force-producing conformational changes or movements of the motors and the spring-like or elastic elements predicted to be required for force production. This information will be essential to understand both the kinesin and myosin motor mechanisms and their similarities and differences to one another.

References


