Nucleotide switches in molecular motors: structural analysis of kinesins and myosins

Elena P Sablin and Robert J Fletterick*

Recent breakthroughs in the structural biology of cytoskeletal motor proteins show that two distinct families of motors — kinesins and myosins — use a similar mechanism of conformational switching for converting small structural changes in their nucleotide-binding sites into larger movements to provide force generation and motion. This mechanism is found to be similar to that employed by G proteins, the well-known molecular switches that regulate protein–protein interactions in many biological systems.

Addresses
Department of Biochemistry and Biophysics, University of California, 513 Parnassus Avenue, San Francisco, California 94143, USA
*e-mail: flett@msg.ucsf.edu

Current Opinion in Structural Biology 2001, 11:716–724

0959-440X/01/$ – see front matter

Abbreviations
EM electron microscopy
NEF nucleotide exchange factor
PDB Protein Data Bank

Introduction

Only a few years have passed since the first atomic-resolution structures of kinesin [1,2] and myosin [3] motors were determined. These years, however, separate the era when these motor families could not be compared to each other from today, when nearly every finding for one motor family prompts us to search for and often find the analogous phenomenon in the other family. Indeed, we have recently learned that not only kinesins [4,5], but also myosin [6••,7••] motors can move processively along their respective polymer track. Furthermore, kinesins (summarized in [8]) and myosins [9••] can translocate in either the 'plus' or the 'minus' direction along their cellular tracks. With atomic resolution structures now available for various kinesins [1,2,10–13,14••,15•] and myosins [3,16–21,22••,23,24], we understand that the functional similarity of these motors is a reflection of their shared evolutionary ancestry and similar structural organization [25].

Both kinesin and myosin have catalytic domains (catalytic cores, for details see [26•,27,28•]), which, although different in size, are structurally similar [1,25] and have both the nucleotide- and biopolymer-binding sites (Figure 1). In conjunction with their track polymers (microtubules for kinesins and actin filaments for myosins), both kinesin and myosin catalytic cores execute the nucleotide hydrolysis cycle, which consists of ATP binding, hydrolysis and release of the products of hydrolysis (ADP and inorganic phosphate [Pi]). Depending on their nucleotide state (with either no nucleotide, or a diphosphate or triphosphate moiety in the bound nucleotide), both kinesin and myosin catalytic domains have been recently shown to switch between different structural conformations [14••,28•,29••]. Attached to the cores, there are smaller domains in both motors. Although structurally divergent and differently named (neck or neck linker [26•,27,30] and converter [28•] domains in kinesins and myosins, respectively; Figure 1), these structural parts perform identical functions [29••]. Interacting closely with the catalytic core, they 'read out' the nucleotide-dependent conformation of the core and, according to their own specific composition and architecture, 'respond' by changing their structure. Numerous structural [3,11,12,14••,21,22••,23] and mutational [31–34,35•] studies indicate that specific neck and converter domains, transmissions in almost a literal sense, are crucial mechanical elements of, respectively, kinesins and myosins, and are essential for the determination of the directionality of these motors. Conformational transitions in the neck or converter regions are amplified into larger motions by the family-specific 'amplifiers' — the 'lever arm' in myosin ([36–38]; summarized in [28•]) and the neck coiled coil in the conventional kinesin (Figure 1). Class-specific domains of unconventional monomeric kinesins [14••] and partner heads in processive dimeric motors assist in this function [7••,26•,29••,39••]. The following sections of this review will focus on the recently discovered mechanisms coordinating the operation of the various structural parts of these molecular motors.

Mechanism of conformational switching

Based on the structural similarity discovered between molecular motors and the molecular switches of the G protein family [2], it was previously proposed that motor proteins and G proteins might use a comparable γ-phosphate-sensing mechanism and a similar strategy for changing their conformations between different nucleotide states [2,40]. To change conformation between the ADP and ATP states, both kinesins and myosins were predicted to use specific structural elements of their catalytic cores, the 'switch' regions, which are able to sense the presence or the absence of γ-phosphate in the bound nucleotide [2,40]. In both motors, the switch I region consists of a loop (with consensus sequence motif NxxSSR) and the switch II region includes a loop (with conserved sequence motif DxxGxE) and the following helix (Figure 2). It was predicted that, analogous to G proteins, the γ-phosphate-sensing mechanism in motors would rely upon two residues, evolutionarily conserved serine and glycine from the switch I and switch II loops, respectively. In the ATP state of the motors, switch I serine and switch II glycine would converge on ('switch on') the nucleotide to form

hydrogen bonds with the γ-phosphate. The initial movements of these residues were predicted to trigger specific restructuring of the entire switch regions, which would move towards the nucleotide and 'close' the nucleotide-binding pocket. In the ADP state, the absence of bridging interactions with the γ-phosphate would disengage the switch regions, causing them to 'switch off' to their initial conformations and to 'open' the nucleotide-binding cleft.

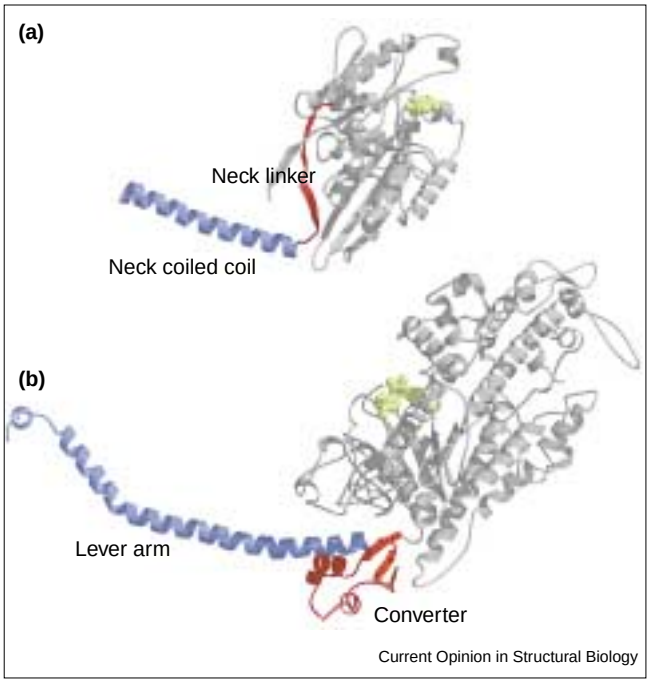
Subsequent structural analysis of available myosin structures showed that they, indeed, form two distinct categories (summarized in [28•]). In the first group of structures, the switch loops are disengaged and the nucleotide-binding pocket is 'opened' (Figure 2a, in yellow). In the second group, the switches are brought together and the nucleotide-binding cleft is 'closed' (Figure 2a, in red). Based on the observed conformations of the switch regions (and not necessarily on the nature of the bound nucleotide [28•], see later discussion), the opened and the closed myosin structures have been proposed to represent, respectively, the ADP/no nucleotide and the ATP/ADP•Pi states of the myosin catalytic core.

Superposition of available kinesin structures [14••] showed that they also form two categories, which have been proposed to represent the ADP and the ATP states of the kinesin catalytic core [14••,29••]. It was demonstrated [14••] that, similar to the myosin structural counterpart, the switch II helix of kinesin undergoes translation and rotational movement between the ADP (Figure 2b, in yellow) and the ATP-like (Figure 2b, in red) states. These movements were shown to be accompanied by the reversible partial unwinding of the switch II helix and lengthening of the switch II loop in the ATP state [14••]. It was noted, however, that, whereas the switch I and switch II loops of myosin motors are well structured and have defined conformations in both ADP and ATP-like states (Figure 2a), the equivalent structural counterparts of kinesin motors show considerable variation in conformation [41] and significant flexibility in both states [14••] (Figure 2b). This difference could be related to distinct mechanochemical cycles of these two motors and will be discussed further in this review.

Conformational transitions in mechanical elements of the motors

The nucleotide-dependent conformational changes in the switch regions result in dramatic rearrangements of their mechanical elements. In kinesins, the nucleotide-induced translation and rotational movement of the switch II helix affect the following helix and their connecting loop, which are all interlocked and, therefore, move together as a structural cluster ('switch II cluster' [14••]; Figure 3a). In the forward-going kinesin motors, the ATP-like conformation of the switch II cluster allows the N-terminal part of the neck domain (the neck linker [26•]) to be 'docked' alongside the catalytic core. A different position

Figure 1

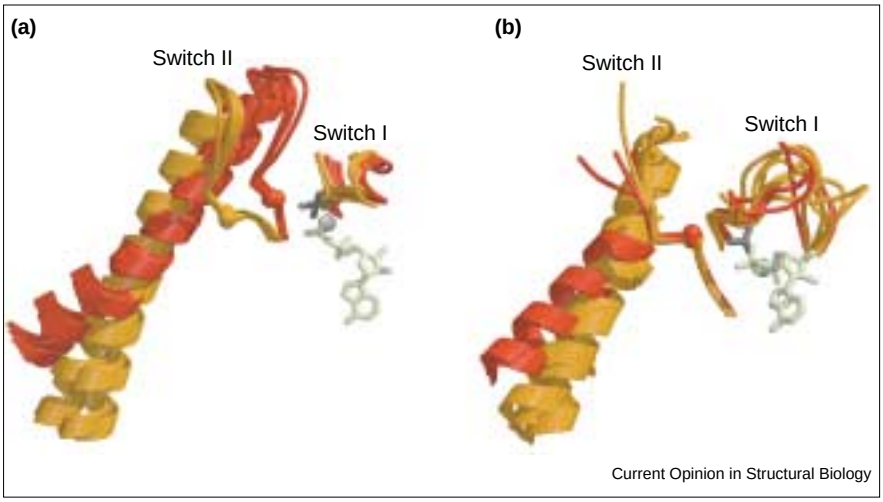


Structural organization of the kinesin and myosin motors. The catalytic domains of (a) kinesin (PDB code 3kin) and (b) myosin (PDB code 2mys) are gray. The neck linker and converter regions of kinesin and myosin, respectively, are red. The amplifier regions (neck coiled coil in kinesin and lever arm in myosin) are blue in both motors. The position of the bound nucleotide is indicated by a yellow space-filling model for both motors.

of the same cluster in the ADP state occludes this docking, pushing the neck away from the core (Figure 3a). As a result, in the forward-going kinesins, the neck linker is docked on the core in the ATP-like state and undocked and freed in the ADP state [14••,29••].

Whereas the 'undocked' neck linkers are found to be flexible and disordered in most kinesin ADP-like crystal structures, the recently solved ADP-like structure of the mitotic spindle kinesin Eg5 [15•] showed the neck linker to be ordered and rotated by ~120° relative to its ATP-like 'docked' conformation. In this study, the authors discuss whether the observed conformation of the neck is related to the specific function this kinesin motor performs in the cell, or whether it represents a preferable but transient conformation employed by all kinesins in the ADP state. The latter proposal does not contradict the results of a previous study [39••], in which the nucleotide-dependent movements of the neck of conventional kinesin have been visualized and measured directly in the combined electron paramagnetic resonance (EPR), fluorescence resonance energy transfer (FRET) and cryo-electron microscopy (cryo-EM) experiments. Based on the complementary results derived from these experiments and mutational studies on kinesin [35•], a model for how nucleotide-induced neck linker 'docking'

Figure 2



The nucleotide-driven conformational changes in the switch regions. The mechanism of conformational switching is exemplified using available atomic coordinates of (a) myosin and (b) kinesin motor domains. All structures were superimposed using the Cα atoms of the conserved nucleotide-binding loop, the P-loop, which is known to remain unchanged throughout the nucleotide hydrolysis cycle. Switch regions of both kinesin and myosin are shown in yellow and red for the ADP and the ATP-like states, respectively. The position of the bound nucleotide is indicated by a model in light gray for both motors. The γ-phosphate is in dark gray. Conserved switch I serine and switch II glycine in all structures are shown as yellow and red stick models and spheres, respectively.

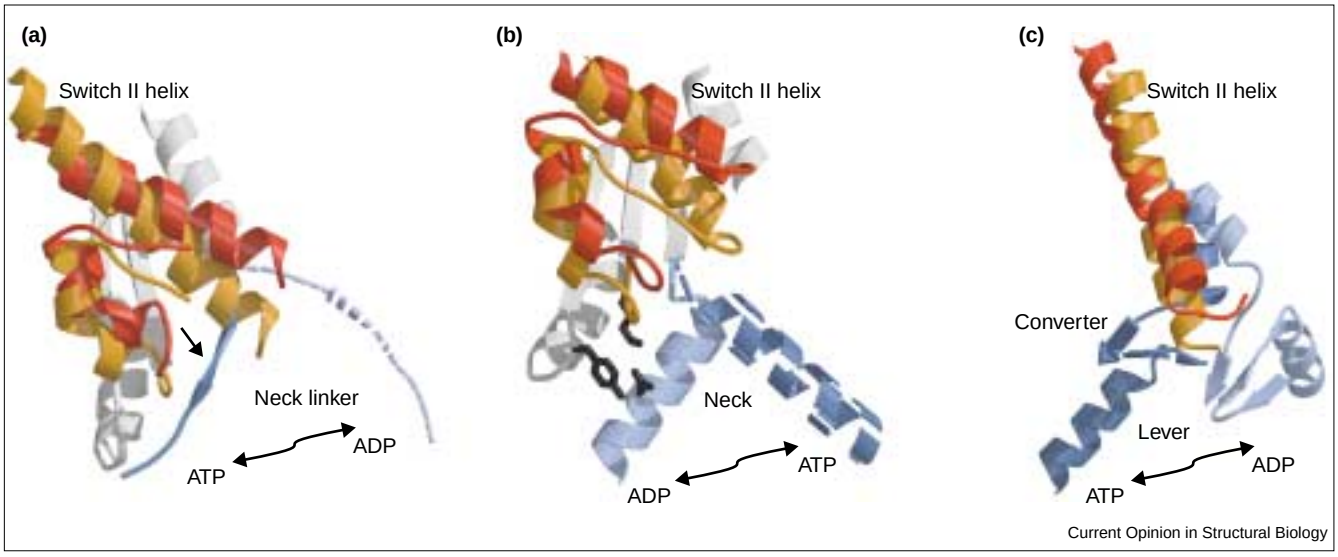
and 'undocking' could drive the processive motion of dimeric conventional kinesin was suggested [39••]. Given the orientation of the bound kinesin head on the microtubule, whereby the tip of the catalytic core points towards the microtubule plus end [39••,42], ATP-induced 'docking' of the neck linker on the core of the bound kinesin head would position the unbound partner head closer to the microtubule plus end. The partner head could then attach to the forward tubulin subunit, provided that its neck linker is in the ADP, 'undocked' conformation. Once the two-head-bound intermediate is formed, the exchange of ATP for ADP in the forward head would again trigger 'docking' of the neck linker. This could assist in tugging on, dissociating and displacing the rear head to the next forward binding site [39••]. The net result of this action would be the observed rapid 8 nm step [5,43,44].

Whereas subsequent biophysical [45,46•] and mutational [47•,48•] studies elaborate this model for the processive movement of conventional kinesin, the recent analysis of the ADP and ATP-like structures of the kinesin motor KIF1A [14••] extends its scope to the processive movement of monomeric kinesins [49••,50•,51•]. This analysis [14••] also suggests that the modular mechanism of conformational switching in the kinesin catalytic core can control the actions of diverse kinesin mechanical elements and power the motility of both plus- and minus-end-directed kinesin motors (Figure 3a,b). A striking symmetry relates the mechanisms of domain movements in these two classes of kinesins. Because of the different location (N-terminal to the catalytic core) and different architecture of the neck of the minus-end-directed kinesin motors [12,52] (Figure 3b), the ADP-like position of the switch II cluster promotes docking of the neck onto the core in the ADP state. Moreover, the docked position of the neck in this case is stabilized by its specific interactions with the switch II cluster in the ADP state [12] (Figure 3b, see figure legend for details). These are the exact opposite of the interactions observed in the forward-going kinesin [14••] (Figure 3a,b). The nucleotide-induced rotation and retraction of the switch II cluster in the ATP state of the minus-end-directed kinesins would disintegrate the neck–core interface and cause the neck to melt away from the core (a hypothetical transition of the neck of the minus-end-directed kinesin motor ncd is shown in Figure 3b). This conformational transition would produce a force vector directed towards the microtubule minus end, opposite to that of the conventional kinesin (Figure 3a,b).

The proposed model [14••] for reversed movement in kinesins is consistent with the recent study in which mutations of conserved residues at the neck–core interface of the minus-end-directed kinesin ncd produced a motor that could move in both directions along the microtubule [53••]. In agreement with the model [14••], such mutations would destabilize the neck–core interface in the ADP state of the motor and cause uncoupling of directionality from the motor's ATPase cycle. Such uncoupling would explain the sudden changes in the motor's directionality and the observed breakage of the microtubules in response to the oppositely directed forces exerted simultaneously by different molecules in the population assayed in the experiment [53••].

As comparative structural analysis shows [29••], the same mechanism of conformational switching that drives the motility of kinesin motors controls movements of the mechanical elements of myosins. The converter domain of myosins, which move towards the 'barbed' (plus) end of the actin filament, appears to be a more complex structural assembly compared to the neck linker of forward-going kinesins (Figure 3a,c). Nevertheless, analogous to the plus-end-directed kinesin motors, the converter region is folded onto the myosin core in the ATP-like state and pushed away from this position in the ADP state [3,21,22••]. These conformations of the converter region are dictated

Figure 3



The switch-controlled movements of the mechanical elements of kinesin and myosin motors. (a) The nucleotide-dependent reorientation of the neck linker between the ADP and ATP-like structures of the kinesin KIF1A [14••]. (b) A hypothetical model for the nucleotide-controlled movements of the neck in the minus-end-directed kinesin motor ncd. Three interacting conserved residues that are essential for the stabilization of the neck–core interface in the ADP state of the motor [12] are shown in black. These are Y426 and K640 from the switch II cluster, and N340 from the ncd neck. Kinesin mutants Y426A and K640A exhibited lower microtubule-gliding velocities than wild-type ncd [12]. More perturbing mutations, N340K, K640N and N340K/K640N, were recently shown [53••] to uncouple the directional bias of the ncd from its ATPase cycle. (c) The nucleotide-induced transitions of the converter region and lever arm between the ADP-like (PDB code 2mys) and the ATP-like (PDB code 1br1) conformations of myosin. For all figure panels, the ADP and ATP-like conformations of the switch II helix (switch II cluster for kinesin and ncd) are shown in yellow and red, respectively. The ADP and the ATP-like conformations of the mechanical elements of all motors are shown in light and dark blue, respectively.

by the same nucleotide-dependent positions of the switch II helix and the following loop as in the forward-going kinesins [29••] (Figure 3a,c). The helical 'lever' arm, which is rigidly attached to the myosin converter domain, amplifies the nucleotide-induced motions of the converter and translates them into angular motions [3,21,22••] (Figure 3c) related to force generation. In kinesins, the structural domains that follow the neck — FHA (fork head associated) domain in monomeric kinesins [14••] or the neck coiled coil [47•,48•] and partner heads in dimeric kinesins [29••,39••] — perform the analogous amplifying function.

Interestingly, the converter domain of myosin VI, the only myosin motor known to move toward the 'pointed' (minus) end of the actin filament [9••], is radically divergent and linked to the lever arm by an insertion of ~50 amino acid residues that is unique to this class of myosin [9••]. It is plausible that, analogous to the minus-end-directed kinesins (Figure 3b), the specific structure of the converter in this case would change the mode of interaction between the switch II region and the converter domain, and reverse the mechanics of these myosin motors. Indeed, the most notable differences between the cryo-EM maps of myosin VI and other myosins attached to the actin filament are in the region corresponding to the mechanical elements — the converter and the lever arm — which, in the case of myosin VI, have an unusual shape and orientation [9••]. Recent mutational studies demonstrated that the unique insertion in the mechanical elements is not critical to determining the directionality of myosin VI [54•]. Positioned N-terminal to the lever arm, it might, therefore, participate in the amplification of the motor's power stroke. However, the authors' [54•] conclusion that the direction of myosin movement is determined by the motor core domain, not by the converter domain, is not fully supported by experimental data and could be misleading.

Switch-dependent interactions of motors with their polymer tracks

A crucial property that transforms a molecular switch into a molecular motor is the ability of a motor to undergo a force-generating conformational change while it is attached to its cellular track and to return to its initial conformation after detaching from it. In kinesins, ATP binding causes the motor to bind tightly to the microtubule and to produce a force-generating motion through its neck domain. For myosins, the release of the products of ATP hydrolysis (Pi and ADP) results in tight binding of the motor to the actin filament and a power stroke of its lever arm. The nucleotide-dependent mechanism of conformational switching offers an explanation for how the two motors coordinate their power strokes with enhanced affinity for their respective tracks.

Recent docking experiments [14••] showed that, in both ADP and ATP-like states, the switch II cluster constitutes

the main microtubule-binding site of kinesins. Staying anchored on the microtubule and committed to undergo rotational movement between the ADP and ATP states (Figure 3a), the switch II cluster causes the kinesin catalytic core to rotate in the opposite direction. This counter rotation of the core was shown [14••] to increase the accessibility of the polymer surface for different microtubule-binding sites of the motor and to result in tighter binding of the core to the microtubule in the ATP state. In addition, the restructuring of both the switch I and switch II loops in the ATP state was proposed [14••] to intensify the interactions of the motor with the microtubule. In the ADP state, the reversed structural transitions in the switch regions would decrease kinesin's affinity for the microtubule and cause the motor to detach from the polymer.

Interestingly, the nucleotide-induced counter rotation of the kinesin core was shown not only to change kinesin's interactions with the polymer, but also to contribute to the power stroke of the forward-going kinesins by both displacing the core and pointing the tip of the core (and the neck linker docked on the tip) towards the plus end of the microtubule in the ATP state [14••]. Remarkably, a rocking motion of the myosin catalytic core relative to the actin filament was detected during the motor's ATP hydrolysis cycle [55•]. Analogous to the kinesin counter rotation, a rotational movement of the myosin catalytic core was proposed to contribute to the myosin power stroke [56,57].

Myosin docking experiments [55•,58–60] indicate that the switch II helix and the following loop of myosin motors do not interact with the actin filament directly. However, the latter loop links the switch II helix to the main stereospecific actin-binding site (helix-turn-helix motif or hydrophobic site) in the lower 50 kDa region of the myosin catalytic domain. Similar to the structural elements of the kinesin switch II cluster (Figure 3a), this site is interlocked with the switch II helix and moves along with the helix while myosin switches between different nucleotide states [3,21,*22*••]. These movements were predicted [61] and recently shown [55•] to restructure the myosin actin-binding interface by opening and closing the large cleft dividing this interface between the myosin upper and lower 50 kDa regions. Independent data suggest that, similar to kinesin motors [14••], the main actin-binding site might be anchored on the actin filament in different nucleotide states of the actomyosin complex [55•,60,62]; the opening and closing of the cleft are achieved by a movement of the upper 50 kDa region of the motor [55•]. Analogous to kinesins [14••], a rotational movement of the myosin catalytic core, as well as cleft opening and closure (both associated with the nucleotide-induced restructuring of myosin switch regions), alters the accessibility of the actin filament surface for other actin-binding sites in the upper and lower 50 kDa subdomains of myosin [55•]. These changes might result in the higher affinity of myosin for the actin filament in the ADP/no nucleotide states of the motor.

Mechanisms regulating conformational switching

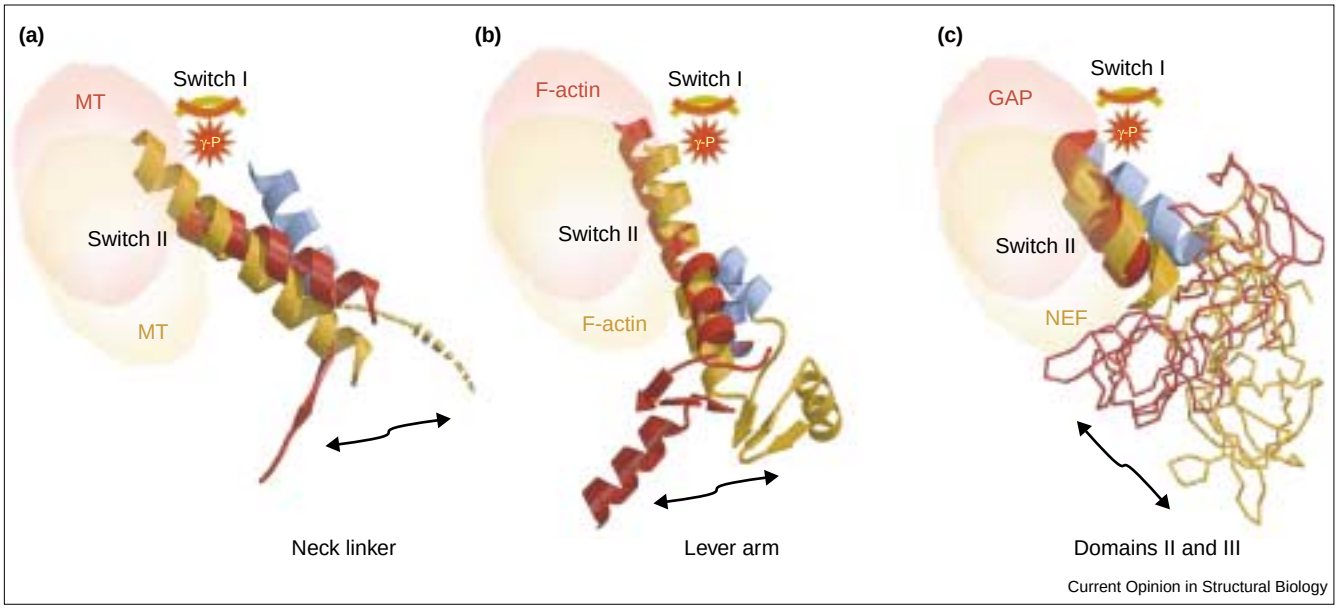
Whereas the conformations of the mechanical elements of both kinesin and myosin are always dictated by the positions of the switch II regions [3,14••,21,*22*••,*29*••], the conformations of the switch regions in the absence of their respective polymer tracks display weak coupling to the nature of the bound nucleotide [14••,*28*•,*29*••]. For both motors, this confusing observation was recently explained [14••,*28*•,*29*••] by a low-energy barrier between different conformations of the switches, which might exist in dynamic equilibrium in the absence of the polymer. It was suggested, therefore, that the choice of the conformations of the switches and the related conformations of the mechanical elements in the working cycles of kinesin and myosin is decided by binding contacts with both the nucleotide and the motors' respective targets — the microtubules and the actin filaments [14••,*29*••].

The suggested coordinating role of the polymer tracks in the switch-dependent mechanism of motor proteins is consistent with the known ability of both microtubules and actin filaments to accelerate nucleotide exchange and hydrolysis at different stages of the enzymatic cycles of kinesins and myosins, respectively. Because of this property, and based on functional similarities between G proteins and motors, it was also proposed [40] that the specific track polymers might function not only as targets of the motors, but could also combine the roles of both nucleotide exchange factors (NEFs) and NTPase-activating proteins (NAPs) for motor proteins (Figure 4). Structural similarities between kinesin–microtubule complexes and available complexes of G proteins with their regulators support and elaborate this proposal [14••]. The analogy between regulation of NTPase activity in G proteins and that in motor proteins appears even more striking since the recently described inhibition of ADP release by the kinesin tail domain [63••], which might function as the 'built-in' analog of the nucleotide dissociation inhibitor (NDI, GDI for G proteins).

Comparative structural analysis of kinesins and myosins also highlights specific structural adaptations that probably accommodate the known differences in the regulation of these motors. It is known that, whereas both kinesins and myosins rely on their polymer tracks to assist in ATP hydrolysis, binding interactions with the microtubule for kinesin and the actin filament for myosin occur at different steps in the ATPase cycle of these motors.

As was proposed for kinesins [14••], the microtubule might disrupt the motors' normally tight interaction with ADP by binding and displacing the motors' switch regions. After ADP is replaced by ATP, the microtubule might accelerate nucleotide hydrolysis by structuring the motor's

Figure 4



Structural and functional similarity shared between different families of molecular switches. In all cases, NDP- and NTP-like conformations of the switches are shown in yellow and red, respectively. Specific macromolecular partners interacting with the switch regions in these two states are shown as surfaces of matching color. The γ-phosphate is shown as a red star. The C-terminal helix of the NTPase domain, to which the mechanical elements of molecular motors or additional domains of G proteins are attached, is shown in blue. **(a)** The nucleotide-driven conformational switching in kinesins results in force-generating rearrangements of the neck domain and movement of kinesin motors along the microtubules (MTs). **(b)** The same mechanism of conformational switching is used by myosin motors for converting small conformational changes in their nucleotide-binding site into larger movements related to their force generation and motion along actin filaments. Part of the converter domain and lever arm helix is shown for myosin in ADP-like (PDB code 2mys) and ATP-like (PDB code 1br1) conformations. **(c)** An identical molecular mechanism is employed by G proteins for their domain rearrangements (domains II and III of EF-Tu in GDP [PDB code 1TUI] and GTP-like [PDB code 1EFT] states are shown) and for controlling affinity for their specific macromolecular partners – NEFs, GAPs (GTPase-activating proteins) and effectors. Macromolecular polymers [microtubule for kinesins (a) and actin filament for myosins (b)] might combine the functions of NEFs, GAPs and effectors.

active site and interacting with both switch regions, assuring the precise positioning of residues involved in ATP hydrolysis [14••]. Because kinesin switch loops accommodate different binding interactions with the microtubules in different nucleotide states of the motor, their structure is expected to be malleable and, in the absence of microtubules, these loops are observed to be flexible and are often disordered (Figure 2b).

In myosin motors, ATP is hydrolyzed while the motor is detached from the actin filament, generating a stable intermediate state of myosin with the products of hydrolysis bound in the nucleotide pocket. To assure efficient hydrolysis and strong binding of the hydrolysis products, the switch loops of myosin are structured and stabilized by binding interactions with the rest of the myosin structure. As a result, they are well defined and stable even in the absence of the actin filament (Figure 2c). The specific details of regulation of the ATPase cycle of kinesins and myosins also explain the availability of numerous myosin structures with bound nucleotide analogs that mimic both the ATP and the transition state of the motor, and the difficulties with incorporating such analogs in the kinesin active site in the absence of the microtubules.

Conclusions and future directions

In summary, the structural findings discussed in this review outline a modular mechanism of nucleotide-driven conformational switching in kinesin and myosin motors. Structural analysis demonstrates that the nucleotide-induced molecular switching in motor proteins is designed to relay the conformational changes in the nucleotide-binding pocket to structurally distant mechanical elements and polymer-binding sites. As discussed, the mechanism of conformational switching used in kinesin and myosin motors for movement is employed by nature in a wide variety of molecular switches for domain rearrangement and for controlling affinity for specific macromolecular partners. The existing data suggest that the specific macromolecular partners of the motor proteins (microtubules for kinesins and actin filaments for myosins) are able to regulate the force-generating conformational switching by accelerating nucleotide exchange and hydrolysis in different nucleotide states of the motor proteins. By delegating functions of the motors' targets and regulators to the specific biopolymers, nature creates an economical, yet efficient system, which ensures the conversion of energy from ATP binding and hydrolysis into mechanical work.

Although numerous structural data have recently focused attention of the field on interactions of molecular motors with their polymer tracks, specific details of these interactions remain unknown. High-resolution structures of both kinesin and myosin motors complexed with their respective tracks at different stages of the motors' mechanochemical cycles would, no doubt, reveal many secrets that nature still keeps away from the curious minds of scientists.

Acknowledgements

As the kinesin and myosin research fields develop and merge, a vast amount of significant papers are published in numerous scientific journals. Space constraints prevent us from citing every paper we would wish to. Work of the authors is supported by the National Institutes of Health.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Kull FJ, Sablin EP, Lau R, Fletterick RJ, Vale RD: Crystal structure of the kinesin motor domain reveals a structural similarity to myosin. *Nature* 1996, 380:550-555.

2. Sablin EP, Kull JF, Cooke R, Vale RD, Fletterick RJ: Crystal structure of the motor domain of the kinesin-related motor ncd. *Nature* 1996, 380:555-559.

3. Rayment I, Rypniewski WR, Schmidt-Base K, Smith R, Tomchick DR, Benning MM, Winkelmann DA, Wesenberg G, Holden HM: Three-dimensional structure of myosin subfragment-1: a molecular motor. *Science* 1993, 261:50-58.

4. Vale RD, Funatsu T, Pierce DW, Romberg L, Harada Y, Yanagida T: Direct observation of single kinesin molecules moving along microtubules. *Nature* 1996, 380:451-453.

5. Visscher K, Schnitzer MJ, Block SM: Single kinesin molecules studied with a molecular force clamp. *Nature* 1999, 400:184-189.

6. •• Mehta AD, Rock RS, Rief M, Spudich JA, Mooseker MS, Cheney RE: Myosin-V is a processive actin-based motor. *Nature* 1999, 400:590-593.

This paper provides direct evidence that myosin-V, which has been implicated in various forms of organelle transport, is a processive motor and can take multiple (up to 40–50) steps along the actin filament before detaching from its track. The authors noted that the spread in the motor's step size measurements (30–38 nm) exceeds the measurement precision (~4 nm). This observation suggests that myosin-V may not move along the 36 nm helical repeat of the actin filament.

7. •• Walker ML, Burgess SA, Sellers JR, Wang F, Hammer JA III, Trinick J, Knight PJ: Two-headed binding of a processive myosin to F-actin. *Nature* 2000, 405:804-807.

Previous [6••] work suggested that myosin-V, the processive myosin motor, might not be able to span the actin helical repeat (13 actin subunits or ~36 nm). Therefore, a succession of its steps would follow a helical path on the filamentous actin. This paper presents results of EM experiments showing that myosin-V is able to span the actin helical repeat. EM images reveal both single- and double-headed binding of myosin-V to F-actin. The bound heads are typically 13 actin subunits apart, but values of 11 and 15 are also found. The authors noted that the doubly attached conformation of myosin-V seen in their work is similar to the atomic model of this complex extrapolated from the crystal structures of myosin II in nucleotide free (rigor) and ADP•AlF_4-bound states [29••].

8. Woehlke G, Schliva M: Directional motility of kinesin motor proteins. *Biochim Biophys Acta* 2000, 1496:117-127.

9. •• Wells AL, Lin AW, Chen LQ, Safer D, Cain SM, Hasson T, Carragher BO, Milligan RA, Sweeney HL: Myosin VI is an actin-based motor that moves backwards. *Nature* 1999, 401:505-508.

This paper presents the exciting finding that, similar to kinesins, myosin motors are able to move in both directions along their cellular tracks, the actin filaments. It reports that the unconventional myosin-VI, unlike all characterized myosins, which move towards the barbed (+) end of actin filaments, moves towards their pointed (–) end. The direction of movement was determined using *in vitro* motility assays. The conformational transition associated with the directed force generation was also visualized in cryo-EM experiments by comparing the nucleotide-bound (ADP) and nucleotide-free (rigor) states of the motor.

10. Sack S, Muller J, Marx A, Thormahlen M, Mandelkow E-M, Brady ST, Mandelkow E: X-ray structure of motor and neck domains from rat brain kinesin. *Biochemistry* 1997, 36:16155-16165.

11. Kozielski F, Sack S, Marx A, Thormahlen M, Schonbrunn E, Biou V, Thompson A, Mandelkow EM, Mandelkow E: The crystal structure of dimeric kinesin and implications for microtubule-dependent motility. *Cell* 1997, 91:985-994.

12. Sablin EP, Case RB, Dai SC, Hart CL, Ruby A, Vale RD, Fletterick RJ: Direction determination in the minus-end-directed kinesin motor ncd. *Nature* 1998, 395:813-816.

13. Gulick A, Song H, Endow S, Rayment I: X-ray crystal structure of the yeast KAR3 motor domain complexed with MgADP to 2.3 Å resolution. *Biochemistry* 1998, 37:1769-1776.

14. •• Kikkawa M, Sablin EP, Okada Y, Yajima H, Fletterick RJ, Hirokawa N: Switch-based mechanism of kinesin motors. *Nature* 2001, 411:439-445.

This paper presents the results of a combined X-ray crystallography and cryo-EM study of the monomeric kinesin motor KIF1A. In this study, the motor is revealed in its two functionally critical states – complexed with ADP or with a non-hydrolyzable analog of ATP. The authors demonstrate that the conformational change observed between these two states is modular, extends to all kinesins and is similar to the conformational change used by myosin motors and G proteins. The combined results of the crystallographic and cryo-EM studies are used to outline a unifying switch-based mechanism employed by various kinesin motors.

15. • Turner J, Anderson R, Guo J, Beraud C, Fletterick RJ, Sakowicz R: Crystal structure of the mitotic spindle kinesin Eg5 reveals a novel conformation of the neck-linker. *J Biol Chem* 2001, 276:25496-25502.

This work describes the crystal structure of the mitotic kinesin motor Eg5 in the ADP state. The structure reveals a novel conformation of the kinesin neck linker, the critical mechanical element of the motor. The authors discuss whether the observed conformation of the neck is related to the specific function this kinesin motor performs in cell or whether it represents a preferable but transient conformation employed by all kinesins in the ADP state.

16. Fisher AJ, Smith CA, Thoden JB, Smith R, Sutoh K, Holden HM, Rayment I: X-ray structures of the myosin motor domain of ***Dictyostelium discoideum*** complexed with MgADP.BeFx and MgADP.AlF4. *Biochemistry* 1995, 34:8960-8972.

17. Smith CA, Rayment I: X-ray structure of the magnesium(II)-pyrophosphate complex of the truncated head of ***Dictyostelium discoideum*** myosin to 2.7 Å resolution. *Biochemistry* 1995, 34:8973-8981.

18. Smith CA, Rayment I: X-ray structure of the magnesium(II).ADP.vanadate complex of the ***Dictyostelium discoideum*** myosin motor domain to 1.9 Å resolution. *Biochemistry* 1996, 35:5404-5417.

19. Bauer CB, Kuhlman PA, Bagshaw CR, Rayment I: X-ray crystal structure and solution fluorescence characterization of Mg.2′(3′)-O-(N-methylanthraniloyl) nucleotides bound to the ***Dictyostelium discoideum*** myosin motor domain. *J Mol Biol* 1997, 274:394-407.

20. Gulick AM, Bauer CB, Thoden JB, Rayment I: X-ray structures of the MgADP, MgATPγS, and MgAMPPNP complexes of the ***Dictyostelium discoideum*** myosin motor domain. *Biochemistry* 1997, 36:11619-11628.

21. Dominguez R, Freyzon Y, Trybus KM, Cohen C: Crystal structure of a vertebrate smooth muscle myosin motor domain and its complex with the essential light chain: visualization of the pre-power stroke state. *Cell* 1998, 94:559-571.

22. •• Houdusse A, Kalabokis VN, Himmel D, Szent-Gyorgyi AG, Cohen C: Atomic structure of scallop myosin subfragment S1 complexed with MgADP: a novel conformation of the myosin head. *Cell* 1999, 97:459-470.

In this work, the crystal structure of scallop myosin subfragment I is presented. The structure reveals a conformation of myosin that has not been seen before. Domains within the structure are only loosely connected and packed. The authors argue that the novel structure does not display any 'ADP-specific' features and represents one of the prehydrolysis (ATP-like) states of myosin. However, structural analysis shows that the nucleotide-binding switch loops are disengaged and the nucleotide-binding pocket is in the 'opened', ADP-like conformation in this myosin structure. Furthermore, both the positions and packing of the converter and the lever arm in the structure are more consistent with the ADP/no nucleotide-like conformation of the mechanical elements, perhaps strained beyond their initial 'post-stroke' angle. Although both interpretations of the novel myosin structure remain hypothetical, the structure certainly extends our previous knowledge of possible myosin conformations.

23. Houdusse A, Szent-Gyorgyi AG, Cohen C: Three conformational states of scallop myosin S1. *Proc Natl Acad Sci USA* 2000, 97:11238-11243.

24. Bauer CB, Holden HM, Thoden JB, Smith R, Rayment I: X-ray structures of the apo and MgATP bound states of ***Dictyostelium discoideum*** myosin motor domain. *J Biol Chem* 2000, 275:38494-38499.

25. Kull FJ, Vale RD, Fletterick RJ: The case of a common ancestor: kinesin and myosin proteins and G proteins. *J Muscle Res Cell Motil* 1998, 19:877-886.

26. • Vale RD, Case R, Sablin E, Hart C, Fletterick R: Searching for kinesin's mechanical amplifier. *Philos Trans R Soc Lond B Biol Sci* 2000, 355:449-457.
The authors give a thorough analysis of available structural, motility and mutagenesis data that collectively implicate the kinesin neck regions as participating in the kinesin force-generating process.

27. Sablin EP: Kinesins and microtubules: structures and motor mechanism. *Curr Opin Cell Biol* 2000, 12:35-41.

28. • Geeves MA, Holmes KC: Structural mechanism of muscle contraction. *Annu Rev Biochem* 1999, 68:687-728.
This review provides a comprehensive structural and functional analysis of myosin and its interactions with the actin filament. The authors discuss recent achievements in the myosin research field and outline possible directions for its future development.

29. •• Vale RD, Milligan RA: The way things move: looking under the hood of molecular motor proteins. *Science* 2000, 288:88-95.
This is an excellent review demonstrating how the evolving modular design of molecular motors could produce a remarkable diversity of kinesin and myosin motors whose properties are optimized for performing distinct biological functions. The review is a true example of how powerful a structural analysis can be when combined with scientific insight.

30. Vale RD, Fletterick RJ: The design plan of kinesin motors. *Annu Rev Cell Dev Biol* 1997, 13:745-777.

31. Henningsen U, Schliwa M: Reversal in the direction of movement of a molecular motor. *Nature* 1997, 389:93-95.

32. Case RB, Pierce DW, Hom-Booher N, Hart C, Vale RD: The directional preference of kinesin motors is specified by an element outside of the motor catalytic domain. *Cell* 1997, 90:959-966.

33. Romberg L, Pierce DW, Vale RD: Role of the kinesin neck region in processive microtubule-based motility. *J Cell Biol* 1998, 140:1407-1416.

34. Endow SA, Waligora KW: Determinants of kinesin motor polarity. *Science* 1998, 281:1200-1202.

35. • Case RB, Rice S, Hart CL, Ly B, Vale RD: Role of kinesin neck linker and catalytic core in microtubule-based motility. *Curr Biol* 2000, 10:157-160.
On the basis of the results of extensive mutational studies, the authors determined the functions of the catalytic core and the following neck linker of conventional kinesin. They demonstrated that the catalytic core is sufficient to regulate both microtubule binding and the ATPase activity of the motor. The core by itself, without any additional kinesin sequence, was also shown to produce very slow plus-end-directed motor activity. Analogous to the converter domain of myosin motor, the neck linker was shown to function as a mechanical amplifier for kinesin motion.

36. Whittaker M, Wilson-Kubalek EM, Smith JE, Faust L, Milligan RA, Sweeney HL: A 35Å movement of smooth muscle myosin on ADP release. *Nature* 1995, 378:748-751.

37. Uyeda TQ, Abramson PD, Spudich JA: The neck region of the myosin motor domain acts as a lever arm to generate movement. *Proc Natl Acad USA* 1996, 93:4459-4464.

38. Corrie JE, Brandmeier BD, Ferguson RE, Trentham DR, Kendrick-Jones J, Hopkins SC, van der Heide UA, Goldman YE, Sabido-David C, Dale RE *et al.*: Dynamic measurement of myosin light-chain-domain tilt and twist in muscle contraction. *Nature* 1999, 400:425-430.

39. •• Rice S, Lin AW, Safer D, Hart C, Naber N, Carragher BO, Cain SM, Pechatnikowa E, Wilson-Kubalek EM, Pate E *et al.*: A structural change in the kinesin motor that drives motility. *Nature* 1999, 402:778-784.
For the first time, a large conformational change of the neck linker in kinesins was detected and visualized by four independent methods. Using EPR, FRET, pre-steady state kinetics and cryo-EM, the authors demonstrated that the neck linker becomes immobilized and extended towards the microtubule plus end when kinesin binds microtubules and ATP, and reverts to a more mobile conformation when γ-phosphate is released after nucleotide hydrolysis. This conformational change explains the direction of kinesin motion, as well as processive movement by the kinesin dimer.

40. Vale RD: Switches, latches, and amplifiers: common themes of molecular motors and G proteins. *J Cell Biol* 1996, 135:291-302.

41. Sack S, Kull FJ, Mandelkow E: Motor proteins of the kinesin family: structures, variations, and the nucleotide binding sites. *Eur J Biochem* 1999, 262:1-11.

42. Hoenger A, Sack S, Thormahlen M, Marx A, Muller J, Gross H, Mandelkow E: Image reconstructions of microtubules decorated with monomeric and dimeric kinesins: comparison with X-ray structure and implications for motility. *J Cell Biol* 1998, 141:419-430.

43. Svoboda K, Schmidt CF, Schnapp BJ, Block SM: Direct observation of kinesin stepping by optical trapping interferometry. *Nature* 1993, 365:721-727.

44. Coy DL, Wagenbach M, Howard J: Kinesin takes one 8 nm step for each ATP that it hydrolyses. *J Biol Chem* 1999, 274:3667-3671.

45. Kawaguchi K, Ishiwata S: Nucleotide-dependent single- to double-headed binding of kinesin. *Science* 2001, 291:667-669.

46. • Nishiyama M, Muto E, Inoue Y, Yanagida T, Higuchi H: Substeps within the 8 nm step of the ATPase cycle of single kinesin molecules. *Nat Cell Biol* 2001, 3:425-428.
Using an assay that resolves nanometer displacements of single kinesin molecules, the authors show that the 8 nm step of kinesin motors can be resolved into fast and slow substeps, each corresponding to a displacement of ~4 nm. The latter observation is consistent with the earlier proposed model for the processive movement of kinesin [39••].

47. • Thorn KS, Ubersax JA, Vale R: Engineering the processive run length of the kinesin motor. *J Cell Biol* 2000, 151:1093-1100.
This work elaborates the earlier proposed model [39••] for the processive motion of dimeric conventional kinesin. The paper shows that the processivity (ability to take multiple steps before detaching from the track) of conventional kinesin is influenced by electrostatic interactions between the kinesin neck coiled coil and the negatively charged C-terminal region of tubulin on the microtubule surface. The authors suggest that these interactions could evolve to control kinesin's run length for its optimal *in vivo* function.

48. • Tomishige M, Vale RD: Controlling kinesin by reversible disulfide cross-linking. Identifying the motility-producing conformational change. *J Cell Biol* 2000, 151:1081-1092.
In this paper, two previously proposed models for the major structural change that would support the processive movement of conventional kinesin have been tested. The first model involves conformational transitions of the kinesin neck linker (its 'docking' and 'undocking' on the core). The second model proposes an unwinding and rewinding of the following neck coiled-coil region. By using disulfide cross-linking of cysteines engineered either at the neck–core interface or inside the neck coiled coil of kinesin, the authors showed that the conformational changes in the neck linker, not the neck coiled coil, are a major driving force for the kinesin processive movement.

49. •• Okada Y, Hirokawa N: A processive single-headed motor: kinesin superfamily protein KIF1A. *Science* 1999, 283:152-157.
This is the first report in which the processivity of a single-headed kinesin construct has been demonstrated. A recombinant chimeric construct of the motor domain of KIF1A, a member of the unc104/KIF1A family of monomeric kinesins, was shown to move processively along the microtubule for more than one micrometer. The movement was stochastic and fitted a biased Brownian movement model. The authors suggested a model that implicates a class-specific, lysine-rich loop ('K-loop') in the catalytic core of monomeric kinesins in the mechanism of their processive movement.

50. • Okada Y, Hirokawa N: Mechanism of the single-headed processivity: diffusional anchoring between the K-loop of kinesin and the C terminus of tubulin. *Proc Natl Acad Sci USA* 2000, 97:640-645.
A model for the processive movement of monomeric kinesins suggested in a previous study [49••] is elaborated in this work. On the basis of the results of mutational analysis, the authors concluded that the nucleotide-dependent interaction between the positively charged, lysine-rich 'K-loop' [49••] in the catalytic core of the monomeric kinesins and the negatively charged flexible C-terminal region of tubulin ('E-hook') on the surface of the microtubule is essential for the single-headed processivity.

51. • Kikkawa M, Okada Y, Hirokawa N: 15 Å resolution model of the monomeric kinesin motor, KIF1A. *Cell* 2000, 100:241-252.
The proposed [50•] interactions between the 'K-loop' of monomeric kinesins and the flexible C-terminal region of tubulin on the microtubule surface are confirmed in this cryo-EM study of kinesin motor KIF1A. The enhanced resolution (15 Å) of cryo-EM data allowed more accurate docking of kinesin atomic models into cryo-EM maps of the motor domain of KIF1A bound to the microtubule.

52. Kozielski F, De Bonis S, Burmeister W, Cohen-Addad C, Wade R: The crystal structure of a minus-end directed microtubule motor protein ncd reveals variable dimer conformations. *Structure Fold Des* 1999, 7:1407-1416.

53. Endow S, Higuchi H: A mutant of the motor protein kinesin that
•• moves in both directions on microtubules. *Nature* 2000, 406:913-916.
In this study, two interacting conserved residues at the neck–core interface of the minus-end-directed kinesin motor ncd have been mutated (N340K, K640N). These mutations produced a motor that could move in both directions along the microtubule. The described phenomenon could be used to test the existing models for the opposite directionality of kinesin motors.

54. Homma K, Yoshimura M, Saito J, Ikebe R, Ikebe M: The core of the
• motor domain determines the direction of myosin movement. *Nature* 2001, 412:831-834.
In this work, the directionality of movement of four chimeric myosin constructs has been determined. Three constructs included the catalytic core of myosin V, which moves towards the 'barbed' end of F-actin. Supplemented with the lever arm of the oppositely directed myosin VI (with or without the unique N-terminal insertion), all these recombinant myosins moved towards the 'barbed' end. The fourth construct, which consisted of the catalytic core and the converter domain of myosin VI without the following unique insertion but with the lever arm of myosin V, moved slowly towards the 'pointed' end of F-actin. Rightfully, the authors concluded that the unique insertion separating the converter domain and the lever arm of myosin VI is not critical for determining the directionality of the motor. However, in the absence of any characterized constructs that would include the catalytic core of myosin-VI but lack its converter domain, the authors' conclusion that the direction of myosin movement is determined by the motor core domain solely, and not by the converter domain, is premature.

55. Volkmann N, Hanein D, Ouyang G, Trybus KM, DeRosier DJ, Lowey S:
• Evidence for cleft closure in actomyosin upon ADP release. *Nat Struct Biol* 2000, 7:1147-1155.
This cryo-EM study offers an extensive analysis of conformational changes accompanying ADP release in smooth muscle myosin bound to an actin filament. The authors present evidence of a complex movement (~34 Å) of the light-chain-binding domain (the lever arm), which is coupled to a rotational motion of the myosin catalytic core relative to the actin filament. The authors also observe closure of the cleft that divides the actin-binding region of the myosin head. The authors suggest that the cleft closure is achieved by the movement of the upper 50 kDa region of the motor, while parts of the lower 50 kDa region are strongly bound to the actin filament.

56. Berger CL, Craik JS, Trentham DR, Corrie JE, Goldman YE: Fluorescence polarization of skeletal muscle fibers labeled with rhodamine isomers on the myosin heavy chain. *Biophys J* 1996, 71:3330-3343.

57. Burghardt TP, Garamszegi SP, Ajtai K: Probes bound to myosin Cys-707 rotate during length transients in contraction. *Proc Natl Acad Sci.* 1997, 94:9631-9636.

58. Rayment I, Holden HM, Whittaker M, Yohn CB, Lorenz M, Holmes KC, Milligan RA: Structure of the actin-myosin complex and its implications for muscle contraction. *Science* 1993, 261:58-65.

59. Schroder RR, Manstein DJ, Jahn W, Holden HM, Rayment I, Holmes KC, Spudich JA: Three-dimensional atomic model of F-actin decorated with ***Dictyostelium*** myosin S1. *Nature* 1993, 364:171-174.

60. Milligan RA: Protein-protein interactions in the rigor actomyosin complex. *Proc Natl Acad Sci USA* 1996, 93:21-26.

61. Fisher AJ, Smith CA, Thoden J, Smith R, Sutoh K, Holden HM, Rayment I: Structural studies of myosin:nucleotide complexes: a revised model for the molecular basis of muscle contraction. *Biophys J* 1995, 68:19S-26S.

62. Yengo CM, Chrin L, Rovner AS, Berger CL: Intrinsic tryptophan fluorescence identifies specific conformational changes at the interface upon actin binding and ADP release. *Biochemistry* 1999, 38:14515-14523.

63. Hackney DD, Stock MF: Kinesin's IAK tail domain inhibits initial
•• microtubule-stimulated ADP release. *Nat Cell Biol* 2000, 2:257-260.
Although the inhibitory effect of the kinesin cargo-binding tail domain on the motor's processive movement was reported previously, this paper demonstrates a pronounced inhibition of kinesin's ATPase activity by the motor's intact tail region. It also shows that, even in the presence of ATP (but in the absence of specific cargo), kinesin with an intact tail domain has weak affinity for microtubules. The observed results are explained by the selective inhibition of the release of ADP upon kinesin's initial interaction with a microtubule.