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Searching for kinesin's mechanical amplifier

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Kinesin, a microtubule-based motor, and myosin, an actin-based motor, share a similar core structure, indicating that they arose from a common ancestor. However, kinesin lacks the long lever-arm domain that is believed to drive the myosin power stroke. Here, we present evidence that a much smaller region of *ca.* 10–40 amino acids serves as a mechanical element for kinesin motor proteins. These 'neck regions' are class conserved and have distinct structures in plus-end and minus-end-directed kinesin motors. Mutagenesis studies also indicate that the neck regions are involved in coupling ATP hydrolysis and energy into directional motion along the microtubule. We suggest that the kinesin necks drive motion by undergoing a conformational change in which they detach and re-dock onto the catalytic core during the ATPase cycle. Thus, kinesin and myosin have evolved unique mechanical elements that amplify small, nucleotide-dependent conformational changes that occur in their similar catalytic cores.

Keywords: kinesin; Ncd; myosin; microtubule

1. INTRODUCTION

Many forms of intracellular motion are generated by interactions between molecular motors with cytoskeletal polymers. To understand such processes in molecular detail, it is essential to decipher how a motor protein changes its three-dimensional structure as it executes its enzymatic cycle in conjunction with the polymeric track. For myosin, a rotating 'lever-arm' model was proposed based upon electron microscopy studies (Huxley 1969). By defining a kinetic pathway for the actomyosin ATPase cycle, Lymn & Taylor (1971) were able to formulate an influential motility model in which the proposed lever-arm motions were connected to specific transitions in the myosin ATPase cycle. In recent years, evidence is accumulating that myosin does indeed contain a lever-arm-like structure (an elongated α-helix surrounded by light chains that emerges from myosin's catalytic core), that can undergo a large angular change in different nucleotide states (Dominguez *et al*. 1998; Rayment *et al*. 1993*a*,*b*; Suzuki *et al*. 1998; Uyeda *et al*. 1996; Whittaker *et al*. 1996). This angular motion produces a 10–15 nm displacement at the C-terminal end of the lever-arm helix, which is sufficient to account for the unitary displacements produced by myosin in optical trap measurements (Finer *et al*. 1994; Ishijima *et al*. 1994). While many details of this mechanism remain to be resolved, the current data supports the general tenet of the Lymn–Taylor model.

Several crystal structures of kinesin motor domains have been obtained recently (Gulick *et al*. 1998; Kozielski *et al*. 1997; Kull *et al*. 1996; Sablin *et al*. 1996, 1998; Sack *et al*. 1997). Kinesin is structurally similar to myosin in its core nucleotide-binding region (Kull *et al*. 1996); however, these microtubule-based motors lack the α-helical lever arm found in myosin, and therefore are likely to operate by a somewhat different mechanism. However, a clear structural model for kinesin-based motility, akin to the work described above for myosin, is missing. In this article, we will discuss structural and mutagenesis data that collectively implicate a region of 10–40 amino acids (termed the 'neck') as a mechanical amplifier for kinesin-based motility. We also discuss potential models for how the neck could amplify small conformational changes in the nucleotide- and microtubule-binding sites.

2. DOMAINS OF KINESIN MOTORS

Before describing models for motility, it is important to introduce and define the different domains of kinesin motors (figure 1). In this review, we use the term 'motor domain' to refer to the functional force-producing element of the protein, which is itself divided into two major elements (Vale & Fletterick 1997). One element is the globular 'catalytic core', which is conserved throughout the superfamily. The second element, which we call the 'neck region', is a region of *ca.* 10–40 amino acids found on either the N- or C-terminus of the catalytic core. In contrast to the catalytic core, the neck is conserved only within certain kinesin classes. The necks of many types of kinesins form coiled-coil structures that are sufficient to dimerize two motor polypeptide chains. A central theme of this article will be to present evidence that the neck works together with the catalytic core to produce movement. Beyond the motor domain, many kinesin proteins contain a very long α-helical coiled-coil domain (called a 'stalk') and other globular domains ('tails') that may target the motor to a particular cargo within the cell (for more information, see the recent reviews by Hirokawa (1998) and Vale (1999)). The catalytic core and the neck domains are described below in more detail.

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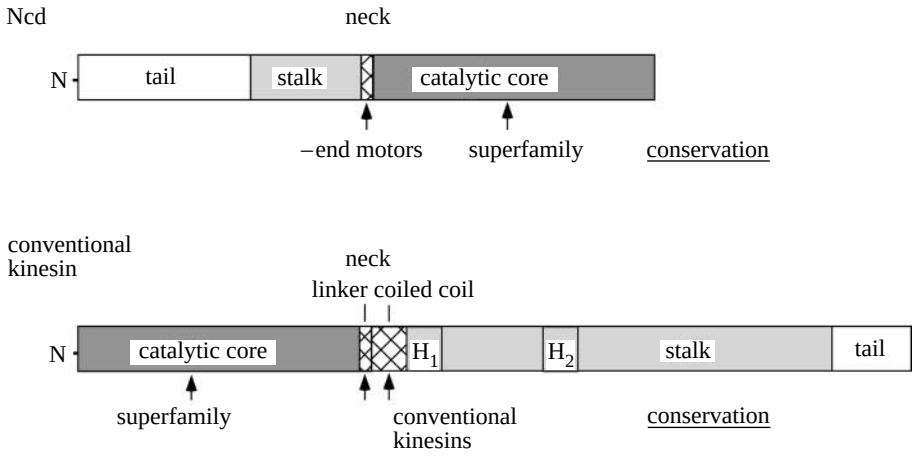

Figure 1. Domain organization of the Ncd and kinesin motor proteins. The polypeptide chain is organized into four domains (upper bar, from C- to N-terminus), as follows. (i) A globular catalytic core (shown in dark grey) that contains the ATP- and microtubule-binding sites and is conserved throughout the kinesin superfamily. The Ncd catalytic core is about 40% identical in sequence to kinesin's catalytic core (lower bar) but, in contrast to kinesin, it is located at the C-terminus of the polypeptide chain. (ii) A 'neck' region (shown in the hatched box) that is adjacent to the catalytic core and is defined by class-specific sequence conservation. In the case of Ncd, the neck contains 13 class-conserved amino acids (R335–G347) that precede the first β-strand of the catalytic core. Kinesin's conserved neck (*ca*. 35 residues) emerges from the C-terminus of the catalytic core and consists of two distinct regions, the first 10–15 residues, termed the 'neck linker', are highly conserved among all plus-end motors and interact with the catalytic core. The subsequent region forms a coiled coil ('neck coiled coil'), does not interact with the core, and is conserved selectively among the conventional kinesin subfamily (Vale & Fletterick 1997). (iii) α-helical 'stalk' domain (shown in light grey) that enhances dimer formation through an extended coiled coil. 'H_1' and 'H_2' indicate flexible hinge regions. (iv) A small C-terminal 'tail' or attachment domain (shown in white). This figure and legend were adapted from Sablin *et al*. (1998).

(a) *Catalytic core*

We define the catalytic core based upon a combination of structural and sequence conservation information. The first secondary structure element of the kinesin catalytic core is a β-strand (termed β1); the precise beginning of this β-strand marks the point at which sequence conservation begins among all motors of the superfamily (I/VxVxxRxRP). Sequence conservation throughout the superfamily terminates towards the end of the last helix of the core globular domain (termed α6; ETxxTLxFAxR). These boundaries of sequence conservation define the compact globular domain seen by crystallographic studies, which we refer to as the catalytic core.

Within the kinesin superfamily, there are three major divisions of related motors termed Kin N, Kin C and Kin I. In addition to being distinguished by amino-acid sequence identity, these three groups of motors differ in the position of the catalytic core with respect to the entire polypeptide chain; the catalytic core is found at the N-terminal, C-terminal, or at an internal position in the motor polypeptide chain in the Kin N, Kin C and Kin I motors, respectively (figure 1; Kin I motors are not shown). Thus far, all motors of the Kin N family move to the microtubule plus-end, while the Kin C motors move in the opposite direction. The Kin I family may not be traditional transport motors at all, but instead act as ATP-dependent microtubule depolymerizing agents (Desai *et al*. 1999).

The catalytic core has helices packed on either side of an eight-stranded, mostly parallel, β-sheet, and is typical of α- and β-class proteins. All six helices and the majority of β-strand elements in the kinesin catalytic core also have counterparts in the myosin motor, indicating that these two motor classes must have evolved from a common ancestor (Kull *et al*. 1998). Moreover, the structures of myosin's and kinesin's ATP-binding sites are similar to the nucleotide-binding site of G-proteins. Kinesins, myosins, and G-proteins are all capable of sensing whether NTP or NDP is bound, and then transmitting a conformational change from the active site. The basic mechanism of this transduction process appears to be similar in all three types of enzymes and involves two loops that form hydrogen bonds with the nucleotide γ-phosphate (termed switches I and II) (Vale 1996). For kinesin, crystal structures of the catalytic core have only been solved for the ADP state, so the exact nature of the conformational change between the ATP and ADP states is unknown. However, by analogy from crystallographic information of the corresponding region in myosin and G-proteins (for which structures in multiple nucleotide states have been obtained), it is likely that motions within kinesin's catalytic core are small (<10 Å motions of certain structural elements). Therefore, we believe that the kinesin catalytic core functions in a manner akin to a G-protein: transitions in the nucleotide-binding site result in small conformational changes that alter the microtubule-binding site and change the motor–polymer affinity. Indeed, we have recently shown that kinesin's catalytic core alone exhibits microtubule-stimulated ATPase activity and can bind and release from the microtubule during its enzymatic cycle (Case *et al*. 2000). Thus, we believe that it is unlikely that the catalytic core undergoes large interdomain motions which are needed to drive efficient unidirectional motility. Direct measurements of catalytic core motility, to be described later, support this notion.

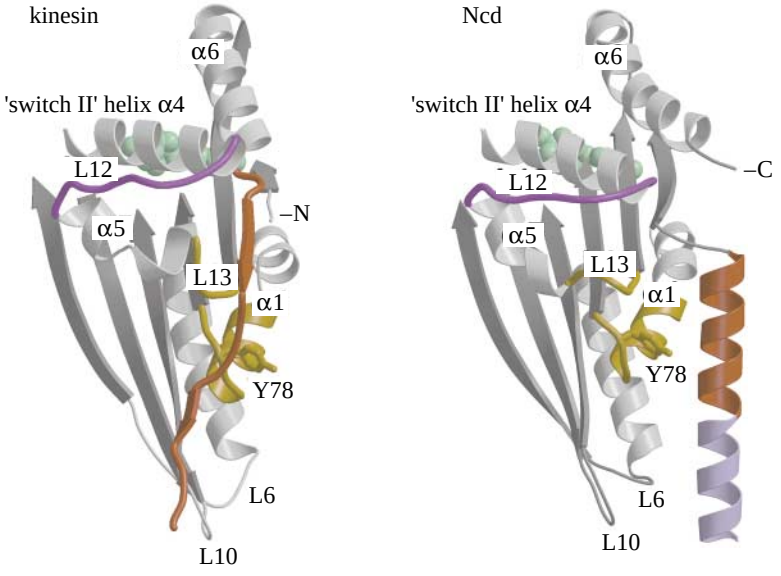

Figure 2. Interaction of the kinesin and Ncd necks with their catalytic cores. Interactions of the neck with the motor heads: the conserved neck is shown in dark orange; important regions of the core that contact the necks are shown in yellow (α1 and L13). Loop L13 is adjacent to the predicted microtubule-binding site—loop L12 (purple) and helix α5. The location of the switch II (α4) helix is shown. L6 and L10 also make contacts with the necks.

(b) *Necks*

Immediately adjacent to the catalytic core is a short stretch of amino acids that exhibits class-specific conservation, which we refer to as the neck (Sablin *et al*. 1998; Vale & Fletterick 1997) (figure 1). For the Kin C motors, there are *ca*. 13 amino acids N-terminal to the catalytic core (immediately preceding β1) that are highly conserved in this group of motors but are not found in the Kin N or Kin I motors. Ncd, a minus-end-directed motor from *Drosophila*, involved in meiotic and mitotic spindle formation, is the best-studied representative of this class. Conversely, for the Kin N motors, there are 10–15 amino acids (termed the neck linker) that emerge from the C-terminal of the catalytic core (immediately following α6), and that are highly and uniquely conserved among the Kin N motors. Conventional kinesin, a plus-end-directed vesicle and intermediate filament transport motor, is the best-studied representative of this class. There are five subclasses of Kin N motors, and the sequence conservation among members of each subclass continues for another 20–30 residues (Vale & Fletterick 1997). Based upon secondary structure predictions, this region appears to form either an isolated helix or a helical coiled coil. Kin I motors also have a well-defined region of conserved residues adjacent to the catalytic core that may be important for its activity as a microtubule-destabilizing enzyme (Vale & Fletterick 1997). Other kinesin motors, termed orphans, do not have one of the highly conserved neck regions found in the Kin N, Kin C or Kin I families.

Surprisingly, the single most class-specific cluster of residues in the kinesin motor domain is found in the neck and not within the catalytic core. Sequence analysis of the conserved neck regions generates a phylogenetic tree that reveals a clear clustering of related motors; such trees are similar to those based upon alignments of the entire 330 residues within the catalytic domain (Case & Vale 2000). This finding suggests that the catalytic cores and necks have coevolved, which we believe reflects the fact that they function as a unit and have a coordinated role in motor activity.

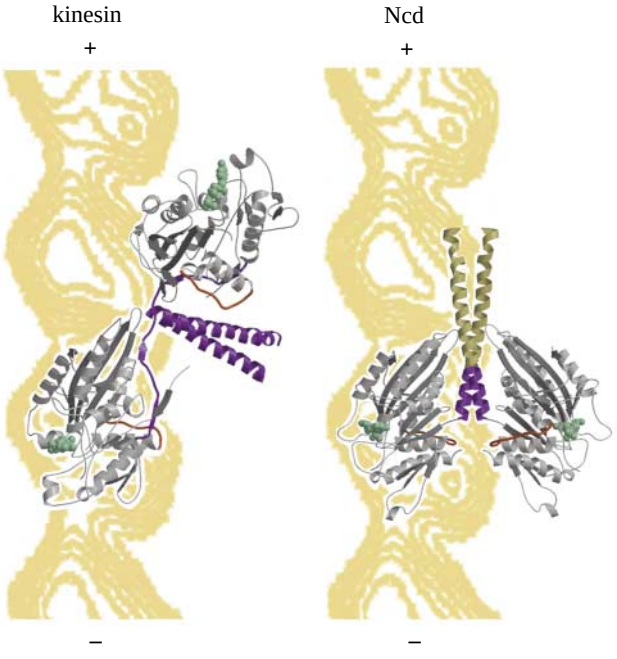

Figure 3. A model showing the Ncd and kinesin dimer structures docked onto a tubulin protofilament. The conserved necks are shown in purple (corresponding to the hatched regions in figure 1). The bound Ncd and kinesin heads are positioned similarly with the microtubule-binding loop L12 (Woehlke *et al*. 1997) docked onto the tubulin. Because of the distinct architectures of the kinesin and Ncd necks, the unbound kinesin head points towards the plus-end, while the unbound Ncd head is tilted towards the minus-end of the protofilament. This figure was reprinted from Sablin *et al*. (1998) with permission from the publishers.

3. STRUCTURES OF KINESIN NECKS

Despite the fact that kinesin and Ncd move in opposite directions along microtubules, their catalytic cores are nearly identical. Given the high degree of amino-acid sequence similarity (*ca*. 40%), this is not necessarily unexpected. Thus, the structures of the two catalytic cores did

not provide a clear answer as to why kinesin and Ncd move in opposite directions. Given the unique and class-conserved sequences of the necks, it is tempting to believe that the necks confer unique motor functions such as directionality. Structural data discussed below provides further support for this idea.

The first structures of kinesin and Ncd did not provide information on the necks, since they were disordered and not visible in the electron density maps. (Interestingly, both polypeptide chains became disordered at the N- and C-terminal boundaries of the catalytic cores.) Later, the structures of the necks were determined by crystallizing a kinesin monomer under different conditions (Sack *et al*. 1997), and kinesin (Kozielski *et al*. 1997) and Ncd (Sablin *et al*. 1998) homodimeric proteins (figures 2 and 3). For kinesin, the neck consists of two short β-strands (β9 and β10; the neck linker) followed by the coiled-coil helix α7 (neck coiled coil); these secondary structural elements are connected by short loops. As described earlier, the neck linker region is conserved among all of the plus-end-directed Kin N motors, and all of these motors probably share a similar β-strand structure. In contrast to the kinesin neck, the structure of an Ncd dimer shows that the Kin C neck (the 13 residues that precede the first β-strand of the catalytic core) is helical and part of a coiled coil. The conserved neck connects to the less-conserved coiled-coil 'stalk' without interruption. Thus, while the Ncd and kinesin catalytic core are similar in structure, the neck regions are structurally distinct and this difference could provide the basis of their opposite directions of motion.

The kinesin dimer in the crystal is asymmetrical, with the two heads related by a 120° rotation (Kozielski *et al*. 1997). In contrast, the Ncd dimer structure has a twofold symmetry due to the coiled-coil neck. The different symmetries of the kinesin and Ncd dimers appear to be determined by their distinct neck architectures. The different symmetries of the motor domains in the Ncd and kinesin dimer structures, in part, could be responsible for their opposite directions of motion (figure 3) (Sablin *et al*. 1998). If the bound kinesin and Ncd head of the dimer are positioned similarly on a microtubule, then the unbound kinesin head would point towards the microtubule plus-end, while the unbound Ncd head would be directed towards the minus-end. Thus, the detached heads in these dimeric motors are positioned in the direction of motor movement. The different positioning of the two heads also agrees with some of the cryo-electron microscopy studies performed on microtubule-bound Ncd and kinesin dimers (Amos & Hirose 1997). The distinct dimer head positioning probably contributes to their opposite directions of movement. However, in addition to positioning of the detached head in the direction of travel, we also believe that Ncd and kinesin necks undergo different types of power strokes, as discussed in § 5.

The necks of kinesin and Ncd interact with the catalytic cores through an extensive network of interactions (figure 2). Many of the interacting residues are class-conserved among Kin N or Kin C motors. Remarkably, despite their different sequences and structures, and the fact that they emerge from opposite ends of the catalytic core polypeptide chain, the kinesin and Ncd necks both interact with similar secondary structure elements on the catalytic core (primarily the α1–β3 turn and L13). These sites of neck–core contacts are near the switch II helix and the main microtubule-binding loop (L12), which are both thought to change conformation during the nucleotidase cycle. Their proximity suggests a possible pathway of communication from the major allosteric elements of the catalytic core to the necks.

4. FUNCTIONAL EVIDENCE FOR A ROLE OF THE NECK IN KINESIN MECHANICS

(a) *Truncations: the catalytic core plus the neck creates a functional motor*

Various truncations of kinesin and Ncd motor proteins have been tested for activity in microtubule gliding assays. For kinesin, a protein containing the catalytic core plus the neck linker produces motility, albeit at fivefold or more reduced velocities (Berliner *et al*. 1995; Stewart *et al*. 1993; Vale *et al*. 1996). These monomeric constructs are also no longer processive in single-molecule motility assays (Berliner *et al*. 1995; Vale *et al*. 1996). Similarly, a truncated Ncd monomer, consisting of the catalytic core plus 13 residues of the helical neck, produces minus-end-directed microtubule gliding with a reduced velocity of motion (Stewart *et al*. 1993). These truncation experiments argue that the catalytic core can cooperate with the neck to produce motion. Additional domains (e.g. adjacent coiled coils and hinge regions) most certainly augment motility in terms of velocity or processivity, but they do not appear to be essential for producing motion.

(b) *Motor chimeras: the necks, and not the catalytic cores, determine directionality*

The opposite directions of motion of kinesin and Ncd have inspired various chimera experiments aimed at mapping a 'directionality element'. Henningsen & Schliwa (1997) and Case *et al*. (1997) prepared chimeras in which the catalytic core of Ncd was joined to the neck and stalk domain of kinesin. Surprisingly, the resultant chimeras moved to the microtubule plus-end, even though the catalytic core belonged to that of the minus-end motor, Ncd. Endow & Waligora (1998) later prepared the converse construct to that of Case *et al*. (1997) in which the catalytic core of kinesin was joined to the neck-stalk of Ncd. This chimera moved to the minus-end, despite the presence of a catalytic core belonging to a plus-end-directed motor. Endow & Waligora (1998) also found that the correct junction between the catalytic core and the neck was important for allowing the motor chimera to move towards the minus-end.

Collectively, the above experiments indicate that the direction of motion is not determined by the catalytic core, but rather by the adjacent neck region. The catalytic core is the enzyme and the juxtaposed neck is the transmission. Moreover, these experiments uncovered the surprising result that necks can communicate, at least to some extent, with catalytic cores from evolutionary distant and even opposite-directed motors. This result is consistent with atomic structures which show that the distinct necks of kinesin and Ncd interact with identical loops on the catalytic core (Kozielski *et al*. 1997; Sablin *et al*. 1998). Thus, it is structurally plausible to imagine

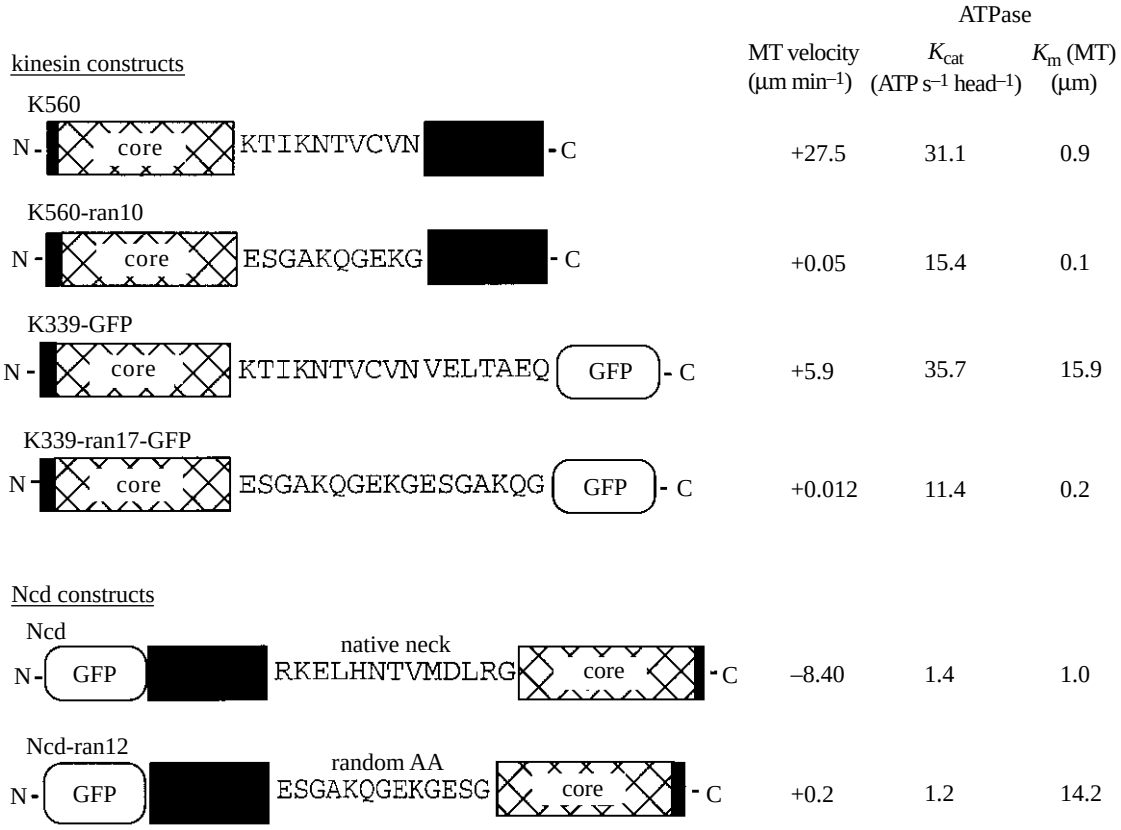

Figure 4. Mutational analysis of the kinesin and Ncd neck regions. The constructs tested are shown on the left. The catalytic core and the sequence of the wild-type or the mutant neck residues are shown. All proteins were analysed for motility and ATPase activity, and results from a typical protein preparation are shown. The mean and s.d. for gliding velocity of > 20 microtubules is indicated. ATPase was measured at several microtubule concentrations, and K_m(MT) and k_{cat} were determined from the fit of the data to a hyperbola. The error of the fit is shown. Additional data for these mutants has been or will be reported elsewhere (Sablin *et al*. 1998; Case *et al*. 2000).

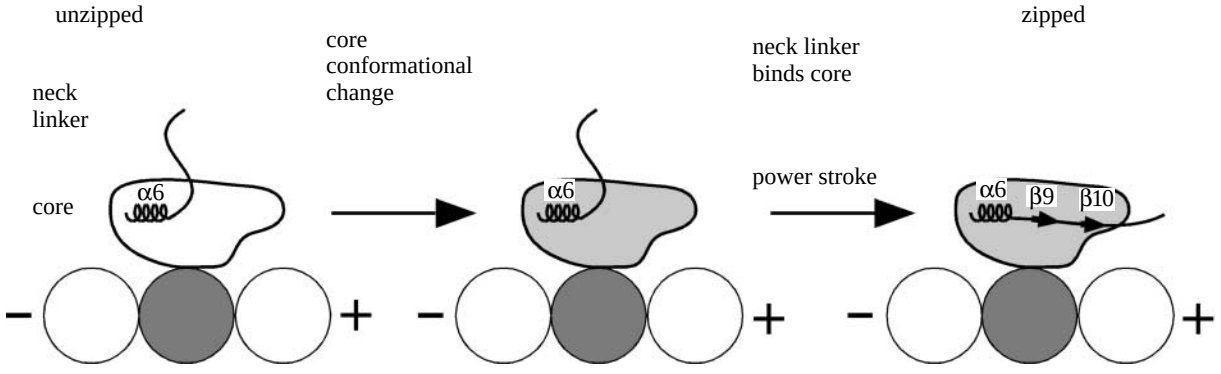

Figure 5. A general model for a plus-end-directed power stroke based upon the docking of the β9–β10 neck linker onto the catalytic core during the enzymatic cycle. Kinesin is orientated with the β9–β10 neck linker directed towards the microtubule plus-end (+), as described in electron microscopy studies. By 'zipping up' and reforming β9 and β10, the neck linker could power stroke towards the plus-end (+) of the microtubule. Rice *et al*. (1999) have shown that the neck linker zipping occurs after ATP binding.

that the kinesin neck could co-function to some extent with the Ncd catalytic core, or vice versa, in the above-described chimeric proteins. Determination of the atomic structure of these chimeric motors should provide further insight into this matter.

(c) *Neck mutations: microtubule-stimulated ATPase activity but inefficient mechanics*

Mutagenesis of the kinesin and Ncd necks also supports the idea that these regions serve as mechanical amplifiers (figure 4). In the case of Ncd, we prepared a drastic mutation that replaced 12 neck residues (amino acids 335–346) with random, hydrophilic residues (Ncd–ran12). This mutation, which should completely disrupt the neck–core interaction and the coiled-coil architecture of the Ncd neck, produced slow motility (50-fold below wild-type Ncd) toward the microtubule plus-end, which is the opposite direction to the intact Ncd motor. This experiment demonstrates that the Ncd neck is essential for producing motion to the microtubule minus-end.

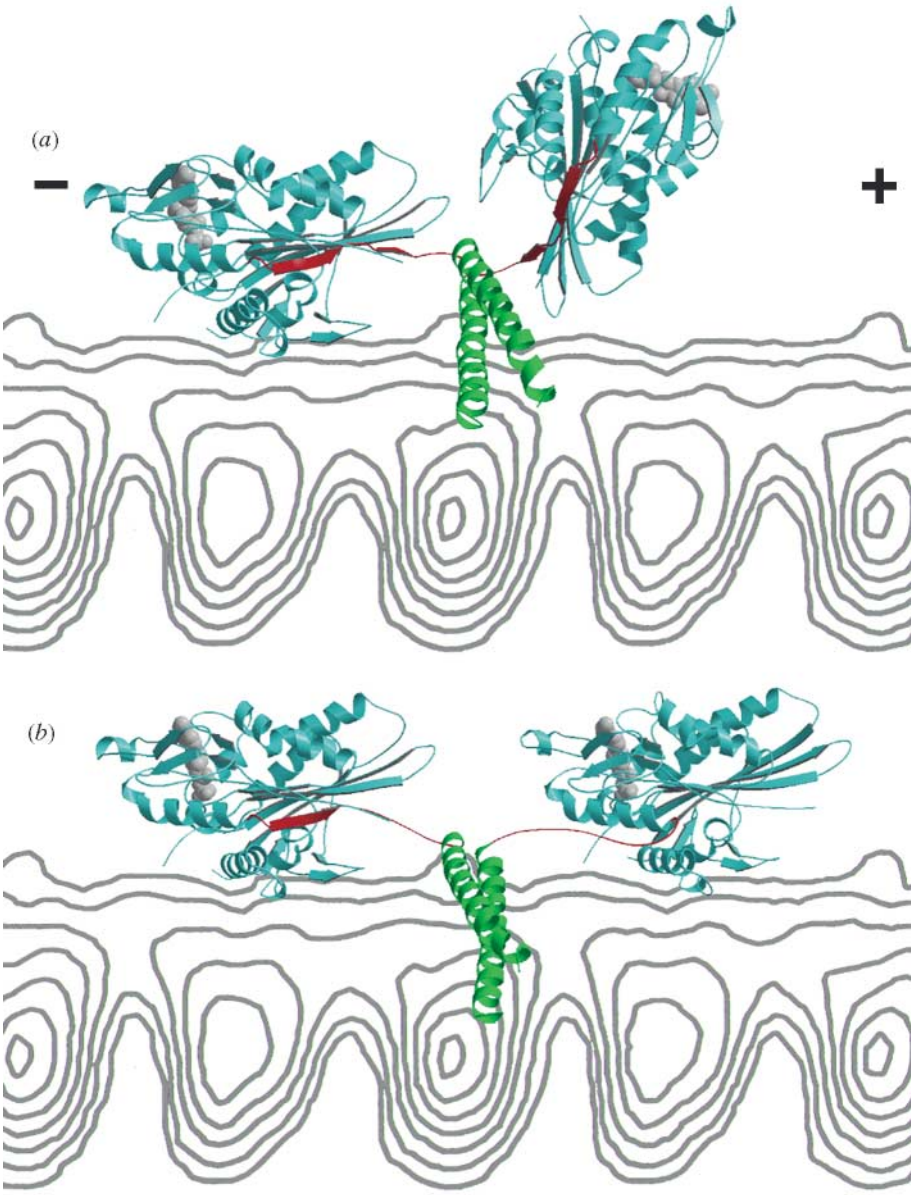

Figure 6. A model for how the kinesin dimer might span the 8 nm between adjacent α and/or β tubulin-binding sites during processive motion. In this crystal structure of the rat kinesin dimer (Kozielski *et al*. 1997), the catalytic core domain is coloured blue, the nucleotide is coloured grey, the β-strand region of the neck (β9 and β10; rat amino acids 321–336) is coloured red, and the neck coiled coil (rat amino acids 337–370) is coloured green (note: the rat kinesin amino acid numbers differ by −2 amino acids compared to human kinesin in this region). A side view of a microtubule protofilament from cryo-electron microscopy reconstructions (Hoenger *et al*. 1995) is shown in grey. The microtubule plus-end (the direction of travel for kinesin) is on the right. In (*a*), the unaltered crystal structure of the rat kinesin dimer is shown with one head docked onto the microtubule. The approximate orientation of the bound head was defined by having the half of the molecule containing the nucleotide pointing towards the minus-end, the 'arrowhead tip' pointing towards the plus-end (Hoenger & Milligan 1997), and the main microtubule-binding loop (L12) in contact with the tubulin surface (Woehlke *et al*. 1997). In the crystal structure, the distance between the two heads is insufficient to enable the second head to dock onto the microtubule. It is important to mention that the structure shown in (*a*) probably does not exactly correspond to one that occurs normally in the motility cycle, since the geometry of the heads could be partially determined by crystal contacts and since microtubule or nucleotide binding can change the solution conformation (Rice *et al*. 1999). In (*b*), the β-strands between amino acids 327 and 336 were separated from the catalytic core in the leading head, obeying restraints of bond distances and geometries. This generates a sufficiently long linker to enable the leading head to dock to the adjacent tubulin-binding site in the identical orientation to the lagging head. Only modest adjustments need to be made to the neck β-strands of the lagging head, since they are already extended and pointing towards the microtubule plus-end. In this model, the neck coiled coil does not unwind, since data from Romberg *et al*. (1998) argues against substantial unwinding of the coiled coil. However, a fraying of the N-terminal end of the coiled coil is possible, although not depicted here. The nucleotide (ADP) from the crystal structure is shown in both heads in these panels, although it is more likely that the two heads are in different nucleotide states during the motility cycle (Hackney 1994). The figure was reproduced from Romberg *et al*. (1998) with permission from the publishers.

A similar mutation was prepared in kinesin by replacing the conserved neck linker (amino acids 323–332) with the same random residues described above. This K560–ran10 mutant was still active and moved to the microtubule plus-end, albeit at much lower velocities compared to parent constructs (0.06 μm min^{-1}; figure 4) (Case *et al*. 2000). This result indicates that the kinesin neck linker, while not essential for plus-end motility, dramatically enhances the rate of movement.

The finding that K560–ran10 and Ncd–ran12 both move very slowly towards the microtubule plus-end suggests that the catalytic cores of both plus- and minus-end-directed motors may undergo a conformational change directed towards the plus-end. To test this idea, we prepared a construct consisting of the kinesin catalytic core connected by a 17 amino-acid linker to green fluorescence protein, which was used to anchor this monomer motor to the glass surface for motility assays (Case *et al*. 2000). This protein (Kin339–ran17–GFP) moved to the microtubule plus-end, indicating that the catalytic core itself has intrinsic motor activity (figure 4). Some of the orphan motors (e.g. *Drosophila* Nod) that have no sequence similarity to the necks of other kinesin motors (Vale & Fletterick 1997) could potentially employ catalytic-core-based motility.

Although the neck-replaced mutants (Ncd–ran12, K560–ran10, Kin339–ran17–GFP) generated slow motility, their ATPase turnover rates were within threefold of normal. ATPase activity depends on the core contact with microtubules and a mechanical linkage between the ATP- and microtubule-binding sites. Therefore, this finding indicates that neck disruption does not greatly alter the normal allosteric activation of the ATPase cycle by microtubules. However, the nucleotide hydrolysis cycle clearly becomes uncoupled from motility in these mutants. The neck mutations also affect the microtubule affinity of kinesin and Ncd, as measured by the apparent tubulin K_m for activation of the ATPase activity. As shown in figure 4, the Ncd–ran12 has a much lower affinity for microtubules (higher K_m(MT)) than wild-type Ncd, while K560–ran10 has a higher affinity for microtubules than wild-type kinesin. These opposite effects of neck mutagenesis on microtubule affinity may be pertinent for understanding the different directions of motion of these two motors.

In summary, these results indicate that motor activity (microtubule gliding velocity) can be uncoupled from ATP turnover by mutating conserved residues in the neck and that an intact neck is essential for generating minus-end-directed motion. These results are consistent with the hypothesis that the neck serves as a mechanical amplifier for motility. In the absence of a neck, a small plus-end-directed conformational change in the catalytic core may account for the residual motion.

5. A GENERAL MODEL FOR NECK-DRIVEN CONFORMATIONAL CHANGES

The neck of kinesin and Ncd bears little structural resemblance to myosin's elongated α-helical lever arm (Rayment *et al*. 1993*b*). The fact that myosin and kinesin differ in structure outside of their catalytic cores argues that they may have evolved different mechanical transducers. How do the kinesin and Ncd necks function to power motility? Below we will present a provisional model, and then consider the available information, albeit limited, that leads us to favour it.

(a) *Models for kinesin and Ncd power strokes*

For kinesin, we propose that the neck–core interface may unzip and zip during the ATPase cycle (figure 5). A hinge point for this motion may exist at the junction of the neck linker with α6, a stable element of the catalytic core. A conserved glycine or alanine (human kinesin G319) at the end of α6 may be important for this hinge motion. Zipping and unzipping of the β9–β10 neck could be generated by enzymatic transitions in the active site and transmitted through α1–β3, L13, and α4 of the catalytic core, which constitute the important elements of the neck–core interface (figure 2). Because of the orientation of the kinesin catalytic core on the microtubule (Hoenger & Milligan 1997), the re-zipping of the neck with the core would generate motion towards the plus-end of the microtubule. Based upon the length of β9–β10 neck linker, the size of the stroke predicted from this model would be expected to be 2 nm, which is close to a size predicted experimentally (Hancock & Howard 1998).

The unzipping of the β9–β10 neck linker may also allow the kinesin to form an intermediate during its cycle, in which both heads are bound to the microtubule during processive motion (Romberg *et al*. 1998) (figure 6). Unzipping of the neck linker from the leading head provides a sufficient tether to allow both heads of the dimer to bind to adjacent tubulin-binding sites 8 nm apart on the protofilament without unwinding the neck coiled coil. However, other groups favour a model in which the neck linker remains bound to the catalytic core and the majority of the neck coiled coil unwinds to allow the two heads to span the 8 nm distance (Hoenger *et al*. 1998). We tend not to favour this latter idea, since complete replacement of the kinesin neck coiled coil with an ultrastable coiled-coil sequence has little effect on the velocity or processivity of kinesin movement (Romberg *et al*. 1998). However, a partial splaying of the N-terminal end of the coiled coil is possible.

For Ncd, we also believe that the neck may detach from the catalytic core and adopt an alternative structure. The necks of Ncd and kinesin interact with the same loops in the catalytic core, and this could allow them to break contacts with the catalytic core through a similar conformational change pathway transmitted from the enzymatic site. The exact conformational changes for the kinesin and Ncd necks are likely to differ, so as to allow them to produce motion in opposite directions. For Ncd, sequence analysis suggests that the neck residues are not predicted to form a stable α-helix (PHD program) or a coiled coil (COILS program), even though they are helical in the crystal structure. Moreover, synthetic peptides containing the Ncd neck (L328–R348) are not helical in solution (T. Shimizu, M. Itoh, H. Morii and M. Tanokura, personal communication; B. Tripet and R. S. Hodges, personal communication). Thus, the helical structure of the neck may require specific interactions with the catalytic core. If the catalytic core–neck interface is weakened during the enzymatic cycle, then the neck helix may melt and adopt another conformation that powers motility to the microtubule minus-end.

Helix–coil transitions have been previously proposed to power myosin motility (Harrington 1979) and are known to underlie dramatic conformational changes in the influenza haemagglutinin protein (Bullough *et al*. 1994).

Several results imply, although do not prove, that the necks of kinesin and Ncd can change structure during the mechanochemical cycle. First, the necks of monomeric kinesin and Ncd in the initial crystal structures were disordered and not docked to the core (Kull *et al*. 1996; Sablin *et al*. 1996), whereas other structures revealed the neck interacting with the catalytic core (Kozielski *et al*. 1997; Sablin *et al*. 1998; Sack *et al*. 1997). Interactions between the neck and catalytic core therefore may be labile and depend upon the crystallization conditions or upon stabilizing interactions in dimeric motors (see note in proof). Second, the neck–core interfaces in Ncd and kinesin are comprised primarily of hydrophilic rather than hydrophobic interactions. This suggests that the interface is designed to be dynamic rather than optimized for maximal stability. Third, electron microscopy images of the Ncd dimer bound to microtubules clearly show that the neck must undergo a conformational change when it binds to the microtubule. While the Ncd dimer is twofold symmetrical in solution, the two heads become asymmetrically orientated in the Ncd–microtubule complex (Hirose *et al*. 1996; Sosa *et al*. 1997). Therefore, a symmetry-breaking conformational change in the neck of the Ncd dimer must occur upon binding of the motor to the microtubule, or upon the subsequent release of ADP from the microtubule-bound head. Fourth, we have found that the kinesin neck linker undergoes a significant change in mobility when the kinesin–microtubule complex is in an AMPPNP (non-hydrolysable nucleotide analogue) versus an ADP state (Rice *et al*. 2000).

6. CONCLUSIONS

Structural as well as mutagenesis data have implicated the neck regions as participating in the kinesin force-generating process. In our opinion, this work has served to focus future efforts on this relatively short stretch of amino acids. However, there is currently a poor understanding of what these neck regions are actually doing to generate motion. The next challenge will be to obtain information on the conformation of the kinesin and Ncd necks at different stages of the enzymatic cycle. New atomic structures (ATP states), cryo-electron microscopy and spectroscopy must all be brought to bear on this important problem. With such techniques, it should be possible to test the models described in this article.

Note in proof. Since this article was written, we have measured directly a conformational change in the kinesin neck linker by electron paramagnetic resonance, fluorescence resonance energy transfer, cryo-electron microscopy and pre-steady-state kinetic measurements (Rice *et al*. 1999). The neck linker is detached and mobile but zippers onto the catalytic core when the microtubule-docked motor binds ATP. We have also published our mutagenesis studies of the kinesin neck linker, preliminary data of which are presented in this review (Case *et al*. 2000). A detailed description and comparison of kinesin and myosin motility cycles is described in Vale & Milligan (2000).

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