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Review

Directionality of kinesin motors[★][✪]

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Kinesins are molecular motors that transport various cargoes along microtubule tracks using energy derived from ATP hydrolysis. Although the motor domains of kinesins are structurally similar, the family contains members that move on microtubules in opposite directions. Recent biochemical and biophysical studies of several kinesins make it possible to identify structural elements responsible for the different directionality, suggesting that reversal of the motor movement can be achieved through small, local changes in the protein structure.

Molecular motors use energy derived from ATP hydrolysis to move unidirectionally along cytoskeletal 'tracks'; myosins use actin filaments, kinesins and dyneins — microtubules. Kinesin motors constitute a large superfamily of motor proteins which participate in numerous biological processes such as transport of vesicles and organelles, organization of spindle microtubules, and chromosome segregation (reviewed by Hirokawa, 1998; Woehlke & Schliwa, 2000). Kinesins share a common force-generating element, called 'motor domain' that hydrolyzes ATP and binds to microtubules. Structural analyses of kinesins have revealed that the topography of their nucleotide-binding pockets is similar to those of myosins and G-proteins. This raises the possibility that all these protein structures evolved from a common ancestor (Kull *et al.,* 1998). Besides the well conserved motor domain kinesins possess a superhelical segment ('stalk') that differs for each of the

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Abbreviation: ncd, non-claret disjunctional.

subfamilies. Some members may also have light chains noncovalently attached to the tail segment of the heavy chain. The motor domain can be found at the N-terminus, C-terminus and also in the middle of the polypeptide chain.

Two properties, discovered for the first time in kinesin motors made these proteins an attractive object of biophysical and biochemical investigation. The first one, processivity, is the ability of a motor protein to undergo many mechanochemical cycles without detaching from the track. The second property of kinesins is the occurrence of superfamily members that move in opposite directions on microtubules. While conventional kinesin translocates to the plus, fast-growing end of the microtubule, ncd (*non-claret disjunctional*) moves towards the (–)-end. Quite recently it has been realized that also in the myosin family one can find motors that move processively (myosin V and VI; Mehta *et al.,* 1999; Rock *et al.*, 2001) and that not all myosins translocate to the (+)-end on actin filaments. For example, myosins VI and IXb move to the (–)-end (Wells *et al.,* 1999; Inoue *et al.,* 2002). In this review we address the question of how it is possible that structurally similar proteins belonging to the same superfamily can generate movement in opposite directions. Several review articles covering other aspects of microtubule-based motility appeared recently (Vale & Milligan, 2000; Woehlke & Schliwa, 2000; Goldstein, 2001; Schief & Howard, 2001; Higuchi & Endow, 2002).

WALKING ALONG MICROTUBULES

The directionality of kinesin motors must be discussed in conjunction with the force-generation mechanism of these proteins. Similarly to myosin, kinesin's affinity to microtubules depends on the type of nucleotide at the active site. However, unlike myosin, kinesin binds strongly to the microtubule when ATP or $ADP \cdot P_i$ occupy the nucleotide-binding pocket. Weak binding occurs for kinesin complexes with ADP. Without a nucleotide, the affinity of kinesin is high. The protein moves along the microtubule protofilament making discrete steps of about 8 nm (Schnitzer & Block, 1997), which corresponds to the separation of α,β-tubulin heterodimers along the microtubule protofilament. The crucial problem in proposing a model of kinesin movement is explanation how small-scale conformational changes (about 0.5 nm) at the motor active site, triggered by breaking the bond between the β- and γ-phosphates of ATP, are converted into much larger displacements (about 8 nm) of the motor head. For skeletal muscle myosin, it has been proposed that the changes at the active site lead to a rotation of a long and stiff lever arm (reviewed by Spudich, 2001). However, the corresponding part in kinesin molecule is quite different. In the most widely accepted model for kinesin movement (Rice *et al.,* 1999), the function of the lever-arm is performed by a dynamic strand of the polypeptide chain, called 'neck-linker'. In the absence of microtubules the neck-linker exists in equilibrium between a disordered and docked conformation (Sindelar *et al.,* 2002). In the kinesin–microtubule complex, the linker appears to be docking on the motor core when the kinesin is free or in the $ADP \cdot P_i$ state. It is disordered and unattached to the core in the ADP state (Rice *et al.,* 1999; Case *et al.,* 2000).

The putative nucleotide-activated mechanism of kinesin movement, depicted in Fig. 1, is based on the article of Rice *et al.* (1999). Free kinesin in solution has an ADP molecule bound to each of the two heads. When one of the heads binds to the microtubule, its nucleotide is rapidly released and replaced by ATP (Fig. 1, Step *2*). Rice *et al.* (1999) proposed that binding of the second head may occur without ATP hydrolysis in the trailing head (Step *3*). However, a different view concerning the sequence of events has also been presented (see Schief & Howard, 2001). The attachment of the leading head to the protofilament (Step *4*) results in the dissocia-

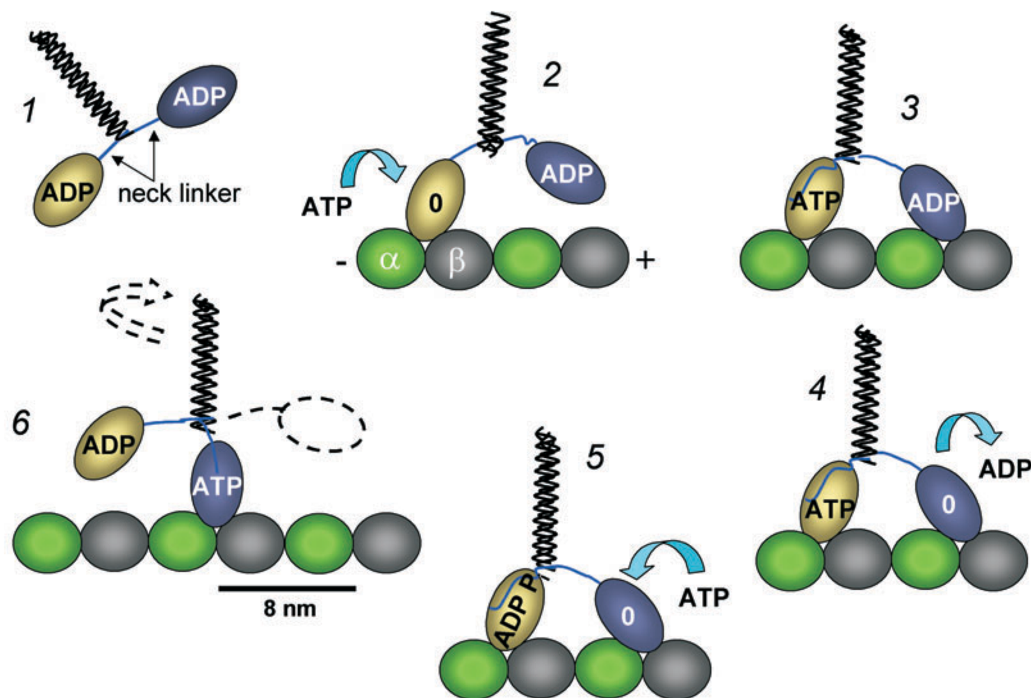

Figure 1. Proposed mechanochemical cycle of kinesin.

The neck linker is docked on the motor-core when the head is in the ATP or ADP · P_i state; it is disordered and mobile in other states. During its processive translocation along the microtubule the kinesin molecule cycles through states 3–6, producing steps of about 8 nm. Full explanation is given in the text and in Rice *et al.* (1999).

tion of ADP from this head (Step *5*) followed by a rapid replacement of this nucleotide by ATP. The leading head docks the linker on its motor core throwing the other head forward (Step *6*). The details of this step are not known; a rotation, marked by a dashed arrow in Fig. 1, is assumed. Nonetheless, no rotation of cargo attached to the kinesin stalk was found experimentally (Hua *et al.,* 2002). This finding has led to the proposal of a revised and controversial version of this model referred to as the 'inchworm model' of kinesin movement where only one of the kinesin's heads is an active ATPase (Hua *et al.,* 2002). Further insights into the force generating mechanism may be obtained using proteins carrying specific mutations in one of the heads (Skowronek & Kasprzak, 2002).

Although there are some uncertainties concerning particular steps of this model, it is an attractive hypothesis since it is based on a disorder-to-order transition found in other biological systems, and can also explain the processivity of kinesin motors: at any given time the two motor domains of kinesin are at different stages of their ATPase cycle. Therefore, at least one of the heads binds tightly to the microtubule preventing dissociation of the whole molecule from the track. The movement is coordinated in a 'hand-over-hand' manner (Hancock & Howard, 1999). One should keep in mind that most of the experiments that led to this model were performed using single-headed kinesin constructs where the conformation of the neck-linker may be substantially different from the one found in the full-length dimeric protein. In addition, there are also alternative models of kinesin movement such as Brownian ratchet supported by some research groups (Astumian & Derényi, 1999; Nishiyama *et al.,* 2002).

For ncd, there is no sufficient experimental data to propose a detailed walking scheme. However, a quite recently published article

suggests a power-stroke (conformational) mechanism of force generation in this protein (Wendt *et al.,* 2002).

MOTOR DIRECTIONALITY

The structures of motor domains of conventional kinesin and ncd have been resolved by X-ray crystallography with 2.5 Å resolution (Kull *et al.,* 1996; Sablin *et al.,* 1996; Fig. 2B). Although the proteins move to the opposite ends of the microtubule and have their motor domains located at the N- or C-terminus of the polypeptide chain (Fig. 2A), the three-dimensional structures of their motor domains are remarkably similar with r.m.s. deviation of 1.21 Å between 146 core α-carbon atoms

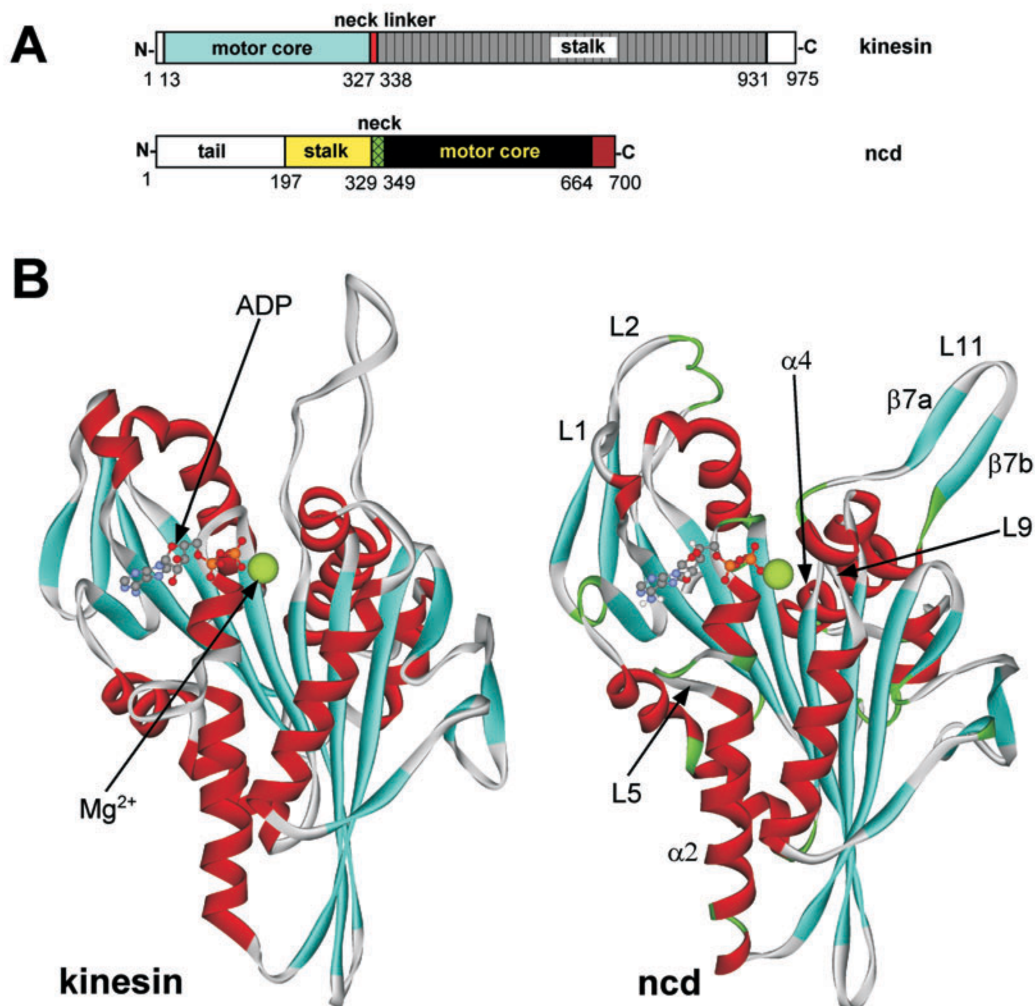

Figure 2A. Domain organization in conventional kinesin and ncd.

The sequence numbers refer to the *Drosophila melanogaster* proteins. In ncd the neck is continuous with the stalk and its definition is based on the presence of two heptad repeats conserved in all (–)-end-directed motors, located between the stalk and the conserved motor core (Endow & Waligora, 1998).

Figure 2B. Structures of motor domains of kinesin and ncd.

The coordinates of human kinesin were obtained from Protein Data Bank, (file 1BG2), and for *Drosophila* ncd, from the Web site http://www.proweb.org/kinesin//NcdCoordinates.html and translated from the 'O' to the PDB format. The structures were rendered using WebLab ViewerLite (Accelrys Corp.).

(Sablin *et al.,* 1996). The central α-helices and β-strands are almost identical. Most of the differences are found in their surface loops. In ncd, loop L11 is stabilized by two small β-strands; in kinesin this loop was not visible probably due to disorder. Loop L5 that interrupts helix $\alpha 2$ is shorter in the case of ncd. In ncd, loop L2 contains 10 additional amino-acid residues. The position of helix $\alpha 4$ is also slightly different. Many lines of experimental evidence demonstrated that both proteins bind to microtubules at the same sites and in the same orientation. How these two almost identical structures can generate movement in opposite directions? The answer was provided by three-dimensional reconstructions of dimeric motors bound to microtubules (Kozielski *et al.,* 1997; Sablin *et al.,* 1998). When the bound head is in the same orientation, for conventional kinesin the unattached head is tilted towards the microtubule (+)-end (Fig. 3A), and that of ncd towards the (–)-end (Fig. 3B). It appears that the motor directionality is controlled by the positioning of the unbound head for the next step. This is possible because the relative orientation of the two heads in dimeric kinesin and in ncd is strikingly different. In kinesin the heads are related by a 120° rotation and are attached to the coiled-coil domain through neck linkers. In contrast, in ncd the heads, related by 180° symmetry, make contacts with the coiled-coil through an array of hydrogen bonds (see also Fig. 5). It is, therefore, possible that specific interactions of the motor domain with the neck domain confer directionality.

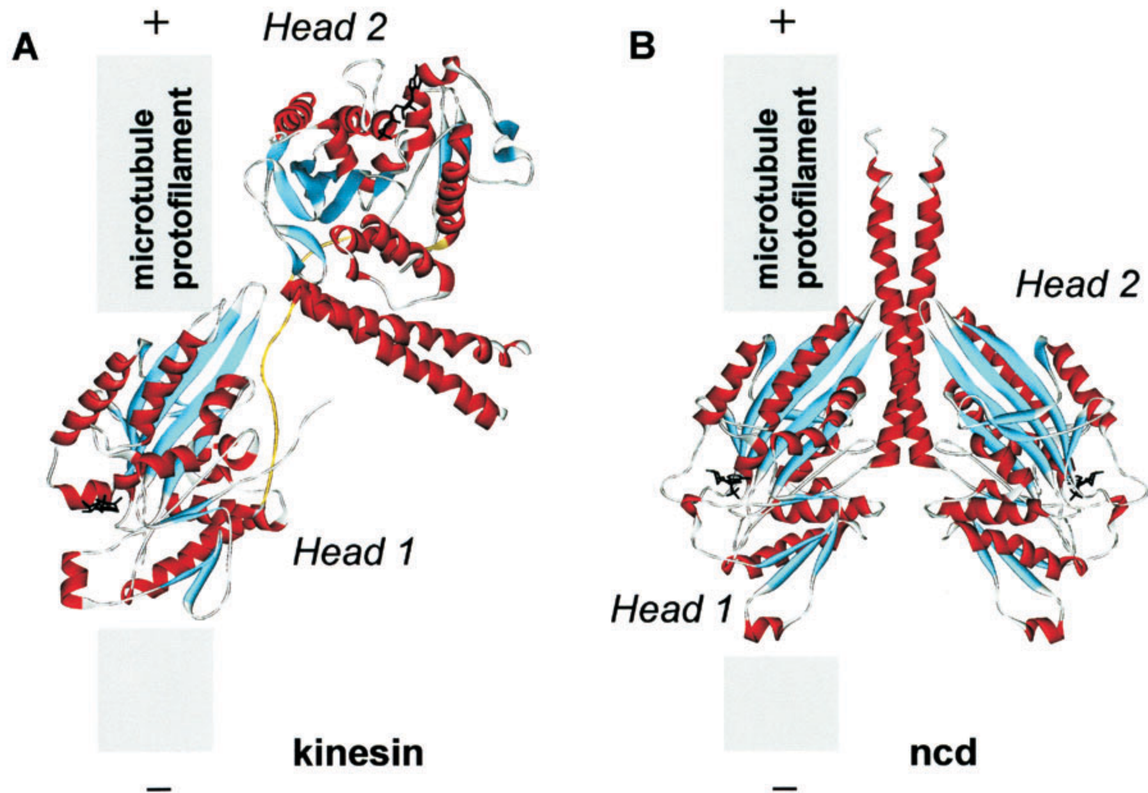

Figure 3. Docking dimeric motors onto microtubule protofilament.

Heads bound to the microtubule protofilament are in approximately similar orientation. The coordinates of rat kinesin and *Drosophila* ncd were obtained from the Protein Data Bank (files 3KIN and 2NCD). The file 2NCD contains coordinates for one molecule of ncd. The second molecule of the dimer was built using the transformation matrix embedded in the file.

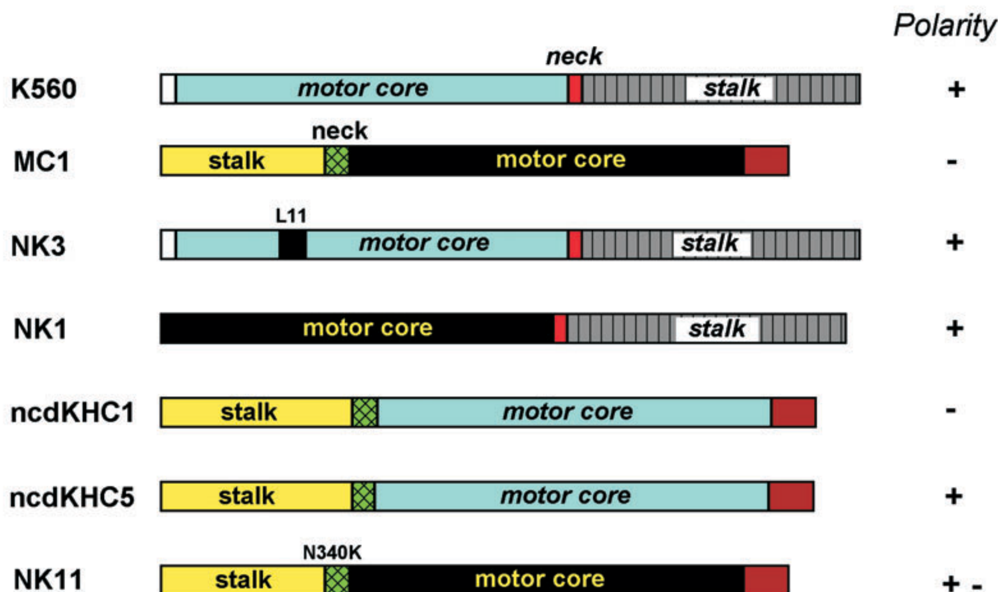

Figure 4. Directionality of movement of chimeras between kinesin and ncd.

K560 is a construct comprising amino-acid residues 1–560 of conventional kinesin. MC1 is an ncd construct containing residues 209–700 of *Drosophila* ncd. Both proteins contain the entire catalytic domain, the neck region and part of the coiled-coil. Human DNA fragment coding for the kinesin part was used to construct NK1 and NK3 whereas the chimeras ncdKHC1 and ncdKHC5 were generated using *Drosophila* kinesin DNA.

REVERSING THE DIRECTION OF MOVEMENT

Numerous mutagenesis studies were undertaken to test the hypothesis that the determinants of motor directionality are located in the head-neck region or can be explained by the known structural differences between the motor domains of conventional kinesin and ncd. In one of those studies the motor core loop L11 of conventional kinesin, the most dissimilar structural element in the kinesin and ncd crystal structures (see Fig. 2), was replaced by its homologue of ncd (Case *et al.*, 1997; Fig. 4). This did not reverse the direction of kinesin movement. In subsequent experiments two research groups constructed chimeric proteins composed of domains belonging to kinesin and ncd (Endow & Waligora, 1998; Henningsen & Schliwa, 1997). In NK1, the entire ncd motor core was fused to kinesin stalk/neck domain. The resulting hybrid still moved to the (+)-end (Case *et al.*, 1997). In ncdKHC1 (Fig. 4) the stalk and neck of ncd were fused to kinesin motor domain (Endow & Waligora, 1998), producing a motor protein that showed (–)-end directed polarity. The velocity of ncdKHC1 was, however, 10-fold lower comparing to wild-type ncd. The construct ncdKHC5 differs from ncdKHC1 only by the absence of two amino-acid residues in the neck-core junction region. The motor moved to the (+)-end on the microtubule with velocities about 6 times slower than ncdKHC1. It is clear from these data that the interaction of the motor core with the neck is of critical importance for the direction of movement. One can also conclude that the ncd motor domain possesses intrinsically (+)-end determinants and transformation of ncd into a (+)-end directed motor is rather simple. The reverse operation, that is converting kinesin into a (–)-end directed motor is more difficult and requires that the entire ncd neck be present in the construct. Even very subtle alterations of this region may result in a protein that still moves to the (+)-end.

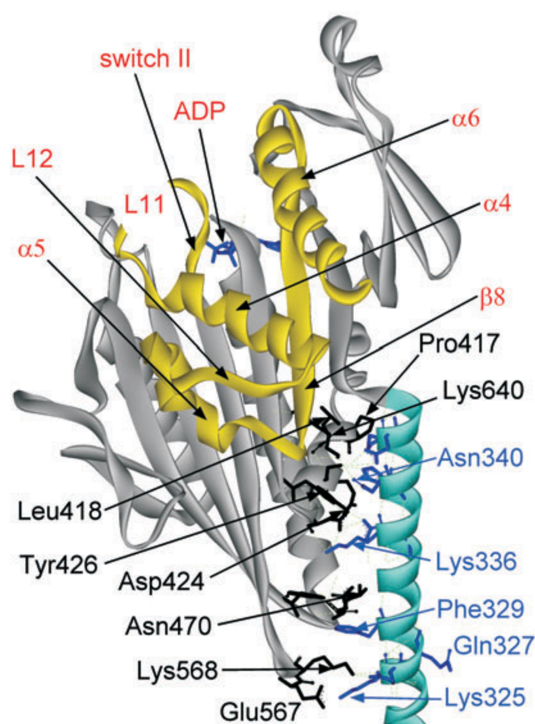

Figure 5. Interactions of the catalytic core with the neck in ncd.

Only one of the ncd heads is shown. The neck segment is colored blue. For the sake of clarity not all hydrogen bonds between the neck and the core were shown. By breaking the hydrogen bond between Asn340 located in the neck and Lys640 located in the catalytic core the conformation of the entire segment marked in yellow may be affected.

WALKING IN BOTH DIRECTIONS

An intriguing observation was made by Endow & Higuchi (2000). They mutated ncd by replacing one of the neck residues, Asn340 with, a lysine (Fig. 4). The mutated protein (NK11) was motile but, surprisingly, could alternate the direction of movement, walking to either the (+) or (–)-end of the microtubule and abruptly reversing the direction of translocation. The protein moved in each direction for relatively long (about 20 μm) distances with velocities only slightly lower than that of wild-type ncd. Although the mutation involved only a single amino-acid residue, it may be extremely disruptive to the neck-head interface of ncd. Lysine is much larger than asparagine and, most importantly, it is a positively charged amino acid. Asn340 forms a hydrogen bond with Lys640 located in the head domain (Fig. 5). This lysine (K640) is the only amino acid that connects the neck region with the segment marked in yellow in Fig 5. This involves large part of the microtubule-binding site, and provides communication — through ‘switch II’ helix — of the neck with the active site.

The simplest explanation of the bidirectional motion of NK11 is that the mutation weakened the neck–core interactions resulting in a conformation where the heads are detached (totally or partially) from the neck. Upon binding to the microtubule, the second head could assume a random orientation and bias the movement of the protein in either direction. To test this hypothesis, Hajdo and Kasprzak (unpublished data) measured the distances between the heads for ncd and ncd-Asn340Lys using fluorescence energy transfer spectroscopy. The results indicated that the mutation had little effect on the interhead distance for free proteins and their complexes with microtubules, therefore it seems unlikely that the head domains of ncd-Asn340Lys were loosely attached to the neck. Thus, the directional bias of the unbound head, shown in Fig. 3, is not sufficient for unidirectional motion which may also require a path of communication between the nucleotide- and microtubule-binding site and the neck/core interface.

The discovery that substitutions of single amino acids can lead to a reversal of motor direction raises the question whether bidirectional motors occur naturally in the cell. Several potential candidates in the dynein family have already been identified (Euteneuer *et al.*, 1998; Shingyoji *et al.*, 1998; Ma & Chisholm, 2002). The direction in which they move can be regulated, for example, by phosphorylation (Euteneuer *et al.*, 1998).

This opens up the possibility of the existence of as yet undiscovered bidirectional kinesin motors in cells.

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