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Structural links to kinesin directionality and movement

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The kinesin motor proteins generate directional movement along microtubules and are involved in many vital processes, including cell division, in eukaryotes. The kinesin superfamily is characterized by a conserved motor domain of ~320 residues. Dimeric constructs of N and C class kinesins, with the motor domains at opposite ends of the heavy chain, move towards microtubule plus and minus ends, respectively. Their crystal structures differ mainly in the region linking the motor domain core to the α-helical coiled coil dimerization domain. Chimeric kinesins show that regions outside of the motor domain core determine the direction of movement and mutations in the linker region have a strong effect on motility. Recent work on chimeras and mutants is discussed in a structural context giving insights to possible molecular mechanisms of kinesin directionality and motility.

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Organelles in eukaryotic cells are transported in specific directions along microtubule networks by two sorts of specialized motor proteins called kinesins and cytoplasmic dyneins. First isolated in the mid 1980s, kinesins now constitute an ever-growing family of enzymes that use ATP hydrolysis to fuel their movement along microtubules. They participate in intracellular transport and in different stages of the cell cycle, especially in mitosis and meiosis. Because of their biological importance, kinesins are the subject of extensive ongoing research, including studies aimed at understanding the molecular mechanisms of motility. Their motor domains, at ~40 kDa, are significantly smaller than those of myosin and dynein (by three and ten times, respectively), and are a convenient size for structural work. The article by Bloom and Endow[1] provides an informative general review on kinesins. Up to date information on kinesin function, motility, transport, superfamily classifications, motor domain alignments and so forth can be found on the internet at the Kinesin and the Kinesin Superfamily Protein (KIF) home pages[2,3].

In its native form, conventional kinesin is an elongated tetramer consisting of two identical heavy and light chains. The heavy chain has three distinct regions: a motor domain, including the ATP and the microtubule binding sites, a stalk and a globular tail. The stalk, an α-helical coiled coil interrupted by nonhelical regions that are believed to act as 'hinges', is responsible for heavy chain dimerization. The C-terminal region of the heavy chain interacts with the light chains, and appears to be involved in regulating the overall activity of the enzyme[4–6] and determining cargo specificity.

The signature sequence of the motor domain core, covering ~320 residues, has now been found in well over 200 proteins mak-

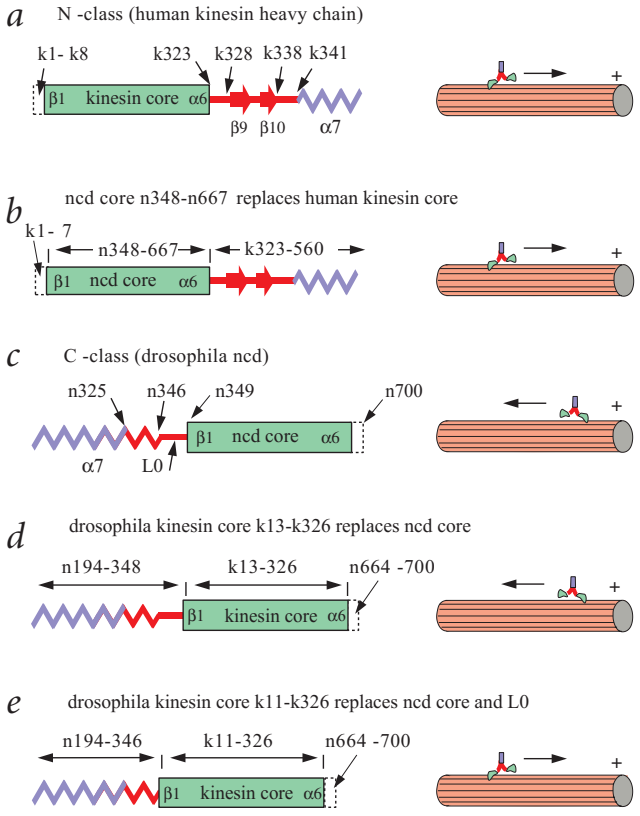

Fig. 1 Motor directionality of kinesin chimeras. Microtubules have a structural polarity and the kinesin superfamily proteins move along them in specific directions. Wild type N and C class kinesins move to the plus and minus ends, respectively. Experiments with kinesin chimeras show that regions outside the motor domain core determine the direction of movement. The constructs shown on the left give functional dimers that move along microtubules (orange cylinder) as shown schematically on the right. In Figs 1–3 the motor domain core regions are green, the coiled coil helices (α7) are blue and the linking regions are red. ***a***, In the presence of ATP, kinesin (N1 class kinesin) moves toward the microtubule plus end, in contrast to ncd (C1 class kinesin, shown in (*c*)) that moves toward the minus end. ***b***, When the kinesin motor domain core is replaced by that of ncd the movement is still towards the microtubule plus end. ***c***, In the presence of ATP, ncd (C1 class kinesin) moves toward the minus end. ***d***, Replacing the motor core of ncd with that of kinesin gives a dimer that moves toward the microtubule minus end, like ncd. ***e***, Deletion of two residues, Gly 347 and Asn 348, in loop L0 next to the ncd core produced a chimera that moves toward the microtubule plus end. These results provide evidence that the motor domain core by itself does not determine the direction of movement and, in ncd, residues in loop L0 are essential for minus end directed movement. The sequence numbers with the n prefix refer to *Drosophila* ncd; the k prefix refers to the human kinesin heavy chain in (*a*) and (*b*), and to the drosophila kinesin heavy chain in (*d*) and (e).

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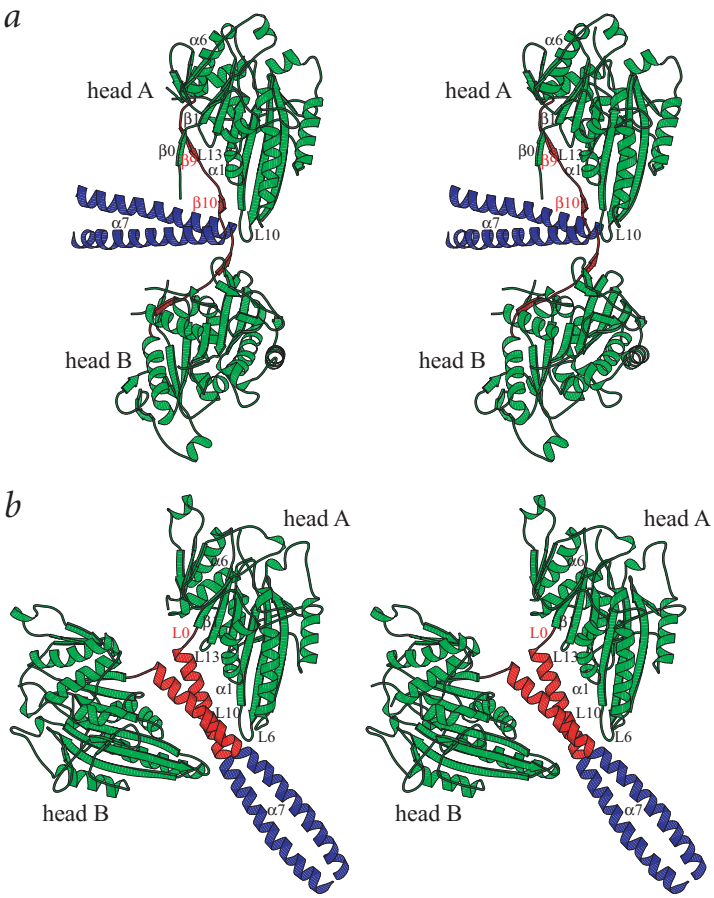

Fig. 2 Crystal structures of kinesin and ncd dimers. Stereo views of ***a***, rat kinesin and ***b***, *Drosophila* ncd. The motor domains A (head A) are aligned by a least squares fit of the core residues. Kinesin and ncd have very similar motor domain core structures but differ considerably in the linker regions, leading to different orientations and positions of the coiled coils and the second motor domain, head B. For rat kinesin the linker region includes the β-strands β9 and β10 as well as the loops covering Lys 325–Glu 340. For ncd the linker region covers L0 (Arg 346–Asn 348) and α7 from Lys 325 to Leu 345. Figs 2, 3 were prepared with the program MOLSCRIPT[38].

ing up the kinesin superfamily. As various genome projects progress, the family continues to grow. The position of the motor domain at the beginning, middle or end of the heavy chain, defines the N, M and C class kinesins, respectively[1,7,8]. These three classes consist of many subclasses with distinct quaternary structures including heterotetramers, homotetramers, heterotrimers, homodimers and monomers. Conventional kinesin is an N class kinesin (subclass N1) that has the motor domain at the N-terminus of the heavy chain, while *Drosophila* ncd, a kinesin with the motor domain at the C-terminus, is the archetype C class kinesin. Both of these kinesins are functional as heavy chain dimers and their movement along microtubules can be tested using *in vitro* motility assays. Microtubules have a structural polarity and all N class kinesins examined so far move towards the microtubule plus end whereas C class motors move towards the minus end. This is quite remarkable considering that the motor domains of kinesin and ncd have similar sequences, structures and overall enzymatic activity.

A major goal of biophysical research in this field is to obtain a detailed molecular description of how the kinesin motors function. In this brief review we highlight the significant ongoing progress in this field. In particular, careful examination of the available structural data provides considerable insight into exciting work using chimeric kinesins to investigate the factors governing the directionality of N and C class kinesins[9–11]. This interpretation of the structural features governing movement and directionality is supported by recent work on the linker region connecting the kinesin motor domain core to the coiled coil[12,13].

Structural studies lead to hybrid motors

Kinesins are minor components of most eukaryotic cells, so bacterial overexpression is usually needed to obtain sufficient amounts for biophysical and structural work. Atomic resolution crystal structures were first obtained for motor domain monomers of human kinesin[14] and *Drosophila* ncd[15]. In both cases the motor domain is an arrow-shaped molecule 70 Å × 45 Å × 45 Å with a core structure composed of an eight-stranded β-sheet flanked on each side by three major α-helices. In view of their movement in opposite directions along microtubules, it was quite a surprise to find that the two structures are practically identical. Another surprise was that kinesin and ncd have a strong structural similarity to the central core of the myosin motor domain, despite the large size difference and the lack of sequence similarity[14,16]. Subsequently, the crystal structure of the motor domain of yeast Kar3, another minus end directed motor, was found to agree closely with ncd[17].

Concurrent with the crystallographic investigations, cryo-electron microscopy was used to obtain low resolution three-dimensional maps of kinesin and ncd dimers interacting with microtubules[18,19]. The dimers were found to attach to microtubules *via* a single motor domain, with the unattached motor domain oriented towards the direction of movement, the microtubule plus end for kinesin and the minus end for ncd. This was an important result, suggesting that the directionality of these motors might be determined by a region outside the motor domain core itself. The most likely candidate appeared to be the region linking the motor domain core and the stalk.

This idea was tested by experiments on chimeric motors. Kinesins constructs that include a sufficient length of the stalk region spontaneously dimerize. By engineering such constructs, the kinesin motor domain core can be replaced by that of ncd to obtain an ncd-kinesin hybrid. In motility assays, although these hybrids have the ncd motor domain they move to microtubule plus ends like wild type kinesin[9,10] (Fig. 1*a*,*b*). In complementary experiments the motor domain of ncd was replaced by that of a conventional kinesin[11]. The resulting chimeras behaved like ncd and move towards microtubule minus ends (Fig. 1*c*,*d*). However, the same construct minus two residues (Gly 347 and Asp 348) immediately preceding the conserved motor domain core moved towards microtubule plus ends (Fig. 1*e*). These experiments show conclusively that the direction of movement depends on regions adjacent to the motor domain core rather than on the core itself. Two residues in ncd immediately preceding the core strand β1 are specifically identified as critical for minus end directed movement.

Crystal structures of dimeric motors

It has proven more difficult to obtain good crystals of kinesin dimers than was the case for the monomers. Fortunately, the crystal structure of dimeric conventional kinesin from rat[20] and two

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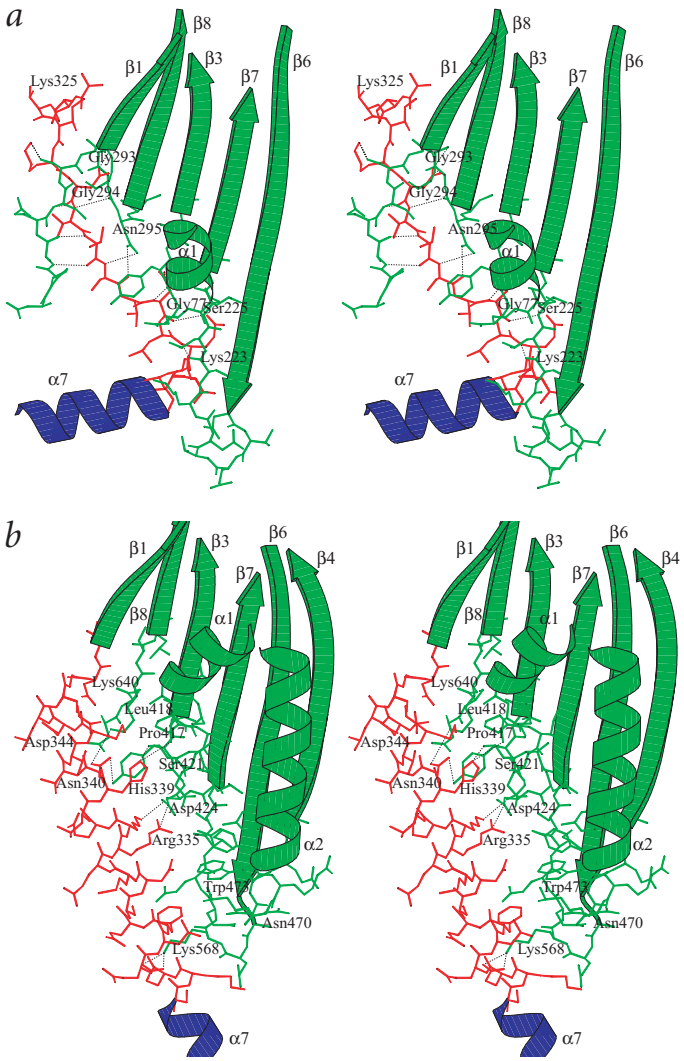

Fig. 3 Structure of the kinesin and ncd linkers. The linker connecting the motor domain core to the free standing coiled coil has many interactions with the core. Stereo view of the linker core interface for ***a***, rat kinesin and ***b***, *Drosophila* ncd. Side chain and main chain interactions (distances below 3.5 Å) are shown as black dotted lines. The kinesin linker also forms main chain interactions with β0 at the N-terminus of the heavy chain. The three ncd dimer structures[21,22] show some variations in the distances between interacting side chains at the interface. An unusual feature is that Asn 470 in L6 of one monomer interacts with Lys 325 in α7 in the other monomer.

structures of the *Drosophila* ncd dimer[21,22] are now available. To date, it has only been possible to obtain crystals of motor domain monomers or dimers in the ADP state, or crystals putatively in other nucleotide states that show no significant conformational differences from the ADP state[23]. However, the available structures reveal that the kinesin and ncd dimers have strikingly different overall conformations (Fig. 2). In both cases, dimerization is mediated by the formation of a coiled coil by the helices α7, one from each monomer as predicted. The virtually identical cores of kinesin and ncd run from strand β1 to helix α6, corresponding to residues 107–324 for rat kinesin and 349–670 for ncd. A distinctive feature of both the monomer and dimer structures is that the 'free' ends of β1 and α6, the N-termini and C-termini of the core, respectively, are only ~7 Å apart.

The crystal structures of rat kinesin monomers[24] and dimers[20] revealed three β-strands, β0, β9 and β10, outside the motor domain core that were not visible in the initial human kinesin structure[14]. β0 is at the N-terminal end of the core and leads into β1, while β9 and β10 link α6 that is at the C-terminal end of the core, to the coiled coil helix α7. β0 interacts with β9, while both β9 and β10 interact with specific regions in the motor domain core, principally through main chain interactions (Figs 2*a*, 3*a*). The coiled coil points away from the motor domains with which it has no direct interactions. The two motor domains are separated by an angle of ~120°.

The *Drosophila* ncd dimer is more compact than kinesin and displays two-fold[21] or near two-fold[22] symmetry with respect to the axis of the coiled coil (Fig. 2*b*). The coiled coil is connected to the core by the short loop L0 (Arg 346–Asn 348) between α7 and β1. The coiled coil region, Lys 325–Arg 346, lies snugly between the motor domains. Many residues in this region interact with the motor domain core through L6, L10, L13 and helix α1 (Figs 2*b*, 3*b*). The coiled coil itself is stabilized by both hydrophobic and ion pair interactions from the side chains.

The regions linking the motor domain core to the free standing part of the coiled coil are completely different in rat kinesin (Lys 325–Glu 340) and ncd (Lys 325–Asn 348) (Figs 2, 3). The sequences covering these linker regions are given in Fig 4*a* and the core residues interacting with the linkers are shown in Fig 4*b*. Interestingly, although the same residues are not involved, three of these core regions, on or close to loops L4a, L10, and L13, are common to kinesin and ncd. The listed sequences suggest that the rat kinesin and *Drosophila* ncd structures can be considered as prototype dimer structures for N1 and C1 class kinesins. For example, the C-terminal end of α6 through to the end of β10 is highly conserved in N1 class kinesins. In the β9–β10 linker region, many residues, including six interacting with the core, are conserved and five of the six core residues involved in main chain interactions with the linker region are conserved throughout the N1 subfamily.

For the C class kinesins the nine residues at the beginning of the first β-strand β1 are conserved within the C1 class and five are strictly conserved throughout all C class kinesins. Seven residues in the proximal stretch (335–345) of the α7 helices are conserved (five strictly conserved in the C1 class) and four of these (Asn 340, Asp 344, His 339 and Arg 335) are involved in side chain interactions with core residues. The loop L0 connecting α7 and β1 is strongly conserved within the C1 class (Lys 346-Gly 347-Asn348; ncd and Kar3 have the conservative substitution of Arg for Lys) and is conserved throughout the C class subfamily. The core regions interacting with α7 have many strongly conserved residues.

A structural rationale for motor directionality

The distinctive conformations of conventional kinesin and ncd dimers are determined by the three-dimensional structures and topographies of the regions linking the core to the free standing part of the coiled coil (Figs 2, 3). This suggests a structural basis for the motor directionality of the dimeric chimeras described earlier (Fig. 1). Since the sequences and structures of kinesin and ncd motor domain cores are almost identical, replacing the kinesin core (β1–α6) with that from ncd, should allow 'kinesin-like' interactions between the β9-β10 kinesin linker region (now connected to the ncd α6) L4a, L10–β7 and L13 in the ncd core. As a result, the ncd-kinesin chimera will have the three-dimensional

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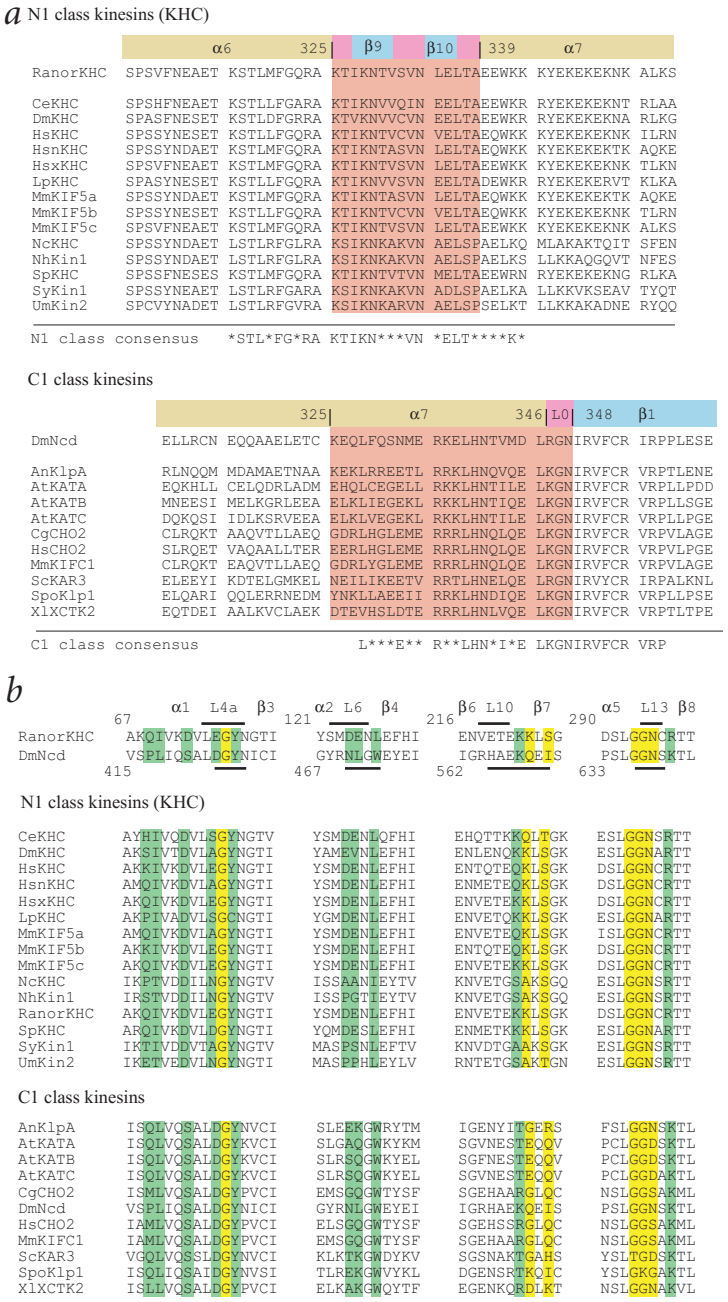

Fig. 4 Sequences of linker and interacting core regions in N and C class kinesins. ***a***, The linker region connecting the motor domain cores to the free standing coiled coils is highly conserved for N1 class kinesins and the sequence of L0 and the proximal region of α7 (Leu 338–Asn 348) is also highly conserved for all C1 class kinesins. The secondary structural elements are indicated in the bar above the sequences: α-helices are brown, β-strands blue and loops pink. The linker region sequences are highlighted in orange and the numbers refer to the rat kinesin and *Drosophila* ncd sequences, respectively. ***b***, Core regions interacting with the linker. The sequences for the rat kinesin heavy chain and for ncd are shown above the aligned sequences for N1 and C1 class kinesins. Loop positions are underscored. Core residues interacting with the linker are overlaid in yellow for rat kinesin and in green for *Drosophila* ncd. The sequences are taken from several databases including the Kinesin and KIF home pages[2,3].

conformation and directional movement of kinesin. Likewise, a construct with the kinesin core replacing that of ncd should be able to adopt an ncd-like structure and have the minus end directionality of wild type ncd. A least squares fit of the kinesin and ncd motor domain cores shows that the N-terminal extremities of β1 are about 1 Å apart and that the C-terminal ends of the more flexible α6 are within 4 Å of each other. Although this computer 'experiment' supports the idea that hybrid dimers can look and behave like wild type ncd and kinesin, X-ray crystallography and motility assays on the hybrids and selected mutants are, of course, required for further verification. As discussed in the following section part of the work has been carried out recently.

The importance of the Loop L0 for C class kinesins11 is corroborated by the crystal structures in which L0 appears to position α7, the coiled coil α-helix, to allow side chain interactions between α7 and the core regions including α1–L4a, L6, L10 and β8–L13, thereby stabilizing a structure in which the proximal region of the coiled coil nests between the two motor domains. This is also supported by observations that, in C class kinesins, the residues immediately upstream of the β1 strand are important for minus end directed movement[25,26].

Links to motility

Although the structural features described above can explain the motility behavior of the chimeras relative to the wild type ncd and conventional kinesin, (Fig. 1), the detailed molecular mechanisms driving the movement of these motors along microtubules are still largely unknown. It was established some time ago that the kinesin dimer moves along the microtubule towards the plus end[27] and kinesin is now known to take an 80 Å step from one tubulin dimer to another for each ATP molecule hydrolyzed[28,29]. Conventional kinesin is processive — that is, an individual dimer can take several hundred steps along a microtubule before releasing — and moves at a velocity of ~1 μm sec^{-1}. Ncd moves more slowly towards the microtubule minus end and is probably not processive[13,30,31]. In the presence of microtubules, the ATPase activity of kinesin and ncd is strongly stimulated. For kinesin, ADP release is the rate limiting factor for ATP hydrolysis[32], showing that the interaction with microtubules catalyzes ADP dissociation and increases ATP turnover.

It has been thought for some time that the proximal region of the coiled coil must unwind at some stage to allow movement along microtubules[19,33,34], but experiments using hybrid kinesin with a very stable coiled coil argue against such models[35]. Recent work on a human kinesin dimer further demonstrates the importance of the β9-β10 linker and its interactions with specific loops in the core in N class kinesin motility[13]. When 10 highly conserved linker residues were replaced by a sequence designed to form a random coil, the velocity of movement along microtubules was reduced 500 fold compared to the wild type dimer. This was accompanied by a mere three-fold reduction in the rate of ATP hydrolysis. Replacing the two glycines in loop L13 by alanines reduced the velocity by a factor of 100 with respect to the wild type with only a marginal effect on ATPase activity.

Conformational changes in the linker when ncd binds to microtubules were first revealed using electron paramagnetic resonance (EPR)[36]. Subsequent studies using a broad range of approaches including ATPase kinetics, EPR, fluorescence resonance energy transfer and cryo-electron microscopy have recently indicated that the linker docks onto the core when the human

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kinesin heavy chain monomer is bound to microtubules in the presence of ATP[12], whereas the linker appears mobile after ATP hydrolysis and γ-phosphate release. If these results also hold for the dimers, they imply that through its interaction with specific loop regions in the core the linker can sense conformational changes at the ATP site situated on the other side of the core. Significantly, loops L4a, L10 and L13 (Fig 3*a*) all connect to β-strands (β3, β6, β7 and β8, respectively), the other ends of which either reach into or are close to the nucleotide binding pocket. Thus, β3 precedes the P-loop (L4); β7 is connected to N-3 (equivalent to switch II in GTP binding proteins); β6 is connected to N-2 (switch I) and β8 is connected to L14, which is close to the nucleotide binding pocket. In addition, L13 connects to α5 which itself connects to L12, which, according to current indications, is likely to be involved in microtubule binding as observed in a recent 15 Å resolution structure of microtubules in complex with the monomeric kinesin KIF1A[37].

Unlike ncd, the linker in kinesin does not involve any stabilizing interactions between the core and the α7 helices in the coiled coil. Consequently, when kinesin interacts with microtubules, the unattached head could be quite flexible and sensitive to thermal agitation. This may explain why the unattached heads of kinesin dimers are less visible than those of ncd in three-dimensional reconstructions of microtubule–motor complexes obtained by cryo-electron microscopy. Such flexibility is possibly an inherent feature of plus end directed movement. This view is supported by the slow plus end directed movements found for an ncd mutant[21] that has random hydrophilic residues, presumably disordered, replacing the region of Arg 335–Arg 346; for the kinesin–ncd chimera[11] with a truncated loop L0; and for a kinesin monomer[13] attached to a random coil linker.

Conclusions

The kinesin motor proteins have given us quite a few surprises so far and undoubtedly have a few more in reserve. Although ncd and kinesin monomers have practically identical structures, their dimers have remarkably different overall conformations. This is due to the different structures of the short linker regions connecting the motor domain cores to the free standing coiled coils. Despite this, the ncd and kinesin linker regions interact with three equivalent loop regions in the cores. Together with the dimer structures, work on hybrid and mutant motors has led to remarkable progress over the past three years. For conventional kinesin, the linker regions immediately adjacent to the motor domains have been shown to determine the direction and speed of movement along microtubules. In addition, the recent demonstration of the nucleotide dependent mobility of the linker in a kinesin monomer implies that this region is intimately involved in the molecular mechanisms of kinesin motility. It appears that the key role of the linker regions depends on their structure and on specific interactions with the core that make them sensitive to subtle changes at the distant nucleotide binding pocket. Basd on our current understanding, we now look forward with enthusiasm to continuing progress that will finally unveil the secrets of directionality, movement and processivity for the wide range of structural organizations adopted by members of the kinesin superfamily.

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