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Motoring down the highways of the cell

Ian P. Trayer and K. John Smith

All eukaryotic cells contain large numbers of motor proteins (kinesins, dyneins and myosins), each of which appears to carry out a specialized force-generating function within the cell. They are known to have roles in muscle contraction, ciliary movement, organelle and vesicle transport, mitosis and cytokinesis. These motor proteins operate on different cytoskeletal filaments; myosins move along actin filaments, and kinesins and dyneins along microtubules. Recently published crystal structures of the motor domains of two members of the kinesin superfamily reveal that they share the same overall fold that is also found at the core of the larger myosin motor. This suggests that they may share a common mechanism as well as a common ancestry.

The old adage that the structure of a protein is the last thing to change as the protein evolves has thrown up some fascinating surprises in recent years. Among the latest is the finding that the motor domains of two members of the kinesin superfamily of motor proteins, human kinesin and *Drosophila* ncd[1,2], share structural features in common with the core of the myosin motor domain[3]. That the motor domains of the two members of the kinesin superfamily have virtually identical topologies is not surprising since they have homologous sequences, despite the motor domain appearing at opposite ends of the molecule (N-terminal in kinesin, C-terminal in ncd). Even with hindsight, however, no significant sequence alignments can be made between these microtubule-based motors and the actin-based myosins. All myosins have large regions of sequence homology in their catalytic domains[4] and so can be confidently predicted to have the same structural scaffold. Thus, although these different classes of motors use different cellular 'railway tracks' and have different kinetic properties and, in the case of kinesin and ncd, move in opposite directions along microtubules, they all appear to have evolved from a common ancestor. This suggests that they share a similar force-generating mechanism that, as we discuss, may have some strategic features in common with molecular switches operated by the signalling GTPases.

Motor protein structure

The motor domains of kinesin and ncd are much smaller than that of myosin – about 350 amino acids compared with about 850 amino acids for myosin. In addition, muscle myosins have two light chains 'clamped' around the long C-terminal helix (that have been omitted in Fig. 1.) In other myosins, this region can be shorter or considerably longer, with calmodulin molecules substituting for light chains[4]. The equivalent region is not seen in the kinesin and ncd structures because of the constructs used for crystallization, but a similar helix, probably forming a coiled-coil structure with another motor, is predicted to exist in the parent proteins[2,5] and is likely to play an important role in the motor processivity. Virtually all of the elements of secondary structure in kinesin and ncd can be overlaid on each other and on the central core of the myosin molecule. Furthermore, the order in which these elements occur in the primary structure of the three proteins is largely preserved[1]. This common fold, comprising seven central (mostly parallel) β-strands and three flanking helices on each side, is highlighted in Figure 1. It forms the majority of the active site; in myosin, this site is less exposed than it is in the kinesins, being covered by two loops unique to myosin. Myosin is a larger molecule by virtue of having substantially longer N- and C-terminal regions either side of the core and, especially, two long insertions that extend from the core. These insertions form a prominent cleft that separates the two actin-binding regions. (This cleft is not visible in the orientation used in Fig. 1 but can be seen in Fig. 3.) It is suggested that the equivalent but much smaller insertions in kinesin and ncd (defined in the legend to Fig. 3) may

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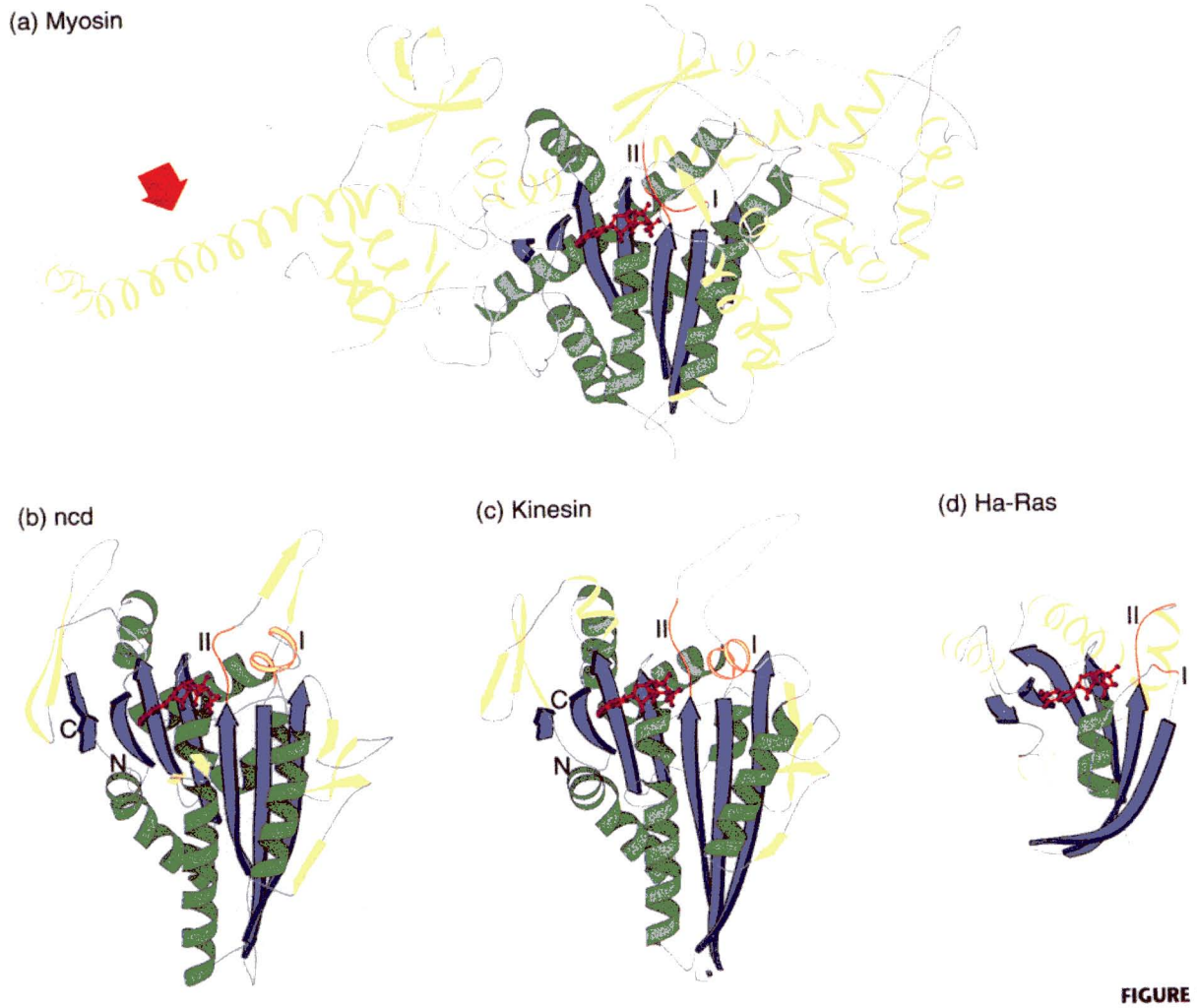

FIGURE 1

Comparison of the common core fold of myosin (a) with that of ncd (b), kinesin (c) and Ha-Ras (d). The features of similar topology are in bolder colours, green for α-helix and blue for β-strands. The rest of the molecular folds are in yellow for secondary structural elements and grey for loops. The four structures were aligned by matching the eight α-carbon atoms of the P-loops (myosin, residues 179–186; ncd, residues 434–441; kinesin, residues 85–92; Ha-Ras, residues 10–17) as described in Kull *et al.*[1] and Sablin *et al.*[2]. The light chains have been removed from the chicken skeletal myosin structure for comparative purposes, and residues 1–852 of the heavy chain are shown (with some mobile regions missing[3]). The arrow in (a) indicates the direction of movement of the myosin 'lever' during contraction. The overall dimensions of the catalytic domain of myosin (excluding the α-helical tail) are 4.5 × 6.0 × 9.0 nm. Constructs containing residues 335–700 of *Drosophila* ncd (representing the C-terminal motor domain of the native protein) and residues 1–349 of human kinesin (representing the N-terminal motor domain of the native protein) were expressed and the proteins crystallized, but only residues 345–667 (ncd) and residues 4–323 (kinesin) are located in the structures shown. These two arrowhead-shaped motor domains have the same dimensions (4.5 × 4.5 × 7.5 nm). Residues 1–166 are shown for Ha-Ras. ATP (red) is shown at the catalytic site of myosin, ncd and kinesin. Its position is based on the coordinates for ADP in the kinesin/ncd structures, and we have added a γ-phosphate. When superimposed on the myosin structure, the β-phosphorus overlies the sulphate ion found in the active site of the chicken myosin head crystal structure[3]. The position of the GTP in Ha-Ras was taken from the crystal coordinates and superimposes exactly on the ATP molecules in the other proteins. As viewed from this angle, the nucleotides lie base-to-γ-phosphate in the left-to-right direction. The six left-hand strands of β-sheet in the motor proteins (five parallel and one antiparallel on the right) have the same orientation and disposition with respect to the nucleotide as the entire β-sheet of the G domain of Ha-Ras. The nucleotide-contacting regions in all four proteins are in the same relative orientation to one another (see Table 1). We have only highlighted the γ-phosphate-binding loops/helices (red) here – the so-called switch I and switch II regions. The N- and C-termini are indicated in the kinesin and ncd structures and lie within I nm of each other, consistent with the fact that the motor domains are located at opposite ends of their native structures. (We thank R. J. Fletterick and R. D. Vale for providing the coordinates of ncd and kinesin, and I. Rayment for providing the coordinates for myosin. The coordinates for Ha-Ras p21 were from 121P. PDB.)

also be responsible for microtubule binding[5], further emphasizing their common evolutionary origins.

The active site

Despite the lack of overall sequence alignment between the two families of motor proteins, their ATP-binding pockets reveal some remarkable similarities of spatially conserved residues making contact with the nucleotide[1,2,6]. This is especially so around the α and β phosphates (the so-called P-loop), and there are also equivalent residues positioned in ncd and kinesin that could make contact

with the γ-phosphate, as occurs in myosin[6] (Table 1). (The crystal structures for kinesin and ncd were solved with ADP in the active site.) It is this enveloping of ATP that draws most parallels with the signalling GTPases[2,5,7] (Fig. 1 and Table 1). These proteins exhibit distinct conformations in their GTP-bound (active) forms and GDP-bound (inactive) forms, and, with nucleotide hydrolysis occurring slowly, the transition between the two nucleotide-bound forms is well characterized for a number of proteins (e.g. see Refs 8 and 9). The ability of the GTPases and the motor proteins to 'sense' the presence or absence of the nucleotide γ-phosphate and adjust their structures accordingly must be a key feature for all of these enzymes. The similarities in amino acid type and topology in all these proteins that do (or could) coordinate the triphosphate moiety and the Mg^{2+} are compelling. In the GTPases, two mobile loops at the rear of the nucleotide-binding cleft (referred to as the switch I and switch II regions) are in close contact with the γ-phosphate in the GTP-bound form but swing out when the hydrolysed γ-phosphate is released. In both myosin and the kinesins, the same switch regions are present and contain similar amino acid side chains in similar orientations to the nucleotide (Fig. 1 and Table 1). The crystal structures of myosin complexed with either ADP-AlF_4 or ADP-VO_4 (analogues of the myosin ADP-Pi state[6]) suggest that the switch regions do envelope the γ-phosphate in the same way. This leads to the conclusion that the mechanism of nucleotide hydrolysis is probably very similar for motors and G proteins and that this is most probably coupled to the same conformational changes in all these proteins. It is the propagation of these changes to the protein-binding faces of these molecules (probably via the switch II region) that must distinguish their different functions. Motors are enzymes that store the potential energy of nucleotide hydrolysis so that the subsequent shape changes can be amplified and harnessed to allow the enzyme to step along filament tracks. Even so, the distinction between the GTPases and the cytoskeletal motor proteins has now become blurred by the recent observation that bacterial elongation factor G can translate the chemical energy of GTP hydrolysis into directional movement on the ribosome, making it the first paid-up member of the GTPase (as opposed to ATPase) motor protein family[10].

TABLE 1 – CONSERVED SEQUENCE MOTIFS AROUND NUCLEOSIDE TRIPHOSPHATES IN MYOSIN, KINESIN, NCD AND Ha-RAS[a]

	α,β-PO_4 (P-loop)	γ-PO_4 (Switch I)	γ-PO_4 (Switch II)
Myosin	**GES**GA**GKT** (179–186)	**NNNSSR** (240–245)	**DIYGFE** (463–468)
Kinesin	**GQ**TSS**GKT** (85–92)	**NEHSSR** (198–203)	**DLAGSE** (231–236)
ncd	**GQ**TGS**GKT** (434–441)	**NERSSR** (547–552)	**DLAGSE** (580–585)
Ha-Ras	**GAGGVGKS** (10–17)	**T** (35)	**DTAGQE** (57–62)

[a]The numbers in brackets refer to the sequence locations of these motifs in the heavy chains of chicken skeletal myosin, human kinesin, *Drosophila* ncd and in human Ras p21. The amino acids in bold are universally conserved in the myosin, kinesin and G-protein superfamilies (see Refs 2, 5 and 7). In addition to the three conserved triphosphate-binding motifs, all families have a purine-binding motif, which is less conserved between families in terms of sequence but occurs spatially in the same relative position in all families.

Mechanisms of action

The most widely held view of the basis for myosin motility (Fig. 2a) is that the motor docks onto actin at a fixed orientation to the filament and that the long helix distal to the filament undergoes a swinging motion in the direction of movement in response to ATP hydrolysis[11,12] (reviewed recently in Ref. 13). Evidence for this has come from direct observation in the electron microscope of nucleotide-free and ADP-bound forms of brush-border myosin I (Ref. 14) and smooth muscle myosin[15]. The length of this 'lever' varies among different members of the myosin superfamily[4], and this suggests that the (potential)

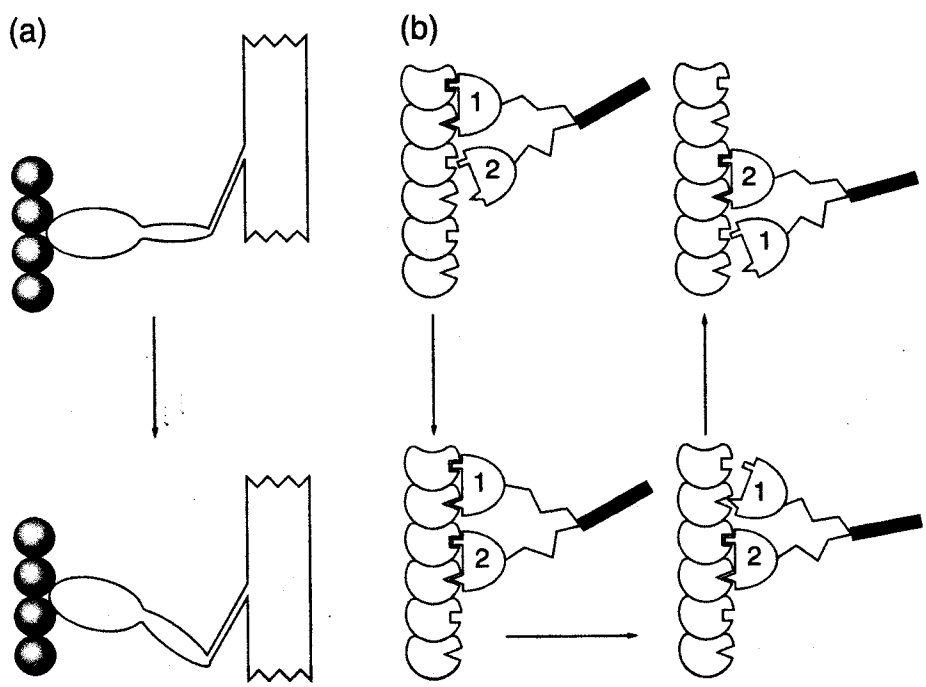

FIGURE 2

Schematic diagrams showing the likely mechanisms of motion adopted by myosins (a) and kinesins (b). In (a), the motor portion of the myosin remains at a fixed orientation to the actin filament (grey spheres) and only the distal part of the myosin crossbridge moves, acting like a swinging lever arm. For processive movement, the myosin head must detach from actin and reattach further down the actin filament. A single head of a muscle myosin is shown in this example, attached to the thick filament (equivalent to the cargo in intracellular transport). In (b), the step-by-step motion of the kinesin motor along a microtubule is shown – the 8-nm step size reflecting the periodicity of the α–β tubulin dimer repeat. Note that, in this model, the kinesin motor does not detach from the microtubule during processive movement.

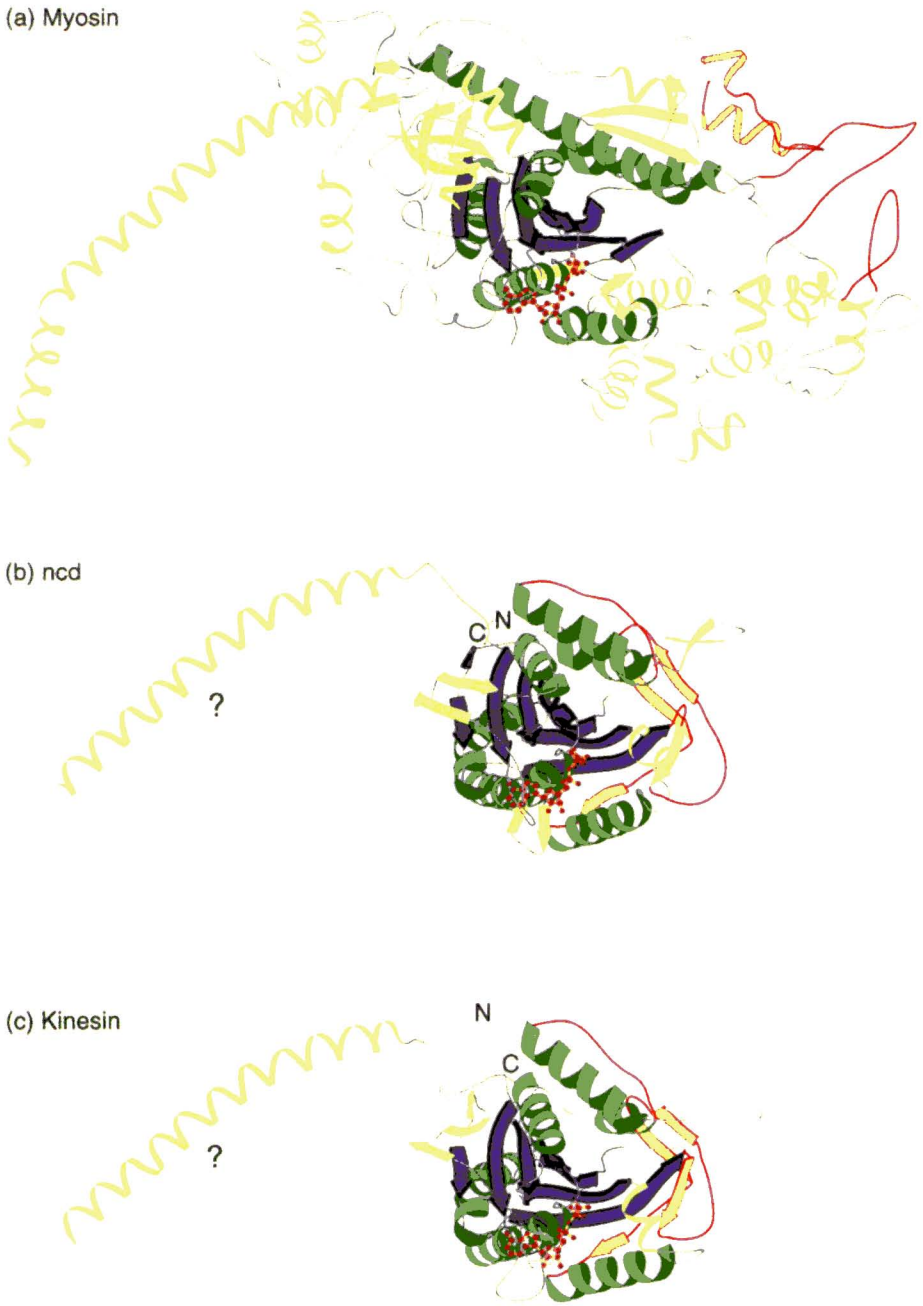

FIGURE 3

View of the myosin (a), ncd (b) and kinesin (c) structures to show potential polymer contact sites. The highlighted common core structures and ATP locations are described in the legend to Fig. 1. The actin contact sites on myosin are shown in red (loops) or edged in red (secondary structure) and are based on actin–myosin docking models[11,12]. A loop containing residues 627–646 has been fitted into the myosin structure and appears as a red 'tongue' on the right-hand side of the molecule. This region is not seen in the crystal structure and is the loop region, joining the 50-kDa and 20-kDa proteolytic segments of myosin, that is protected from tryptic digestion in the presence of actin. This region is contained within the same insertion sequence (see text) as the helix–loop–helix motif (residues 532–558) that is proposed to contact an actin helix, residues 338–349, in the docking model. The only other region close to the actin interface is the loop 405–414 (lower 'jaw' in model shown), which is within the other insertion sequence. It is in this loop that the most common mutation in familial hypertrophic cardiomyopathy occurs (Arg405). In kinesin and ncd, the inserted regions in the core structure are much smaller (in kinesin, residues 137–175 and 271–279, and, in ncd, residues 484–524 and 616–624) and are entirely coloured red – as for the myosin actin-binding sites. (The equivalent insertion regions in myosin are residues 270–450 and 506–649, based on the numbering system in chicken myosin.) It can be seen that, if these regions do contain the microtubule-binding sites[5], their juxtaposition relative to the myosin actin-binding sites, the ATP site and their N- and C-termini is preserved. To complete the analogy, we have added a (hypothetical) α-helical region to the N-terminus of ncd and to the C-terminus of kinesin. Their tentative nature is emphasized by the question marks. Graphics were produced using MOLSCRIPT (Ref. 24).

length of the power stroke can vary accordingly. In an effort to prove this, Spudich and coworkers[16] engineered variants of *Dictyostelium* myosin with different lengths of lever arm and found that the sliding velocity of actin in *in vitro* motility assays increased with increasing length of lever. Even more amazingly, it has been found that this natural α-helical lever can be replaced by an 'artificial' rigid structure (segments of the α-actinin repeat) and still function[17]. This has focused attention on the region of the molecule near the base of the lever responsible for converting the structural changes in the active site into this mechanical movement (becoming known in the myosin field as the 'converter' region). Several conserved structural features that are known to undergo changes in structure during ATP hydrolysis[4,6] converge at the converter, and mutations that interfere with motor function are frequently found in this region[18], giving credence to the converter model. Interestingly, in kinesin and ncd, there are structurally equivalent features coupled to the active site that could also act as a converter, amplifying the changes induced by nucleotide hydrolysis. But, if they do, where is the lever? The truncated kinesins used for crystallization have had this region removed, and the N-terminal (in ncd) and C-terminal (in kinesin) 20 or so residues are mobile and not seen in the crystal structures. Adjacent to these in the two-headed parent molecule are sequences that almost certainly form coiled-coils with the other head (which may extend further towards the motor domain in the native molecule). In Figure 3, an orientation of all three molecules is shown that emphasizes the possible similarities in the juxtaposition of their polymer-binding sites (in the inserted regions), their ATP sites and their putative neck regions. The analogy is striking.

While there is potential for a common mechanism, there must be (subtle) differences between the actin-based and microtubule-based motors. Kinesins can only move along the microtubules for long distances without detaching as double-headed molecules[19] (Fig. 2b), advancing in increments of 8 nm (Ref. 20). This is a huge step size for such a small motor (7.5 nm in its longest dimension in Fig. 1) and suggests that the neck region must be involved in coordinating the step-by-step action[21] of the two motor domains in the native molecule. In other words, the interaction of one head with the microtubule determines the direction in which the other head will

search for the next binding site 8 nm away – perhaps more akin to a directed diffusion rather than a mechanical drive. How these neck regions contribute to generating opposite polarities of movement in ncd and kinesin remains a mystery. It may be that the neck region interacts transiently with the motor domain during the mechanochemical cycle, imposing a directional impetus.

Biological implications

The lever arm in myosins is found attached to each motor domain, whether in two-headed or single-headed myosin molecules. It should be noted that, of the 13 subfamilies of myosins so far described[4], about half are single headed and half double headed. The equivalent region in the kinesin family is shared by two motor domains, and, as noted above, kinesins require two heads to move processively along the microtubule (Fig. 2). The kinesins, therefore, are designed to be able to carry their cargo individually or with relatively few molecular motors involved. Myosins, on the other hand, appear to have short duty cycles (i.e. they spend a small amount of the ATPase cycle attached to actin); this is true of even the single-headed myosin I (Ref. 22). There is also no evidence of cooperativity between heads in the double-headed myosin II molecules. If these kinetic properties are found in all myosins, then this imposes limitations in the way that they can operate. Single motors (and their cargo) would diffuse away from the actin filaments, making it imperative that myosins 'hunt in packs' in the cell to maintain multiple, but independent, contact with the filament along which they are moving (much in the way of the thick filament in muscle). The consequence of this in the cell may be that kinesins are called upon to carry out the long-distance haulage, whereas myosins specialize in more local deliveries.

Future perspectives

That the basic machinery allowing conversion of energy stored in ATP into directed movement of a motor along a filament is conserved in the kinesin and myosin superfamilies, and has similarities to the molecular-switching mechanism in GTPases, has come as something of a surprise. We await with great interest to see whether the same design features occur in the dynein motors that move on microtubules or in helicases that track along nucleic acids. Several years ago, before the discovery of ncd, the idea that kinesins moved material along microtubules to the cell periphery and dyneins made the return journeys was appealing but made it seem unlikely that their respective motor domains would have any structural similarity. The present knowledge that different members of the kinesin superfamily can move in opposite directions along their tracks, with virtually identical motors, removes this inhibition. It has also been found that cytoplasmic dynein works in conjunction with an actin-related protein (ARP1, homologous to actin) to drive microtubule-based motility, although it does not appear to interact with this actin homologue directly[23]. It would not be too surprising, therefore, if tubulin were found to share an actin-like fold, so that both the filamentous tracks and their motors evolved hand-in-hand from some primitive single system. We await with great interest to see whether these cellular transport systems can be adapted for practical applications as we enter the 'nanotechnology' revolution.

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Addendum

The figures in this article are available through the authors' web page: `http://www.bham.ac.uk/biochemistry/trayer/`