Cell. Mol. Life Sci. 65 (2008) 509 – 515
1420-682X/08/040509-7
DOI 10.1007/s00018-008-7518-3

Cellular and Molecular Life Sciences

Visions & Reflections (Minireview)

Molecular motors: not quite like clockwork

L. A. Amos

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 0QH (United Kingdom),
e-mail: laa@mrc-lmb.cam.ac.uk

Received 9 November 2007; received after revision 7 December 2007; accepted 11 December 2007
Online First 19 January 2008

Abstract. Models commonly used to explain the mechanism of myosin motors typically include a power stroke that is attributed to a conformational change in the motor domain and amplified by a long lever arm that connects the motor domain to the cargo. Similar models have proved less enlightening in the case of microtubule motors, for which it may be more helpful to consider models involving thermally driven mechanisms.

Keywords. Molecular motors, kinesin, dynein, myosin, microtubules.

Introduction

Cytoplasmic molecular motors are enzymes driven by cycles of nucleotide triphophosphate hydrolysis to move cargo along filamentous polymeric tracks such as microtubules (MTs) or F-actin. Researchers use a range of different models to explain the mechanisms in everyday terms. Motor protein molecules mostly consist of a globular motor domain that possesses the enzymatic activity and binds cyclically to the track and another domain that connects to the cargo. Often the molecules dimerise by means of a coiled-coil region, and the simplest mechanistic analogy used is to a biped walking over a series of stepping-stones that correspond to the equally spaced binding sites on an MT or actin filament. This basic model illustrates, for example, that alternate steps must differ to avoid a rotation of the cargo. The stepping analogy can also be usefully extended to larger ensembles of motor molecules by replacing the biped with a millipede. However, the walking analogy is unsatisfactory because at a molecular level there is no force equivalent to gravity to keep the motor on the track. This problem can be avoided by progressing to a hand-over-hand model, where the track becomes analogous to a rope.

The hand analogy is apt since molecular motors do appear to move along their tracks by alternating between strongly binding and weakly binding conformations. But it is not, of course, intended to provide an explanation of the actual motor mechanism. For this purpose, it is possible to imagine the hand replaced by a clockwork motor, built using springs, catches and levers. A simple mechanical model has greatly aided our understanding of the myosin motor; a conformational change in the motor domain is proposed to produce a small power stroke that is amplified into a much larger movement by a long lever arm between the motor domain and the cargo-binding domain. The prediction that the net speed of a motor will be in proportion to the length of its lever arm has been experimentally confirmed and fits observations even at a single-molecule level [1]. Moreover, the step in the cycle of ATP hydrolysis that would drive the power stroke has been identified. For myosin, it corresponds to the process of losing the hydrolysis products, phosphate (Pi) and then ADP, whilst the motor is attached to the track and is proposed to actively swing the lever arm. The subsequent binding of another ATP molecule in the enzymatic site leads to weak binding, detachment and a return to the initial

conformation. A number of different crystal structures of myosin motor domains have supplied details of possible conformational changes at different stages in the cycle [2, 3], so the myosin power-stroke model has remained fairly robust despite some notable objections [4]. However, in this review, I will consider the conceptual difficulties that have been encountered when similar models are applied to the MT motors kinesin, ncd and dynein and suggest that these problems arise when the analogies with macroscopic machines, such as mechanical clocks, are followed too literally.

Thermal models

A model in which movement of a molecular motor is driven by thermal energy was first suggested by A. F. Huxley [5], and such models continue to be developed [6]; the same approach has successfully been extended to MT motors [7, 8]. A thermal ratchet model is further from everyday experience than clockwork-like levers and springs but can nevertheless be appreciated without mathematics. A small motor domain can be brought into contact with a new binding site along the track by a series of random 'Brownian' movements resulting from bombardment by fast-moving molecules in the solution. Instead of directly producing power, the binding and splitting of ATP control the affinity between the motor and the track, while conformational changes between the motor and its tail bias the direction of movement. A longer tail allows a wider search for a new binding site, and a stiffer tail also biases the search towards longer distances. Thus, a long extension that is assumed to act as a strong lever in a power-stroke model acts more like a fishing rod in a thermal model. A coiled-coil or a single helix supported by other proteins, such as calmodulin bound to a myosin tail, might be a good compromise, being extended but flexible enough to allow the motor domain to search around.

If the main movement of a motor domain and its cargo occurs during the part of each cycle when it is detached from the track, progress depends on cooperation between motor domains. Dimerisation may provide the means of cooperation, but two or more motor domains connected independently to the same cargo can move it by taking turns in binding to the track. Finding a new place on the track during the weakly binding phase and exerting force during the tightly binding phase can be regarded as separate processes for each individual motor domain. This is an important difference from the power-stroke model, in which the movement of the cargo is actually driven by the conformational change in the motor domain. In a thermally driven motor, no individual conformational change is required to account for all the work done. For example, the 4-nm movement detected for an individual myosin molecule [9] may be regarded as a manifestation of the structural rearrangement that occurs within the motor domain to change its affinity to the track, rather than the stroke that carries out all of the work done; it may then be easier to understand how steps much longer than 4 nm can be taken.

At a submolecular level, there is only sparse information about how signals are transmitted from one region of a motor domain to another to control its conformation in accordance with the nucleotide cycle. Thus, internal changes still tend to be modelled in terms of springs, levers and plungers [7], but this, too, will change as more detailed protein features are considered [10]. Molecular biologists may eventually model molecular motors in terms of the quantum states of individual atoms and electrons.

MT motors

The motor proteins that move along MTs are more diverse in structure than myosins but basically similar. The three main functional regions of the motor domain that exchange information are the track-binding interface, the nucleotide-binding site and the region that connects the motor domain to the cargo-binding domain (Fig. 1). Like myosin, the motor domains move along their track in response to the cycle of enzymatic activity (ATP binding, hydrolysis and product release) by alternating between strong binding states and weak binding states, with the latter leading to detachment. Strong and weak binding occur at different stages in the ATPase cycles of different motors, as outlined below.

Kinesin-1

Conventional kinesin, now known as kinesin-1, is a dimer that can move towards the plus ends of MTs, without detaching, using a processive hand-over-hand mechanism [11–15]. The dimer follows a single protofilament in 8-nm steps corresponding to the periodicity of the tubulin heterodimers. Its remarkably continuous attachment to the track appears to be helped by secondary interactions with the acidic C-termini of the tubulin subunits [16, 17], which extend out from the MT surface and hold on to the kinesin molecule even if an accident causes both motor domains to become detached. Each motor domain binds weakly when the nucleotide pocket is occupied by ADP. But the interaction with tubulin opens up the

pocket, releasing ADP, and the motor binds tightly. The interaction remains strong whilst ATP binds in the pocket and is hydrolysed. Kinesin fails to conform to a myosin-like model by lacking a lever arm, having instead a flexible 'neck linker' between the motor domain and the cargo-binding tail: a fishing line without a rod! The neck linker binds to the side of the motor domain in the ATP-bound state [18, 19] but no fixed position has been detected in other states [20, 21] (Fig. 1A). Instead of a power-producing conformational change near the point of attachment of the neck linker, a plus-end-directed force generated by the docking of the neck linker onto its site on the motor domain was proposed. Subsequent experiments suggested that neck-linker docking cannot provide a sufficient free-energy change for an 8-nm movement against the maximum loads that have been measured, though its role in producing directional bias is still proposed to be vital [22–26]. Thus the function of the neck linker is to control movement, but the 8-nm shift of the cargo is seen as being thermally driven. As soon as the neck is docked in position, the second head is in a position to bind to the next site on the MT and prevent a reversal of the movement.

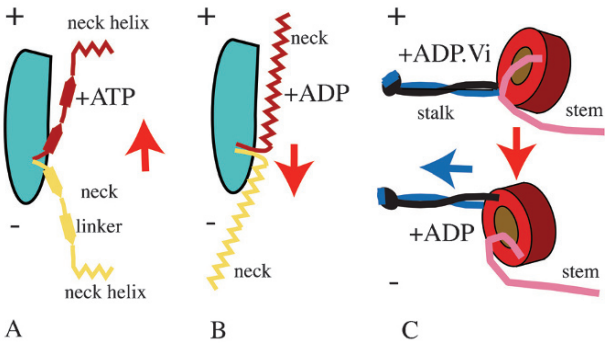

Figure 1. Models of microtubule (MT)-associated molecular motors. (*A*) Alternative positions for the cargo-binding tail of a plus-end-directed kinesin. The core of the motor domain is represented in cyan; in this view the back surface would interact directly with tubulin. When ATP is bound to the core, the neck linker (red) docks on to the surface [18, 19] so that it points towards the MT plus end (+). When ADP is bound, the neck linker (yellow) is free to move [20, 21]. When kinesin binds to its track, a cargo attached to the end of the neck linker should be free to move forward and back until trapped in a forward position by docking of the neck linker (and the subsequent binding of another motor domain). (*B*) Alternative positions for the coiled-coil neck (which connects to the cargo) of a minus-end-directed kinesin [27]. The neck is docked (red) [28, 29] on to the core when ADP is bound but is thought to be undocked (yellow) under other conditions [30]. The neck is angled towards the MT plus end when the motor domain binds to the track, so when the neck is undocked any net movement of the cargo must be towards the minus end. (*C*) Alternative conformations of a dynein monomer (based on images of Burgess et al. [32]). The globular ATPase domain (AAA) is shown as a red ring. The pink C-terminal tail interacts with the AAA ring and then, augmented by various associated polypeptides (not shown), forms the 'stem' or cargo-binding domain. The coiled-coil 'stalk' (black and blue) ends in a small globular domain that interacts with tubulin [33]. When no nucleotide is added to EM samples, the stalk and stem appear distant from each other; but when ADP and vanadate (Vi) are present, the AAA domain apparently rotates relative to the stem, bringing the stalk much closer [32]. Only 2D projected views of the two conformations are known at present, so it is unclear whether there is a big change in the shape of the motor domain or whether the difference is mainly a rotation to give a different view. In the stalk, the two stretches of polypeptide (represented here in black and blue) that associate as an antiparallel coiled-coil are thought to slide relative to one another, by half of a heptad repeat [40], to modulate the conformation of the tubulin-binding domain and its binding affinity, depending on the nucleotide bound to the P1 site of the AAA domain [38, 39].

Ncd (a kinesin-14)

Kinesin-14 motors that move towards the minus ends of MTs have a tail connected directly to the N-terminus of the motor domain. Since the fold of the kinesin-family motor domain brings its two termini quite close together, the tails of minus-end-directed and plus-end-directed kinesins emerge from similar points and interact with similar regions of the motor domain surface [27] (Fig. 1A, B). The tail of an ncd or kar3 dimer is a coiled-coil rod, more like the tail of a myosin than the neck linker of kinesin-1, but appears to rotate about a simple hinge rather than having its base embedded in a myosin-like 'converter region'. In crystal structures, the ncd neck has been observed to dock against the side of the ADP-bound motor domain, which is when the motor domain is detached or only weakly associated with tubulin. 3D electron microscopy (EM) images [28, 29] have suggested that the neck is free to move when the motor domain contains ATP or an ATP analogue (Fig. 2, transition from stage 1 to stage 2). On the basis of a crystal structure in which the nucleotide pocket was partially empty, Yun et al. [30] proposed that the neck may undock at an earlier stage, at the same time as ADP is lost (i.e. it may no longer be as firmly docked at stages 1 and 6 as it is at stage 5 in Fig. 2). In either case, the proposed 'power stroke' would correspond to undocking of the lever arm. An active stroke would require the presence of a spring under tension in the region of the hinge, but the latter appears to consist of a single polypeptide link. Even without detailed calculations, it is hard to see how sufficient force to move a cargo could be generated by a free-energy change due either to undocking of the coiled-coil or to an active rotation at the simple hinge.

Dynein

The largest molecular motors, cytoplasmic and flagellar dyneins, also move towards MT minus ends. Recent work has revealed many interesting features.

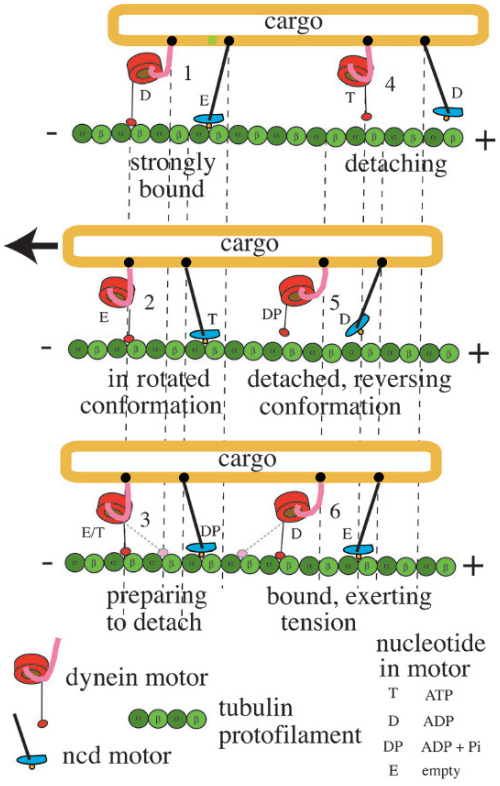

Figure 2. Transport of cargo by cooperation between pairs of motor domains. Schemes for a pair of kinesin family motors and a pair of dynein motors are shown superimposed to allow easy comparison. Six stages in the nucleotide hydrolysis cycles are depicted. A pair of dynein monomers (in red) and a pair of ncd monomers (in cyan) are each shown moving cargo towards the minus end of an MT (represented by just one protofilament – tubulin heterodimers being shown as pairs of 4-nm monomer subunits, in dark and light green). While one motor is strongly bound (steps 1–3), the other detaches (steps 4–5) and readjusts its conformation ready to rebind (step 6). Each bound motor domain changes conformation in response to a specific change in the active site (loss of ADP from dynein, binding of ATP to ncd) in such a way that the thermally agitated cargo is more likely to move to the minus end (see legend to Fig. 1). If another motor domain then binds tightly, the move will be established. The shift of 8 nm for each stalk domain between the top and middle stages is indicated by black dots on the cargo and dashed vertical lines. The conformational change in each motor domain is reversed after detachment (between steps 4 and 5) in response to further steps in the ATPase cycle, so that rebinding is likely to occur closer to the minus end. Note the 16-nm shift of the tubulin-binding domains between steps 4 and 6, though the cargo is shifted only 8 nm [54, 55]. The centre of the head would appear to move an even shorter distance; 3.7 nm has been measured for axonemal dynein [48]. However, if the dynein stalk were to flex towards the minus end while first making an attachment (conformation shown dotted in step 6) instead of pointing at 90° to the MT, the cargo could subsequently be moved by 16 nm; 24-nm shifts also reported during movement under low loads [59] could possibly be produced by simultaneously flexing the bound stalk in the other direction (conformation shown by dotted line in step 3).

An N-terminal tail domain of the heavy chain, along with several associated polypeptides (the 'stem' [31]), interacts directly or indirectly with the cargo, either an A-tubule of a flagellar doublet or a membranous organelle in the case of cytoplasmic dynein. A heavy chain also encompasses a series of 6 or 7 AAA (ATPases Associated with diverse cellular Activities) domains arranged in a ring [32–37], though only four of the AAA domains (P1–P4) bind ATP [38, 39]. The region apparently responsible for direct interaction with the track loops out from the motor domain between P4 and P5, forming a small globular domain at the end of an antiparallel coiled-coil [33, 34], the 'stalk' [31]. Nucleotide-dependent conformational shifts in the coiled-coil have been predicted to change the affinity of the globular domain for tubulin [40]. There has been significant progress in defining the complex enzymatic behaviour of P1–P4 [41–45]. The P1 domain hydrolyses ATP and controls the coordination [38, 39]. ATP hydrolysis also occurs in P3, while nucleotide binding to P2 and P4 may play a regulatory function [38–45]. At low ATP levels, binding to P1 occurs preferentially, then to P3 and finally to P2 and P4 [39, 43–45].

Meanwhile, EM of isolated axonemal dynein c monomers that form part of an axonemal inner arm complex [32] has shown them in two apparently very different conformations (Fig. 1C). The analysis of the images also showed that both the stalk and tail (pink feature in Fig. 1C) are quite flexible. Since only 2D projected views of the nucleotide-free and ADP+Vi-filled structures can currently be compared, it is not clear whether there is a big change in 3D conformation or whether the main difference is due to a rotation. It is assumed, though not yet shown, that other dynein motor domains have similar substructures and change in a similar way. 3D models reconstructed from EM images of whole axonemes [46, 47] or of purified axonemal dyneins bound to pure MTs [48] have provided lower-resolution views of dynein dimers and trimers, and shown their general orientation on the MT.

Figure 2 includes a scheme for the cycle of structural changes involved in dynein stepping movements, in comparison with the cycle for a kinesin family member that moves towards the MT minus end. There is no structural information about the ADP-bound state of dynein, although it is known to bind strongly to MTs [41]. However, it seems likely that the change from this state to the strongly bound empty state (from 1 to 2 in Fig. 2) is essentially the reverse of the change that takes place during ATP hydrolysis (from 4 to 5 in Fig. 2) after the motor has been detached by binding ATP. Dynein is peculiar in having the site that binds directly to tubulin at the end of a long extension. The coiled-coil stalk may provide a flexible search capability in order to compensate for the large size of the whole motor domain, which must slow its diffusion. Flexibility is likely to be especially important for

axonemal dynein arms to find their MT binding sites in the axonemes of cilia and flagella, where the doublet MTs whip around during swimming. Observations of random diffusion by axonemal dynein [49–51] suggests that weakly bound MT-binding domains may be able to slide along protofilaments and thereby maintain contact under stress. It has even been reported that axonemal dynein c can move processively on its own [52]; in this case, there may be a secondary interaction between tubulin and dynein in addition to that occurring at the tip of the stalk. Interestingly, Koonce [33] found that a peptide corresponding to part of the P5 AAA domain bound weakly to microtubules. Possibly the tubulin C-termini reach out to contact the AAA ring and hold it while the tip either detaches and rebinds or slides along the protofilament to the next strong binding site. This would be similar to the mechanism proposed for KIF1A, a kinesin monomer that can move processively by making a secondary contact with tubulin C-termini [53]. Both sliding and the use of secondary 'tethers' to aid processivity are understandable in a thermal model context. In contrast to dynein c, cytoplasmic dynein needs to be dimeric to move processively [54, 55] and is far less processive than kinesin-1. It even fails to follow individual protofilaments faithfully [54, 56]. To achieve an adequate level of processivity in cells, it makes a secondary, indirect contact with the MT via the dynactin complex [57, 58]. This striking difference between axonemal and cytoplasmic dyneins requires further investigation.

The data on dynein are becoming ever more complex as researchers gradually overcome the great experimental difficulties. Most surprisingly, a variable step size, with a dependence on load and ATP concentration, has been seen for cytoplasmic dynein by optical trapping [59]. Under a high load, the dynein dimers moved forwards in 8-nm steps if plenty of ATP was available, but larger steps were reported for small or zero loads. It is not yet clear whether the observed 16- and 24-nm steps were actually 2 or 3 × 8-nm steps in rapid succession, since other groups [60, 61] did not observe any steps longer than 8 nm, or whether under some circumstances the stalk is flexible enough to swing up to 24 nm between specific binding sites on tubulin dimers (Fig. 2). If so, the whole dynein motor domain may become effectively shorter and/or less flexible when all the ATP binding sites are occupied and the load is high.

Summary

Macromolecular machines work in a chaotic environment but still produce highly reliable results, operating in a way that is quite remote from our normal experience. Thus, molecular biologists apply a variety of different models to the molecular motors that transport cargoes along actin filaments or MTs, depending on the level of detail they hope to explain (according to Einstein, a model should be as simple as possible, but no simpler). Just as a hand-over-hand model is very useful in describing the movement of processive dimeric motors, though no-one would think of extending the analogy to the molecular mechanism, the popular lever-arm model provides much valuable insight but has some serious limitations. It clearly fails to explain certain aspects of each of the MT motors. A wealth of recent data is revealing the interesting individuality of each of these molecular machines.

1 Geeves, M. A. and Holmes, K. C. (1999) Structural mechanism of muscle contraction. Ann. Rev. Biochem. 68, 687–728.
2 Coureux, P. D., Sweeney, H. L. and Houdusse, A. (2004) Three myosin V structures delineate essential features of chemo-mechanical transduction. EMBO J. 23, 4527–4537.
3 Yang, Y., Gourinath, S., Kovàcs, M., Nitray, L., Reutzel, R., Himmel, D. M., O'Neall-Hennessey, E., Reshetnikova, L., Szent-Györgyi, A. G., Brown, J. H. and Cohen, C. (2007) Rigor-like structures from muscle myosins reveal key mechanical elements in the transduction pathways of this allosteric motor. Structure 15, 553–564.
4 Yanagida, T. and Iwane, A. H. (2000) A large step for myosin. Proc. Natl. Acad. Sci. USA 97, 9357–9359.
5 Huxley, A. F. (1957) Muscle structure and theories of contraction. Prog. Biophys. Biophys. Chem. 7, 255–318.
6 Becker, E. W. (2000) Kinetic equilibrium of forces and molecular events in muscle contraction. Proc. Natl. Acad. Sci. USA 97, 157–161.
7 Cordova, N. J., Ermentrout, B. and Oster, G.F. (1992) Dynamics of single-motor molecules: The thermal ratchet model. Proc. Natl. Acad. Sci. USA 89, 339–343.
8 Mather, W. H. and Fox, R. F. (2006) Kinesin's biased stepping mechanism: amplification of neck linker zippering. Biophys. J. 91, 2416–2426.
9 Molloy, J. E., Burns, J. E., Kendrick-Jones, J., Tregear, R. T. and White, D. C. (1995) Movement and force produced by a single myosin head. Nature 378, 209–212.
10 Grant, B. J., McCammon, J. A., Caves, L. S. and Cross, R. A. (2007) Multivariate analysis of conserved sequence-structure relationships in kinesins: coupling of the active site and a tubulin-binding sub-domain. J. Mol. Biol. 368, 1231–1248.
11 Asbury, C. L, Fehr, A. N. and Block, S. M. (2003) Kinesin moves by an asymmetric hand-over-hand mechanism. Science 302, 2130–2134.
12 Carter, N. J. and Cross, R. A. (2005) Mechanics of the kinesin step. Nature 435, 308–312.
13 Hackney, D. D. (2002) Highly processive microtubule-stimulated ATP hydrolysis by dimeric kinesin head domains. Nature 377, 448–450.
14 Schief, W. R., Clark, R. H., Crevenna, A. H. and Howard, J. (2004) Inhibition of kinesin motility by ADP and phosphate supports a hand-over-hand mechanism. Proc. Natl. Acad. Sci. USA 101, 1183–1188.
15 Yildiz, A., Tomishige, M., Vale R. D. and Selvin P. R. (2004) Kinesin walks hand-over-hand. Science 303, 676–678.
16 Thorn, K. S., Ubersax, J. A. and Vale, R. D. (2000) Engineering the processive run length of the kinesin motor. J. Cell Biol. 151, 1093–1100.

17 Wang Z, Sheetz M. P. (2000) The C-terminus of tubulin increases cytoplasmic dynein and kinesin processivity. Biophys. J. 78, 1955–1964
18 Rice, S., Lin, A. W., Safer, D., Hart, C. L., Naber, N., Carragher, B. O., Cain, S. M., Pechatnikova, E., Wilson-Kubalek, E. M., Whittaker, M. et al. (1999) A structural change in the kinesin motor protein that drives motility. Nature 402, 778–784.
19 Rosenfeld, S. S., Jefferson, G. M. and King, P. H. (2001) ATP reorients the neck linker of kinesin in two sequential steps. J. Biol. Chem. 276, 40167–40174.
20 Kull, F. J., Sablin, E. P., Lau, R., Fletterick, R. J. and Vale, R. D. (1996) Crystal structure of the kinesin motor domain reveals a structural similarity to myosin. Nature 380, 550–555.
21 Sindelar, C. V., Budny, M. J., Rice, S., Naber, N., Fletterick, R. and Cooke, R. (2002) Two conformations in the human kinesin power stroke defined by X-ray crystallography and EPR spectroscopy. Nat. Struct. Biol. 9, 844–848.
22 Sugata, K., Nakamura, M., Ueki, S., Fajer, P. G. and Arata, T. (2004) ESR reveals the mobility of the neck linker in dimeric kinesin. Biochem. Biophys. Res. Commun. 314, 447–451.
23 Vale, R. D. and Milligan, R. A. (2000) The way things move: looking under the hood of molecular motor proteins. Science 288, 88–95.
24 Tomishige, M., Stuurman, N. and Vale, R. D. (2006) Single-molecule observations of neck linker conformational changes in the kinesin motor protein. Nat. Struct. Mol. Biol. 13, 887–894.
25 Asenjo, A. B., Weinberg, Y. and Sosa, H. (2006) Nucleotide binding and hydrolysis induces a disorder-order transition in the kinesin neck-linker region. Nat. Struct. Mol. Biol. 13, 648–654.
26 Rice, S., Cui, Y., Sindelar, C., Naber, N., Matuska, M., Vale, R. and Cooke, R. (2003) Thermodynamic properties of the kinesin neck-region docking to the catalytic core. Biophys. J. 84, 1844–1854.
27 Sablin, E. P., Case, R. B., Dai, S. C., Hart, C. L., Ruby, A., Vale, R. D. and Fletterick, R. J. (1998) Direction determination in the minus-end-directed kinesin motor ncd. Nature 395, 813–816.
28 Endres, N. F., Yoshioka, C., Milligan, R. A. and Vale, R. D. (2006) A lever-arm rotation drives motility of the minus-end-directed kinesin Ncd. Nature 439, 875–878.
29 Wendt, T. G., Volkmann, N., Skiniotis, G., Goldie, K. N., Muller, J., Mandelkow, E., and Hoenger, A. (2002) Microscopic evidence for a minus-end-directed power stroke in the kinesin motor ncd. EMBO J. 21, 5969–5978.
30 Yun, M., Bronner, C. E., Park, C. G., Cha, S. S., Park, H. W. and Endow, S. A. (2003) Rotation of the stalk/neck and one head in a new crystal structure of the kinesin motor protein, Ncd. EMBO J. 22, 5382–5389.
31 Goodenough, U. W. and Heuser, J. E. (1982) Substructure of the outer dynein arm. J. Cell Biol. 95, 798–815.
32 Burgess, S. A., Walker, M. L., Sakakibara, H., Knight, P. J. and Oiwa, K. (2003) Dynein structure and power stroke. Nature 421, 715–718.
33 Koonce, M. P. (1997) Identification of a microtubule binding domain in a cytoplasmic dynein heavy chain. J. Biol. Chem. 272, 19714–19718.
34 Asai, D. J. and Koonce, M. P. (2001) The dynein heavy chain: structure, mechanics and evolution. Trends Cell Biol. 11, 196–202.
35 Neuwald, A. F., Aravind, L., Spouge, J. L., and Koonin, E. V. (1999) AAA+: a class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes. Genome Res. 9, 27–43
36 Mocz, G. and I. R. Gibbons (2001) Model for the motor component of dynein heavy chain based on homology to the AAA family of oligomeric ATPases. Structure 9, 93–103.
37 Samso, M. and Koonce, M. P. (2004) 25 Å resolution structure of a cytoplasmic dynein motor reveals a seven-member planar ring. J. Mol. Biol. 340, 1059–1072.
38 Gibbons, I. R., Lee-Eiford, A., Mocz, G., Phillipson, C. A., Tang, W.-J. Y. and Gibbons, B. H. (1987) Photosensitized cleavage of dynein heavy chains. Cleavage at the 'V1 site' by irradiation at 365 nm in the presence of ATP and vanadate. J. Biol. Chem. 262, 2780–2786
39 Kon, T., Nishiura, M., Ohkura, R., Toyoshima, Y. Y. and Sutoh, K. (2004) Distinct functions of nucleotide-binding/hydrolysis sites in the four AAA modules of cytoplasmic dynein. Biochemistry 43, 11266–11274.
40 Gibbons, I. R., Garbarino, J. E., Tan, C. E., Reck-Peterson, S. L., Vale, R. D. and Carter, A. P. (2005) The affinity of the dynein microtubule-binding domain is modulated by the conformation of its coiled-coil stalk. J. Biol. Chem. 280, 23960–23965.
41 Imamula, K., Kon, T., Ohkura, R., Sutoh, K. (2007) The coordination of cyclic microtubule association/dissociation and tail swing of cytoplasmic dynein. Proc. Natl. Acad. Sci. USA 104, 16134–16139.
42 Mogami, T., Kon, T., Ito, K. and Sutoh, K. (2007) Kinetic characterization of tail swing steps in the ATPase cycle of Dictyostelium cytoplasmic dynein. J. Biol. Chem. 282, 21639–21644.
43 Reck-Peterson, S. L. and Vale, R. D. (2004) Molecular dissection of the roles of nucleotide binding and hydrolysis in dynein's AAA domains in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA.101, 1491–1495.
44 Takahashi, Y., Edamatsu, M. and Toyoshima, Y. Y. (2004) Multiple ATP-hydrolyzing sites that potentially function in cytoplasmic dynein. Proc. Natl. Acad. Sci. USA 101, 12865–12869.
45 Silvanovich, A., Li, M. G., Serr, M., Mische, S. and Hays, T. S. (2003) The third P-loop domain in cytoplasmic dynein heavy chain is essential for dynein motor function and ATP-sensitive microtubule binding. Mol. Biol. Cell 14, 1355–1365.
46 Nicastro, D., Schwartz, C., Pierson, J., Gaudette, R., Porter, M. E. and McIntosh, J. R. (2006) The molecular architecture of axonemes revealed by cryoelectron tomography. Science 313, 944–948.
47 Ishikawa, T., Sakakibara, H. and Oiwa, K. (2007) The architecture of outer dynein arms in situ. J. Mol. Biol. 368, 1249–1258.
48 Oda, T., Hirokawa, N. and Kikkawa, M. (2007) Three-dimensional structures of the flagellar dynein–microtubule complex by cryoelectron microscopy. J. Cell Biol. 177, 243–252.
49 Vale, R. D., Soll, D. R. and Gibbons, I. R. (1989 One-dimensional diffusion of microtubules bound to flagellar dynein. Cell 59, 915–925.
50 Wang, Z. and Sheetz, M. P. (1999) One-dimensional diffusion on microtubules of particles coated with cytoplasmic dynein and immunoglobulins. Cell Struct. Funct. 24, 373–383.
51 Shima, T., Kon, T., Imamula, K., Ohkura, R. and Sutoh, K. (2006) Two modes of microtubule sliding driven by cytoplasmic dynein. Proc. Natl. Acad. Sci. USA 103, 17736–17740.
52 Sakakibara, H., Kojima, H., Sakai, Y., Katayama, E. and Oiwa, K. (1999) Inner-arm dynein c of Chlamydomonas flagella is a single-headed processive motor, Nature 400, 586–590.
53 Okada, Y., Higuchi H, and Hirokawa, N. (2003) Processivity of the single-headed kinesin KIF1A through biased binding to tubulin. Nature 424, 574–577.
54 Reck-Peterson, S. L., Yildiz, A., Carter, A. P., Gennerich, A., Zhang, N. and Vale, R. D. (2006) Single-molecule analysis of dynein processivity and stepping behavior. Cell 126, 335–348.
55 Shima, T., Imamula, K., Kon, T., Ohkura, R. and Sutoh, K. (2006) Head-head coordination is required for the processive motion of cytoplasmic dynein, an AAA+ molecular motor. J. Struct. Biol. 156, 182–189.
56 Wang, Z., Khan, S. and Sheetz, M. P. (1995) Single cytoplasmic dynein molecule movements: characterization and comparison with kinesin. Biophys. J. 69, 2011–2023.
57 King, S. J. and Schroer, T. A. (2000) Dynactin increases the processivity of the cytoplasmic dynein motor. Nat. Cell Biol. 2, 20–24.

58 Culver-Hanlon, T. L., Lex, S. A., Stephens, A. D., Quintyne, N. J. and King, S. J. (2006) A microtubule-binding domain in dynactin increases dynein processivity by skating along microtubules. Nat. Cell Biol. 8, 264–270.
59 Mallik, R., Carter, B. C., Lex, S. A., King, S. J. and Gross, S. P. (2004) Cytoplasmic dynein functions as a gear in response to load. Nature 427, 649–652.
60 Kural, C., Kim, H., Syed, S., Goshima, G., Gelfand, V. I. and Selvin, P. R. (2005) Kinesin and dynein move a peroxisome in vivo: a tug-of-war or coordinated movement? Science 308, 1469–1472.
61 Toba, S., Watanabe, T. M., Yamaguchi-Okimoto, L., Toyoshima, Y. Y. and Higuchi, H. (2006) Overlapping hand-over-hand mechanism of single molecular motility of cytoplasmic dynein. Proc. Natl. Acad. Sci. USA 103, 5741–5745.