Cell, Vol. 93, 5–8, April 3, 1998, Copyright ©1998 by Cell Press

Kinesin: What Gives?

Minireview

Steven M. Block
Department of Molecular Biology
Princeton University
Princeton, New Jersey 08544

Kinesin shares one or more properties with various mechanoenzymes. Like myosin, kinesin is the eponymous member of an entire superfamily of ATP-driven motors in eukaryotes: yeast has six kinesin-related proteins, and over two dozen relatives have been identified in the mouse. Like dynein, kinesin is a protein that binds to and moves along a microtubule substrate, powering a variety of transport processes, such as vesicle movement. Like many nucleic acid–based enzymes (polymerases, helicases, nucleases, etc.), but unlike myosin or dynein, kinesin functions processively, translocating through multiple enzymatic cycles before releasing from its substrate (Gelles and Landick, 1998; Lohman et al., 1998 [both in this issue of *Cell*]). However, kinesin enjoys one distinction that sets it apart from the pack. Each of its two globular heads, responsible for both enzymatic and motor activity, is formed from a single polypeptide only ~345 amino acids long. Kinesin's motor domain weighs three times less than that of myosin (which carries two additional light chains), and ten times less than that of dynein (which carries both light and intermediate chains). As such, kinesin is the smallest molecular motor, by far—and quite possibly the simplest (for reviews, see Bloom and Endow, 1995; Howard, 1996; Vale, 1996; Vale and Fletterick, 1997). Although kinesin's discovery lagged two decades after dynein and nearly a century after myosin, our understanding of kinesin today rivals or surpasses that of any other motor protein, thanks to advances in molecular and structural biology, biophysics, and the not inconsiderable groundwork laid by prior studies of myosin. But despite the wealth of data accumulated for kinesin in just over a decade, we still don't understand the molecular mechanism by which it, or any other biological motor, moves.

Much of what we've learned about kinesin is so new that researchers have scarcely had time to digest it. Crystal structures have now been determined for the motor domains of kinesin (Kull et al., 1996; Sack et al., 1997) and family relatives ncd (Sablin et al., 1996) and Kar3p (Gulick et al., 1998). Remarkably, all three structures share a folding motif that is identical to one present in the core of the myosin head, and also to a lesser degree in G proteins, such as ras and α-transducin, raising the possibility of a common mechanism (Vale, 1996). Recently, the structure of a dimeric kinesin construct was solved, revealing how its two heads are connected (Figure 1; Kozielski et al., 1997), as well as the long-sought structures for α- and β-tubulin (Nogales et al., 1998). High-resolution electron microscopic reconstructions by several groups have shown how kinesin, a plus-end directed motor, is situated when bound to microtubules, as compared to ncd, its minus-end directed cousin (Amos and Hirose, 1997). These reconstructions, and studies of the directions moved by genetically engineered kinesin-ncd chimeras, have furnished early hints about the origin of directional polarity in kinesin proteins (Case et al., 1997; Henningsen and Schliwa, 1997). Although ATP hydrolysis by kinesin has many similarities to myosin biochemistry, there is at least one crucial difference: the two heads of kinesin are by no means independent, but act instead in a coordinated fashion, such that the binding and hydrolysis of ATP by one head promotes ADP release by its counterpart (Lohman et al., 1998, and references therein). This, and the finding that single-headed kinesin constructs do not sustain processive movement (Berliner et al., 1995; Hancock and Howard, 1998; Young et al., 1998), have lent support to the notion that dimeric kinesin moves "hand-over-hand," advancing its heads in strict alternation. In this way, at least one head stays bound to the substrate at any given time, explaining how single

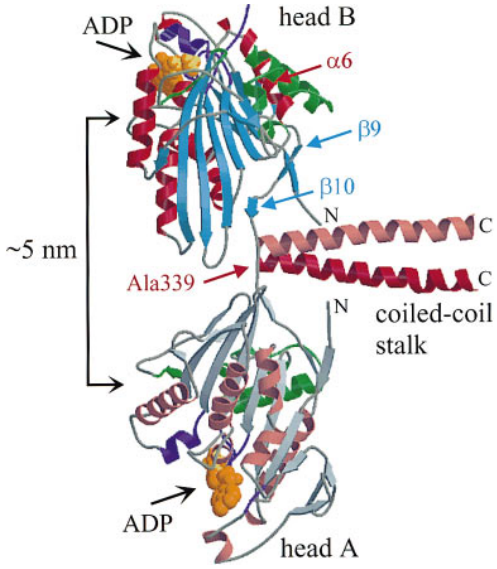

Figure 1. The Crystal Structure of the Dimeric Kinesin Motor Bound to ADP

Adapted from Figure 2A of Kozielski et al., 1997. The N-terminal portion of the kinesin heavy chain from residues 2 through 370 is visible in the structure, with the exception of residues 241–255 (loop L11), which are disordered. The core of kinesin consists of an antiparallel β sheet sandwiched between α helices. The color scheme follows (Kozielski et al., 1997): nucleotide (yellow-orange), nucleotide-binding regions (purple), putative microtubule-binding regions (green), loops and turns (gray), all other β strands (blue), all other α helices (red). The view shows the N-terminal head domains A and B on the left and the initial stalk region on the right, with the core β sheet of head A (lower) and the axis of the coiled-coil lying roughly in the plane of the paper (there is a dihedral angle of ~120° between the heads, seen looking down the long axis of the coiled-coil). Both ADP molecules and the N and C termini are indicated, along with the three structural elements (α6, β9, β10) that complete the head and serve to connect it to the coiled-coil stalk, beginning at residue Ala339. The two putative microtubule-binding regions of the heads (green) are separated by roughly 5 nm (scale bar, arrows) and do not lie in the same plane (see text). The author thanks Eckhard Mandelkow and Stefan Sack for kindly furnishing the key elements of this figure.

kinesin molecules can move steadily against sustained loads approaching the stall force. An important corollary of this is that both heads must be capable of binding to a microtubule simultaneously, at least transiently.

We've also learned a great deal about the biophysics of movement. Kinesin advances at random times through fixed distances, moving in discrete steps of 8 nm, which corresponds directly to the lattice spacing of tubulin dimers along the microtubule (Block, 1995). Kinesin molecules travel along paths that are parallel to single protofilaments in the microtubule lattice (Ray et al., 1993). Individual molecules release at random from these paths, typically making ~100 steps before disengaging. The kinesin motor is impressively fast, capable of speeds of ~800 nm/s (100 steps/s) in vitro, and is quite powerful, continuing to move against loads up to ~6 pN. (Scaled up to our own dimensions, a motor with corresponding properties would travel at similar speeds and produce as much horsepower per unit weight as the jet engines of the Thrust supersonic car, which recently broke the sound barrier.) As such, kinesin expends a mechanical energy equal to $6 \times 8 = 48$ pN · nm per step near stall, an amount corresponding to 60% or more of the available chemical free energy in a single ATP molecule (reviewed in Block, 1995). Two important questions (among many) raised by these findings are the following. First, how is the energy of ATP hydrolysis coupled to mechanical motion? Could kinesin undergo multiple steps per ATP hydrolysis at low loads, as postulated for myosin (Block, 1996; Ishijima et al., 1998), or conversely, use up several molecules of ATP at every step? Or is ATP hydrolysis coupled one-to-one with stepping? Second, how can a protein the size of kinesin, whose heads measure just $\sim 7.5 \times 4.5 \times 4.5$ nm, execute 8 nm displacements while still exerting force? Can a processive mechanism somehow be reconciled with kinesin structure? These questions are intimately linked to one another, and incomplete answers have begun to emerge. Inevitably, more questions have been raised in the process.

Two recent studies independently addressed the mechanochemical coupling issue for kinesin, in the limit of negligible load (Hua et al., 1997; Schnitzer and Block, 1997). In principle, coupling can be determined by scoring both mechanical steps and ATP hydrolysis at the same time for a single molecule. An experiment akin to this has, in fact, been attempted for myosin; however, the work posed significant technically difficulties, and its interpretation remains controversial (Ishijima et al., 1998). It turns out, however, that for the special case of processive movement, coupling can be inferred without recourse to direct ATPase measurement. This works because at extremely low ATP concentrations, the acquisition of ATP by a kinesin head becomes rate-limiting, and therefore stochastic ATP-binding events are directly reflected in the stepping statistics. Analysis of displacements from recordings of individual motors obtained at limiting ATP levels showed kinesin's motion to be consistent with a tight, 1:1 coupling scenario, with one ATP hydrolyzed per 8 nm step. It remains to be seen if 1:1 coupling will continue to hold at finite load: the effect of mechanics on biochemical kinetics remains one of the outstanding problems in the motility field (Howard, 1997).

The coupling results at low load came as something of a surprise. There are only a limited number of ways that a kinesin molecule can step in apparent increments of 8 nm on a microtubule following a protofilament track, dubbed the "waddle models" (Block and Svoboda, 1995). Some of these invoke a single-headed mechanism, or require binding to both α- and β-tubulin subunits, and seem untenable in light of various recent findings, so they are not described here. The remaining candidates (Figure 2A) all proceed hand-over-hand, and incorporate individual (Long Stride) or pairwise (Two Step) head displacements. To remain consistent with the coupling findings, one hydrolysis must move an individual head by 16 nm in the Long Stride model (advancing the centroid, or "center of gravity," of the molecule by 8 nm), whereas in the Two Step model, one hydrolysis must not only bring one head forward by 8 nm, but also must supply additional energy (perhaps indirectly, through elastic strain) to displace its partner head by an identical amount. Examination of the kinesin dimer structure (Figure 1), where the two putative tubulin-binding regions (green) are separated by ~5 nm, makes it clear just how demanding such motions really are.

Evidently, something in the kinesin structure must give to separate its heads by 8 nm, perhaps more. One idea involves the "neck" of kinesin, which is defined as the first ~20–40 amino acids beyond the catalytic motor domain, spanning a conserved peptide sequence that serves to link each head of the dimer to the coiled-coil "tail," known as the stalk (Vale and Fletterick, 1997). It is proposed that this neck contains within it some element analogous to the α-helical "lever arm" of myosin, which is thought to produce movement by amplifying small motions inside the head (Block, 1996; Goldman, 1998 [this issue of *Cell*]). However, the dimer structure shows this neck to be something of a misnomer: the domain actually begins with two successive β strands (Figure 1, β9 and β10) directly associated with the motor core over their entire length, leaving under 1 nm of free polypeptide chain (~3–4 amino acids) beyond the heads to reach the coiled-coil junction. Thus, nothing obvious whatsoever in the kinesin structure resembles myosin's 9 nm-long "lever."

The most straightforward way to separate kinesin's two heads would be to unwind ("melt") the initial portion of the coiled-coil stalk. However, in a recent study of engineered mutants in which the coiled-coil junction zone was either strengthened (by replacing it with a coil-forming heptad consensus sequence) or otherwise altered (by deletion, or by inserting a Gly-Gly-Gly swivel), kinesin processivity was affected—but not entirely abolished (Romberg et al., 1998). In its simplest interpretation, this experiment implies that nothing about the initial coiled-coil region is fundamental for generating steps, although it may fine tune the process. How, then, can the two kinesin heads separate by 8 nm and simultaneously bind a microtubule? One speculation may make structural biologists wince (Figure 2B): it involves unpacking and remodeling portions of the early neck (Romberg et al., 1998). This represents a possible resolution of a well-known quandary, namely, that microtubules have translational symmetry, whereas a kinesin dimer composed of identical heavy chains is expected to have

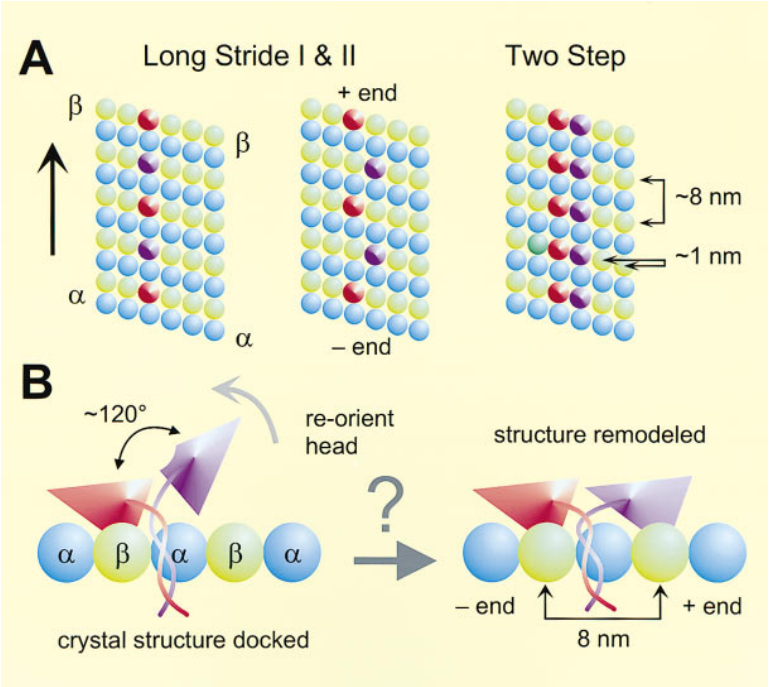

Figure 2. Ways that a Dimeric Kinesin Molecule Might Move Along a Microtubule

(A) Candidate "waddle models," illustrating positions for successive placements of each of the two kinesin heads (red and violet) on the helical microtubule surface lattice, such that the dimeric molecule advances parallel to a single protofilament in steps of ~8 nm. The lattice, consisting of alternating α- and β-tubulin monomers (blue and green, respectively), is seen in projection, with each protofilament offset from its neighbor by ~1 nm; kinesin binds to the β subunit. (Left) In the "Long Stride" models, the rearmost head moves ahead 16 nm, passing the foremost head and advancing the centroid of the molecule by half the length of the stride. Long Stride I tracks along a single protofilament, whereas Long Stride II uses two adjacent protofilaments. (Right) In the "Two Step" model, each head steps forward by 8 nm alternately. To account for the observation of 8 nm steps, rather than 4 nm, the timing of the head motions must be asymmetric and pairwise, with one of the heads (but not the other) advancing in rapid succession immediately after its counterpart moves, so that the molecule does not dwell for any detectable time at an intermediate position. For discussion of additional possibilities, see Block and Svoboda, 1995.

(B) A scheme for binding both kinesin heads to the microtubule with minimal distortion (Romberg et al., 1998). The view is from the side, looking at a single protofilament, with the same color scheme as (A). The shape of heads corresponds to the distinctive "arrowhead" outline of the motor domain structure identified by crystallography. (Left) The dimeric structure of Kozielski et al., 1997, has been docked without distortion against the protofilament, with the tubulin-binding site of one head (red) in contact with the β subunit. The coiled-coil tail of kinesin (double helix) is nearly perpendicular to the protofilament axis and emerges from the plane of the paper in the general direction of the viewer. This orientation leaves the tubulin-binding domain of the second head (violet, curved face) pointing up and away from the protofilament, given an ~120° dihedral angle between the heads. (Right) Counterclockwise rotation of the unbound head through this same angle, around an axis into the page (curved gray arrow), and accompanied by remodeling of the peptides corresponding to the neck region of kinesin, could bring this head into a parallel alignment, suitable for binding to a site 8 nm distal.

2-fold rotational symmetry. Actually, the heads in the published structure are related by an angle of ~120°, not 180°, but this may be due to crystal packing interactions, and therefore not representative of the protein in solution. Thus, there is no way to place both heads in (quasi-equivalent) contact with the microtubule without significant rearrangement. This observation led Howard (1996) to suggest a radical rotary model for movement, where the heads are splayed 8 nm apart while the entire molecule undergoes a rotation about a vertical axis (the axis of the coiled stalk), marking off steps in much the same way as a navigator marks off distance on a map, turning a compass divider. But the rotary model is beset by its own share of problems, among them a way to get the heads 8 nm apart in the first place, some means to relieve the twist that builds up, and the fact that the axis of the coiled stalk seems to run nearly horizontally, not vertically, when the tubulin-binding site of one head is placed against a microtubule surface.

For now, the resolution of the dilemma posed by kinesin's structure and function is unclear. If kinesin and myosin share a common motif, and move by a related mechanism, why is the kinesin head so compact? Could something in the neck region function as a sort of "stealth" lever arm, flipping one head forward transiently, then spring back rapidly against the core motor domain and seem to vanish? Alternatively, could the two heads function together to wag kinesin's stiff tail, acting on the initial coiled-coil segments at the base of the neck? Lastly, even if it's possible to pry the kinesin heads apart and align them with the microtubule lattice, it isn't obvious how they could generate sizeable force and displacement from these apparently strained positions. What is becoming increasingly apparent is that static crystal structures don't tell the full story, and it will take a combination of clever biophysics and biochemistry to fill in the gaps. Interesting challenges lie ahead.

Selected Reading

Amos, L.A., and Hirose, K. (1997). Curr. Opin. Cell Biol. *9*, 4–11.

Berliner, E., Young, E.C., Anderson, K., Mahtani, H.K., and Gelles, J. (1995). Nature *373*, 718–721.

Block, S.M. (1995). Trends Cell Biol. *5*, 169–175.

Block, S.M. (1996). Cell *87*, 151–157.

Block, S.M., and Svoboda, K. (1995). Biophys. J. *68*, 230S–241S.

Bloom, G., and Endow, S. (1995). Protein Profile *2*, 1109–1171.

Case, R.B., Pierce, D.W., Hom-Booher, N., Hart, C.L., and Vale, R.D. (1997). Cell *90*, 959–966.

Gelles, J., and Landick, R. (1998). Cell *93*, this issue, 13–16.

Goldman, Y. (1998). Cell *93*, this issue, 1–4.

Gulick, A.M., Song, H., Endow, S.A., and Rayment, I. (1998). Biochemistry *37*, 1769–1776.

Hancock, W.O., and Howard, J. (1998). J. Cell Biol., in press.

Henningsen, U., and Schliwa, M. (1997). Nature *389*, 93–95.

Howard, J. (1996). Annu. Rev. Physiol. *58*, 703–729.

Howard, J. (1997). Nature *389*, 561–567.

Hua, W., Young, E.C., Fleming, M.L., and Gelles, J. (1997). Nature *388*, 390–393.

Ishijima, A., Kojima, H., Funatsu, T., Tokunaga, M., Higuchi, H., Tanaka, H., and Yanagida, T. (1998). Cell *92*, 161–171.

Kozielski, F., Sack, S., Marx, A., Thormählen, M., Schönbrunn, E., Biou, V., Thompson, A., Mandelkow, E.-M., and Mandelkow, E. (1997). Cell *91*, 985–994.

Kull, F.J., Sablin, E.P., Lau, R., Fletterick, R.J., and Vale, R.D. (1996). Nature *380*, 500–555.

Lohman, T.M., Thorn, K., and Vale, R.D. (1998). Cell *93*, this issue, 9–12.

Nogales, E., Wolf, S.G., and Downing, K.H. (1998). Nature *391*, 199–203.

Ray, S., Meyhöfer, E., Milligan, R.A., and Howard, J. (1993). J. Cell Biol. *121*, 1083–1093.

Romberg, L., Pierce, D.W., and Vale, R.D. (1998). J. Cell Biol., in press.

Sablin, E.P., Kull, F.J., Cooke, R., Vale, R.D., and Fletterick, R.J. (1996). Nature *380*, 555–559.

Sack, S., Müller, J., Marx, A., Thormählen, M., Mandelkow, E.-M., Brady, S.T., and Mandelkow, E. (1997). Biochemistry *36*, 16155–16165.

Schnitzer, M.J., and Block, S.M. (1997). Nature *388*, 386–390.

Vale, R.D. (1996). J. Cell Biol. *135*, 291–302.

Vale, R.D., and Fletterick, R.J. (1997). Annu. Rev. Cell Dev. Biol. *12*, 745–777.

Young, E.C., Mahtani, H.K., and Gelles, J. (1998). Biochemistry, in press.