

3D Electron Microscopy of the Interaction of Kinesin with Tubulin

Keiko Hirose^{1,*}, Jan Löwe², Maria Alonso³, Robert A. Cross³, and Linda A. Amos²

¹Natl Inst Advanced Interdisciplinary Res, Tsukuba 305, Japan, ²MRC Lab of Molecular Biology, Hills Road, Cambridge CB2 2QH, and ³Marie Curie Institute, Oxted, U.K.

ABSTRACT. We have studied the structure of microtubules decorated with kinesin motor domains in different nucleotide states by 3D electron microscopy. Having docked the atomic coordinates of both dimeric ADP.kinesin and tubulin heterodimer into a map of kinesin dimers bound to microtubules in the presence of ADP, we try to predict which regions of the proteins interact in the weakly binding state. When either the presence of 5'-adenylyimidodiphosphate (AMP-PNP) or an absence of nucleotides puts motor domains into a strongly-bound state, the 3D maps show changes in the motor domains which modify their interaction with beta-tubulin. The maps also show differences in beta-tubulin conformation compared with undecorated microtubules or those decorated with weakly-bound motors. Strongly-bound ncd appears to produce an identical change.

Key words: Cryo-electron microscopy/3-D image reconstruction/atomic structure/motility

Introduction

The microtubule-based molecular motors, kinesin and ncd have functions in organelle transport and chromosome separation (5, 8, 10, 34). Although they have opposite directions of movement along microtubules, the motor domains are structurally homologous. X-ray crystallography has been used to solve the atomic structures of monomeric and dimeric constructs of the two motor domains, all with bound ADP (18, 21, 28, 29, 30). However, the way in which the motor domains interact with microtubules is still controversial (15, 17, 19, 32).

We have shown recently (15) that it is possible to dock atomic coordinates into a map of microtubules decorated with dimeric kinesin in the presence of ADP so that the directly-bound motor domain (head) and the tethered head have the same asymmetric relationship as in the X-ray structure (18). Ncd coordinates can also be docked into a map of microtubules decorated with dimeric ncd.ADP so that the directly-bound head interacts with tubulin in the same manner as the directly-bound head of kinesin. In the structure solved for crys-

tals of dimeric ncd (29), the two heads were related by a 2-fold; when ncd dimers bind to microtubules in the presence of ADP, the heads still appear to be roughly 2-fold related, though our docking suggests that a small conformational change causes them to rotate relative to one another (15). Now we investigate in more detail the interaction with tubulin that is predicted from our docking orientation.

Materials and Methods

Microtubules were assembled from purified pig brain tubulin in a polymerizing solution (80 mM PIPES (pH 6.8), 1 mM EGTA, 5 mM MgCl₂, 1 mM DTT, 0.5 mM GTP, 5% DMSO), stabilizied with taxol, centrifuged, and resuspended in a solution without GTP. DMSO was added in order to increase the proportion of 15-pf microtubules (27). K Δ 430 was expressed and purified as described for K Δ 401 (22). Microtubules diluted in the MES solution (60 mM MES, 5 mM MgSO₄, 1 mM EGTA, 1 mM DTT, 10 μ M taxol, pH 6.5) were applied to a EM grid coated with a holey carbon film, and K Δ 430 was added to give a final concentration of 10 μ M. For freezing in the no nucleotide state, the microtubule-K Δ 430 mixture was incubated on the grids with 2 U/ml apyrase and then rapidly frozen by plunging the grids into an ethane slush. For the ADP state, the microtubule-ncd mixture was put on

* To whom correspondence should be addressed: National Institute for Advanced Interdisciplinary Research, 1-1-4 Higashi, Tsukuba, Ibaraki 305-8562, Japan.

Tel: +81-298-54-2556, Fax: +81-298-54-2565

to the grid first and 1 mM ADP, 1 U/ml hexokinase, and 0.01% glucose were added just before freezing.

The grids were examined using a Gatan cold stage in a Philips EM 420 electron microscope operating at 120 kV and with a defocus of 1,300–1,600 nm. Images were photographed at a magnification of 36,000 and then scanned in 28 μ m steps using a Zeiss Phodis scanner. Images of microtubules with 15 or more protofilaments were selected by measuring their diameters and then by inspecting their Fourier transform patterns, as described (14). Micrographs were recorded with a consistent exposure and the images were scanned in the same manner. The digitized images were boxed and floated consistently before their Fourier transforms were calculated. Phases in the transform data from different images were adjusted to match, as well as possible, those of a reference images, by allowing for a phase origin shift and a rotation about the axis. Averaging was carried out in reciprocal space. The final average included only images for which both sides were completely consistent with the consensus, in

order to be sure of avoiding microtubules with “seams” in the helical lattice (31). Maps were displayed as previously (e.g., 14). Fitting of the α -carbon backbone of the atomic coordinates into the EM density maps was carried out visually using the program MAIN (33). Crystal structures were drawn using Rasmol (Roger Sayle, rasmol@ggr.co.uk) and Molscript (20).

Results

3-D EM maps of microtubule-motor complexes

Figures 1(a, b) show typical electron microscope images of reassembled brain microtubules decorated with dimeric kinesin (K Δ 430); some specimens were frozen after incubation with apyrase to remove traces of ATP and ADP (Fig. 1(a)), others had 1 mM Mg. ADP added immediately before freezing (Fig. 1(b)). Density maps calculated from the averaged data (14, 15) are shown in Figures 1(c–f). In each complex, one of the two heads of a dimer appears to be bound directly to the microtubule surface, whilst the other is tethered to it

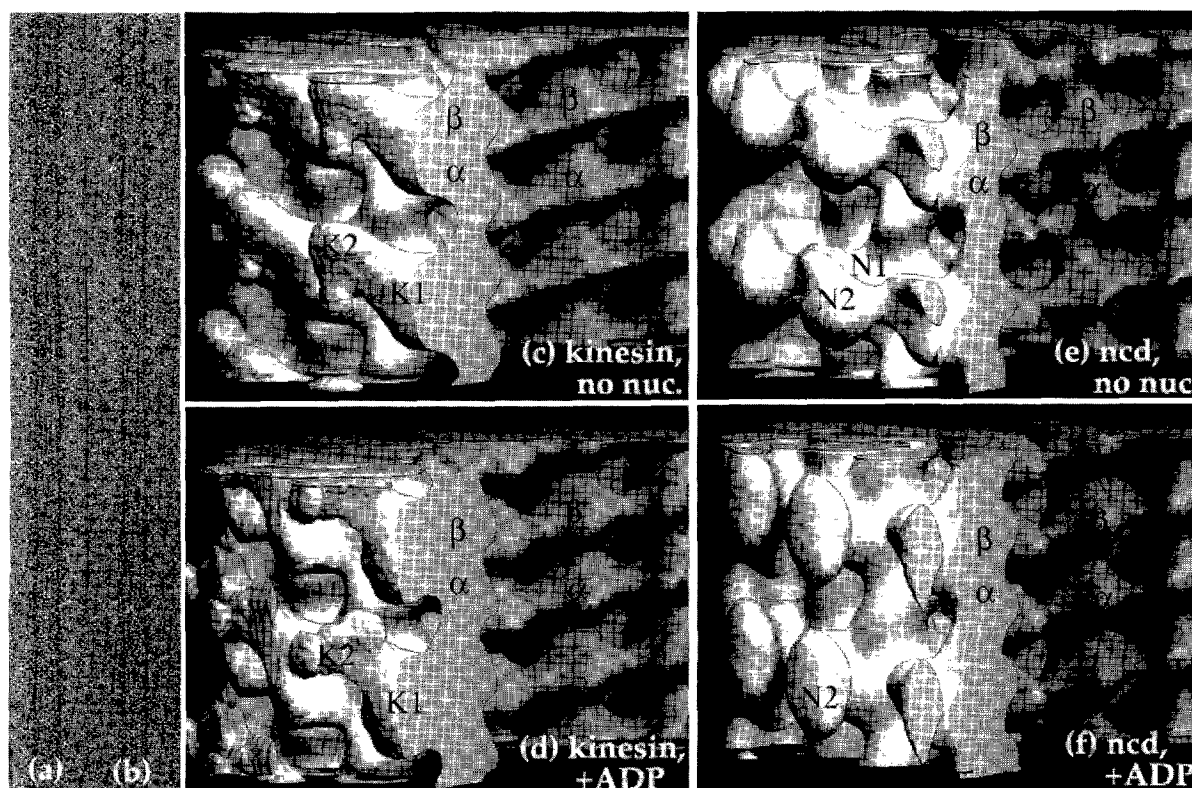


Fig. 1. (a–b). Electron microscope images of 15-protofilament brain microtubules decorated with the double-headed kinesin motor construct (K Δ 430): (a), treated with apyrase, to remove all free nucleotides; (b), in the presence of ADP and hexokinase. (c–f), Surface representations of 3-D density maps (oriented with the microtubule plus end at the top of the page) computed from averaged data (14, 15): microtubules are decorated with kinesin in the absence of nucleotide (c) or presence of ADP (d) or ncd in the absence of nucleotide (e) or presence of ADP (f). Subunits of α - and β -tubulin can be distinguished on the inside and cut surfaces of the microtubule. Attached heads (K1 for kinesin, N1 for ncd) and tethered heads (K2 and N2) are labelled.

in a relatively fixed position. In all nucleotide conditions studied, microtubule polarity is clear from previously established features (2, 11, 13).

The most obvious difference between different complexes is in the position of the tethered head relative to the bound head. However, there are also small structural changes in the shapes of the bound heads, including a difference in the way they interact with the microtubule. A comparison of pairs of longitudinal sections (Figs. 2(a–d)) through maps of kinesin- or *ncd*-decorated microtubules in strong (nucleotide-free) and weak (ADP-containing) states, shows a weakening of the contact with β -tubulin when either motor is complexed with ADP (Figs. 2(b, d)). Cross-sections (Figs. 2(e, f)) show that the contact right across the top of the head appears to be weaker with ADP. Equivalent sections through directly-attached motor domains complexed with AMP-PNP (not shown here) resemble the nucleotide-free structures.

Both pairs of maps also show a structural change within the microtubule that depends on the state of the bound motor domains. This is evident in the apparent tilt of the β -tubulin subunit, which is different from that of the α -tubulin subunit when kinesin or *ncd* is strongly-bound (Figs. 2(a, c)), whilst α and β -tubulin appear very similar to each other when the motors are weakly-bound (Figs. 2(b, d)).

Fitting atomic coordinates into the EM density maps

All the atomic structures solved to date for kinesin or *ncd* motor domains have ADP bound to them. We have therefore docked the coordinates of monomeric kinesin (21, 30) into the directly-bound heads in our maps of the kinesin.ADP-microtubule complex. Figure 3(a) shows the best fit, as judged by eye, to best satisfy the shape of the outer boundary of the directly attached head (crystal structure colored yellow). This orientation is also the best for placing a second head (pink structure in Fig. 3(a)) in the remaining density, so that the relationship between the attached and tethered heads resembles the relationship between the two heads in the kinesin dimer crystals. We were also able to dock *ncd* in an equivalent orientation into the directly-attached head in a map of microtubules decorated with *ncd*.ADP (15).

We have docked tubulin protofilament coordinates (Nogales *et al.*, 1998) into the microtubule part of the map, in the same orientation as Nogales *et al.* (1999) docked them into an undecorated microtubule. The result leads to a good complementarity between the shapes of the molecules (Fig. 3).

Discussion

Interacting Surfaces

3-D EM images show that kinesin and *ncd* bind to essentially the same sites on tubulin (11, 12, 13, 16) in agreement with the equivalent way in which microtubules protect the two motors from proteolysis (1). The changes seen in the directly-attached heads in different nucleotide states (14, 15) also appear to be similar for both motors, despite the fact that they move along microtubules in opposite directions. This is consistent with the fact that the direction in which chimaeric constructs move depends ultimately on the position and composition of added neck domains, rather than the source of the globular catalytic domain itself (3, 6, 9, 29).

Although each motor domain interacts with both monomers of an α/β -tubulin dimer, motor domains complexed with ADP (see Figs. 2, 3) appear to interact most directly with α -tubulin, the subunit that lies nearer to the microtubule minus end (7, 23). There is a broad area of contact between the bottom half of the kinesin motor domain and α -tubulin, while the upper region makes a looser contact with the surface of β -tubulin. The upper contact includes loop L11 of kinesin, which is mobile in all of the motor protein crystals but seems likely to be in a suitable position to interact with the ridge on the outermost surface of β -tubulin (see Fig. 3(b)).

The structure of tubulin is illustrated in Figures 3, 4. Tubulin protofilaments are oriented in a microtubule so that, when viewed from outside with the plus end upwards, the M loop on one protofilament contacts helix H3 of its right-hand neighbour (26). Two long helices, H11 and H12, occupy the ridge on the outer surface of each tubulin monomer (see Figs. 3(b, c)). The motor-binding site on each tubulin monomer covers a section of this ridge and spreads over to the left, where it involves part of the surface of the GTPase domain between the ridge and helix H3.

The acidic C-terminal segments are also somewhere on the outer surface but residues 438–453 of α - and β -tubulin are apparently completely disordered in the 2-D crystals. These segments may also be involved in the interaction with kinesin or *ncd*, since there is an excess of basic residues on the tubulin-binding surfaces (36), especially near the tops of the motor molecules. In particular, there are conserved arginines or lysines in loop L12 and in helix α -5. These lie on the right-hand side of the top of the motor domain, when viewed from outside with the plus end upwards, near to the H11/H12-containing ridge on the tubulin protofilament, and may interact more directly in the strongly-bound states than in the weakly-bound ADP state. We have predicted (15) that changes in the L12/ α -5 region of kinesin, which

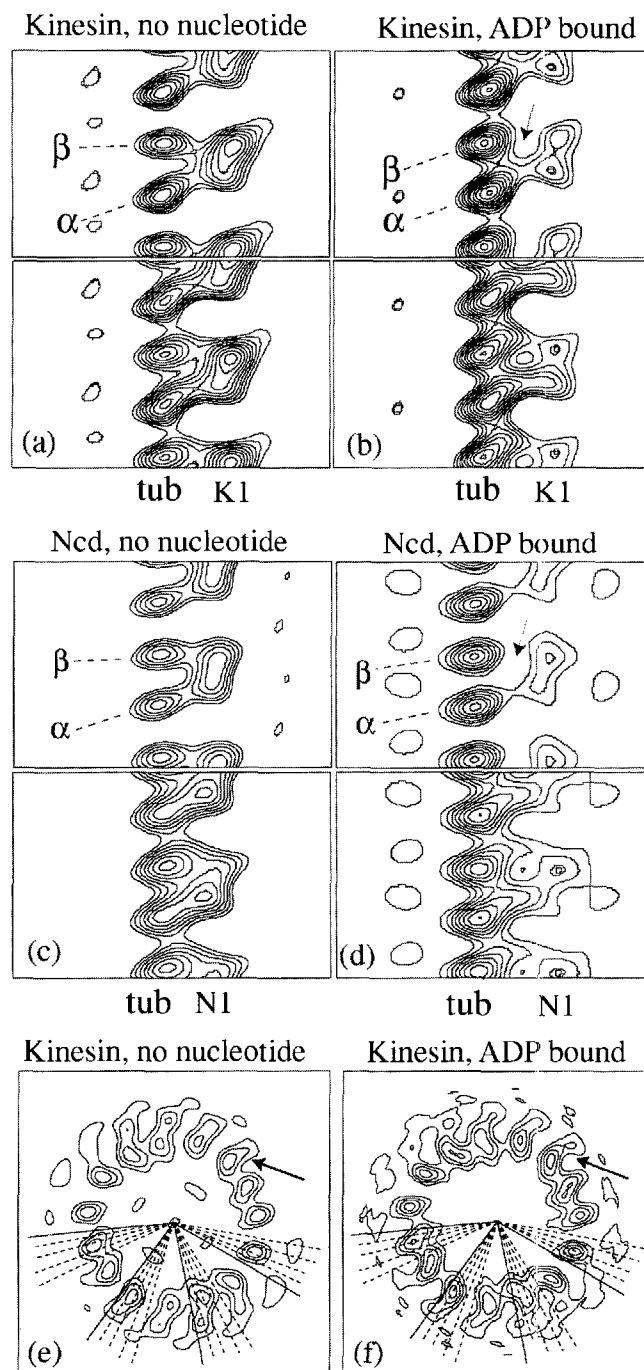


Fig. 2. (a–d) Pairs of sections cut through the microtubule axis at rotational angles differing by 2.4 degrees; they pass through the left side of a tubulin protofilament (tub) and then through the middle of the directly attached heads (K1 or N1). The interaction between tubulin and kinesin (a, b) changes in the same way as that between tubulin and ncd (c, d). With ADP in the nucleotide-binding site, the association with β -tubulin appears weaker for both species of attached heads. Ncd may make an additional bond to α -tubulin via the L2 loop, which is very short in kinesin, and this may compensate for ncd's apparently weaker interaction with β -tubulin (evident as a greater tilt of this motor domain). Strong binding by either motor appears to induce the same change in the tubulin dimer, as indicated by dashed lines through the tubulin monomers; in the nucleotide-free state, the outer tip of the β -tubulin subunit tilts closer to that of the α -tubulin subunit, whereas in the ADP state both monomers appear equal. (e–f) Cross-sections through maps of microtubules decorated with kinesin in the absence of nucleotide (e) or in the presence of ADP (f). Each group of 5 radial lines shows equivalent longitudinal sections. Figs. (a–b) correspond to sections close, on either side, to the solid radial lines. The arrows point to a level where the difference in the strength of the contact is clear.

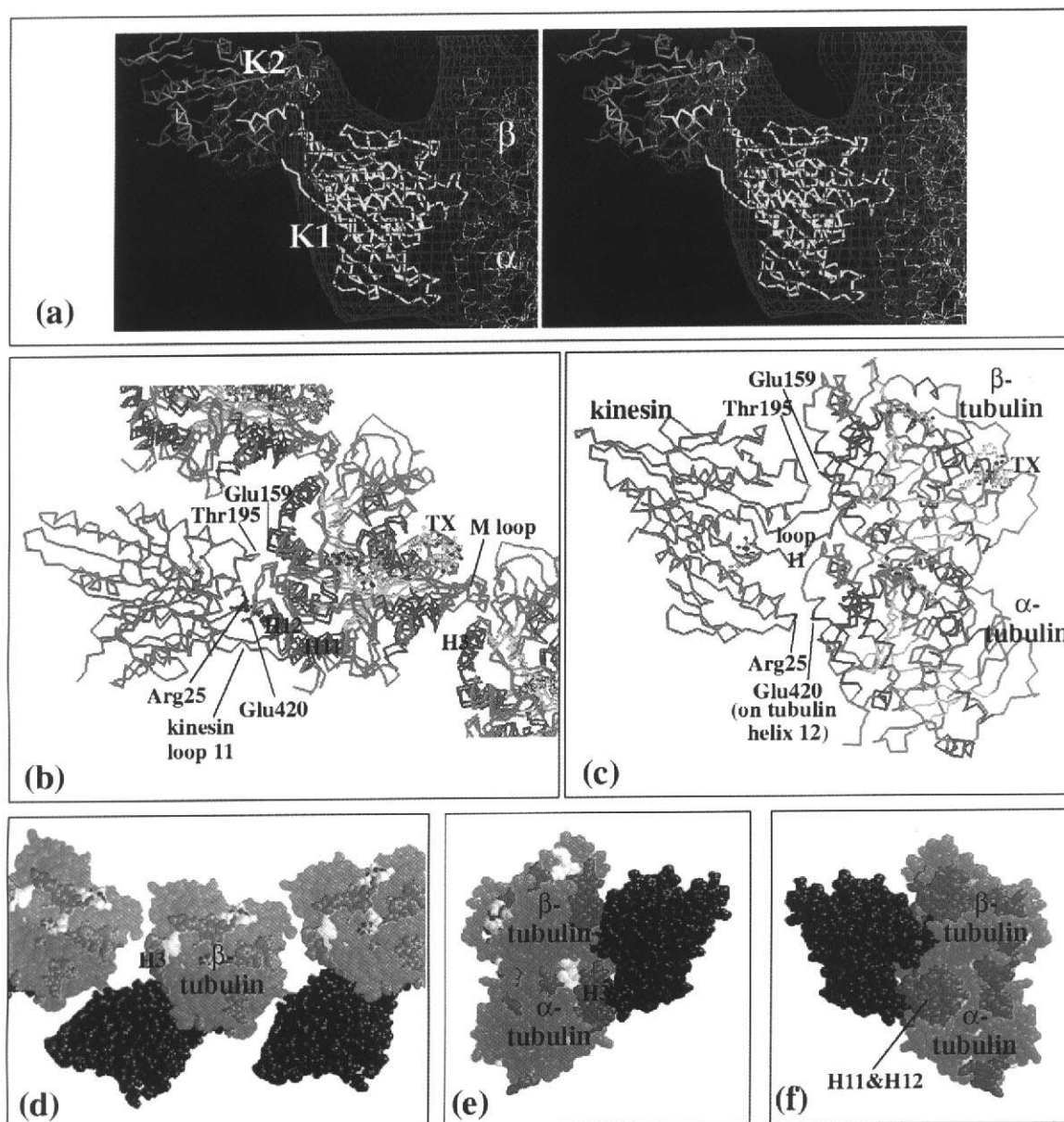


Fig. 3. (a) Stereo view of two kinesin monomers (30) and a tubulin dimer (25, 26) oriented inside part of the EM map of a microtubule decorated with dimeric kinesin-ADP. The map is represented as a surface net (red lines). α - and β -tubulin are docked into the microtubule density in the same orientation as found by Nogales *et al.* (26). One kinesin molecule (shown in yellow) occupies the directly attached head density, another (shown in pink) is positioned in the tethered head density (15). The tethered head density appears less than required because of random motion in the specimens. The relative orientation of the pink and yellow molecules mimics the asymmetric arrangement of heads in crystals of dimeric kinesin (18). (b–f) Views of kinesin and tubulin molecules interacting as predicted from the docking shown in (a). Molecular structures are represented by their α -carbon backbones (b, c), or by spacefilling models (d–f). The secondary structure of tubulin is shown in orange (α -helix) and yellow (β -sheet); GDP and paclitaxel (TX) are shown in cpk colours. In (d–f), kinesin is coloured in black. The residues brought into closest contact by this docking are labelled. The view from the top of a microtubule (b, d) shows a central tubulin protofilament interacting with two neighbouring protofilaments and one attached kinesin monomer. (c) and (f) are side views. (e) is a side view from the opposite side of kinesin. Kinesin fits closely to the protofilament surface in all views.

has been shown by mutagenesis to be important for tubulin-activated ATPase activity (36), may bring these structures on the right-hand side into closer contact

with the microtubule.

The surface of β -tubulin's GTPase domain is in contact with the left-hand side of the top of the motor do-

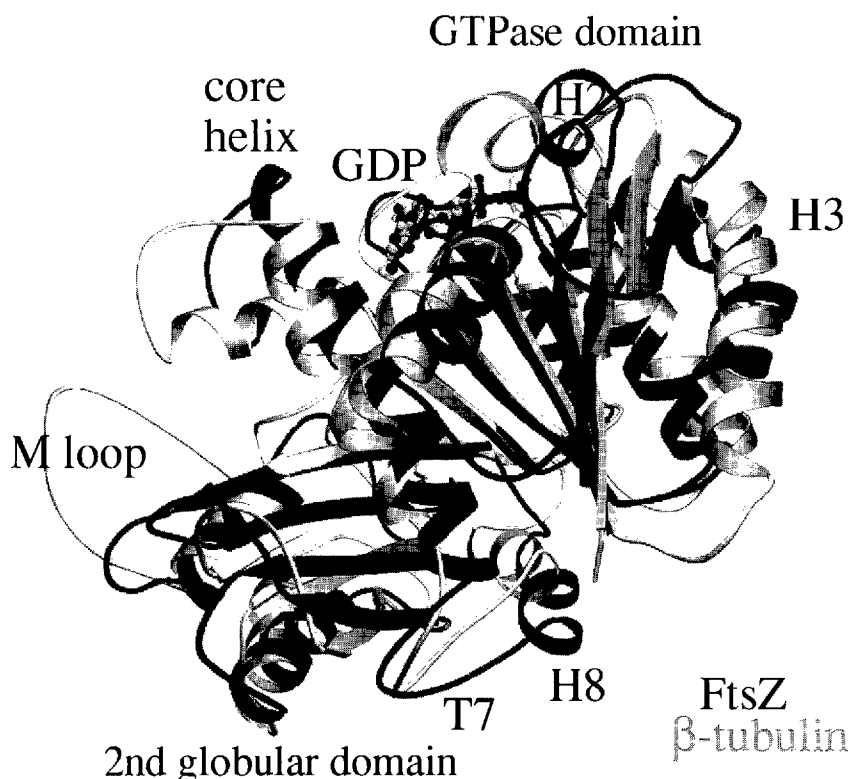


Fig. 4. Superimposed ribbon diagrams of β -tubulin and FtsZ (Nogales *et al.*, 1998), showing tubulin as seen from inside a microtubule. Both structures consist of two globular domains on either side of the core helix. Tubulin has an extra pair of long helices on its outer surface that are not found in FtsZ. Some of the loops and helices involved in making contacts in the microtubule lattice are labelled. Helix H3 makes lateral contact with the M loop (26). Loop T7 makes a longitudinal contact with the loops surrounding the nucleotide in the next monomer. Compared with either tubulin monomer, the β -strands in the second globular domain of FtsZ are rotated by about 11° and there are movements in loop T7 plus helix H8, at the bottom of the subunit, and in helix H2, at the top of the subunit.

main. One of the residues we predict to sit nearest to tubulin is a conserved threonine (T195 in human kinesin) in loop L9 (see Figs. 3(b, c)), which is thought to be analogous to the switch I loop of a G-protein's nucleotide-binding site (35). The direct contact between tubulin and the putative switch loop on the left may be as important as the interaction with the right-hand side. The linkages between the ATPase site and the track binding sites need to be complex in order to work reciprocally; nucleotide turnover causes the motor to cycle between weak and strong binding conformations, while binding to tubulin produces a signal that switches the affinity of the active site for nucleotides, speeding up the process of product release.

There is no evidence from EM maps for nucleotide-dependent conformational changes in the lower part of a kinesin or ncd motor domain (14, 15). This region interacts closely with α -tubulin, which also appears constant in structure (see below). Figure 3(b, c) shows that kinesin-Arg25 in loop L1 lines up with α -tubulin-Glu420. This matching of charged residues may not be highly significant, however, since Arg25 is not highly

conserved across the kinesin family. In fact, all residues that are absolutely conserved throughout the kinesin family are confined to the top of the molecule. The broad area of contact between the bottom half of the kinesin motor domain and α -tubulin (see space-filling models in Figs. 3(d-f)), may not require individual residues to be conserved.

Effects of motor binding on tubulin subunits

Comparing the 3-D maps of dimeric kinesin complexed to microtubules in the presence of ADP, AMP-PNP, or without nucleotides, we have found similar structural differences in the ways kinesin and ncd heads bind to tubulin. In the strongly bound states, the interaction with β -tubulin is strong enough to alter its conformation, a change which shows up at low resolution as a tilting of the whole subunit (Fig. 2). At atomic level it probably represents a change in the relative positions of different domains; The atomic structures of α - and β -tubulin and FtsZ, the bacterial homologue of tubulin, show some differences (Fig. 4) that may indicate possible types of movement, since the FtsZ mono-

mer structure may provide an approximate model of disassembled β -tubulin. Compared with assembled tubulin, there is a relative rotation of about 11° between the second domain of FtsZ and the GTPase domain plus the core helix (Fig. 4). Thus, it seems reasonable to suggest that binding of a motor domain to the GTPase domain of β -tubulin might cause it to move relative to the second globular domain, by pivoting around the core helix.

The role of tubulin in the motile mechanism of normal kinesin or *ncd* is unknown. The tilting of the β -tubulin subunit could be important in providing feedback to the motor domain, in storing energy or in sending a signal along the protofilament to potentiate subsequent interactions. The conformational change we have observed is possibly a mild version of the one produced by destabilising motors in the kinesin family, such as those whose catalytic domain lies in the middle of the polypeptide (including XKCM1 and XKIF2), which apparently cause microtubules assembled with GMP-CPP to depolymerise into coiled protofilaments (4).

Acknowledgments. We are grateful to Juan Fan for providing purified tubulin and to R. Fletterick and colleagues for the atomic coordinates of monomeric *ncd*. Coordinates for 1KIN, 2KIN and 3KIN were obtained from the Brookhaven data-base. J.L. was the recipient of a long-term EMBO fellowship during this project.

References

- ALONSO, M.C., VANDERKERCKHOVE, J., and CROSS, R.A. 1998. Proteolytic mapping of kinesin/*ncd*-microtubule interface: nucleotide-dependent conformational changes in the loops L8 and L12. *EMBO J.*, **17**: 945–951.
- AMOS, L.A. and HIROSE, K. 1997. The structure of microtubule-motor complexes. *Curr. Opin. Cell Biol.*, **9**: 4–11.
- CASE, R.B., PIERCE, D.W., HOM-BOOHER, N., HART, C.L., and VALE, R.D. 1998. The directional preference of kinesin motors is specified by an element outside of the motor catalytic domain. *Cell*, **90**: 959–966.
- DESAI, A., VERMA, S., MITCHISON, T.J., and WALCZAK, C.E. 1999. Kin 1 kinesins are microtubule-destabilising enzymes. *Cell*, **96**: 69–78.
- ENDOW, S.A., HENIKOFF, S., and SOLER NIEDZIELA, L. 1990. Mediation of meiotic and early mitotic chromosome segregation in *Drosophila* by a protein related to kinesin. *Nature*, **345**: 81–83.
- ENDOW, S.A. and WALIGORA, K.W. 1998. Determinants of kinesin motor polarity. *Science*, **281**: 1200–1202.
- FAN, J., GRIFFITHS, A.D., LOCKHART, A., CROSS, R.A., and AMOS, L.A. 1996b. Microtubule minus ends can be labelled with a phage display antibody specific to α -tubulin. *J. Mol. Biol.*, **259**: 325–330.
- GOLDSTEIN, L.S.B. 1993. With apologies to Scheherazade — tails of 1001 kinesin motors. *Ann. Rev. Genetics*, **27**: 319–351.
- HENNINGSEN, U. and SCHLIWA, M. 1997. Reversal of the direction of movement of a molecular motor. *Nature*, **389**: 93–96.
- HIROKAWA, N. 1998. Kinesin and dynein superfamily proteins and the mechanism of organelle transport. *Science*, **279**: 519–526.
- HIROSE, K., LOCKHART, A., CROSS, R.A., and AMOS, L.A. 1995. Nucleotide-dependent angular change in kinesin motor domain bound to tubulin. *Nature (London)*, **376**: 277–279.
- HIROSE, K., LOCKHART, A., CROSS, R.A., and AMOS, L.A. 1996. Three-dimensional cryoelectron microscopy of dimeric kinesin and *ncd* motor domains on microtubules. *Proc. Natl. Acad. Sci. USA*, **93**: 9539–9544.
- HIROSE, K., AMOS, W.B., LOCKHART, A., CROSS, R.A., and AMOS, L.A. 1997. Three-dimensional cryoelectron microscopy of 16-prot filament microtubules: structure, polarity and interaction with motor protein. *J. Struct. Biol.*, **118**: 140–148.
- HIROSE, K., CROSS, R.A., and AMOS, L.A. 1998. Nucleotide-dependent structural changes in dimeric *ncd* molecules complexed to microtubules. *J. Mol. Biol.*, **278**: 389–400.
- HIROSE, K., LÖWE, J., ALONSO, M., CROSS, R.A., and AMOS, L.A. 1999. Congruent docking of dimeric kinesin and *ncd* into 3-D electron cryo-microscopy maps of microtubule-motor. ADP complexes. *Mol. Biol. Cell*, **10**: 2063–2074.
- HOENGER, A. and MILLIGAN, R.A. 1997. Motor domains of kinesin and *ncd* interact with microtubule protofilaments with the same binding geometry. *J. Mol. Biol.*, **265**: 553–564.
- HOENGER, A., SACK, S., THORMÄHLEN, M., MARX, A., MÜLLER, J., GROSS, H., and MANDELKOW, E. 1998. Image reconstructions of microtubules decorated with monomeric and dimeric kinesins: comparison with x-ray structure and implications for motility. *J. Cell Biol.*, **141**: 419–430.
- KOZIELSKI, F., SACK, S., MARX, A., THORMÄHLEN, M., SCHÖNBRUNN, E., BIOU, V., THOMPSON, A., MANDELKOW, E.M., and MANDELKOW, E. 1997. The crystal structure of dimeric kinesin and implications for microtubule-dependent motility. *Cell*, **91**: 985–994.
- KOZIELSKI, F., ARNAL, I., and WADE, R.H. 1998. A model of the microtubule-kinesin complex based on electron cryo-microscopy and X-ray crystallography. *Current Biology*, **8**: 191–198.
- KRAULIS, P.J. 1991. Molscript — a program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallogr.*, **24**: 946–950.
- 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 (London)*, **380**: 550–555.
- LOCKHART, A., CREVEL, I.M.-T.C., and CROSS, R.A. 1995. Kinesin and *ncd* bind through a single head to microtubules and compete for a shared MT binding site. *J. Mol. Biol.*, **249**: 763–771.
- MITCHISON, T.J. 1993. Localization of an exchangeable GTP-binding site at the plus end of microtubules. *Science*, **261**: 1044–1047.
- NOGALES, E., DOWNING, K.H., AMOS, L.A., and LÖWE, J. 1998. Tubulin and FtsZ form a distinct family of GTPases. *Nature Structural Biology*, **5**: 451–458.
- NOGALES, E., WOLF, S., and DOWNING, K.H. 1998. Structure of the $\alpha\beta$ -tubulin dimer by electron crystallography. *Nature*, **391**: 199–203.
- NOGALES, E., WHITTAKER, M., MILLIGAN, R.A., and DOWNING, K.H. 1999. High resolution model of the microtubule. *Cell*, **96**: 79–88.
- RAY, S., MEYHÖFER, E., MILLIGAN, R.A., and HOWARD, J. 1993. Kinesin follows the microtubule's protofilament axis. *J. Cell Biol.*, **121**: 1083–1093.

28. SABLIN, E.P., KULL, F.J., COOKE, R., VALE, R.D., and FLETTERICK, R.J. 1996. Crystal structure of the motor domain of the kinesin-related motor ncd. *Nature (London)*, **380**: 555–559.
29. SABLIN, E.P., CASE, R.B., DAI, S.C., HART, C.L., RUBY, A., FLETTERICK, R.J., and VALE, R.D. 1998. Direction determination in the minus-end-directed kinesin motor ncd. *Nature*, **395**: 813–816.
30. SACK, S., MÜLLER, J., MARX, A., THORMÄHLEN, M., MANDELKOW, E.M., BRADY, S.T., and MANDELKOW, E. 1997a. X-ray structure of motor and neck domains from rat brain kinesin. *Biochemistry*, **36**: 16155–16165.
31. SONG, Y.-H. and MANDELKOW, E. 1995. The anatomy of flagellar microtubules: polarity, seam, junctions, and lattice. *J. Cell Biol.*, **128**: 81–94.
32. SOSA, H., DIAS, D.P., HOENGER, A., WHITTAKER, M., WILSON-KUBALEK, E., SABLIN, E., FLETTERICK, R.J., VALE, R.D., and MILLIAGN, R.A. 1997. A model for the microtubule-ncd motor protein complex obtained by cryo-electron microscopy and image analysis. *Cell*, **90**: 217–224.
33. TURK, D. 1992. Weiterentwicklung eines programms für molekülgraphik und elektrondichte-manipulation und seine anwendung auf verschiedene protein-strukturaufklärungen: Technische Universität, München.
34. VALE, R.D., REESE, T.S., and SHEETZ, M.P. 1985. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell*, **42**: 39–50.
35. VALE, R.D. and FLETTERICK, R.J. 1997. The design plan of kinesin motors. *Annu. Rev. Cell Dev. Biol.*, **13**: 745–777.
36. WOHLKE, G., RUBY, A.K., HART, C.L., LY, B., HOMBOOHER, N., and VALE, R.D. 1997. Microtubule interaction site of the kinesin motor. *Cell*, **90**: 207–216.