

# Tubulin Protofilaments and Kinesin-dependent Motility

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**Abstract.** Microtubules are built of tubulin subunits assembled into hollow cylinders which consist of parallel protofilaments. Thus, motor molecules interacting with a microtubule could do so either with one or several tubulin subunits. This makes it difficult to determine the structural requirements for the interaction. One way to approach the problem is to alter the surface lattice. This can be done in several ways. Protofilaments can be exposed on their inside (C-tubules or "sheets"), they can be made antiparallel (zinc sheets), or they can be rolled up (duplex tubules). We have exploited this polymorphism to study how the motor protein kinesin attached to a glass surface interacts and moves the various tubulin assemblies.

Microtubules glide over the surface along straight paths and with uniform velocities. In the case of C-tubules, ~40% glide similarly to microtubules, but a major fraction do not glide at all. This indicates (a) that a full cylindrical closure is not necessary for movement, and (b) that the inside surface of microtu-

bules does not support gliding. With zinc sheets, up to 70% of the polymers move, but the movement is discontinuous, has a reduced speed, and follows along a curved path. Since zinc sheets have protofilaments alternating in orientation and polarity, this result suggests that in principle a single protofilament can produce movement, even when its neighbors cannot. Duplex microtubules do not move because they are covered with protofilaments coiled inside out, thus preventing the interaction with kinesin. The data can be explained by assuming that the outside of one protofilament represents the minimal track for kinesin, but smooth gliding requires several parallel protofilaments.

Finally, we followed the motion of kinesin-coated microbeads on sea-urchin sperm flagella, from the flagellar outer doublet microtubules to the singlet microtubule tips extending from the A-tubules. No change in behavior was detected during the transition. This indicates that even if these microtubules differ in surface lattice, this does not affect the motility.

ONE of the most important functions of microtubules is to provide paths for the active transport of vesicles within eukaryotic cells. The motion depends on motor proteins such as kinesin (Vale et al., 1985; Brady, 1985; Cohn et al., 1987) or cytoplasmic dynein (Paschal and Vallee, 1987) which interact with microtubules, hydrolyze ATP, and generate a force in a direction specified by the microtubule polarity. In the case of kinesin this direction is towards the plus-end or anterograde (for recent reviews see Vallee and Shpetner, 1990; Goldstein, 1991; Sawin and Scholey, 1991).

The motile machinery consists of several components the motor protein (kinesin), the fuel (ATP), the track (microtubules), and the load (vesicles). To study the motile mechanism it would be desirable to decrease the number of components. A number of earlier studies have shown that movement is generated in the head domain of kinesin while the stalk may act as a force transducer (Ingold et al., 1988; Scholey et al., 1989; Kuznetsov et al., 1989; Yang et al., 1990).

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In the case of microtubules it is less clear what constitutes the minimal unit required for the motility. The COOH-terminal domain of  $\alpha$  and  $\beta$  tubulin is generally considered to contain the binding site for motor proteins, although there is a debate on the exact location and on the relationship with the binding sites of other MAPs (Paschal et al., 1989; Rodionov et al., 1990; Goldsmith et al., 1991). More specifically, it seems that the motility is not affected by MAPs, suggesting that motors and MAPs generally do not interfere with one another (except perhaps MAP2, von Massow et al., 1989). Independently of MAPs, the state of polymerization of tubulin is critical. Microtubules, but not tubulin, enhance the kinesin ATPase (Kuznetsov and Gelfand, 1986). The tracks of beads are laterally confined to the width of a protofilament, suggesting that one or two adjacent protofilaments are sufficient for movement. Moreover, there are 4-nm steps in the axial direction, implying that the periodicity of tubulin monomers along a microtubule was important (Gelles et al., 1988). A similar conclusion follows from the combination of 8-nm and 4-nm step sizes observed in the dynein-dependent movement of flagellar outer doublet microtubules (Kamimura and Kamiya, 1992). Finally, motor proteins move in a de-

1. *Abbreviations used in this paper:* MAP, microtubule-associated protein; PC-tubulin, phosphocellulose-purified tubulin.

finer direction, presumably because they can make use of the inherent polarity of the microtubule; but on the other hand different members of the kinesin superfamily can move in opposite directions (McDonald et al., 1990; Walker et al., 1990).

These observations suggest that the surface lattice of tubulin subunits is important for microtubule-based motility, but they do not reveal how the motor proteins use the lattice. We therefore approached the problem from a new direction by varying the surface lattice of the tubulin polymer rather than the components of kinesin. Tubulin can be assembled into a remarkable variety of polymorphic states (for review, see Amos and Eagles, 1987). In this study we asked which of these states support kinesin-based motility. The results can be interpreted by assuming that the outside of a single protofilament can be regarded as a minimal track for kinesin.

## Materials and Methods

### Preparation of Tubulin

Tubulin was prepared by phosphocellulose chromatography preceded by a MAP-depleting step as described (Mandelkow et al., 1985). The buffer used for microtubule assembly was 0.1 M Pipes, pH 6.9, 20  $\mu$ M taxol, 1 mM  $MgCl_2$ , 0.5 mM EGTA, and 1 mM GTP. DTT was always used at 0.1 mM (to prevent precipitation in the presence of zinc, see below). Microtubules were polymerized from the solution containing 0.5 mg/ml of PC-tubulin by incubation at 30°C for 10–20 min. Taxol served to stabilize microtubules at low concentrations.

### Preparation of C-tubules

C-tubules were polymerized from PC-tubulin (0.5 mg/ml) in standard assembly buffer plus 15% DMSO. The incubation time was 10–20 min at 30°C.

### Preparation and Stabilization of Zinc Sheets

Zinc sheets were polymerized in a buffer containing 1 mM  $ZnCl_2$ , 0.5–2 mg/ml PC-tubulin, 100 mM MES, pH 6.0, 20  $\mu$ M taxol, 0.5 mM  $MgCl_2$ , 0.5 mM EGTA, 0.1 mM DTT, and 3 mM GTP (see Larsson et al., 1976; Baker and Amos, 1978; McEwen et al., 1983). The incubation time was 15–20 min at 30°C. In this buffer they were stable for more than 5 h. However, when mixing this preparation with the motility assay buffer (see below) the zinc ions were chelated by the EGTA, leading to the disappearance of the zinc sheets within 2 min. Taxol did not stabilize the structure even at 100  $\mu$ M concentration. On the other hand, when zinc was not chelated it completely inhibited microtubule gliding by kinesin. Because of these constraints we selected 15% DMSO in the assay buffer to stabilize zinc sheets even though this reduced the gliding velocity of microtubules. In these conditions there was no significant change of morphology for more than 30 min. The concentrations of metal ions and EGTA were calculated from the algorithm of Goldstein (1979), using the dissociation constants of  $10^{-12.91}$  M between  $Zn^{++}$  and  $EGTA^{-4}$  at pH 7 (compared to  $10^{-10.97}$  and  $10^{-5.20}$  for  $Ca^{++}$  and  $Mg^{++}$ , respectively).

### Preparation of Duplex Microtubules

Duplex tubules were prepared by adding 0.1 mg/ml protamine-Cl to the suspension of microtubules (2 mg/ml) just after the polymerization (see Jacobs et al., 1975).

### Preparation of Kinesin

Kinesin was prepared according to the methods of Kuznetsov and Gelfand (1986) or Vale et al. (1985), with modifications (Von Massow et al., 1989; Kuznetsov and Gelfand, 1986).

### Video Microscopy

Observation with differential interference contrast microscopy was done fol-

lowing the principles of Allen (1985). We used an inverted Zeiss microscope (IM35) in DIC equipped with a  $\times 63$  Plan-Apo objective (NA 1.4) and a chalnicon video-camera (C2400-01; Hamamatsu-Photonics, Herrsching/München, Germany). Background subtraction and digital enhancement were done using image processors DVS-3000 (Hamamatsu Photonics) or BM90 (B&M Spektronik, Gernlinden/München, Germany). For dark-field video microscopy we used a Zeiss Axioplan with a  $\times 100$  objective (Plan Aplanachromat, NA 0.6–1.3) and a dark-field condenser (NA 1.2–1.4), equipped with an SIT camera (AVT-9222, AVT-Horn, Aalen, Germany). Processed images were recorded on a super-VHS video cassette recorder (AG-7330; Panasonic).

### Microtubule Gliding Assay

The motility was observed by coating a glass surface with kinesin and observing the gliding of microtubules (Vale et al., 1985). The buffer used for the assay was 0.1 M Pipes, pH 6.9, 1 mM  $MgCl_2$ , 0.5 mM EGTA, 0.1 mM DTT. 5  $\mu$ l of brain kinesin (final concentration about 100  $\mu$ g/ml) was placed on a clean coverslip ( $24 \times 50$  mm<sup>2</sup>) and kept for 5–10 min in a moist chamber at room temperature (19–22°C). Subsequently 6  $\mu$ l of assay buffer with 2 mM ATP (with or without 4 M DMSO) and 1  $\mu$ l of sample solution containing microtubules or polymorphic forms of tubulin were added and mixed. The final tubulin concentration was 0.05 mg/ml in the case of microtubules, duplex-tubules, and C-tubules. In the case of zinc sheets the final concentration of tubulin was 0.05–0.2 mg/ml. Immediately after the mixing another coverslip ( $22 \times 22$  mm<sup>2</sup>) was placed on the specimen. The motility was observed under the microscope and recorded within 20 min after mixing; during this period there was no apparent change of motility and shapes of the filaments.

### Bead Movement Assay

The motility of kinesin-coated silica or polystyrene microbeads on demembrated sea-urchin sperm flagella was assayed following the procedures of Gilbert et al. (1985). Dry sea-urchin sperm was washed once with natural sea-water and collected by centrifugation. 5  $\mu$ l of the precipitate was mixed with 100  $\mu$ l of assay buffer containing 0.1% Triton X-100 and kept on ice for >1 min. The suspension of demembrated spermatozoa was introduced into a space between two glass slides. After washing once with assay buffer containing ATP a mixture of brain kinesin (5  $\mu$ l), BSA (100 mg/ml, 2  $\mu$ l), assay buffer (12  $\mu$ l of 2 mM ATP), and suspension of silica particles (0.1% wt/vol, 1  $\mu$ l) or polystyrene microbeads (Estapor No. PSI-68, 0.1% wt/vol, 1  $\mu$ l; Rhone-Poulenc, Paris, France) was added.

### Determination of Velocities

A cursor which could be controlled by a mouse interfaced to an Atari 1040ST computer was superimposed through a genlock adaptor (GST30; Atari) onto video sequences played in real time (final magnification on the TV monitor was  $\sim \times 5000$ ). The filament positions were determined in 2–5-s intervals and used later for the analysis of velocities.

### Electron Microscopy, Optical Diffraction, and Densitometry

Specimens were negatively stained with 2% uranyl acetate on carbon-coated grids and examined on a Philips CM12 microscope at magnifications of 8,000–45,000. The surface lattices typical of microtubules or polymorphic forms were ascertained by optical diffraction, using a diffractometer equipped with a He-Ne laser and an f-100 cm lens. Densitometry of selected areas of micrographs was done with an EPSON GT-6000 laser scanner.

## Results

### Kinesin-dependent Motility along Flagellar Outer Doublet Microtubules

All tubulin assembly forms are made up of protofilaments of alternating  $\alpha$  and  $\beta$  tubulin subunits, but the relative stagger of protofilaments may vary. Two main lattice forms have been proposed (Amos and Klug, 1974), one where monomers of the same type are staggered by  $\sim 3.1$  nm (A-lattice),

the other with a stagger of 0.9 nm (B-lattice). Microtubules reassembled *in vitro* have predominantly the B-lattice (or, strictly speaking, a mixed lattice with mainly B and some A arrangement; see Mandelkow et al., 1977, 1986; McEwen and Edelstein, 1980). But in the case of intact flagella the two lattices are thought to occur in different microtubules, the complete A-subfiber and the incomplete B-subfiber, both of which make up the outer doublet microtubules (Fig. 1).

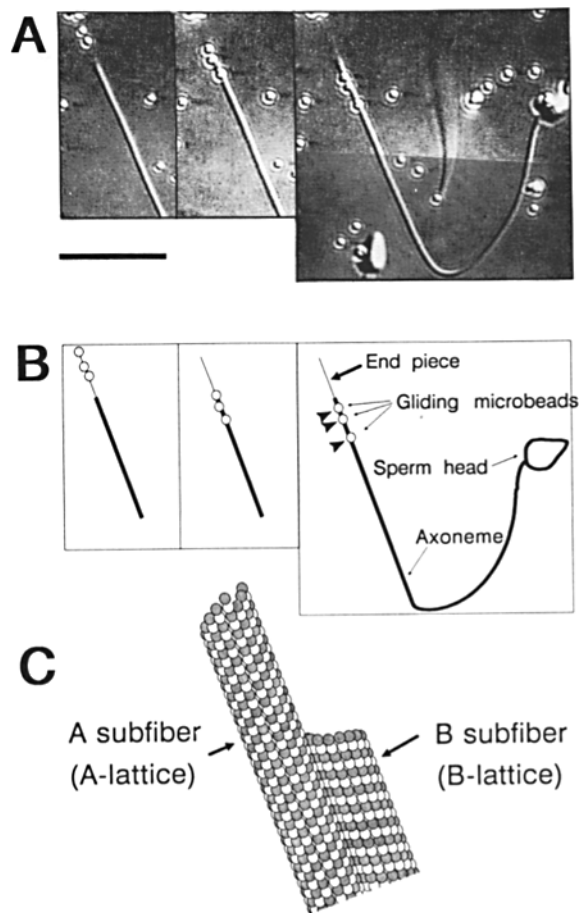
One question was therefore: Does the A- or B-lattice make a difference to kinesin-based movement? This point can be studied by observing the motion of kinesin-coated polystyrene beads on demembranated sea urchin flagella. The main part of the flagellum (9 + 2 region) has both A and B microtubules, but the tips contain protrusions which originate almost exclusively from the A-tubules (Satir, 1968; Allen and Borisy, 1974). Fig. 1 *A* shows snapshots of beads moving distally on a flagellum (high contrast) and continuing on the faintly visible microtubule extensions. Fig. 1 *B* is a diagram illustrating the results. The transitions are smooth, and there is no change in speed ( $\sim 0.6 \mu\text{m/s}$ ). This means that whatever structural change there may be in the microtubule track, it makes no detectable difference to the kinesin-microtubule interaction.

#### Structure of Polymorphic Assembly Forms of Tubulin by Electron and DIC Microscopy

The next step was to analyze the interaction of kinesin with different tubulin lattices. The polymorphic forms we studied here have been described by several authors in the past; but in order to make use of them we had to relate their ultrastructure (as seen by EM and optical diffraction) to their appearance and motility as seen by video microscopy. We first survey the structural features (see Figs. 2–4) before describing the motility results (see Figs. 5–7). The relationship between structure and motility will be summarized and discussed using the diagrams of Figs. 8 and 9.

In Fig. 2 we compare electron and video microscopic images of microtubules, C-tubules, zinc sheets, and duplex tubules; Fig. 3 shows higher magnification electron images and their optical diffraction patterns. Microtubules (Fig. 2, *A* and *B*) are long and straight, with typical lengths of 10–20  $\mu\text{m}$  in a motility experiment. C-tubules (Fig. 2, *C* and *D*) are also straight but shorter,  $\sim 5 \mu\text{m}$ . Their DIC contrast is roughly comparable to that of microtubules. Zinc sheets (Fig. 2, *E* and *F*) and duplex tubules (Fig. 2, *G* and *H*) have a higher contrast, they are usually shorter than microtubules ( $\sim 1\text{--}5 \mu\text{m}$ ), they tend to aggregate, and they are often somewhat curved. Given these features it is usually possible to distinguish preparations of the polymorphic assembly forms on the basis of the video images alone, although the final analysis rests on the electron images and optical diffraction patterns. Apart from the higher resolution of the EM the two microscopies differ in one important aspect: In the EM the structures tend to become flattened by the adsorption and drying process, while in solution they retain their curvature. This is particularly apparent for C-tubules and the zinc-induced aggregates, hence they are often referred to as sheets and zinc sheets (Fig. 3, *C* and *E*).

Microtubules are hollow cylinders consisting of parallel protofilaments, usually 13 or 14 when reassembled *in vitro* (Fig. 3 *A*). The front and back surface are superimposed in opposite orientation, hence the optical diffraction pattern is

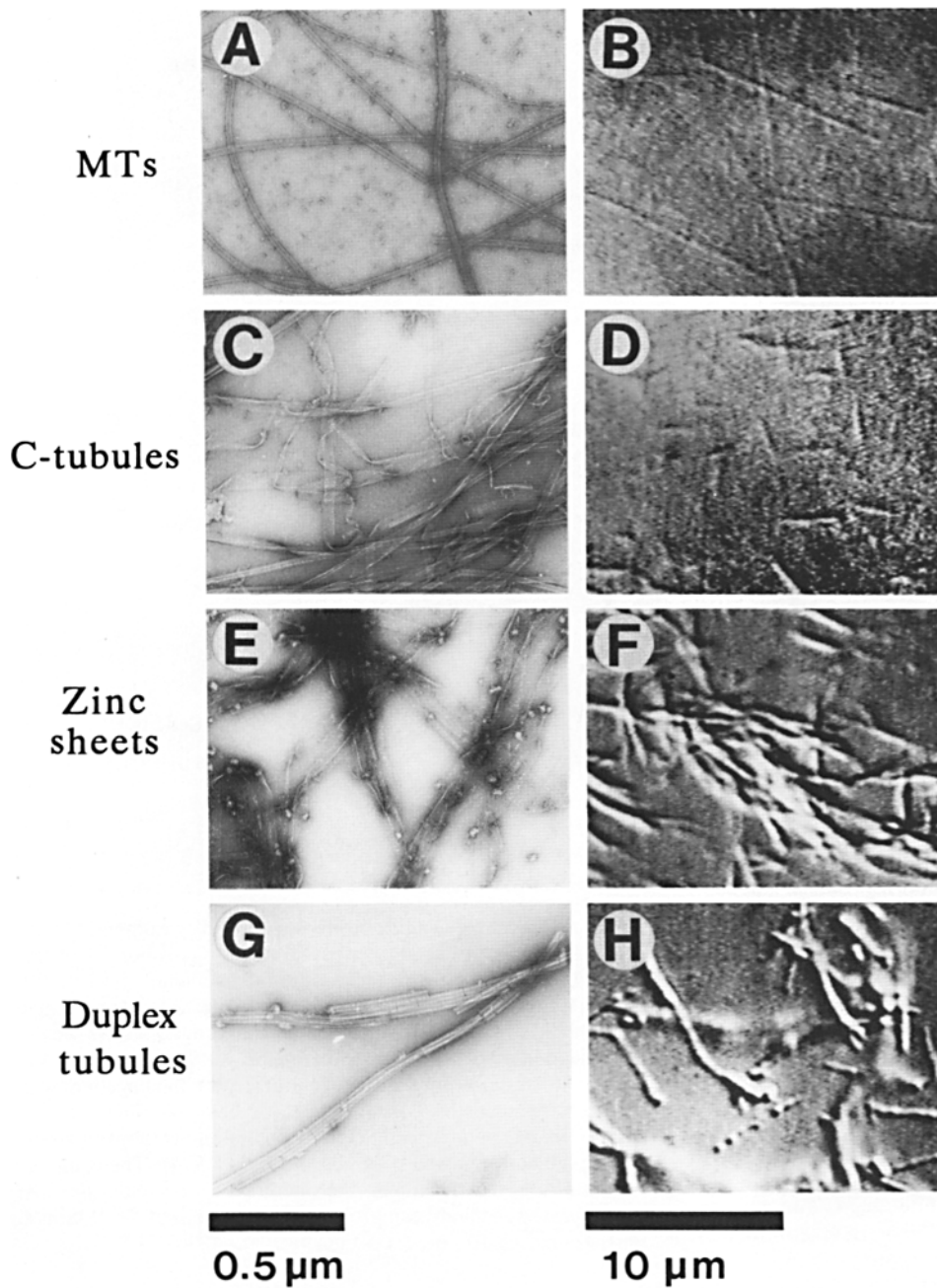


**Figure 1.** Movement of silica beads along flagellar axonemes of sea urchin sperm and protruding microtubules. (*A*) Sequential photographs (7 s apart). The bright lines are full axonemes (9+2 microtubules); the faint protrusion on the axoneme arises from single microtubules. Beads move distally and continuously across the tip. (*B*) Diagram illustrating the experiment, showing a sea urchin sperm with its demembranated flagellum (*right*), the flagellum with its extension, and the positions of three beads at different time points (*middle* and *left*). (*C*) Flagellar outer doublet tubule with the surface lattices proposed by Amos and Klug (1974). The complete A-tubule has the A-lattice, the incomplete B-tubule has the B-lattice. The A-tubule can protrude distally beyond the B-tubule. Bars: (*a* and *b*) 10  $\mu\text{m}$ ; (*c*) 50 nm.

roughly left/right symmetric (Fig. 3 *B*; for details see Amos and Klug, 1974). An important consequence of the cylindrical structure is that only the outside surface of protofilaments is available for interactions with other molecules, whereas the inside surface is shielded away (see Fig. 8 *A*).

C-tubules or sheets (Erickson, 1974) also consist of parallel protofilaments (typically 5–15, Fig. 3 *C*), but they fail to close into a cylinder. In solution they retain C-shapes whose curvature is usually less than that of microtubules (as seen by thin section EM, not shown). When they are flattened on a surface (as in negative stain EM) they form a single protein layer whose diffraction pattern is no longer symmetric about the meridian (Fig. 3 *D*). This means that both the inside and outside surfaces of a C-tubule are accessible for interactions (see Fig. 8 *B*).

Zinc sheets are more complex (Amos and Baker, 1979; McEwen et al., 1983). They may consist of many protofila-

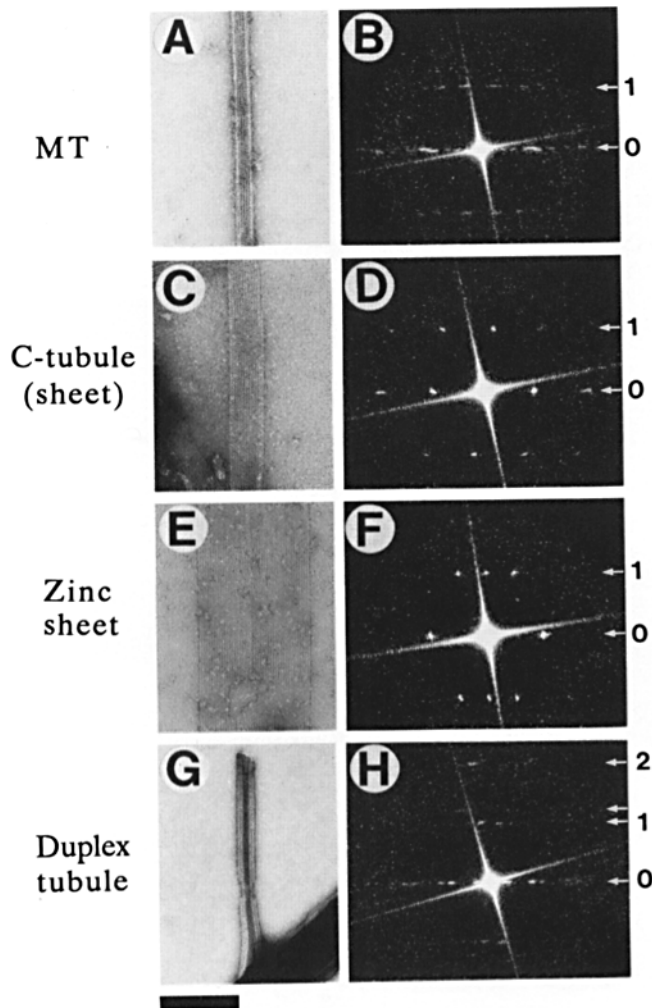


*Figure 2.* Electron micrographs at low magnification (*left*) and DIC images (*right*) of polymorphic assembly forms of tubulin. (*A* and *B*) Microtubules; (*C* and *D*) C-tubules; (*E* and *F*) zinc sheets; and (*G* and *H*) duplex tubules. Scale bar 0.5  $\mu\text{m}$  in EM and 10  $\mu\text{m}$  in DIC images.

ments (up to 100), often subdivided into two domains joined at a seam (left and right halves in Fig. 3 *E*). Each domain consists of protofilaments, but in contrast to microtubules or C-tubules they alternate in polarity (ends pointing up or down) and orientation (outside surface towards or away from the observer, see *arrows* and *shades* in Fig. 8 *C*). The optical diffraction pattern (Fig. 3 *F*) is left/right symmetric and contains a new set of reflections arising from the antiparallel pairing of protofilaments. Clearly, if kinesin required two or more protofilaments of equal orientation and polarity, then one would not expect motility with zinc sheets.

The presence of two domains represents another level of complexity (Fig. 4). The polarities of the domains are opposite, as deduced from Fourier transform image processing (see McEwen et al., 1983, and compare Fig. 9 *D*). The orientations of the protofilaments can be inferred from the

staining; those whose outside surface faces towards the carbon are darker on the EM print (marked with *dots* in Fig. 4 *A*). This analysis has two consequences. The two protofilaments forming the seam (*long arrows* below Fig. 4 *C*) are difficult to recognize clearly, but the structure of the domains implies that the rule of antiparallel alternation is broken; these protofilaments are only opposite in orientation but not in polarity (both pointing down in Fig. 4 *C*). Note that this is the same interaction as the one leading to the polymorphic forms of S-shapes or hooks which can be used for determining microtubule polarities (see Mandelkow and Mandelkow, 1979; Heidemann and McIntosh, 1980). The second implication is that kinesin molecules would push in opposite directions in the two domains (say, towards the *black arrows* in Fig. 4 *C*). Thus, they would neutralize one another, or possibly induce rotations (see Fig. 9 *D*).

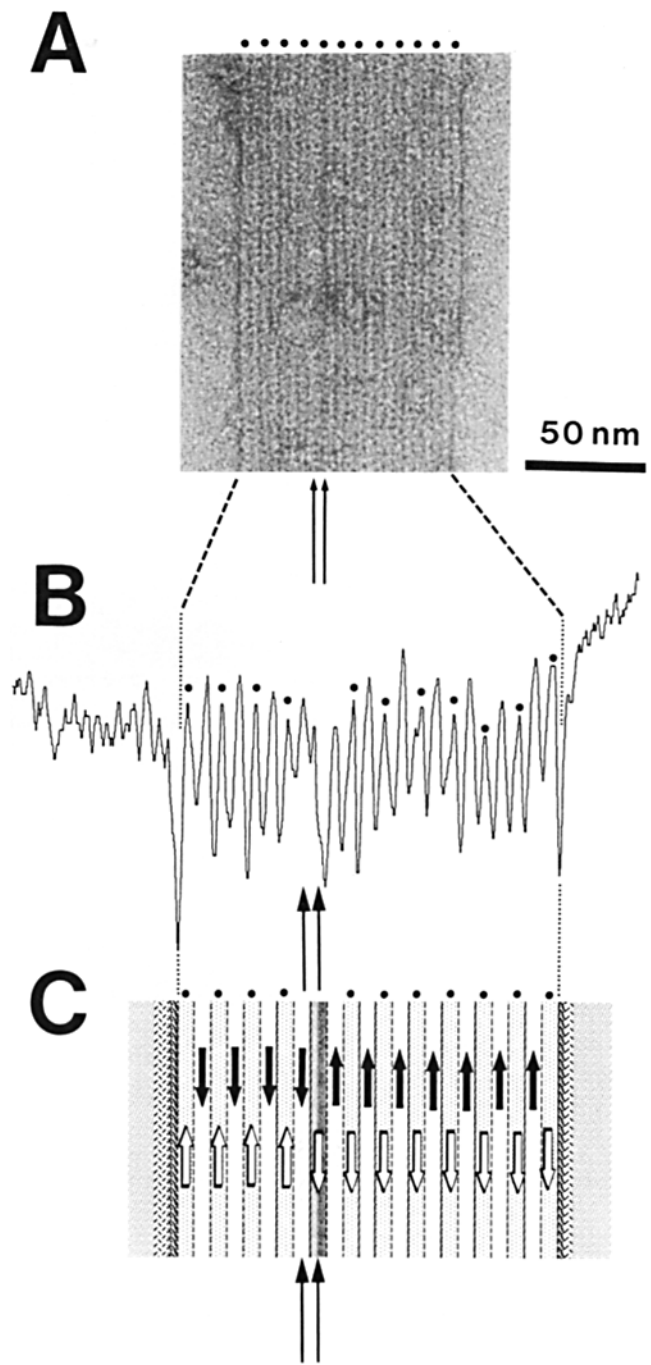


**Figure 3.** Electron micrographs at higher magnification (*left*) and optical diffraction patterns (*right*) of polymorphic assembly forms of tubulin. (*A* and *B*) Microtubules; (*C* and *D*) C-tubules; (*E* and *F*) zinc sheets; and (*G* and *H*) duplex tubules. The white arrows on the right of *B*, *D*, *F* indicate layer lines at the order of 4 nm, arising from the axial repeat of tubulin monomers along protofilaments. In *H* the arrows 1 and 2 show orders of 4.8 nm, the spacing of protofilaments on the outer coat. The 4-nm layer line from the innercoat is just above the arrow 1. Bar, 0.15  $\mu\text{m}$ .

Finally, duplex tubules (Jacobs et al., 1975; Fig. 3 *G*) and the related double-walled tubules (not shown, see Erickson and Voter, 1976) are structures consisting of a central microtubule and protofilaments wrapped around the outside, so that the two sets of protofilaments touch each other at nearly right angles (see Fig. 8 *D*). Theoretically, kinesin should cause this structure to move sideways, if it interacted with it at all.

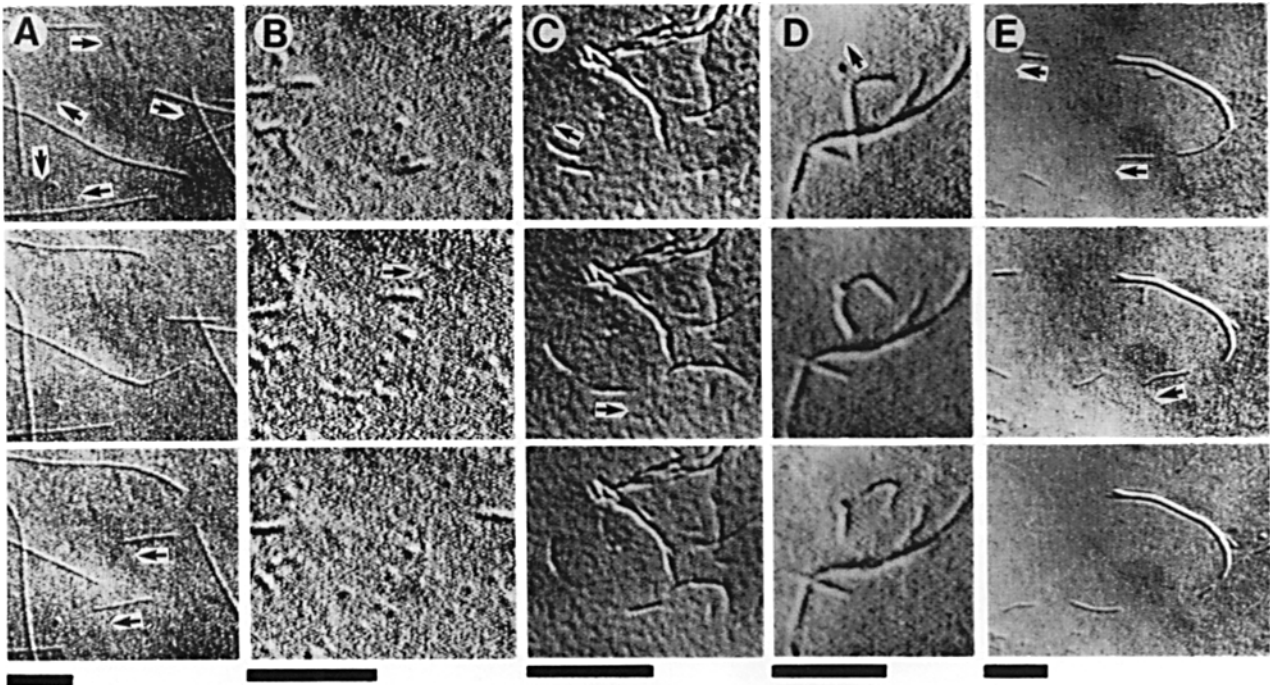
#### **Kinesin-induced Motility of Microtubules and Polymorphic Forms**

The motion of single microtubules on a kinesin-coated glass surface has been studied by a number of authors and is shown here as a standard of reference. The gliding is smooth, continuous, with a nearly constant velocity (0.6  $\mu\text{m/s}$  in our conditions), and usually along a straight path (Figs. 5 *A* and 6 *A*). Moreover, practically all microtubules were gliding



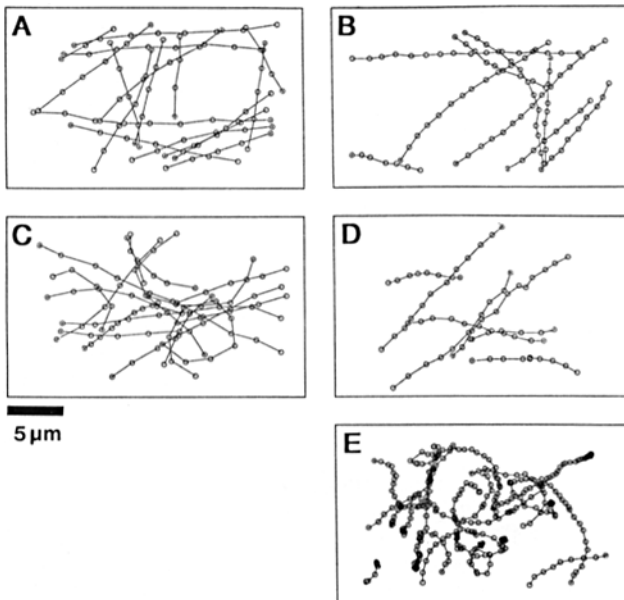
**Figure 4.** Domains and seam in zinc sheet. (*A*) Electron micrograph of negatively stained zinc sheet containing a seam (*long arrows below*). Every other protofilament has a lower brightness (best seen by glancing down the structure) and is labeled by a dot above. The separation of two protofilaments is  $\sim 5$  nm. (*B*) Densitometer trace of the electron micrograph in *A*. The dots indicate the same protofilaments as above. (*C*) Diagram showing protofilaments orientation and polarity in the two domains. White or shaded protofilaments, outside surface towards or away from observer. Arrows show polarity and direction of interaction with kinesin. The seam structure (*long arrows*) is unknown in detail, but note that the positions of shaded protofilaments (*dots*) could be extrapolated across the seam. This implies that the seam protofilaments have the same polarity. Bar, 50 nm.



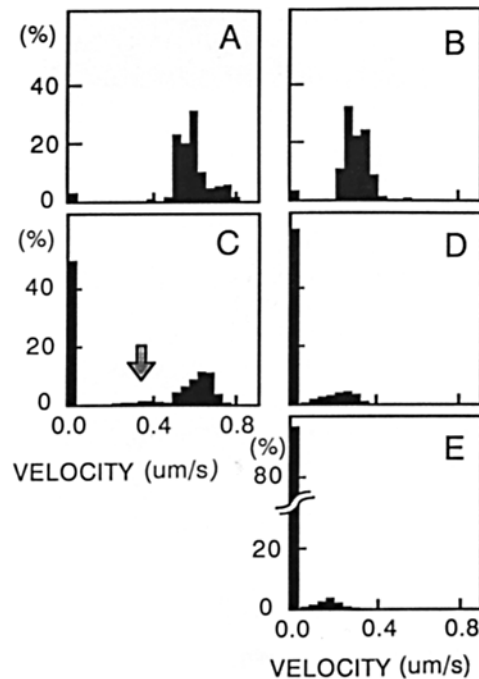


**Figure 5.** Sequential photographs showing the motion of polymorphic structures on kinesin-coated surfaces of glass. Arrows indicate direction of motion. (A) Taxol-stabilized microtubules, with intervals of 10 s; (B) C-tubules, 7-s intervals; (C) zinc-induced sheets, 20-s intervals; (D) zinc-induced sheets, 20-s intervals. For the bent structure refer to Fig. 9 c. (E) Immobile duplex-tubule bundle from which short microtubules are sliding out at different times. Bars, 5  $\mu\text{m}$ .

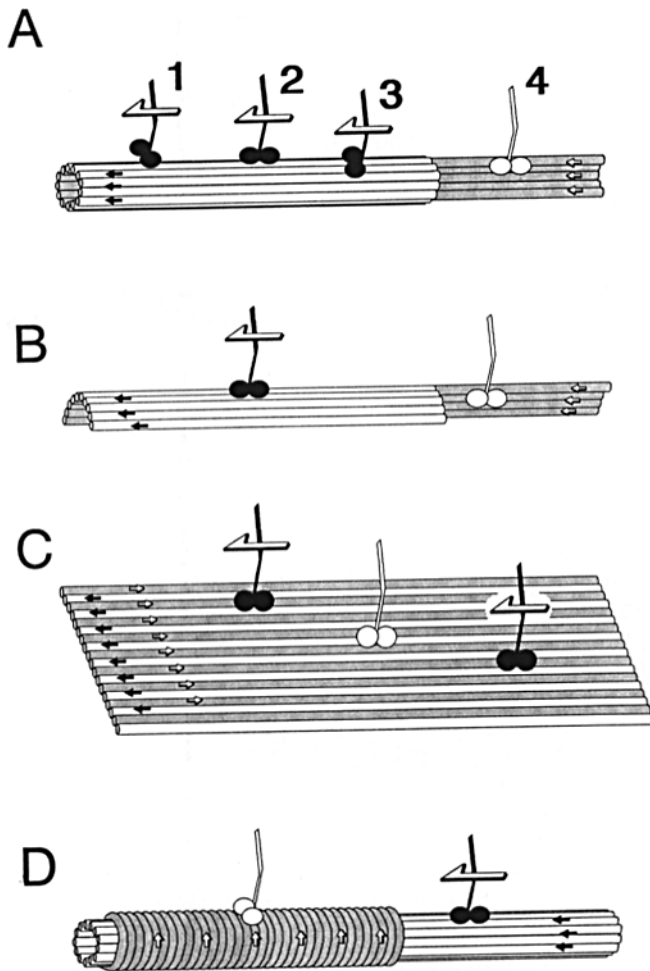
(see histogram of velocities, Fig. 7 A). Since the experiments with zinc sheets required DMSO we tested its effect on microtubule gliding as well. The overall appearance was similar (smooth, continuous), except that the speed was re-



**Figure 6.** Tracks of motion of polymorphic forms, with positions marked every 5 s. (A) Microtubules; (B) microtubules in 15% DMSO (note closer spacing of marks, indicating slower motion); (C) C-tubules; (D) C-tubules in 15% DMSO; (E) zinc sheets in 15% DMSO (note curved tracks and uneven speed). Bar, 5  $\mu\text{m}$ .



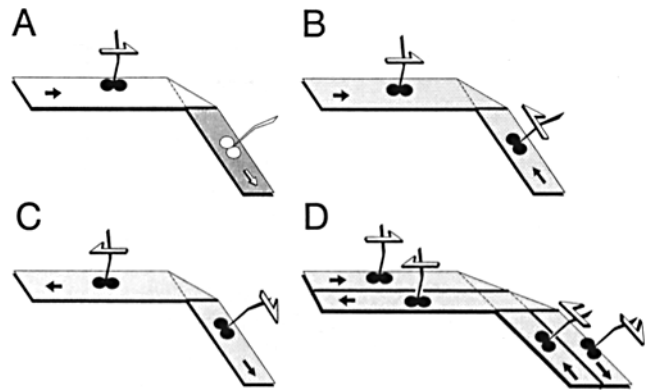
**Figure 7.** Velocity distributions of sliding structures. Bars at zero velocity (left in each histogram) indicate the percentages of immobile ones. (A) Microtubules (mean speed  $0.58 \pm 0.07 \mu\text{m/s}$ ,  $n = 163$ ); (B) microtubules in 15% DMSO (mean speed  $0.30 \pm 0.05 \mu\text{m/s}$ ,  $n = 148$ ); (C) C-tubules (mean speed  $0.58 \pm 0.10 \mu\text{m/s}$ ,  $n = 171$ , note higher proportion of immobile structures and fraction of slowly moving ones, arrow); (D) C-tubules in 15% DMSO (mean speed  $0.26 \pm 0.07 \mu\text{m/s}$ ,  $n = 91$ ); (E) zinc sheets in 15% DMSO (mean speed  $0.17 \pm 0.06 \mu\text{m/s}$ ,  $n = 135$ ).



**Figure 8.** Diagrams illustrating protofilament arrangements and possible interactions with kinesin. The small arrows on protofilaments indicate polarities (black arrow towards plus end on outside surface, white on inside surface). Kinesin molecules are shown with two heads, and moving in the direction of the one-sided arrows (same direction as *small black arrows* since kinesin is an antero-graduate motor). Black molecules can move, white ones cannot. The direction of motion shown corresponds to the bead movement assay. When kinesin is fixed on a glass surface, as in the microtubule gliding assay, the movement of the structures would be in the opposite direction. (A) Microtubule, all protofilaments have same polarities and orientations (outside out). A cutaway is shown on right. (B) C-tubule, all protofilaments have same polarities, but cylinder is not closed. (C) Zinc sheet, with alternating polarities and orientations, (D) duplex tubule.

duced to  $\sim 0.3 \mu\text{m/s}$ , suggesting an interference of DMSO with some step affecting the kinesin power cycle (Figs. 6 B and 7 B). When plotting the gliding tracks at equal time intervals one obtains straight lines, with widely spaced points for fast movement (Fig. 6 A) and more densely spaced points for slow movement (Fig. 6 B).

C-tubules also showed kinesin-dependent gliding along straight paths and with similar velocities (Figs. 5 B, 6 C, and 7 C). Moreover, adding DMSO halved the velocity, as with microtubules (Figs. 6 D and 7 D). The main difference compared to microtubules is the high percentage of immobile structures (50%, and up to 80% in DMSO). The obvious interpretation is that kinesin generates normal motility on the



**Figure 9.** Motility of kinesin on folded structures. Only black kinesin molecules can move (in direction of one-sided arrow), white ones cannot. White surface in *a* represents outsides of protofilaments, dark shade represents inside surface. Medium shade in *b-d* indicates mixture of alternating orientations in zinc sheets. (A) C-tubules; only the left part generates motion. If the black kinesin were fixed, the C-tubule would glide to the left. (B) Zinc sheet, one domain folded over. Both parts can generate motion in opposite directions. If the kinesin molecules were fixed, they would stretch the structure. (C) Folded zinc sheet in an opposite direction to B. In the gliding assay the two parts would tend to move towards one another, the result of which might be a motion in an intermediate direction (see Fig. 5 c). (D) Folded zinc sheet with two domains, creating a situation intermediate between B and C.

outside of a C-tubule, but not on the inside (see Fig. 8 B). The histograms also reveal a fraction of polymers that move distinctly slower than the bulk, generating a "tail" in the velocity distribution (Fig. 7 C, arrow). We interpret these polymers as folded ones, where only that part of the structure moves actively whose outside surface is oriented towards the glass (Fig. 9 A, left); but when it is folded the inside surface faces the glass, it does not contribute to active movement and must be dragged along (Fig. 9 A, right). Such folded C-tubules were frequently observed in the EM (e.g., Fig. 2 C).

Theoretically, the failure of some C-tubules to move might be explained by an alternative mechanism. If the inside surface of protofilaments adhered strongly to the glass surface, this might prevent any motion by kinesin, independently of whether kinesin interacted with the inside or not. We consider this possibility unlikely for two reasons. One is that the affinity of microtubules or polymorphic structures to the glass surface is weak, so that one can detach and remove them by perfusing the chamber with buffer. Secondly, we will show later that a duplex tubule can move when it is partially uncoated, again arguing that the inside surface of protofilaments (exposed on the outer coat; Fig. 8 D) interacts only weakly with the glass. Thus, we assume that the failure of a polymer to move can be accounted for by the way it does or does not interact with kinesin.

In the case of zinc sheets the motility assay could only be done in DMSO. The reason was that zinc inhibits the motility of kinesin, so that it had to be removed by chelation with EGTA before starting the motility experiment. On the other hand, the zinc sheets depolymerized quickly when zinc was removed, but they could be stabilized by 15% DMSO. This was the reason for the control experiments in DMSO in the cases of microtubules and C-tubules described above.

Zinc sheets attached to kinesin-coated glass showed active

gliding, but the percentage of moving structures at any given time was low,  $\sim 10\%$  (Figs. 5 C and 7 E). However, the paralysis was not permanent: When observing for extended periods gliding could be detected with the majority of the structures (50–70%). In other words, a relatively high percentage of zinc sheets was capable of movement, but the motion was discontinuous. This is illustrated for selected structures in Fig. 6 E where the clustering and overlap of marks indicates periods of little or no gliding. This behavior was not due to contamination of zinc ions ( $\sim 0.6$  nM after chelating with 0.5 mM EGTA) since microtubules showed their normal motility when mixed with the largely paralyzed zinc sheets. The mean velocity of zinc sheets is  $0.17 \mu\text{m/s}$ , comparable but somewhat less than that of C-tubules or microtubules in the same buffer conditions. Smaller zinc sheets tended to glide more continuously than larger ones. Considering that the observed motion might average out brief pauses undetectable under the light microscope, the mean velocity of Fig. 7 E would be an underestimate. Consistent with this we found that the maximal velocity of zinc sheets was roughly the same as that of microtubules ( $\sim 0.3 \mu\text{m/s}$  in the buffer conditions).

A notable feature of zinc sheets was that almost all tracks were curved (Fig. 6 E). This included even sharp turns with a radius of curvature below  $1 \mu\text{m}$  (i.e., a magnitude comparable to the size of the zinc sheets), creating the appearance of a rotatory motion. Another frequent pattern of motion was bending and looping. Fig. 5 D shows an example of a kinky zinc sheet gliding initially in the direction of the kink, i.e., at  $45^\circ$  to the straight sections. It appears as if each of the straight sections pushed towards the kink so that the observed direction was the resulting addition of the two forces (see Fig. 9 C for an interpretation in terms of a folded structure). Some zinc sheets grew very long (up to  $20 \mu\text{m}$ ). In these cases they usually lay paralyzed on the glass, but they could also show bending motion and a tendency to break into smaller pieces which then moved independently. All of these features can be interpreted by assuming that a single protofilament suffices for kinesin-induced movement without requiring adjacent ones with equal polarity. However, the domain structure and the possibility of folds in zinc sheets creates complicated patterns.

Duplex tubules were the easiest structures to analyze: They adsorbed onto the kinesin-coated glass but showed no motility. Only when the protamine concentration was reduced by dilution, thin filaments began to emerge from the duplex tubules, resulting in normal gliding (Fig. 5 E). These filaments had a contrast similar to microtubules, as if the duplex tubules had lost some of their coat by endwise stripping. This interpretation was confirmed by EM. The observations imply that there is no motility when long and straight protofilaments are not available, or when only the "inside" surface of protofilaments is exposed to the environment.

## Discussion

The gliding of kinesin-coated beads, or the gliding of microtubules over a kinesin-coated surface has been described by several authors (for reviews see Vale, 1987; Vallee and Shpetner, 1990; Sawin and Scholey, 1991). Most studies have analyzed the structural requirements for gliding in one of two ways, by altering kinesin, or by altering the microtu-

bule surface. Kinesin can be manipulated by cleaving the head (Kuznetsov et al., 1989), construction of various recombinant forms (Yang et al., 1990), changing the nucleotide cofactor (Bloom et al., 1988; Shimizu et al., 1991), binding of specific antibodies (Ingold et al., 1988; Hirokawa et al., 1989), or by comparing different members of the kinesin superfamily (Goldstein, 1991). These studies have shown that the head is responsible for force generation and ATPase activity and that the movement usually corresponds to anterograde axonal transport, although there are exceptions (e.g., the retrograde motor *ncd*, Walker et al., 1990; McDonald et al., 1990). The microtubule surface was altered by the presence or absence of MAPs, or by proteolytic cleavage of tubulin. The data showed that MAPs generally do not interfere with motility, except MAP2 in certain conditions (Von Massow et al., 1989; Heins et al., 1991), and that the COOH-terminal domain of tubulin is important for the interaction with motor proteins (Paschal et al., 1989; Rodionov et al., 1990; Goldsmith et al., 1991).

In most of these studies the question of the microtubule surface lattice was not addressed directly, but there are several sets of experiments which are relevant in our context. One is that the kinesin ATPase is activated by microtubules but not tubulin (Kuznetsov and Gelfand, 1986; Hackney, 1988), implying that the assembled state is necessary for motility. Secondly, high precision analysis of beads powered along microtubules by kinesin showed a step size of 4 nm and a narrow lateral confinement (Gelles et al., 1988), suggesting that one or two adjacent protofilaments and the spacing of tubulin monomers along protofilaments were determining factors. Furthermore the displacement of flagellar microtubules driven by dynein contains components of 8 and 4 nm (Kamimura and Kamiya, 1992), emphasizing the role of tubulin dimers and monomers along protofilaments.

The questions to be answered can be derived from the microtubule structure (Fig. 8 A): Microtubules have straight protofilaments, the protofilaments have the same polarity and orientation ("outside out"), they are juxtaposed with a cylindrical curvature, and the protofilaments can be staggered in different ways, giving rise to the A- or B-lattices (Fig. 1 C). We can therefore ask: Is the stagger between protofilament important for kinesin-dependent motility? Is the juxtaposition of protofilaments important? If so, does the cylindrical curvature play a role? Which surface of tubulin does kinesin interact with? Do protofilaments need to be straight for kinesin-induced motility?

The experiments with the polymorphic assembly forms of tubulin offer some answers to these questions. The structures are diagrammed in Figs. 1 C, 8, and 9. In outer doublet microtubules (Fig. 1 C) the A- and B-tubules have the same monomer lattice, but they are thought to differ in the stagger of protofilaments which creates different dimer lattices. Otherwise the protofilaments are identical, i.e., they consist of an alternating chain of  $\alpha$  and  $\beta$  tubulin (we disregard here the seam between A- and B-tubules). This substructure of protofilaments is implicit in the diagrams of Figs. 8 and 9. Fig. 8 A is a microtubule, with all protofilament polarities identical (*arrows* towards plus end) and surfaces outside out (*white*) while the inside surface (cut open on the right, *shaded*) is shielded away from the environment. Two-headed kinesin molecules are shown to move in the anterograde direction (*one-sided arrows*). One could imagine four possi-



bilities: (1) only one head interacts with the microtubule; (2) both heads interact with the same protofilament; (3) the two heads interact with different protofilaments; or (4) kinesin might interact with surfaces other than the outside, such as the inside. These possibilities cannot be distinguished by observing just microtubules.

Cytoplasmic microtubules generally have a surface lattice of the B-type (apart from a discontinuity or seam; see McEwen and Edelstein, 1980; Mandelkow et al., 1986). However, in the case of flagellar outer doublet microtubules it has been proposed that there are two lattices, A and B (Amos and Klug, 1974, illustrated in Fig. 1 C). Single microtubules may protrude from the tip (Satir, 1968; Allen and Borisy, 1974); these emerge from the A-tubules and are thus thought to have the A-lattice. This structure is therefore suitable to test possible differences between the two lattices with regard to motility.

Using the bead movement assay, we have consistently observed a smooth transition from the bulk of the flagellum to the protruding tip. This could be interpreted in three ways. Firstly, given a choice of A- and B-lattices in the flagellum, kinesin might prefer the A-lattice over the B-lattice and thus transit smoothly to the protruding A-tubule. We consider this unlikely since kinesin has no difficulty moving along cytoplasmic microtubules from different sources which have mostly the B-lattice. Secondly, A- and B-tubules might actually not differ in terms of tubulin arrangement; in this case the smooth transition would be the expected result (the question of whether the A-tubule in fact has the A-lattice may be debated; see Linck and Langevin, 1981). Thirdly, kinesin might not care what type of microtubule it moves on; what matters is only that both consist of protofilaments. As discussed below we consider this the most likely interpretation.

C-tubules differ from microtubules in two ways (Fig. 8 B). They are opened up cylinders, so the inside and outside surfaces of protofilaments are both accessible. Secondly, their curvature is less than that of microtubules (this can be enhanced by DMSO and is one reason for the lack of closure). Otherwise the protofilaments have the same polarity and surface lattice. The characteristics of motion are very similar to microtubules in terms of speed and straightness of tracks (Figs. 6, C and D and 7, C and D), suggesting that the cylindrical curvature is not very important for gliding. However, there is a high proportion of immobile polymers (50% or more; Fig. 7, C and D). This is consistent with the assumption that the inside surface of C-tubules cannot interact with kinesin in a force-producing manner. This result is not surprising, considering that motor proteins bind to a region of tubulin near the COOH-terminus which is available on the outside surface but not on the inside. More generally speaking, if one considers the usual specificity of protein-protein interactions one would expect only one site of interaction between tubulin and kinesin, but not two on opposite sides. Thus, in Fig. 8 we have drawn the kinesin molecules facing the shaded inside of a protofilament in white to indicate their inability to move. One consequence is that when a C-tubule folds over, only one of the two sections can generate active gliding, while the other would be dragged along (Fig. 9 A).

This lack of interaction with the inside surface also explains the behavior of duplex tubules (Fig. 8 D). Their outer coat consists of one or more protofilaments coiled around the inner microtubule in an inside-out fashion (Jacobs et al.,

1975). This is similar to other forms of coiled protofilaments such as rings (Voter and Erickson, 1979), double walled microtubules (Erickson and Voter, 1976), hoops (Mandelkow et al., 1977), or fraying microtubule ends during disassembly (Mandelkow et al., 1991). Since kinesin cannot move along an inside surface it cannot move on the outer coat of duplex tubules either (white kinesin on the left of Fig. 8 D). Whether or not the coiling as such plays a role cannot be decided. Interestingly, when part of the outer coat is stripped away, exposing the inner microtubule, movement commences again in the usual fashion (black kinesin on the right of Fig. 8 D). This shows that the outer coat of duplex tubules can attach to the glass surface, but otherwise presents little resistance when pulled or pushed.

Finally, zinc sheets are the most versatile and complicated structures (Fig. 8 C). The basic building principle is that protofilaments alternate in polarity and orientation, that is up/down (black and white arrows) and at the same time are oriented with "outside" or "inside" towards the observer (white and shaded) (Baker and Amos, 1978; McEwen et al., 1983). Thus every other protofilament is unavailable for kinesin (see white kinesin molecule on shaded protofilament). If kinesin required two or more juxtaposed protofilaments with the same polarities one would not expect any movement of zinc sheets. The observation is that a large fraction of zinc sheets are indeed immobile, but not permanently; and those that move do so at speeds which are only moderately slower than microtubules or sheets in the same buffer conditions. Our interpretation is that kinesin can indeed move along single protofilaments, without requiring neighboring ones (black kinesins in Fig. 8 C). This would be consistent with the options 1 and 2 in Fig. 8 A.

The jerky and curved movement of zinc sheets requires additional interpretation; it is probably related to two other structural features. Firstly, zinc sheets often consist of two domains which have opposite polarities. This means that kinesin molecules pushing on neighboring domains would work against one another, and the net movement might depend on the excess number of kinesin molecules on one of the domains. Secondly, zinc sheets often fold over at an angle (Fig. 9, B-D). Because of the substructure of each domain this means that the effective polarities are also inverted. Thus, in contrast to folded C-tubules, both parts of a folded zinc sheet can glide. If there is only one domain, both parts would tend to glide either away from the fold (as in Fig. 9 B; this might lead to breakage of the structure), or they could tend to glide towards the fold (Fig. 9 C), with a net movement in some other direction, e.g., in the direction of the kink (Fig. 5 D). When the zinc sheet has two domains, as in Fig. 9 D, the movement becomes even less predictable. Note that the black arrows in Fig. 9 indicate the migration of kinesin or of a kinesin-loaded bead (bead motility assay); in the corresponding gliding assay (where kinesin is fixed to the glass surface) the gliding direction would be opposite to the black arrows.

Two caveats should be borne in mind when interpreting the observations. First, kinesin has two heads, and therefore it is conceivable that the heads reach across and attach to two parallel protofilaments of a zinc sheet, leaving the intervening one (with the wrong orientation) out. In this case, the motile unit would involve two heads and two protofilaments whose orientation is the same (similar to microtubules) but

whose lateral separation is 10 nm instead of the usual 5 nm (see Fig. 8 C). Secondly, a single kinesin molecule is strong enough to carry an entire microtubule (Howard et al., 1989). This could conceivably lead to the amplification of irregularities in the surface lattice, especially in the case of zinc sheets. Ideally these possibilities should be checked with single headed kinesin and single protofilaments which are however not available for experimentation at present.

In summary, the simplest interpretation of our studies is that kinesin will move along polymers of tubulin if they contain (nearly) straight protofilaments with their "outside" surface available for interaction. Kinesin does not care about the difference between A- and B-lattices, it does not need the cylindrical curvature of a microtubule, and in principle it can move along the track of one protofilament, even when the neighbors have the wrong orientation and polarity. Thus the minimal functional motile unit is probably made up of one protofilament (as shown here) and one kinesin head (see Yang et al., 1990, and compare option 1 in Fig. 8 A). The existence of this minimal unit does however not imply that nature uses only this unit. Nature has provided for redundancy: Two kinesin heads and 13 parallel protofilaments make sure that the movement is smooth and straight if necessary.

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