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Evidence for a Novel Affinity Mechanism of Motor-assisted Transport Along Microtubules[V]

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In microtubule (MT) translocation assays, using colloidal gold particles coupled to monoclonal tubulin antibodies to mark positions along MTs, we found that relative motion is possible between the gold particle and an MT, gliding on dynein or kinesin. Such motion evidently occurred by an affinity release and rebinding mechanism that did not require motor activity on the particle. As the MTs moved, particles drifted to the trailing edge of the MT and then were released. Sometimes the particles transferred from one MT to another, moving orthogonally. Although motion of the particles was uniformly rearward, movement was toward the (−) or (+) end of the MT, depending on whether dynein or kinesin, respectively, was used in the assay. These results open possibilities for physiological mechanisms of organelle and other movement that, although dependent on motor-driven microtubule transport, do not require direct motor attachment between the organelle and the microtubule. Our observations on the direction of particle drift and time of release may also provide confirmation in a dynamic system for the conclusion that β tubulin is exposed at the (+) end of the MT.

INTRODUCTION

To study in detail how molecular motors, in particular dyneins, exert a force to move a microtubule (MT), we have been developing an in vitro MT translocation assay system with high spatial and temporal resolution. In principle, this assay should allow us to collect large amounts of data by computer-aided motion analysis to make robust measurements of motor molecule parameters, such as step size or duty cycle. Our approach was to place colloidal gold particles of ~40 nm diameter (Frens, 1973), very bright objects in dark-field microscopy whose position could be accurately determined, onto an MT and to trace this position as a marker when the underlying MT moved. To ensure stable binding to the MT lattice, the gold particles were first coated with a secondary antibody that binds anti-tubulin antibodies, and then they were incubated with various monoclonal anti-tubulin antibodies. The particles were then perfused into a microchamber under a video-enhanced dark-field microscope, where motor-driven MT translocation could be activated by ATP perfusion. By choosing appropriate antibodies, we were able to observe that the antibody-coated particles became attached to and moved together with the MTs; however, unexpectedly, rather than remaining bound in a fixed position, many particles started changing position, drifting systematically toward the trailing edge of the attached MT. Although these particles were unsuitable for our original purpose, aspects of the phenomenon of drifting seemed worth exploring further. This study describes certain quantitative observations about this phenomenon and our conclusions.

The structure of the tubulin has recently been solved at high resolution using stabilized tubulin sheets and electron crystallography (Nogales *et al.*, 1998, 1999). Although originally the conclusions were controversial (Hoenger *et al.*, 1995; Song and Mandelkow, 1995; Fan *et al.*, 1996; Wade and Hyman, 1997), a consensus has now arisen that β-tubulin is at the (+) or fast polymerizing end of the MT. Our observations regarding the direction of particle drift and the time of release from the end of the MT may provide confirmation of this conclusion. In turn, these considerations lead to a hypothesis regarding a mechanism by which nonmotor-driven particle movement may occur and a discussion of the consequences of such a mechanism for cell function.

[V] Online version of this article contains video material for Figures 2, 3, and 7. Online version available at www.molbiolcell.org.

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Abbreviations used: MAPs, microtubule-associated proteins; MT, microtubule.

MATERIALS AND METHODS

MTs, Dynein, and Kinesin

MTs were polymerized from phosphocellulose column-purified twice-cycled calf brain tubulin in the presence of 1 mM GTP and stabilized using 5 μM taxol (Williams and Lee, 1982). Ciliary dy-

neins (22S dynein: outer arm dynein; 14S dynein: inner arm dynein) were purified from *Paramecium tetraurelia* or *Tetrahymena thermophila* (Hamasaki *et al.*, 1991; Barkalow *et al.*, 1994). Kinesin was expressed in *Escherichia coli* and purified according to procedures of Jiang *et al.* (1997) and Hancock and Howard (1998), or purchased from Cytoskeleton (Denver, CO). The kinesin constructs were a kind gift from Drs. David Hackney (Carnegie Mellon University, Pittsburgh, PA) and Jonathon Howard (University of Washington, Seattle, WA). The proteins were aliquoted and stored at −80°C until use.

Other Chemicals and Methods

Taxol (paclitaxel) was purchased from Calbiochem (La Jolla, CA). Dimethylsulfoxide is the water-free grade and was obtained from Aldrich (Milwaukee, WI). ATP disodium salt was obtained from Boehringer Mannheim (Mannheim, Germany). All chemicals were reagent grade.

Tubulin Antibody-coated Gold Particles

Gold particles with an average diameter of ~40 nm were made after the method of Frens (1973). The gold particles were then coated with 1:500 volume of anti-mouse IgG + IgM (Jackson ImmunoResearch, West Grove, PA) by incubation for 20 min at room temperature, followed by a blocking by 2 mg/ml each casein and BSA; 1% carbowax was added after 10 min incubation. After another 10 min, excess antibodies and blockers were washed away using several centrifugations. The particles were resuspended in 1 mM phosphate buffer, in the presence of 0.02% sodium azide and blocking reagents described above, then stored at 4°C. Just before experiments, an aliquot of the particles was taken out and mixed with anti-tubulin antibodies. Mainly, B-5–1-2 (a monoclonal antibody against α-tubulin [Sigma, St. Louis, MO]; LeDizet and Piperno, 1987) and TUB 2.1 (a monoclonal antibody against β-tubulin [Sigma]; Gozes and Barnstable, 1982) were used. Other anti-α- and anti-β-tubulin, anti-γ-tubulin antibodies, anti-kinesin antibody, and anti-actin antibodies were used as controls. Anti-actin was a rabbit antibody. The anti-tubulin or control antibodies were appropriately diluted (usually 1:5 for B-5–1-2 and 1:25 for TUB 2.1, which varied depending on the batch of antibody obtained and on storage conditions) and mixed with the particles for at least 30 min at room temperature. The mixture was further diluted (1:30) with in vitro translocation assay motility buffer (composition shown below) with or without ATP and perfused into the in vitro MT translocation assay chamber. In some cases, the excess tubulin antibodies were removed from the particle suspension by centrifugation; however, this process generally made the particles very unstable. The tubulin antibody-coated particles were usable for the assays only on the day of preparation or on the following day.

In Vitro MT Translocation Assays

In vitro MT translocation assays were performed using our standard procedures (Hamasaki *et al.*, 1991, 1995). Briefly, as illustrated in Figure 1, assays were performed in microchambers that were constructed from a glass slide (3010 or 3011; Gold Seal, Portsmouth, NH) for dynein assays and Fisherfinest 12–544-1 (Fisher Scientific, Pittsburgh, PA) for kinesin assays, number 0 cover glass (Gold Seal), and 3M (St. Paul, MN) 198 or 39 double-stick tape. Slides were cleaned thoroughly using acetone followed by ethanol and then water before chambers were constructed. The applicable motor molecule, dynein or kinesin, was perfused into a chamber and allowed to absorb onto the glass surfaces for a few minutes, followed by washing several times with motility buffer (10 mM $MgSO_4$, 1 mM EGTA, 2 mM dithiothreitol, 10 mM Tris-acetate, pH 7.5) to remove excess motors. MTs, together with the motility buffer containing 1 mM ATP (ATP buffer), were perfused into the chamber to initiate translocation. After the excess MTs were removed by a further perfusion of ATP buffer, the same buffer now containing tubulin antibody-coupled gold particles was perfused into the chamber. After a few minutes to permit the particles to attach to MTs, more ATP buffer was perfused through the chamber to remove excess particles. An appropriate field that showed moving MTs with attached particles was selected for video recording. MTs and particles on the glass slide surface were visualized using a dark-field Zeiss microscope (with Ultrafluar 100× optic lens [Zeiss, Thornwood, NY]) with a 200-W mercury-arc lamp and CIT-68LX camera (Dage-MTI, Michigan City, IN) and recorded with a Panasonic (Secaucus, NJ) 7300 S-VHS recorder. The recorded images were examined frame by frame, then either hand-traced onto transparent plastic sheets placed on the video monitor or printed out using a video copy processor (Mitsubishi, Cypress, CA, P71U) for qualitative analysis, or digitized into motion JPEG files and then processed using Adobe Premier (ver 5.0), Photoshop (ver 5.0), and Illustrator (ver 8.0) for motion-image presentations. All statistics were calculated using Sigmastat (ver 2.0; Jandel Scientific, Corte Madera, CA).

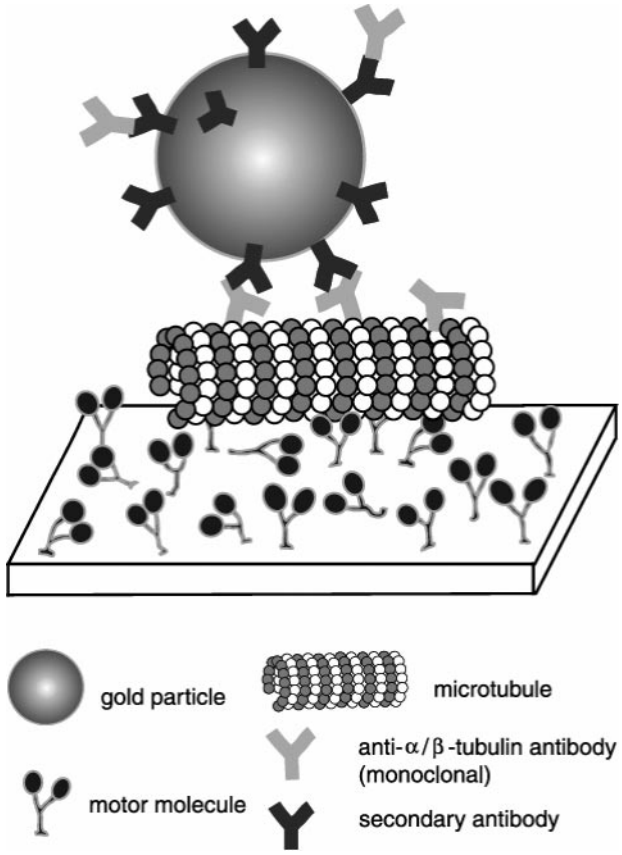

Figure 1. Scheme of the experiments. A 40-nm gold particle was attached via monoclonal anti-tubulin antibody and secondary antibody to a microtubule (MT) that was gliding on a motor molecule substratum, such as dynein or kinesin.

RESULTS

Particles Drift on Moving MTs and Transfer between MTs

When the solution containing particles labeled with anti-α(B-5–1-2)- or anti-β(TUB2.1)-tubulin antibodies was per-

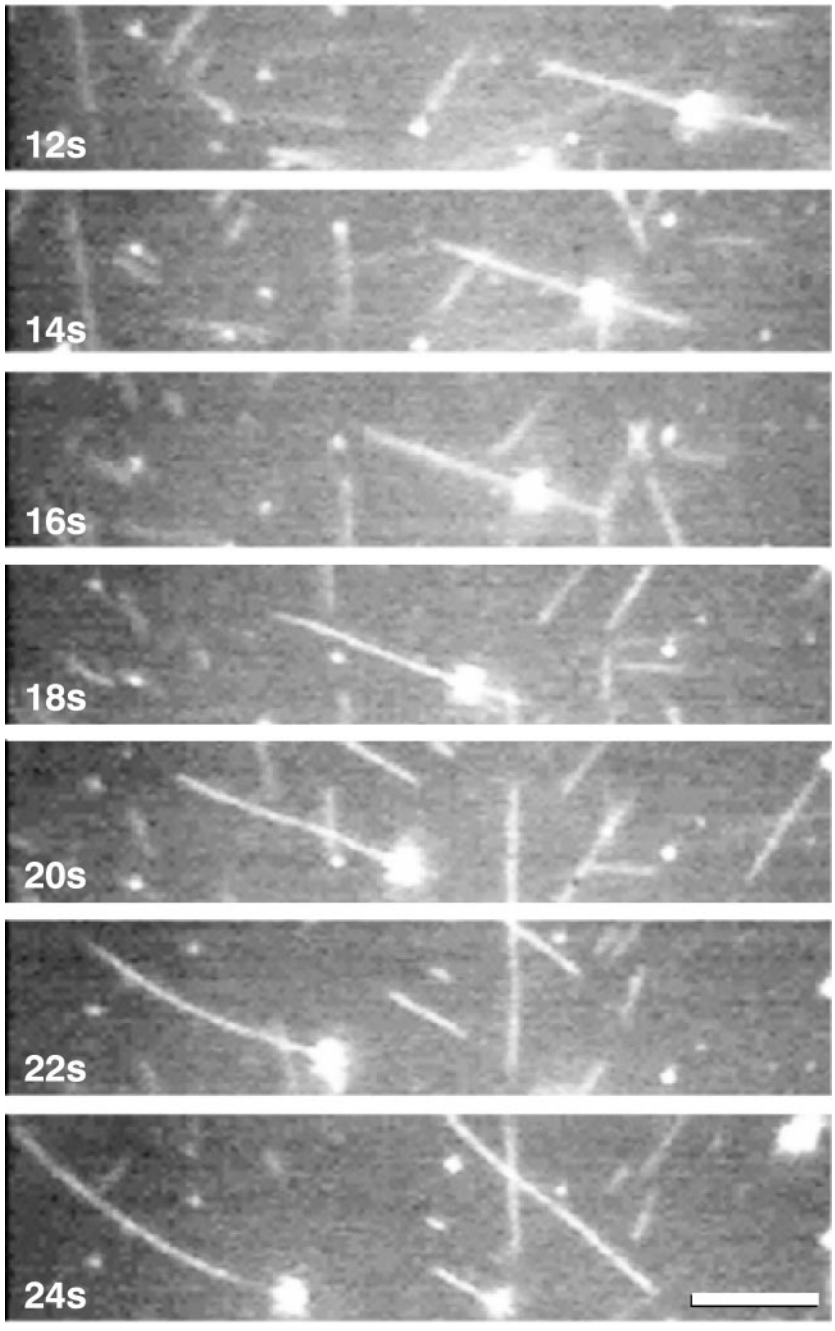

Figure 2. Movement of an MT and a gold particle attached using TUB 2.1 (anti-β-tubulin antibody) by 14S dynein; portion of a video image captured at 2-s intervals. The MT was moving from lower right to upper left. The position of the attached particle gradually shifted toward the trailing edge of the MT. At 20 s the particle reached the trailing edge, stayed until 22 s, and then dropped onto the surface at 24 s. Bar, 10 μm.

fused into the translocation chamber, some particles attached to MTs. Other α- and β-tubulin antibodies tended to cross-link MTs or did not produce consistent particle attachment, whereas anti-γ-tubulin antibodies produced some attachment, but only at MT ends, which indicates that some γ-tubulin may be present in our polymerized MT preparation. When particles were labeled with anti-kinesin mouse antibodies or anti-actin rabbit antibodies, no attachment of particles to MTs was observed. In all experiments, some particles attached to the surface of the protein-coated glass slide, presumably by nonspecific hydrophobic and electrostatic interactions, and remained stationary with no observable Brownian movement, while the rest were washed away with perfusion.

As shown in Figure 2, when ATP was perfused into the chamber, the MTs moved, and the particles coated with anti-tubulin attached to MTs moved together with them; however, instead of remaining fixed in position along the MT, many of these particles gradually drifted back along the moving MT toward its trailing edge. Although subtle

changes could perhaps be undetected, neither the particles nor the MTs showed obvious rotation. Of the particles that attached along the length of an MT, more than half showed drift. Often the particles drifted down to the very end of the MT, stayed there for a few seconds, and then released into the surrounding solution or dropped onto a surface of the glass slide. Particles released into solution moved rapidly away from the MT by Brownian motion. Similar movements were seen on the kinesin substratum. As expected, no MT or particle movement was observed when the chamber was coated with anti-α (B-5–1-2) antibody instead of dynein or kinesin, although ATP was present. When 14S dynein was later perfused into these chambers, <1% of MTs moved. As seen in Figure 3, sometimes while moving along the MT the particles transferred from one MT to another, moving orthogonally. Among the anti-tubulin antibodies that we have used, anti-α-tubulin B-5–1-2 and anti-β-tubulin TUB 2.1 gave particles that showed these drifts and transfers most often.

We traced the positions and calculated the velocities of the moving MTs and particles. Figure 4 shows the result of tracing the MT and particle of Figure 2. A 20-μm-long MT was translocated by *Tetrahymena* 14S dynein at a relatively rapid velocity that varied from ~2 to 5 μm/s. Measurement error is shown by the differences between the velocities or positions of the leading versus trailing edge of the MT. Both the MT and the particle moved in the same direction with reference to the glass surface, but the particle moved with slower average velocity than the MT, which produced the gradual drift from a position near the leading edge toward the trailing edge of the MT. For example, particles coated with anti-α-tubulin antibody moved on 14S dynein on average at 0.81 $\pm$ 0.07 (SD) of MT velocity (n = 7). After a few seconds, the particle was released from the end of the MT and remained on the slide while the MT continued forward. The same phenomenon—drifting of particles toward the trailing edge of the MT followed by release—was seen, regardless of whether the MT was translocating on 14S dynein, on 22S dynein, or on kinesin (Figure 5, B–D).

When a particle attached to and moving with an MT encountered a second MT moving orthogonally (Figure 3), the particle remained at the intersection of the MTs for variable periods of time. It then either continued moving with the original MT or transferred and moved with the orthogonal MT, eventually drifting toward the trailing edge of the latter MT. The amount of time spent at the intersection was up to 6 s when anti-α-tubulin was attached to the particle, but never more than 2 s when anti-β-tubulin was used. In the absence of ATP, MT movement was absent, and attached particles did not move with respect to the MT (Figure 5A).

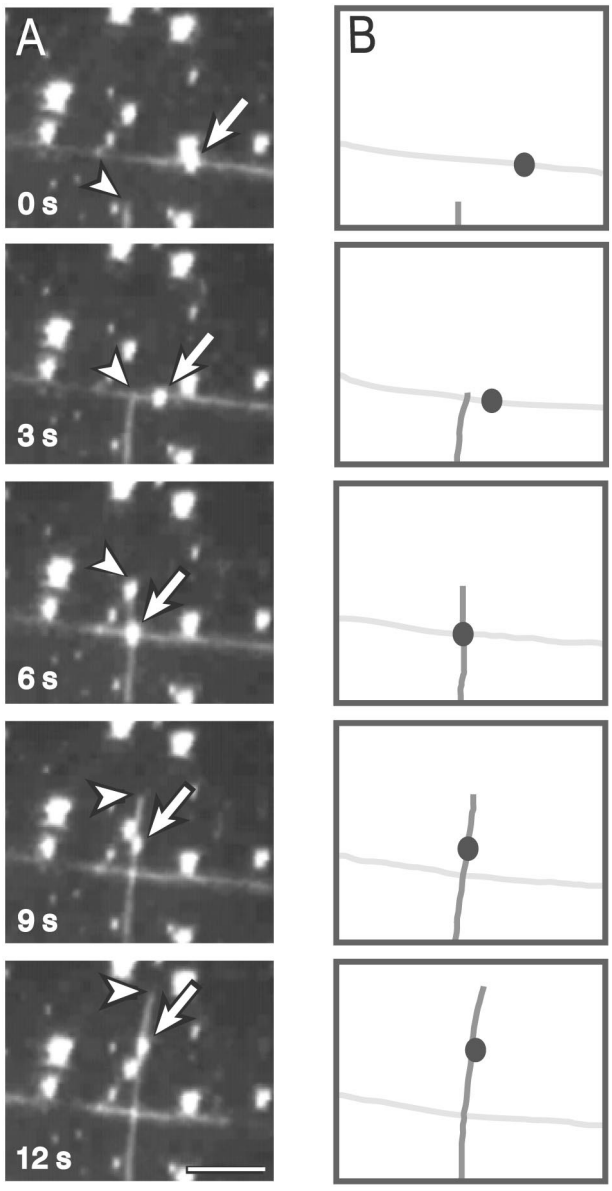

Figure 3. A particle (arrow) transfers from one MT (which is moving horizontally from right to left) to another (which is moving up). (A) Sequence from video recording captured every 3 s. Arrowheads show positions of the leading edge of the second MT. (B) Tracings of the positions of the MTs and the particle from the sequence shown in A. Bar, 10 μm.

Evidence That Particle Movement Along MTs Is Not Motor-based

Although particle drifting depends on gliding of the underlying MT, it evidently does not require motor molecules on the particles themselves to produce motion. In a microscope field where some MTs were moving and others were not, the particles never moved on a stopped MT. Particles never moved faster than MTs, but MTs often moved past stopped particles. In orthogonal transfers, the particles remained in position, while both MTs moved forward. When an MT transiently slowed or stopped, as often occurred when the motor used was 22S dynein, the attached particles correspondingly slowed or stopped.

Figure 6 shows a record of the movement of two particles attached to the same MT. Both particles drifted away from the leading edge of the MT during the time traced, and both particles slowed or stopped when the MT slowed or stopped. The particles, which started out next to one another, moved apart, because for several seconds, particle 2 remained nearly stationary, while particle 1 continued to move with the MT. Therefore, we conclude that although

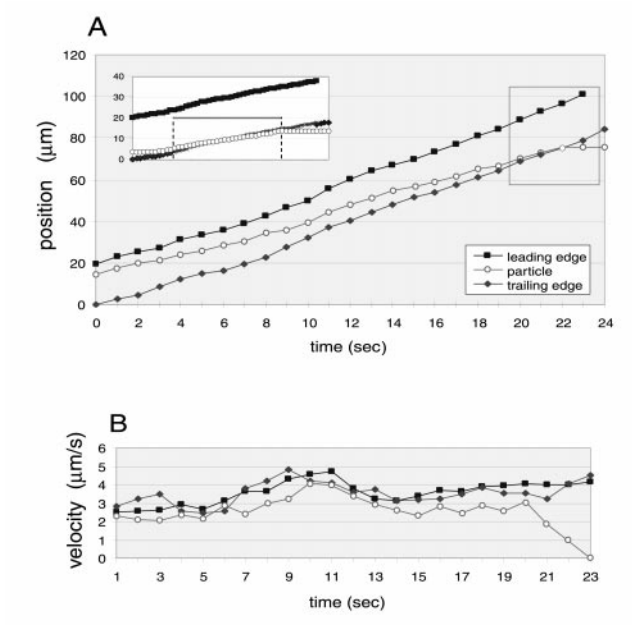

Figure 4. Representative tracking of MT and attached particle movements, measured from the video used in Figure 2 with corresponding time points. (A) Positions of two ends of the MT and of the attached particle are traced. Inset, frame-by-frame analysis of the time at which the particle reaches and leaves the trailing MT end. The 3-s measurement of length of stay (horizontal bar) is detected from the point at which the end of the MT is no longer separately discernable to the point where the end reappears (broken lines). (B) Velocities of the MT and particles calculated using a moving average of three successive measurements of position. The differences between measurements of moving leading and trailing MT ends indicate the measurement error, exaggerated by the moving average. Note that the particle is constantly moving at slightly slower velocity than the MT. The rapid drop off of particle velocity beginning at 20 s reflects the time at which the particle leaves the MT end.

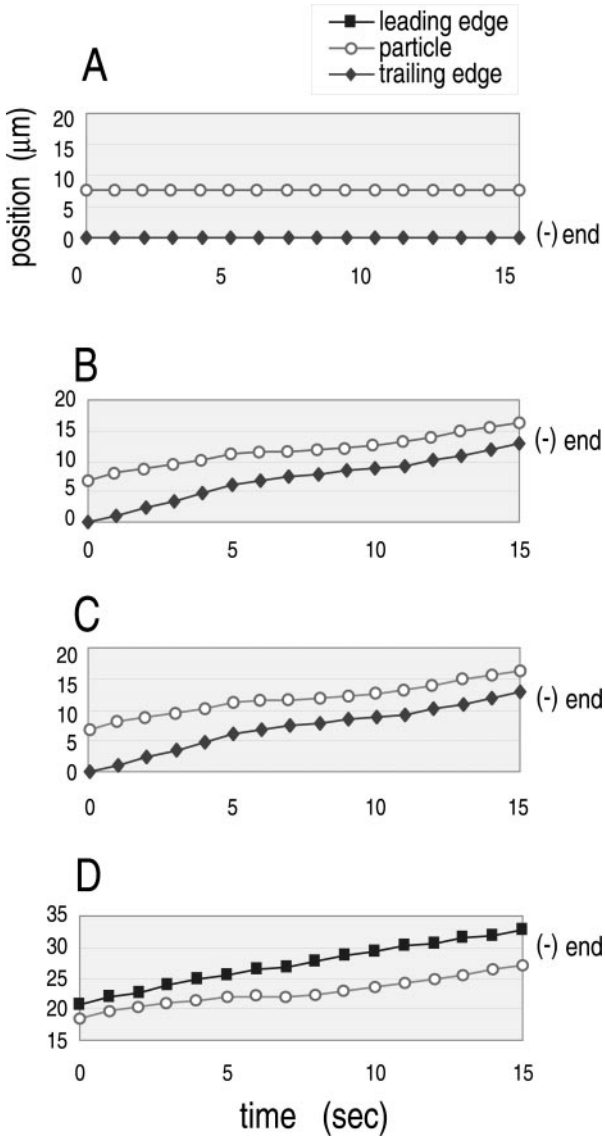

Figure 5. Gold particle drifting movements observed in association with in vitro MT motilities driven by various motors: (A) 14S dynein control: no Mg^{2+}-ATP supplied. Neither MT nor particle move. (B) 14S dynein; (C) 22S dynein; (D) kinesin, all with 1 mM Mg^{2+}-ATP. In B and C, the particle drifts toward the trailing, (−) polarity end. In D the particle drifts away from the leading, (−) polarity end. B-5–1-2 (anti-α-tubulin antibody) was used in all cases.

particles did not move independently of MT movement, particles could attach and transiently detach from the moving MT, moving with the MT when robustly attached, to maintain their positions along the MT, or moving with a lower velocity in the same direction as the MT, when transiently detached.

Detachment during drifting or transfer was not prolonged or permanent, because the particle stayed with the MT and did not exhibit Brownian movement. Repeated perfusion with ATP buffer resulting in a strong fluid flow within the chamber never detached particles from MTs. Such perfusion, either in the same direction as or in the opposite direction from MT movement, did not obviously change particle drifting velocity, although unattached particles moved rapidly with the flow (our unpublished results).

To test the effect of ionic strength on particle movement, we used tetraethylammonium acetate because salts as KCl interfere with motor molecule function (Vale and Toyoshima, 1989). When 250 mM tetraethylammonium acetate in ATP buffer was perfused through the chamber, the particles detached from the MTs within a few seconds and moved away by Brownian movement, whereas MT movement was unaffected (Figure 7). When the tetraethylammonium acetate concentration was 100 mM, particle detachment took longer to occur. This suggests that particle–MT attachment is more sensitive to ionic strength than is motor–MT mechanochemistry. Particles attached to the substratum did not detach under these conditions.

To test the effect of viscosity on particle movement, 2% wt/vol polyvinyl–pyrrolidone (PVP-360; Sigma) was added to ATP buffer. Movement of an MT and its attached particle was recorded before and after the addition of PVP. Addition of PVP usually slowed both MT and particle movement but had a greater effect on the particle movement (Figure 8). A

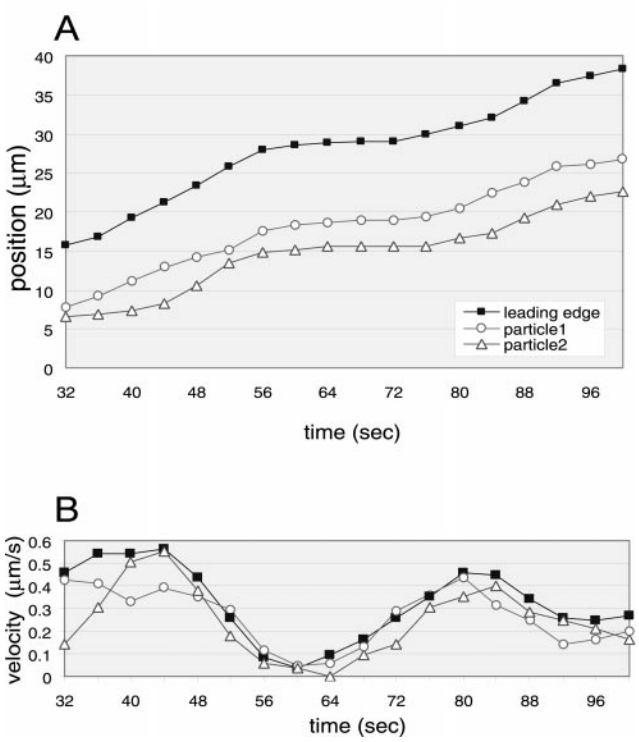

Figure 6. Analysis of movement of two particles attached to the same MT. (A) Tracking of leading edge of the MT and attached particle movements on a 22S dynein substratum. (B) Average velocities, using a three-point moving average of position. Particles move independently of each other with maximum velocity equal to MT velocity, indicating robust attachment, and minimal velocity of zero, indicating transient detachment from the MT.

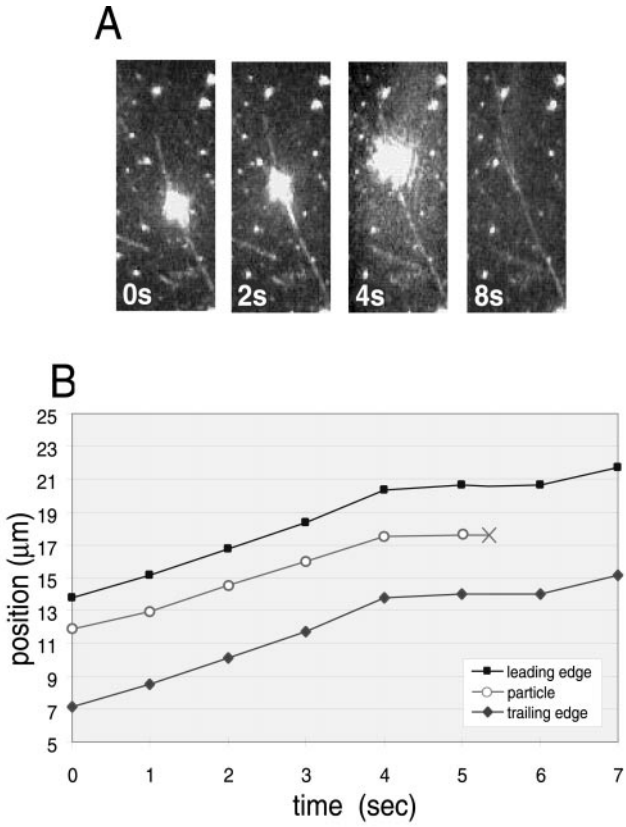

Figure 7. Analysis of particle detachment in a high salt experiment. (A) Video sequence showing the effect of application of high salt (250 mM tetraethylammonium acetate). (B) Tracing shows abrupt disappearance of the particle from A. Perfusion is begun at 0 s. At 4 s, the particle image expands, presumably as the high salt perfusion reaches this field while MT movement is still seen. X indicates the point when the particle is detached from the MT.

particle that previously moved mainly with the MT now remained longer in one position as the MT moved onward, so that the apparent velocity of rearward drifting of the particle increased. After addition of 2% PVP, movement of particles on 14S dynein coated with α-tubulin antibody was reduced on average by 15% to 0.69 $\pm$ 0.23 (SD) of MT velocity (n = 4). In 4% wt/vol PVP-360, the gliding velocity of longer MTs was sometimes reduced, and sometimes the MT and its attached particle stopped completely (our unpublished results).

Although each of these features suggested that the mechanism by which particles drift along MTs is different from motor–MT mechanochemical interaction, and that the particles moved passively along the MTs, the direction of drift both in chambers with dynein and with kinesin substrata was that expected if some motor molecules bound to the particle and actively moved the particle. We next considered whether motor molecules from the substratum might solubilize and bind to the particles to produce the observed particle movements. To simulate this condition, we first perfused particles into a kinesin-coated chamber and incubated for 45 min. Because the activity of one kinesin molecule can override that of 10 or more dynein molecules (Vale *et al.*, 1992), and because a kinesin molecule is able to support continuous particle movement on an MT (Block *et al.*, 1990), we reasoned that if a particle picked up even a few kinesin molecules, its movement would predominantly be due to the attached kinesins. The particles were then collected from the first chamber and perfused into a second chamber where MTs were translocating by surface-coated dynein activity in the presence of 1 mM Mg^{2+}-ATP. If a particle picked up kinesin molecule(s) in the first chamber, it should move forward toward the leading (+) end of the moving MT in the dynein chamber because of the opposite directionalities of the motors. We measured movement of 21 attached particles in three different experiments. Of these, seven particles drifted, and all of these drifted toward the trailing edge of the MTs (Figure 9). This result indicates that the particle motion is not carried out by motors attached to the particle.

Dynamic interactions between tubulin dimers within a moving MT might play a role in producing particle drifting. We therefore tested whether MTs whose tubulin subunits were cross-linked to each other would support the particle movement. MTs, which were incubated in the presence of 0.1% glutaraldehyde for 2 min at room temp, were still translocated on a dynein surface, but we were unable to attach gold particles onto the MTs, whereas exactly the same

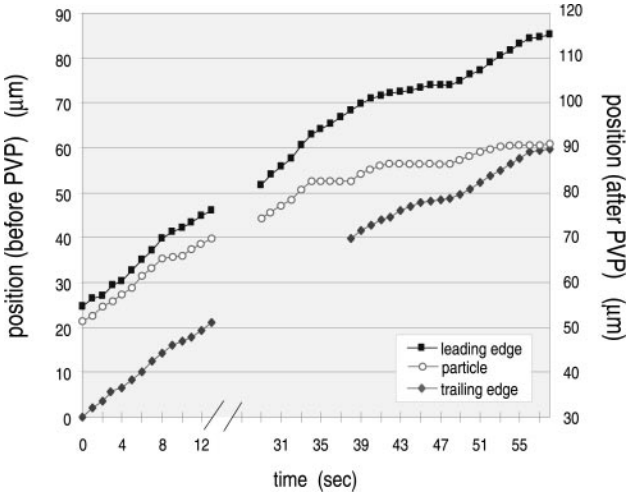

Figure 8. Effect of increased viscosity on particle movement. Tracing curves for MT ends and attached particle before versus after perfusion of 2% PVP into the translocation chamber. Break in record shows time of PVP addition. PVP addition slowed MT movement somewhat and increased velocity of rearward drift of the particle. Left *y*-axis corresponds to before PVP and right *y*-axis corresponds to after PVP.

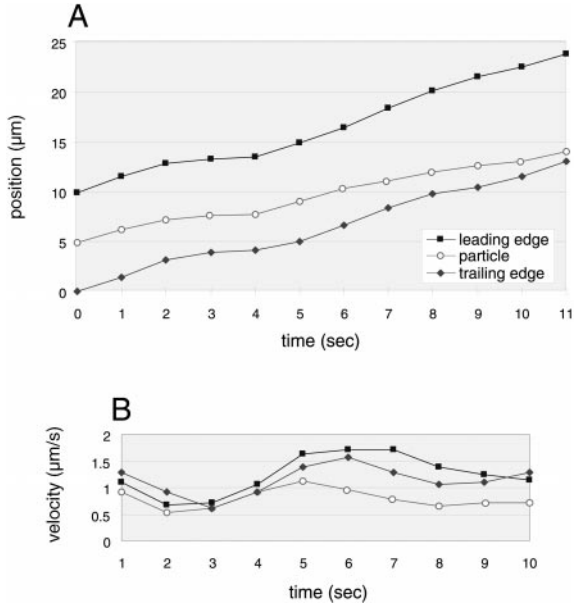

Figure 9. Preincubation experiment to test absorption of motors to particles. Gold particles with TUB 2.1 anti-β-tubulin antibody were preincubated 45 min in a kinesin-coated chamber, then transferred to a second chamber coated with 14S dynein. (A) Tracking curves for MT ends and attached particle in the dynein-coated chamber. (B) Velocity profile. The particle moved toward the trailing (−) end of the MT.

components without glutaraldehyde treatment exhibited normal particle attachments and movements. A greater degree of cross-linking (0.5% glutaraldehyde for 5 min) affected dynein-driven MT translocation. This result may mean either that the surface modification of tubulin by glutaraldehyde inhibits antibody binding (although it does not inhibit dynein binding or mechanochemistry) or that tubulin dynamics within an MT are necessary for particle attachment.

Release of Particles from the MT Ends

As part of our measurements, we determined by frame-by-frame analysis (Figure 4, inset) the length of time between when a drifting particle initially attached to the middle of the MT arrived at the trailing edge of the MT and when it released to the solution or dropped onto the glass surface. At frame rates of 30 s^{-1}, the arrival time—as indicated by the disappearance of the MT end below the particle—could be determined with an accuracy of better than 10 frames (0.3 s), whereas the release—indicated by the reappearance of the MT end and either the beginning of Brownian motion or the drop of particle velocity to zero with continued MT movement—could be measured to approximately 3 frames (0.1 s).

The time measured (several seconds or longer) varied from particle to particle, but there were differences in the average time of stay at the MT ends 1) between particles coated by α-tubulin antibody versus particles coated by β-tubulin antibody moving on the 14S dynein substratum and 2) between particles coated by β-tubulin antibody moving along MTs driven by kinesin versus 14S dynein. For unknown reasons in our experiments, too few particles coated with α-tubulin antibodies attached to the MTs moving on kinesin substrata (n = 18 vs. n = 97–190 scored for other conditions) to permit us to measure the time that particles remained at the trailing edge under this condition. In the first instance, when moving on a dynein substratum, i.e., drifting toward the (−) end of the MT, particles coated with antibody to α-tubulin remained at the end significantly longer ($p < 0.001$) than those coated with antibody to β-tubulin. In the second instance, in kinesin substrata where the trailing edge of the MT is the (+) end, particles coated with β-tubulin antibody remained significantly longer at the trailing edge ($p < 0.005$) than similarly coated particles did in dynein substrata.

DISCUSSION

From these observations under simplified in vitro conditions, we have concluded that relative (passive) motion is possible between a moving MT and an object that is attached to it, without direct involvement of a motor on the object itself. This conclusion is based on the behaviors of refractive particles coated with specific IgGs capable of binding to a tubulin, but we believe that the phenomenon described has relevance to cellular processes such as organelle, vesicle, or nucleoprotein transport and to the aggregation of signaling molecules on MTs, as recently reviewed by Gundersen and Cook (1999). The binding of the IgGs to MTs mimics that of MAPs, although MAPs are usually considered to bind to a particular site on an MT and to stay bound there without drifting. The previous prevailing view has been that diffusion of proteins along the MT was very restricted and that

motor molecules had to be attached to cargo if the cargo was to move on the MT; we suggest that this may not always be the case. Although all of our results support the conclusion that our moving cargo does not bind motor molecules directly, the relative movement we describe requires MT motility. In our experiments, MT motility is produced by motor molecules of the chamber substratum. Cargo movement is in the direction of MT movement, but with slower average velocity, which leads to the cargo moving backward along the MT, independent of MT polarity or flow conditions in the chamber.

We considered the possibility that the relative movement we have observed is the result of MT treadmilling or differential dynamic shortening and re-extension under our experimental conditions; however, treadmilling should yield reverse directions of particle movement when kinesin versus dynein substrata are used, which is not the case. In addition, when two particles move along a single MT, they move independently of one another (Figure 6), whereas a treadmilling mechanism would probably require coupling of the movement. Within measurement errors, lengths of the moving MTs as directly measured at intervals of <0.1 s remain constant for >5 min of recording, which indicates that there is almost no dynamic instability occurring in our experiments in which taxol-stabilized MTs were used. We have not ruled out the possibility that local dynamic changes within the MT that do not affect overall length could contribute to the observed particle movement and our results whereby glutaraldehyde fixation of the MT prevents particle attachment could be interpreted to support this hypothesis.

As an alternative explanation for particle movement, it seems probable that multiple anti-tubulin antibodies attach to a single particle and that in the absence of treadmilling, procession down the MT relies on transient detachment of some, but probably not all, of the binding sites. As the MT moves forward, the weakly attached particle could experience a drag that brought new sites into reach of the antibodies. The particle would then remain on the MT but be slowly pushed backward. A change in viscosity of the medium might then be expected to increase the velocity of drifting, which seems to be the case. In some respects, this phenomenon could be the equivalent of that producing one-dimensional diffusion of MTs described by Vale *et al.* (1989) or of one of the possibilities described for the weak binding state of C351 to MTs by Okada and Hirokawa (1999), where C351 is anchored by an electrostatic potential that restricts its movement away from the MT while allowing free movement along the MT. In our case, drag created by the MT movement would bias the direction of particle drift. Unlike Vale *et al.* (1989) or Okada and Hirokawa (1999), however, we have never observed that the drifting particles exhibit back-and-forth movement. Alternatively, the antibodies could interact transiently with the substrate or another passing MT. The latter event clearly must occur when orthogonal transfer of a particle occurs between MTs (Figure 3). Transient attachment probably involves electrostatic binding, because particle binding seems highly sensitive to ionic strength.

Along the length of the MT, anti-α-tubulin– and anti-β-tubulin–coated particles generally behave similarly, although the affinities of the antibodies for the MT or the number of antibodies coating the particles are not the same. Differences in antibody affinities and/or in number of antibodies per particle are reflected by the different rates of transfer between orthogonal MTs that are found with anti-α-tubulin– versus anti-β-tubulin–coated particles and possibly by different lengths of stay of anti-α-tubulin– versus anti-β-tubulin–coated particles at the end of MTs under different conditions; however, the differences in lengths of stay might also be due to differences in the structure of MT ends. Because anti-β-tubulin–coated particles drifting along the MT remain attached to the MT significantly longer when the trailing edge of the MT is the (+) end than when it is the (−) end, and anti-α-tubulin–coated particles remain attached to the trailing (−) end significantly longer than anti-β-tubulin–coated particles do on the comparable substratum, our results might simply confirm in a dynamic system that α-tubulin lies at the (−) end and β-tubulin forms the (+) end of the MT.

This affinity mechanism of particle drifting could have significance for intracellular movement in at least three situations:

Vesicle, Organelle, or Protein Complex Movement or Aggregation in the Absence of Bound Motor Molecules in Moving Cells

Essentially, if a vesicle or complex had an attached protein whose transient attachment to an MT permitted drifting, as a cell moved the vesicle would be transported relative to the moving MT as the frame of reference changed. This mechanism would also permit transfer of vesicles from one cytoskeletal element to another, without specific motor activation or deactivation.

Increasing the Apparent Duty Phase Ratio or Lowering the Numbers of Motors Attached to a Vesicle or Complex

This may be significant for dynein-mediated transport, and perhaps for myosin-based transport as well, because as measured, some of these motors have low duty phase ratios (0.01–0.05) (Uyeda *et al.*, 1990; Hamasaki *et al.*, 1995). It has been a paradox that with such ratios and the small numbers of motors that could physically bridge vesicle or complex and MT or microfilament, movement is evident. One possibility, which would resolve this paradox, would be to add to the complex a small number of molecules capable of transient attachment and drifting along the MT or microfilament. For dynein, dynactin may be such a molecule. Dynactin, a nonmotor protein that binds to MTs, and cytoplasmic dynein work together to produce cytoplasmic dynein-mediated vesicle motility (Gill *et al.*, 1991; Vaughan and Vallee, 1995). We suggest that dynein, which has a short duty cycle and cannot hold a vesicle on an MT, might move the vesicle actively during its duty phase, whereas dynactin would hold the vesicle to the MT during the remaining time and also contribute to its movement via the affinity drift mechanism described here.

Kinetochore Attachment and Movement

A second paradox is found at the (+) ends of MTs in mitosis, where MTs must shorten and move while maintaining their hold on the kinetochore (Koshland *et al.*, 1988; Rieder and Salmon, 1998). If one or more of the centromeric proteins now localized (Grancell and Sorger, 1998) were capable of

transient attachment and drifting in the way we describe, a cytoplasmic dynein that interacted with the spindle MTs would affect translocation to the poles without kinetochore detachment.

These applications remain speculative, but the unanticipated particle drifting mechanism we have described here clearly opens new possibilities for study of mechanisms of movement along cytoskeletal elements in cells.

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REFERENCES

Barkalow, K., Avolio, J., Holwill, M.E.J., Hamasaki, T., and Satir, P. (1994). Structural and geometrical constraints on the outer dynein arm *in situ.* Cell Motil. Cytoskeleton *27*, 299–312.

Block, S.M., Goldstein, L.S.B., and Schnapp, B.J. (1990). Bead movement by single kinesin molecules. Studied with optical tweezers. Nature *348*, 348–352.

Fan, J., Griffiths, A.D., Lockhar, A., Cross, R.A., and Amos, L.A. (1996). Microtubule minus ends can be labeled with a phage display antibody specific to alpha-tubulin. J. Mol. Biol. *259*, 325–330.

Frens, G. (1973). Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. Nature *241*, 20–22.

Gill, S.R., Schroer, T.A., Szilak, I., Steuer, E.R., Sheetz, M.P., and Cleveland, D.W. (1991). Dynactin, a conserved, ubiquitously expressed component of an activator of vesicle motility mediated by cytoplasmic dynein. J. Cell Biol. *115*, 1639–1650.

Gozes, I., and Barnstable, C.J. (1982). Monoclonal antibodies that recognize discrete forms of tubulin. Proc. Natl. Acad. Sci. USA *79*, 2579–2583.

Grancell, A., and Sorger, P.K. (1998). Chromosome movement: kinetochores motor along. Curr. Biol. *8*, R382–R385.

Gundersen, G.G., and Cook, T.A. (1999). Microtubules and signal transduction. Curr. Opin. Cell Biol. *11*, 81–94.

Hamasaki, T., Barkalow, K., Richmond, J., and Satir, P. (1991). A cAMP-stimulated phosphorylation of an axonemal polypeptide that copurifies with the 22S dynein arm regulates microtubule translocation velocity and swimming speed in *Paramecium.* Proc. Natl. Acad. Sci. USA *88*, 7918–7922.

Hamasaki, T., Holwill, M.E.J., Barkalow, K., and Satir, P. (1995). Mechanochemical aspects of axonemal dynein activity studied by in vitro microtubule translocation. Biophys. J. *69*, 2569–2579.

Hancock, W.O., and Howard, J. (1998). Processivity of the motor protein kinesin requires two heads. J. Cell Biol. *140*, 1395–1405.

Hoenger, A., Sablin, E.P., Vale, R.D., Fletterick, R.J., and Milligan, R.A. (1995). Three-dimensional structure of a tubulin-motor-protein complex. Nature *376*, 271–274.

Jiang, W., Stock, M.F., Li, X., and Hackney, D.D. (1997). Influence of the kinesin neck domain on dimerization and ATPase kinetics. J. Biol. Chem. *272*, 7626–7632.

Koshland, D.E., Mitchison, T.J., and Kirschner, M.W. (1988). Polewards chromosome movement driven by microtubule depolymerization *in vitro.* Nature *331*, 499–504.

LeDizet, M., and Piperno, G. (1987). Identification of an acetylation site of *Chlamydomonas* α-tubulin. Proc. Natl. Acad. Sci. USA *84*, 5720–5724.

Nogales, E., Whittaker, M., Milligan, R.A., and Downing, K.H. (1999). High-resolution model of the microtubule. Cell *96*, 79–88.

Nogales, E., Wolf, S.G., and Downing, K.H. (1998). Structure of the $\alpha\beta$ tubulin dimer by electron crystallography. Nature *391*, 199–203.

Okada, Y., and Hirokawa, N. (1999). A processive single-headed motor: kinesin superfamily protein KIF1A. Science *283*, 1152–1157.

Rieder, C.L., and Salmon, E.D. (1998). The vertebrate cell kinetochore and its roles during mitosis. Trends Cell Biol. *8*, 310–317.

Song, Y.H., and Mandelkow, E. (1995). The anatomy of flagellar MTs: polarity, seam, junctions, and lattice. J. Cell Biol. *128*, 81–94.

Uyeda, T.Q.P., Kron, S.J., and Spudich, J.A. (1990). Myosin step size estimation from slow sliding movement of actin over low densities of heavy meromyosin. J. Mol. Biol. *214*, 699–710.

Vale, R.D., Malik, F., and Brown, D. (1992). Directional instability of microtubule transport in the presence of kinesin and dynein, two opposite polarity motors. J. Cell Biol. *119*, 1589–1596.

Vale, R.D., Soll, D.R., and Gibbons, I.R. (1989). One-dimensional diffusion of microtubules bound to flagellar dynein. Cell *59*, 915–925.

Vale, R.D., and Toyoshima, Y.Y. (1989). Microtubule translocation properties of intact and proteolytically digested dyneins from *Tetrahymena* cilia. J. Cell Biol. *108*, 2327–2334.

Vaughan, K.T., and Vallee, R.B. (1995). Cytoplasmic dynein binds dynactin through a direct interaction between the intermediate chains and p150Glued. J. Cell Biol. *131*, 1507–1516.

Wade, R.H., and Hyman, A.A. (1997). Microtubule structure and dynamics. Curr. Opin. Cell Biol. *9*, 12–17.

Williams, R.C., and Lee, J.C. (1982). Preparation of tubulin from brain. Methods Enzymol. *85*, 376–381.