End-to-end Annealing of Microtubules In Vitro

Stephen W. Rothwell, William A. Grasser, and Douglas B. Murphy
The Department of Cell Biology and Anatomy, The Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205

Abstract. Mixtures of pre-formed microtubules, polymerized from chicken erythrocyte and brain tubulin, rapidly anneal end-to-end in vitro in standard microtubule assembly buffer. The erythrocyte tubulin segments in annealed heteropolymers can be distinguished by an immunoelectron microscopic assay that uses an antibody specific for chicken erythrocyte beta-tubulin. An annealing process is consistent with the following observations: (a) Microtubule number decreases while the polymer mass remains constant. (b) As the total number of microtubules declines, the number of heteropolymers, and the number of segments contained in each heteropolymer, increases. (c) The size of the segments determined after annealing and antibody labeling is the same as the original microtubule polymers. (d) Points of discontinuity in the annealing heteropolymers can be observed directly by electron microscopy, and correspond to type-specific polymer domains. The junctions probably represent initial contact points during the annealing process. Microtubule annealing occurs rapidly in vitro and may be significant for determining properties of microtubule dynamics in vivo.

I t is generally accepted that the dynamic properties of microtubules, such as the redistribution of microtubule lengths at steady state, can be explained in terms of the association of tubulin dimers with microtubule ends (1-4) rather than by the fragmentation and reannealing of microtubule polymers. Carlier and co-workers (5, 6) proposed a mechanism for length redistribution based on the appreciable differences in the rate constants for the association and dissociation of GTP-tubulin vs. GDP-tubulin with the ends of microtubules. Once the GDP-tubulin cores of small polymers are exposed to solvent, they depolymerize quickly (5, 7), and, due to the low rate of association of GTP-tubulin subunits with microtubules containing GDP-tubulin ends, the conversion back to growing polymers containing GTP-tubulin caps occurs infrequently (8). As the microtubule number decreases, the remaining microtubules with GTP-tubulin caps can lengthen because of an increase in the concentration of the tubulin dimer pool. The result of this activity is fewer but longer polymers. Although several investigators have observed microtubules to undergo changes in length (4, 9), the observations on length redistribution are also consistent with a redistribution mechanism based on microtubule annealing.

In studies of the kinetics of actin polymerization, the significance of fragmentation and annealing has long been an area of interest. Experiments by Kawamura and Maruyama (10), Nakaoka and Kasai (11), and Kondo and Ishiwata (12) suggest that small F-actin fragments associate end-to-end to yield longer filaments in a concentration-dependent fashion, and recent models for actin polymerization (13-15) were found to fit the observed assembly kinetics better when terms for fragmentation and annealing of F-actin were included. Nevertheless, annealing of actin filaments or microtubules has not yet been directly observed, and recent studies by Carlier et al. (16) have explained the redistribution of actin filament lengths solely in terms of the association and dissociation of actin monomers. Thus, the role that annealing may play in the redistribution of polymer lengths is unclear.

In this study we present evidence that in fact end-to-end annealing of microtubules contributes significantly to the alteration of microtubule length in vitro. With an immunoelectron microscopic assay developed for the study of tubulin flux (17), we document the end-to-end joining of microtubules. The efficiency and magnitude of this process suggest that both microtubule annealing and subunit addition may determine microtubule length in vitro.

Materials and Methods

Preparation of Chicken Brain and Erythrocyte Tubulin

Chicken brain tubulin was isolated by the method of Dentler et al. (18) in 0.1 M PIPES buffer containing 1 mM MgCl₂, 2 mM EGTA, 1 mM GTP, and 4 M glycerol.

Chicken erythrocyte tubulin was prepared from chicken blood by the method of Murphy and Wallis (19). Tubulin was purified free of microtubule-associated proteins (MAPs)³ by ion exchange chromatography using Whatman P-11 phosphocellulose (PC) (20) and cycled once before use to remove any inactive subunits. Unless stated otherwise, microtubule assembly buffer was 0.1 M Na-PIPES at pH 6.94, containing 1 mM MgCl₂, 1 mM GTP, and supplemented with 5% glycerol.

Abbreviation used in this paper: PC, phosphocellulose; MAP, microtubule-associated protein.
Preparation of Microtubule Seeds and Subunits Used to Study Microtubule Annealing

Microtubule seeds with and without MAPs were prepared by polymerizing PC tubulin or microtubule protein in 0.1 M PIPES, pH 6.94, containing 10 mM MgCl₂, 1 mM GTP, and 20% glycerol, and sedimenting and resuspending the polymers to 5-13 mg/ml in assembly buffer. Polymers were sheared by 10 passes through a 27 gauge needle (21) to prepare microtubules with a mean length of 1.35 μm. Alternatively, taxol-stabilized seeds were prepared by adding 50 μM taxol to assembled polymers before shearing. Erythrocyte PC-tubulin subunits were diluted to the desired concentration in assembly buffer at 5°C containing 0.25 U/ml acetyl kinase and 50 mM acetyl phosphate as a GTP regenerating system (22). The maintenance of GTP levels (>95% for periods up to 3 h) was previously confirmed (17) by chromatography on polyethyleneimine plates in LiCl/formic acid (23). The subunit preparations were lightly sonicated before use to disperse any oligomers (19).

Two methods were used to prepare microtubules for the analysis of annealing. In the first method, preparations of brain and erythrocyte microtubules, mixed in a 1:1 ratio at 10 mg/ml, were incubated at 37°C for up to 4 h. In the second method, brain microtubule seeds were added to erythrocyte tubulin subunits to produce heteropolymers, each with a short length of erythrocyte tubulin polymer at each end. Brain microtubule seeds (25 μl) were added to preparations of erythrocyte subunits (700 μl, 1 mg/ml) that were preincubated at 30°C in a 1-ml cuvette for 60 s, a length of time that had been determined to be sufficient for complete temperature equilibration. After 90 s, aliquots were removed and mixed with prewarmed assembly buffer, which decreased the tubulin subunit concentration and rapidly brought the polymers close to steady state as determined by monitoring the turbidity of the sample at 350 nm (plateau turbidity reached within 30 s). The preparation of heteropolymers was then incubated for up to 3 h. In both cases, aliquots of these incubation mixtures were fixed at various times in assembly buffer with 1% glutaraldehyde and prepared for electron microscopy.

Analysis of Microtubule Annealing

Annealing was monitored in three ways: (a) We examined the patterns of immunogold labeling on heteropolymers. We visualized the decrease in microtubule number resulting from annealing by counting fixed microtubules that were centrifuged onto electron microscope grids. Glutaraldehyde-fixed microtubules from the annealing experiments were diluted 10^-fold and then spun onto electron microscope grids for 10 min in a Beckman Airfuge (Beckman Instruments, Inc. Palo Alto, CA). The grids were negatively stained with uranyl acetate and the number of microtubules per grid square was counted (20 squares per time point; 400-mesh grids). (b) We monitored the decrease in microtubule number by examining the initial rate of polymerization after the addition of aliquots of the annealing microtubules as seeds to samples of tubulin subunits (21). Typically, 20-μl seeds were added to 700-μl subunits, resulting in a linear increase in turbidity of 0.02-0.04 absorbance units within 2 min. In control experiments without seeds, no self-assembly occurred for up to 5 min. Initial rates were determined from the slopes of the spectrophotometer tracings within the first 90 s after the addition of seeds. The error for this method was determined to be ~4%.

Procedures for Immuno Electron Microscopy

Electron microscopic grids containing glutaraldehyde-fixed microtubules were stained according to the method of Rowlatt et al. (17, 24) using an antibody specific for the beta-subunit of chicken erythrocyte tubulin (25 μg/ml) or MAP2 (284 μg/ml) (25) and Protein A-gold colloid (26).

Measurements of the lengths of decorated microtubules were made using a Zeiss EM 10A electron microscope. At least 25 microtubules from each grid were measured with respect to the length of the seed and the extent of polymerization at each end. For the calculation of mean seed length during annealing, at least 50 seeds were analyzed for each time point.

Determination of Protein Concentration

Total protein concentrations were determined by the Bradford protein assay (26). Bovine serum albumin was used as a standard.

Biochemical Materials

PIPES sodium salt was obtained from Calbiochem-Behring Corp. (La Jolla, CA). Other chemicals and nucleotides were obtained from Sigma Chemical Co. (St. Louis, MO).

Results

Antibody Labeling of Chicken Erythrocyte Tubulin

Using an antibody specific for the beta-subunit of chicken erythrocyte tubulin and Protein A-gold, erythrocyte microtubules were observed to be distinctly and specifically labeled as determined by electron microscopy. The specificity of the antibody for just the beta-subunit of erythrocyte tubulin is shown in the immunoblot in Fig. 1. The antibody also specifically recognizes the erythrocyte beta-subunit in microtubules. Fig. 1 shows that the density of labeling of heteropolymers prepared by polymerizing mixtures of erythrocyte and brain tubulin subunits is proportional to the amount of erythrocyte tubulin contained in the subunit mixtures. This dependence was quantified by counting gold particles per micrometer microtubule length on polymers containing different proportions of erythrocyte tubulin. The linear portion of this curve (Fig. 2) may be used to estimate the amount of erythrocyte tubulin found in mixed polymers. The assay is most sensitive in the range of 5-50% erythrocyte tubulin and can detect microtubules containing only 50-80 erythrocyte subunits/μm length of brain microtubule.

Annealing of Pre-formed Microtubule Seeds

We mixed brain and erythrocyte seeds together (10 mg/ml each species) to determine if pre-formed microtubules were capable of end-to-end annealing. We used seeds that had been prepolymerized to steady state so that there would be little or no growth of new polymer at the microtubule ends during the first minutes after mixing. 1 min after mixing, we observed heteropolymers composed of alternating labeled and unlabeled segments that corresponded to the density of labeling observed for brain and erythrocyte microtubules. After 1 min and 60 min, the percent of heteropolymers observed was 21% and 54%, respectively, indicating that annealing was both rapid and efficient. The number of distinct domains increased with longer incubation time. Examples of "annealed heteropolymers" are shown in Fig. 3.

We also examined annealing in microtubule preparations containing MAPs or taxol. (a) Annealing of microtubule seeds in the presence and absence of MAPs was qualitatively similar, indicating that MAPs are not required for annealing to occur. (b) When microtubules with MAPs were mixed with microtubules without MAPs, heteropolymers containing alternately labeled and unlabeled segments were observed after staining with MAP antibody. No significant redistribution of MAPs was observed for up to 30 min. Therefore, the redistribution of MAPs on microtubule surfaces does not appear to be required for annealing. (c) Microtubules stabilized with taxol (1:1 molar ratio of taxol/tubulin) also exhibited annealing although at a slightly reduced rate. After 30 min, the number of annealed heteropolymers was only 15% of the total microtubule population. Therefore, annealing occurs both in the absence of MAPs and in the presence of taxol.

Annealing of Heteropolymers

To test whether annealing actually occurred, we prepared microtubule heteropolymers, each of which contain a central brain segment that was flanked at each end with erythrocyte tubulin polymer. Since the brain polymer is enclosed at each end by erythrocyte tubulin caps, we hypothesized that the
Figure 1. Demonstration of the specificity of the beta tubulin antibody. Chicken erythrocyte (E) and brain (B) tubulin were fractionated by electrophoresis (29) and transferred to nitrocellulose paper (30). The left lanes show the Amido black-stained pattern, and the right lanes show the corresponding autoradiogram after incubation with antitubulin serum (1/500 dilution) and ~2~l-protein A. A–E show that the extent of antibody labeling is directly related to the proportion of erythrocyte tubulin contained in co-polymers prepared by mixing erythrocyte and brain tubulin subunits together before assembly. Microtubules containing various ratios of erythrocyte/brain tubulin were fixed in 1% glutaraldehyde in assembly buffer and incubated with affinity-purified tubulin antibody (25/~g/ml) followed by protein A–gold. The proportions of erythrocyte tubulin contained in the microtubules are the following: A, 100%; B, 33%; C, 25%; D, 10%; E, 0% (100% brain tubulin). Bar, 0.05 ~m.

Figure 2. The relationship of the density of gold labeling to the proportion of erythrocyte tubulin present in co-polymers. Gold particles/unit length of microtubule were counted on microtubules (n =

lengths of brain polymer segments within annealed polymers should remain constant throughout the annealing process. Microtubule heteropolymers made from brain tubulin seeds and erythrocyte tubulin subunits were prepared as described in Materials and Methods. 30 min after dilution of the subunits to 0.3 mg/ml, we observed longer microtubules, each with multiple labeled and unlabeled regions. The generation of these annealed polymers is shown in Fig. 4. Each set of horizontal lines in Fig. 4 represents a single microtubule. Initially all microtubules contained three distinct regions; by 60 min, long microtubules with as many as ten alternating domains were observed. The density of gold labeling in many domains was equivalent to the level of labeling observed for homologous brain and erythrocyte microtubules as determined by counting gold particles per unit length. Some regions of intermediate labeling were also observed, which probably resulted from subunit addition that occurred before the time that annealing happened. Although the overall length

10) polymerized from various mixtures of erythrocyte and brain tubulin as shown in Fig. 1.
Figure 3. Heteropolymers formed by annealing of pre-formed microtubules. Microtubules polymerized from erythrocyte PC-tubulin and brain microtubule protein, both at 10 mg/ml, were mixed together and incubated for 60 min at 37°C. Samples were fixed in 1% glutaraldehyde in assembly buffer and prepared for electron microscopic examination as described in the text. (A) Bar, 0.11 μm. (B) Bar, 0.1 μm.
Heteropolymers were formed by the elongation of erythrocyte PC-tubulin subunits (1.0 mg/ml) from the ends of seeds polymerized from brain microtubule protein. The heteropolymers were diluted into warm assembly buffer and the incubation was continued at 30°C. Samples were taken at various times and prepared for electron microscopic examination. The lengths of the brain and erythrocyte tubulin segments within individual heteropolymers were measured and plotted as segmented horizontal lines with each set of lines representing one microtubule. At 1 min after dilution heteropolymers contained three segments per line, representing a middle brain tubulin domain (B) and two growth regions of erythrocyte tubulin (E) flanking it. With time, the number of alternating segments increased as the heteropolymers annealed to form more complex patterns of erythrocyte and brain tubulin.

Figure 5. The constancy of brain tubulin domains in annealed heteropolymers with time. Brain tubulin domains were measured from the microtubules shown in Fig. 4. 0 min, 1.02 ± 0.55 μm; 30 min, 0.82 ± 0.55 μm; 60 min, 1.13 ± 0.59 μm; 120 min, 1.20 ± 0.98 μm; 180 min, 0.99 ± 0.53 μm; and 240 min, 1.13 ± 0.60 μm. The sample size (n) is approximately 50 tubulin domains for each time point.

Evaluation of Polymer Mass and Number Concentration during Annealing

Microtubule annealing was indicated by the following three observations: (a) Polymer mass should remain constant. Fig. 7 shows that for >5 h the mass of heteropolymers, elongated from brain microtubule seeds in an experiment similar to that shown in Fig. 4, remained constant as measured by absorbance at 350 nm. (b) Microtubule number concentration should decline. We documented a decline in microtubule number by counting negatively stained microtubules after quantitative pelleting of microtubules onto electron microscope grids (Fig. 8) and by measuring the decrease in the initial rate of elongation in which the annealing polymers were used as seeds for subunit addition (Figs. 9 and 10). The rate of elongation has been shown to be a direct measurement of the concentration of tubule ends available for growth (21), a point that we have also confirmed (28). We observed that the rate of elongation decreased with time in agreement with the results obtained by electron microscopy. (c) Overall microtubule length should increase. This was confirmed by inspection of microtubule samples as described above (Fig. 4).

Discussion

We observed that microtubule polymers anneal end-to-end and that this process contributes significantly to the redistribution of microtubule lengths in vitro. Evidence for annealing is based on the following observations: (a) Mixtures of short length polymers composed of erythrocyte and brain tubulin rapidly generate longer polymers with alternating regions characteristic of erythrocyte or brain tubulin domains. (b) Short length heteropolymers with brain tubulin cores and erythrocyte tubulin ends produce long polymers whose brain tubulin segments are the same length as the brain tubulin segments in the original heteropolymers. (c) The immunoelectron microscopic assay indicates that no subunit mixing occurs within tubulin-specific domains in heteropolymers even after several hours. (d) During annealing at steady state, polymer mass remains constant, while microtubule length increases and microtubule number declines. (e) When mixtures of brain and erythrocyte microtubules are examined by electron microscopy, heteropolymers in the process of joining are observed to contain discontinuities at their original contact points.

Documentation of Initial Microtubule Contacts during Annealing

Electron microscopic examination of long annealed polymers revealed no discontinuities between domains in the microtubule wall. This agrees with our previous observations that both brain and erythrocyte microtubules contain the same number of protofilaments (mean number 13.6) and that no discontinuities were observed between domains when erythrocyte subunits were allowed to elongate off brain tubulin seeds (17). However, examination of microtubules in the process of joining just 1 min after mixing pre-formed microtubules revealed many end-to-end microtubule contacts and discontinuities in the microtubule wall at the joint between brain and erythrocyte segments (Fig. 6). These configurations may represent instances of initial microtubule contacts during the annealing process.
Figure 6. Discontinuities at the points of contact between annealing brain and erythrocyte microtubules. Samples were fixed 1 min after mixing pre-formed microtubules polymerized from erythrocyte PC-tubulin and brain microtubule protein and labeled with antitubulin antibody for electron microscopic examination. The discontinuities between microtubules in the process of joining are indicated with arrows. Soon after joining, irregularities in the microtubule wall disappear (example shown, lower left). Bar, 0.05 μm.

Figure 7. Constancy of polymer mass with time. The turbidity of a heteropolymer preparation formed from the elongation of erythrocyte PC-tubulin subunits from brain microtubule protein seeds was recorded and found to remain constant for at least 5 h.

Figure 8. Decline in microtubule number as determined by direct counting. Microtubules polymerized from erythrocyte and brain PC tubulin were sheared, mixed together, and incubated at 37°C. Samples from various times were centrifuged onto electron microscopic grids, counted, and plotted as the percentage of the initial time that was designated 100%.

These discontinuities later become "filled in" and disappear with time, suggesting that annealing consists of a rapid phase of direct end-to-end contact of the polymers followed by a slower second phase of alignment and disappearance of irregularities.

No simple polymerization scheme based on polymer growth by subunit addition (including the phenomenon of dynamic instability) can account for the formation of alternating regions of brain and erythrocyte tubulin observed within individual microtubules. Homopolymers consisting of pure erythrocyte or brain tubulin are always uniformly labeled along their lengths. Preparations of erythrocyte and brain
tubulin subunits mixed before assembly produce mixed heteropolymers that are also uniformly labeled along their lengths to an extent dependent on the proportion of erythrocyte tubulin contained. Mixtures of brain and erythrocyte subunits do not exhibit strong preferential assembly (subunit sorting) onto a given type of microtubule. It is therefore unlikely that the alternating regions of antibody labeling that we observed are due to patchy labeling by the antibody or to tubulin sorting. We conclude that they are due instead to microtubule annealing.

The appearance of heteropolymers immediately after mixing pre-formed microtubules occurred at a rate that is inconsistent with an elongation mechanism that is based solely on subunit addition to microtubule ends. From the rate constants that we have determined for erythrocyte-tubulin subunit association and dissociation with microtubule ends (\(k^+ = 8.4\) and \(2.5 \times 10^6\) M\(^{-1}\)s\(^{-1}\) and \(k^- = 14\) and \(5\) s\(^{-1}\) for the plus and minus ends, respectively [23]), and from an equilibrium subunit concentration of 3 \(\mu\)M, we calculate that microtubules would elongate by 0.2 \(\mu\)m during the first 30 s after mixing. However, for the same time interval we observed lengths of 1.2–1.5 \(\mu\)m, or six to seven times the rate expected for elongation by subunit addition alone.

Microtubules polymerized from brain microtubule protein containing MAPs were often used in the annealing experiments in preference to the brain PC tubes because of their tendency to shear to shorter lengths and to yield fewer anomalous polymers upon self-assembly. The use of microtubule protein could potentially complicate the analysis of annealing due to the inclusion of MAPs. However, annealing does not appear to depend on MAPs, since (a) microtubule preparations prepared from PC tubulin anneal efficiently in the absence of MAPs, (b) observations of microtubule mixtures labeled with a MAP2 antibody showed that the MAP2 molecules do not redistribute over the entire microtubule population, which suggests that the annealing process is not associated with the binding of MAPs over microtubule surfaces, and (c) microtubule seeds containing MAPs are competent to support elongation by tubulin subunits, which indicates that the ends of the microtubules are not blocked. Therefore, MAPs are not required for annealing; but, at the present time, their effect on microtubule annealing cannot be ruled out.

As microtubules anneal, they become longer while their number decreases. This phenomenon of redistribution of microtubule length has been reported previously (4, 9). During taxol-initiated assembly of tubulin subunits, Carlier and Pantaloni (9) observed an initial rapid phase of nucleation and polymerization resulting in many short polymers. This rapid phase was then followed by a second, slower phase during which the microtubule distribution shifted to fewer, longer tubules. Our experiments, which show that taxol-stabilized polymers also are able to anneal, indicate that the redistribution in length that Carlier and Pantaloni (9) reported could be attributed to annealing. In the three experiments performed using taxol, the rate of annealing was less than in the absence of taxol, suggesting that the dynamic properties of microtubule ends are important for annealing. The disappearance of the discontinuities between initial contact points supports this idea.

In complex biological phenomena, it is often useful to determine the rate-limiting events, and, as the first step in this analysis, we examined whether or not annealing was a diffusion-limited process. We calculated the relative mean lengths of heteropolymers from our annealing experiments (such as...
those shown in Fig. 10) and plotted mean length versus the square root of the time (Fig. 10, inset) according to the method of Oosawa and Asakura (31) and as applied by Carlier and Pantaloni (9). The markedly nonlinear relationship suggests that annealing is complex and is not simply a diffusion-limited process. This is not unreasonable, given the complexity of bringing two microtubules into alignment, each of which has 13 protofilaments.

Our intuition suggests that the annealing process is a second order reaction in which the rate of annealing (R) is the product of the annealing rate constant (k*) and the square of the microtubule number concentration. From the experiment shown in Fig. 10, we determined the rate of annealing by counting the number of annealed microtubules with time by electron microscopy and estimated the microtubule molarity (6 x 10^{-8} M) from the mean microtubule length. The rate constant calculated from the percentage of annealed polymers at the 1- and 60-min time points was determined to be 5.4 x 10^{-4} M^{-1}s^{-1} and 2.5 x 10^{-5} M^{-1}s^{-1}, respectively. These rate constants are two to three orders of magnitude lower that the constant calculated from the percentage of annealed polymers produced by the elongation of brain PC-tubulin subunits from erythrocyte tubulin seeds (the reverse of the process). This is not unreasonable, given the complexity of the annealing rate constant (k*) and the square of the microtubule number concentration. From the experiment shown in Fig. 10, we determined the rate of annealing by counting the number of annealed microtubules with time by electron microscopy and estimated the microtubule molarity (6 x 10^{-8} M) from the mean microtubule length. The rate constant calculated from the percentage of annealed polymers at the 1- and 60-min time points was determined to be 5.4 x 10^{-4} M^{-1}s^{-1} and 2.5 x 10^{-5} M^{-1}s^{-1}, respectively. These rate constants are two to three orders of magnitude lower that the constant calculated from the percentage of annealed polymers.

Annealing activity may be a unique property of erythrocyte microtubules. Preliminary observations indicate that brain microtubules will anneal with themselves in the absence of any erythrocyte tubulin. Populations of tyrosinated and detyrosinated brain microtubules displayed significant annealing within 30 min of mixing (Rothwell, S. W., and D. B. Murphy, manuscript in preparation). However, when heteropolymers produced by the elongation of brain PC-tubulin subunits from erythrocyte tubulin seeds (the reverse of the system described in Fig. 4) were incubated for long periods of time, no significant annealing was seen before 180 min. The basis for this difference could be due to differences in the magnitudes of their respective association and dissociation rate constants (24), the concentration of microtubule ends, or differences in experimental conditions. Thus, brain microtubules anneal with themselves just as erythrocyte microtubules do, although possibly at a slower rate.

Since the rate of microtubule annealing appears to be concentration-dependent, annealing activity may be most significant in vivo in regions in cells containing a high density of aligned microtubules, such as in marginal bands in blood cells, in fibers of the mitotic spindles in dividing cells, or in processes of neurons. In blood platelets, the microtubule bundle comprising the marginal band is thought to consist of a coiled hoop of a single microtubule (32, 33) and in other blood cells to be composed of only a few polymers (34). This configuration may be generated by microtubule annealing and may function to provide increased mechanical rigidity to the marginal band. In spindle fibers and nerve cell processes, microtubule annealing may constitute a repair mechanism to maintain structural integrity of microtubule-containing structures. It is interesting that photobleaching of fluorescent tubulin derivatives in the spindles of living cells are consistent with a process of breaking and annealing of the microtubules (35). Annealing activity may, therefore, be important for microtubule length changes that occur during mitosis. Analysis of microtubule annealing in cells containing broken microtubules will be of value in assessing the significance of microtubule annealing in vivo.

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