Differentiation between Dynamic Instability and End-to-end Annealing Models for Length Changes of Steady-state Microtubules*

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Short microtubules can be formed by shearing a sample at polymerization steady state of microtubules formed by glycerol-induced assembly of pure tubulin dimer. Such short microtubules show a rapid increase in mean length. The rate of this increase is too fast to be accounted for by statistical redistribution of subunits between microtubules. We propose that the fast length changes are a result of the end-to-end annealing of microtubules demonstrated by Rothwell et al. (Rothwell, S. W., Grassner, W. A., and Murphy, D. B. (1986) J. Cell Biol. 102, 619-627). This proposal has been tested by measuring the rate of annealing of free microtubules to Tetrahymena axonemes under conditions identical to those used for the lengthening of sheared microtubules. That free microtubules anneal to axonemal microtubules is indicated by the following observations. (a) Axonemes elongate at both ends in the presence of steady state microtubules, as predicted for a symmetrical annealing process; (b) under conditions where the microtubule number concentration is greater than that for axonemes, the initial rate of axoneme elongation is more rapid with a low concentration of long microtubules at steady state than with a high number concentration of short microtubules at steady state. These observations are inconsistent with the predictions of a model based on microtubule dynamic instability (Mitchison, T., and Kirschner, M. (1984) Nature 312, 237-242). The annealing rate observed with axonemes can account for the rate of elongation of sheared steady state microtubules.

The dynamic instability model for microtubules proposes that a sample of microtubules at steady state is composed of two subpopulations, one growing and one shrinking, that interconvert infrequently (1). This model was proposed to account for two experimental results. First, when microtubules undergoing presteady-state elongation were diluted, so that the tubulin subunit concentration fell below the critical subunit concentration, the ensuing disassembly resulted in a decrease in the number concentration of microtubules. However, when the number concentration was reduced by about half (10 min), the mean microtubule length had increased from 18.3 to 21.5 μm. Although one might expect the mean length of the surviving microtubules would increase during the initial phase for microtubule disassembly, since the calculated mean is no longer influenced by the population of very short microtubules which are preferentially lost in an endwise subunit dissociation mechanism (2, 3), the observed length distribution was not that which would be expected from an uncomplicated endwise disassembly (2, 3) of the initial population of microtubules. Second, when assembling microtubules which had almost attained a steady state were sheared and then allowed to reach a steady state, there was a relatively rapid decrease in the microtubule number concentration, as the mean microtubule length dramatically increased (from about 12 to 70 μm) (1). This increase was more rapid than could be accounted for by statistical fluctuation in lengths (4). Both of these surprising observations were accounted for (1, 5) by a reaction scheme which assumes that: (a) microtubules have a cap of tubulin-GTP subunits at each end; (b) a tubulin-GTP cap stabilizes microtubules, since the rate for tubulin-GTP subunit dissociation is much slower than that for tubulin-GDP subunits; (c) tubulin-GTP subunits are unable to recap a microtubule which has lost tubulin-GTP caps at both ends. We have recently provided evidence that (c) may not be correct (6). This does not, however, rule out the GTP cap model, since it is still possible that uncapped microtubules will undergo essentially irreversible disassembly, if the rate for tubulin-GDP subunit loss is sufficiently high relative to that for tubulin GTP subunit loss, i.e. if the rate constants in element (b) have appropriate values.

Although the studies implicating microtubule dynamic instability were done with pure tubulin in the absence of glycerol (1), the GTP cap model for interpreting these results is based on studies of the GTPase and assembly kinetics with microtubules formed in a glycerol-induced reaction (7-9). Differences in reaction conditions may alter the relative significance of various reaction paths and we, therefore, thought that it would be of value to determine whether dynamic instability is manifested with microtubules formed in a glycerol-induced reaction. We find that there is a rapid length redistribution with sheared microtubules that had been formed from pure tubulin in a glycerol-induced assembly but that this apparently results from microtubule end-to-end annealing, rather than dynamic instability. It has recently been demonstrated that preformed microtubules polymerized from chicken brain and erythrocyte tubulin anneal end-to-end, so that the lengths of in vitro microtubules can be influenced by this process (10).

EXPERIMENTAL PROCEDURES

Pure tubulin dimer, prepared from pig brain as described previously (6), was polymerized at 37 °C in pH 6.8 assembly buffer containing 0.1 M Mes, 6.5 mM MgCl₂, 3.4 M glycerol, 1.0 mM EGTA, 1 mM acetylphosphate, and acetate kinase (0.0750 units/ml). Unless stated otherwise, axonemes were from Tetrahymena thermophila (strain B-256, kindly provided by Dr. K. A. Johnson, Pennsylvania State University), isolated as described (1). Axonemes from Strongylocen-
**RESULTS**

Dynamic Instability and/or Annealing Is Important with Free Microtubules in Glycerol Buffer—Electron microscopic analysis of sheared steady state microtubules revealed that after shearing the mean length of microtubules increased from 1.12 μm (standard deviation 0.66) to 2.55 μm (standard deviation 1.47) in 10 min, and to 3.32 μm (standard deviation 1.70) after 30 min (Fig. 1). The length of steady state microtubules is predicted to increase progressively as a result of statistical redistribution of tubulin subunits (4). To determine whether the rates for subunit addition and loss at microtubule ends are sufficiently rapid for this mechanism to generate the observed doubling in length in only 10 min, we studied the rate of dilution-induced disassembly. The observed rate for dilution induced disassembly following a dilution such that during which the number of subunit addition and subtraction events at microtubule ends is equal to the square of the resultant tubulin subunit concentration is very much less than the critical concentration provides an estimate of the rate for subunit loss at steady state. Results from a typical experiment (Fig. 2) show that microtubules disassemble at a rate of about 0.5% of polymer mass/s. Based on a mean microtubule length of 12.9 μm (i.e. 21,000 subunits (13)), this corresponds to a rate constant equal to 105 subunits/microtubule/s. For a random subunit dissociation mechanism the time necessary for a doubling in mean length corresponds to that during which the number of subunit addition and subtraction events at microtubule ends is equal to the square of...
the number of tubulin subunits in mean-length microtubules (14). Thus, for the reaction shown in Fig. 2, a doubling in length by a random process is expected to require about 2.25 h; since the mean length more than doubles in only 10 min, such a mechanism is ruled out. As a result, we conclude that as is the case with glycerol-free buffer (1), an additional reaction path, such as that encompassed in the microtubule dynamic instability phenomenon and/or a facile end-to-end annealing reaction, contributes significantly to the dynamic properties of free microtubules in glycerol-containing buffer.

**Dynamic Instability and/or Annealing Is Important for Microtubule Growth of Axonemes in Glycerol Buffer**—That annealing and/or dynamic instability is important for axoneme elongation is indicated by our observation that the rate of microtubule growth at axoneme ends at a fixed tubulin subunit concentration was dramatically enhanced by the presence of microtubules (cf. Reactions A and B in Table I). It has been predicted that net polymerization on a stable nucleating site will occur at tubulin subunit concentrations equal to (and less than) the critical concentration for free microtubules (15). Such behavior has been observed previously with both centrosomes (16) and *Tetrahymena* axonemes (1). The important point is that the same tubulin subunit concentration is present for the reaction with (A in Table I) and without (B in Table I) microtubules (see below), so that the enhanced rate of growth must result from the presence of microtubules. It should be noted that the lower rate in Reaction B does not result from microtubules not being present to buffer a decrease in tubulin concentration that results from axoneme elongation. The reactions contain only $5 \times 10^{-12}$ M axonemes, so the addition of a 1-μm length of microtubule on every axoneme would consume about $8 \times 10^{-3}$ M tubulin, which is less than 0.5% of the tubulin subunits in the reaction mixture (the critical subunit concentration is 1.7 μM, determined by the method described in Table I of Ref. 6).

As noted above, the tubulin subunit concentration is presumed to be the same in the reaction of axonemes with sheared microtubule (Reaction A) and with the supernatant derived from centrifuging unsheared microtubules (Reaction B). The existence of a higher subunit concentration with sheared microtubules would invalidate our conclusion that annealing and or dynamic instability contribute to axoneme elongation. Our concern that the subunit concentration is increased by shearing is based on the observation that there is a transient decrease in the turbidity of a microtubule solution after shearing (1). Such a decrease in microtubule mass is predicted by the dynamic instability model, since shearing will generate uncapped microtubules. The following three experiments provide evidence that the increase in elongation in Reaction A does not result from the reaction having a higher tubulin subunit concentration. First, instead of diluting steady-state microtubules before the shearing step, these were sheared and then diluted 6-fold. The dilution will tend to offset an increase in subunit concentration that might result from shearing. We found in a 10-min reaction that was initiated 30 s after shearing and dilution there were 5 (with *Tetrahymena* axonemes) to 10 (with *Strongylocentrotus* axonemes) times as many axonemes elongated, as compared to a reaction with steady state tubulin subunits. Second, we measured the fraction of axoneme ends that were elongated when the reaction with sheared microtubules was initiated at different times after shearing. The rationale here is that any perturbation in the steady state produced by shearing would dissipate rapidly. With *Strongylocentrotus* axonemes 52 of 148 axoneme ends

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**TABLE I**

<table>
<thead>
<tr>
<th>Reaction of axonemes with</th>
<th>Fraction of axoneme ends with microtubules</th>
<th>Fraction of axonemes with microtubules at both ends</th>
<th>Microtubules/axoneme end</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Sheared steady-state microtubules, 10 min</td>
<td>0.69 (n = 110)</td>
<td>0.44</td>
<td>1.24</td>
</tr>
<tr>
<td>B Steady-state tubulin subunits, 10 min</td>
<td>0.028 (n = 106)</td>
<td>0</td>
<td>0.028</td>
</tr>
</tbody>
</table>

*The 69% figure reported here does not indicate that 31% of axonemes are damaged so that they cannot be elongated. In a separate experiment it was found that 99% of axonemes were elongated with at least one (generally more than 5) microtubule when these were incubated with 10 μM tubulin subunits for 45 min.

Addition of axonemes results in a 5% dilution of the steady-state supernatant, and one might wonder how it is possible to observe any axoneme elongation at 0.95 times the critical subunit concentration. It would appear that the effect of the dilution is compensated for by disassembly of microtubules that are observed to contaminate the supernatant obtained by centrifuging the steady-state microtubules. Also, the initial assembly to steady state is at 37°C, and the centrifugation is at 25°C. As a result, it is likely that there is some disassembly during the centrifugation step, so that the subunit concentration probably slightly exceeds the critical concentration before the axoneme addition.

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2 Calculated by assuming that the two microtubule ends are equally reactive, so that at steady state there are about 105/2 disassembly (and assembly) events at each end, per s. Subunit diffusion into each end to a depth of 1.12/2 μm would require (900)$^2$ events.

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**Fig. 2.** Dilution-induced disassembly of microtubules. Steady-state microtubules, that had been assembled in the presence of a trace amount of $[^3H]GTP$, were diluted 2500-fold into a solution containing 3.4 M glycerol, 6.5 mM MgCl₂, the amount of remaining radioactive polymer was analyzed as a function of time, as described previously (6).

**TABLE I**

*Effect of free microtubules on the rate of axoneme elongation*

Axonemes (number concentration, approximately $5 \times 10^{-12}$ M) were reacted with sheared steady-state microtubules (number concentration, about $6.9 \times 10^{-14}$ M, initial mean length 0.76 μm) or with the supernatant obtained by centrifuging microtubules that were at steady state.
(35%) were elongated after a 10-min reaction that was initiated 30 s after shearing. In a reaction initiated 5 min later, 29% of the axoneme ends were elongated. When this experiment was done with Tetrahymena axonemes 66 and 54% of axoneme ends were elongated in reactions initiated 30 s and 5 min after shearing. Finally, we compared the pattern for axoneme elongation in a reaction where microtubules were sheared in a syringe and the final pass was squirted into a solution containing axonemes, with that in a reaction of axonemes with tubulin subunits at a concentration that slightly exceeded the critical subunit concentration. In the former reaction 19% of Strongylocentrotus axonemes were elongated in 5 min, and in 93% of these there were one or two microtubules/axoneme end. In contrast, 45 of 47 Strongylocentrotus axonemes were elongated after a 5-min reaction with 3 μM tubulin. Elongation took the form of a short tuft (4 or more microtubules at one end) for 70% of these, and a single microtubule at an axoneme end was only observed twice. The 3 μM tubulin subunit concentration used for axoneme elongation exceeds the 1.7 μM critical subunit concentration, and it represents the concentration that would exist if about 15% of the microtubules disassemble as a result of shearing. The different pattern for axoneme elongation observed in Reaction A in Table I and in a reaction of 3 μM tubulin suggests that axoneme elongation in Reaction A does not result from a dramatic shear-induced increase in the tubulin subunit concentration.

Rate of Elongation of Axoneme Ends—Axonemes were incubated for 10 min with tubulin that had been assembled to steady state and contained a high number concentration of short microtubules (Reaction I), or with a low number concentration of long microtubules (Reaction II); there were about 300 times more microtubules and 20 times as much microtubule mass in Reaction I as compared to Reaction II. Results are summarized in Table II, and representative electron micrographs used in our analysis are given in Fig. 3. Incubation with a high number concentration of short microtubules in the steady state concentration of tubulin (Reaction I) increased the frequency of microtubule elongation on axonemes (Table II, line B), the total mass of microtubules at axoneme ends (line D), and the fraction of axonemes with microtubules at both ends (line E), as compared to a reaction with a low concentration of long microtubules in steady state tubulin (Reaction II). A substantial fraction (27%) of axonemes in Reaction I had one or more microtubules at both ends. Equivalent reactivity of both axoneme ends requires the presence of free microtubules, since when axonemes were reacted with tubulin subunits at the critical concentration, there is very little elongation at both ends, as compared to a reaction with a mixture containing steady state microtubules (Table I). Equivalent reactivity of both axoneme ends is not observed even when the tubulin subunit concentration is about 3 times the critical concentration; only 1% of axonemes had comparable microtubule elongation of both ends when these were incubated for 5 min with 5 μM tubulin; 68% of axonemes had growth at only one end and 30% displayed the pattern shown in Fig. 3C.

Discussion

Differentiating between Microtubule Dynamic Instability and End-to-end Annealing Mechanisms for Elongation of Axonemes—That dynamic instability and/or annealing is important under the reaction conditions used here is indicated by the fact that microtubule elongation at axoneme ends is not identical in Reactions A and B (Table I) and I and II (Table II). Dynamic instability was proposed to be important for elongation of free microtubules, axonemes, and centrioles in glycerol-free buffer (1, 16). Murphy and co-workers (10) have shown by immunogold labeling and electron microscopy that segments of preformed microtubules can be joined together end-to-end to form a longer microtubule. We now analyze whether this path is the predominant one for elongation of sheared microtubules in glycerol buffer.

Table III describes how annealing and dynamic instability models for axoneme elongation can be differentiated. The predictions described here are for the initial phase of the reaction, i.e., before a significant fraction of axoneme microtubules have been elongated and before there is significant mass transfer from free microtubules to axoneme ends. The most surprising prediction for the dynamic instability model is that Reaction I's 300-fold higher free microtubule number concentration and 20-fold greater mass of free microtubules, as compared to Reaction II, will only result in 2.0-fold greater axoneme-elongated microtubule length and mass. To understand this consider the fate in Reaction I and II of subunits released by rapid quantitative disassembly of a dynamically unstable microtubule. In Reaction I the subunits are widely

<table>
<thead>
<tr>
<th>Table II Microtubule elongation at axoneme ends</th>
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<tbody>
<tr>
<td>Reaction</td>
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<tr>
<td>A. Mean length of elongated microtubules (μm)*</td>
</tr>
<tr>
<td>B. Fraction of axoneme ends with microtubules*</td>
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<tr>
<td>C. No. microtubules/axoneme end</td>
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<tr>
<td>D. Total polymer mass (A x B x C)</td>
</tr>
<tr>
<td>E. Fraction axonemes with microtubules at both ends*</td>
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* Measured from photographs of axoneme-associated microtubules; results plotted in Fig. 4

Determined by microscopic inspection of axoneme ends.

41% of 110 axonemes that were photographed had microtubules at both ends. This value is higher than the 0.27 value derived from scoring directly from the electron microscope, and we believe that this comes from the fact that photographs are usually taken of axonemes where both ends are clearly visible. In contrast, when scoring from the electron microscope image, we ignored axonemes that were so tangled with microtubules that a clear connection could not be discerned. This difference would tend to decrease the reported fraction of axoneme ends with microtubules (Column B) from the true value and from the value derived from photographs of clearly discernable axonemes.

It should be noted that equivalent reactivity of both axoneme ends does not predict that under conditions where only a fraction of axonemes have microtubules at ends, 100% of axonemes with microtubules will be elongated at both ends.
Fig. 3. Reaction of microtubules and tubulin subunits with *Tetrahymena* axonemes. A, axonemes from Reaction I; B, axoneme from Reaction II at low (top) and high (bottom) magnification; C, axoneme from reaction of 5 μM tubulin subunits for 5 min, under the conditions described in the legend to Fig. 1.
Differentiation between Models for Length Changes of Microtubules

Table III

<table>
<thead>
<tr>
<th>Observation</th>
<th>Dynamic instability model</th>
<th>Annealing model</th>
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<tbody>
<tr>
<td></td>
<td>Reaction I/Reaction II</td>
<td>Reaction I/Reaction II</td>
</tr>
<tr>
<td>Calculated ratio</td>
<td>Observed ratio(^a)</td>
<td>Calculated ratio</td>
</tr>
<tr>
<td>Mean length of microtubules on axonemes</td>
<td>3.0(^b)</td>
<td>0.2(^e)</td>
</tr>
<tr>
<td>Total microtubule mass on axonemes</td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td>Fraction axonemes with microtubules at both ends</td>
<td>1(^f)</td>
<td>27(^f)</td>
</tr>
</tbody>
</table>

\(^a\) Results from Table II.

\(^b\) For a dynamic instability model the initial rate of increase in microtubule length at axoneme ends is equal to: (number of subunits released by net microtubule disassembly/time)/(sites for accepting released subunits). This is equal to \(f_0 \times \alpha_{ad} \times (\text{microtubule number concentration}) \times (\text{length of microtubule})/(\text{number of sites for accepting released subunits})\), where \(f_0\) is the fraction of microtubules that are uncapped/time and \(\alpha_{ad}\) is the rate constant for dissociation of tubulin-GDP subunits from the end of an uncapped microtubule. Both \(f_0\) (see Equation 2 in Ref. 17 for an analysis of factors that influence the steady-state value of \(f_0\) and \(\alpha_{ad}\) are expected to be independent of microtubule length. For Reactions I and II in Table II the dynamic instability model predicts rates for axoneme elongation equal to: 

\[
(3.3 \times 10^{-10} \text{ M}, \text{ i.e. average of initial and final microtubule number concentration}) = 1.35 \times f_{ad} \mu M \text{ and } f_{ad} = (2.1 \times 10^{-10})/(2.1 \times 10^{-12}) = 0.45 \text{ f}_{ad} \mu M, \text{ respectively. Thus, for Reactions I and II, the ratio of lengths and microtubule mass is expected to be 1.35/0.45 = 3.0, respectively. It should be noted that if the axoneme concentration were made lower, which we found difficult since there were insufficient axonemes to score or the concentration at long microtubules increased, which we found not possible since microtubules bound to axonemes were difficult to score in a background of tangled microtubules, then the microtubule number concentration would cancel in the denominator and numerator of the equation describing the rate for axoneme elongation. If the microtubule number concentration had cancelled, then the ratio of the rates in Reactions I and II would be equal to the ratio of the microtubule lengths (i.e. 1.55/12.1 = 0.13). It is emphasized that the predictions given have only correspond to the initial phase of the reaction before the microtubule number concentration changes significantly. That the microtubule number concentration does not change in Reaction I, as a result of axoneme elongation, is indicated by the fact that the microtubule number concentration (6.3 \times 10^{-10} \text{ M}) was 138 times that for axonemes and the incubation period was sufficiently short that there were only 0.91 microtubule/axoneme. With Reaction II, the microtubule number concentration was only 42% that for axonemes, so that we are more concerned about the question of whether we, in fact, measured the initial rate. Our conclusion that we measured the initial rate is based on the following. (a) The microtubule number concentration did not change significantly, since only 13.5% of axoneme ends had microtubules at the end of the 10-min incubation. (b) In a control reaction, axonemes were incubated for 45 min with a steady-state microtubule supernatant (derived by centrifugation of a mixture of steady-state microtubules) that was supplemented with a concentration of tubulin subunits that was identical to that contained in the microtubules added in Reaction II. It is expected that this reaction will rapidly generate an end-point equivalent to that which would be reached in Reaction II. It was found that under these conditions 48% of axonemes had microtubules (mean length, 1.26 \mu m, \(n = 116\)) and there were as many free microtubules, formed by \textit{de novo} nucleation or possibly by breakage of microtubules from axonemes, as axoneme-bound microtubules. This result indicates that when we saw only 13.5% of axoneme ends elongated with microtubules in Reaction II, we were following the initial rate.

\(^c\) For an annealing reaction in which the initial rate is studied, so that multiple annealing reactions do not occur, the ratio of lengths at axoneme ends is expected to be equal to that for free microtubules; this ratio was equal to 2.35/12.1 = 0.2.

\(^d\) For an annealing mechanism the rate of increase in microtubule ends will be proportional to the total mass of reacting microtubules. Only the more reactive plus end, with a lower critical subunit concentration (1, 18), is expected to be significantly elongated at the low tubulin concentration corresponding to the critical concentration. Also significant elongation at both ends is not expected since the treadmilling phenomenon will disassemble microtubules at the axoneme’s minus end, leaving the higher tubulin subunit critical concentration. Since the tubulin subunit concentration is the same during the 10-min incubation of Reactions I and II, the tendency for elongation at both axoneme ends is expected to be identical in both reactions.

\(^e\) Annealing of microtubules at either axoneme end is a perfectly symmetrical reaction, and more axonemes are expected to have microtubules at both axoneme ends when a greater fraction of axonemes has reacted with microtubules (i.e. in Reaction I).

shared by the large population of short microtubules and the low concentration of axonemes. The former process drains off most of the subunits, so that the axonemes are little elongated. In contrast, in Reaction II there is less competition by microtubules for released subunits, and consequently the axonemes will be elongated relatively rapidly. Footnote b in Table III quantitatively analyzes the predictions of the dynamic instability model for the reactions described in Table II.

As indicated in Table III, our observation that incubation of axonemes with a low number concentration of long microtubules (Reaction II) results in longer microtubules and 14-rather 3.0-fold less total microtubule mass at axoneme ends, as compared to the reaction with a high number concentration of short microtubules, is consistent only with an annealing mechanism for axoneme elongation under these reaction conditions. Further proof of this point comes from our observation of significant fraction of axonemes with microtubules at both ends (compare Reactions A and B in Table I and see column E in Table II). It is well established that the two axoneme ends react at dramatically different rates with tubulin subunits (1, 18), and we have confirmed this for our reaction conditions (Fig. 3C). The symmetry of the annealing reaction requires equivalent reactivity at the two axoneme ends.

There is an additional prediction for an annealing mechanism not considered in Table III: the length distribution for
microtubules should be the same for axoneme-associated and free microtubules. This prediction is not fully met; the mean length for free and axoneme-associated microtubules is 2.35 and 1.72 μm, respectively, in Reaction I, and 3.74 and 12.1 μm for Reaction II (Table II). To account for the discrepancy between the lengths of axoneme-associated and free microtubules we suggest the following. (a) There may be a greater tendency for shear-induced breakage of long microtubules with an axoneme at an end. (b) The mean microtubule length is not a perfect measure of the length distribution, since it is disproportionately influenced by a relatively small population of very long microtubules (the median length for the free microtubules in Reaction II was 10.3 μm). (c) About 20% of the observed 0.135 microtubule/axoneme end (column C in Table II) are apparently derived from reaction of subunits (there are 0.028 microtubule/axoneme end in Reaction B, Table I), and the so-generated short microtubules will decrease the observed mean length.

In summary, we suggest that in the presence of glycerol the predominant route for elongation of axonemal microtubules involves end-to-end annealing. This conclusion is supported by: (a) the observations of the bipolar axoneme elongation predicted for a symmetrical annealing process; (b) the different pattern for axoneme elongation on reaction with steady state microtubules (Fig. 3) and with tubulin subunits at a concentration that exceeds the critical concentration (Fig. 4); (c) the fact that the initial rate of axoneme elongation is more rapid in the presence of a low as compared to a high number concentration of steady state microtubules (Table III).

The Annealing Rate Measured with Axonemes Can Account for the Elongation of Free Microtubules—The observed doubling in length of sheared microtubules in only 600 s (Fig. 1) can be accounted for by an annealing mechanism. This conclusion is next justified by a qualitative and then by a semi-quantitative analysis. The reaction conditions for the study described in Fig. 1 were identical to those in Reaction I (Table II), except that the microtubule number concentration was about 9-fold higher. In Reaction I, 1.8 (i.e. 2 X 0.91, Table II) of the 11 microtubules in each axoneme were elongated by annealing to a microtubule end. If we assume that the annealing rate is proportional to the microtubule number concentration, we would expect about 16 (i.e. 9 X 1.8) of the 11 axoneme microtubules to be elongated under the conditions described in Fig. 1. If we further assume that annealing of free microtubules with axoneme microtubules is not specially facilitated, the prediction that every axoneme microtubule will be elongated once corresponds to the prediction that every free microtubule, as well, will react about one time with one other microtubule under these conditions; that is, a doubling in length of free microtubules is predicted.

In a semiquantitative analysis of the results we calculate an approximate rate constant for microtubule annealing and then show that this rate constant predicts the results in Fig. 1. The attachment of 0.91 microtubules at the 11 potential axoneme reaction sites, in the reaction of 6.9 X 10^{-10} M microtubules (Reaction I, Table II), corresponds to a rate constant for a pseudo first-order process equal to 1.4 X 10^{-4} s^{-1} and a second-order rate constant equal to 3.2 X 10^{-10} M^{-1} s^{-1}. To obtain a rough estimate of the predicted annealing rate for the reaction described in Fig. 1, we use the value for the second-order rate constant derived from Reaction I and assume that annealing is a simple second-order process in which the microtubule number concentration decreases, as it does in a simple dimerization process. The latter assumption will underestimate the annealing rate, since unlike dimerization, the reaction product from annealing is able to react further. For a simple second-order dimerization mechanism the half-time for the reaction is equal to 1/(second-order rate constant)(microtubule number concentration). This is equal to 1/(3.2 X 10^{-5} M^{-1} s^{-1}) (1.0 X 10^{-10} M tubulin subunits in microtubules) (1.14 μm^{-1} (1640 tubulin subunits/μm^{-1})^{-1}) = 580 s. Thus, the doubling in length of sheared microtubules

![Fig. 4. Length distribution of microtubules at axoneme ends after incubation with steady-state microtubules. Reactions I and II are represented by the dotted and solid lines, respectively.](image-url)
is also indicated by results from a recent study (23). It was
found that the mean length of steady state microtubules only
increased to 3 h 14.0 ± 1.7 μm at 3°C (0.57 μm/min). This rate
is about 50 times slower than in the absence of glycerol (1, 23).
The observed increase in mean microtubule length and
decrease in microtubule number concentration (23) can be
accounted for if 15-20% of the microtubules undergo anneal-
ing; about 10% of the tubes had an appearance consistent
with their having undergone an annealing reaction. It should
be noted that the observation that dynamic instability is
reduced in glycerol buffer (23) controverts the interpretation of
the experiments (also done in glycerol buffer, Fig. 1 in Ref.
9) that provide the basis for the GTP cap as the source of
dynamic instability behavior. That is, the interpretation of
results from studies of the GTPase and assembly kinetics in
glycerol buffer (7-9) would appear to predict that dynamic
instability will be manifested in glycerol buffer. Finally the
observation of annealing is only 10.6% of microtubules after
3 h (23) would appear to conflict with our conclusion that
annealing is a facile process under these experimental con-
ditions. However, although the solvent conditions are similar
in the two studies, our results are for short microtubules
(mean length about 1 μm) in relatively dilute solution (micro-
tubule numbers concentration = 1/(microtubule length)3, see Table
IV). In the study by Kristofferson et al. (23) the microtubules
were much longer (mean length, 14 μm) and the microtubule
concentration extremely high (concentration = 500/(length)3,
see Table IV), relative to that required for free rotation and
diffusion. It has been concluded that microtubule annealing
rates in dilute solution will be extremely sensitive to length
(24), and the high concentration used in these experiments
likely hinder end-to-end contacts by both bimolecular diffusion
and by reaction within tangles of microtubules. Thus, the recent
results (23) appear to be in accord with those presented here.

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REFERENCES