Sliding of STOP Proteins on Microtubules†

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ABSTRACT: Microtubules are stabilized against cold temperature disassembly by 145-kilodalton proteins [stable tubule only polypeptides (STOPs)] that block the end-wise dissociation of subunits from the polymers. We describe here several kinetic parameters of the interaction of STOPs with microtubules. STOPs will bind to microtubules either during assembly of the polymer or at steady state. The addition appears random on the polymers and does not require the mediation of tubulin subunits. Tubulin subunits compete with microtubules for STOP binding, but binding to the polymers is apparently irreversible. We demonstrate that STOPs do not exchange measurably between polymers at steady state. Nonetheless, a displacement of STOPs within a single polymer is readily demonstrable. We have determined that the displacement is apparently due to a surface translocation, or "sliding", of STOPs on microtubules.

Microtubules are central to the establishment of several motility functions within eucaryotic cells [for a review, see Dustin (1978)]. Further, their capacity to establish motility is most likely interwoven with the specific regulation, both temporal and spacial, of their assembly state.

To establish a better understanding of the control of the microtubule assembly state, we have been studying the substoichiometric stabilization of microtubules to disassembly by a protein factor isolated from mammalian brain. In the presence of this factor, microtubules are stable to cold temperature (4 °C) (Webb & Wilson, 1980; Margolis & Rauch, 1981; Job et al., 1981, 1982) and to millimolar calcium levels (Job et al., 1981). Such microtubules, designated "cold stable", are, however, labile to micromolar calmodulin levels (Job et al., 1981) and to an apparent protein kinase activity (Job et al., 1983). A protein which we have recently isolated (R. L. Margolis and C. T. Rauch, unpublished results) is responsible for cold stability. It has been designated STOP protein (stable tubule only polypeptide). We have established that STOPs bind randomly on microtubules and protect regions of the polymer that lie between two blocks against end-wise depolymerization (Job et al., 1982).

We present here evidence that STOPs bind to microtubules by a random surface binding mechanism and that the binding reaction is apparently irreversible under our assay conditions. We cannot compete the activity off of bound microtubules nor find any exchange from cold-stable microtubules that will stabilize cold-labile microtubules. Nonetheless, we find that, within a single polymer, STOPs appear to migrate so that previously cold-labile regions become progressively cold stable while previously cold-stable regions become cold labile. This sliding of STOPs on microtubules is potentially of substantial interest, since such sliding behavior may represent a prototype for the general behavior of microtubule-bound proteins. Further, there is also the possibility that STOPs may be involved directly in microtubule-dependent motility.

Materials and Methods

Materials. All chemicals unless otherwise indicated were purchased from Sigma Chemical Co. [^{3}H]GTP (25–50 Ci/mmol) was obtained from New England Nuclear; nucleotides and acetate kinase were products of Boehringer-Mannheim; podophyllotoxin, purified from the crude form supplied by Aldrich, was the kind gift of L. Wilson; the filtration assay used GF/C glass fiber filters from Whatman. The buffer used throughout, for protein purification and for assay, was 100 mM 2-(*N*-morpholino)ethanesulfonic acid (Mes), 1.0 mM $MgCl_2$, 1.0 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), and 0.02% NaN_3, pH 6.75 (designated MME).

Protein Preparation. Cold-stable microtubule protein was obtained from beef brain by two cycles of assembly and disassembly by minor modification for beef brain (Job & Margolis, 1984) of procedures previously demonstrated to give high yields in sheep brain extracts (Pirollet et al., 1983). An active fraction of partially purified STOP protein, when used to reconstitute cold stability, was purified away from tubulin by passage of disassembled cold-stable microtubule protein through DEAE-cellulose (Whatman) columns, as described previously (Job et al., 1982; Pirollet et al., 1983). The column elution buffer was MME, supplemented with 0.1 M NaCl. The active fraction, present in the flow-through peak from the column, was concentrated by ammonium sulfate precipitation (50% of saturation) and a final desalting step on a G-25 (Pharmacia) column into MME buffer.

STOP proteins used for the assays in this report represent only a small fraction of the diethylaminoethyl (DEAE) column eluate. Elsewhere, we report on the purification of the STOP protein (R. L. Margolis and C. T. Rauch, unpublished results). For the pure protein, we find that 0.015 mg of STOP protein will 50% stabilize 0.56 mg of tubulin in an assembly assay. Assuming molecular weights of 145 000 for STOPs and 100 000 for tubulin, and that all the STOPs bind to microtubules and that approximately 50% of the tubulin is assembly competent, the molar ratio of STOPs to tubulin in microtubules at 50% stabilization is 1:110. This is several times more than theoretically required according to our model (Job et al., 1982), but we do not know if all the STOPs are active or if they act independently or cooperatively on the microtubule. By comparison of dose–effect curves corresponding either to the purified material or to the DEAE column eluate, it can be estimated that STOPs represent approximately 5–10% of the DEAE eluate proteins.

Assays. The microtubule assembly state was monitored by turbidity measurement as described (Pirollet et al., 1983). The filter assay, to determine radioactive regions of microtubules

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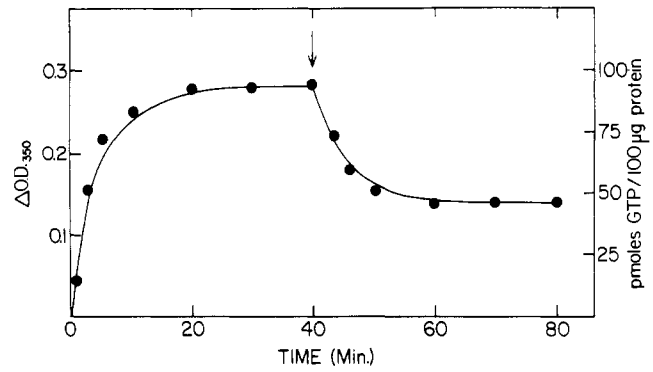

FIGURE 1: Comparison of spectrophotometric and filter assays of microtubule assembly state. Assembly of cold-stable microtubules was monitored by the turbidity change at a wavelength of 350 nm (solid line). The arrow indicates the point at which the samples were rapidly cooled to 7 °C. A portion of the same sample was incubated with [^{3}H]GTP, and aliquots were filter assayed at time points as described under Materials and Methods. Radioactivity incorporation into microtubules and retention in cold-stable regions are shown (closed circles), expressed as specific picomoles of GTP incorporated. Assembly, at 30 °C, was conducted with 2 mg/mL protein and 50 μM GTP. Acetyl phosphate and acetate kinase were also present.

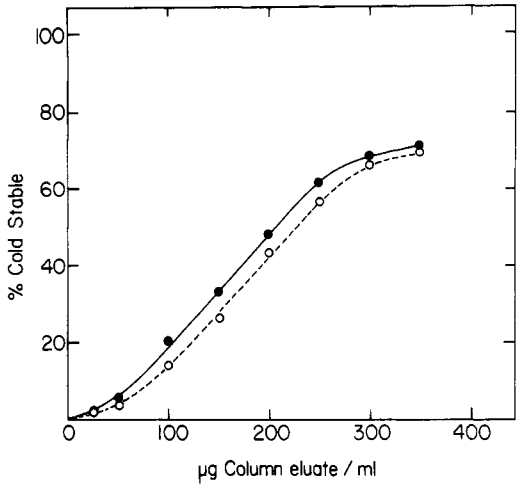

FIGURE 2: Comparison of cold stability induced by STOP protein addition prior to or after microtubule assembly. Recycled (3×) cold-labile microtubule protein (1 mg/mL) was assembled to steady state with [^{3}H]GTP (see Materials and Methods) and filter assayed for total label incorporation and for cold-stable level at each data point. The DEAE column flow-through eluate with cold-stabilizing activity was added at different concentrations, as indicated, either prior to assembly (●) or after cold-labile microtubules had attained steady state by preassembly at 30 °C for 50 min (○). For samples where the active fraction was added prior to assembly, microtubules were assembled at 30 °C for 50 min, total label incorporation was determined, and then an aliquot was chilled to 7 °C for 40 min after which the cold-stable level was assayed. For the steady-state addition experiments, microtubules assembled for 50 min were mixed with the active fraction, incubated a further 20 min at 30 °C, and assayed for total incorporation. Aliquots were cooled to 7 °C for 40 min and then assayed for cold stability.

labeled with [^{3}H]GTP, was performed essentially as previously described (Wilson et al., 1982). Assay conditions, unless otherwise noted, were 2.0 mg/mL protein, 50 μM GTP, 10μCi/mL [^{3}H]GTP, 0.05 unit/mL acetate kinase, and 10 mM acetyl phosphate, when used to maintain constant GTP concentration. Time points were taken for filter assay by removing 50-μL aliquots into 500 μL of a stop buffer. This buffer, composed of MME, 10% dimethyl sulfoxide (Me_2SO) 25% glycerol, and 50 μM podophyllotoxin (PLN), was always maintained at 30 °C. The buffer was designed to prevent further assembly or disassembly reactions of the polymer after taking a time point. Samples in stop buffer were applied to GF/C filters under negative pressure and processed as previously reported (Wilson et al., 1982). All measurements were the average of three replicates and are reported as net values after subtraction of blanks. The blanks, usually about 1000 cpm each, were generated by incubating microtubule protein with PLN from the time assembly was initiated, to prevent assembly, or were residual counts after disassembling microtubules (10 min at 7 °C in MME buffer plus 2 mM $CaCl_2$). The blanks obtained by both methods are equivalent.

Results

Verification of the Filter Assay. In much of the work reported here, we use a filter assay (Wilson et al., 1982) to quantify specific radioactive regions of microtubules. The assay measures the presence of [^{3}H]GDP in the microtubule polymer. [^{3}H]GTP incorporates into the polymer during the assembly reaction with an accompanying hydrolysis to GDP (Weisenberg et al., 1976). [^{3}H]GDP remains bound nonexchangeably in the polymer until subunit release and therefore serves as a marker for the presence and position of its bound tubulin subunit (Margolis & Wilson, 1978). The concentration of [^{3}H]GDP-containing regions of microtubules can be readily determined by trapping of polymers on glass fiber filters and subsequent radioactive assay. It has been determined (Wilson et al., 1982) that the bulk of unassembled subunits and free GTP passes through filters, giving modest backgrounds, and that the trapping of radioactivity varies linearly with the concentration of fully labeled polymers. Further, accurate time points can be reliably determined with this method (Wilson et al., 1982; Farrell et al., 1983), and the method is fully comparable in result with both turbidity measurements of the assembled state (Wilson et al., 1982; Farrell et al., 1983) and microtubule sedimentation assay (Farrell et al., 1983).

We have extensively reverified the validity of the filter assay for the purpose of this work. We show the result of one such determination which compares the microtubule assembled state as determined by turbidity measurement and filter assay (Figure 1). Solution turbidity has previously been shown to linearly covary with the microtubule assembled state (Gaskin et al., 1974). For this experiment, microtubules were assembled in [^{3}H]GTP and assayed simultaneously by turbidity measurement and filter trapping. It is clear that there is a one to one correspondence of radioactivity to turbidity both during assembly and during cold-induced disassembly of partially cold-stable microtubules.

The lack of a threshold for detection of radioactivity during the early stages of assembly (when short microtubules predominate) indicates there is no detectable bias for longer microtubules in the filter assay. We have confirmed this possibility directly in assays which show sheared and unsheared microtubules are both retained to the same extent on filters (not shown).

Parameters of STOP Protein Addition to Microtubules. We wished to determine the nature of STOP protein binding to microtubules. We have previously modeled STOP protein addition to be random along the length of the polymer (Job et al., 1982). If the binding sites are accessible on the polymer (surface binding sites rather than within the polymer lattice or interior), then STOP protein addition should stabilize previously assembled microtubules as efficiently as it stabilizes assembling polymers. Upon titration of STOPs, in increasing concentration, into polymers during assembly or at steady state, we find (Figure 2) that the steady-state microtubule stabilization has a STOP protein concentration dependency that is superimposible on that generated by addition during assembly.

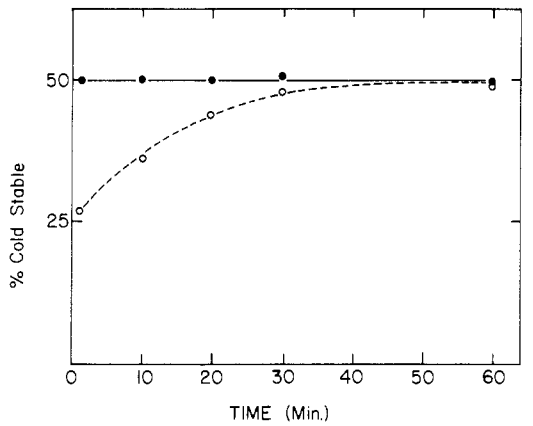

FIGURE 3: Kinetics of the binding of STOPs onto preformed microtubules: the influence of free tubulin. Cold-labile (3× purified) microtubules were assembled for 40 min at 30 °C with [^{3}H]GTP under the standard conditions for filter assay, and total assembly and blank values were obtained. At time zero, a 200 μg/mL (final concentration) sample of the DEAE column derived active fraction was added with 10 μM PLN. Aliquots were cooled to 7 °C at the indicated time points, and cold-stable levels were assayed (●). An identical experiment was performed, except for the addition of unpolymerized tubulin at time zero, to a final concentration of 4 mg/mL (○). Final dilutions of microtubules and of STOP protein were the same in both experiments.

Both curves are close matches to the hypothetical curve generated by statistical prediction based on random STOP protein addition to microtubules along their length (Job et al., 1982). The result indicates that addition of STOPs is random and that the binding sites are accessible on the polymer surface.

The close match of results obtained on titration of STOPs onto steady-state and assembling microtubules (Figure 2) requires that STOPs not bind rapidly to polymers during assembly. Otherwise, the STOPs would bind to saturation on initiation of microtubule fragments and yield very little measurable cold stability. We therefore tested to determine the time course of STOP protein binding on microtubules. Surprisingly, the time course of STOP protein addition to steady-state microtubules is very rapid (Figure 3) and is essentially complete at the first time point taken. However, a large excess of unpolymerized tubulin subunits (such as would be present during assembly) effectively competes for STOP protein and delays its binding to polymers (Figure 3). The random addition of STOP proteins on polymers generated during assembly is therefore most likely due to tubulin subunit competition for STOP binding.

Since STOP proteins add randomly and rapidly to polymers at steady state, tubulin is not a necessary carrier of the STOP protein onto the microtubule. Further, it is evident that podophyllotoxin [PLN, a microtubule assembly inhibiting drug (Wilson et al., 1976)], which is present throughout this assay (Figure 3), does not interfere with STOP protein binding to microtubules.

Taken together, the correspondence of titration results on assembling and steady-state microtubules (Figure 2) and the tubulin subunit competition with microtubules for the binding of STOPs (Figure 3) suggest that cold stability could be generated randomly during assembly. We have directly tested this possibility. As measured by turbidity, cold stability is a constant fraction of the total assembled polymer when assayed at various time points during assembly (Figure 4). In addition to tubulin competition for STOP binding to microtubules, the random addition of STOPs observed during assembly may be generated by coaddition of STOPs with carrier tubulin subunits. This possibility has not been tested.

STOP Proteins Do Not Exchange between Microtubules.

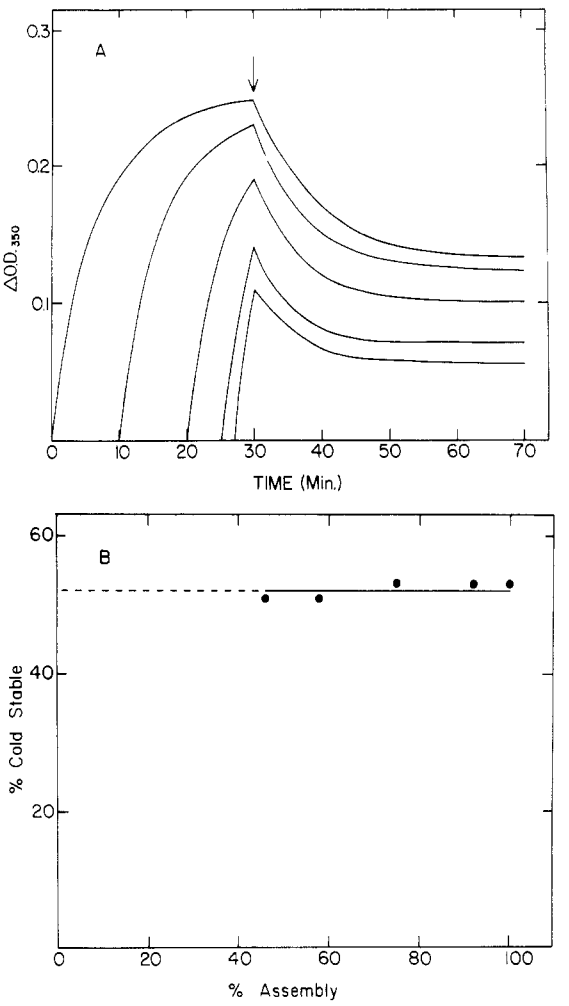

FIGURE 4: Cold stability level as a function of the extent of assembly. (A) Disassembled cold-stable microtubule protein was preincubated at 30 °C, and assembly was initiated by addition of 1.0 mM GTP at the indicated time points. At the arrow, the samples were rapidly cooled to 7 °C. Both assembly and disassembly were monitored continuously by turbidity measurement. (B) Measured cold stability is plotted as a function of the extent of the assembly attained prior to chilling.

We have found that tubulin subunits can compete with polymers for STOP protein binding but that eventually all STOPs absorb to the polymers (Figure 3). It appears that STOP protein binding to polymers is not a readily reversible reaction. In order to test for binding reversibility, we have performed experiments to determine the equilibrium exchange of STOPs between microtubules.

These binding competition experiments were conducted by assembling cold-stable microtubules with [^{3}H]GTP and assaying to determine whether a 3-fold excess of cold-labile (3× recycled) microtubules could compete STOPs off of stable polymers. Competition would be detected as an increased loss of cold stability from the radioactive polymers in the presence of competing polymers. Podophyllotoxin was present to prevent mixing between the two polymer populations by subunit exchange. The concentration of protein and the assembled state of the polymers were unchanged by mixing, so that there was no perturbation of the steady state. We find that, when compared with controls, the total assembled state and the cold-stable level of the cold-stable microtubules are both completely insensitive to the presence of competing polymers (Figure 5A). The slow loss of the assembled state is the result of the presence of PLN and the continuing steady-state loss of subunits from the polymer (Margolis & Wilson, 1978).

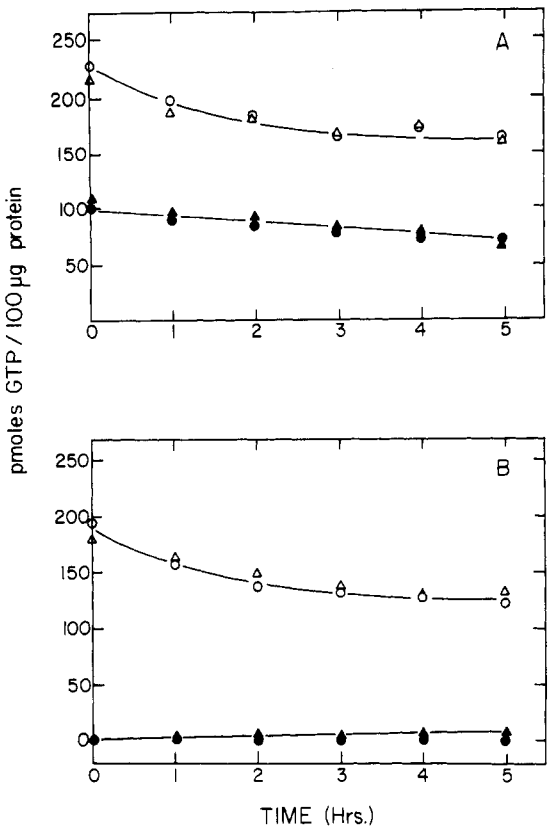

FIGURE 5: Assay of the exchange of STOPs between microtubules. (A) Competition of cold-labile microtubules for STOPs on cold-stable microtubules. For the control, cold-stable microtubules (2 mg/mL) were assembled for 50 min at 30 °C under the standard conditions for filter assay (including 50 µM [^{3}H]GTP). At steady state (time zero), 10 µM PLN and 1 mM GTP were added, and total (O) and cold-stable (●) microtubule levels were filter assayed as a function of time. For the competition experiment, at steady state, preassembled cold-labile microtubules, also at 2 mg/mL, were added with PLN. Final PLN, GTP, GTP-regenerating system, and protein concentrations were the same as those for the control. Three volumes of cold-labile microtubules were added for 1 volume of cold-stable microtubules. Total incorporation (Δ) and cold-stable levels (▲) were assayed as a function of time. Aliquots of 50 µL of control samples and 200 µL of competition samples were loaded onto filters to keep the quantity of cold-stable microtubule protein constant. One can expect a maximum 4-fold drop in the cold stability of labeled microtubules, relative to controls, if competition were effective. (B) The reverse experiment: induction of cold stability by exchange between microtubules. For the control sample, cold-labile (3 × cycled) microtubules (2 mg/mL) were assembled for 50 min at 30 °C under the standard conditions for filter assay (including 50 µM [^{3}H]GTP). At steady state (time zero), 10 µM PLN and 1.0 mM GTP were added. Total (O) and cold-stable (●) levels were filter assayed at the indicated time points (50 µL/filter). For the competition samples, at steady state, preassembled cold-stable microtubules at 2 mg/mL were added, 3 volumes to 1 volume, along with 10 µM PLN. Final concentrations of all materials were the same as those for controls. Aliquots (200 µL/filter) were assayed at time points for total label (Δ) and cold-stable levels (▲).

The reverse experiment was also done. Cold-labile microtubules were assembled in the presence of [^{3}H]GTP, and at steady state, they were mixed with a 3-fold excess of unlabeled cold-stable microtubules in the presence of GTP chase and PLN. Again, equal protein concentrations and equivalent assembled states were mixed together in order to prevent disturbing the steady state. We find no evidence (Figure 5B) that blocks from cold-stable microtubules were capable of exchanging onto cold-labile microtubules, since we find no more cold stability appearing in cold-labile microtubules incubated in the presence rather than in the absence of excess cold-stable microtubules. Overall, we find no evidence that STOP proteins can exchange between microtubules.

The experiments to assay competition between microtubules for STOPs were conducted with PLN present, since, in the absence of drug, the addition of cold-labile microtubule protein can create unlabeled polymeric "tails" on labeled cold-stable microtubules. This would create a condition in which both lateral migration of STOPs on a polymer and competition between polymers could, in theory, occur. We can exclude the possibility that PLN influences the exchange of STOPs between microtubules, however, by examining whether STOPs would exchange from cold-stable microtubules onto labeled cold-labile polymers in the absence of PLN. The result obtained was indistinguishable from that shown in Figure 5B; thus, PLN does not influence the competition experiment result. We have elsewhere shown (Figure 3) that PLN does not interfere with STOP protein binding to microtubules.

Sliding of STOPs upon the Polymer Surface. We have established that STOPs do not measurably exchange between polymers. If they change their positions relative to the tubulin subunits in a cold-stable polymer, they must be migrating on the polymer rather than exchanging through equilibrium. To determine if migration of STOPs occurs, the experiments diagrammed in Figure 6A were performed. Cold-stable microtubules were labeled by assembly in [^{3}H]GTP, chilled to preserve only cold-stable regions, and rewarmed in a GTP chase (Figure 6A). Following this procedure, only the stable region of the polymer is labeled. If STOPs move along the polymer relative to the position of subunits, then the labeled tubulin subunits should become progressively cold sensitive. This change in cold sensitivity of the labeled subunits could be determined by chilling aliquots of such protein at time points and filter assaying the residual labeled polymer. We also assayed the same protein at warm temperature to show that no label has been lost from the polymer during the experiment except during cold temperature treatment.

We show one such experiment in which the protection against cold temperature disassembly of the labeled polymer region declines with kinetics that may be first order or diffusional (varying as the square root of time) (Figure 6B, open circles). Assay of the same protein at warm temperature shows that no label has been lost with time from the initially labeled region (Figure 6B, closed circles), except upon chilling. We also performed a control in which the entire polymer was labeled by assembly with [^{3}H]GTP and maintained in label for the entire time course of the experiment to show that neither the overall assembled state (Figure 6B, closed triangles) nor the extent of cold stability of the polymers (Figure 6B, open triangles) changes with time under these experimental conditions. We conclude that the overall assembled state and the extent of cold stability do not vary with time, and no label is lost from the initially cold-stable region of the polymer when assayed at warm temperature. The loss of cold stability of the labeled polymer region can therefore best be explained by translocation of STOPs relative to tubulin subunits on the polymer.

We have also performed the reverse experiment, in which cold-stable microtubules were assembled without label, chilled to preserve their stable regions (7 °C, 20 min), and then rewarmed in [^{3}H]GTP to label the cold-labile regions only (Figure 7A). Microtubules were then maintained in the presence of GTP label for the course of the experiment. Filter assay of the microtubules after exposure to cold temperature at the indicated time points would reveal any cold stabilization of the initially cold-label polymer regions. As a control, the same microtubules were assayed without chilling at the in-

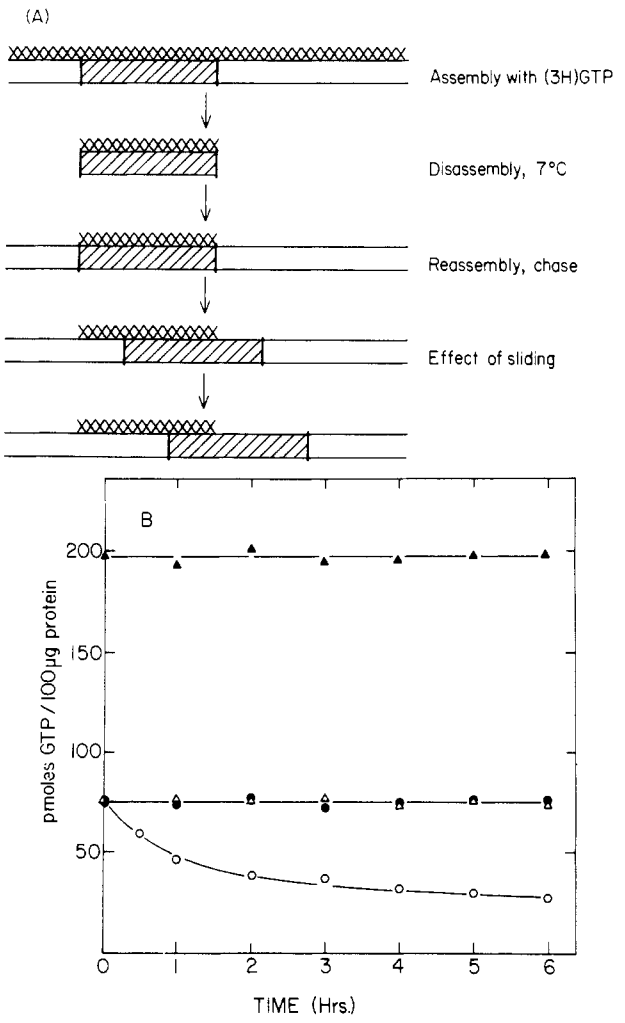

FIGURE 6: Assay of the movement of STOP proteins on microtubules. (A) Diagram of the experimental protocol and expected result if sliding occurs. Cold-stable microtubules are assembled with [^{3}H]GTP, disassembled at cold temperature to leave only residual cold-stable regions, and reassembled in 20-fold excess GTP so that only cold-stable regions are labeled. If STOP proteins move relative to labeled subunits, the subunits that come to lie in regions of the polymer external to STOPs will become cold labile. Diagonal lines indicate the cold-stable region of the polymer, delimited by heavy vertical lines representing STOPs; XXX indicates labeled subunits. (B) Sliding experiment. Cold-stable microtubules (2 mg/mL) were assembled in [^{3}H]GTP under standard conditions for filter assay. After 50 min at 30 °C, the protein was exposed to 7 °C for 40 min and separated into two parts. The first sample, the control, was reassembled under the same conditions (the polymer remained fully labeled) and filter assayed at the indicated time points (time zero being the time of rewarming) for total label incorporation (▲) and for cold stability (△) after aliquots were cooled to 7 °C for 20 min. The second part, the experimental sample, was reassembled at the indicated time zero in the presence of 2 mM unlabeled GTP. At the indicated time points, the total label at 30 °C (corresponding to the initial cold-stable part) was assayed (●), and the residual cold stability of the labeled region was assayed (○) after aliquots were cooled to 7 °C for 20 min.

dicated time points to determine if there was any change in the overall labeled state of the polymers with time.

We show the result of one such experiment (Figure 7B). Assay of the labeled polymer regions for cold stability shows a distinct increase in cold stability with time (Figure 7b, open circles). Assay of the same protein at warm temperature shows that the overall extent of labeling of the polymer does not change during the course of the experiment (Figure 7b, closed circles).

As a control, another sample of the microtubule protein used in this experiment was assembled in the presence of [^{3}H]GTP

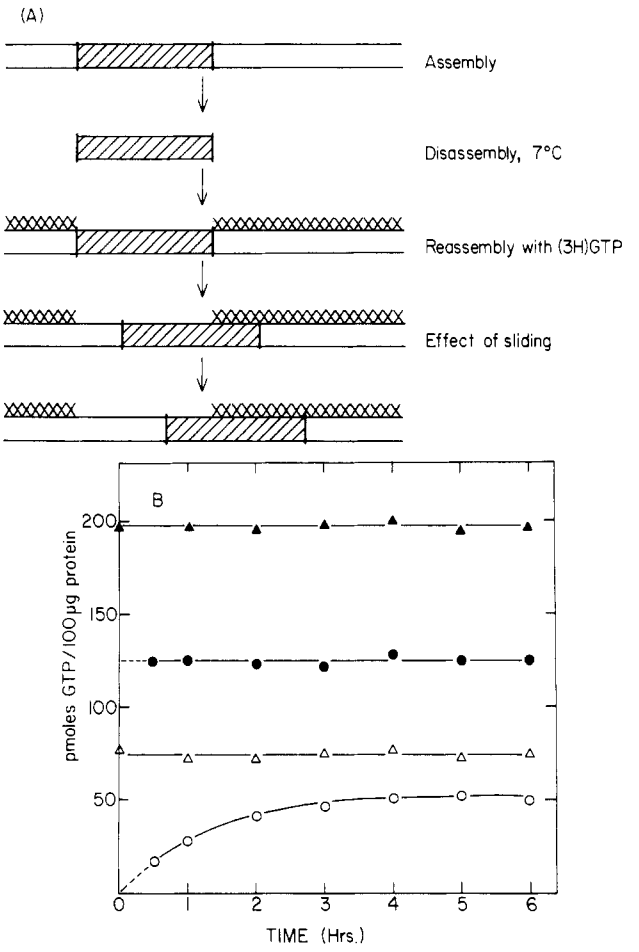

FIGURE 7: Reciprocal experiment, demonstrating movement of STOP proteins into cold-labile regions of microtubules. (A) Diagram of the experimental protocol and expected result if sliding occurs. Cold-stable microtubules are assembled in the absence of label, disassembled to residual cold-stable regions at 7 °C for 20 min, and reassembled in [^{3}H]GTP to label, specifically, the labile regions of the polymer. If STOPs move relative to labeled subunits, the subunits that come to lie in regions of the polymer between STOP proteins will become cold stable. Diagonal lines indicate the cold-stable region of the polymer, delimited by heavy vertical lines which represent STOPs; XXX indicates labeled subunits. (B) Sliding experiment. Cold-stable microtubules were assembled under standard conditions for filter assay but omitting [^{3}H]GTP. They were then cooled to 7 °C for 40 min and rewarmed (indicated time zero) for a second cycle of assembly in the presence of [^{3}H]GTP to specifically label cold-labile regions of polymers. Time points were then taken to assay for total label in the polymers, corresponding to the initial cold-labile regions (●), and for label in cold-stable regions (○), determined by chilling aliquots to 7 °C for 20 min prior to filter assay. A control was conducted for this experiment by assaying fully labeled microtubules for total label with time and for label in cold-stable regions. The procedure was identical with that for the control shown in Figure 6B. Total label incorporation (▲); cold-stable region (△).

and was consequently fully labeled. Time points taken with this protein show that the overall assembled state (Figure 7B, closed triangles) and the overall extent of cold stability (Figure 7B, open triangles) do not change with time. The most likely explanation for the data obtained is that STOP proteins are migrating along microtubules into previously cold-labile regions.

The increase in stability that we have observed in this experiment is roughly reciprocal in both time course and extent with the loss of stability observed in the assay of initially cold-stable regions (compare Figures 6B and 7B, open circles), as would be expected of migration, without loss of STOP proteins from the microtubule. The creation of hybrid microtubules which are partially cold stable is essential to the

migration assay. There is no doubt that they are produced. First, only portions of microtubules are cold stable, as determined by length measurement before and after chilling (Job et al., 1982). Second, addition of STOPs has been modeled as random along a microtubule length (Job et al., 1982), and we verify here that experimental results match the statistical prediction. Third, cold-stable microtubules are capable of acting as seeds for the assembly of cold-labile regions that will otherwise not initiate assembly (Job & Margolis, 1984). Fourth, in experiments where only the cold-stable region of the polymer is labeled initially (Figure 6), we see no warm temperature loss of this label from the polymer over the time course of the experiment, although it comes to reside in a cold-labile region. The labile region of the polymer has to have assembled onto the labeled region bidirectionally, since otherwise the label would remain at the net disassembly end of the polymer and would be lost to treadmilling (Margolis & Wilson, 1978).

The drug PLN was included in competition experiments for reasons explained previously and left out of the translocation experiments for the purpose of creating stable steady-state controls (no overall gain or loss of tubulin from polymers) against which changes in subunit cold stability could be readily seen.

Further controls have been conducted to distinguish clearly between sliding and equilibrium exchange of STOPs on microtubules. Microtubules assembled to label only the cold-labile region (as in Figure 6) have been incubated, after assembly, both with and without PLN. Both populations exhibit an identical degree of cold stabilization of previously cold-labile regions with time even though the overall assembly state of PLN-blocked microtubules is diminishing. The presence of PLN does not, therefore, influence the observed migration results. In another control experiments, microtubules labeled in their cold-stable region only (as in Figure 7) were further incubated after full assembly in either the presence or the absence of a 3-fold excess of cold-labile microtubules and with no drug. There was no difference in the time course of cold labilization of cold-stable regions despite the presence of a large excess of competing microtubules in one case. Thus, the cold labilization of stable regions had no apparent component of equilibrium exchange of STOPs.

Discussion

We have established some interesting aspects of the behavior of STOP proteins on microtubules. STOPs bind rapidly to the microtubule surface, and apparently in a random manner. This binding reaction is irreversible under our assay conditions and yields the same concentration-dependent binding curve whether under microtubule assembly initiation or steady-state conditions. We are unable to detect any competition between microtubules for bound STOPs nor any equilibrium exchange of STOPs on a microtubule. Nonetheless, we find that STOPs appear to migrate upon a single microtubule, so that previously cold-stable regions become cold labile as cold-labile regions become cold stable.

The protein that confers cold stability on microtubules and which has the above characteristics has recently been purified in small quantities. It is apparently a protein of 145 kDa (R. L. Margolis and C. T. Rauch, unpublished results). It should soon be possible to begin describing the molecular mechanisms underlying the binding reactions that the STOP protein displays. For instance, calmodulin and protein kinase activities disrupt cold stability. These agents may act in a straightforward manner by causing a shift in the equilibrium of STOPs on microtubules, or they may instead increase the rate of migration with no loss of STOPs from the polymer.

The translocation of STOP proteins on microtubules is reminiscent of the "sliding" of many proteins on polynucleotide substrates. Many such proteins, capable of translocating on chromatin or polynucleotide polymers without equilibrium release, have been described. As examples, DNA helicase (Yarronton et al., 1979; Kuhn et al., 1979), T4 DNA polymerase (Winter et al., 1981), DNA methylase (Drahovsky & Morris, 1971), recBC (Rosamund et al., 1979a), restriction endonuclease (Rosamund et al., 1979b), and the eucaryotic 40S ribosomal subunit (Kozak, 1980) all exhibit processivity on their bound polynucleotide strand without equilibrium exchange.

A molecular mechanism for the observed translocation of proteins on linear substrates has been offered (Winter et al., 1981; Berg et al., 1982). For a more general treatment of high-affinity binding reactions in protein systems with barriers to free ligand diffusion or "retention binding", see Silhary et al. (1975) and Schwartz (1976). The mechanism basically involves two interactions of the protein with its polymer substrate: a high-affinity binding reaction at specific sites on the polymer and low-affinity interactions upon the entire polymer surface which serve to create a thermodynamic barrier to free diffusion of the ligand from the polymer surface.

The model, developed for the case of *lac* repressor binding to *Escherichia coli* DNA, explains how sliding may occur without loss of the *lac* repressor from the DNA substrate. Nonspecific binding of *lac* repressor to chromatin is visualized as electrostatic, resulting from 11 charge–charge interactions per repressor molecule. The repressor may move along the DNA with no net ion displacement. Since it is moving on an isopotential surface, there is no thermodynamic barrier to such movement. However, "dissociation of the repressor from the DNA requires net counterion replacement and is thermodynamically unfavorable (Berg et al., 1982)". With little or no activation energy barrier to sliding, the rate of sliding should approach a calculated hydrodynamically limited value of approximately 1.0 μm/s over a nonspecific binding surface. STOP proteins, we believe, have a potential high-affinity binding site on each tubulin dimer. Retention at high-affinity sites would serve to substantially retard sliding motility, and, indeed, the rate of sliding we observe appears many orders of magnitude below the theoretical maximum.

The profusion of proteins that translocate in a sliding manner on chromatin may have its correlate in the cytoskeletal system. If Berg et al. (1982) are correct, sliding requires no special property of STOPs other than a low-affinity electrostatic interaction with microtubules. A large number of proteins, even vesicles and organelles, can share this property. We note that the hydrodynamically limited rate of sliding, as calculated by Berg et al. (1982), is approximately the rate of fast axonal transport, and of saltatory organelle movement, processes which are believed to involve microtubules. Interaction of proteins with high-affinity binding sites on microtubules can serve to retard the flow, as may be the case with the STOP proteins.

Several predictions may be generated by invoking a mechanism of translocation due to nonspecific electrostatic interactions. (1) The apparent affinity of a protein ligand for the microtubule will be substantially greater than that for the tubulin subunit. This appears to be the case for STOPs, as shown in Figure 3. (2) There is no energy requirement for sliding nor any required directionality, although directionality could be induced by the specific mode of release of a particular

protein from its high-affinity binding site. (3) Any protein or ligand that binds to the microtubule surface has the potential to slide. (4) Those proteins that bind to the microtubule surface and slide will ride the polymer surface as if they were a "sleeve", without equilibrium loss, although the polymer is treadmilling beneath them. (5) For the case of STOPs, where protein kinase activity and calmodulin cause the loss of cold stability, these factors would be predicted to disrupt the high-affinity binding but not necessarily cause the release of STOPs from the polymer. These predictions are all testable and will all be examined in due course.

Although the molecular mechanism of the observed translocation of STOPs on microtubules remains to be described, the utility to the cell of such translocating molecules on microtubules cannot, we think, be overestimated. Microtubules control the directional movement of various bound vesicles and organelles [for reviews, see Dustin (1978) and Hyams & Stebbings (1979)] and can therefore direct their movement to specific cell loci and may dramatically improve diffusional efficiency.

Directed movement, requiring energy expenditure, can be superimposed on the passive lateral diffusion system proposed here. In this manner, the directed vectoral movement of materials can be generated. Alternatively, microtubule-associated proteins can be firmly anchored to fixed cell loci, past which they cause microtubules to move. Such a laterally bound translocatory apparatus, may, for example, serve to drive the progressive shortening of chromosome-bound microtubules during mitotic anaphase (Margolis & Wilson, 1981).

If the ability of proteins to absorb to and concentrate on cytoskeleton is a common property and if such concentration is accompanied by sliding behavior, then the capacity of the cytoskeleton to passively organize and compartmentalize the cytosol may be profound. Enzymes of a particular metabolic pathway could then rapidly form stable complexes on polymers and increase metabolic efficiency. A number of cytosolic enzymes are known to bind microtubules [see references in Margolis & Rauch (1981)]. Therefore, the possibility of such enhancement is not remote. Given the fact that microtubules are an unusually labile cytosolic component, their assembly state may rapidly lead to substantial changes in the metabolic state of the cell. The substantial effect of the microtubule assembly state on the capacity of cells to enter a mitotic cycle has already been well documented (Teng et al., 1977; Friedkin et al., 1979; Otto et al., 1979; Crossin & Carney, 1981). We offer, here, the possibility that these microtubule-dependent metabolic changes can be accounted for in a relatively straightforward manner, if the microtubule assembly state alters the capacity of enzymes to interact.

The translocation of STOPs on microtubules is not necessarily, as we have argued, unique. Rather, it is a prototype of sliding behavior which we believe will be found to be commonplace in all the cytoskeletal systems (microtubules, intermediate filaments, and actin filaments). The uniqueness of STOPs is that their exceptional stabilization of the microtubule has made their location relative to defined regions of the polymer assayable. The physiological role of the migration of STOPs cannot be assessed at present, and it may be irrelevant to their actual cellular function. They will, however, we believe, serve as a useful experimental system to help unravel the nature of microtubule-dependent intracellular motility.

Acknowledgments

We thank Francoise Gilles for technical assistance and Charles T. Rauch for preparing figures for publication.

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