Effects of the Tubulin–Colchicine Complex on Microtubule Dynamic Instability†

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ABSTRACT: The effects of the tubulin–colchicine complex (Tu-Col) on the dynamic behavior of microtubules have been examined under steady-state conditions *in vitro*. The addition of Tu-Col to tubulin microtubules at steady state results in only partial microtubule disassembly. Nevertheless, both the rate and the extent of tubulin exchange into microtubules are markedly suppressed by concentrations of Tu-Col which are low relative to the total amount of free tubulin. In addition, the time-dependent changes in microtubule length distribution, characteristic of dynamic instability, are suppressed by the addition of Tu-Col. Examination by video-enhanced dark-field microscopy of individual microtubules in the presence of Tu-Col shows that the principal effect of this complex is to reduce the growth rate at both ends of the microtubule. We have used computer simulation to rationalize the action of Tu-Col in terms of its effects on the experimentally observable parameters, namely, the rates of growth and shortening and the mean lifetimes of growth and shortening, which provide an empirical description of the dynamic behavior of microtubules. The results have been interpreted within the framework of the lateral cap formulation for microtubule dynamic instability [Martin, S. R., Schilstra, M. J., & Bayley, P. M. (1993) *Biophys. J. 65,* 578–596]. The simplest model mechanism requires only that Tu-Col binds to the microtubule end and inhibits further addition reactions in *either* the 5-start or the $\bar{8}$-start direction of the microtubule lattice. Monte Carlo simulations show that Tu-Col can, in this way, cause major suppression of the dynamic transitions of microtubules without inducing bulk microtubule disassembly. This type of mechanism could be important for the regulation of microtubule dynamics *in vivo*.

The discovery of tubulin as the major constituent protein of microtubules was closely related to the cytostatic effect of colchicine (Weisenberg *et al.*, 1968; Dustin, 1978). The drug is known to affect microtubule growth (Sternlicht & Ringel, 1979; Lambeir & Engelborghs, 1980) through the prior formation of a soluble tubulin–colchicine (Tu-Col)[1] complex (Margolis & Wilson, 1977; Keates & Mason, 1981; Skoufias & Wilson, 1992). Substoichiometric concentrations of the drug relative to the total amount of tubulin cause a disproportionately high suppression of microtubule assembly (Olmsted & Borisy, 1973). However, the addition of suprastoichiometric amounts of colchicine to preformed microtubules does not necessarily induce complete disassembly (Olmsted & Borisy, 1973; Sternlicht *et al.*, 1980; Deery & Weisenberg, 1981; A. Vandecandelaere and Y. Engelborghs, in preparation). These observations, which cannot be reconciled with simple models in which Tu-Col acts merely as a pool of assembly-incompetent tubulin, suggest that Tu-Col is directly involved in reactions at the microtubule ends. This has been confirmed by the observations that Tu-Col binds rapidly and reversibly to microtubule ends with affinity similar to that of unliganded tubulin (Lambeir & Engelborghs, 1980; Keates & Mason, 1981) and that it inhibits growth at both ends of axoneme-seeded microtubules (Bergen & Borisy, 1982). Furthermore, the steady-state exchange of tubulin into microtubules is strongly suppressed by incorporation of substoichiometric amounts of the Tu-Col complex (Skoufias & Wilson, 1992).

Several models have been proposed to explain the inhibitory effects of Tu-Col [see, for example, Hamel (1990)]. The two principal models are as follows: (1) Tu-Col binds to the microtubule end and blocks it, thereby preventing or suppressing further growth (Margolis & Wilson, 1977; Margolis *et al.*, 1980). This model has emphasized the sensitive response of microtubule growth to the binding of as little as one Tu-Col complex per end, though more extensive addition could occur (Margolis *et al.*, 1980; Skoufias & Wilson, 1992). (2) Tu-Col can copolymerize with unliganded tubulin (Tu-GTP) to form normal microtubules (Sternlicht *et al.*, 1983). The copolymerization model was formulated to account for the observation that substantial incorporation of labeled Tu-Col into microtubules can occur under certain conditions (Sternlicht & Ringel, 1979; Sternlicht *et al.*, 1983; Farrell & Wilson, 1984).

These models need not necessarily be mutually exclusive, however, as their relative importance may well depend on the exact experimental conditions employed (Farrell & Wilson 1980).

Much previous work on the effects of colchicine on microtubules *in vitro* has been performed with microtubule protein, i.e., tubulin plus microtubule-associated proteins

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[1] Abbreviations: Col, colchicine; Tu-Col, tubulin–colchicine complex; Tu-GTP, the tubulin $\alpha\beta$ heterodimer with GTP at the exchangeable nucleotide binding site (E-site); Tu-GDP, the tubulin $\alpha\beta$ heterodimer with GDP at the E-site; MAPs, microtubule-associated proteins; Mes, 2-(*N*-morpholino)ethanesulfonic acid; PEM, microtubule assembly buffer containing 100 mM 1,4-piperazinediethanesulfonic acid (Pipes), 0.1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), and 1.7 mM $MgCl_2$, at pH = 6.5; PEMG, PEM buffer containing 1 M glycerol; EGS, ethylene glycol bis(succinic acid *N*-hydroxysuccinimide ester).

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(MAPs). Because these MAPs are known to influence the dynamic properties of the microtubules (Hotani & Horio, 1987), we have chosen to examine the effects of Tu-Col on microtubules assembled from pure tubulin. A population of such microtubules is known to consist of two subpopulations of growing and shortening microtubules. This property is known as dynamic instability (Mitchison & Kirshner 1984a,b). At steady state of assembly, interconversions between the slowly growing majority state (G) and the rapidly shortening minority state (S) occur rather infrequently. At any tubulin concentration, C_t, the *population* behavior may be completely described by four empirical parameters: the mean rates of growth and shortening (R_g and R_s) and the mean lifetimes of the growing and shortening states (T_g and T_s) (Bayley *et al.*, 1989). At steady state of assembly, the polymer is in equilibrium with free tubulin (the critical concentration, C_c) and $R_gT_g = R_sT_s$. The behavior of a single microtubule over long times may be described by the same growth and lifetime parameters. The average length changes undergone during growth and shortening excursions (ΔL_{avg}) are given by R_gT_g for growth and R_sT_s for shortening. At steady state of assembly $\Delta L_{avg} = R_gT_g = R_sT_s$.

Dynamic processes in microtubules have been studied by three different methods: (1) By making direct observations of the behavior of individual dynamic microtubules in the optical microscope in order to determine values of R_g, R_s, T_g, and T_s [e.g., Walker *et al.* (1988)]. This approach has the advantage of being direct and does allow values of R_g, and to a lesser extent R_s, to be determined with considerable accuracy. The principal disadvantage is that one requires a very large number of observations to obtain reliable averages for the mean state lifetimes, T_g and T_s (Bayley *et al.*, 1991). Furthermore, the approach is not suitable for making comparisons of a large range of different solution conditions [see Schilstra *et al.* (1991)]. (2) By making measurements of changes in the length distribution of a steady-state microtubule population as a function of time. The disappearance of (shorter) microtubules during large shortening excursions results in an increase in the average length of the population, as originally observed by Mitchison and Kirschner (1984a,b). The principal disadvantage of this approach is that the accurate determination of length distributions is difficult. (3) By using a double-labeling experiment to monitor the incorporation of labeled tubulin into the microtubules. This incorporation depends upon the addition of Tu-GTP to the microtubule ends and may be monitored using a technique that depends upon the nonexchangeability of nucleotide (GDP) bound to polymerized tubulin, plus a fast enzymic GTP-regenerating system to convert all non-polymer-bound GDP into GTP (Schilstra *et al.*, 1989, 1991).

For a given steady-state microtubule population the extents of fractional exchange and length redistribution occurring in a given time, t, will depend upon the following (Bayley *et al.*, 1989): (1) The length distribution of the initial population. If the initial mean length is high, then the change in mean length after time t, and the extent of tubulin incorporation, will be low. (2) The average excursion occurring during periods of growth and shortening, ΔL_{avg}. If ΔL_{avg} is small, then the change in average length and both the rate and the extent of labeling (after time t) will be low. ΔL_{avg} depends upon the individual values for mean state lifetimes and growth/shortening rates as noted above.

These experimental approaches are complementary. In this work we have used them to assess the effects of the tubulin–colchicine complex (Tu-Col) on microtubule dynamics. The critical concentration has been determined in the presence of different concentrations of Tu-Col (and free colchicine). We have measured the exchange of tubulin into microtubules, the length redistribution of microtubules, and the growth rates for individual microtubules in the presence of different concentrations of Tu-Col. We have used computer simulations to rationalize the results in terms of observable parameters.

The possible underlying molecular mechanism has also been considered within the framework of the lateral cap formulation for microtubule dynamic instability (Bayley *et al.*, 1990; Martin *et al.*, 1993). This model has been extended to treat explicitly the interactions of Tu-Col with a microtubule end, describing the action of the complex in terms of its effect on the helical growth properties of the microtubule lattice. We used Monte Carlo simulations to evaluate how observable dynamic properties of microtubules are affected by the presence of substoichiometric concentrations of Tu-Col.

MATERIALS AND METHODS

Materials. Colchicine, GTP, and acetyl phosphate were from Sigma Chemical Co.; [^{3}H]GTP and [^{14}C]GTP were from Amersham International; and acetate kinase was from Boehringer Mannheim.

MAP-free tubulin was prepared as described (Clark *et al.*, 1981) and was stored at −70 °C in 50 mM Mes buffer, pH 6.5, 0.1 mM EGTA, 7 mM $MgCl_2$, and 3.4 M glycerol. Before each experiment a suitable sample was assembled and the microtubules were pelleted and resuspended in 100 mM Pipes, pH 6.5, 0.1 mM EGTA, 1.7 mM $MgCl_2$, and 1 M glycerol (PEMG buffer). The Mg^{2+} concentration in solution was kept low (1.7 mM) in order to avoid the possibility of extensive copolymerization of Tu-GTP and Tu-Col into non-microtubular structures (Andreu & Timasheff, 1983; Saltarelli & Pantaloni, 1983). The tubulin–colchicine complex (Tu-Col) was prepared by mixing stoichiometric amounts of the drug with tubulin and allowing the mixture to react for 90 min at room temperature. Seeds for seeded-assembly studies were prepared by cross-linking small microtubule fragments with ethylene glycol bis(succinic acid *N*-hydroxysuccinimide ester) (EGS) (Koshland et al., 1988). Seeds were stored at room temperature in PEM buffer (plus 10 mM glutamic acid, 1.5 M sucrose, and 6 mM NaN_3). Prior to each experiment the seeds were sedimented (100000*g*) and resuspended in the appropriate buffer.

All experiments were carried out in the presence of a GTP-regenerating system (1 IU/mL acetate kinase, 2.5 mM acetyl phosphate) without adding extra nucleotide, other than the small amounts added as radiolabel. Control measurements showed that this GTP-regenerating system remained fully active throughout the experiments.

Concentrations of colchicine were estimated spectrophotometrically, using an extinction coefficient of 16.6 mM^{-1} cm^{-1} at 350 nm. The tubulin and nucleotide contents of the samples were calculated from the absorption of the protein solution at 278 and 255 nm with extinction coefficients for tubulin of 1.2 (278 nm) and 0.646 mg^{-1} mL cm^{-1} (255 nm) and values for guanine nucleotide (GDP and GTP) of 7.663 (278 nm) and 12.167 mM^{-1} cm^{-1} (255 nm) (Engelborghs *et al.*, 1993).

Determination of the Apparent Critical Concentration under Different Conditions. The effect of colchicine or Tu-Col on the apparent critical concentration was assessed as follows. Tubulin (≈40 μM) was assembled into microtubules in PEMG buffer (37 °C) in the presence of the GTP-regenerating system and a trace amount of [^{3}H]GTP. After steady state had been

reached (40 min), the solution was divided into aliquots and each of these was mixed with either colchicine ([Col] = 0–120 μM) or Tu-Col ([Tu-Col] = 0–6 μM). These samples were then incubated for a further 60 min before quenching with 2.5% (v/v) $HClO_4$. After neutralization with KOH, the precipitate was spun down, the nucleotides were separated on a Waters Partisil SAX column using 0.65 M ammonium phosphate buffer (pH 5.5), and the distribution of radioactive label in GMP, GDP, and GTP was determined. The radioactivity (cpm) in the GDP peak divided by the sum of the radioactivities (cpm) in the GMP, GDP, and GTP peaks is equal to the fraction of nucleotide present as GDP, $f_{GDP(3H)}$. This fraction of GDP was corrected for background levels of GDP (usually $\approx$5% of the total radioactivity). This background level was determined by subjecting a sample of the protein solution to the same treatment under nonassembly conditions (0 °C).

All experiments included a control to which no drug was added. The concentration of polymer in this control solution, C_p, may be calculated directly from the known values of C_t (total tubulin concentration) and C_c (critical concentration) using the equation $C_p = C_t - C_c$. We have used $C_c = 4.1 \pm 0.5$ μM (Schilstra *et al.* 1991). Because the polymer mass is directly proportional to the measured $f_{GDP(3H)}$ value, the polymer mass in the presence of the drug, C_p', may be calculated using the equation

$$C_p' = C_p f'_{GDP(3H)}/f_{GDP(3H)}$$

where $f'_{GDP(3H)}$ and $f_{GDP(3H)}$ are the values measured in the presence and absence of drug, respectively. The critical concentration in the presence of drug, C_c', is given by

$$C_c' = C_t' - C_p'$$

where C_t' is the total concentration of tubulin in the presence of drug. This equals C_t (experiments with free colchicine) or {[Tu-GTP] + [Tu-Col]} (experiments with Tu-Col).

Study of Tubulin Exchange into Microtubules. Tubulin exchange into microtubules at steady state was studied in a double-label experiment, essentially as described elsewhere (Martin *et al.*, 1987; Schilstra *et al.*, 1991). Briefly, microtubules assembled from free tubulin in the presence of [^{14}C]GTP become labeled with [^{14}C]GDP, and the ratio R_{14} = [[^{14}C]GDP]/[total ^{14}C] can be related to the polymer mass. The incorporation of tubulin into microtubules *at steady state* is an exchange process that can be monitored using a second radiolabel. Thus, if [^{3}H]GTP is added to preformed microtubules, then [^{3}H]GDP only becomes incorporated through the exchange reaction involving addition of [^{3}H]GTP–tubulin. The ratio R_3 = [[^{3}H]GDP]/[total ^{3}H] is a measure of the extent of exchange. The fraction of total polymer labeled through exchange reactions, the fractional exchange, equals R_3/R_{14}.

Tubulin exchange as a function of time was measured as follows. Tubulin ($\approx$40 μM) was assembled at 37 °C in the presence of the GTP-regenerating system and a trace amount of [^{14}C]GTP. After 40 min the solution was divided into aliquots, and different amounts of Tu-Col (0–6 μM) were added to each. Ten minutes after the addition of Tu-Col, a trace amount of ^{3}H[GTP] was added to each sample. Aliquots (30 μL) were removed from each sample at predetermined time intervals and quenched with $HClO_4$ prior to determination of $f_{GDP(3H)}$ and $f_{GDP(14C)}$. The effect of Tu-Col on the polymer mass (and hence the apparent critical concentration) was assessed from the value of $f_{GDP(14C)}$ as described above. The fractional exchange at any time t, F_t, was determined using the equation $F_t = f_{GDP(3H)}/f_{GDP(14C)}$ (Schilstra *et al.*, 1991).

Exchange as a function of [Tu-Col] was measured as follows. Tubulin ($\approx$40 μM) was assembled at 37 °C in the presence of the GTP-regenerating system and a trace amount of [^{14}C]-GTP. After 40 min the solution was divided into several separate samples, and different amounts of Tu-Col (0–6.0 μM) were added to each sample. Thirty minutes after the addition of Tu-Col, a trace amount of [^{3}H]GTP was added to each sample. The samples were then incubated at 37 °C for a further 30 min before quenching with $HClO_4$. Values of $f_{GDP(3H)}$ and $f_{GDP(14C)}$ were determined and used to calculate the fractional exchange at t = 30 min, F_{30}.

Determination of Microtubule Length Distribution. Samples (typically 10 μL) for the determination of length distributions were diluted ten-fold in warm PEMG containing 0.25% glutaraldehyde. After $\approx$30 min (room temperature) this solution was diluted 30-fold in PEMG. Pictures of fixed microtubules were taken as soon as possible in dark-field videomicroscopy. The images were enhanced by integrating 256 frames and were stored for subsequent analysis. A typical field contained between 50 and 120 microtubules and several fields were collected for each sample. Several fields were chosen at random for the analysis; the only restriction applied was that the fields did not overlap. The lengths of all the microtubules in a single field were measured, providing that microtubule bundelling did not make it impossible to discriminate between two or more microtubules. Between 250 and 500 microtubules were measured in order to construct a length distribution histogram for the population.

Determination of Growth Rates for Individual Microtubules. The effect of Tu-Col on the growth rate of individual microtubules was measured using dark-field video microscopy (Nikon Diaphot microscope and Sony CCD camera) (Bayley *et al.*, 1993). Microtubules were grown on EGS-cross-linked seeds at 37 °C in the presence of the GTP-regenerating system. EGS seeds were deposited on a clean coverslip and allowed to attach at room temperature. The seeds were then placed in a flow cell, washed with buffer, and exposed to different mixtures of Tu-GTP and Tu-Col prior to examination in the microscope. Real-time images were sent to a Hamamatsu Argus-10 image processor for background subtraction and averaging. The enhanced images were stored on S-VHS videotape for subsequent analysis.

Computer Simulation. The behavior of *a population of microtubules* with a known initial length distribution was simulated as a function of time using defined values of R_g, T_g, R_s, and T_s and an experimentally determined initial length distribution essentially as described in Bayley *et al.* (1989).

The behavior of a *single microtubule* was simulated using the lateral cap formulation described by Martin et al. (1993), with modifications to allow for the presence in solution of Tu-Col.

RESULTS AND DISCUSSION

(1) Effect of Col and Tu-Col on the Apparent Critical Concentration. The addition of free colchicine to microtubules at steady state results in a significant increase in the apparent critical concentration, C_c', as shown in Figure 1A. The addition of high concentrations of colchicine ([Col] >> C_t') does not, however, cause the complete disassembly of microtubules under the conditions employed here. Thus, for concentrations of colchicine in the range 50–120 μM, the apparent critical concentration, C_c', remains effectively constant at $\approx$22 μM. A qualitatively similar dependence of the critical concentration

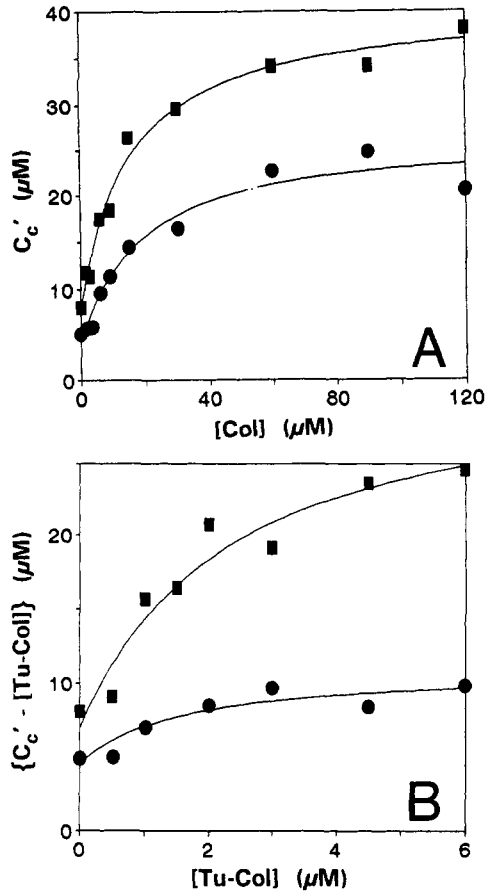

FIGURE 1: Effect of colchicine and Tu-Col on the apparent critical concentration for microtubule assembly. Tubulin (≈40 μM) was assembled into microtubules at 37 °C in the presence of a fast GTP-regenerating system and a trace amount of [^{3}H]GTP. After steady state had been reached (40 min), the solution was divided into aliquots and each of these was mixed with either colchicine ([Col] = 0–120 μM) or Tu-Col ([Tu-Col] = 0–6 μM). These samples were then incubated for a further 60 min, after which period they were quenched with 2.5% (v/v) $HClO_4$, and their nucleotide composition was determined by HPLC. The critical concentration was calculated from the fraction of nucleotide present as GDP, $f_{GDP(3H)}$, and the critical concentration of the control experiments (see Materials and Methods). (A) Effect of colchicine in PEM buffer (■) and in PEMG buffer (●). (B) Effect of Tu-Col in PEM buffer (■) and in PEMG buffer (●). Note the different ordinates used in these plots. The continuous lines represent the smoothest representation of the experimental data.

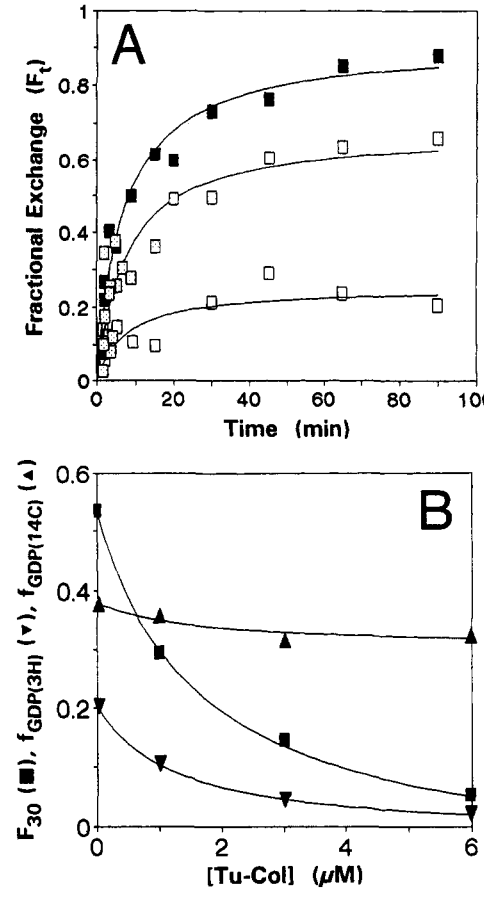

FIGURE 2: Effect of Tu-Col on dimer exchange into microtubules in PEMG buffer. (A) Fractional exchange plotted as a function of time in the absence (filled boxes) and presence of Tu-Col (open boxes: middle, 0.65 μM; bottom, 2.1 μM). Tubulin (≈40 μM) was assembled at 37 °C in the presence of a fast GTP-regenerating system and a trace amount of [^{14}C]GTP. After 40 min the solution was divided into aliquots, and different amounts of Tu-Col were added to each. Ten minutes after the addition of Tu-Col, a trace amount of [^{3}H[GTP] was added to each sample. Aliquots (30 μL) were removed from each sample at predetermined time intervals and quenched with $HClO_4$ prior to determination of the fractions of [^{3}H]GDP ($f_{GDP(3H)}$) and [^{14}C]GDP ($f_{GDP(14C)}$). The fractional exchange at any time t, F_t, was determined using the equation $F_t = f_{GDP(3H)}/f_{GDP(14C)}$ (see Materials and Methods). (B) Fractional exchange after 30 min, F_{30} (■), plotted as a function of [Tu-Col]. The experiment was performed as described in (A) with one modification: 40 min after the start of self-assembly, Tu-Col (0–6 μM) was added and the aliquots were incubated for 30 min before the addition of [^{3}H]GTP. Thirty minutes after the addition of the tritium label, the samples were quenched for nucleotide analysis. Also shown are the values of $f_{GDP(3H)}$ (▼) (a measure of exchange) and $f_{GDP(14C)}$(▲) (a measure of the polymer mass). The errors on these values typically are ±0.03 on $f_{GDP(3H)}$ and ±0.04 on $f_{GDP(14C)}$.

on colchicine concentration was observed in the absence of glycerol (in PEM buffer). In this case the C_c' was ≈36 μM at colchicine concentrations in the range 50–120 μM.

Figure 1B shows that the addition of Tu-Col to microtubules in PEMG buffer also causes a small increase in the apparent critical concentration, C_c'. The main change in C_c' with [Tu-Col] in PEMG buffer occurs in the concentration range 0–2 μM. This is consistent with the value of 0.3 μM that has been determined for the *apparent* dissociation constant for the interaction of the Tu-Col complex with the microtubule end (Lambeir & Engelborghs, 1980; Keates & Mason, 1981). In the absence of glycerol (in PEM buffer), the effect of Tu-Col is significantly greater (see Figure 1B).

(2) Effect of Tu-Col on Tubulin Exchange Kinetics. The results for the variation of fractional exchange with time for 0, 0.65, and 2.1 μM Tu-Col are compared in Figure 2A. This figure clearly shows that the incorporation of tubulin into microtubules in PEMG buffer is suppressed in the presence of Tu-Col, even at very low concentrations. Higher concentrations cause much greater suppression (see below). Differences between experiments could occur if the initial microtubule length distributions are significantly different. However, direct measurements of length distributions (see below) show that this is not, in fact, the case. Furthermore, the scatter between different experiments is no greater than that within a single experiment.

The dependence of F_{30}, the fractional exchange parameter (see Materials and Methods), on [Tu-Col] in PEMG buffer is shown in Figure 2B. Because of the relatively high error on these measurements, several separate experiments were averaged to produce this plot. This figure shows that the incorporation of tubulin into microtubules is strongly suppressed by increasing [Tu-Col]. In the absence of added Tu-Col the fractional exchange at t = 30 min is 0.53 ± 0.09, in agreement with previous results for this buffer system (Schilstra et al., 1991). In the presence of 1 μM Tu-Col, F_{30}

is reduced to 0.29 ± 0.05. Thus, the addition of relatively low concentrations of Tu-Col causes major suppression of exchange processes without having a major effect on the apparent critical concentration (see Figure 1B). Similarly, the addition of 6 μM Tu-Col reduces F_{30} by approximately 1 order of magnitude, to 0.06 ± 0.03.

When significant length redistribution occurs in a microtubule population, the mean length will increase with time. Therefore, the experimentally determined fractional exchange should *decrease* if the microtubule population is incubated for longer times prior to addition of the radioactive label. In the absence of Tu-Col there was indeed a small, but significant, decrease in F_{30} when an extra 60 min was allowed before addition of the label in the experiment shown in Figure 2B (data not shown). However, in the presence of 3 μM Tu-Col, incubating for an additional 60 min had little effect on the measured F_{30}. This suggests that significant microtubule length redistribution does not occur in the presence of Tu-Col. We address this point explicitly below.

(3) Effect of Tu-Col on Microtubule Length Redistribution. Samples for the determination of microtubule length distributions were taken for fixation during the experiment described in Figure 2B, immediately before the addition of Tu-Col (at t = 40 min) and 1 h after the addition of Tu-Col (at t = 100 min). Figure 3A shows a typical dark-field image of the microtubule population sampled 40 min after the initiation of assembly (no Tu-Col); the corresponding length histogram is shown in Figure 3B. This sample contained a total of 470 microtubules with a mean length $L(40) = 7.9 \pm 2.8$ μm. Owing to the limited resolution of the optical microscopy techniques, the contribution of very short microtubules (length < 1 μm) is probably underestimated and this mean length is therefore only an upper estimate.

The effect of different concentrations of Tu-Col on microtubule length redistribution is shown in Figure 3C. In the absence of added Tu-Col, the mean length increases from $L(40) = 7.9 \pm 2.8$ (t = 40 min) to $L(100) = 11.9 \pm 3.4$ (t = 100 min). The extent of length redistribution occurring in this buffer (which contains 1 M glycerol) is thus rather small, consistent with the known effect of glycerol in suppressing microtubule length redistribution (Kristofferson et al., 1986). The extent of length redistribution is significantly reduced by the addition of low concentrations of Tu-Col (<2 μM) and appears to be eliminated entirely at [Tu-Col] > 3 μM.

(4) Effect of Tu-Col on the Growth Rates of Individual Microtubules. Growth rates (R_g) for individual microtubules at 37 °C were determined in dark-field microscopy using small EGS-cross-linked microtubule fragments as seeding structures. The growth rates were measured in PEMG buffer for both the fast- and slow-growing ends of the seeds in the presence of different concentrations of Tu-Col. For a particular concentration of Tu-Col, the total concentration of protein used in the experiment, C_t', was chosen so that the following relationship was satisfied:

$$C_t' = [\text{Tu-GTP}] + [\text{Tu-Col}] = C_c' + 3\ \mu\text{M}$$

where C_c' is the apparent critical concentration determined in PEMG buffer in the presence of Tu-Col (see Figure 1B). Figure 4 shows the values of the growth rate as a function of [Tu-Col] (measured at $C_t' = C_c' + 3$ μM) for the two ends. For both ends, the growth rate is reduced dramatically as [Tu-Col] increases. At [Tu-Col] = 3 μM, the growth rate is reduced by almost an order of magnitude at both ends.

The value of R_g at the appropriate C_c' was estimated by assuming that the growth rate is proportional to the total tubulin concentration and that it extrapolates to zero at $C_t' = 0$. This approximation neglects any contribution from Tu-GTP (or Tu-Col) dissociation occurring during growth, and the calculated value is therefore only an upper estimate of the

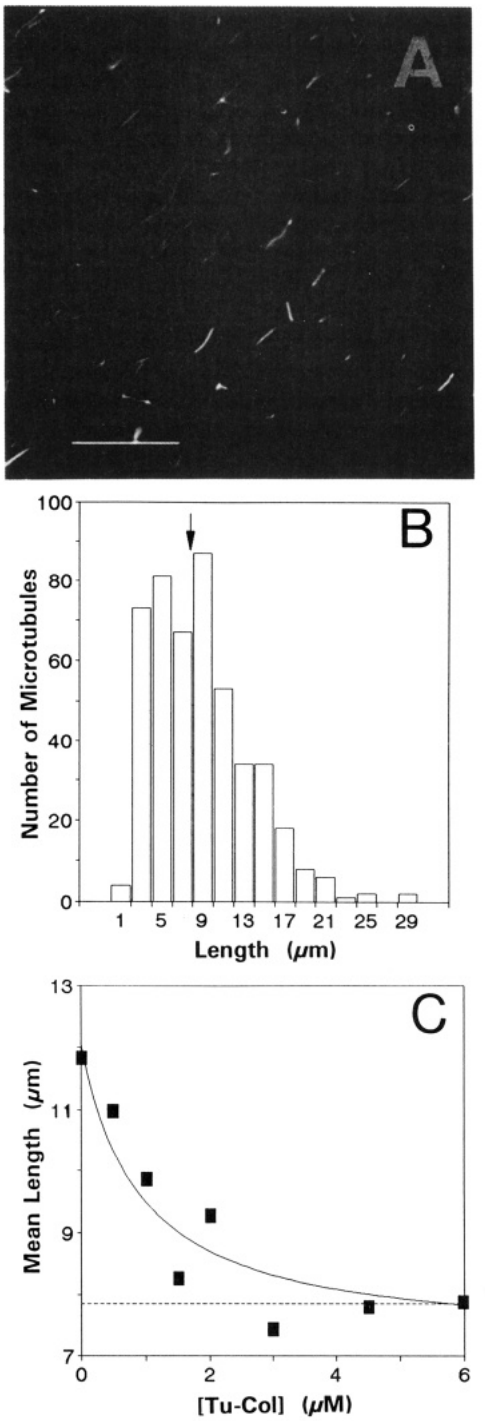

FIGURE 3: Effect of Tu-Col on microtubule population length redistribution in PEMG buffer. In the course of the experiment in Figure 2B samples were taken just before and 60 min after the addition of Tu-Col for the determination of the microtubule length distributions. The samples (typically 10 μL) were diluted 10-fold in warm PEMG containing 0.25% glutaraldehyde. After ≈30 min (room temperature) this solution was diluted 30-fold in PEMG. Pictures of fixed microtubules were taken as soon as possible in dark-field video microscopy. (A) A typical image of the microtubule population prior to the addition of Tu-Col; the bar represents 25 μm. (B) Length histogram for the population prior to the addition of Tu-Col. The mean length was 7.9 ± 2.8 μm (arrow) for a total of 470 microtubules. (C) Mean length 60 min after the addition of Tu-Col, plotted as a function of [Tu-Col]. The dashed line gives the mean length of the initial population as determined in (B).

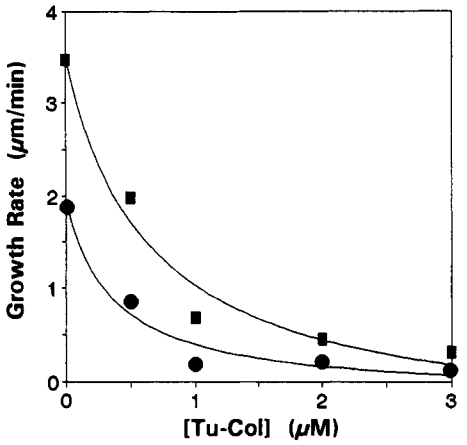

FIGURE 4: Effect of Tu-Col on the growth rates of individual microtubules in PEMG buffer. Microtubules were grown on EGS-cross-linked seeds at 37 °C in the presence of the GTP-regenerating system. Growth of both ends of EGS seeds exposed to different mixtures of Tu-GTP and Tu-Col was monitored by dark-field video microscopy. The total tubulin concentration in solution, C_t' (=[Tu-GTP] + [Tu-Col]) was adjusted to C_c' + 3 μM, where C_c' is the apparent critical concentration as determined in Figure 1B. In these conditions no transitions of the seeds between growth and shortening were observed within the limits of observation. Growth rates for the fast-growing (■) and slow-growing ends (●) of the EGS-cross-linked microtubule seeds are shown as a function of [Tu-Col]. At least six ends were measured to provide an average. The standard deviation on the measured rates is between 20 and 80%.

growth rate at C_c'. Thus, in the absence of Tu-Col we calculate growth rates of R_{gf} = 2.2 μm min^{-1} (for the fast-growing end) and R_{gs} = 1.2 μm min^{-1} (for the slow-growing end). The values for the steady-state growth rates in the presence of Tu-Col were calculated in the same way.

(5) Computer Simulation of Tubulin Exchange into Microtubules. If the length distribution of a microtubule population is known, then computer simulation can be used to estimate the extent of fractional exchange as a function of time for particular values of R_g, T_g, R_s, and T_s (Bayley et al., 1989). The length distribution of the population shown in Figure 3B (t = 40 min, no Tu-Col) was smoothed and adjusted for the underestimation of the number of short microtubules seen in the dark-field assay by creating a Poisson-like distribution. This population (numbering 544 microtubules) was used as the starting population in simulations. We assume that R_s is unaffected by the presence of Tu-Col [cf. Deery and Weisenberg (1981)] and use R_s = 5.9 μm/min for these buffer conditions (Schilstra *et al.*, 1989). This is based on the observation that only very little Tu-Col is incorporated in the microtubules at steady state (Skoufias & Wilson, 1992; A. Vandecandelaere and Y. Engelborghs, in preparation). Under these conditions we infer that the average rate of shortening is not significantly perturbed. The simulations were performed by fixing R_g and T_g at selected values and calculating T_s through $T_s = R_g T_g / R_s$. A length versus time plot was derived for all microtubules in the population, and this enabled calculation of F_{30} values (Bayley et al., 1989).

The *extent* to which a particular microtubule population becomes labeled after very long (experimental) times clearly depends only upon the size of the average length excursion, ΔL_{avg} (=$R_g T_g$). However, the *rate* at which the fractional labeling changes with time (and hence the value of F_{30}) is high when the average duration of an excursion is short. The average duration of an excursion is equal to $\Delta L_{avg}/R_g$ (=T_g). Hence, for any given value of ΔL_{avg}, the calculated value of F_{30} will be high for high R_g values. We therefore calculated F_{30} values from the simulations as a function of the average excursion length, ΔL_{avg} (=$R_g T_g$), for several different values of R_g. The values of R_g used in the simulations were selected as described below.

Table 1: Experimental and Calculated Parameters for Microtubule Dynamic Instability in the Presence of Tu-Col[a]

[Tu-Col] (μM)	F_{30}	R_g^{max} (μm min^{-1})	ΔL_{avg} (μm)	T_g (min)	T_s (min)
0	0.44–0.62	3.4	>3	>0.88	>0.50
1	0.24–0.34	0.63	0.4–1.7	0.63–2.7	0.07–0.18
3	0.10–0.20	0.35	0.1–0.5	0.28–1.4	0.02–0.08
6	0.03–0.09	*b*	<0.1[c]		

[a] The values of the fractional exchange (F_{30}) and the microtubule growth rate (R_g^{max}) were determined as described in the text. Using the experimentally determined R_g^{max} values, F_{30} of a starting population as shown in Figure 3B is calculated by computer simulation for different average length excursions (ΔL_{avg}) and compared with the observed fractional exchange. T_g and T_s (the mean lifetimes of the growing and shortening states) were calculated according to the relationship $\Delta L_{avg} = R_g^{max} T_g = R_s T_s$ using R_s = 5.9 μm/min (Schilstra *et al.*, 1989). [b] No growth is observed under these conditions. [c] Estimation based on the observations of Figure 3C.

The experimental values of F_{30} in the absence and the presence of Tu-Col are given in Table 1. In order to determine the value of ΔL_{avg} that is consistent with these values, one needs to know the appropriate growth rate at the C_c' (see above). An upper limit for the growth rates (R_g^{max}) was obtained as the sum of R_{gf} and R_{gs} (see above), because the contribution of both ends may be taken to be additive as far as the fractional dimer exchange is concerned. Thus, in the absence of the Tu-Col, R_g^{max} was estimated to be 3.4 μm min^{-1}, and this requires average length excursions larger than 3 μm to account for the observed fractional dimer exchange (see Table 1). Similarly, with 1 μM Tu-Col, R_g^{max} is 0.63 μm min^{-1}; thus only values for ΔL_{avg} between 0.4 and 1.7 μm can account for a relative dimer exchange in the range 0.29 ± 0.05. At 3 μM Tu-Col R_g^{max} is 0.35 μm min^{-1} and ΔL_{avg} is between 0.1 and 0.5 μm. With higher concentrations of Tu-Col difficulties exist in the estimation of R_g^{max} because of the strong inhibition of growth by the protein–drug complex. However, from the strong reduction of the length changes in these conditions (see Figure 3C) it is clear that ΔL_{avg} is reduced still further.

From the values of ΔL_{avg} estimated in this way one can use the relations $\Delta L_{avg} = R_g T_g = R_s T_s$ (at steady state) to estimate probable values for the growing state lifetime, T_g, and the shortening state lifetime, Ts, assuming the constancy of R_s. Table 1 summarizes the results. Although these are only very approximate calculations, the results may be understood in terms of the effects of Tu-Col on the apparent critical concentration (see Figure 1B). The increased C_c' in the presence of drug means that the concentration of Tu-GTP in solution is increased. Both experimental results (Walker et al., 1988) and theoretical studies (Bayley et al., 1990; Martin et al., 1993) predict that this increase in [Tu-GTP] should result in an increase in T_g and a decrease in T_s. The increase in T_g is somewhat less than expected for an increase in [Tu-GTP] of the order shown in Figure 1B (Bayley et al., 1990; Martin et al., 1993). This is most probably due to the interference of Tu-Col in the growth process, leading to an increased probability of the microtubules undergoing a transition into the shortening state and therefore to a lower value of T_g.

(6) Monte Carlo Simulation of Microtubule Dynamics in the Presence of Drugs. We have developed a quantitative numerical treatment, the lateral cap model, which allows simulation of the dynamic properties of microtubules by

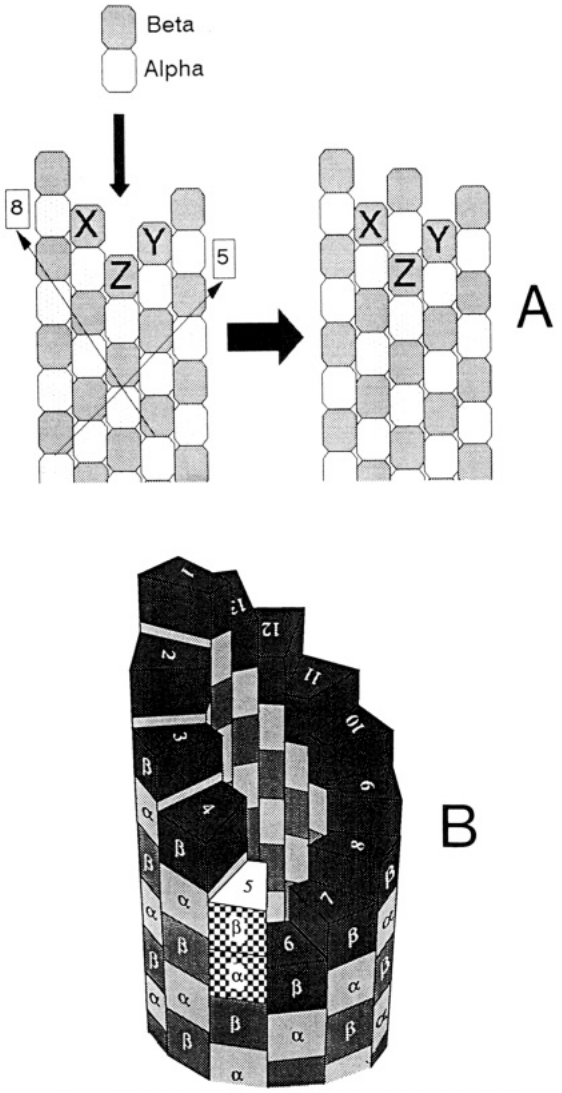

FIGURE 5: Lateral cap model: effects of Tu-Col. (A) Addition of a tubulin $\alpha\beta$-heterodimer to a site at the β-out end of a 13-protofilament A-lattice. Only a part of the structure is shown in a two-dimensional representation. Heavy shading = β-subunit; light shading = α-subunit. The addition reaction shown causes the hydrolysis of any GTP in the β-subunit labeled **Z**. The affinity of a dimer for the end is determined by the nucleotide content of β-subunits labeled **X** and **Y** and is high when these contain GTP. In the model employed here the reaction shown inhibits further addition at position **Y** *when the dimer added is Tu-Col*. The 5- and 8-start dimer helices are indicated. (B) Possible mechanism of action of Tu-Col: 13-protofilament A-lattice in a three-dimensional representation. Black = β-tubulin-GTP; medium gray = β-tubulin-GDP; light gray = α-tubulin; checkered = Tu-Col. The Tu-Col complex is visualized as binding to the lattice but inhibiting further addition reactions in the 5-start direction. The microtubule has adopted the characteristic configuration, frequently observed in simulations, in which the only remaining addition site (on protofilament 6) is strongly inhibited by the presence of Tu-Col.

treating events at the microtubule end (Bayley *et al.*, 1990; Martin *et al.*, 1993). In this section we suggest one possible way in which the lateral cap model can be extended to explain the influence of species such as Tu-Col on microtubule dynamics. The basic postulates of the model are as follows: (1) The affinity of a tubulin $\alpha\beta$-dimer for a microtubule end is determined by the number of contacts formed upon binding and by the nucleotide content of the adjacent β-subunits (**X** and **Y** in Figure 5A). When the adjacent subunits contain GTP, the affinity of a Tu-GTP molecule for the site is high and the dissociation rate constant is correspondingly low. When the adjacent subunits contain GDP, the affinity of the site for

Table 2: Simulation Parameters (Lateral Cap Model)[a]

site (xy)	$k_{off(T/xy)}$ (s^{-1})	$k_{off(D/xy)}$ (s^{-1})
TT	0.4	2.0
DD	100.0	364
TD	5.4	27.4
DT	7.5	27.4

[a] Shown are the chosen values for the dissociation rate constant for Tu-GTP (T) or Tu-GDP (D) from the terminal site of the protofilament labeled Z (Figure 5A) where the indices xy refer to the nucleotide composition of subunits in positions X and Y; the value of k_{on} (2.0×10^6 M^{-1} s^{-1}) is the same for all sites, irrespective of nucleotide content. In the presence of the tubulin–drug complex, all dissociation rates remain the same as in the table, even when the dissociating species is Tu-Drug (with nucleotide GTP or GDP as above). When the complex of Tu-GTP with drug (Tu-Drug) adds at Z, for type 1 inhibition k_{on} is reduced 100-fold for addition at X ($\bar{8}$-start inhibition) or Y (5-start inhibition), and, for type-2 inhibition k_{on} is reduced 100-fold for addition at both X and Y (5-start and $\bar{8}$-start inhibition). The tabulated values derive from affinities as described in Martin *et al.* (1993); the parameters for the affinity and kinetic effects of Tu-Drug are chosen to illustrate the principles of the mechanism, and as discussed in the text (section 6 under Results and Discussion), they are interdependent.

Tu-GTP is low and the dissociation rate constant is high. We generally assume that association rate constants are the same for all sites, irrespective of structure and nucleotide content (Martin et al., 1993). (2) The addition of a Tu-GTP molecule to the microtubule end is coupled to the hydrolysis of a GTP located in a previously terminal β-subunit (**Z** in Figure 5A). This tight coupling restricts Tu-GTP to a terminal layer, the lateral cap, at the microtubule end.

If Tu-Col adds to the microtubule lattice and then inhibits further addition of Tu-GTP, it could clearly have a substantial effect on the growth rate, even at a low concentration relative to the total amount of Tu-GTP. The detailed behavior will depend on the specific properties of the Tu-Col complex at a terminal position of the microtubule lattice. Two obvious possibilities are that Tu-Col inhibits further additions in either the 5-start or the $\bar{8}$-start lattice direction *or* that it inhibits further addition in both the 5-start and the $\bar{8}$-start direction (see the caption to Figure 5A). These have been designated as type 1 and type 2 inhibition, repectively (Martin et al., 1993; Bayley et al., 1993a). One distinction between these mechanisms is that inhibition of further addition in both lattice directions appears to induce complete microtubule disassembly when the drug is added at sufficiently high concentrations. We have therefore chosen to simulate the effect of Tu-Col as type 1 inhibition, inhibiting further addition of Tu-GTP in the 5-start direction. Addition of Tu-GTP at the inhibited site is then significantly reduced. Alternatively, the Tu-Col can dissociate and normal growth could then resume.

Simulations were performed as described in Martin et al. (1993) for the β-out end of a 13-protofilament A-lattice with some modifications to allow for the presence of Tu-Col. Rate constants for addition and dissociation at different sites were calculated as described (Martin et al., 1993) and are illustrated in Table 2. In addition, we assume that Tu-Col adds to the lattice with the same affinity as Tu-GTP does [cf. Lambeir and Engelborghs (1980) and Keates and Mason (1981)], but then reduces the association rate constant for any species at the 5-start related neighboring site (see Figure 5A) by a factor of 100. The action of Tu-Col is entirely attributed to its effect on this adjacent lattice site. The specific hydrolysis rule involved in the addition reaction (Bayley et al., 1990; Martin et al., 1993) is assumed to be unaffected. This is consistent with the effects of Tu-Col on preformed microtubules assembled from either Tu-GTP or Tu-GMPPNP, suggesting that the differences in the effects of Tu-Col on these different

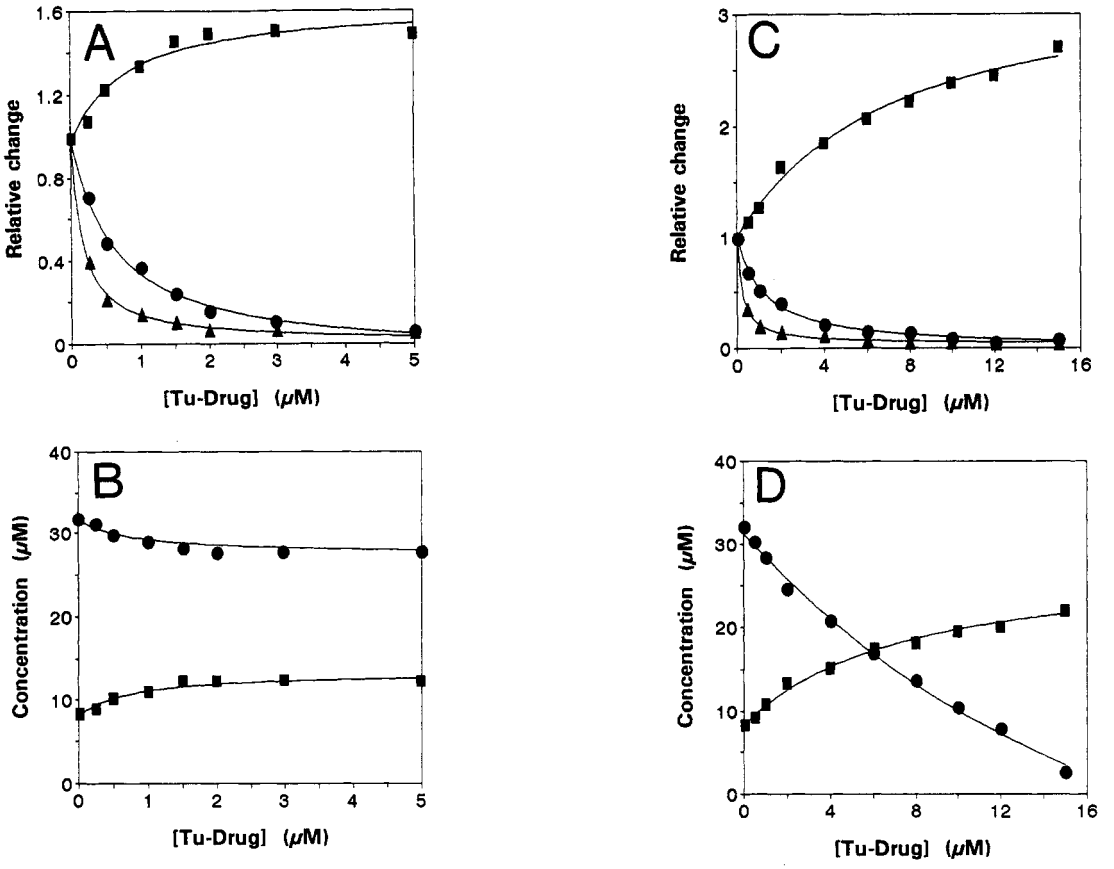

FIGURE 6: Simulation of the effects of a Tu-Drug complex on microtubule dynamics. The effects of Tu-Drug on several parameters were simulated for the β-out end of the 13-protofilament microtubule A-lattice. The values in panels A and C have been normalized by dividing by those observed in the absence of Tu-Col. The simulation was performed for both a type 1 drug (A, B) and a type 2 drug (C, D). The Tu-Drug complex reduces the on-rate constant k_{on} for the adjacent inhibited site(s) in the lattice by a factor of 100. The total concentration of tubulin is set at 40 μM. Panels A and C: (■) The apparent critical concentration, plotted as C_c' – [Tu-Drug] and normalized to an initial value of 8.25 μM (Martin et al., 1993); (▲) The growth rate, simulated at $C_t' = C_c' + 3$ μM (see text) and normalized to an initial value of 2.67 μm/min; (●) The fractional exchange, calculated in terms of average length excursion (ΔL_{avg}) and normalized to an initial value of 4.2 μm. Panels B and D: (■) The apparent critical concentration, plotted as C_c' – [Tu-Drug], unnormalized; (●) the polymer mass calculated as $C_t' - C_c'$.

microtubule types can be attributed to the relative stability of the polymers, without requiring any effect of the drug on the microtubule end-dependent GTP hydrolysis (A. Vandecandelaere and Y. Engelborghs, in preparation). Although colchicine is known to induce a low GTPase activity in dimeric tubulin (David-Pfeuty *et al.*, 1979; Heusèle & Carlier, 1981; Andreu & Timasheff, 1981), this property does not seem to contribute to the dynamics of assembled microtubules.

An important qualitative result of the simulations is that the microtubule is frequently seen to adopt the characteristic inhibited configuration shown in Figure 5B. This configuration typically has a single (normal) dissociation site (protofilament 1, Figure 5B) and a single (inhibited) site (protofilament 6, Figure 5B). In this configuration, the probability of Tu-Col dissociation is greatly reduced, owing to its partially buried position requiring successive loss of terminal Tu-GTP molecules from protofilaments 1–4. Growth is therefore interrupted for long periods, since the only addition site remaining is that inhibited by the presence of Tu-Col. Thus, small amounts of Tu-Col can have a major effect on microtubule growth rates. Note, however, that this inhibited structure has an extended lifetime because any terminal Tu-GTP lost is easily replaced by addition reactions at normal uninhibited sites. The continued presence of normal addition sites also explains why increasing concentrations of Tu-Col do not cause complete microtubule disassembly. Although microtubule growth can become completely inhibited by the presence of Tu-Col in the terminal layer of the microtubule, the microtubule is prevented from entering the shortening state provided that there is a sufficient number of uninhibited sites available for Tu-GTP addition.

The quantitative results of a series of simulations of the effects of Tu-Drug complexes are summarized in Figure 6. Figure 6a shows simulation according to the type 1 mechanism which produces results in good qualitative agreement with the experimental observations for Tu-Col. Simulations of the effect of Tu-Drug on the apparent critical concentration were performed at a total Tu-GTP concentration of 40 μM, with different amounts of Tu-Drug. The apparent critical concentration, C_c', increases for [Tu-Drug] in the range 0–2 μM, as observed experimentally (Figure 1B). Furthermore, both the growth rate (determined in simulations at $C_t' = C_c' + 3$ μM) and the size of the average length excursion (measured in simulations at the appropriate C_c') are suppressed by increasing concentrations of Tu-Drug, as observed experimentally (Figures 2 and 4). Figure 6B shows that this type 1 mechanism for the Tu-Drug action causes only partial disassembly of the microtubule mass under these conditions.

In these simulations the effect of Tu-Col is due to two principal factors: (a) the affinity of Tu-Col for the tubulin lattice and (b) the effect of Tu-Col in the lattice on the properties of the adjacent binding site. For simplicity (and consistent with existing evidence) we assign an affinity of Tu-Col for the lattice identical to that of Tu-GTP. A lower affinity of Tu-Col [e.g., Sternlicht *et al.* (1983)] would make it a less effective inhibitor; however, this could be compensated by a larger effect on the adjacent site in the lattice, and vice versa. Thus, the modeling can, in principle, accommodate a

value for the affinity of Tu-Col within a substantial range without necessarily affecting the predicted behavior.

Although the model depends upon unfavorable interactions between tubulin and Tu-Drug in the microtubule lattice, the finite probability that is attributed to the addition of a dimer to an inhibited site allows for a low but significant incorporation of Tu-Drug in the polymer mass. Experimentally, in the presence of MAPs, the incorporation of Tu-Col in the polymer phase increases progressively with time (Sternlicht *et al.*, 1983; Skoufias & Wilson, 1992), and values of up to 5% of the polymer mass have been reported after such assembly in the presence of Tu-Col (Sternlicht & Ringel, 1979; Sternlicht *et al.*, 1983; Farrell & Wilson, 1984; Skoufias & Wilson, 1992). Incubation of tubulin microtubules with very high levels of Tu-Col for 1 h gave no more than approximately 0.3% incorporation of Tu-Col into the polymer mass (A. Vandecandelaere and Y. Engelborghs, in preparation). The probability for an addition reaction at the inhibited site increases with the concentration of Tu-GTP and with the ratio of Tu-GTP to Tu-Col. Hence, the relative efficiency of the incorporation is expected to be higher at low concentrations of Tu-Col. This has indeed been observed by several authors (Sternlicht & Ringel, 1979; Sternlicht *et al.*, 1983; Farrell & Wilson, 1984; A. Vandecandelaere and Y. Engelborghs, in preparation). The simulations described in Figure 6 for determining the apparent critical concentration were performed at a total Tu-GTP concentration of 40 μM (as used experimentally). At steady state, 15% of the added Tu-Drug is incorporated at 0.5 μM Tu-Col, compared with 5% at 5 μM Tu-Drug. As a percentage of the total amount of polymerized tubulin, these values correspond to 0.25% (0.5 μM Tu-Drug) and 0.95% (5 μM Tu-Drug). Low levels of incorporation are expected because the addition of Tu-Col causes (limited) microtubule disassembly and abolishes the length excursions characteristic of dynamic instability. It should be noted that with this mechanism fully stoichiometric incorporation of Tu-Drug does not occur, and the extent of relative incorporation of Tu-Drug is strongly dependent upon the average length of the microtubule population prior to the addition of the Tu-Drug complex.

Figure 6C shows a similar simulation for the effects of a type 2 drug, which is seen (Figure 6D) to cause significantly greater microtubule disassembly. Growth rate and mean excursion length are both markedly reduced by low concentrations of the tubulin–drug complex. These effects correspond to experimentally observed results for the tubulin–podophyllotoxin system (Schilstra et al., 1989). A clear distinction between type 1 and type 2 mechanisms clearly requires these properties to be evaluated at different Tu-GTP concentrations.

General Discussion. The effects of the tubulin–colchicine complex (Tu-Col) on the dynamic instability of microtubules have been examined. The addition of either free colchicine or Tu-Col to polymerized tubulin induces significant microtubule disassembly. However, in agreement with previous observations (Sternlicht *et al.*, 1980; Deery & Weisenberg, 1981; A. Vandecandelaere and Y. Engelborghs, in preparation), the disassembly is found to be incomplete, even at high concentrations of colchicine or Tu-Col, where the apparent critical concentration approaches a limiting value (see Figure 1). This behavior may be contrasted with that of drugs such as podophyllotoxin, which cause complete microtubule depolymerization when added at levels stoichiometric with tubulin under identical buffer conditions (Schilstra *et al.*, 1989). This difference suggests that these two drugs have significantly different modes of action.

Amounts of Tu-Col which cause only limited polymer disassembly do, however, have major effects on the dynamic behavior of the microtubules. Thus, relatively low concentrations of Tu-Col suppress both tubulin exchange processes (see Figure 2) and the characteristic microtubule length redistribution (see Figure 3). These effects derive from a dramatic reduction in the size of the average length excursion during periods of growth and shortening. Examination of individual microtubules using dark-field video microscopy has shown that the reduction in the size of the average length excursion is associated with a large reduction in values for the growth rates (R_g) of individual microtubules (see Figure 4). Consistent with a report by Bergen and Borisy (1982), these reduced growth rates are observed for both ends of the microtubules.

Colchicine is an extremely potent inhibitor of microtubule growth, a property often referred to as substoichiometric inhibition since only a small amount of colchicine relative to the total amount of tubulin in the system (about 5%, mol/mol) is sufficient for complete inhibition of growth (Olmsted & Borisy, 1973; Margolis & Wilson, 1977). The lateral cap model (Martin et al., 1993) has been modified to treat the effects of colchicine on the dynamics of the microtubule ends. Colchicine is assumed to exert its effect through the formation of Tu-Col, as indicated by experimental evidence (Margolis & Wilson, 1977; Skoufias & Wilson, 1992). Again, consistent with existing evidence (Lambeir & Engelborghs, 1980; Keates & Mason, 1981), Tu-Col is assumed to have an affinity for the sites in the lattice similar to that of Tu-GTP. However, when Tu-Col adds to the lattice, one of the neighboring lattice sites is considered to have a (very) low affinity for subsequent addition reactions of Tu-GTP or Tu-Col (see Figure 5A). The dramatic effect of Tu-Col on microtubule ends can be rationalized using the lateral cap model as formulated here. Owing to the helical symmetry of the microtubule lattice, a microtubule with Tu-Col bound in a terminal position frequently enters an inhibited configuration such as that shown in Figure 5B. Further growth is then possible only if the Tu-Col dissociates (after a series of Tu-GTP dissociations) or if the Tu-Col is buried by an energetically unfavorable (and hence slow) addition of a dimer to the adjacent inhibited site. Although unfavorable, the latter event explains why substantial amounts of Tu-Col may be incorporated into the polymer under certain conditions (see above).

Given the simplicity of its assumptions, the modified version of the lateral cap model described here explains and reproduces the observed effects of the interactions of Tu-Col with dynamic microtubules. Owing to its numerical iterative approach, this model can potentially provide a quantitative treatment of nonequilibrium properties of the microtubule system, and offers a means to examine possible interactions of potential regulating molecules with the microtubule ends. This treatment shows how any molecule with an affinity for the microtubule end similar to that of Tu-GTP can act substoichiometrically to suppress microtubule dynamics. It therefore provides a possible general mechanism for the end-dependent action of any regulator molecule acting to switch the state of microtubules between dynamic activity and a quiescent state. While definite evidence for such regulatory molecules is lacking, the advantages of such a control process are evident.

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