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Some Thoughts on the Partitioning of Tubulin Between Monomer and Polymer Under Conditions of Dynamic Instability

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ABSTRACT

We have considered the partitioning of tubulin between monomer and polymer in the cell under conditions of dynamic instability. Dynamic instability adds to the on and off rate constant of steady-state dynamics' new parameters: (1) the rate at which growing microtubules transit to a shrinking phase; and (2) the rate at which shrinking microtubules transit to the growing phase. Under these conditions the free-monomer concentration in the cell increases with total tubulin if the number of nucleating sites is fixed. If the number of nucleating sites increases at fixed total tubulin, subunits shift from the monomer to the polymer phase. These important properties deviate from the traditional equilibrium and steady-state theories and have important implications for the biosynthetic regulation of tubulin.

INTRODUCTION

Microtubules are helical polymers of the subunit protein tubulin. They play important roles in shaping cells and directing intracellular motility, and these functions require their polymerization into highly organized spatial arrays, such as the mitotic spindle [for reviews see, refs. (*1–5*)]. Microtubules coexist with a pool of soluble subunits within cells, and in many situations there is rapid exchange between monomeric and

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polymeric tubulin, which can be visualized directly (*6*) or through the effect of agents that perturb the balance between monomer and polymer (*7*). We need to understand the nature and mechanism of this dynamic equilibrium in order to explain at least two important aspects of microtubule biology: The spatial organization of microtubule assembly, since this is intimately connected to the dynamics (*8*), and the regulation of tubulin biosynthesis. In several cell types the rate of tubulin synthesis of the tubulin polypeptides is regulated by feedback from the level of monomer (*9,10*); increased monomer tends to destabilize tubulin mRNA (*11,12*). However, for most aspects of tubulin biology the polymer is presumably more important than the monomer. The experiments on feedback of tubulin synthesis show that the cell monitors the mass of microtubule polymer by sensing the monomer concentration. For this to work, the monomer concentration must vary as some function of the total polymer concentration. In a purely equilibrium system, this would not be the case, since the monomer concentration would be fixed, and any change in total tubulin would cause a change in polymer.

For many years our understanding of the assembly of linear polymers has been strongly influenced by the theory of Oosawa (*13*). In this view subunit assembly and disassembly reach a true equilibrium, characterized by a fixed monomer concentration in equilibrium with polymer; this monomer concentration is called the critical concentration. Most polymerizing systems, such as actin, tubulin, flagellin, and tobacco mosaic virus, roughly conform to this theory. However, microtubules are not in a true equilibrium with their subunits; they require GTP for assembly and hydrolyze it stoichiometrically (*8,14*). Thus, both in vitro and in the cell, microtubule assembly is a steady-state process that can resemble in some respects the true equilibrium process described by Oosawa. Various models have been proposed for the dynamic processes in microtubules that continue to occur at steady state. These models differ mainly in the kinetics and consequences of GTP hydrolysis. Theoretical ideas have often preceded experimental observations in this area, and Terrell Hill has played an important role in their development. Treadmilling, in which nucleoside triphosphate hydrolysis causes a difference in critical concentrations at the ends of a polymer, was first postulated as a theoretical idea by Wegner for actin filaments (*15*) [and independently by Margolis and Wilson for microtubules (*16*)]. The consequences of this type of dynamics were then explored exhaustively in a series of papers by Hill and Kirschner (*17–19*) for biologically relevant situations. The consequences of a cap of GTP liganded subunits at the end of a growing polymer were first realized theoretically by Hill and Carlier (*20*) and subsequently shown experimentally (*21*). These ideas were crucial to the development of the dynamic instability model for microtubule assembly, in which the microtubule population consists of two slowly interconverting subpopulations of growing and shrinking microtubules. This model (*22,23*) was borrowed from the GTP cap model of Hill and Carlier (*20*) to

explain the coexistence of growing and shrinking microtubules. Chen and Hill went on to show that the GTP cap model with altered rate constants could potentially explain the experimental observations of dynamic instability (*24*), though experiment is still lagging behind theory in confirming or refuting this explanation. Direct visualization of microtubule growth in real time has confirmed the more indirect in vitro experiments that microtubule ends interconvert apparently in a stochastic manner between episodes of growing and shrinking (*25*). In vivo experiments also strongly suggest that rapid growth and shrinking of microtubules with infrequent interconversion also occurs in vivo in what is presumably a steady-state situation (*26*).

The view that microtubules are in true equilibrium with their subunits is clearly an incorrect one, but the simplification and elegance of the equilibrium theory of Oosawa still dominates thinking in the field. Most of our ideas about microtubule physiology in vivo are based on these ideas. We need to rethink the nature of the dynamic equilibrium between tubulin and microtubules and examine the consequences of dynamic instability within the cell.

In order to examine the partitioning of tubulin between monomer and polymer we will first discuss the development of theoretical models of microtubule dynamics in vitro, and the factors that determine the concentration of monomer present at steady state. We will then proceed to a quantitative model for dynamics in vivo to examine the same question in a more biological context. The quantitative model is highly simplified and is not intended as an exercise in curve fitting, but, rather (taking a leaf from Terrell Hill), to use real numbers to illustrate theoretical points. We shall see that the concept of critical concentration—which has dominated most previous thinking in this area—is of limited utility in understanding the dynamic equilibrium between tubulin and microtubules within cells. Although under some conditions the behavior of the monomer–polymer system is similar to that expected for true equilibrium, in detail, it departs from the simple Oosawa model. Under typical in vivo conditions the partitioning of tubulin between monomer and polymer deviates from that predicted for polymers at equilibrium; this deviation has important consequences for the regulation of tubulin biosynthesis.

THE TUBULIN MONOMER CONCENTRATION UNDER CONDITIONS OF DYNAMIC INSTABILITY

In the simplest kind of polymerization reaction, the addition or loss of monomers from the end of the polymer is a reversible reaction, which can come to a true equilibrium (*13*). The behavior of the polymerization reaction is governed by the critical concentration, c_c, which is the concentration of monomer present at equilibrium in the presence of polymer. The c_c is the ratio of the off to the on rate constant (Fig. 1,A) and also the

dissociation constant for the addition of monomer to the end of the polymer. The polymerization of tubulin into microtubules does not come to equilibrium. The hydrolysis of bound GTP at the E site to GDP (*14*) is an irreversible reaction in a thermodynamic sense, because the GTP–GDP system is maintained far from equilibrium by the cell's energy metabolism. Obligate coupling of polymerization to an irreversible process means that the polymer reaches a steady state with continuous GTP hydrolysis when assembly and disassembly are balanced. In a simple system in which polymerization and depolymerization occur from the same end, this steady state still has a unique critical concentration, c_s, defined by the ratio of the off to the on rate constant (Fig. 1,B). Because the off reaction is no longer the simple reverse of the on reaction, c_s is not a true equilibrium or dissociation constant, but is instead a ratio of rate constants of different reactions. This makes c_s subject to variation with changes in the system that would not effect c_c: For example, a capping protein that differentially affected the on and off rate constants would change c_s (*18*), and c_s can be different for the two ends of the polymer [treadmilling, refs. (*18,27*)]. However, c_s still retains many of the properties of a true equilibrium constant, such as insensitivity to the polymer number concentration and the total amount of monomer + polymer in a closed system.

Recently, it has been shown that microtubule ends in vitro can exist in two states that differ greatly in assembly properties and interconvert infrequently (*22,23*). This behavior has been termed dynamic instability, and evidence is accruing that suggests that microtubules in living cells behave similarly (*26*). So far the difference between growing and shrinking ends has not been determined; one possibility is the stabilization of growing ends by a cap of subunits that have not yet hydrolyzed their bound GTP (*8,23*). In vitro mixtures of microtubules, tubulin, and GTP come to a sort of steady state, with the majority of polymers growing slowly, balanced by a minority shrinking rapidly and a constant monomer concentration. It seems likely that a similar steady state exists in vivo (*26,28*).

In order to derive steady-state equations for a dynamic instability system (Fig. 1,C), we assume the existence of two populations of polymer, with n growing, and N-n shrinking, where N is the total polymer number concentration. A steady state is produced where monomer concentration = c. The growing and shrinking polymer states have on rates kc and lc, and off rates k' and l', respectively. At steady state, production of polymer is balanced by loss, so that:

$$knc + l(N-n)c = k'n + l'(N-n)$$
$$c = [k'n + l'(N-n)]/[kn + l(N-n)] \quad [1]$$

If we assume that at steady state the great majority of assembly occurs onto growing ends, and disassembly from shrinking ends (i.e., that the two states differ greatly in their equilibrium critical concentration), then we can ignore k' and l, so that:

DYNAMIC SYSTEM	Steady State Monomer Concentration
A. Equilibrium Polymer (polymer ⇄ monomer; k, k′)	$Cc = \frac{k'}{k}$
B. Simple Steady State Polymer (P_i; k, l′; GDP, GTP)	$Cs = \frac{l'}{k}$
C. Dynamic Instability (P_i; k, l′; Ω′, Ω; GDP, GTP)	$C = \frac{l'}{k} \times \frac{\Omega}{\Omega'}$

Fig. 1. Dynamic models for the assembly of subunits to a single end of a microtubule.

$$c = (l'/k)(N-n)/n \quad [2]$$

The ends can interconvert at rates given by the first-order rate constants, Ω (growing-to-shrinking) and Ω' (shrinking-to-growing). The rate of conversion of growing ends-to-shrinking is given by Ω multiplied by the fraction of ends in the growing state, and, similarly, shrinking-to-grow-

ing is given by Ω' multiplied by the shrinking fraction. At steady state, there must be a balance in the interconversion between growing and shrinking ends. Thus:

$$\Omega n = \Omega'(N-n)$$
$$c = (l'/k)\,\Omega/\Omega' \qquad [3]$$

Note that this equation contains the same ratio of rate constants as the simple steady-state situation shown in Fig. 1,B, but is now multiplied by a new factor, which can be expressed either as the ratio of shrinking-to-growing end concentrations or the ratio of end-interconversion rates. The two ratios in Eq. [3], l'/k and Ω/Ω' differ in a fundamental way: The rate constants, l' and k, are true kinetic constants governed only by properties intrinsic to the polymer and monomer themselves, defined under a given set of solvent conditions and temperature and reflect the stability of the lattice, diffusion rate of monomer, solvation of the protein, and the like. These rate constants are independent of tubulin concentration or polymer-number concentration. Ω and Ω' are also influenced by the intrinsic properties of the lattice, but they are not independent of c. Thus, although Eq. [3] is generally useful empirical equation, for quantitative analysis we must rewrite it, replacing l'/k by a constant, K, and Ω/Ω' by a general expression:

$$c = Kf(c) \qquad [4]$$

In order to derive Eq. [3] we assumed the existence of a steady state containing both growing and shrinking microtubules. For this to be possible puts constraints on $f(c)$ in Eq. [4], in that it must be either a constant or a decreasing function of c. In reality, it is probably a decreasing function, as will be discussed in the two subsequent sections. If the ratio of shrinking-to-growing ends increased with c, the only steady state would have no polymer. Equation [4] obviously calls for simplification, but to do this we must know the form of $f(c)$. This is model dependent, and in the rest of this paper we will consider the situation first for pure microtubules in vitro and, subsequently, for an approximately realistic model for dynamics within the cell. We shall show that the behavior of these systems conforms in some respects to the simple equilibrium model of Oosawa, but departs in other, important ways. In particular, the expression for $f(c)$ may contain factors that would not appear in an equilibrium or simple steady-state model, such as the number of nucleating sites and total amount of tubulin in a closed system.

MICROTUBULE DYNAMICS IN VITRO

The simplest situation in the test tube consists of long microtubules at a steady state in polymer concentration. These microtubules undergo rounds of shrinking and growing, but are long enough to escape com-

plete disassembly, so that number concentration is constant, a situation similar to that observed directly by Horio and Hotani [25]. k and l' are constants, fixed by the solution conditions and temperature, so in order to determine c we need only to find the steady state value of Ω/Ω'. Experimentally, Ω has been found to decrease as c increases, as expected for the GTP cap model (*8,23*). Less is known about Ω', but it is likely to be constant or increase with c (*24*). In the next section we adopt a specific function for Ω, but here it is only necessary to suppose that Ω/Ω' is a decreasing function of c. If more tubulin monomer is added to such a system at constant volume, it will transiently perturb the system toward an increased fraction of growing ends. Once the extra monomer is incorporated into polymer, Ω/Ω' will come back to the steady-state level, with increased polymer concentration, but the original monomer concentration. If the number concentration of microtubules is changed, but they remain long enough to resist complete disassembly, there is no change in average behavior of a given microtubule, and thus no change in c. Thus, if only bulk properties were assayed, this system would appear Oosawa-like. Ω/Ω', like l'/k, will be determined by properties of the lattice, and a unique value of c at steady state can be described for a given set of solution conditions—although it is worth noting that experimentally Ω/Ω' is likely to be much more sensitive than l'/k to variations in the solution conditions and purity of the protein. Whether this steady state is ever reached in a real experiment is problematical. If the rate of spontaneous nucleation is very slow, and if microtubule disassembly leads to complete loss of microtubules, there will be a decrease in microtubule number concentration with time (*23,29*). Loss of individual microtubules from complete disassembly effectively raises the monomer concentration to an extent dependent on the rate of loss.

In practice, the deviation from Oosawa-type behavior is probably small, given a slight dependence of c on average polymer length (c decreasing with polymer length), but it will be interesting to look for experimentally. However, in general, the bulk behavior of free, long microtubules in solution under conditions of dynamic instability will be quite similar to those predicted by previous models. More striking departures would be seen for nucleated microtubules, such as the existence of transiently stable microtubules below the steady bulk critical concentration, which has been observed experimentally (*22*). The behavior of nucleated microtubules under conditions of dynamic instability, a situation more relevant biologically, will be considered in detail in the next section.

MICROTUBULE DYNAMICS WITHIN THE CELL

A Quantitative Model

Microtubule dynamics within living tissue culture cells have been probed by microinjecting tubulin subunits labeled with chemical tags

and then following their incorporation into microtubules either directly (*26,28*), or following a photobleach (*4*). The results of such studies are congruent with observations of the dynamic instability of pure microtubules in vitro [reviewed in ref. (*8*)] and lead to the model for in vivo dynamics shown in Fig. 2. Microtubules are nucleated at the centrosome with their plus ends distal (*30,31*) and grow out toward the plasma membrane at about 4 μm/min at 37° (*26*). At some point they stop growing, and start to shrink. The cause of this reversal is not known. In some cases, particularly in mitotic cells, it is apparently stochastic, occurring before the microtubule reaches the cell periphery. This leads to a highly polydisperse array of microtubules, which are short relative to the cell dimensions and turn over very rapidly (*32*). We shall use this situation, in which turnover is controlled only by the polymer dynamics, as the basis for quantitative analysis. We will first need to introduce the following simplifications:

(1) All microtubules are nucleated at the centrosome, with their plus ends distal. Minus ends are anchored and nondynamic.
(2) Nucleation is highly efficient, so that nucleating sites are never empty. As soon as a shrinking microtubule has depolymerized to the nucleating site, a new growing one is initiated. In fact, centrosomes are very efficient at nucleating in vitro and show no detectable lag phase (*22*).
(3) Microtubule shrinkage is very rapid (compared to growth) so that it may be treated as instantaneous. This, combined with (1) and (2) above, means that all nucleating sites are occupied

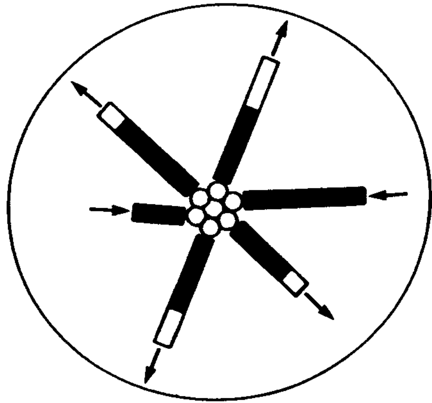


Fig. 2. Growth of microtubules from the centrosome in vivo. Arrows denote direction of growth or shrinking. For a GTP cap model the empty segments denote GTP containing subunits; the filled segments denoted GDP containing subunits.

by growing microtubules. In fact, up to 20% of the microtubules in the cell may be shrinking at any time (*28*), but this should not greatly affect the outcome of the analysis.
(4) Ω' is effectively zero, so that growing ends are produced only by new nucleation. This is consistent with the rapid incorporation of labeled subunits into microtubules at the centrosome in vivo (*26,28*). Assumptions (1)–(4) are needed for quantitative analysis, but are probably inappropriate under some circumstances. We will discuss below the qualitative effects of altering these assumptions.
(5) Ω is given by a simple function of c. In vitro studies suggest that the probability of growing microtubules starting to shrink decreases as the growth rate increases (*23*), and this is expected from models in which growing ends are stabilized by a lag between assembly and GTP hydrolysis [GTP cap models (23,24)]. The exact dependency of Ω on c is not known in any situation and may depend on factors other than growth rate—for example, Ω is apparently larger for microtubule plus ends than minus ends at steady state, even though the former grow faster (*25*). However, in general, we expect Ω to be large at low c and decrease as c increases. Therefore, we adopt an empirical function relating Ω to c of the form $\Omega = \Omega_\gamma / c^\gamma$, where Ω_γ is a constant, and γ is an arbitrary exponent. Based on modeling data, Hill estimated γ = 2.4 (*33*). We will consider the situation for γ = 1, 2, and 2.4, as well as the special case, γ = 0, in which microtubule depolymerization is initiated independently of growth rate. In principle, any dependency on concentration can be used.

We also need to define the parameters (and units) used in the model and justify approximate values. These values will then be used as the reference point for numerical calculation of the effects of perturbation of single parameters. Parameters and reference values are summarized in Table 1. The total concentration of tubulin (monomer and polymer) in a fibroblast cell, c_T, has been estimated by Hiller and Weber to be 20 μ*M* (*34*). The fraction as polymer has been estimated to be between 40 and 80% (*34–37*). We shall assume that 50% is in polymer; therefore, c_P = 10 μ*M*, where c_P is the polymer concentration and 50% is in monomer (c = 10 μ*M*). The number of microtubules in a fibroblast cell has been estimated at 120–500 (*28,34,38*). We assume that the number of microtubules represents the total number of nucleating sites N, and that N = 250. The average length of these microtubules expressed as the number of subunits, n, can be calculated from the cell volume, V [which we fix at 1.25×10^{-12}L, about half that assumed by Hiller and Weber (*34*), c_p, and N. This value of the cell volume was chosen somewhat smaller than that estimated by Hiller and Weber to make consistent, independent calculations of n. Thus, we calculate n to be 18.8 μm. The microtubule assembly

Table 1
Values of the Parameters Used in the Model[a]

Parameters	Units	Value
c_T = Total tubulin concentration	μM	20
c = Monomer tubulin concentration	μM	10
c_P = Polymer tubulin concentration	μM	10
N = Number of nucleating sites in cell	—	250
V = Cell volume	liters	1.25×10^{-12}
n = Average microtubule length in subunits		3×10^4 (18.8 μm)
$\mathcal{N}$ = Avogadro's number	μmol^{-1}	6×10^{17}
K = On-rate constant	$\mu M^{-1}s^{-1}$	10
Ω = First-order rate constant from growing to shrinking	s^{-1}	3.3×10^{-3}

[a]The values are estimates for a typical tissue culture cell (*see* text).

rate in vivo has been estimated by Schulze and Kirschner to be 3.7 μm/min (*26*). Assuming about 1600 subunits/μm (*39*), this corresponds to an on-rate constant, k, of 10 $\mu M^{-1}s^{-1}$ at c = 10 μM. The first-order rate constant for a transition from growing to shrinking, Ω, we estimate to be $3.3 \times 10^{-3}s^{-1}$, which corresponds to a half-time for turnover of 3.85 min. The half-life of microtubules in vivo has been reported to be between 2 and 10 min in interphase (*4,26*).

Tubulin is partitioned in the cell between monomer and polymer, so the basic conservation equation is:

$$c_T = c + c_P \qquad [5]$$

The polymer fraction can be considered to be made up of N microtubules of average length, n, so that c_P (in units of μM) is given by:

$$c_P = \frac{Nn}{\mathcal{N}V} \qquad [6]$$

At steady state we have assumed that individual microtubules spend all their life growing, since time spent in the shrinking state is negligible. Thus, we can consider that n, the mean length, is the product of the growth rate for a single polymer, kc, and its average lifetime in the growing state. Since the rate of entry into the shrinking state is given by the first-order rate constant, Ω, the average lifetime of a single polymer is simply $1/\Omega$. Thus:

$$n = \frac{kc}{\Omega} \qquad [7]$$

This equation connects the average size of a polymer to its lifetime and growth rate. Combining Eqs. [4–6] yields:

$$c_P = \frac{Nkc}{\mathcal{N}\Omega V} \qquad [8]$$

Considering now the concentration dependence of Ω discussed above, we can write:

$$\Omega = \frac{\Omega_\gamma}{c^\gamma} \qquad [9]$$

which gives:

$$c_T = c + \frac{Nk}{\mathcal{N}\Omega_\gamma V} c^{\gamma+1} \qquad [10]$$

or:

$$c_P = \frac{Nk}{\mathcal{N}\Omega_\gamma V}(c_T - c_P)^{\gamma+1} \qquad [11]$$

This equation will allow us to see how c_P (or c) varies as c_T is changed in a fixed cell volume. Similarly, we can consider how c_P varies with the number of nucleating sites, N, at constant c_T.

Partitioning of Tubulin as a Function of c_T

We consider first the simple case where $\gamma = 0$, that is, where microtubule depolymerization is initiated at a constant rate independent of free tubulin concentration. This could be a realistic case if the transition was governed by some enzymatic or other regulatory process. For $\gamma = 0$, Eq. [11] reduces to:

$$c_P = \frac{\frac{Nk}{\mathcal{N}\Omega_\gamma V} C_T}{1 + \frac{Nk}{\mathcal{N}\Omega_\gamma V}} \qquad [12]$$

Under these conditions, c_P and c are simply proportional to c_T. Equation [10] may be solved for c_P as a function of c_T for any γ by numerical analysis. In Fig. 3a we plot c against c_T for various values of γ. The parameters are chosen (i.e., Ω_γ is forced) so that all the curves pass through the point given by the values in Table 1. This plot demonstrates how extra subunits would be partitioned between monomer and polymer under various conditions. A familiar way to consider this type of partitioning is to consider the extent to which the monomer concentration is buffered by the presence of polymer. The degree of buffering can be quantitated as the rate of change of monomer with polymer. From Eq. [11]:

$$\frac{dC_P}{dC_T} = \frac{(\gamma + 1)(C_T - C_P)^\gamma}{(\gamma + 1)(C_T - C_P)^\gamma + \frac{\mathcal{N}\Omega_\gamma V}{Nk}} \qquad [13]$$

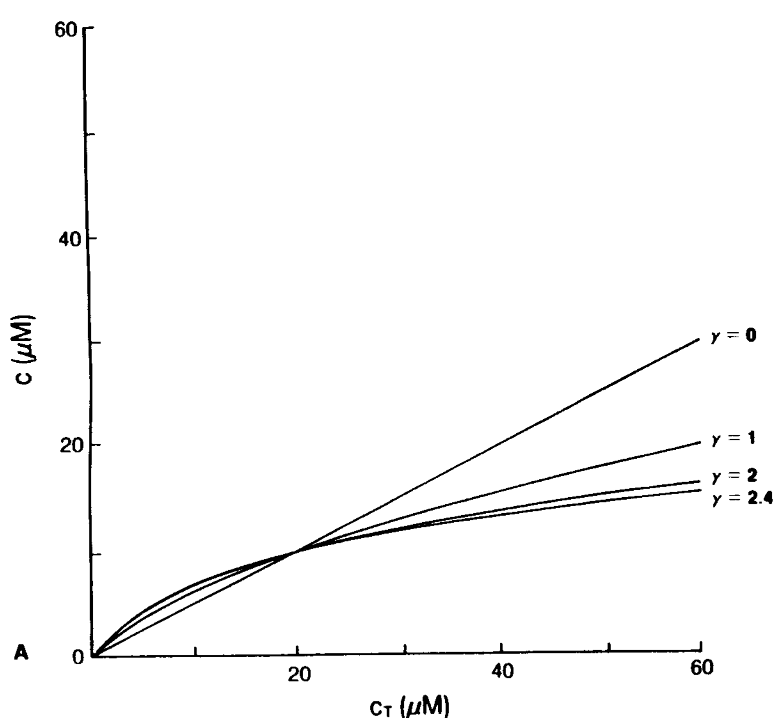
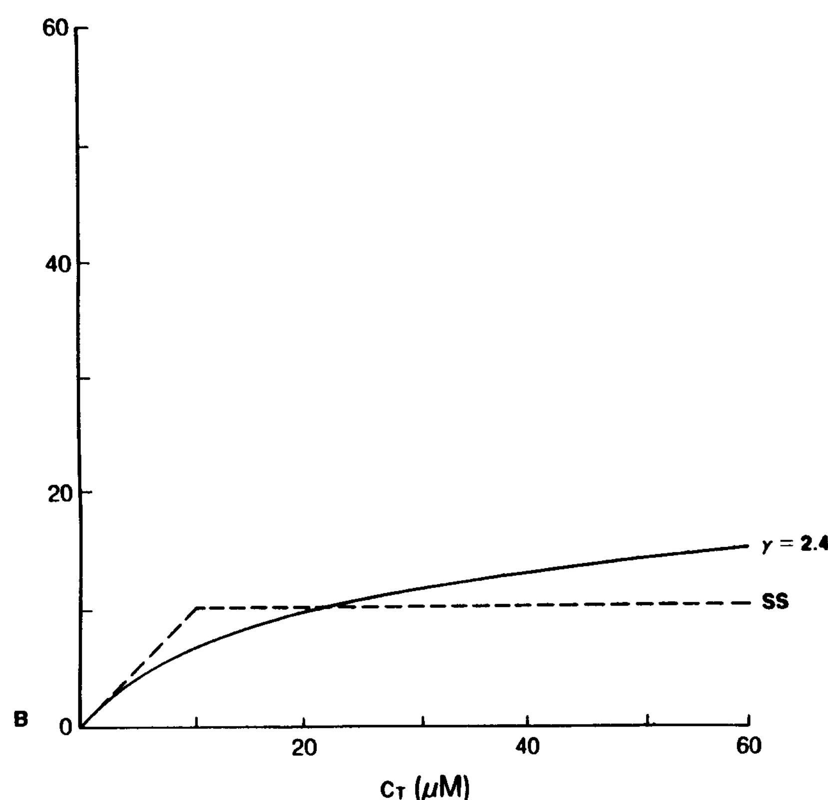


Fig. 3. In (a) (top) we plot the monomer concentration, c, versus the total concentration, c_T for various values of γ. In (b) the plot for $\gamma = 2.4$ is compared to the value for a steady-state polymer with a critical concentration of 10 μM. In (c) the polymer concentration c_P, is plotted versus c_T for $\gamma = 2.4$ and for a steady-state polymer.

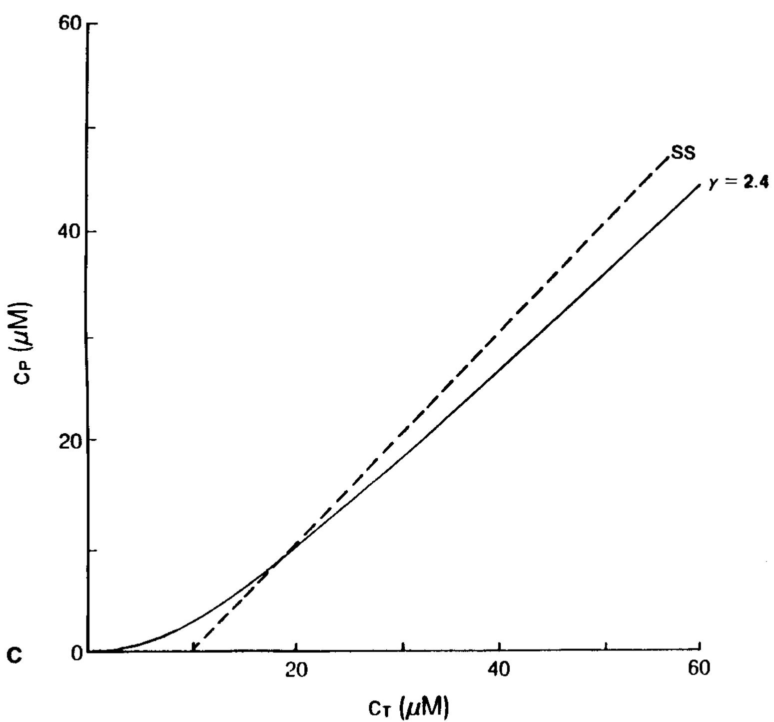
Perfect buffering would be the case if $dc/dc_T = 0$, and no buffering if $dc/dc_T = 1$. When $\gamma = 0$, dc/dc_T decreases as c_T increases—that is, the system is buffered better when there is more tubulin in the cell, and a greater fraction is in polymer.

It is interesting to compare the partitioning of tubulin in the model with a simple steady-state system showing a defined critical concentration. This is done graphically for the $\gamma = 2.4$ case in Fig. 3b, where c is plotted versus c_T, fixing the critical concentration from the values of c_T and c in Table 1. In the simple steady-state system, there is no buffering below c_c, when all added tubulin goes into monomer, and complete buffering above c_c, when all the additional tubulin goes into polymer. For dynamic instability the system is buffered at all tubulin concentrations, but completely buffered as c_T approaches infinity. Thus, for each value of c_T, there is a unique value of c_P and c in the dynamic instability model. At



conditions estimated as realistic (Table 1), for $\gamma = 2.4$, $dc/dc_T = 0.33$, so that changes in c_T around 20 μM tubulin would partition 33% into monomer and 77% into polymer.

These conclusions may be relevant to the regulation of tubulin synthesis, which is thought to be controlled in the cell by monitoring the tubulin monomer concentration (*9,10,37*). In the model based on dynamic instability, the monomer concentration, c, varies continuously with the total tubulin concentration, c_T, as shown in Fig. 3b and Fig. 3c. Thus, c can be used as a sensor for total microtubule mass. Biosynthesis will be exactly balanced by degradation and dilution (from cell growth) at a unique value of c through feedback inhibition, and Eq. [11] shows that a unique value of c specifies a unique value of c_T and, hence, c_P. Note that for the simple steady-state polymer with a fixed critical concentration, c is independent of c_T, the cell could not use the monomer concentration to monitor its polymer concentration. This analysis may explain why microinjecting exogenous tubulin into cells suppresses biosynthesis (*40*),



Partitioning of Tubulin as a Function of N

For an equilibrium system, the polymer mass is independent of the number of nucleating sites as equilibrium. No such constraints exist for dynamic instability, because c itself is not fixed. Since changes in the number of nucleating sites, N, are likely to be of biological importance, it is of interest to examine how the partitioning tubulin between monomer and polymer varies with N.

One obvious example for such a change in N is mitosis. When cells enter mitosis, c_T is probably unchanged, but average microtubule length generally decreases. Labeled tubulin microinjection experiments show that the rate of microtubule turnover is dramatically increased (4,32). We do not know if there are changes in the intrinsic properties of the microtubule system, but there is usually a large increase in the number of microtubules, probably mediated by an increase in centrosome nucleating sites (41). It is interesting to consider whether the change in length and dynamics (n and Ω decrease) can be accounted for solely as a result

of the increase in N without changes in intrinsic parameters of the microtubule system, or whether we must also invoke changes in k, Ω_γ, or γ. Equation [11] shows that changes in N will affect the partitioning of c_T into c_P and c; changes in c will in turn affect both turnover rate, Ω, and n. c_P is shown as a function of N, with c_T held fixed at 20 μM in Fig. 4a, for $\gamma = 0, 1, 2,$ and 2.4. When $\gamma = 0$ (Eq. [12]), c_P increases hyperbolically with N, and asymptotes at $c_P = c_T$. This would be the case if depolymerization were initiated by some enzymatic process that was independent of the concentration of tubulin. For more realistic values of γ, c_P increases more slowly with N, but still asymptotes to c_T: Given sufficient nucleating sites, all the tubulin could be driven into polymer. At low values of N, there is an approximately linear relationship between N and c_P for all γ. A plot of both c_P and c versus N is given in Fig. 4b for $\gamma = 2.4$, illustrating that only at very high values of N is c relatively independent of N. When

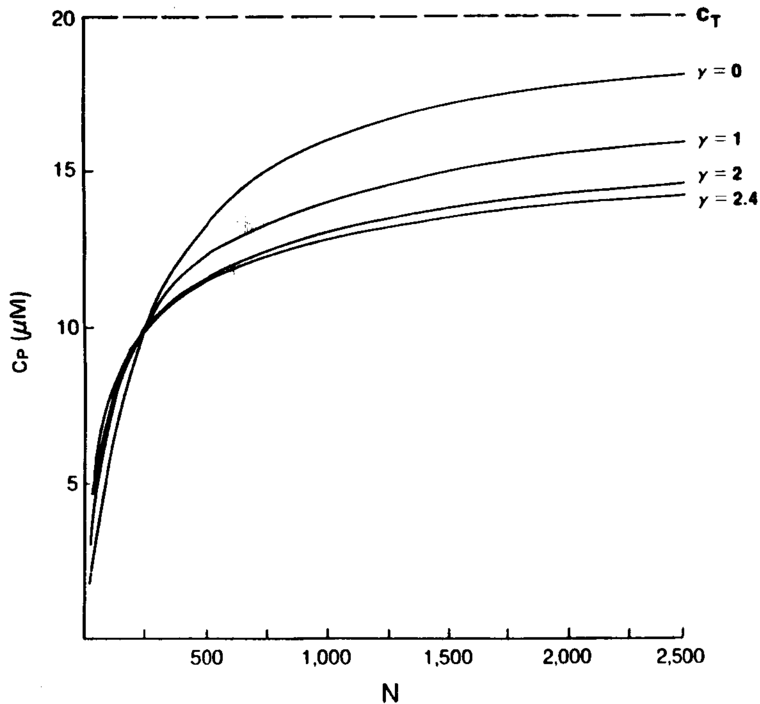
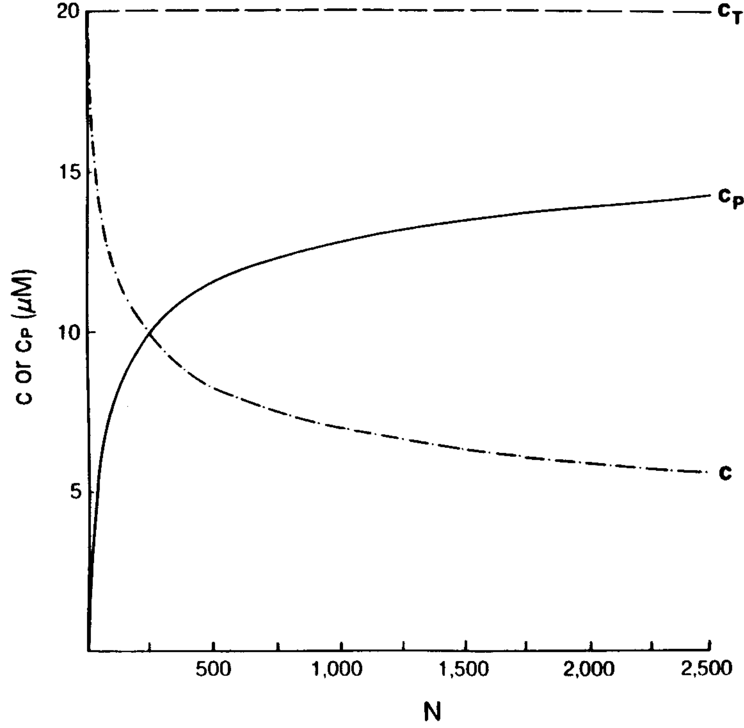


Fig. 4. In (a) (top) the polymer concentration, c_P, is plotted versus the number of nucleating sites, N, for various values of γ. The total concentration, c_T, is noted. In (b) (next page) both c and c_P are plotted versus N for $\gamma = 2.4$.



nucleation off centrosomes was studied in vitro, the amount of polymer formed was dependent on the number of centrosomes present, even below the apparent bulk critical concentration (22), confirming the view that this parameter is of little relevance when considering nucleated microtubules under dynamic instability conditions.

In Table 2 we calculate for some realistic values the effect of increasing or decreasing N on polymer mass, free monomer concentration, average polymer length, and half-time for turnover. As before, the parameters are initially set at the standard values of Table 1, and then N is varied. We have considered two cases, $\gamma = 0$ and $\gamma = 2.4$. If the number of nucleating sites is increased from 250 to 2000, which is approximately reasonable for the interphase-to-mitosis transition in animal cells, there is a corresponding increase in c_P and decrease in c for both cases. For $\gamma = 0$, the increase in c_P is greater, because there is no corresponding decrease in half-time with decreasing c. For $\gamma = 2.4$, the average length of the microtubule, n, decreases with increasing N due to, first, the effect of the decrease in c and, hence, the decrease in the growth rate, kc, and second, the increase in Ω (expressed as a decrease in half-time, $\frac{\ln 2}{\Omega}$) according to Eq. [9]. Increasing values of γ make n and half-time more sensitive to changes in N, although changes in c_P are damped.

TABLE 2
Effect of the Number of Nucleating Sites on the Dynamics of Microtubules and Their Extent of Assembly[a]

	$\gamma = 0$			$\gamma = 2.4$		
N	c_P	$t_{1/2}$	n	c_P	$t_{1/2}$	n
50	3.3	208	31	6.1	455	57
125	6.7	208	25.1	8.4	301	31.5
250	10.0	208	18.8	10.0	208	18.8
500	13.3	208	12.2	11.5	141	10.8
1000	16	208	7.5	12.8	92	6.0
2000	17.8	208	4.2	14.0	61	3.3

[a]We have assumed the same set of standard conditions with c_T = 20 μM and the other parameters listed in Table 1. The values of c_P are given in μM units, $t_{1/2}$ in seconds, and n in microns.

Can we explain the in vivo changes in dynamics using our simple model? At $\gamma = 2.4$, going from 250 to 2000 nucleating sites changes n from 19 to 3.3 μm, which may be a reasonable estimate of the change in vivo (mean microtubule length in mitosis is notoriously difficult to estimate). Half-time goes from 208 to 61 s, a very significant decrease, but considerably less than that experimentally observed (4). To account for the extremely rapid microtubule turnover at mitosis simply in terms of our model, we would need a larger value for γ, but this would lead to unrealistically short mitotic microtubules. In addition, the dynamic changes are accomplished by lowering c and decreasing growth rate, whereas in vivo growth rate probably, if anything, increases at mitosis (4,32). Thus, to fully account for the dynamic changes at the interphase-to-mitosis transition probably requires changes in the intrinsic properties of microtubules—for example, Ω_γ may increase, so microtubules become inherently more unstable by an unknown mechanism. In fact, Karsenti et al. (42) have presented evidence that microtubule assembly is inherently less favorable during mitosis in *Xenopus* eggs. However, we feel that simply increasing N alone is likely to be a significant factor in the dynamic changes that occur at the onset of mitosis.

QUALITATIVE EXTENSIONS OF THE MODEL

In real cells in interphase, the situation is certainly more complicated than our model, and under various circumstances the assumptions behind it may become invalid. Some possible considerations (not an exhaustive list) are given below, together with likely effects on the quantitative conclusions from the model. These examples were chosen because they seem likely to occur under some biologically relevant conditions.

(1) Many microtubules may reach the cell periphery before starting to shrink, and the transition could be caused by reaching this barrier. In this case, turnover would be strongly influenced by the size and geometry of the cell, but would become rather independent of intrinsic dynamics and Ω_T. Under these circumstances, there is an upper limit to n, and, thus, to c_P at a given N. As c_T increases, c_P will approach this limit, and further tubulin will have to remain as monomer. Thus, the system becomes less buffered than expected, and c_T is still specified by a unique value of c.

(2) Microtubules may not shrink at all the way back to the nucleating site. Borisy has termed this possibility "tempered instability" (pers. communication). In general, this will lead to some fraction of each microtubule being effectively nondynamic. The greater this fraction the more it resembles the situation described earlier for very long microtubules in vitro, where a unique critical concentration is produced, and c becomes completely buffered to changes in c_T.

(3) Subsidiary noncentrosomal nucleating sites may exist. If these are as efficient as centrosomal sites, they will simply contribute to N. However, they may be less efficient, so that they come into play only at higher values of c. For example, one could postulate N' extra sites, where N' is an increasing function of c. In general, this will tend to buffer c more than expected to an increase in c_T, although it will not lead to c becoming independent of c_T. Similarly, changes in c_P or $t_{1/2}$ in response to changes in N will be buffered more than expected. Karsenti et al. (*43*) studied the effect of removing the centrosome from fibroblast cells (decreasing N) on microtubule assembly. In rapidly growing cells, most of the microtubules were of a centrosomal origin, and removal of the centrosome caused a major decrease in polymer mass. In cells near confluence, subsidiary nucleating sites became prevalent; here, removal of the centrosome had a much smaller effect on polymer mass.

RETROSPECTIVE

Oosawa, Hill, and the Critical Concentration

The pioneering studies of Oosawa established an important fundamental theoretical understanding of the assembly of linear polymers (*13*). These concepts have been rigorously applicable to a number of systems

and describe in a general way the behavior of all linear polymers. The most powerful concept has been that of the critical concentration or equilibrium concentration, which is a measure of the dissociation constant of the monomer for the end of the polymer. Despite the evidence over the past 15 years that microtubules are not in equilibrium and that they consume GTP during assembly, the overall model of Oosawa has still been applied in most studies. Measurement of polymer mass or monomer concentration may not be particularly sensitive in distinguishing the behavior of polymers that reach true equilibrium from the behavior of polymers that display other extreme nonequilibrium properties—see, for example, Fig. 3b and 3c. Nevertheless, the differences could be very significant for processes, such as the regulation of tubulin biosynthesis, or the onset of mitosis.

There has been an understandable reluctance to abandon rigorous physical–chemical models even in the face of conflicting data. It is for that reason that experimentalists have been so indebted to theoreticians like Oosawa and Hill. The understanding of the nonequilibrium nature of microtubule assembly depended on the development of believable and rigorous theoretical models as much as on the experimental results. Wegner provided the first theoretical suggestion of the role of ATP hydrolysis in actin assembly (*15*). This modified the concept of critical concentration as a purely equilibrium property, but did not lead to its abandonment. Hill generalized these results and coupled the thermodynamics to the kinetics and showed many ways in which treadmilling could be made to do work or have a biological function (*17–19*). The discovery of an apparent phase transition in microtubule assembly was anticipated by attempts by Hill theoretically to understand the effect of unhydrolyzed GTP at the end of the polymer. Finally, dynamic instability would not have been proposed without the stochastic ideas about GTP caps, and it would not have been considered so seriously had it not been proven theoretically feasible as well as experimentally demonstrable by Hill, Carlier, and their colleagues. However, this congruence of theory and experiment does not prove the existence of GTP caps—that must await direct experimental testing.

Following the clear demonstration of the stochastic behavior of individual microtubules in solution, the concept of a critical concentration that would determine the behavior of each microtubule had to be abandoned. However, this did not cause the abandonment of the critical concentration as a bulk parameter, namely, a monomer concentration toward what the bulk microtubule system would tend. In this paper we have shown that the critical concentration is not fixed when nucleating sites are present. Even for the study of bulk properties there is little value in retaining the concept of the critical concentration. For the microscopic behavior of individual polymers the concept of a critical concentration is virtually without meaning.

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