

Beyond Self-Assembly: From Microtubules to Morphogenesis

Review

Marc Kirschner and Tim Mitchison*

Department of Biochemistry and Biophysics
University of California
San Francisco, California 94143

Introduction

The goal of modern cell biology is to explain the structure and behavior of the whole cell in terms of the biochemical properties of its individual components. This has been strikingly successful for many cellular components to which the principles of enzymology and self-assembly have been directly applicable. However, larger, more complex systems still pose formidable problems. In this respect, a major remaining challenge is the satisfactory biochemical description of cell morphology and the related problems of cell movement, cell division, and cell differentiation. Until recently, such a description seemed beyond reach, since the morphology of cells was thought to depend on an intractably large number of specific interactions.

A breakthrough in our understanding of cell morphology came in the 1970s, when it was first generally appreciated that cells contained extensive arrays of linear polymers that could span great distances and were perhaps responsible for organizing and integrating cell behavior (Goldman et al., 1976). Since these polymers were rather simple structures, one could hope to develop general principles of cell morphology, not by studying specific short-range interactions peculiar to individual cell types, but by studying ubiquitous simple polymers made up of repeated units of globular proteins. The three major cytoplasmic polymers—microtubules, intermediate filaments, and actin filaments—can assemble spontaneously from subunits to give long uniform polymers held together by noncovalent interactions. Polymerization follows the principle of self-assembly, which was established from the study of proteins and viruses. This principle directly connects the linear information in the genome with the structure of complex three-dimensional components of cells (Fraenkel-Conrat and Williams, 1955; King, 1980; Inoue, 1982). In this review we shall consider the application and extension of this principle, not just to the assembly but also to the organization of microtubules in cells.

The difficulty in extending the simple picture of self-assembly to the general question of cell morphology or even to the specific question of the microtubule arrangement in cells is illustrated in Figure 1. Here, a monolayer of fibroblast cells in culture has been wounded by making a scratch across the dish and their microtubules visualized by immunofluorescence. The cells at the boundary of the wound respond by changing their shape and migrating into the empty space. Despite the overall similarity of the

response in all cells, careful examination of the microtubule distributions shows each cell to be different. Given this complexity in the detailed structure of the microtubule arrays, it is hard to imagine how we would ever be able to predict from simple principles the specific organization of microtubules in a single cell. Fortunately, our goal as cell biologists is not necessarily to predict a specific microtubule distribution, but to understand the rules that govern the overall organization of microtubules and how this organization solves a functional problem, such as the production of directed cell movement. In the case of the wounded monolayer experiment, we note that the symmetric distribution of microtubules of cells in the interior of the cell group is transformed into an array polarized towards the wound in cells at the margin. This polarization of the microtubule cytoskeleton precedes a functional polarization of the cell that results in directed cell movement (Gottlieb et al., 1981). It is not the goal of this review to explain directed cell movement. However, if we can begin to understand how the wounding stimulus generates a functional polarization of the microtubules, we will be close to solving one of the most difficult problems in cell biology—that of overall cellular organization.

In this review we approach the problem of the spatial organization of microtubules by considering the biochemical properties of the microtubule polymers themselves. We first discuss what can be achieved using the properties of simple linear polymers driven to equilibrium by self-assembly. Next we consider the complications of the GTP hydrolysis activity of tubulin and what new advantages could accrue from operating at steady state. We then review new experimental results on microtubule dynamics *in vitro* and *in vivo* and consider the consequences of these dynamics for morphogenesis. In the end, we examine what has been added to the simple rules of self-assembly and how far we have progressed towards explaining the morphogenesis of the microtubule cytoskeleton.

Microtubules Resemble Equilibrium Polymers

Microtubules are formed by the polymerization of tubulin monomers (reviewed by Kirschner, 1978; Timasheff and Grisham, 1980; McKiethan and Rosenbaum, 1984; Dustin, 1984; Purich and Kristofferson, 1984). Tubulin polymerization has much in common with the assembly of other large molecular aggregates such as viruses, detergent micelles, or even simple salt crystals. When assembly is followed by a parameter that reflects the polymer level, such as turbidity or viscosity, there is first a lag phase when no microtubules form, then a phase of exponential growth, and finally a stable plateau (Figure 2A). A plot of the polymer concentration in the plateau region versus the total concentration of tubulin produces the pattern in Figure 2B. At low concentrations, no microtubules are formed; above a certain concentration (the critical concentration, C_c), microtubules increase proportionate-

*Present address: Division of Virology, National Institute for Medical Research, London N.W. 7, England

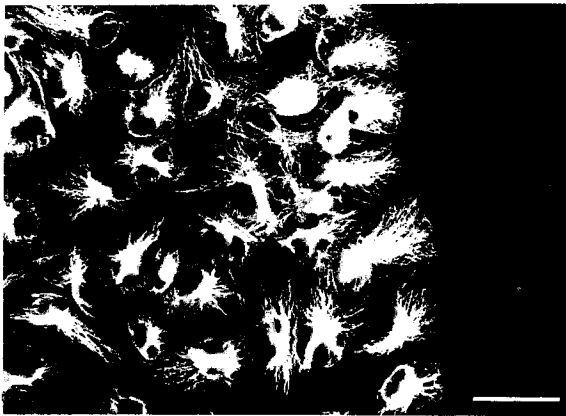


Figure 1. Microtubule Arrays in a Wounded Monolayer of Fibroblast Cells

BSC-1 cells grown to confluence were scraped with a glass needle, fixed 20 min later, and stained with mouse anti-tubulin IgG and rhodamine anti-mouse IgG according to the procedures of Schulze and Kirschner (1986). Note the randomly oriented microtubule arrays in the interior of the cell group and the polarized arrays in cells at the margin. Bar represents 50 μm .

ly to the total tubulin concentration and behave as though they are in equilibrium with a pool of unassembled monomer equal to C_c . Proteins that polymerize to a true equilibrium will be referred to as "equilibrium polymers."

For microtubules in solution, the mechanism of nucleation during the lag phase is complicated and not relevant to the *in vivo* situation, where assembly is usually initiated from organizing centers such as centrosomes (McIntosh, 1983). The elongation reaction for several linear polymers, however, is a much simpler reaction (Oosawa and Asakura, 1975). The simplicity results from the assumptions that subunits add only at the polymer's end and that a polymer of $n + 1$ subunits behaves exactly the same as one of n subunits. Considering only one end of the polymer, subunits add at a rate (α), which is proportional to the monomer concentration (c), and come off at a rate (α'), which is independent of the monomer concentration. This is illustrated in Figure 2C. Thus, the net rate of assembly at one end of a polymer (dn/dt) is:

$$dn/dt = \alpha c - \alpha' \quad (1)$$

At equilibrium, the net rate of assembly is zero. This again defines the critical concentration, C_c , which from the above equation is:

$$dn/dt = 0, \text{ and } C_c = \alpha'/\alpha = K_{\text{diss}} \quad (2)$$

The critical concentration is therefore equal to the rate constant for disassembly divided by the rate constant for assembly, and is formally the dissociation constant for reversible subunit addition to the end of a polymer.

Microtubules are not true equilibrium polymers since they hydrolyze GTP. If they were, they might not be able to fulfill all their biological roles. Eqs. (1) and (2) put important limitations on the behavior of a linear polymer. First, the rate of assembly is relatively insensitive to monomer

concentration. Second, even the maximum rate of disassembly (α') at zero free monomer concentration could be too slow for some cellular processes, since α' is constrained by values of α and K_{diss} . For microtubules, α is near the diffusion-controlled limit and K_{diss} must be small enough to give reasonable levels of polymer mass, which in a cell would be about 2.5 μM (Hiller and Weber, 1978). These values would give $\alpha' = 12.5 \text{ sec}^{-1}$, or about 0.5 $\mu\text{m}/\text{min}$. In a *Xenopus* egg, however, microtubules of the sperm aster, which are 200 to 500 μm long, must depolymerize within 10 min (Ubbels et al., 1983). Third, polymers that come to true equilibrium would be rather static structures, exhibiting only diffusional exchange of subunits at both polymer ends.

The Complication of GTP Hydrolysis

The description of microtubules as equilibrium polymers is incorrect because tubulin is an enzyme that binds and hydrolyzes GTP to GDP during assembly. Pure tubulin hydrolyzes one mole of reversibly-bound GTP concomitantly with assembly (David-Pfeuty et al., 1977). (Another mole of GTP is bound irreversibly at a separate site and is not hydrolyzed in the assembly reaction [Spiegelman et al., 1977].) After assembly, GDP on the polymer no longer exchanges with GTP in solution (Kobayashi and Simizu, 1976; Weisenberg et al., 1976). The plateau of Figure 2A is therefore not a true equilibrium state but a steady state, since there is continual GTP hydrolysis as monomer units assemble and disassemble. If all the GTP were exhausted, the system would come to equilibrium, but under these circumstances the microtubules would disassemble (Lee et al., 1982). A similar situation holds for actin (Pollard, 1984).

In reality, there is no energy requirement for assembly of tubulin and actin, since microtubules and actin filaments form when nonhydrolyzable analogues substitute for GTP and ATP (Arai and Kaziro, 1976; Penningroth et al., 1976; Cooke and Murdoch, 1973). Therefore, the following paradox exists: GTP binding is required for microtubule assembly, GTP hydrolysis accompanies assembly, but GTP hydrolysis is not required for assembly.

In theory, the coupling of assembly to GTP hydrolysis relieves the obligatory relationship between α , α' , and the K_{diss} for an equilibrium polymer (eq. 2). Historically, the first implication considered was the ability to have a different C_c at each end of the polymer. As discussed by Wegner (1976), this could lead to net polymerization at one end of the polymer balanced by net depolymerization at the other end—a process called treadmilling (reviewed by Margolis and Wilson, 1981). Microtubule treadmilling has been reported (Margolis and Wilson, 1978; Rothwell et al., 1985), but it does not seem to be a major source of subunit flux for pure tubulin assembled to steady state (Kristoffer-son et al., 1986). As discussed below, treadmilling may be an interesting special case of the nonequilibrium behavior of microtubules.

In the most general sense, the introduction of GTP hydrolysis into the assembly reaction potentially endows the system with some interesting properties. Instead of a sin-

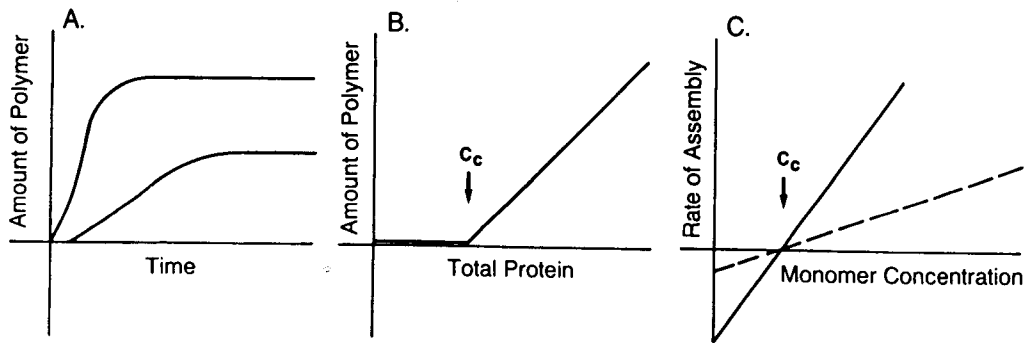


Figure 2. Properties of Tubulin Polymerization

(A) A plot of the amount of polymer formed versus time at two concentrations of protein. The parameters usually measured are turbidity, viscosity, or polymer mass by sedimentation. (B) A plot of the total amount of polymer at equilibrium versus the total amount of protein. This plot is generated by measuring the plateau values in (A) at various initial protein concentrations. Below the critical concentration, C_c , no polymer forms. (C) A plot of the net rate of assembly–disassembly versus concentration of monomer for elongation of an equilibrium polymer. The two lines (solid and dashed) represent curves for the two ends of the polymer. Both intersect at the same critical concentration C_c .

gle reversible reaction at a microtubule end, there are now at least three separate reactions to consider: the addition and loss of GTP-tubulin, the conversion of GTP-tubulin to GDP-tubulin, and the addition and loss of GDP-tubulin. Since an essentially irreversible step (GTP hydrolysis) separates the assembly–disassembly reactions, these reactions could in principle be regulated independently. To understand how important this segregation of assembly and disassembly could be for the cell, let us consider a simple heuristic model (Figure 3). We consider two types of assembly–disassembly events, separated in time. In the first, GTP-tubulin assembles rapidly to form stable microtubules with strong intersubunit bonds. This reaction, governed by eq. (2), is essentially unidirectional. It has a fast on-rate and a slow off-rate; hence, microtubules grow rapidly and subunits are used efficiently. Later, should the cell need to remodel its cytoskeleton, it could trigger GTP hydrolysis. This would cause a conformational change in all subunits that would destabilize intersubunit bonds. The GDP-tubulin would dissociate rapidly from the microtubule end—again, an essentially irreversible reaction. The advantages of this model over the equilibrium situation are that, here, the on-rate is limited in principle only by the rate of diffusion of the subunit to the polymer, the off-rate can be extremely rapid, and the amount of polymer formed can be regulated independently of the on- and off-rates by the hydrolysis of GTP. In the remainder of this review, we see how the cell can come close to realizing these theoretical advantages using real mechanisms.

Evidence for Different Assembly and Disassembly Reactions in Microtubules

Although attempts were made to show that microtubule assembly was a simple equilibrium between subunits and polymer, comparison of the work from several laboratories revealed an incompatibility of the off-rates, on-rates, and C_c , as demanded by eq. (2). Most results suggested that for tubulin plus associated proteins, the on-rate constant

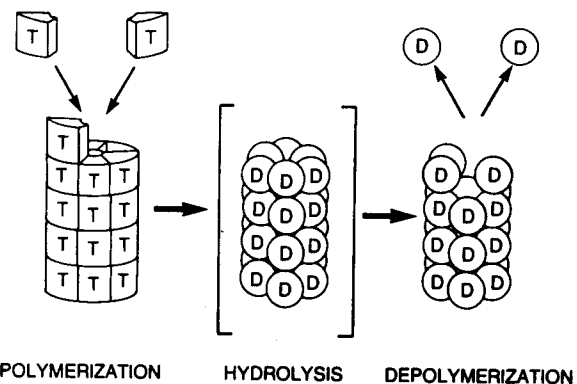


Figure 3. Heuristic Model for Microtubule Assembly

Subunits liganded with GTP, denoted by T, are shown in a conformation having strong intersubunit bonds, drawn as cylindrical sectors. After hydrolysis to the GDP form, denoted by D, the subunits undergo conformational change to a structure with weak intersubunit bonds, shown as spheres. The unstable GDP-containing structure shown in brackets depolymerizes from the end of the microtubule, as shown on the right.

was $1-7 \times 10^6 \text{ M}^{-1}\text{sec}^{-1}$, and C_c was about $2-5 \times 10^{-6} \text{ M}$ (Johnson and Borisy, 1977; Summers and Kirschner, 1979; Bergen and Borisy, 1980; Mitchison and Kirschner, 1984b); this would require an off-rate constant of about 10 sec^{-1} . Direct experimental determination of off-rates usually gave values of about 150 sec^{-1} (Karr et al., 1980; Zeeburg et al., 1980). When Carlier et al. (1984) and Farrell et al. (1983) reexamined the entire plot of dn/dt versus c to see if it was linear, as predicted by eq. (1), they found a sharp downward slope below C_c (Figure 4). These results indicated that the assembly reaction could not be a simple equilibrium.

An explanation for this unusual phenomenon came from earlier observations of Carlier and Pantaloni (1981), and from theoretical studies of Hill and Carlier (1983). While it was previously assumed that GTP hydrolysis was so rapid that the polymer consisted entirely of GDP-tubulin, these authors showed that at very high rates of

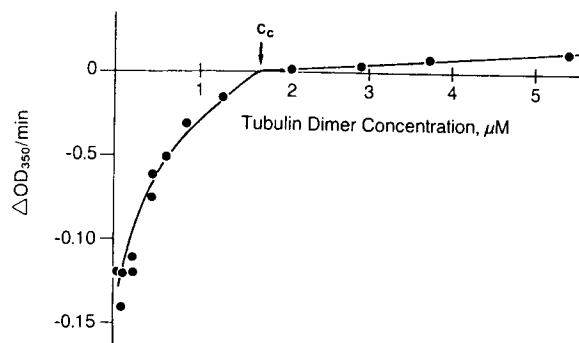


Figure 4. Effect of Tubulin Dimer Concentration on Microtubule Assembly-Disassembly

The data, redrawn from Carlier et al. (1984), show the net rate of assembly-disassembly, measured as change in turbidity per min, versus the tubulin dimer concentration.

assembly (attained at high tubulin concentration), the hydrolysis rate is slower than the assembly rate. This produces a chimeric polymer with stretches of unhydrolyzed GTP at the ends and GDP in the interior. This means that growing polymers could be different from shrinking polymers. Growing polymers have some GTP subunits at their ends. Shrinking polymers quickly lose their "GTP cap" and expose GDP subunits at the ends, so that their disassembly is governed by the fast off-rate of GDP-tubulin. Hence, in this model microtubules exist in two phases: a growing phase (occurring above C_c), in which both ends of the polymer have GTP caps, and a shrinking phase (occurring below C_c), in which both ends of the polymer consist of GDP-tubulin. In Figure 4, C_c would represent a true phase transition in a thermodynamic sense. A real innovation of the GTP cap model is that the stability of the entire polymer could be controlled by the presence of GTP-tubulin at the microtubule ends.

Behavior of Individual Microtubules in Solution

In previous studies of microtubule assembly, the behavior of individual microtubules was inferred from the average properties of the bulk population, monitored by methods such as viscometry, turbidity, and sedimentation. To analyze the growth of microtubules from purified centrosomes, we examined individual microtubules using light microscopic immunocytochemistry and electron microscopy (Mitchison and Kirschner, 1984a,b). At relatively high concentrations of pure tubulin, microtubules grow steadily. Surprisingly, when the concentration of tubulin is reduced to below C_c , some microtubules continue to grow while others appear to depolymerize quickly from the free end. The fraction of microtubules that depolymerize per unit time is dependent on the concentration. When microtubule fragments are used as seeds to initiate assembly, tubulin polymerization proceeds to a plateau, as shown in Figure 5. The total polymer mass remains constant at the plateau. However, the microtubule population in this region does not consist of a fixed number of

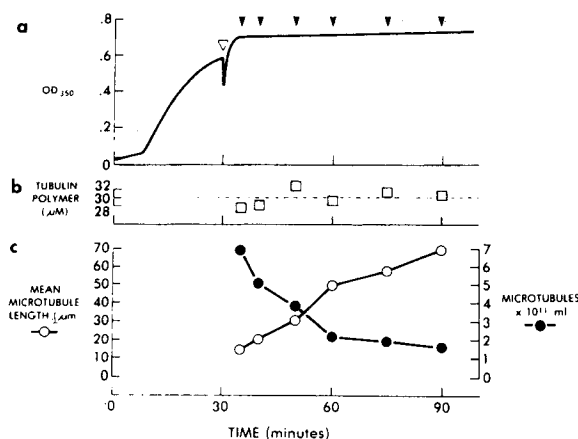


Figure 5. Length Redistribution of Microtubules at Steady State

(a) shows the assembly of microtubules from pure tubulin as monitored by turbidity. Microtubule seeds are added to initiate the reaction. At the time shown by the open triangle, microtubules are sheared to hasten the approach to steady state. At the times indicated by arrows, the population is sampled, fixed, and examined for length and number by anti-tubulin immunofluorescence; (b) shows the concentration of tubulin polymer, obtained by multiplying the average length by the average number; (c) shows the average length (O) and average number (●) of the microtubules. (Data redrawn from Mitchison and Kirschner [1984a].)

microtubules having the same average length; rather, the number of microtubules declines steadily, and their average length increases steadily. Thus, turbidity measurements accurately reflect the constancy of polymer mass but conceal dramatic microtubule dynamics. Microtubules thus appear to exist in two populations: the majority growing at an appreciable rate and a minority shrinking very rapidly to provide new subunits for growth. Since long microtubules disappear completely, depolymerization and growth must be very persistent, with only a small probability that a shrinking microtubule will revert to growth and vice versa. Recent studies show that persistent growth at constant polymer mass is a property of both ends of a microtubule (Kristofferson et al., 1986).

Separate growing and shrinking phases of microtubules in the same population, with rather infrequent transitions between them, has been called "dynamic instability." At present, the most reasonable model for dynamic instability is based on the GTP cap, as outlined in Figure 6. Growing microtubules are thought to have a cap of tubulin subunits containing unhydrolyzed GTP. Since the GTP hydrolysis rate per subunit in the polymer is independent of the concentration of tubulin, whereas the rate of assembly is proportional to the concentration, the size of the cap increases with the concentration of tubulin (or the rate of assembly). At any concentration, the size of the cap fluctuates in a stochastic manner. The faster the growth rate, the larger the GTP cap, and the lower the probability of the cap disappearing and the microtubule depolymerizing. When the cap disappears (or falls below some minimum size), GDP-containing subunits are exposed and the polymer enters a rapid depolymerizing phase. Since the

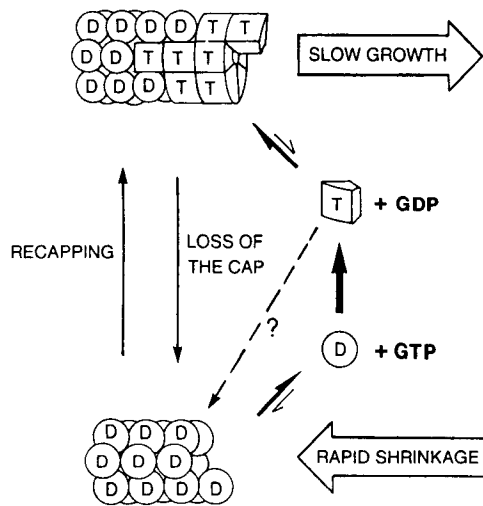


Figure 6. GTP Cap Model for Dynamic Instability
Microtubules with a cap of GTP-containing subunits, denoted by T, are shown growing slowly by the addition of T-containing subunits. The free T subunits are shown to be in equilibrium with the T-containing end, with a very low K_{diss} . At some probability, GTP hydrolysis catches up with assembly, the T cap disappears, and the polymer transits to the "rapid shrinkage" phase (all D) shown at the bottom. This polymer rapidly loses subunits from its end (high K_{diss}). The GDP-containing subunits that are released, denoted by D, exchange with free GTP in solution and form T subunits. It is not known whether T subunits can add to a depolymerizing end to recap the shrinking polymer.

microtubule contains thirteen parallel protofilaments with many possible structures at each end, it is not clear how many GDP- or GTP-containing subunits at the end are required for stability. In addition, the rate of GTP hydrolysis probably would depend on whether the neighboring subunits were in the GTP- or GDP-form. The effect of these neighboring interactions has recently been stressed in regard to actin polymerization (Pantaloni et al., 1985).

Figure 7 connects the behavior of individual microtubules to that of the bulk population, to give a plot of assembly rate against monomer concentration for microtubules showing dynamic instability (Hill and Chen, 1984). This should be compared to Figures 2 and 5. The behavior of the bulk population is complex and requires a reassessment of the use of the term "critical concentration." For an equilibrium polymer, a single critical concentration governs the behavior of both ends of individual microtubules and the behavior of the population. Treadmilling introduced the idea of separate critical concentrations for the two ends of the microtubule, and a bulk critical concentration, which is an appropriately weighted average of the two (Kirschner, 1980; Hill and Kirschner, 1983). With dynamic instability, there are several "critical concentrations": For each end of the microtubule, there is the (low) critical concentration of the GTP phase, and the (high) critical concentration of the GDP phase, corresponding to where the dotted lines in Figure 7 intersect or would intersect the X axis. In addition, there is the critical concentration for the bulk population, denoted as C_c in this figure. This represents an appropriately weighted average of the

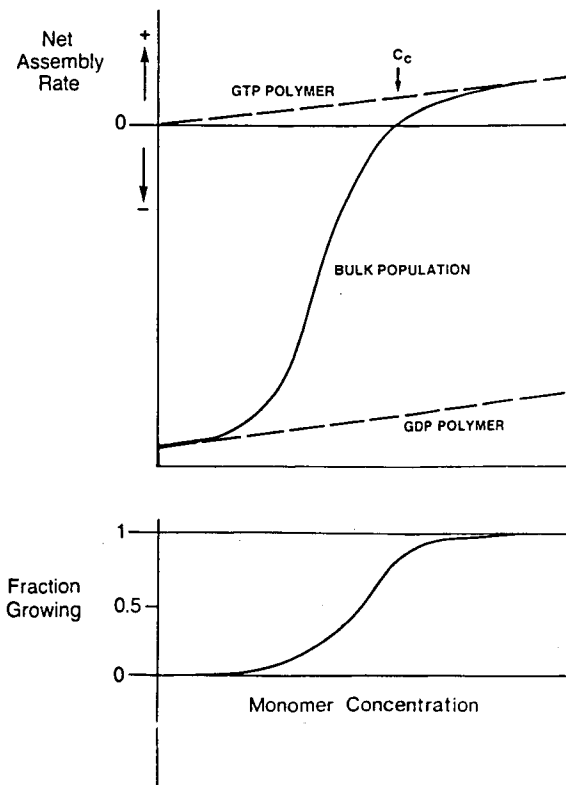


Figure 7. Population Behavior of Microtubules
The assembly rate of microtubules is shown as a function of concentration. The behavior of microtubules with a GTP cap (labeled GTP polymer) and the behavior of microtubules without a GDP cap (labeled GDP polymer) are shown with dashed lines. The behavior of the bulk population is shown as a solid line. The bulk behavior is calculated as the fraction of microtubules in the GTP phase (shown in the lower panel) times their net rate of polymerization, minus the fraction of microtubules in the GDP phase times their net rate of depolymerization.

parameters of the individual ends. It is still the concentration below which free microtubules are ultimately unstable. To avoid ambiguity in the rest of this review, we will use the term critical concentration to refer only to this bulk C_c .

The major characteristic that distinguishes dynamic instability from other models for polymer assembly is the coexistence, over a wide concentration range, of growing and shrinking phases that interconvert infrequently. This produces two essentially separate populations of polymers. The transition rate between the two phases is governed by the probability of a growing microtubule starting to shrink and vice versa. These transition probabilities are the most important parameters governing the overall behavior of the system. The probability of a growing microtubule starting to shrink is thought to be governed by the stochastic loss of the fluctuating GTP cap. This would be promoted by agents that retard growth, such as capping factors or physical barriers to elongation. The rapid depolymerization of GDP-liganded microtubules could in principle be reversed by reconstitution of the GTP

cap caused by an encounter with GTP-containing subunits in the interior of the polymer, the exchange of GTP in solution with GDP at the polymer terminus, or the binding of a GTP-containing tubulin subunit from solution to an exposed GDP end. With pure tubulin in aqueous buffers, all of these reactions appear to have a low probability, and hence microtubules frequently depolymerize to completion. Conditions that stabilize the polymer along its length (as opposed to end stabilization) and reduce the depolymerization rate would be expected to increase the probability of recapping, damping out length fluctuations at steady state. This may explain why length fluctuations are less dramatic for tubulin in glycerol buffer (Kristofferson et al., 1986), and for pure tubulin containing microtubule-associated proteins (MAPs) in aqueous buffer (Kristofferson and Purich, 1981). Under sufficiently stabilizing conditions, microtubules should oscillate around some average length, and there might be an observable net addition of subunits on one end and net loss on the other end (treadmilling).

Can the GTP cap model really generate dynamic instability? In a series of theoretical papers, Hill and Chen have tested the model and shown that, with a reasonable set of parameters, microtubules can grow and shrink in the same population and generate the distribution of microtubule lengths found experimentally (Hill and Chen, 1984; Chen and Hill, 1985a; Chen and Hill, 1985b; Hill, 1984). Although there is no direct evidence for the role of GTP in microtubule stabilization, indirect evidence for the increased stability of the GTP polymer comes from the stabilizing effects of nonhydrolyzable analogues of GTP (Weisenberg and Deery, 1976; Penningroth and Kirschner, 1977). The existence of GTP caps on growing microtubules at steady state has yet to be demonstrated, so at present the GTP cap explanation of dynamic instability must be considered a reasonable hypothesis.

Are there any alternatives to the GTP cap model to explain dynamic instability? There is strong evidence that growing and shrinking microtubules coexist, and it seems inescapable that these must represent structurally different polymers. Job et al. (1985) have suggested that individual microtubules in a population could bind MAPs to differing extents at substoichiometric MAP concentrations. Although this could generate microtubules of differing stability, it is hard to see how it could account for continuous redistribution at steady state. Furthermore, the existence of dynamic instability in highly purified tubulin preparations containing no measurable MAPs argues against MAP redistribution as a cause of dynamic instability (Kristofferson and Kirschner, unpublished).

It is conceivable that growing and shrinking microtubules could differ by some structural feature other than the GTP cap. For example, hydrolysis could be rapid, but the protein could undergo a slow conformational change after hydrolysis. In this case, hydrolysis would be a prerequisite for the loss of the conformational cap. In an equilibrium polymer, it is also possible for the conformation at the end to fluctuate or for the end to be transiently capped by some other molecule. Without input of energy, however, it

is impossible on thermodynamic grounds for persistently growing and shrinking polymers to coexist at steady state.

Dynamics of Microtubules In Vivo

For many years, the in vivo behavior of microtubules was better understood than their in vitro behavior. This was primarily because the high density and favorable geometry of the microtubules in the mitotic spindle allowed their assembly to be monitored by polarization microscopy in living cells (Inoue, 1953; 1964). These studies showed that drugs and physical parameters could induce rapid changes in spindle mass. To account for these rapid perturbations, Inoue and Ritter (1975) proposed a cylindrical micelle model whereby subunits could be inserted or withdrawn anywhere along the microtubule. Such a model could account for the extremely rapid dynamics in vivo, but was irreconcilable with in vitro models that restricted reactions to the ends of the microtubule. Salmon et al. (1984b) also suggested that extremely rapid depolymerization of microtubules induced by microinjection of colchicine into sea urchin eggs might require multiple sites of disassembly along microtubules.

Recently, microtubule dynamics in vivo has been studied by microinjection of fluorescently labeled tubulin into living cells (Keith et al., 1981). McIntosh, Salmon, and their colleagues used this technique to measure the rate of tubulin incorporation into the spindle (Salmon et al., 1984a, Saxton et al., 1984). After the fluorescent tubulin equilibrated with the microtubules, laser photobleaching was used to measure the rate of exchange of tubulin into microtubules. These methods showed a half-life of exchange of 18 sec in the sea urchin spindle and 13 sec in spindles in cultured mammalian cells. The half-life of exchange in the interphase cytoskeleton of cultured cells was found by similar techniques to be about 4.5 min. Although this was much slower than the rates in the spindle, many of the microtubules in these cells are 20 μm long or longer. Such rapid rates of exchange could not be accommodated by diffusional exchange at equilibrium or by treadmilling.

Two interrelated questions are not addressed by conventional polymer assembly models: 1) How can microtubules shrink so quickly in response to perturbations, yet maintain an appreciable mass of polymer at steady state? and 2) How does the monomer pool exchange with polymer so rapidly? Both these problems can be satisfactorily explained by dynamic instability if it is assumed that microtubules in vivo grow out from the centrosome, elongate, and then shrink back suddenly.

A real test of the predictions of dynamic instability would require visualization of the mode of subunit incorporation into individual microtubules, which so far exceeds the resolution of the fluorescent photobleaching experiments. Recently, two methods of pulse labeling and static imagery have given a more detailed picture of individual microtubule dynamics in interphase and mitotic cells.

Soltys and Borisy (1985) injected fluorescein-labeled tubulin into interphase fibroblast cells, visualized its incor-

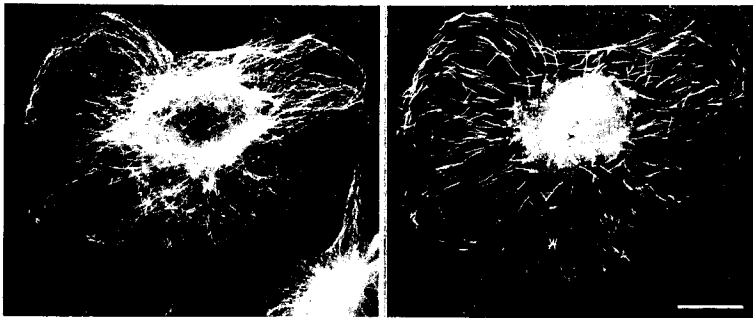


Figure 8. Injection of Biotinylated Tubulin into BSC-1 Fibroblasts

Cells were injected 50 sec before fixation and staining. Left: the total microtubule distribution visualized with antibody to tubulin. Right: biotin-tubulin visualized with antibody to biotin. Bar represents 20 μm .

poration into polymer using an anti-fluorescein antibody, and visualized the total polymer distribution with an anti-tubulin antibody. Since the labeled tubulin was incorporated only at microtubule ends and at the centrosome, these authors concluded that subunits were not likely to add along the polymer wall. In a similar experiment with biotin-labeled tubulin, Schulze and Kirschner (1986) provided a quantitative analysis of dynamics at the single microtubule level. Microtubules grew out continuously from the centrosome at the very rapid rate of 3.7 $\mu\text{m}/\text{min}$. At short times, this leads to a striking image of labeled microtubule segments shown in Figure 8. Unlabeled microtubules disappeared with a half-life of less than 10 min. Thus, there is a continuous cycle of microtubule growth out to the periphery, followed by depolymerization. Mitchison et al. (1986) obtained similar results for astral microtubules in mitotic cells, except that the rate of microtubule turnover was much faster (less than 30 sec). Such patterns of subunit incorporation are in complete agreement with the predictions of dynamic instability and are totally contrary to the predictions of treadmilling or diffusional exchange models. Interestingly, a few microtubules in interphase cells were inert to exchange over 1–2 hr; these must somehow be stabilized and segregated from the dynamic pool (Schulze and Kirschner, 1986).

Biological Consequences of Dynamic Instability

Rapid Depolymerization of Microtubules. GTP hydrolysis weakens the intersubunit bonds in a stable polymer to allow rapid disassembly. Cells require rapid microtubule depolymerization in order to adapt to changing conditions. For an equilibrium polymer, such rates of disassembly would require a very high K_{diss} , which would lead to very low levels of polymer since the on-rate constant cannot exceed about $10^7 \text{ M}^{-1} \text{ sec}^{-1}$ due to the limitations of diffusion. Dynamic instability allows both high turnover and high levels of polymer.

Coexistence of Shrinking and Growing Microtubules. A unique feature of the dynamic instability model is the segregation of polymer into growing and shrinking phases that coexist over a wide range of monomer concentrations. Cells may frequently require one set of microtubules to grow while another, perhaps spatially intermingled, set

shrinks—for example, during anaphase, when astral and midzone microtubules elongate while kinetochore microtubules shorten (Nicklas, 1971). Although the dynamic instability model does not explain what regulates these subsets (a point discussed later), it does provide a thermodynamic basis for the coexistence of shrinking and growing microtubule populations.

Sensitivity to Tubulin Concentration. Dynamic instability allows the net extent of assembly to be very sensitive to assembly conditions. In order to drive an equilibrium polymer from extensive polymerization to extensive depolymerization, a massive decrease in monomer concentration would be required. For a polymer showing dynamic instability, however, a small change in monomer concentration can produce a large change in polymer mass (Figure 7). This is true because it is not only the on-rate that is affected, but also the size of the GTP cap, and hence the proportion of polymers in the growing phase. Such subtle regulation of polymer mass occurs in the interphase mitotic conversion in *Xenopus* eggs (Karsenti et al., 1984).

Growth below the Critical Concentration. With dynamic instability, the term “critical concentration” loses the special significance it had for equilibrium polymers. The bulk critical concentration is that concentration at which subunit addition into growing microtubules is exactly balanced by subunit loss from shrinking microtubules. This concentration has little significance for an individual microtubule since, provided it retains its GTP cap, an individual microtubule can grow extensively below the critical concentration. Persistent growth below the critical concentration has been observed off centrosomes and axonemes in vitro (Mitchison and Kirschner, 1984a,b).

Predominance of Nucleated Microtubules in the Cell. In many cells, most if not all of the microtubules arise from the centrosomes and grow towards the cell periphery with uniform polarity (Euteneuer and McIntosh, 1981), a configuration probably important for organizing intracellular transport. Even though these microtubules are dynamic, the overall distribution is quite stable. What preserves this distribution and prevents spontaneous and disorganized microtubule assembly? DeBrabander et al. (1981) showed that artificially produced random polymerization will revert in a few minutes to the usual centrosomally focused

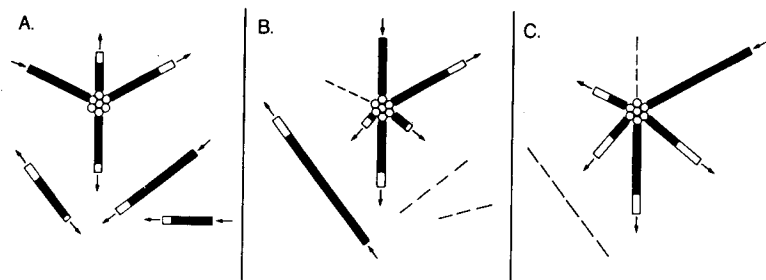


Figure 9. The Persistence of Nucleated Microtubules below C_c

(A) shows microtubules nucleated from a centrosome and free microtubules grown above C_c . The direction of the arrows show whether the microtubules are growing or shrinking. The unfilled microtubule segments signify the GTP cap. In (B), the concentration of tubulin has dropped below C_c . Some microtubules have completely depolymerized and are shown as dashed lines. (C) shows a later time. Further microtubule depolymerization has occurred and no free microtubules are left. However, new nucleation of unstable microtubules continues on the centrosome.

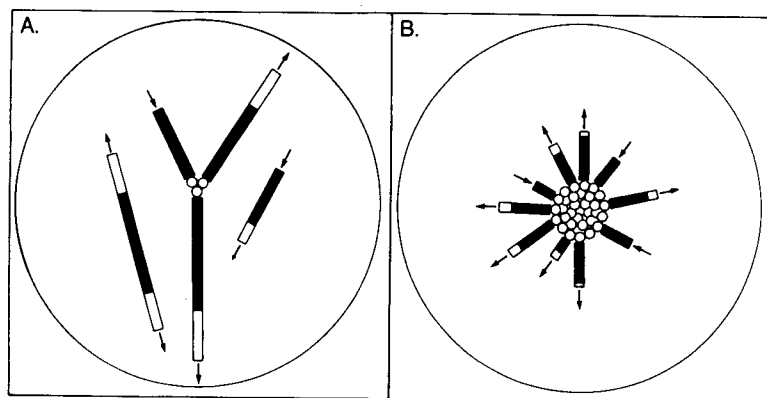


Figure 10. Nucleated Assembly in a Finite Cell Volume

(A) shows a cell with a small number of nucleation sites and excess tubulin. Spontaneous assembly is possible and free microtubules coexist with nucleated ones. At this high concentration of tubulin, GTP caps (shown as unfilled rectangles) are relatively large, and microtubule growth is persistent, producing long microtubules. In (B), there has been a large increase in the number of nucleating sites in a cell with the same volume and content of tubulin. The increased nucleation increases both the number of microtubules and the total polymer mass, necessitating a decrease in the free tubulin concentration. This is reflected in shorter GTP caps, more frequent transitions to depolymerization phase, and, as a result, shorter microtubules.

array. Clearly, the cell has a means for selective retention of centrosomal microtubules. One explanation for this selective retention is based on the existence of different critical concentrations at the two ends of the microtubule (Kirschner, 1980). Dynamic instability offers another, perhaps more generally applicable, explanation. As shown in Figure 9, the effects of dynamic instability are different for free and centrosomal microtubules. When a free microtubule depolymerizes below C_c , it is gone, whereas when a centrosomal microtubule depolymerizes, it leaves a free nucleation site that can initiate a new polymer. At steady state, the centrosome will continually nucleate unstable microtubules. Nucleation sites are therefore like permanently stabilized GTP ends. Two things make nucleation sites special: the kinetic advantage they give to assembly at low tubulin concentration, and their permanence. It is the latter characteristic that allows them to generate a stable array of individually unstable microtubules at concentrations of tubulin well below C_c .

Nucleated Assembly in a Finite Cell Volume. With dynamic instability, appreciable polymerization can occur

below C_c , provided nucleation sites are present. Under these circumstances, nucleation sites are not just catalysts—they determine the number of microtubules and hence the total polymer mass. For a cell with a fixed content of tubulin, this has special consequences: in a finite volume, an increase in polymer mass decreases the free tubulin concentration, which in turn leads to a slower rate of polymerization and to shorter, more unstable microtubules. Thus, an increase in the number of nucleating sites will result in an increased number of more dynamic microtubules (Figure 10). This could play a role in the interphase-mitosis transition (see below).

Morphogenesis by Selective Stabilization. In addition to understanding the steady-state distribution of cytoskeletal elements, we ultimately would like to understand the reorganization of the cytoskeleton, which is often seen as a polarization of the cell. There are numerous examples of such polarization: the extension of axons and dendrites, the elongation of cells in the neural ectoderm, and the formation of pseudopods in locomotory cells. Typically, a spatial cue is presented at the cell periphery, and this

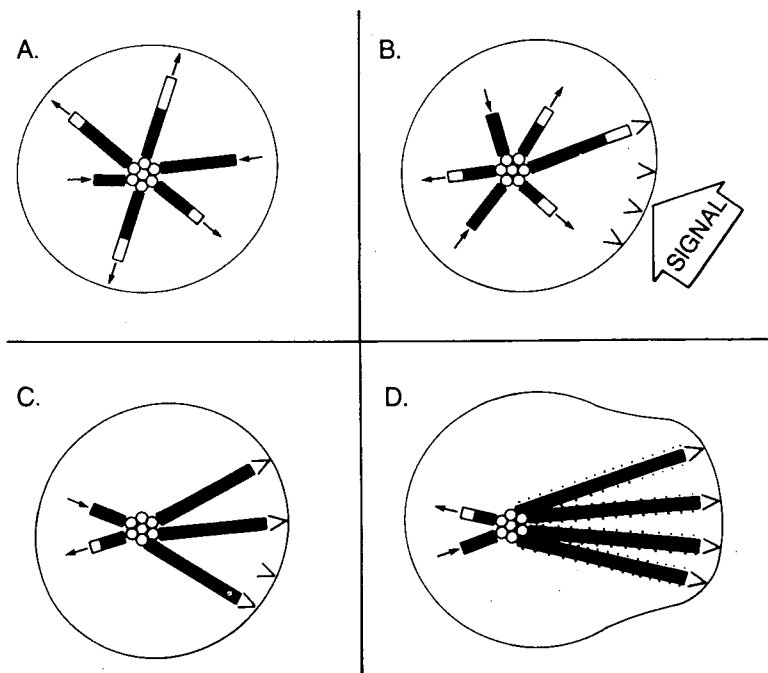


Figure 11. Morphogenesis by Selective Stabilization

In (A), we depict an unpolarized cell with microtubules that are growing out with no preferred direction and that are spontaneously shortening by dynamic instability. In (B), a local extracellular signal activates some capping structures near the cell periphery. In (C), the selective stabilization of these capping structures gradually leads to a reorientation of the microtubule arrays. In (D), the polarization is complete, with capped microtubules much less dynamic than unpolarized microtubules. Here we also show chemical modification of the less dynamic microtubules (as indicated by the dots).

leads to an asymmetrization of the microtubule cytoskeleton and an overall polarization of the cell. This presents an archetypical problem for the coordination of cellular behavior: How can a peripheral cue lead to reorganization deep in the cell center?

One possibility is that a signal is relayed to the microtubule organizing center, leading to a change in its structure and directed nucleation of microtubules. A simpler idea is that a signal at the periphery affects the distribution directly. Since the whole array is very dynamic, it would only be necessary to stabilize a particular subset of microtubules for the array to be rapidly transformed. The continuity of a microtubule means that the entire structure can be stabilized by effects at its ends, as we saw for the GTP cap. Thus, preferential stabilization of a microtubule could be mediated by interactions at the cell periphery, close to the site receiving the environmental information.

In Figure 11, we suggest that stabilization of the desired microtubules could be achieved by capping their ends against disassembly. Capping could occur by blocking the end of the lattice directly, or by a lateral interaction near the end. Preventing GTP hydrolysis along a short stretch of polymer could also effectively stabilize an end.

By using the dynamics of the microtubule array to probe many regions at random, and by stabilizing certain conformations as they arise, the cell can arrive at a structure that is not precisely defined by genetic information, but that nevertheless fulfills a particular functional role. Unstable

microtubules offer many possibilities for controlling the distribution of microtubules in the cell by selective stabilization; this may have been the fundamental advantage that caused unstable microtubules to evolve (see below).

Temporal Differentiation of Microtubules. When a microtubule is stabilized, it soon starts to differ in age from its dynamic cousins. If a slow, time-dependent process acts on the polymer, then this age difference will be reflected in a chemical change. Such a modifying mechanism would allow chemical differentiation of microtubules even if they were in physical proximity, and the whole microtubule would be affected. For example, a microtubule initially stabilized by an end interaction could become further stabilized along its entire length by a time-dependent process, as shown in Figure 11. This may be a molecular example of a common developmental phenomenon where an initial transient stabilization is gradually converted to a more permanent form.

One possible mechanism for differentially modifying older microtubules could be the removal of the C-terminal tyrosine of α -tubulin by a specific carboxypeptidase. Gunderson et al. (1984) used antibodies specific for the two C-terminal peptides (one containing the terminal tyrosine and the other with the penultimate amino acid, glutamate) and found by immunofluorescence analysis that the two forms segregated into different microtubules; i.e., there were microtubules uniformly labeled with anti-glu-tubulin in the same vicinity as microtubules uniformly labeled

with anti-tyr-tubulin. Since deetyrosination is a time-dependent reaction that acts preferentially on polymerized tubulin (Thompson, 1982), the simplest explanation is that glumicrotubules are more stable, and thus older and more highly modified. The consequences of this specific modification are not yet known.

Control of Dynamic Behavior. The highly dynamic microtubule cytoskeleton of fibroblasts may not be an appropriate model for all cells. We might expect to find similar dynamics in other cell types that are continually exploring different morphologies, or responding to a changing environment. However, in cells having a definitive morphology, it seems appropriate for dynamics to be suppressed. Indeed, microtubules in such cells are frequently much less sensitive to depolymerizing drugs (Black and Greene, 1982; Heidemann et al., 1985). Other circumstances may arise when it would be advantageous for a cell to increase dynamics—for example, during the interphase–mitosis transition. For any model of microtubule assembly, it is possible to modulate assembly and disassembly rates with proteins that interact with the lattice. What is special about dynamic instability is that not only are subunit addition and loss reactions affected, but so too are the transition probabilities between growing and shrinking microtubules. This means that the nature as well as the rate of dynamic changes can be modulated. For example, in principle the cell could go from a regime of rapid microtubule turnover to one of treadmilling. We expect that microtubule-associated proteins will have an important role in modulating endogenous microtubule dynamics in order to achieve a behavior and rate appropriate for different cell types. Of course, microtubule dynamics is only one part of any morphogenetic process; typically, these processes also involve interaction with other components of the cytoskeleton.

Morphogenesis of the Mitotic Spindle: A Case Study

An interphase cell faces several structural problems at the onset of mitosis. The unipolar cytoskeleton must be transformed into the bipolar arrangement that foreshadows the organization of two new unipolar daughters. Interphase microtubules must be replaced by more numerous spindle microtubules. Kinetochores must become connected to poles by microtubule bundles, and arranged at the metaphase plate. During these changes, the inevitable mistakes in organization must be continually corrected. (For a review of these events, see Nicklas [1971].)

The onset of prophase is characterized by a rapid transition from few, relatively long microtubules that stretch to the cell periphery to many short microtubules near the centrosome and nuclear periphery (Bajer and Mole-Bajer, 1969; Vandre et al., 1984). The photobleaching data of Salmon, McIntosh and coworkers, interpreted in terms of dynamic instability, indicate that the microtubule turnover rate is increased at the onset of mitosis (Salmon et al., 1984a; Saxton et al., 1984). This is most easily explained by an increase in the probability that a newly nucleated microtubule will transit to the depolymerizing phase. As a result of this increased turnover, interphase microtubules

will be destabilized, and newly nucleated microtubules will grow out a shorter distance from the centrosome before they depolymerize.

We do not yet know how the cell can increase the probability that a newly nucleated microtubule will start to depolymerize, but dynamic instability suggests several possibilities: Because of the increased nucleation capacity in prophase (Kuriyama and Borisy, 1981), the polymer mass would increase, with a resultant decrease in the stability of each microtubule. However, this mechanism predicts a concomitant decrease in microtubule growth rate, whereas in fact this rate may actually increase during mitosis (Saxton et al., 1984; Mitchison et al., 1986). Other possibilities include an increase in the inherent GTP hydrolysis rate by the microtubule, a reduction in the size of the GTP cap at a given growth rate, or a structural change, which in some other way increases the probability that a growing microtubule starts to shrink. Perhaps changes in the composition of microtubule-associated proteins could do this. We feel the increase in dynamics serves two purposes: first, to promote depolymerization of the interphase cytoskeleton; and second, to allow rapid spindle morphogenesis by selective stabilization mechanisms.

After initiating spindle morphogenesis, the next problem is the attachment of kinetochores to the poles by microtubule bundles. We assume that the centrosome is the major site of microtubule nucleation in the spindle. (For discussion of the evidence behind this assumption, see Rieder [1982], Sluder and Rieder [1985], and Mitchison and Kirschner [1985b].) The pole appears to nucleate with spherical symmetry (Mitchison et al., 1986), and early in prometaphase, chromosomes can be found throughout the cell. Since the kinetochore is only a small part of the chromosome, a growing microtubule may have difficulty finding it. We propose that, given their rapid dynamics, it would be sufficient for the microtubules to grow through the cell at random. Those microtubules that accidentally contact a kinetochore would become capped, while those that do not would soon depolymerize. In support of this idea, we have shown that the kinetochores of isolated chromosomes can stabilize the ends of microtubules *in vitro* (Mitchison and Kirschner, 1985). Furthermore, we have demonstrated directly that kinetochore microtubules turn over less rapidly than astral microtubules *in vivo* (Mitchison et al., 1986). The role of the kinetochore in capping and stabilizing microtubules *in vivo* has been elegantly demonstrated by Nicklas and Kubai (1985), who showed that detachment of the chromosome from the kinetochore fiber with a microneedle leads to rapid depolymerization of the kinetochore fiber.

A model for kinetochore fiber formation based on capture and capping makes the extraordinarily rapid (and energetically expensive) microtubule turnover rate in the spindle seem more reasonable. Since the half-time exchange of spindle polymer with subunits is about 15 sec (Saxton et al., 1984), the average spindle microtubule persists for 30 sec. Thus, a nucleating site will send out 2 microtubules per min, or about 1000 per aster per min. Assuming the average spindle microtubule length is 4 μm , half of these microtubules will grow through an imaginary

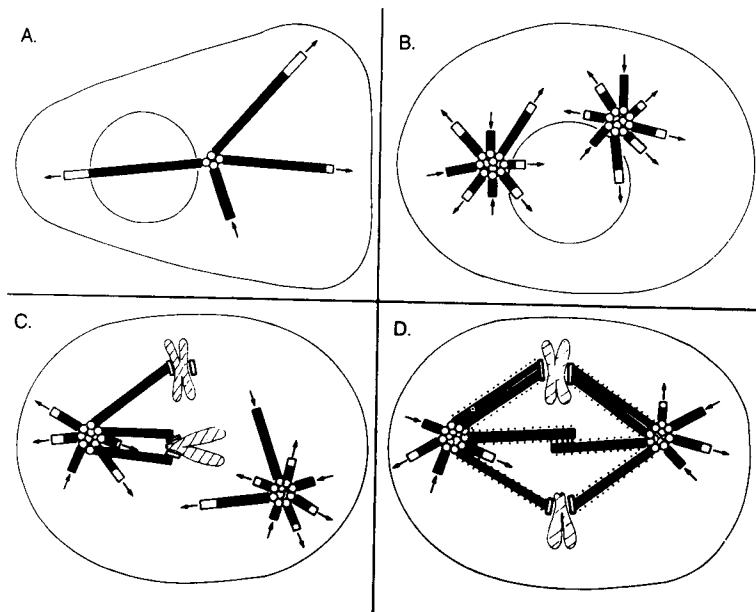


Figure 12. Simplified Model of Spindle Morphogenesis, Incorporating Features of Dynamic Instability

In (A), the interphase cell has long microtubules with large GTP caps and a small nucleating center. In prophase, (B), the centrosomal nucleation center increases in size, producing a larger number of shorter and more dynamic microtubules. In prometaphase, (C), some of the astral microtubules interact with kinetochores, causing them to be capped and partially stabilized. Some errors are made, such as the connection of both kinetochores of the same chromosome to one pole; however, since these microtubules are only transiently stable, the errors can be corrected. In metaphase, (D), microtubules that have made proper connections to the kinetochores or that interact properly in the overlap zone are further stabilized by unknown modifications, shown as dots along the length of the microtubules.

sphere of radius $4 \mu\text{m}$ per min. If the kinetochore is a disc $0.4 \mu\text{m}$ in diameter, it will subtend $1/800\text{th}$ of this sphere, which means that a microtubule connection would probably be made within 2 min. Although these numbers are approximate, they suggest that the model may quantitatively account for the attachment of chromosomes to the fibroblast cell spindle in prometaphase, or the rapid reattachment of a chromosome mechanically detached from a meiotic spindle (Nicklas and Kubai, 1985). (See Hill [1985b] for a more mathematical treatment of this problem.)

The model we have described for microtubule capture by kinetochores covers only the initial interaction; several other steps are required to form the mature metaphase plate. During these processes it is common for incorrect configurations to arise—for example, both kinetochores of sister chromatids may be connected to a single pole (Figure 12). However, since such incorrect configurations almost invariably rearrange, they must be less stable than correct ones. Nicklas (1986) has hypothesized that the kinetochore–microtubule connection is stabilized by tension when the chromosome has made optimal mechanical connections to the spindle. This type of fine-tuning mechanism is crucial for the overall fidelity of chromosome segregation, and here one could regard dynamic instability as a sort of proofreading mechanism that ensures the disappearance of incorrect orientations.

Although we have concentrated on the kinetochore fiber as an example of selective stabilization, this type of mechanism could also account for the correct placement of

other asymmetrically disposed microtubules in the spindle. Overlap zone microtubules could be stabilized by cross-linking interactions between antiparallel microtubules; this would be an example of capping by multiple lateral interactions (Figure 12). Selective stabilization mechanisms achieve the desired microtubule configurations because these configurations are more stable. To find the most stable state, the system must be able to search rapidly through intermediate configurations (avoiding the danger of being trapped in metastable configurations), and be able to detect a gradient of stability. Rapid microtubule dynamics and inherent instability coupled to progressive stabilization seem well tailored to these requirements.

Microtubule Assembly in Perspective

In this review, we have looked in detail at the physicochemical properties of microtubule assembly to determine whether there are interesting or novel features that may be related to the function of microtubules in cells. Although microtubule assembly bears a resemblance to simple well-studied equilibrium systems such as virus assembly, it in fact possesses several important differences. First, microtubules use the energy of GTP hydrolysis during assembly. The input of energy keeps polymer assembly very dynamic, so that the microtubule population in the cell turns over with amazing rapidity. Second, polymer stability can be controlled by events at the end of the polymer. In freely growing microtubules, the stability of the

polymer is most likely controlled by the presence or absence of subunits containing unhydrolyzed GTP. When microtubules interact with specific structures such as kinetochores or centrosomes, the stability of the polymer is regulated by these interactions. Third, special biological properties arise because microtubules are very long. Since interactions at the end determine their stability, microtubules are a means of communicating information from one part of the cell to another.

As discussed above, there are several consequences of the dynamics, end interactions, and length of microtubules that can be exploited by adding to the monomer-polymer system of pure tubulin other rather simple components, such as nucleation sites, enzymatic modifications of the polymer, associated proteins, or capping factors. With these additional components, the overall shape of the microtubule distribution can be regulated in a rather sophisticated manner. We can think of these additional components as simply modulating the dynamics of the polymer, whose properties are determined by the enzymatic and structural features of tubulin itself.

The picture that emerges of how a cell rearranges its microtubules is quite different from the pattern of morphogenesis found in systems such as bacteriophage. This is most easily seen in the morphogenesis of the mitotic spindle. The structure of the spindle, like that of the bacteriophage, is determined by the sum of all protein interactions and physiological parameters, and both structures in principle could have arisen by similar mechanisms. However, the pathways leading to the desired structures are fundamentally different. One can construct a map of bacteriophage assembly that shows an obligate pathway for protein interactions (Edgar and Wood, 1966). Although thermal fluctuations will produce limited statistical variation in the detailed structures of intermediates, the overall sequence of events is hard-wired into the amino acid sequence of the proteins involved. Presumably, evolutionary pressure has made this pathway most efficient and invariant; for example, the pathway often includes proteolytic cleavages to prevent reversal of particular steps (Laemmli, 1970).

It is not possible to construct a comparable assembly map for spindle morphogenesis. Dynamic instability produces a rapid turnover of microtubule configurations and the intermediate structures produced during prometaphase in genetically identical cells are highly variable. This variability goes far beyond thermal fluctuations, so that in each cell division a different sequence of structures is produced in the pathway of spindle morphogenesis. The most stable configuration at metaphase is reached not by following a map, but by following a gradient of increasingly more stable structures.

Spindle assembly is certainly less efficient than phage morphogenesis. Energy is consumed in microtubule turnover and time is wasted in exploring inappropriate configurations. What is lost in efficiency, however, is gained in adaptability. The extreme adaptability of mitosis is demonstrated by the ease with which the spindle can recover from perturbations that the cell would not normally see, such as complete microtubule depolymerization by drugs.

In a more physiological context, this adaptability allows the cell to carry out mitosis from many different starting configurations. This is important since cells at different sites in the body may begin mitosis from different morphologies. Furthermore, during embryonic development, the cell can change its structure and still use the same basic machinery for division. Finally, adaptability allows the cell to resolve the unpredictable errors that occur during mitosis. Thus, the adaptability produced by dynamic instability is probably indispensable for the evolution of multicellular organisms. Variability of intermediates and the resulting adaptability of pathways are also seen in processes such as directed cell movement, and probably occur as general features of most cytoplasmic reorganizations.

The principle that seems to drive the morphogenesis of microtubule arrays is selection from a complex and dynamic population. Selection is a familiar concept in biology, and perhaps is its most general organizing principle. On the time scale of evolution, the fittest macromolecules have been selected from a variable population. On the time scale of an organism's life, selection of cells can occur from variable populations; one example is clonal selection in the immune system (Burnett, 1962). Even bacterial chemotaxis can be regarded as a selective stabilization of appropriate trajectories from random locomotory behavior (Macnab and Koshland, 1972). In the case of microtubules, selection probably occurs continually. It is clear that deliberate generation of diversity, coupled with selective stabilization, can produce exquisite structures in biology. It seems likely that other pathways which appear to be highly deterministic will in fact turn out to make use of these same principles.

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