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Modulation of Microtubule Dynamics by Drugs: A Paradigm for the Actions of Cellular Regulators

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ABSTRACT. **Microtubules are intrinsically dynamic polymers. Two kinds of dynamic behaviors, dynamic instability and treadmilling, are important for microtubule function in cells. Both dynamic behaviors appear to be tightly regulated, but the cellular molecules and the mechanisms responsible for the regulation remain largely unexplored. While microtubule dynamics can be modulated transiently by the interaction of regulatory molecules with soluble tubulin, the microtubule itself is likely to be the primary target of cellular molecules that regulate microtubule dynamics. The antimitotic drugs that modulate microtubule dynamics serve as excellent models for such cellular molecules. Our laboratory has been investigating the interactions of small drug molecules and stabilizing microtubule-associated proteins (MAPs) with microtubule surfaces and ends. We find that drugs such as colchicine, vinblastine, and taxol, and stabilizing MAPs such as tau, strongly modulate microtubule dynamics at extremely low concentrations under conditions in which the microtubule polymer mass is minimally affected. The powerful modulation of the dynamics is brought about by the binding of only a few drug or MAP molecules to distinct binding sites at the microtubule surface or end. Based upon our understanding of the well-studied drugs and stabilizing MAPs, it is clear that molecules that regulate dynamics such as Kin 1 and stathmin could bind to a large number of distinct tubulin sites on microtubules and employ an array of mechanisms to selectively and powerfully regulate microtubule dynamics and dynamics-dependent cellular functions.**

Key words: microtubules/dynamics/antimitotic drugs/microtubule-associated proteins/mitosis

Microtubule dynamics

Treadmilling and Dynamic Instability

Microtubules are not simple equilibrium polymers (*e.g.*, 6, 14, for recent reviews). Soluble tubulin binds GTP reversibly at a site in the β subunit and the GTP becomes hydrolyzed to GDP and P_i as or shortly after the tubulin polymerizes onto a growing microtubule end. The irreversible hydrolysis of GTP during tubulin addition to the microtubule creates two unique dynamic behaviors. One such behavior, treadmilling (11, 13, 14), characterized by net growth at the plus end of a microtubule and net shortening at the minus end, is due to the difference in critical tubulin subunit concentrations at plus and minus ends. The second behavior, dynamic instability (10, 15, 28), is characterized by switching at microtubule ends between episodes of relatively slow sustained growth and rapid shortening. At or near steady state, both behaviors can coexist in microtubule populations. Even when an individual microtubule continually changes its overall length due to extensive dynamic instability excursions, net tubulin addition occurs at plus ends and loss at minus ends (Fig. 1). The extent to which individual microtubule populations display treadmilling and dynamic instability behaviors appears highly dependent upon the conditions (7).

Spindle microtubule dynamics

Treadmilling and dynamic instability behaviors appear to be critical to many microtubule-dependent cell functions. Rapid microtubule dynamics are especially prominent in mitosis and are essential for proper spindle assembly and function. In interphase cells, microtubules exchange their tubulin with soluble tubulin in the cytoplasmic pool with half times of several min to sev-

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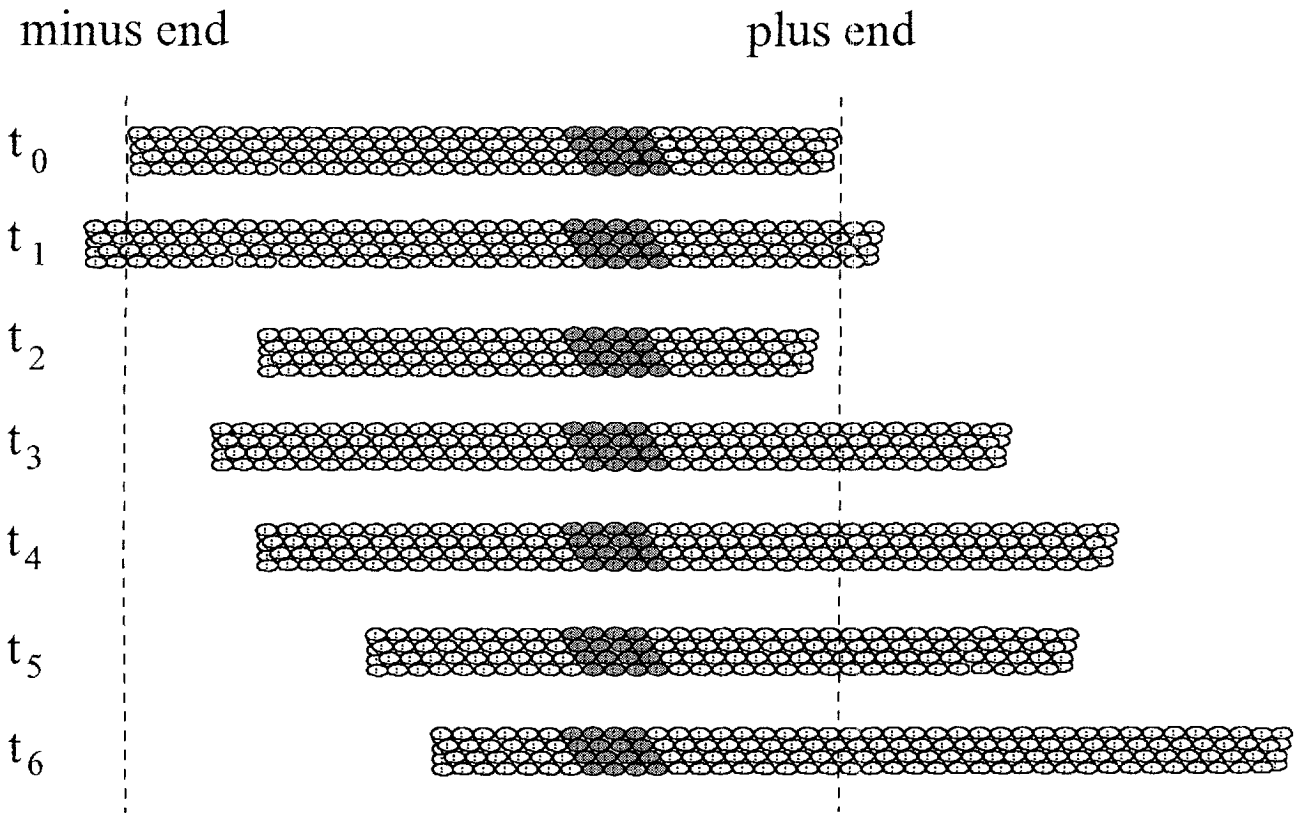

Fig. 1. Simultaneous treadmilling and dynamic instability, producing a net flow of tubulin subunits from plus to minus ends. Shown are consecutive "snapshots" of a microtubule exhibiting growth and shortening at plus and minus ends, with net growth at the plus end and net shortening at the minus end. The shaded subunits represent a marked segment. T_0=zero time; t_1 to t_6 represent arbitrary equal units of time.

eral hr. However, with the onset of mitosis, a population of highly dynamic mitotic spindle microtubules replaces the interphase microtubules. These microtubules are 10 to 100 times more dynamic than microtubules in interphase cells (1, 23). The extremely rapid dynamics of mitotic spindle microtubules, which are highly sensitive to modulation by antimitotic drugs, play a crucial role in the intricate movements of the chromosomes. For example, extensive growth and shortening excursions appear to be responsible for the initial attachment of the chromosomes at their kinetochores to the forming spindle. Rapid treadmilling of microtubules also occurs during metaphase and anaphase (16) where it may be involved in the flow of signals from kinetochores to the poles (14).

Kinetic modulation of microtubule dynamics by antimitotic drugs as paradigms for the actions of cellular regulatory molecules

A large and chemically diverse number of natural product drugs inhibit cell proliferation by acting on spindle microtubules. Most of the known antimitotic agents including colchicine and the anticancer drug vinblastine inhibit microtubule polymerization. In contrast, the anti-cancer drug taxol has an opposite action on microtubules; stimulation of polymerization and stabilization of spindle microtubules. Not surprisingly, the actions of these antimitotic drugs on spindle function were thought to be caused by reduction or enhancement of the spindle microtubule mass. However, a sophisticated understanding is now emerging at the molecular level of the mechanisms by which these compounds act on microtubule polymerization and dynamics. The early idea that these agents acted on microtubule-dependent cell functions only by destroying the microtubules or preventing their polymerization was simplistic. These drugs bind to the surfaces or ends of microtubules at discrete sites and modulate polymerization dynamics at drug concentrations well below those required to change the polymer mass (Table I). It now seems likely that the natural product drugs have evolved to mimic the actions of endogenous cellular molecules whose functions involve regulation of microtubule dynamics. The drugs can be used as tools to probe the roles of microtubule dynamics in cell func-

Table I. EFFECTS OF COLCHICINE, VINBLASTINE, TAXOL, AND TAU ON THE DYNAMIC INSTABILITY OF INDIVIDUAL MICROTUBULES AT STEADY STATE

Concentration (μM)	Colchicine 0.05	Vinblastine 0.1	Taxol 0.1	Tau 0.15
	Percent of Control			
Growing rate	67	66	69	75
Shortening rate	53	52	39	56
Catastrophe frequency	71	71	100	100
Rescue frequency	195	295	60	200
Dynamicity	63	39	53	38
Polymer mass	95	92	136	117

Colchicine data are taken from Panda *et al.* (19); vinblastine data are from Panda *et al.* (21); taxol data are from Derry *et al.* (4); and the tau data are from Panda *et al.* (20).

tions. However, their mechanisms can also be considered as paradigms for the mechanisms of action of cellular molecules that regulate microtubule dynamics. The mechanisms of some of the best understood drugs are described below in the context of their serving as such paradigms.

Colchicine

Colchicine has played a fundamental role in elucidation of the properties and functions of tubulin and microtubules since it was first found to bind to "the subunits of microtubules" in cell extracts more than 30 years ago (2, 3, 29). The binding reaction between colchicine and tubulin is a two-step process that begins with formation of a reversible, low-affinity pre-equilibrium complex. This is followed by one or more slow steps in which conformational changes in tubulin lead to formation of a poorly reversible final-state tubulin-colchicine (TC) complex (reviewed in 8, 31). While the nature of the slow conformational changes that occur during formation of the final state TC complex remain unknown, it seems likely that the conformational changes are responsible for the powerful effects of TC complex on tubulin exchange at microtubule ends. The specific location of the colchicine binding site in tubulin is not known precisely, but appears to be located at the interface between the α and β tubulin subunits in tubulin dimers (18).

Colchicine inhibits microtubule polymerization *in vitro* at concentrations well below the concentration of tubulin free in solution (reviewed in 31), indicating that it inhibits microtubule polymerization by binding to microtubule ends rather than to the soluble tubulin pool. Free colchicine itself either may not bind directly to microtubule ends or it does so very inefficiently. Instead, it must first bind to soluble tubulin and form a final-state TC complex, which then incorporates at the ends in small numbers along with large numbers of free tubulin molecules (25, 26). When TC complexes are incorporated at microtubule ends, the ends remain competent to grow.

Significantly, low concentrations of TC complex inhibit tubulin exchange at microtubule ends at concentrations far below those required to induce microtubule disassembly. For example, in an experiment using 0.1 μM TC complex, incorporation of only 1–2 molecules of colchicine per plus end inhibited by 50% the rate of tubulin incorporation by treadmilling. As time progressed TC-complexes were continuously incorporated with very little change in inhibition (25). Inhibition occurred in the absence of significant microtubule depolymerization.

Early experiments with radiolabeled GTP-tubulin indicated that incorporation of TC complex at microtubule ends stabilized the ends; a surprising action for a drug that inhibits microtubule polymerization. Direct evidence that TC complex kinetically stabilizes plus ends of microtubules has been obtained by video microscopy (19). Specifically, when added at low concentrations to steady-state MAP-free microtubules, TC complex strongly reduces the rate and extent both of growing and shortening (Table I). In addition, it strongly increases the percentage of time the microtubules remain in an attenuated state, neither growing nor shortening detectably. It also strongly increases the frequency of switching from shortening to growth or to an attenuated state (the rescue frequency) and strongly decreases the frequency of switching from growth or attenuation to shortening (the catastrophe frequency) (Table I). Thus, the switching mechanism responsible for gain and loss of the stabilizing cap at plus ends appears to be affected by colchicine.

We hypothesize that the conformational changes induced in tubulin by the binding of colchicine play a major role in the ability of TC complexes to stabilize microtubule ends. TC complexes may assume a conformation that disrupts the tubulin lattice at or near the end in a way that impairs the efficiency of new tubulin

addition but does not destroy the ability of tubulin to be incorporated. Importantly, the incorporated TC complex must bind more tightly to its tubulin neighbors so that the normal rate and extent of tubulin dissociation is reduced. Finally, because TC complexes strongly reduce the catastrophe frequency and increase the rescue frequency, we hypothesize that TC complex may modulate the mechanism responsible for gain and loss of the stabilizing GTP cap.

Vinblastine

Vinblastine binds to tubulin dimers at a site that appears to be located on the β subunit. The site appears to be exposed at the plus ends of the microtubule (18). It is distinct from the colchicine site. Vinblastine binds with high affinity to tubulin at the microtubule ends and with markedly reduced affinity to tubulin buried in the tubulin lattice (30). Vinblastine exerts two distinct actions on microtubule polymerization and dynamics that appear to be due to binding of the drug to these two classes of sites. Vinblastine inhibits tubulin exchange at plus ends of MAP-rich microtubules by 50% when only 1–2 vinblastine molecules are bound at the end (see 31). Like TC complex, low concentrations of vinblastine (in the sub-micromolar range) powerfully suppress both dynamic instability and treadmilling behaviors without appreciably depolymerizing the microtubule.

Vinblastine suppresses dynamic instability behavior selectively at plus ends (21, 27). The drug strongly reduces the rate and extent of growing and shortening and increases the percentage of time microtubules spend in an attenuated state, neither growing nor shortening detectably. Also, like TC complex, vinblastine increases the rescue frequency and decreases the catastrophe frequency, indicating that its action may involve stabilization of the GTP cap. Vinblastine induces a conformational change in tubulin that increases the affinity of tubulin for itself (12, 17) an action that undoubtedly is responsible for the increased stability of the microtubule ends. It is quite interesting, that at low concentrations, vinblastine and colchicine, which act by different molecular mechanisms at different tubulin binding sites, exert similar stabilizing effects on microtubule dynamics. Consistent with the postulated exposure of the vinblastine binding sites at the extreme plus ends and absence at the minus ends (18), vinblastine does not suppress dynamics at minus ends but in contrast, it destabilizes these ends (21).

Binding of vinblastine to microtubules at somewhat higher concentrations (>5 micromolar) depolymerizes the microtubules by peeling of protofilaments at both microtubule ends (Fig. 2). Thus, binding of vinblastine to the low affinity sites appears to weaken the lateral interactions between protofilaments. At still higher concentrations (>100 μM), the drug induces formation of paracrystalline arrays composed of tubulin in the form of oriented protofilaments and bound vinblastine, both in cells and *in vitro* (see 31).

Thus, the vinblastine binding site represents another site in tubulin involved in possible regulation of dynamics. It is of potential significance in the context of anticipating how cellular regulators of microtubule polymerization and dynamics might act that microtubule polymerization and dynamics respond differently to vinblastine, depending upon the location of the drug binding sites in the microtubule lattice. Interestingly, recent evidence indicates that Kin 1, a kinesin-like protein, induces microtubule depolymerization by inducing protofilament peeling at microtubule ends in a manner that appears similar to that of vinblastine (5). Conceivably, this protein, postulated to be a regulator of microtubule polymerization, may be acting at the vinblastine binding site and inducing depolymerization by a mechanism similar to that of vinblastine.

Taxol

The taxol binding site is yet another distinct site in microtubules that may play an important role in the regulation of dynamics. The site appears to be located on the inside microtubule surface, and access to it by taxol might occur through small pores in the surface lattice (18). Taxol profoundly affects the polymerization characteristics and stability of microtubules. It stimulates microtubule polymerization *in vitro*, promoting nucleation and reducing the critical tubulin subunit concentration (*i.e.*, soluble tubulin concentration at steady state) to near zero (9, 24).

Analysis of the effects of low taxol concentrations on dynamic instability indicates that the drug powerfully suppresses the rate and extent of shortening and greatly increases the percentage of time the microtubules spend in the attenuated state in the absence of appreciable change in the polymer mass (4). Like the actions of TC complex and vinblastine, a small ratio of bound taxol molecules to total tubulin in a microtubule is sufficient to produce a high degree of stabilization. For example, one taxol molecule bound per 100 tubulin molecules inhibits the shortening rate 50% (4). Such data suggest that small numbers of bound regulatory molecules that act by stabilizing microtubule dynamics might act in analogy to taxol by binding to only a very few tubulin molecules in the microtubule polymer. In support of this idea, very low ratios of the microtubule-stabilizing MAP tau bound to tubulin in a microtubule are sufficient to induce a powerful suppression of dynamics (20). Interestingly, the suppressive action of small numbers of tau molecules on microtubule dynamics is remarkably similar to that of taxol. In addition, small peptides representing the tubulin binding domains of tau suppress dynamics in a manner qualita-

Fig. 2. Vinblastine-induced protofilament peeling. A microtubule end with attached spirals, negatively stained without agitation after incubation of a steady-state suspension of microtubules with vinblastine (200 μM) for 2 min (1.1 mg microtubule protein/ml). This high vinblastine concentration rapidly induces extensive protofilament peeling. A drop of microtubule suspension was placed directly onto a parlodion and carbon-coated grid to which one drop of buffered 40% sucrose had previously been applied. Following aspiration of the liquid, cytochrome c (1 mg/ml in water) followed by 3 drops water and 1% w/uranyl acetate were applied sequentially for 15–20 s and then aspirated. The bar = 100 nm.

tively similar to that of full-length tau. Because the tau binding site and taxol binding site are almost certainly distinct, the data indicate that interaction with tubulin in two distinct domains can bring about a similar stabilization of dynamics.

Lessons to be learned from understanding drug mechanisms

Several lessons can be learned from analysis of the actions of drugs on microtubule dynamics. Microtubules have many distinct regions along their surfaces

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