Microtubule dynamics: taking aim at a moving target

Antitumor drugs of the vinca alkaloid and taxane classes function by suppressing the dynamics of microtubules in spindles, blocking cell division at metaphase. The drugs bind to various sites on the tubulin dimer and at different positions within the microtubule, suggesting that there are many unexplored targets for the design of novel drugs of this type.

Chemistry & Biology September 1995, **2**:569–573

Microtubules are major structural components in cells. They are important in the development and maintenance of cell shape, in cell reproduction and division, and in cellular movement (reviewed in [1,2]). Microtubules are highly dynamic assemblies of heterodimers of α and β tubulin, arranged parallel to a cylindrical axis to form tubes of diameter 25 nm that may be several to many μm long (Fig. 1). Polymerization of microtubules occurs by a nucleation–elongation mechanism in which the formation of a short microtubule 'nucleus' is followed by elongation of the microtubule at its ends by the reversible, noncovalent addition of tubulin dimers.

Microtubules are not simple equilibrium polymers. Tubulin dimers bind guanosine triphosphate (GTP) reversibly at a site in the β subunit and the GTP becomes hydrolyzed to guanosine diphosphate (GDP) and orthophosphate (P_i) during polymerization. Loss of phosphate is not immediate, however, so that after polymerization there is a 'cap' of tubulin subunits bound to either GTP or GDP plus P_i at the end of the microtubule; because the subunits in the cap have a higher affinity for each other than the tubulin-GDP subunits in the body of the microtubule, the presence of the cap encourages growth, while loss of the cap leads to shortening [1].

The fact that hydrolysis of GTP is irreversible thus creates unusual non-equilibrium dynamics. One consequence of this is that microtubule ends can stochastically switch between growing and shortening states (Fig. 2) in cells and *in vitro*; this is called dynamic instability [3]. The two ends of the microtubule are not equivalent; one end, called the plus end, is kinetically more dynamic than the other (the minus end). Although both ends can either

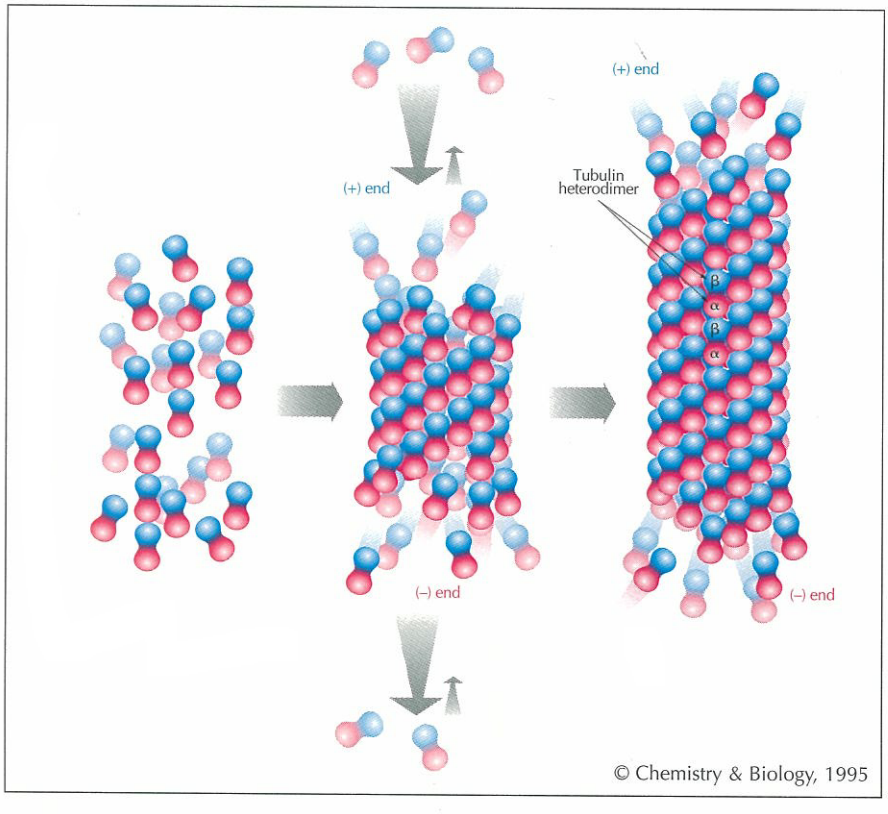

Fig. 1. Polymerization of microtubules. Heterodimers of α and β tubulin aggregate to form a short microtubule nucleus. Nucleation is followed by elongation of the microtubule at both ends by the reversible, noncovalent addition of tubulin dimers. Both ends can also shorten. The plus (+) end of the microtubule is kinetically more dynamic than the opposite or minus (–) end, growing and shortening over a wider range than the (–) end.

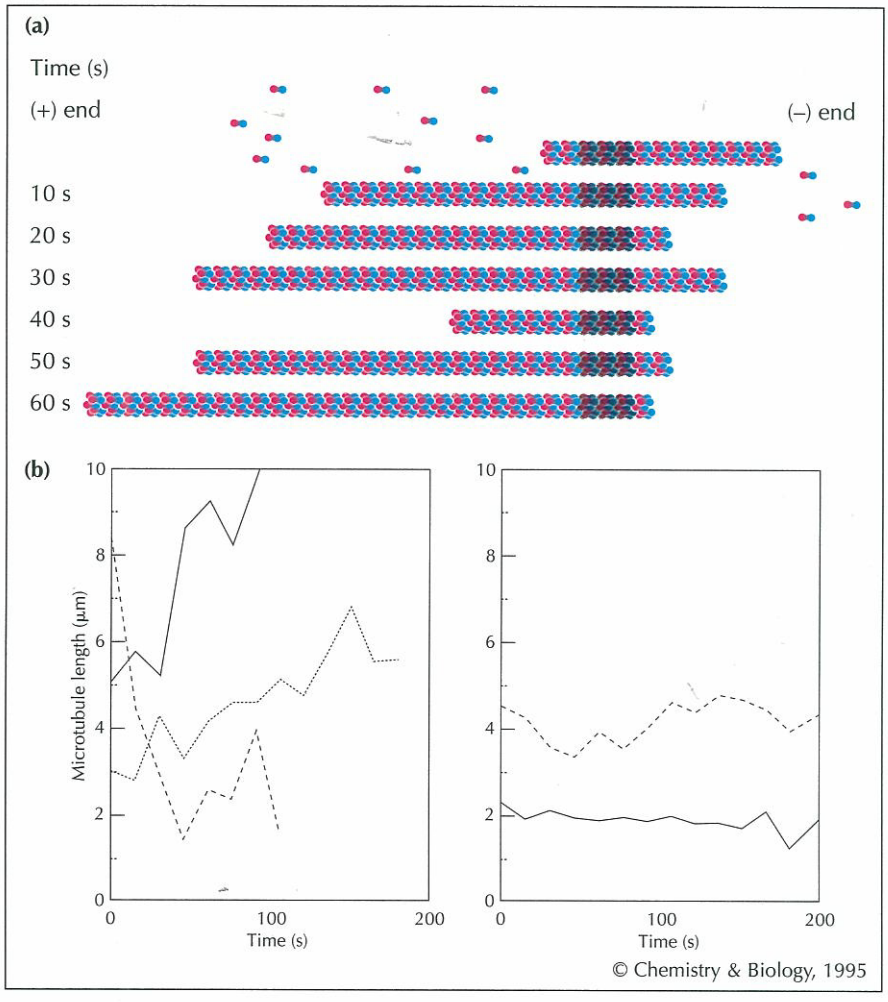

Fig. 2. Vinblastine suppresses the dynamic instability of microtubules. **(a)** Schematic showing how dynamic instability is observed. The dark-shaded section of the microtubule is marked (for example by fluorescence) or fixed, and the distance from this fixed point to the two ends of the microtubule is measured at different times. Both ends show stochastic switching between shortening and growing phases, with a wider dynamic range being shown by the (+) end. **(b)** Suppression of dynamic instability length changes of individual microtubules by vinblastine, measured by video microscopy. Left, three control microtubules; right, two microtubules in the presence of 0.5 μM vinblastine.

grow or shorten, the change in length at the plus end is much larger than the change in length at the minus end. Net growing of microtubules in a population *in vitro* or in cells can occur at plus ends and net shortening can occur at minus ends; when both of these occur at once, the microtubule is said to be treadmilling [4].

Dynamic instability is responsible for many microtubule-dependent processes in cells, the most dramatic of which is mitosis. Mitosis is the process during cell reproduction in which the replicated genetic material in the form of chromosomes is partitioned equally between two new 'daughter' cells. When cells enter mitosis, the cytoskeletal microtubule network is dismantled and a bipolar spindle-shaped array of microtubules is assembled outwards from the centrosome. Microtubules from the spindle become attached to the chromosomes and move them to the two spindle poles (Fig. 3). Microtubule growing and shortening dynamics are relatively slow in interphase cells. When cells enter mitosis, however, the rate of growing and shortening increases 20 to 100-fold so that spindle microtubules exchange their tubulin with tubulin in the soluble pool with half-times of ~10 s (see [1]). These extremely rapid dynamics, which are highly sensitive to

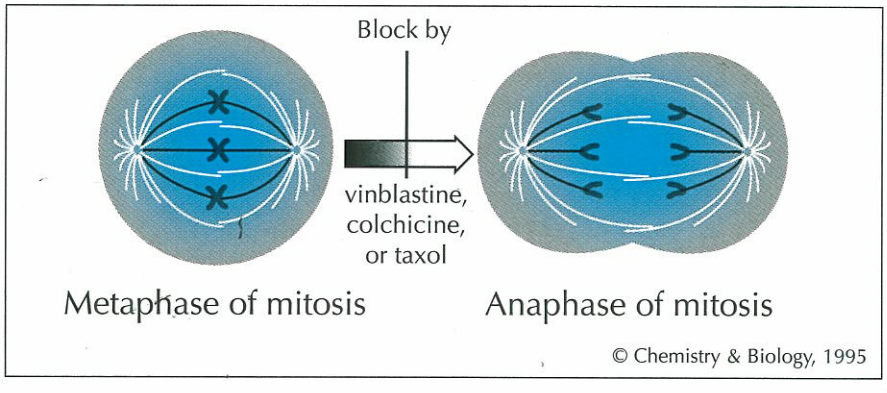

Fig. 3. Partitioning of chromosomes to two daughter cells in mitosis. At metaphase, the chromosomes have been transported to an equatorial position on the mitotic spindle by dynamic microtubules. At anaphase, microtubule dynamics change and the chromosomes partition and move to the two spindle poles on dynamic spindle microtubules.

modulation by antimitotic drugs, appear to be crucial in the intricate movements of the chromosomes.

Antimitotic drugs

A large number of chemically diverse substances, many of which are derived from natural products, bind to tubulin or microtubules and inhibit cell proliferation by acting on spindle microtubules [1,2]. Most such drugs inhibit microtubule polymerization at high drug concentrations; these include colchicine (Fig. 4a) from the plant *Colchicum autumnale* and the antitumor drugs vinblastine (Fig. 4b) and vincristine from the plant *Catharanthus roseus*. Other diverse compounds with similar actions include maytansine, rhizoxin, phomopsin, dolastatin and its derivatives [5,6], the cryptophycins [7], benzimidazole compounds such as nocodazole (see [1,2]), and the curacins [8]. At appropriately high concentrations, these drugs inhibit the polymerization of spindle microtubules or depolymerize existing spindle microtubules. They have therefore been thought to inhibit cell proliferation at mitosis by destroying the spindle microtubules required for mitosis [1,2]. More recently, the important new antitumor drug taxol (Fig. 4c) was isolated from the Pacific yew tree *Taxus brevifolia* (reviewed in [9]), which also inhibits cell replication by acting on microtubules. But taxol, in contrast to the other antimitotic drugs, can stimulate microtubule polymerization and stabilize microtubules. The remarkable success of taxol in the treatment of ovarian and breast cancer has stimulated an extensive search for new molecules that, like taxol, stimulate microtubule polymerization.

Kinetic suppression of microtubule dynamics: a common mechanism of antimitotic effects

A twist in the story came from our own recent demonstration that, like taxol, vinblastine, colchicine, and other compounds that depolymerize microtubules can also stabilize microtubule dynamics at relatively low concentrations ([10–12]; reviewed in [13]). Although both colchicine and vinblastine were found some years ago to suppress the rate of microtubule disassembly caused by dilution (called 'kinetic capping', [13]), their stabilizing effects on dynamics were only fully recognized with the introduction of real-time differential-interference contrast video microscopy. This technique allowed direct visualization of the stabilizing action of the drugs on the growing and shortening dynamics of individual microtubules (Fig. 2b) with high resolution. For example, vinblastine (0.5–1 μM) added to bovine brain microtubules at steady state greatly decreases the rate and extent of both growing and shortening at microtubule plus ends, and increases the fraction of time that the microtubules spend in an attenuated (or pause) state, in which they neither grow nor shorten detectably ([10]; see Fig 2b). Vinblastine binds to microtubule ends with relatively high affinity (Fig. 5a) and the powerful suppressive action of the drug on microtubule dynamics appears to be due to the presence of a very small number (one or two) of vinblastine molecules at the ends of the microtubule (reviewed in [3]).

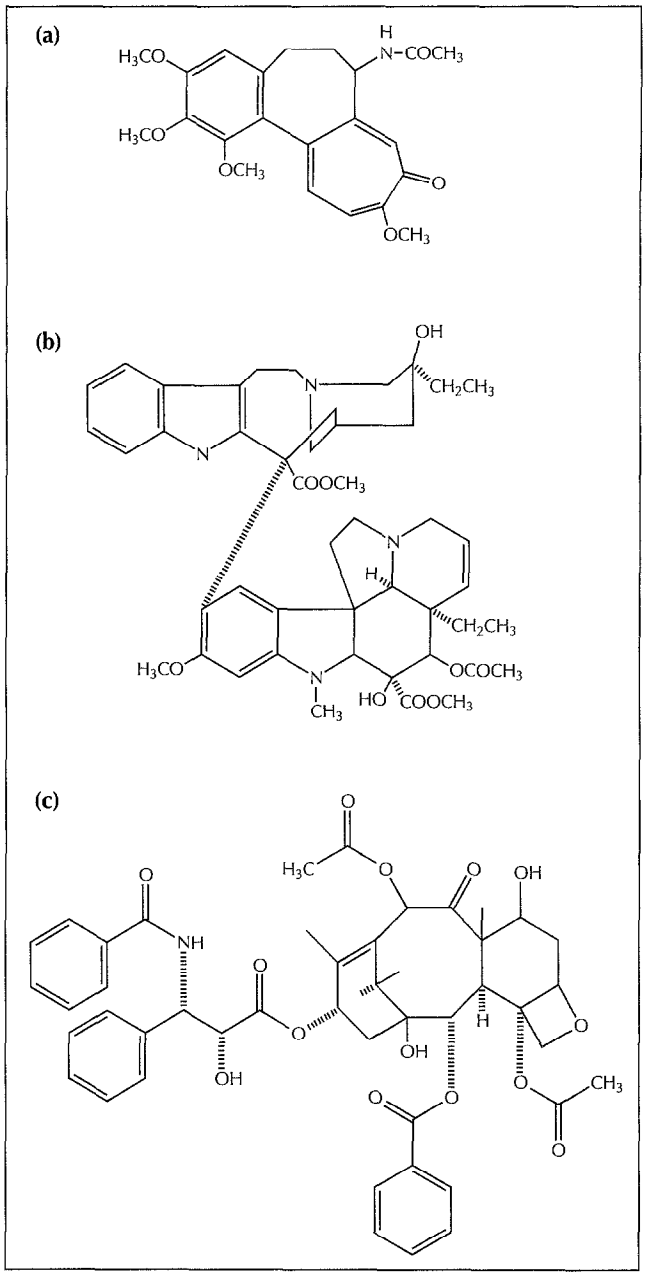

Fig. 4. Drugs that affect microtubule dynamics have diverse structures. Structures are shown for **(a)** colchicine, **(b)** vinblastine, and **(c)** taxol.

One of the more surprising results that emerged from these *in vitro* studies was the finding that, at low concentrations, the drug suppresses dynamics without appreciably depolymerizing microtubules. A qualitatively similar effect has recently been observed in living cells [14].

Colchicine also stabilizes microtubule ends but by a different molecular mechanism from that of vinblastine. Unlike vinblastine, colchicine must first bind to soluble tubulin to act. Relatively small numbers of tubulin–colchicine complexes then become incorporated along with tubulin at the microtubule ends (Fig. 5b) [11]. The incorporated tubulin–colchicine complexes again show profound effects on the rate and extent of growing and shortening, and powerfully increase the percentage of

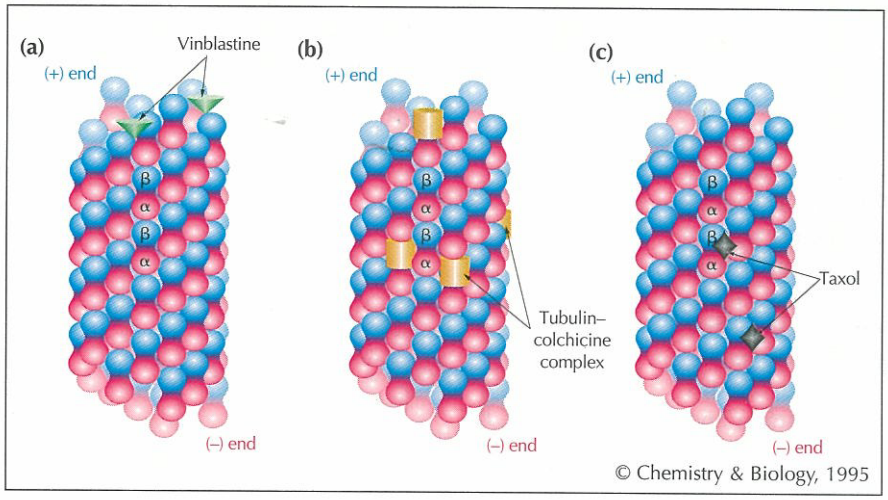

Fig. 5. Antimitotic drugs interact with microtubules at diverse sites. **(a)** A few (one or two) molecules of vinblastine bound to high-affinity sites at the microtubule end are sufficient to affect microtubule dynamics. **(b)** Complexes of colchicine with tubulin monomers are incorporated into the microtubule instead of the normal α–β tubulin dimers. **(c)** Taxol binds along the surface of the microtubule.

time that the microtubules spend in the attenuated state. Like vinblastine, low concentrations of tubulin–colchicine complex also kinetically stabilize microtubules without causing appreciable depolymerization.

Vinblastine and colchicine have similar effects to taxol

The effects of the vinca alkaloids and colchicine on microtubule dynamics are much more similar to those of taxol than previously recognized. Taxol stimulates microtubule polymerization by binding directly to tubulin along the length of the microtubule (Fig. 5c) (see [9, 11–13,15]). There is a taxol-binding site on each molecule of tubulin in microtubules, and the ability of taxol to increase microtubule polymerization is associated with nearly stoichiometric binding of taxol to tubulin in microtubules [15]. Binding of a small number of taxol molecules to microtubules stabilizes the microtubules [12]. At relatively low ratios of bound taxol to tubulin in microtubules (e.g., 1 molecule of taxol per ~200–600 molecules of tubulin in microtubules) taxol inhibits the rate and extent of shortening, an action similar to that of colchicine and vinblastine. Like vinblastine and colchicine at low concentration, taxol at very low concentrations kinetically suppresses microtubule dynamics without significantly changing the polymer mass [12].

Microtubule dynamics can also be suppressed by cellular proteins, the microtubule-associated proteins (MAPs), both *in vitro* and *in vivo* [1,2]. Among the best studied stabilizing MAPs are the neuronal proteins MAP2 and tau, which stabilize the microtubules in neuronal processes [1,16,17]. Interestingly, tau suppresses steady-state dynamic instability *in vitro* in a manner that is qualitatively indistinguishable from taxol [18], consistent with the idea that antimitotic drugs mimic the actions of natural regulatory substances. Tau and MAP2 have common microtubule-binding domains consisting of three or four imperfect repeats of 18 amino acids [16,17]. A synthetic 18 amino acid peptide representing the first binding repeat of bovine brain tau, and an 8 amino acid peptide representing the region between the first and second repeats, both bind weakly to microtubules *in vitro* (see [19], for example) and stabilize microtubule dynamics in a manner that is qualitatively indistinguishable from that of full-length tau [18]. These small peptides are thought to bind near the carboxyl-terminus of tubulin, presumably to different regions, while taxol appears to bind near the amino-terminus of β (and perhaps α) tubulin [20]. The effects of these peptides are qualitatively similar to that of taxol.

Kinetic stabilization, inhibition of proliferation and cell death

Given the above findings, the mechanism underlying the ability of antimitotic compounds to inhibit cell proliferation and to kill tumor cells may be the kinetic stabilization of spindle microtubule dynamics, not the depolymerization or excessive polymerization of spindle microtubules. While investigating the antimitotic actions of vinblastine, taxol, and other antimitotic compounds in human tumor cells (HeLa), we discovered that at their lowest effective concentrations most of these compounds inhibit cell proliferation at mitosis; although the microtubule and chromosome organization of the spindles is only subtly altered and the microtubule polymer mass is similar to that in control cells [13, 21–23]. Because low concentrations of these drugs kinetically stabilize microtubule dynamics without significantly changing the microtubule mass *in vitro* and in cells, it is reasonable to suppose that the drugs also block spindle function by kinetically stabilizing the microtubules.

As indicated previously, microtubule growing and shortening dynamics increase dramatically when cells progress from interphase into mitosis, and it is probable that these rapid dynamics are critical for mitosis. It is remarkable that cells can construct a normal or nearly normal bipolar spindle in the presence of antimitotic drugs; therefore assembly of the spindle is not as critically dependent upon the dynamics of its microtubules as is spindle function after it is constructed. HeLa cells are blocked by these drugs at the metaphase–anaphase transition of mitosis (Fig. 3); thus, it appears that rapid microtubule dynamics are especially important at metaphase. The transition from metaphase to anaphase is an important checkpoint in the cell cycle [24,25], which prevents cells from progressing into anaphase until the spindle is fully assembled and the chromosomes are properly poised for separation. Drugs

that suppress spindle microtubule dynamics may exert their antiproliferative and cytotoxic effects at this cell-cycle checkpoint. In support of this notion, we recently showed that prolonged blockage of HeLa cells at metaphase by low concentrations of vinblastine or taxol triggers apoptosis, a form of programmed cell death [26].

Lessons for the development of antimitotic antitumor drugs

The emerging awareness that the dynamic properties of the microtubules in the spindle are critically important in cell proliferation and mitosis, and that suppressing spindle microtubule dynamics may be sufficient to induce cell death, is crucial for the design of novel drugs of this class. Furthermore, the response of a particular tumor cell to suppression of spindle microtubule dynamics appears to be the factor that determines whether that tumor cell will live or die. As two of the most successful antimitotic anti-tumor drug classes discovered thus far (the vinca alkaloids and taxanes) may exert their antitumor actions by suppressing microtubule dynamics, it seems clear that the synthesis and/or identification of compounds that suppress microtubule dynamics is a potentially fruitful avenue for future drug development. An understanding of what determines the response of a tumor cell to treatments of this kind might also, eventually, lead to expansion of the usefulness of these compounds. The antitumor specificities of the vinca alkaloids and taxanes are strikingly different; this might be due to differences in their pharmacokinetics, or to other differences that are not yet understood. Other compounds that act by stabilizing spindle microtubule dynamics might have entirely new tumor specificities.

Finally, it is worth noting that microtubule dynamics can be suppressed by the interaction of diverse drugs, MAPs, and small peptides with tubulin at a large number of different sites. Given the importance of microtubule dynamics in mitosis, it is plausible that cells have available a sophisticated array of molecules and mechanisms to regulate microtubule dynamics. Understanding these mechanisms may lead to development of yet more new drugs. Because only a few molecules are necessary for the effect, it might be possible to find drugs of very high potency with selectivity for diseased cells. Cancer is not the only target; diseases such as schistosomiasis and malaria are caused by parasites whose microtubule dynamics may be controlled in ways distinct from those in human cells. The possibilities for collaboration between chemists and biologists in these areas are many and varied, and the potential for progress is tremendous.

Acknowledgements: The work described from our laboratories was supported by American Cancer Society grant DHP-43G, and NIH grants CA57291, and NS13560.

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