We have characterized the effects of vinblastine on the growing and shortening dynamics at opposite ends of individual bovine brain microtubules at steady state \textit{in vitro} by video microscopy. Vinblastine exerted strikingly different effects on the dynamics and polymer mass at the plus and minus ends of microtubules. At concentrations between 0.1 and 0.4 \textmu M, the drug strongly depolymerized microtubules at minus ends, whereas it did not significantly depolymerize microtubules at plus ends. Vinblastine stabilized plus ends by suppressing the rate and extent of growth and shortening, decreasing the catastrophe frequency, and increasing the rescue frequency. In contrast, vinblastine destabilized minus ends by increasing the catastrophe frequency and decreasing the rescue frequency, whereas it had no effect on the rate or extent of growth or shortening. Thus, vinblastine moderately increased the overall dynamicity at minus ends while strongly suppressing dynamicity at plus ends. Both the kinetic destabilization of microtubules at minus ends and the stabilization at plus ends may contribute to the altered function of mitotic spindle microtubules of cells blocked in mitosis by low concentrations of vinblastine.

Vinblastine, an indole-dihydroindole compound from the plant \textit{Catharanthus roseus}, is one of several vinca alkaloids important for the treatment of cancer (1). It is a potent inhibitor of cell proliferation that acts by disrupting spindle microtubule function (2–4). At low nanomolar concentrations, vinblastine arrests mitosis in HeLa cells at the transition from metaphase to anaphase in the absence of significant spindle microtubule depolymerization or spindle disorganization (3, 5, 6). These results have indicated that low concentrations of vinblastine inhibit spindle function by kinetic stabilization of microtubule dynamics and that the rapid dynamics of spindle microtubules, not just their presence in spindles, are critical for proper spindle function.

Microtubules are dynamic tube-shaped polymers composed of the heterodimeric protein tubulin (7). Polymerization of tubulin into microtubules occurs by a nucleation-elongation mechanism in which formation of a short microtubule “nucleus” composed of tubulin heterodimers is followed by growth of the microtubule at its ends by the reversible addition of tubulin subunits. However, microtubules do not attain a true equilibrium at its ends by the reversible addition of tubulin subunits. However, microtubules do not attain a true equilibrium. GTP, which binds reversibly to tubulin dimers in microtubules, is irreversibly hydrolyzed to GDP and Pi, as (or shortly after) the tubulin polymerizes onto the growing microtubule end (8, 9); this creates polymers with unique non-equilibrium dynamics. At microtubule ends, stochastic transitions occur between phases of relatively slow growth and rapid shortening (10–12). The opposite ends of the microtubule differ kinetically, with one end, called the plus end, being more dynamic than the opposite minus end. Regulation of the transitions between growing and shortening at both microtubule ends appears to be due to the stochastic gain and loss of a stabilizing “cap” consisting of a short region of tubulin-GTP or tubulin-GDP-Pi (8, 13). Loss of the cap is thought to be required for initiation of a shortening phase, and the rate-limiting step has been postulated to be a conformational change in tubulin that is associated with GTP hydrolysis or Pi release.

During mitosis, microtubule dynamics are greatly increased as compared with the dynamics during interphase. Spindle microtubules exchange their tubulin with soluble tubulin in the cytoplasmic pool with half-times of 10–15 s, 20–100-fold faster than during interphase (14–18). The rapid dynamics of microtubules during mitosis play an essential role in the formation of the spindle and in movement of the chromosomes. At prometaphase the plus ends of microtubules rapidly grow out from the centrosomes, probing the cytoplasm by continuous excursions of growing and shortening until they encounter and become attached to the kinetochores of the chromosomes (19, 20). In addition, rapid treadmilling occurs during mitosis (17). Growth occurs at the plus ends of microtubules tethered to the kinetochores of the chromosomes, and balanced shortening occurs at the minus ends that are embedded in the centrosomes. The function of treadmilling during metaphase is unknown but may involve the development of tension on the kinetochores that may function in the signal to transition from metaphase to anaphase (21) or it may mediate the translocation of signaling molecules poleward from kinetochores to centrosomes (22).

Vinblastine binds to tubulin in intact microtubules with two widely different affinities depending upon whether the tubulin binding site is located at the microtubule ends or is situated along the microtubule surface. The binding sites on the microtubule surface have low affinity for vinblastine (1–2 sites per molecule of tubulin dimer in microtubules; \(K_d\approx 0.25–0.3\) \textmu M (23, 24)). Binding of vinblastine at high concentrations to these sites \textit{in vitro} depolymerizes the microtubule at both ends by the peeling of protofilaments and leads to formation of tubulin-vinca alkaloid paracrystals in cells (see Ref. 4). Suppression of tubulin exchange at microtubule ends, which occurs at low vinblastine concentrations in the presence of significant microtubule depolymerization, appears to be due to the reversible binding of vinblastine to high affinity binding sites located uniquely at one or both microtubule ends (\(\approx 16\) binding sites per microtubule, \(K_d\approx 1–2\) \textmu M (25)). In experiments with popula-
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Experimental Procedures

Purification of Tubulin—Microtubule protein was isolated from bovine brain by three cycles of polymerization and depolymerization; tubulin was purified from the microtubule protein by phosphocellulose chromatography (27). The tubulin solution was quickly frozen as drops in liquid nitrogen and stored at −70°C until used. Protein concentration was determined by the method of Bradford (29) using bovine serum albumin as the standard.

Assembly of Microtubules and Determination of Steady-state Microtubule Polymer Mass—Tubulin pellets were thawed and centrifuged at 4°C for 30 min. Tubulin protein was isolated from bovine brain tubulin. The tubulin (17 μM) was added to S. purpuratus flagellar axonemal seeds in 75 mM Pipes, 1.8 mM MgCl₂, 1 mM EGTA, pH 6.8 (PME buffer) containing 1.5 mM GTP and incubated to steady state (35–45 min) at 37°C. To determine the microtubule mass, microtubules were assembled as described above in the presence of vinblastine and pelleted by centrifugation for 1 h. Microtubule pellets were solubilized in PME buffer at 0°C for protein determination.

Effects of Vinblastine on Preformed Microtubules—Tubulin was polymerized to steady state as described above. Vinblastine at a range of concentrations was added to the microtubule suspensions and incubation continued for an additional 15 min. The number of microtubules at the opposite ends of the seeds was determined by video microscopy immediately after fixation with 0.25% glutaraldehyde (30).

Determination of Microtubule Dynamics by Video Microscopy—Tubulin was polymerized as described above in the absence of presence of vinblastine. The seed concentration was adjusted to achieve 3–6 seeds per microscope field. After 35 min of incubation, samples of microtubule suspensions (4 μl) were prepared for video microscopy, and the dynamics of individual microtubules were recorded at 37°C as described previously (30). The microtubules were observed for a maximum of 45 min after reaching steady state. Under the conditions used microtubules grew from both the plus and minus ends of the axonemes, and thus, we were able to analyze the kinetic parameters simultaneously at both microtubule ends.

Designation of Plus and Minus Ends in the Absence and Presence of Vinblastine—In the absence of vinblastine, the lengths of the excursions and the growing rates were much greater at one end of the seeds than the other. Consistent with previous experiments, the end with the greater excursion lengths and higher growing rates was considered the plus end (12, 31). In the absence of vinblastine, all seeds had one, two, or three microtubules at one end, and approximately 75% of the seeds contained a single microtubule at the other end. The microtubules at both ends were of similar length. Addition of vinblastine to initiation of microtubule polymerization induced a striking change in the relative numbers of microtubules at the two ends. In the vinblastine concentration range examined, one end retained from one to three microtubules, but the number of microtubules at the other end was strongly reduced in a vinblastine concentration-dependent manner. At vinblastine concentrations <0.4 μM, microtubules formed predominantly, but not exclusively, at one end. At vinblastine concentrations >0.4 μM, microtubules formed exclusively at one end. The end at which microtubules predominated was considered to be the plus end because vinblastine stabilizes microtubule plus ends in vitro (27) and in vivo (28). Using our present buffer conditions and low tubulin concentrations (11 μM), at which microtubules grew exclusively at one end of the seeds (therefore the plus end), we verified the previous findings that microtubule dynamics were strongly stabilized by 0.4 μM vinblastine (data not shown).

Microtubule length changes were measured in real time at 3–6-s intervals until microtubules underwent complete depolymerization to the axoneme seed or until the microtubule end became obscured. The length changes undergone by a particular microtubule as a function of time were used to create a “life history” plot, and the growing and shortening rates were determined by least squares regression analysis of the data points for each growing or shortening phase. The reported mean growing and shortening rates represent the average values for all growing or shortening events observed for a particular reaction condition. We considered a microtubule to be in a growth phase if the microtubule increased in length by >0.2 μm at a rate >0.15 μm/min and in a shortening phase if the microtubule decreased in length by >0.2 μm at a rate >0.3 μm/min. Length changes equal to or less than 0.2 μm over the duration of six data points were considered as attenuation phases. An average of 15–25 microtubules was measured for each experimental condition.

We calculated the catastrophe frequency (a catastrophe is a transition from the growing or attenuated state to shortening (12)) by dividing the number of catastrophes by the sum of the total time spent in the growing plus attenuated states for all microtubules for a particular condition. The rescue frequency (a rescue is a transition from shortening to growing or attenuation, excluding new growth from a seed (12)) was calculated by dividing the total number of rescue events by the total time spent shortening for all microtubules for a particular condition. Dynamicity is the total tubulin exchanged at a microtubule end during all measurable growing and shortening events divided by the total time of observation (27).

Results

Effects of Vinblastine on the Polymer Mass—Tubulin (1.7 mg/ml) was polymerized at the ends of axonemal seeds in the absence and presence of vinblastine (see “Experimental Procedures”). The microtubules were collected by centrifugation, and the polymeric protein in the pellets was determined. As shown in Fig. 1, vinblastine reduced the microtubule polymer mass in a concentration-dependent manner. No significant decrease in microtubule mass occurred at vinblastine concentrations <0.1 μM. Polymerization was reduced 25% at 0.2 μM vinblastine, and half-maximal inhibition of polymerization occurred at ~0.5 μM vinblastine.

Vinblastine differentially reduced the microtubule polymer mass at opposite ends of the seeds. In control suspensions, all seeds had microtubules at one end, and ~75% of the seeds had microtubules at both ends. Addition of vinblastine prior to initiation of microtubule polymerization strongly reduced the number of seeds that had microtubules at both ends. For example, at 0.6 μM vinblastine no seeds contained microtubules at both ends, whereas most of the seeds contained at least one or more long microtubules at one end (data not shown).

Addition of Vinblastine to Preformed Microtubules Differentially Depolymerized the Microtubules at Opposite Ends of the Axonemes—The effects of vinblastine on preformed microtubule-axoneme constructs were determined by counting the number of microtubules remaining at the opposite ends of the seeds 15 min after adding the drug (see “Experimental Procedures”). The number of microtubules at one end of the axon-
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Vinblastine had a higher catastrophe frequency and a lower rescue frequency at the plus ends compared to the minus ends. Figure 2 illustrates the changes in length of individual microtubules with time at both ends in the absence and presence of vinblastine. The effects of vinblastine on the polymer mass were determined (see “Experimental Procedures”). Error bars, S.E.

The catastrophe frequency and the rescue frequency are shown in Figure 2. Visual inspection of the traces clearly indicates that vinblastine strongly suppressed growing and shortening events more significantly at the plus ends (Table I). The plus ends of microtubules also spent a somewhat larger fraction of time in the attenuated state than the minus ends. For example, the mean shortening rate at minus ends was 3-fold higher at the plus ends than the minus ends (Table I). The plus ends of microtubules also spent a somewhat larger fraction of time in the attenuated state.

### Table I

| Parameter                  | Plus end | Minus end |
|----------------------------|----------|-----------|
| Rate (μm/min)              |          |           |
| Growing                    | 1.0 ± 0.1| 0.46 ± 0.07|
| Shortening                 | 14.2 ± 1.2| 13.2 ± 4.7 |
| Length change (μm)/event   |          |           |
| Growing                    | 2.3 ± 0.2| 1.0 ± 0.1  |
| Shortening                 | 5.8 ± 0.7| 2.8 ± 0.7  |
| Percent time in phase      |          |           |
| Growing                    | 80.4     | 76.7      |
| Shortening                 | 11.9     | 6.9       |
| Attenuation                | 7.7      | 16.4      |
| Transition frequencies (min⁻¹)|        |           |
| Catastrophe                | 0.31 ± 0.06| 0.19 ± 0.05|
| Rescue                     | 0.78 ± 0.26| 2.0 ± 0.6  |
| Dynamicity (μm/min)        | 2.28     | 0.78      |

The catastrophe frequency and the rescue frequency are considered to reflect the loss and gain of the stabilizing GTP or GDP-P, cap at the microtubule ends (12). Microtubule plus ends had a higher catastrophe frequency and a lower rescue frequency than the minus ends (Table I). The plus ends of microtubules also spent a somewhat larger fraction of time growing and shortening than the minus ends, and the minus ends spent significantly more time in the attenuated state than the plus ends (Table I). The dynamicity (a measure of total detectable tubulin dimer exchange at a microtubule end) was 3-fold higher at the plus ends than the minus ends.

### Effects of Vinblastine on Microtubule Dynamics at Opposite Microtubule Ends

Analysis of the effects of vinblastine on dynamics at plus and minus ends indicated that vinblastine modulates dynamics very differently at the opposite microtubule ends. Life history traces of individual microtubules at plus and minus ends in the absence and presence of 0.4 μM vinblastine are shown in Figure 2. Visual inspection of the traces clearly indicates that vinblastine strongly suppressed growing and shortening at plus ends (Figure 2, A and C) and that the microtubules remained in an attenuated state for a large fraction of total time. In contrast, 0.4 μM vinblastine had little perceptible effect on growing and shortening dynamics at the minus ends (Figure 2, B and D).

Vinblastine does not significantly affect the rates or extents of growing or shortening events at minus ends. For example, the mean shortening rate at plus ends was 13.2 μm/min for control microtubules and 12.7 μm/min for microtubules treated with vinblastine.
Differential Effects of Vinblastine at Opposite Microtubule Ends

**Table II**

Effects of vinblastine on the dynamic instability parameters of microtubules at their opposite ends

| Vinblastine concentration (μM) | 0  | 0.1 | 0.2 | 0.4 | 0.6 |
|-------------------------------|----|-----|-----|-----|-----|
| **Plus ends**                |    |     |     |     |     |
| Rate (µm/min)                |    |     |     |     |     |
| Growing                      | 1.0±0.1 | 0.66±0.08 | 0.48±0.05 | 0.44±0.04 | 0.35±0.04 |
| Shortening                   | 14.2±1.2 | 7.5±1.1 | 6.25±1.9 | 3.55±1.3 | 2.25±0.5 |
| Percent time in phase        |    |     |     |     |     |
| Growing                      | 80.4 | 54.1 | 60.4 | 44.4 | 35.9 |
| Shortening                   | 11.9 | 7.9 | 8.5 | 7.1 | 6.2 |
| Attenuation                  | 7.7 | 38.0 | 31.1 | 48.5 | 57.9 |
| Dynamicity (µm/min)          | 2.28 | 0.89 | 0.41 | 0.51 | 0.22 |
| **Minus ends**               |    |     |     |     |     |
| Rate (µm/min)                |    |     |     |     |     |
| Growing                      | 0.46±0.07 | 0.57±0.07 | 0.58±0.1 | 0.60±0.09 | ND |
| Shortening                   | 13.2±4.7 | 13.1±2.7 | 14.6±3.0 | 12.7±3.3 | ND |
| Percent time                 |    |     |     |     |     |
| Growing                      | 76.7 | 72.2 | 73.0 | 57.9 | ND |
| Shortening                   | 6.9 | 13.0 | 12.9 | 10.9 | ND |
| Attenuation                  | 16.4 | 14.8 | 14.1 | 31.2 | ND |
| Dynamicity (µm/min)          | 0.78 | 1.45 | 1.19 | 1.0 | ND |

±, standard error of the mean. ND, not determined.

**Fig. 3.** Microtubule length changes per growing (A) or shortening (B) event at microtubule plus ends (squares) and minus ends (triangles) as a function of vinblastine concentration. The mean length a microtubule grew during growing events was calculated by dividing the summed growing lengths for all microtubules for a particular condition by the total number of growing events measured for that condition. The shortening length per shortening event was calculated similarly. Error bars, S.E.

The catastrophe and rescue frequencies are believed to be important determinants of microtubule function in cells (32, 33). The effects of vinblastine on these parameters were very different at the plus and minus ends (Table III). At minus ends, vinblastine significantly increased the catastrophe frequency and perhaps slightly reduced the rescue frequency. Specifically, the catastrophe frequency was increased 1.8-fold by 0.4 μM vinblastine, and the rescue frequency was reduced by 17%. In contrast, at plus ends vinblastine strongly reduced the catastrophe frequency and increased the rescue frequency. For example, 0.4 μM vinblastine reduced the catastrophe frequency 58% and increased the rescue frequency 2.2-fold.

The rescue frequency per μm of length shortened was determined by dividing the total number of rescue events by the total length shortened during shortening events. The rescue frequency/μm of shortening was not significantly altered by vinblastine at the minus ends (Fig. 4). For example, the rescue frequency was 0.28 μm⁻¹ for control minus ends and 0.26 μm⁻¹ in the presence of 0.4 μM vinblastine. These results indicate that minus ends are not stabilized by vinblastine during rapid depolymerization. In contrast to the lack of effect at minus ends, the rescue frequency per μm of shortening at the plus ends was strongly increased by vinblastine. For example, the rescue frequency per μm was increased 13-fold at 0.6 μM vinblastine. Thus, vinblastine appears to strongly induce recapping of plus ends before significant polymer loss can occur at these ends (see “Discussion”).

Vinblastine slightly increased dynamicity at minus ends, whereas the drug strongly reduced dynamicity at plus ends (Table III). For example, 0.4 μM vinblastine increased dynamicity 1.25-fold at minus ends, whereas it reduced dynamicity 7.4-fold at plus ends. Taken together the results demonstrate that at steady state, vinblastine modulates dynamics differently at the opposite microtubule ends.

**DISCUSSION**

We found in the present study that vinblastine exerted strikingly different effects on microtubule polymer mass and on dynamics at opposite microtubule ends. Specifically, vinblastine did not alter the rate or extent of growth or shortening at minus ends. In contrast, the drug strongly suppressed the rate and extent of growth and shortening at plus ends. In addition, vinblastine moderately increased the dynamicity at minus ends, while in contrast it strongly suppressed dynamicity at plus ends. Thus, with the minus end microtubules that persisted at 0.4 μM vinblastine, the overall dynamics were the
Differential Effects of Vinblastine at Opposite Microtubule Ends

**TABLE III**

|                  | Plus ends, frequency (min^-1) | Minus ends, frequency (min^-1) |
|------------------|-------------------------------|-------------------------------|
|                  | 0 | 0.1 | 0.2 | 0.4 | 0.6 | 0 | 0.1 | 0.2 | 0.4 | 0.6 |
| **Catastrophe**   | 0.31 ± 0.06 | 0.22 ± 0.06 | 0.17 ± 0.04 | 0.13 ± 0.03 | 0.10 ± 0.02 | 0.19 ± 0.05 | 0.42 ± 0.1 | 0.42 ± 0.09 | 0.35 ± 0.085 | ND |
| **Rescue**        | 0.78 ± 0.28 | 2.3 ± 0.64 | 1.62 ± 0.35 | 1.72 ± 0.38 | 1.5 ± 0.34 | 2.03 ± 0.58 | 1.44 ± 0.45 | 1.42 ± 0.41 | 1.69 ± 0.53 | ND |

Vinblastine decreased the catastrophe frequency and increased the rescue frequency at plus ends, transitions that appear to be due to the loss and gain of a stabilizing GTP or GDP-Pi cap (8, 41). Thus, vinblastine may increase the stability of the cap at plus ends. The binding of vinblastine to tubulin at plus ends may stabilize the cap by increasing the affinity of tubulin for itself (38). Alternatively, vinblastine may reduce the rate of cap loss by decreasing the rate of GTP hydrolysis and/or the subsequent rate of Pi release. The high affinity reversible binding of vinblastine to depolymerizing plus ends may increase the rescue frequency indirectly by lowering the rate of shortening, thus allowing more time for the recapping process to occur. Transient vinblastine binding to a depolymerizing microtubule end could directly stabilize tubulin-GTP addition by increasing the association of tubulin at the microtubule end through its ability to induce a stabilizing conformational change.

**FIG. 4. Effects of vinblastine on the rescue frequency per micrometer of length shortened at plus ends (squares) and minus ends (triangles).** The rescue frequencies were calculated by dividing the total number of rescues by the total shortening lengths for all microtubules. Error bars, S.D.

Vinblastine decreased the catastrophe frequency and increased the rescue frequency at plus ends, transitions that appear to be due to the loss and gain of a stabilizing GTP or GDP-Pi cap (8, 41). Thus, vinblastine may increase the stability of the cap at plus ends. The binding of vinblastine to tubulin at plus ends may stabilize the cap by increasing the affinity of tubulin for itself (38). Alternatively, vinblastine may reduce the rate of cap loss by decreasing the rate of GTP hydrolysis and/or the subsequent rate of Pi release. The high affinity reversible binding of vinblastine to depolymerizing plus ends may increase the rescue frequency indirectly by lowering the rate of shortening, thus allowing more time for the recapping process to occur. Transient vinblastine binding to a depolymerizing microtubule end could directly stabilize tubulin-GTP addition by increasing the association of tubulin at the microtubule end through its ability to induce a stabilizing conformational change.

This is known from previous studies that at low concentrations vinblastine binds reversibly and with relatively high affinity directly to at least one and perhaps both microtubule ends, without being incorporated into the core of the polymer (25). Binding of vinblastine to tubulin is also known to induce conformational changes in tubulin (34–37). Thus binding of vinblastine to tubulin at microtubule plus ends may inhibit growth by altering the tubulin conformation and lattice structure at the end in a manner that makes future addition of tubulin-GTP energetically unfavorable. The vinblastine molecule is large ($M_\text{r}$ 811), and an alternative possibility is that inhibition of growth occurs simply by steric hindrance at the microtubule end. The rate and extent of shortening at microtubule plus ends are probably reduced because vinblastine appears to strengthen longitudinal tubulin-tubulin interactions along protofilaments, making tubulin dissociation less favorable. In support of this hypothesis, vinblastine binding to soluble tubulin is known to induce an isodesmic self-association of tubulin (38, 39), which may contribute to formation of stabilized spiral protofilaments (23, 40).

Vinblastine decreased the catastrophe frequency and increased the rescue frequency at plus ends, transitions that appear to be due to the loss and gain of a stabilizing GTP or GDP-Pi cap (8, 41). Thus, vinblastine may increase the stability of the cap at plus ends. The binding of vinblastine to tubulin at plus ends may stabilize the cap by increasing the affinity of tubulin for itself (38). Alternatively, vinblastine may reduce the rate of cap loss by decreasing the rate of GTP hydrolysis and/or the subsequent rate of Pi release. The high affinity reversible binding of vinblastine to depolymerizing plus ends may increase the rescue frequency indirectly by lowering the rate of shortening, thus allowing more time for the recapping process to occur. Transient vinblastine binding to a depolymerizing microtubule end could directly stabilize tubulin-GTP addition by increasing the association of tubulin at the microtubule end through its ability to induce a stabilizing conformational change.

**How Might Vinblastine Destabilize Minus Ends?**—Tubulin in the core of the microtubule is believed to exist in a strained conformation (35, 36). It is possible that weak binding of vinblastine to low affinity sites at or near the minus ends induces a conformational change in the tubulin that exaggerates the strain and weakens lateral bonding between the protofilaments of microtubule thus promoting depolymerization (23, 24). Because of the reversibility of vinblastine binding to its high affinity sites at microtubule ends, it was not possible in the previous work to determine the distribution of the sites between the two ends. At least some of the high affinity sites must be at the plus ends because low concentrations of vinblastine stabilize these ends (27, 28). It is reasonable to think that the high affinity sites might be exposed exclusively at the plus ends of the microtubule but not at the minus ends. Vinblastine at low concentrations stabilizes plus ends but not minus ends. The maximum number of high affinity vinblastine sites at microtubule ends determined by extrapolation of binding data to infinitely high vinblastine concentration is small (16 sites per microtubule (25)); this value is within experimental error of the protofilament number in reconstituted microtubules in

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**FIG. 4. Effects of vinblastine on the rescue frequency per micrometer of length shortened at plus ends (squares) and minus ends (triangles).** The rescue frequencies were calculated by dividing the total number of rescues by the total shortening lengths for all microtubules. Error bars, S.D.
vitro (approximately 14–15 protofilaments (42)). In addition, the tubulin dimer has structural polarity and the microtubule ends differ kinetically. Whether the α or β subunit of the tubulin dimer is exposed at the plus end is not clear (9, 16). However if the β subunit is exposed at plus ends then the α subunit must be exposed at minus ends and vice versa. Thus the immediate surroundings of the vinblastine binding site will be different in the tubulin molecules located at the two ends, which could lead to differences in binding or to differences in the effects of vinblastine binding on tubulin-tubulin interactions.

Vinblastine increased the catastrophe frequency, and it may have slightly reduced the rescue frequency at minus ends. These results may explain why vinblastine preferentially depolymerized preformed microtubules and reduced microtubule formation at minus ends. A catastrophe is believed to occur upon loss of the stabilizing cap at a microtubule end, which may be due to loss of the last molecule of tubulin-GDP-Pi or tubulin GTP at the end. A rescue may occur by successful rebinding of one or more tubulin-GTP molecules at the end of a depolymerizing microtubule. Vinblastine may increase the catastrophe frequency at minus ends by increasing the probability of cap loss. The drug may collide transiently with the terminal tubulin cap at the minus end and, somehow, cause dissociation of the cap. Such an action is clearly opposite of the action of vinblastine at plus ends. The presence of vinblastine bound to low affinity sites along the microtubule surface during rapid shortening may decrease the probability of recapping and, thus, decrease the rescue frequency.

Implications for Cell Function—We previously found that mitosis in HeLa and BSC cells is blocked or slowed by low concentrations of vinblastine, with spindles that contain a normal mass of microtubules organized in a nearly normal bipolar manner (3, 6, 28, 43). We also found that vinblastine potently suppresses dynamics at the plus ends of microtubules both in vitro and in living cells (27, 28). These results suggest that at low concentrations vinblastine induces its powerful mitotic block by kinetically stabilizing the plus ends of mitotic spindle microtubules. The results presented here suggest that a destabilizing effect of vinblastine on the minus ends of spindle microtubules may also play a role in mitotic block by vinblastine. For example, alteration of minus end dynamics may be responsible for the observed disruption of the tight association between mother and daughter centrioles at the centrosomes or spindle poles of HeLa cells blocked in mitosis by low concentrations of vinblastine (6).

Centrosomes are the major microtubule nucleating centers in cells, and the minus ends of microtubules are tethered at the centrosomes. Vinblastine inhibited microtubule formation selectively at the minus ends at low drug concentrations, and it is possible that vinblastine may be mimicking the action of natural regulatory molecules in cells that suppress microtubule nucleation at centrosomes in a vinblastine-like manner. In support of this idea, a protein present in sea urchin egg extracts selectively inhibits microtubule assembly at minus ends by increasing the critical concentration at these ends (44).

Spindle microtubules are highly dynamic, and their rapid dynamics appear to be essential in mitosis (19, 20). In the present work we found that at 0.4 μm vinblastine the minus ends of microtubules were kinetically more dynamic than the plus ends. Such a reversal of dynamics at the opposite ends of spindle microtubules may alter the normal tension on kinetochores or the movements of motor molecules along microtubules, resulting in mitotic block. The mitotic block induced by low concentrations of vinblastine in HeLa cells results in cell death by apoptosis. Thus, the kinetic stabilization of mitotic spindle microtubule plus ends and destabilization of minus ends in the absence of overall changes in the spindle microtubule mass may be the most potent chemotherapeutic mechanism of vinblastine.

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