Interphase microtubule dynamics are cell type-specific

P. Wadsworth, University of Massachusetts - Amherst
M. McGrail, University of Massachusetts - Amherst
Interphase microtubule dynamics are cell type-specific

P. WADSWORTH\textsuperscript{1,2} and M. McGRAIL\textsuperscript{1}

\textsuperscript{1}Department of Zoology and \textsuperscript{2}Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, MA 01003, U.S.A.

Summary

The rate and pattern of microtubule polymer loss in interphase cells have been examined using nocodazole to block microtubule assembly. Cells were incubated with high concentrations of nocodazole for various times and the pattern of microtubule disassembly was determined using tubulin immunofluorescence. Polymer loss was quantitated by measuring the decrease in percentage of cell area occupied by microtubules. The results demonstrate that microtubules in diverse cells disassemble individually and asynchronously. In addition, these quantitative measurements reveal that epithelial and fibroblast cells display strikingly different kinetics of polymer loss. In fibroblasts, polymer loss is rapid, with a half-time of 4 min at 37°C. In epithelial cells, loss of 60% of the microtubules occurs with a half-time of 18 min; the remaining 40% of the microtubules disassemble much more slowly (average half-time of 72 min). To demonstrate that these differences were not due to species differences among various cells assayed in these experiments, epithelial and fibroblast cells derived from primary cultures of newt lung have been examined. Again, fibroblast and epithelial cell microtubule dynamics could be readily distinguished. To determine if modifications to epithelial cell microtubules contribute to their stability, microtubules were completely disassembled and allowed to regrow. The rate of polymer loss for recently regrown microtubules was more rapid than microtubules in control cells, indicating that stability increases with time after assembly.

Key words: interphase, microtubules, nocodazole.

Introduction

A variety of recent observations demonstrate that microtubules are dynamic polymers that continually assemble and disassemble in living cells (Saxton et al. 1984; Wadsworth and Salmon, 1986; Cassimeris et al. 1987). The majority of microtubules in a population continually elongate while a subset rapidly, or catastrophically, disassembles (Mitchison and Kirschner, 1984a, b). This behavior has been termed dynamic instability, and has been observed \textit{in vivo} as well as \textit{in vitro}. For example, direct imaging of microtubules, using DIC or darkfield microscopy, or imaging of fluorescent microtubules in living cells microinjected with labeled tubulin, demonstrates that individual microtubules grow and shrink (Horio and Hotani, 1986; Cassimeris et al. 1988; Sammak and Borisy, 1988; Schultz and Kirschner, 1988). However, only a very limited region of a cell is examined in these experiments, in particular the thin lamellar region of the cell. Numerous microtubules in cells examined in this way were not dynamic over the time course of the experiment, so their contribution to the kinetic behavior of the cell's microtubule population is not clear.

To date, few experiments have addressed the function and regulation of microtubule dynamic behavior in interphase cells. It has been suggested, however, that selective stabilization of microtubules may occur in various cells during morphogenesis (Kirschner and Mitchison, 1986). The modified microtubules might then be utilized in specific cellular functions. If this is the case, then the dynamic behavior of interphase microtubules may display cell type-specific characteristics.

In the following experiments, microtubule disassembly behavior has been examined in a variety of cell types using high concentrations of the drug nocodazole (Hoebeke et al. 1976) to block microtubule assembly. A novel quantitation technique has been developed that permits rapid measurement of microtubule disassembly kinetics for the population of a cell's microtubules. Our results clearly demonstrate that microtubule polymer loss is cell type-specific. In addition, these quantitative measurements of microtubule polymer loss can now be utilized to monitor changes in microtubule turnover during morphogenetic events in various cells and tissues. Portions of this work have been reported previously in abstract form (Nison et al. 1988).

Materials and methods

Cell culture
All established cell lines were obtained from the American Type
Culture Collection and grown at 37°C in the following media all containing 10% fetal bovine serum: PtK₁, Ham's F-10; MDCK, modified Eagle's medium; CHO, Ham's F-12; 3T3, BSC-1, Dulbecco's modified Eagle's medium. Chick embryo fibroblasts were prepared from 10-day-old chick embryos (Paul, BSC-1, Dulbecco's modified Eagle's medium. Chick embryo Culture Collection and grown at 37°C in the following media all grown as previously described (Wadsworth and Salmon, 1986).

Immunofluorescence
Cells were rinsed in saline and then incubated in 20 μM nocodazole (Sigma Chemical Co., St Louis, MO) at 37°C for various intervals and before processing for immunofluorescence. Cells were lysed in buffer containing 80 mM-Pipes, 5 mM-EGTA, 1 mM-MgSO₄ and 0.5% Triton X-100, pH 6.9 (Cassimeris et al. 1986), for 20 s. Cells were then fixed for 15 min in 2% paraformaldehyde, 0.1% glutaraldehyde in PBS pH 7.3, and rinsed in PBS containing 0.1% Tween and 0.02% azide (PBS-Tw-AZ). Cells were incubated for 2 h with 40 μl of anti-tubulin monoclonal antibodies (generous gift from Dr J. R. McIntosh), rinsed extensively in PBS-Tw-AZ and incubated with a rhodamine-conjugated secondary antibody (Organon Teknika, West Chester, PA) for 30 min. Antibody staining was performed in humid chambers at room temperature; antibodies were diluted in PBS-Tw-AZ containing 1% bovine serum albumin (BSA). Coverslips were mounted in 5% «-propyl gallate. For regrowth experiments, cells were incubated in 4 μM-nocodazole for 4 h, rinsed and allowed to regrow in inhibitor-free medium for various intervals. Disassembly was then examined in cells incubated in 20 μM-nocodazole for 20 min.

Microscopy
Cells were imaged using a 63× objective lens, NA 1.4, on a Zeiss IM-35 microscope equipped for epifluorescence. Cells were photographed using TMAX 400 film developed in HC110 dilution B. For quantitative measurements, cells were imaged using a Nikon fluorescence microscope with a 60× lens, NA 1.4. Images were collected and processed using the BioRad MRC 500 confocal scanning microscope. The pin-hole was fully open for these measurements and 30 frame running averages were collected. The MRC 500 software package was then used to quantitate the area occupied by microtubules in each cell. The area of the cell was estimated in two dimensions by outlining the circumference of the cell using a mouse and the area function of the software package. The area occupied by microtubules within each outlined cell was then determined using the band function. With this function, structures of a particular intensity range can be selected and highlighted. Because these images are collected using an averaging technique, little background fluorescence was present and the microtubules were the brightest objects in the field. A cutoff intensity was selected so that all the microtubules were highlighted (selected) but no nuclear or diffuse staining was included. The highlighted objects appear in color on the monitor. The intensity of the appropriate intensity cutoff value can be selected, by highlighting only the structures of interest. The computer then calculates the area occupied by the highlighted structures within the cell's area. In control experiments, a test object was used to vary the number of objects in a constant area. A linear relationship between the % of the area that was highlighted and the area of the highlighted objects was obtained. This area technique was particularly useful because variations in the overall staining intensity from day to day did not effect the results, provided that the microtubules were brighter than the background. For fibroblasts, the majority of microtubules were close to the substratum, so this focal plane was selected. In the thicker epithelial cells microtubules were distributed more uniformly throughout the cell, so a variable proportion of the microtubules were not in focus. It was possible, however, to select a focal plane that included the majority of microtubules in each cell. Because not every microtubule is included in the selected plane of focus, and because microtubules overlap, our measurements may be an underestimate of the actual number of microtubules, especially in thicker cells. To determine half-times, the natural log of the normalized % area occupied by microtubules was plotted as a function of time. The half-time was determined using the relationship: \( t_1/2 = \ln 2/k \) where \( k \) is the first-order rate constant.

Microinjection of biotin–tubulin
Biotin-tubulin was prepared as previously described (Mitchison et al. 1986; Wadsworth et al. 1989). Cells were pressure microinjected using an Eppendorf microinjector and Narishige micromanipulator; pipettes were pulled using a Sutter Instruments micropipette puller and back loaded using a Hamilton syringe (see Wadsworth and Salmon, 1986; Wadsworth et al. 1989, for details). Following injection cells were rinsed in saline, lysed and fixed as described above. Cells were double-labeled using rabbit anti-biotin antibodies (Enzo Biochemicals, NY), followed by goat anti-rabbit and rabbit anti-goat fluorescein-labeled secondary antibodies. Finally, cells were labeled for total tubulin as described above.

Stained, injected cells were photographed and the lengths of the labeled segments were measured by enlarging the photographic negative and tracing the biotin-labeled segments. Alternatively, negatives were projected onto a digitizing pad coupled to a PC and measured using appropriate software functions.

Results
Rate and pattern of microtubule disassembly
When cells are incubated with high concentrations of nocodazole, microtubule assembly is abruptly blocked and the rate and pattern of disassembly can be analyzed (Cassimeris et al. 1986). In the following experiments, cultured mammalian cells have been incubated with 20 μM-nocodazole at 37°C for various times and the pattern of microtubule disassembly revealed using anti-tubulin immunofluorescence. The results of experiments using two epithelial cell lines, MDCK and PtK₁, and CHO fibroblasts are shown in Fig. 1. In all cases, microtubules disassembled individually and asynchronously; in no case has a wave of synchronous disassem-
Interphase microtubule dynamics
bly, for example from the periphery to cell center, been observed. As seen in Fig. 1, microtubule density decreased relatively uniformly throughout the cell during incubation in nocodazole for all cell lines examined: MDCK, PtK₁, CHO (Fig. 1) and BSC-1 and 3T3 (data not shown). In CHO and CEFs (see below), nearly all of the cell's microtubules were completely disassembled following 20 min in 20 μM-nocodazole. Remaining microtubules were usually straight and often centrosomal. In epithelial cells a very different pattern was observed. Numerous microtubules remained after a 20-min incubation in nocodazole, and many of these microtubules were wavy and peripheral to the cell centrosome (see Bre et al. 1987). Incubation for longer periods, however, did result in complete microtubule disassembly (see below), demonstrating that these microtubules do disassemble, but with slower kinetics (see Fig. 1).

Microtubule disassembly has also been measured in primary cultures of chick embryo fibroblasts (CEF). As shown in Fig. 2, microtubule disassembly in these cells was very similar to that in fibroblasts growing in continuous culture: microtubule disassembly was rapid and nearly complete within a 20-min incubation period. The remaining microtubules were straight and frequently centrosomal, as observed in CHO cells.

One possible explanation for these observations is that the average microtubule length is greater in epithelial cells than fibroblasts, and that longer microtubules require more time to disassemble. Although it is difficult to estimate the average microtubule length in these cells, numerous microtubules extended from the perinuclear region to the periphery in all cell types. On average, epithelial cells were somewhat larger than fibroblasts, although the fibroblasts often displayed a more extended morphology. Thus, the slow loss of polymer in epithelial cells is probably not simply due to an increase in average microtubule length.

Quantitative analysis of microtubule disassembly
These initial observations demonstrate that microtubule disassembly is cell type-specific, but quantitative information concerning the kinetics of disassembly could not be readily or accurately obtained from visual inspection of the microtubule staining pattern. To obtain quantitative information, a novel technique has been developed. Briefly, the microtubule staining pattern was digitally imaged, using frame averaging to reduce non-specific background staining, and then the percentage of the cell's area occupied by microtubules was determined from these images (see Materials and methods).

The results of these quantitative measurements are shown in Figs 3 and 4. In fibroblasts, the average area occupied by microtubules in control cells was 68%. In the presence of 20 μM-nocodazole, a rapid and complete disassembly of the microtubules is apparent. Semi-log plots of the data reveal a half-time disassembly of 4 min...
for the microtubule population in CHO and CEF cells (Fig. 3A,B).

In epithelial cells, the average percentage of area occupied by microtubules in control cells was 85%. In these cells, the kinetics of disassembly were more complex, as suggested by previous experiments (see Shultze and Kirschner, 1986, 1987). About 60% of the microtubules were disassembled during a 20-min incubation in nocodazole (Fig. 4A,B). When longer periods of time were analyzed, complete disassembly was observed (Fig. 4). The half-time for disassembly of the more rapid population is 18 min, about fourfold slower than the microtubule population in fibroblasts. The more-stable microtubules, which represent about 40% of the microtubules in these cells, disassemble with a half-time of 72 min (Fig. 4, insets).

**Microtubule disassembly in primary cultures of newt lung cells**

Although these data clearly demonstrate that the rate of polymer loss is cell type-specific, the observed variation could be due to differences in the tissue or species sources of these various cell lines. In addition, cell type-specific features of cells are often lost in continuous culture (Zuk et al. 1989), and this could influence the results from established cell lines. To address these concerns, primary cultures of newt lung tissue have been prepared. In these cultures, ciliated epithelial sheets grow from the tissue explant. In addition, some cultures contain motile fibroblasts, which become dispersed over the surface of the coverslip. Immunofluorescence observations of these cells confirm our observations: fibroblasts contain fewer microtubules and these microtubules disassemble more rapidly than microtubules in cells within the epithelial sheet (Fig. 5). In addition, the microtubules in epithelial cells were frequently curved while those in fibroblasts were straight. Thus, cells in primary cultures derived from a single species and tissue display differences in microtubule disassembly behavior. These observations were not quantitated, owing to the small number of cells available.

**Microtubule disassembly following regrowth**

Careful examination of epithelial cells incubated with nocodazole for 10 or 20 min revealed that daughter cells, identified by the presence of a midbody, contained fewer microtubules than cells lacking a midbody (Fig. 6). This observation indicated that the newly assembled interphase microtubule array was more labile than an older interphase array, and suggested that time-dependent changes to the interphase microtubule network contrib-
Fig. 5. Immunofluorescence micrographs of the pattern of microtubule disassembly in primary cultures of newt lung cells. Microtubule disassembly in 20 μM-nocodazole was examined in epithelial sheets (A, B) and false sheets (fibroblasts; C, D). A, C, Controls; B, D, 20 μM-nocodazole for 20 min.

Fig. 6. Immunofluorescence micrographs of the pattern of microtubule disassembly in daughter cells. A. MDCK, and B. PtK1 cells that have been incubated with 20 μM-nocodazole for 10 min; note that neighboring cells contain more microtubules than cells joined by a mid-body.

To test whether time-dependent modifications of epithelial cell microtubules contribute to the slow kinetics of polymer loss, the following experiment was performed. Microtubules were totally disassembled by incubating MDCK cells in 4 μM-nocodazole for 4 h. When cells were incubated with higher concentrations of nocodazole for long times the effect of the drug was not as reversible as when lower concentrations were used (McGrail and Wadsworth, unpublished data). After total disassembly, the nocodazole was removed and microtubules
Table 1. Microtubule disassembly following regrowth in MDCK cells

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>% of cell area occupied by MTs after 20 min in 20 μM-nocodazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85±3</td>
</tr>
<tr>
<td>2h regrowth</td>
<td>84±7</td>
</tr>
<tr>
<td>60 min regrowth</td>
<td>80±7</td>
</tr>
<tr>
<td>30 min regrowth</td>
<td>76±5</td>
</tr>
</tbody>
</table>

allowed to regrow for various times at 37°C. The regrown microtubules were then examined in the nocodazole disassembly assay (Table 1). When cells were allowed 2 h for recovery, the percentage area occupied by microtubules was 84%, similar to that observed in control preparations, and they displayed disassembly kinetics that were nearly indistinguishable from endogenous microtubules in these cells. However, when microtubule disassembly kinetics were examined at time points before regrowth was complete, the partially regrown microtubules disassembled more rapidly than microtubules in control preparations (Table 1).

Microtubule assembly measurements

Microtubule assembly has been examined in epithelial and fibroblastic cells by microinjection of biotin–tubulin (Mitchison et al. 1986; Wadsworth et al. 1989) and measurement of the rate of individual microtubule elongation (Schultze and Kirschner, 1986). An example of the pattern of microtubule elongation in CHO cells is shown in Fig. 7. Numerous segments of new microtubule growth are visible; these microtubule segments elongate with time (Fig. 7). At short times following injection of biotin–tubulin and incubation at room temperature no difference in the rate of microtubule elongation in epithelial and fibroblastic cells was detected (Table 2). Cells were also examined following a 5-min post-injection incubation at 37°C. In CHO cells, all of the cells' microtubules appear to have fully incorporated the biotin–tubulin subunits. In PtK₁ cells some unlabeled microtubules could be detected in many cells, although in some cells all the microtubules appeared fully labeled. Because of the density of labeled microtubules in these cells, quantitative measurements of the number of unlabeled microtubules were not attempted.

Discussion

The experiments reported here demonstrate that microtubule disassembly in a variety of established cell lines and primary cell cultures occurs on an individual micro-

---

**Fig. 7.** Microtubule assembly in CHO fibroblasts. Cells were injected with biotin–tubulin and stained with anti-tubulin (A,C) or anti-biotin (B,D) antibodies. Cells were fixed 70 s (A,B) or 120 s (C,D) post-injection. Bar, 10 μm.
tubule basis as previously reported for other cell types (Cassimeris et al. 1986, 1988; Schultz and Kirschner, 1988; Sammak and Borisy, 1988). A uniform wave of microtubule disassembly, from the periphery towards the cell center or from the center towards the periphery, was not detected in these experiments. Rather, the density of microtubules gradually decreased throughout the entire cytoplasm in all cells examined. These observations are consistent with recent observations of microtubule dynamics in living cells, which reveal the individual and stochastic nature of microtubule behavior.

Differences in the appearance of the microtubules during disassembly were apparent in the different cell types examined. Microtubules in fibroblasts were frequently straight, while epithelial cell microtubules were often wavy or curved (Bre et al. 1987). While this difference in the morphology of the microtubules could be detected in control cells, it was more obvious in cells in which partial disassembly had occurred, because individual microtubules could be more easily resolved. In addition, although microtubules were lost relatively uniformly throughout the cytoplasm, those microtubules that remained after 20 min in nocodazole in epithelial cells were often periferal and non-centrosomal. In fibroblasts, however, more of the microtubules that remained after partial disassembly were centrosomal. This pattern is consistent with recent observations (Bre et al. 1987) describing numerous non-centrosomal microtubules in MDCK cells and few in Vero (fibroblast) cells. One possible explanation for this morphological difference between microtubules in epithelial and fibroblastic cells is that traction forces produced during cell motility influence the arrangement of microtubules (Harris, 1987). In addition, our preliminary observations suggest that a more dynamic microtubule network correlates with more rapid cellular locomotion (Wadsworth, unpublished observations).

A striking result of these experiments is that the rate of disassembly for the population of microtubules is cell type-specific. Visual inspection of the microtubule staining pattern of various cells during disassembly clearly reveals this difference (see also Bre et al. 1987). Quantitative analysis of the rate of polymer loss further substantiates this observation. In fibroblasts, polymer loss occurs with a half-time of 4 min, while in epithelial cells two populations of microtubules could be distinguished kinetically. The rate of polymer loss from each of these two classes was slower than microtubules in fibroblasts, with average half-times of 18 and 72 min.

Other recent observations have also suggested that the rate and pattern of microtubule polymer loss vary in different cells (Bre et al. 1987; Khawaja et al. 1988), but a systematic analysis has not been reported. In most experiments, only one or two cell lines were analyzed, and often using only one or two time-points during disassembly. In addition, results from various laboratories are difficult to compare directly because experiments were performed using different incubation times and different concentrations of nocodazole. To overcome these problems, a variety of cells was examined throughout the time course of microtubule disassembly. In addition, we chose to analyze disassembly using a high concentration of nocodazole (20 μM), so that more than sufficient nocodazole was present to bind the free tubulin in these cells (Hiller and Weber, 1978). In addition, experiments in which lower concentrations of nocodazole were used (2 and 5 μM, data not shown) gave similar results, indicating that nocodazole was not limiting in the experiments presented here.

Our analysis of disassembly further reveals that interphase microtubules, in all cells examined thus far, could be completely disassembled if sufficiently long incubations in nocodazole were utilized. Thus, even though many microtubules remain in epithelial cells following a 20-min incubation in nocodazole, these microtubules are not permanently stabilized. Rather, our results demonstrate that complete disassembly occurs after a 4-h treatment with nocodazole. This observation demonstrates that the relative stability of microtubules varies for different cell types.

The nature of cellular factors that regulate the kinetics of microtubule dynamic behavior are not known. One possibility is that the more-persistent microtubules contain elevated levels of post-translationally modified forms of alpha-tubulin. Various reports have indicated that a subset of microtubules in cultured cells contain tyrosinated alpha-tubulin, and that these microtubules are more resistant to nocodazole-induced disassembly (Bulinski et al. 1988). However, additional recent experiments reveal that, although tyrosination of alpha-tubulin is correlated with increased microtubule stability, it is not sufficient for increased stability (Khawaja et al. 1988). A second modified form of alpha-tubulin, acetylated alpha-tubulin, has also been implicated in enhanced microtubule stability (Le Dizet and Piperno, 1986). However, PtK1 cells, which contain many microtubules after 20 min in nocodazole (Fig. 3), do not contain detectable levels of acetylated alpha tubulin (Schulze et al. 1987; Wadsworth and McGrail, unpublished observations) while both CHO and CEF fibroblasts do (Schulze et al. 1987; Wadsworth and McGrail, unpublished observations). Thus, factors other than, or in addition to, these post-translational modifications must act to modify the rate of microtubule polymer loss.

An alternative possibility is that microtubule-associated proteins (MAPs) could regulate microtubule dynamic behavior. For example, experiments utilizing MAP2 in vitro have demonstrated that this MAP can increase microtubule stability by decreasing the k_diss for tubulin (Burns et al. 1984). Furthermore, the stabilizing

### Table 2. Rate of microtubule elongation in vivo determined by injection of biotin–tubulin

<table>
<thead>
<tr>
<th>Cell type</th>
<th>No. cells analyzed</th>
<th>Pipe-</th>
<th>Rate of elongation at 22°C (μm min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>3</td>
<td>0.3</td>
<td>1.45±0.3</td>
</tr>
<tr>
<td>PtK1</td>
<td>9</td>
<td>3.0</td>
<td>1.68±0.2</td>
</tr>
</tbody>
</table>

Other recent observations have also suggested that the rate and pattern of microtubule polymer loss vary in different cells (Bre et al. 1987; Khawaja et al. 1988), but a systematic analysis has not been reported. In most experiments, only one or two cell lines were analyzed, and often using only one or two time-points during disassembly. In addition, results from various laboratories are difficult to compare directly because experiments were performed using different incubation times and different concentrations of nocodazole. To overcome these problems, a variety of cells was examined throughout the time course of microtubule disassembly. In addition, we chose to analyze disassembly using a high concentration of nocodazole (20 μM), so that more than sufficient nocodazole was present to bind the free tubulin in these cells (Hiller and Weber, 1978). In addition, experiments in which lower concentrations of nocodazole were used (2 and 5 μM, data not shown) gave similar results, indicating that nocodazole was not limiting in the experiments presented here.

Our analysis of disassembly further reveals that interphase microtubules, in all cells examined thus far, could be completely disassembled if sufficiently long incubations in nocodazole were utilized. Thus, even though many microtubules remain in epithelial cells following a 20-min incubation in nocodazole, these microtubules are not permanently stabilized. Rather, our results demonstrate that complete disassembly occurs after a 4-h treatment with nocodazole. This observation demonstrates that the relative stability of microtubules varies for different cell types.

The nature of cellular factors that regulate the kinetics of microtubule dynamic behavior are not known. One possibility is that the more-persistent microtubules contain elevated levels of post-translationally modified forms of alpha-tubulin. Various reports have indicated that a subset of microtubules in cultured cells contain tyrosinated alpha-tubulin, and that these microtubules are more resistant to nocodazole-induced disassembly (Bulinski et al. 1988). However, additional recent experiments reveal that, although tyrosination of alpha-tubulin is correlated with increased microtubule stability, it is not sufficient for increased stability (Khawaja et al. 1988). A second modified form of alpha-tubulin, acetylated alpha-tubulin, has also been implicated in enhanced microtubule stability (Le Dizet and Piperno, 1986). However, PtK1 cells, which contain many microtubules after 20 min in nocodazole (Fig. 3), do not contain detectable levels of acetylated alpha tubulin (Schulze et al. 1987; Wadsworth and McGrail, unpublished observations) while both CHO and CEF fibroblasts do (Schulze et al. 1987; Wadsworth and McGrail, unpublished observations). Thus, factors other than, or in addition to, these post-translational modifications must act to modify the rate of microtubule polymer loss.

An alternative possibility is that microtubule-associated proteins (MAPs) could regulate microtubule dynamic behavior. For example, experiments utilizing MAP2 in vitro have demonstrated that this MAP can increase microtubule stability by decreasing the k_diss for tubulin (Burns et al. 1984). Furthermore, the stabilizing
effect of MAP2 is modified by the extent of MAP2 phosphorylation (Burns et al. 1984; Murthy and Flavin, 1983). However, in recent experiments, microinjection of fluorescent MAP2 did not stabilize cellular microtubules (Olmsted et al. 1989), although microinjection of another brain MAP, tau protein, into cultured cells did stabilize microtubules to nocodazole-induced disassembly (Drubin and Kirschner, 1987). Less is known about MAPs in cultured cells (Olmsted, 1986), but it is likely that they have a similar range of effects on microtubules as brain MAPs. If this is the case, then changes in the quantity or phosphorylation state of stabilizing MAPs could account for cell type-specific differences in microtubule turnover. Our data also reveal that the kinetics of polymer loss in epithelial cells varies with the age of the microtubules: the rate of polymer loss for recently regrown microtubules was more rapid than for older microtubules. Perhaps the accumulation and/or modification of cell-type specific MAPs contributes to this increased stability.

The rates of polymer loss reported here can be compared with other recent measurements of the dynamic behavior of the interphase microtubule population. For example, approximately 80% of the microtubules in the interphase network of fibroblasts were labeled within 15 min of injection of labeled tubulin subunits (Schulze and Kirschner, 1986). This corresponds to an estimated half-time for turnover of 5–10 min, similar to the half-times reported here for fibroblasts. Using photobleaching methods, a half-time for microtubule turnover of 4.5 min in the interphase array of PtK1 cells has been measured (Saxton et al. 1984), which is much faster than our values obtained from disassembly measurements. However, since the recovery of fluorescence in these cells was incomplete, turnover of the more-persistent microtubules may not be measured in this value. In human monocytes, a half-time for nocodazole-induced disassembly of 36 s has been calculated (Cassimeris et al. 1986). In these cells all of the microtubules are centrosomal and are much shorter than the microtubules observed in the cultured cells used here. Although these morphological differences are likely to contribute to the very rapid disassembly observed in these experiments (Cassimeris et al. 1986), these data also strongly suggest that blocking assembly with nocodazole can reveal the intrinsic rate of microtubule disassembly.

Real-time observations of individual microtubule dynamics have also been reported recently. In cells injected with fluorescent tubulin, similar average growth rates were measured for BSC-1 (epithelial-like) and PtK1 cells (4.5–5.0 μm min⁻¹), although a broad range of rates was noted (0.4–12.5 μm min⁻¹) (Schulze and Kirschner, 1988). Using DIC optics to image microtubules directly in newt lung epithelial cells (NLCs) in real time, less variation, and a faster rate of elongation, have been reported (7.2 μm min⁻¹; Cassimeris et al. 1988). A bimodal distribution of depolymerization rates was observed in PtK1 and BSC-1 cells; (mean rates of 7.4 and 14.0 μm min⁻¹); also a faster average rate of disassembly was measured from direct observation of NLC microtubules (17.3 μm min⁻¹; Cassimeris et al. 1988).

Although the very rapid kinetics of individual microtubule behavior in a variety of different cell types are in conflict with the slow, cell type-specific rates of polymer loss measured here, differences in the assays most likely account for the disparate results. In the disassembly assay used here, average microtubule disassembly behavior throughout the entire cell is measured. Our measurements include areas other than the leading lamella, which is known to have more dynamic microtubules than elsewhere in the cell (Sammak et al. 1987), and our measurements include the more-persistent microtubules, which are not observed to grow or shrink when individual microtubule behavior is monitored for short periods (Schulze and Kirschner, 1988; Sammak and Borisy, 1988; Cassimeris et al. 1988). Thus individual microtubules are likely to disassemble more rapidly than our population measurements indicate. In fact, the similar rates of disassembly measured in vitro and by direct observation of NLCs suggest that individual microtubule depolymerization may occur at a similar rate in diverse cells (Walker et al. 1988; Cassimeris et al. 1988). Together, these observations suggest that the rate obtained in the population assay reflects the probability that a microtubule will initiate disassembly (the frequency of catastrophe), and not the rate of individual microtubule disassembly (Walker et al. 1988). Our results revealing similar growth rates for microtubules in different cell types also suggest that cells may modulate dynamics by modulating the frequency of transitions from growing to shrinking, not the rate of individual microtubule growth. This explanation is only valid, however, if nocodazole blocks assembly without altering other aspects of in vitro microtubule dynamics such as the frequencies of rescue and catastrophe. A variety of experiments, both in vitro and in vivo, indicate that nocodazole blocks microtubule assembly (Hoebeke et al. 1976), but direct measurements of the action of nocodazole on individual microtubule dynamics have not been reported. Therefore, the possibility that the differential response of various cells to nocodazole might be due to additional factors cannot be eliminated.

In summary, our data demonstrate that the dynamic behavior of the microtubule population in fibroblasts differs from that in epithelial cells. Thus, our data strongly suggest that individual cell types must modulate microtubule dynamics in specific ways.

The expert technical assistance of Laura Chen is gratefully acknowledged. We also thank M. Nison, who contributed greatly to the initial phase of the project, and Dr D. Asai for the gift of anti-acetylated tubulin antibodies. We thank our colleagues E. Connor, E. Shelden and R. Weis for comments and suggestions, and L. Cassimeris for a critical reading of the manuscript.

References


(Received 2 August 1989 – Accepted 25 September 1989)