

# Observation and Quantification of Individual Microtubule Behavior In Vivo: Microtubule Dynamics Are Cell-Type Specific

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**Abstract.** Recent experiments have demonstrated that the behavior of the interphase microtubule array is cell-type specific: microtubules in epithelial cells are less dynamic than microtubules in fibroblasts (Pepperkok et al., 1990; Wadsworth and McGrail, 1990). To determine which parameters of microtubule dynamic instability behavior are responsible for this difference, we have examined the behavior of individual microtubules in both cell types after injection with rhodamine-labeled tubulin subunits. Individual microtubules in both cell types were observed to grow, shorten, and pause, as expected. The average amount of time microtubules remained within the lamellae of CHO fibroblasts, measured from images acquired at 10-s intervals, was significantly shorter than the average amount of time microtubules remained within lamellae of PtK<sub>1</sub> epithelial cells. Further analysis of individual microtubule behavior from images acquired at 2-s intervals reveals that microtubules in PtK<sub>1</sub> cells undergo multiple brief episodes of growth and shortening, resulting in little overall change in the microtubule network. In contrast, microtubules in lamellae of CHO fibroblasts are observed to undergo fewer transitions which are of longer average duration, resulting in substantial changes in the microtubule network over

time. A small subset of more stable microtubules was also detected in CHO fibroblasts. Quantification of the various parameters of dynamic instability behavior from these sequences demonstrates that the average rates of both growth and shortening are significantly greater for the majority of microtubules in fibroblasts than for microtubules in epithelial cells ( $19.8 \pm 10.8 \mu\text{m}/\text{min}$ ,  $32.2 \pm 17.7 \mu\text{m}/\text{min}$ ,  $11.9 \pm 6.5 \mu\text{m}/\text{min}$ , and  $19.7 \pm 8.1 \mu\text{m}/\text{min}$ , respectively). The frequency of catastrophe events (1/interval between catastrophe events) was similar in both cell types, but the frequency of rescue events (1/time spent shrinking) was significantly higher in PtK<sub>1</sub> cells. Thus, individual microtubules in PtK<sub>1</sub> lamellae undergo frequent excursions of short duration and extent, whereas most microtubules in CHO lamellae undergo more extensive excursions often resulting in the appearance or disappearance of microtubules within the field of view. These observations provide the first direct demonstration of cell-type specific behavior of individual microtubules in living cells, and indicate that these differences can be brought about by modulation of the frequency of rescue. These results directly support the view that microtubule dynamic instability behavior is regulated in a cell-type specific manner.

**M**ICROTUBULES are dynamic polymers that alternate stochastically between periods of assembly and rapid disassembly (Cassimeris et al., 1988; Horio and Hotani, 1986; Mitchison and Kirschner, 1984; Sammak and Borisy, 1988a; Schulze and Kirschner, 1988; Walker et al., 1988). This behavior, termed dynamic instability (Mitchison and Kirschner, 1984), has been directly observed both in living cells (Cassimeris et al., 1988; Sammak and Borisy, 1988a; Schulze and Kirschner, 1988) and in vitro using microtubules nucleated from axonemes or centrosomes (Belmont et al., 1990; Walker et al., 1988). Quantification of the various parameters of dynamic instability, however, reveals striking differences between microtubule dynamic behavior in vivo and in vitro. First, microtubules are rarely seen to pause in vitro (Walker et al., 1988), whereas microtubules in vivo have been observed to remain

in a metastable state, during which neither growth or shrinking can be observed, for many minutes (Sammak and Borisy, 1988a; Schulze and Kirschner, 1988). Second, the average rate of microtubule growth is significantly higher when measured in living cells than when measured at similar tubulin concentrations in vitro (Cassimeris et al., 1988). Finally, the transition from growing to shrinking, catastrophe, and the reverse transition, rescue, occur much more frequently in vivo than in vitro (Walker et al., 1988). These differences are likely to reflect the complex environment found in living cells and further indicate that analysis of microtubules in living cells is necessary to fully understand the mechanisms which cells use to regulate the dynamic behavior of microtubules.

Experiments in which the behavior of the population of microtubules in living cells is measured further reveal that

microtubule dynamic behavior is cell-type specific. For example, when rhodamine-labeled tubulin subunits are injected into living cells and the resulting fluorescent microtubule array photobleached (Pepperkok et al., 1990), fluorescence is recovered in fibroblasts much more quickly than in confluent epithelial cells. Other studies in which microtubules in different cell types are exposed to the microtubule destabilizing drug nocodazole have shown that disassembly of the microtubule population is more rapid in fibroblasts than in epithelial cells (Wadsworth and McGrail, 1990). Interestingly, in both studies two kinetically distinct populations of microtubules are detected in epithelial cells but not in fibroblasts. Although these previous studies have demonstrated that microtubule populations in fibroblasts are more labile than those found in epithelial cells, the behavior of individual microtubules was not examined. Therefore, these results do not reveal which aspects of individual microtubule dynamic instability behavior are regulated to generate the observed cell-type specific behavior of the microtubule population. Finally, although microtubules have been directly observed *in vivo* in both epithelial cells and fibroblasts (Cassimeris et al., 1988; Sammak and Borisy, 1988a); Schulze and Kirschner, 1988), these direct observations have failed to detect cell-type specific regulation of microtubule dynamic behavior.

To determine which parameters of individual microtubule dynamic instability behavior are regulated to produce cell-type specific differences in microtubule population behavior, we have injected rhodamine-labeled tubulin subunits into PtK<sub>1</sub> epithelial cells and CHO fibroblasts (Wadsworth and McGrail, 1990) and examined individual microtubules within thin lamellar regions at intervals of 2 or 10 s. Quantitative analysis confirms that individual microtubules in fibroblasts turnover more rapidly than in epithelial cells, as measured by the average amount of time microtubules remain within an approximately equivalent field of view. The average rates of microtubule growth and shortening were also found to be significantly higher in fibroblasts. The frequency of rescue is higher in PtK<sub>1</sub> epithelial cells than CHO fibroblasts, whereas the frequency of catastrophe is similar in both cell types. In addition, a small number of more stable microtubules was detected in CHO fibroblasts; such meta-stable microtubules have been reported previously from a wide variety of cell sources (Cassimeris et al., 1988; Sammak and Borisy, 1988a; Schulze and Kirschner, 1988). These results provide the first direct demonstration of cell-type specific behavior of individual microtubules *in vivo* and indicate that differences in interphase microtubule dynamics may be brought about by regulating the frequency of rescue events.

## Materials and Methods

### Cell Culture and Microinjection

PtK<sub>1</sub> epithelial cells and CHO fibroblasts were cultured as described previously (Wadsworth and McGrail, 1990), plated on glass coverslips, and allowed to grow for a minimum of 48 h before use. Cells at the margin of subconfluent cell monolayers were injected with rhodamine-labeled tubulin prepared as described by Vigers (Vigers et al., 1988), using injection techniques described previously (Wadsworth et al., 1989). After microinjection, cells were returned to a 37°C incubator for at least 1 h before observation.

### Microscopy and Image Acquisition

Cells were held in a Rose chamber (Rose, 1958) modified as described previously (Shelden and Wadsworth, 1992), and examined using a Zeiss IM-35 inverted microscope. All observations were made at 35–37°C; temperature was maintained as described previously (Shelden and Wadsworth, 1992). Cells were observed using a Nikon 100× 1.4 NA objective lens, and epifluorescent illumination was provided with a 100-W mercury arc lamp. To obtain the greatest light transmission, several objective lenses were tested before the lens used in this study was selected. Illumination from the mercury arc lamp was filtered using a narrow band width Zeiss filter cube for rhodamine excitation/emission, and greatly attenuated by defocusing the collector lens and placing neutral density filters in the illumination pathway. Illumination intensity was measured using a photodetector (IR Industries Inc., Waltham, MA) and locally constructed circuitry, and was typically 0.007 mW/cm<sup>2</sup>. For experiments in which the interval between images was ≥10 s, the epifluorescent illumination was shuttered using Uniblitz shutters controlled by a Masscomp computer to further reduce the total amount of illumination received by the cell under observation. A small amount of ascorbic acid (1 mM final concentration) was added to the cell culture medium to increase the resistance of living cells to photodamage (Borisy, G., personal communication). Finally, the field diaphragm of the microscope was reduced to ~150 μm<sup>2</sup> to further reduce the total amount of illumination received by each cell. Images were collected using an DAGE ISIT video camera operated at full gain and sensitivity settings, digitized using the Masscomp computer, and stored, along with the time and date, on optical discs using a Panasonic optical memory disc recorder (OMDR). 32 video frames (~2 s) were averaged before the final image was written to the OMDR. Unfortunately, the sensitivity of the ISIT camera increases slowly when an image is first placed on the camera faceplate. Thus, for observations made at 10-s intervals, a short delay (typically 0.3–0.5 s) between illumination of the specimen and the start of image acquisition was necessary. For examination at 2-s intervals, cells were observed using continuous illumination for several minutes, and images were collected using a 32-frame running average. After some observation periods, cells were held on the microscope stage without illumination for several minutes and then subsequently reexamined. No long-term change in cell morphology or microtubule behavior resulting from the original observation period was detected using these methods (data not shown).

### Quantification and Analysis of Microtubule Dynamics

Microtubule dynamic behavior was quantified using data generated from a locally written computer program running on the Masscomp computer, and further analyzed using software written within the lab. Microtubules in sequential images were traced using a mouse-driven cursor, and the computer maintained a record of each trace, the interval between sequential images, and any microtubule length changes which occurred between images. During quantification of these image sequences, microtubule traces from a previous image could be overlaid onto the current image to facilitate the identification of individual microtubules from one image to the next. Only microtubules that could be clearly distinguished as single microtubules (based on fluorescent intensity and behavior; Sammak and Borisy, 1988) were analyzed in these experiments.

The average amount of time microtubules spent within lamellar regions (lifespan) was measured from images obtained at 10-s intervals using intermittent illumination. To minimize the possible effects of photobleaching and photodamage on the quantitative data, and to increase the number of cells that could be analyzed, only the first 3 min of each sequence was used for most analysis. Some sequences obtained from epithelial cells were reanalyzed for 6 min to determine if stable subsets of microtubules could be detected with these longer periods of observation (see Results).

Microtubule dynamic instability parameters were quantified from image sequences obtained at 2-s intervals. Due to the limit of resolution of the light microscope, and the presence of electronic noise in images generated by the ISIT camera, microtubule length changes of <0.5 μm were considered to be pause events (Sammak and Borisy, 1988a). Because of this limitation, the minimum growth or shrinking rate detectable over a 2-s interval would be 15 μm/min. It should be noted that this limitation would be more severe at shorter intervals between images. For example, if images were collected using a 0.5-s interval between observations, a microtubule growth event would have to occur at 60 μm/min over this interval to be detected. Thus, although microtubule growth and depolymerization events of <2 s in duration may not be detected using our techniques, increasing the number of images collected would not necessarily increase the number of detected growth

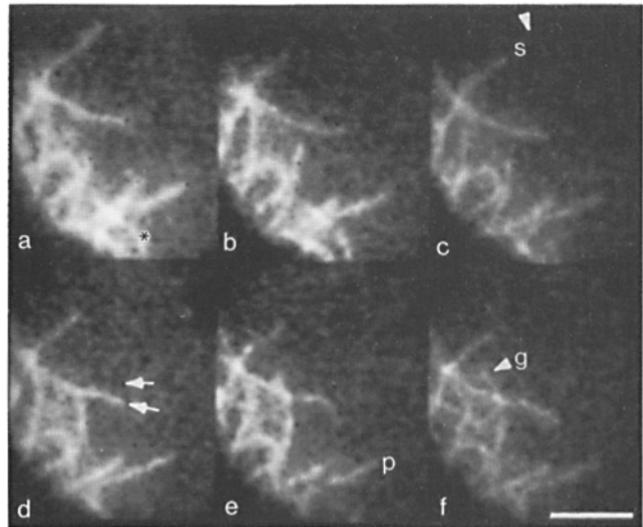
and shrinking events. To detect growth or shrinking events of  $<15 \mu\text{m}/\text{min}$ , image sequences collected at 2-s intervals between observations were quantified in the following manner. Two separate measurements of the microtubule's behavior were obtained as described previously, and the results of these two trials were averaged by the computer to minimize errors in measurement during the tracing of each microtubule. The difference in measurements made at each time point for the two trials was  $3.9 \pm 3.5$  pixels or  $0.2 \pm 0.2 \mu\text{m}$ , approximately the resolution of the light microscope. A plot of the average length changes (a "life-history" plot) was then placed on a video monitor, and reference lines representing the scale in microns were overlaid onto this graph. Regions of the graph that represented periods of microtubule elongation, pause, or depolymerization were selected using a mouse-driven cursor, and the rate, duration, and extent of these events were calculated by the computer using appropriate scaling parameters and linear regression techniques. Although only a limited number of microtubules were analyzed in this manner, this method allowed examination of a microtubule's entire life history and made detection of slow but extended growth and depolymerization events possible. Unfortunately, slow growth and depolymerization events of short duration may not be detected using these methods.

Previous investigators have defined rescue frequency as the inverse of the average duration of microtubule depolymerization events, and catastrophe frequency as the inverse of the average duration of microtubule growth events (Walker et al., 1988; Cassimeris et al., 1988). However, in these previous studies microtubules rarely paused. In contrast, microtubules in PtK<sub>1</sub> epithelial cells undergo frequent pause events (see Results). Because microtubules in these cells could undergo several short growth and pause events before undergoing a single catastrophe event, the average duration of growth events inaccurately describes the interval between catastrophe events. We have therefore reinterpreted the original definition of catastrophe events to mean the inverse of the average duration of time between the cessation of one rapid shortening event and the initiation of the next rapid shortening event regardless of whether the microtubule grows or pauses during this time. In a system where microtubules do not pause, the duration of time described in this manner is equivalent to the average duration of growth events, and thus, the values reported here can be directly compared to the values obtained from previous work. Similarly, we have defined a rescue event as the cessation of rapid shortening, regardless of whether the microtubule then undergoes a pause or a growth event.

Values for percent time paused were calculated by computing the total time spent paused for all microtubules, and dividing this figure by the total observation time for all microtubules. The frequency of transitions per microtubule was calculated by dividing the average number of excursions made per microtubule by the average microtubule lifespan. Finally, statistical comparisons were made using two-tailed *t* tests.

## Results

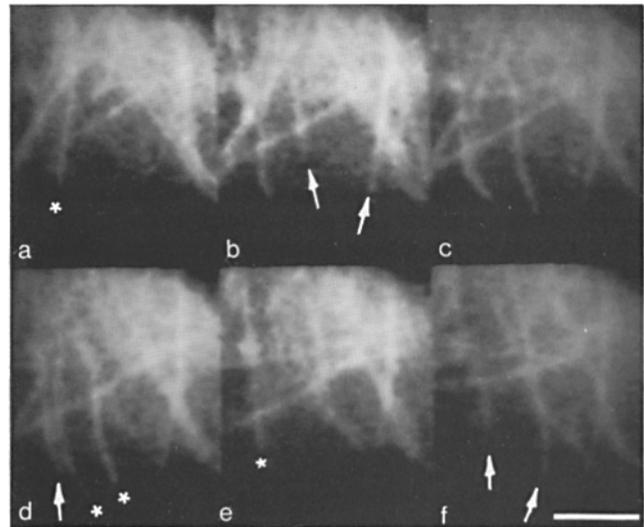
When image sequences of fluorescent microtubules are collected at 10-s intervals and examined, microtubules in lamellar regions of PtK<sub>1</sub> epithelial cells and CHO fibroblasts are observed to grow (g), shrink (s) and pause (p), as expected (Figs. 1 and 2). Careful inspection of microtubule behavior in PtK<sub>1</sub> epithelial cells (Fig. 1) reveals that microtubules which begin depolymerization frequently stop depolymerization and either pause or resume growing (rescue). Only one of the microtubules visible within the PtK<sub>1</sub> lamella shown in Fig. 1 can be observed to depolymerize out of the field of view during this 50-s period (*asterisk*, Fig. 1), and the overall arrangement of microtubules remains remarkably constant (compare Fig. 1 *a* with *f*. Note that sideways movement (parallel to the edge of the lamella) of some microtubules is occasionally detected (*arrows*, Fig. 1). In contrast, much more extensive changes in the microtubule arrangement are observed during similar periods of observation in CHO fibroblasts (Fig. 2, compare with Fig. 1). These changes result from microtubules depolymerizing out of (*asterisks*, Fig. 2) and entering into (*arrows*, Fig. 2) the field



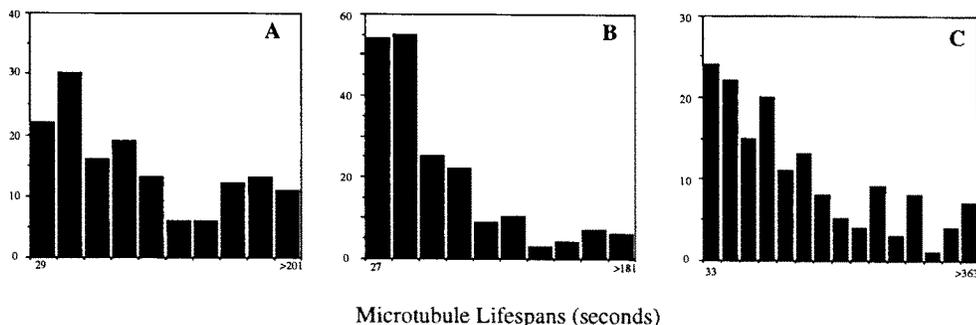
**Figure 1.** Individual microtubule dynamic behavior in PtK<sub>1</sub> cells examined at 10-s intervals. Individual microtubules are clearly observed to grow (g), shrink (s), and pause (p) in the lamella of this PtK<sub>1</sub> cell during the 50-s interval shown. A microtubule that moves sideways (laterally) is also observed within this lamella (*arrows* point to the microtubule and the original location of its plus end). Only one of the microtubules present in the first panel leaves the field of view during the interval shown (*asterisk*). Bar, 4  $\mu\text{m}$ .

of view during the interval shown (Fig. 2, compare with Fig. 1).

Quantitative analysis of these and other image sequences reveals that the average lifespan of microtubules in lamellar regions (measured as the average amount of time a microtubule spends within the  $\sim 150 \mu\text{m}^2$  field of view) is  $59 \pm$



**Figure 2.** Individual microtubule dynamic behavior in a CHO lamella examined at 10-s intervals. Many of the individual microtubules detected in this lamella leave the field of view during the 50-s interval shown (*asterisks*). In addition, several new microtubules are observed to enter the field of view during this interval (*arrows*). Many microtubules appear and disappear completely during the 10-s interval between two images. Bar, 4  $\mu\text{m}$ .



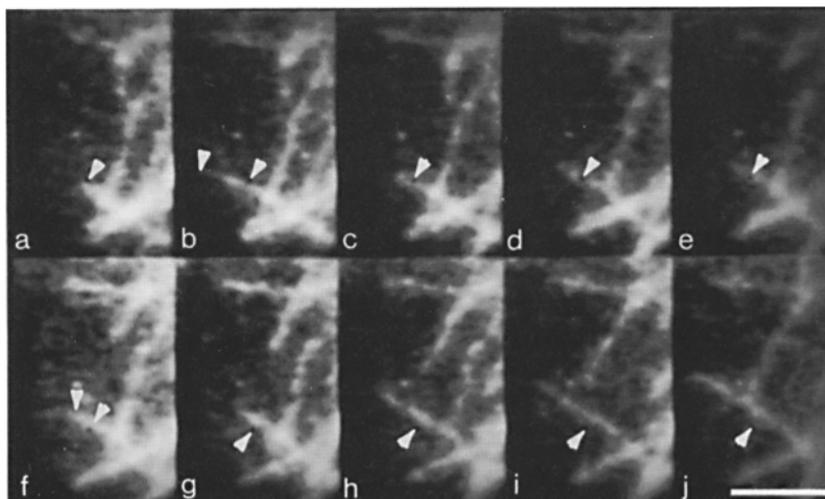
**Figure 3.** Distributions of microtubule lifespans in PtK<sub>1</sub> epithelial cells and CHO fibroblasts demonstrate that most microtubules in PtK<sub>1</sub> epithelial cells remain within the field of view longer than microtubules in CHO fibroblasts. MT lifespans were obtained from images acquired at 10-s intervals. Most microtubules in PtK<sub>1</sub> epithelial cells (a) spend more time within

the field of view than microtubules in CHO fibroblasts (B) when the first 3 min of each sequence is examined. Many microtubules in PtK<sub>1</sub> cells remain within the field of view for the entire 3-min sequence (a); however, analysis of the first 6 min of these sequences (c) demonstrates that only a single distribution of microtubules is detected in PtK<sub>1</sub> epithelial cells using these methods.

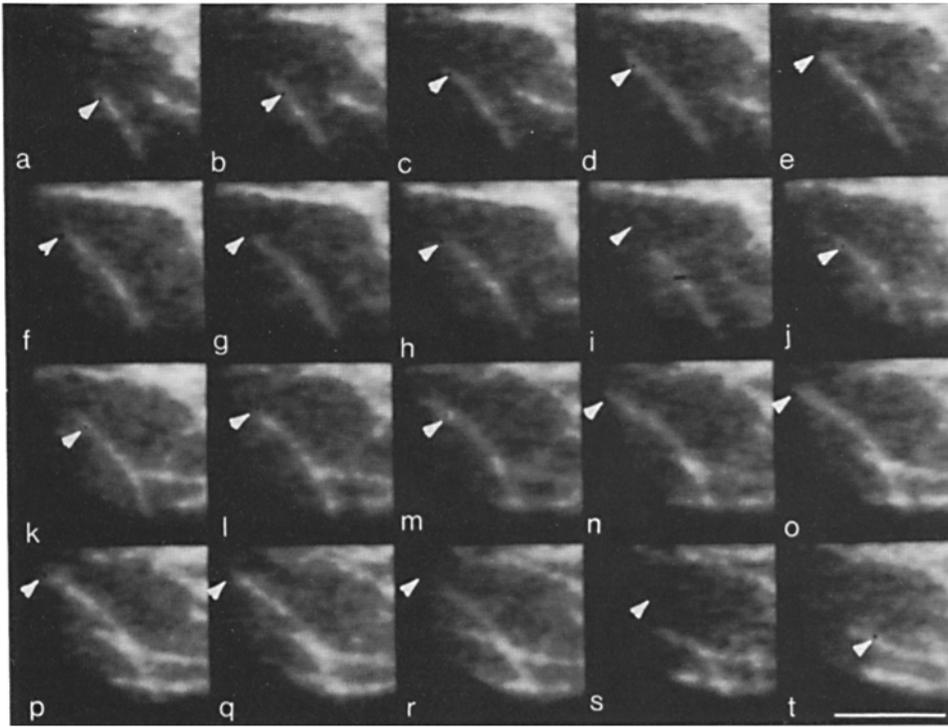
51 s for 195 microtubules in five CHO cells and  $88 \pm 57$  s for 148 microtubules in five PtK<sub>1</sub> cells. For this analysis, images of microtubules in living cells were obtained at 10-s intervals, and the first 3 min of each sequence were examined. Distributions of the amount of time individual microtubules spent within the field of view further demonstrate that most microtubules remained within the field of view longer in PtK<sub>1</sub> epithelial cells than in CHO fibroblasts (Fig. 3, a and b, respectively). In addition, these distributions reveal that many microtubules in the lamellar region of PtK<sub>1</sub> epithelial cells remain within the field of view for the entire 3-min observation period (Fig. 3 a). To determine if these microtubules represent a separate and more stable population of microtubules, image sequences from four PtK<sub>1</sub> epithelial cells were requantified using the first 6 min of each sequence. The average amount of time microtubules remained within the field of view, when measured in this manner, was  $125 \pm 93$  s. However, a histogram of the time individual microtubules remained within the field of view during these longer image sequences reveals that only a single population of microtubules can be detected in the lamellae of PtK<sub>1</sub> epithelial cells using these methods (see Discussion) (Fig. 3 c).

When individual microtubules in the lamellar region of PtK<sub>1</sub> cells are examined at 2-s intervals, multiple, brief episodes of growth and shortening, which are not detected in

images obtained at 10-s intervals, are observed (Figs. 4 and 5). For example, the single microtubule near the bottom of each panel in Fig. 4 grows  $\sim 1.9 \mu\text{m}$  (Fig. 4, a to b), and depolymerizes  $\sim 1.2 \mu\text{m}$  (Fig. 4, b to c). This microtubule therefore appears to grow at a rate of  $28 \mu\text{m}/\text{min}$ , and depolymerize at a rate of  $17 \mu\text{m}/\text{min}$ . Importantly, if the images of this microtubule had been taken at 8-s intervals, the apparent change in microtubule length between a and c would have been  $0.7 \mu\text{m}$ , and the measured growth rate would have been  $5.3 \mu\text{m}/\text{min}$ . Furthermore, the depolymerization event detected between b and c is followed by a second growth event (c-d), a pause event of  $\sim 12$  s in duration (e-h), and a third growth event (h-i). Therefore, five discrete events of growth, shrinking, or pause are exhibited by this microtubule in the 36-s interval shown in Fig. 4. Similar behavior of microtubules in the lamella of another PtK<sub>1</sub> epithelial cell is shown in Fig. 5. In this example, images are shown at 2-s intervals. An individual microtubule is clearly detected in the lower region of each panel, and has been marked in each case with the location of its plus end in the previous image to more clearly reveal the changes in MT length which occurred between images. Although the final depolymerization event occurs over the duration of several images (r-t), many growth (a-b, j-k, l-m) and depoly-



**Figure 4.** Individual microtubules are observed to undergo frequent episodes of growth, shortening and pause when images of microtubules in PtK<sub>1</sub> cells are obtained at 2-s intervals. The interval between displayed images is 4 s. An individual microtubule can be observed to undergo three growth events (a-b, c-d, g-j), a depolymerization event (b-c), and a pause event (d-g) during the 36-s interval shown (see also Fig. 6 a). The arrowhead marks the starting location of this microtubule's plus end, and a second arrowhead marks its plus end in b and f. Other microtubules can be observed to enter or leave this lamellar region during the interval shown, Bar,  $4 \mu\text{m}$ .



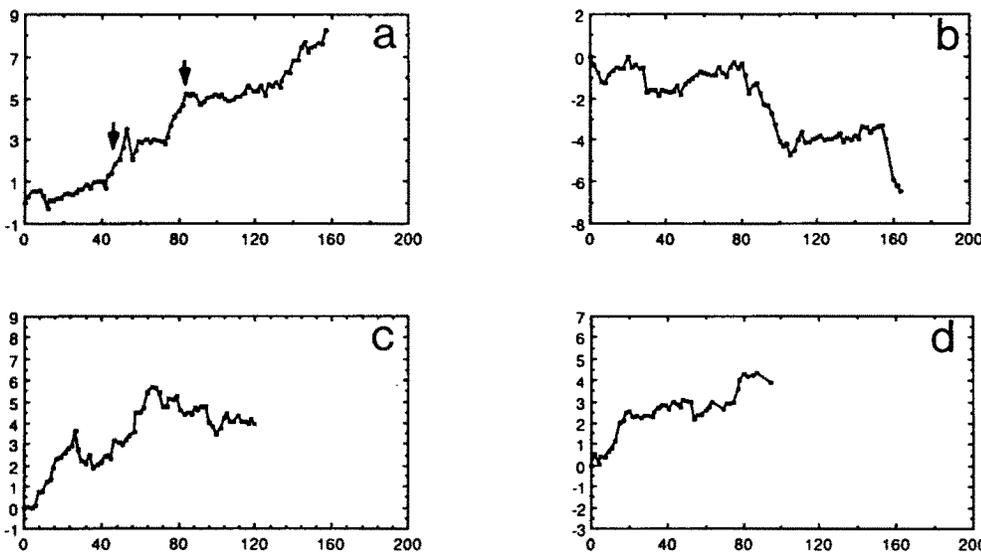
**Figure 5.** Some microtubule growth and shrinking events observed in PtK<sub>1</sub> epithelial cells are  $\leq 2$  s in duration. Images of individual microtubules in a PtK<sub>1</sub> cell obtained at 2-s intervals are shown. An individual microtubule is clearly detected in the lower region of each panel and has been marked in each case with the location of its plus end obtained from the previous panel. Growth events (*a-b*, *j-k*, *l-m*) and depolymerization events (*h-i*, *k-l*) of  $\leq 2$  s in duration are observed. Bar, 4  $\mu$ m.

merization (*h-i*, *k-l*) events of  $\leq 2$ -s interval between images are observed. A microtubule is considered to have paused when the location of the microtubule in a previous image coincides with its present location (*a*, *c*, *n-p*). Thus, microtubules in PtK<sub>1</sub> epithelial cells appear to undergo frequent growth, depolymerization, and pause events of short duration.

The behavior of microtubules in PtK<sub>1</sub> lamella has also been examined by plotting the change in microtubule length as a function of time (Fig. 6) (see Methods). The trace shown in Fig. 6 *a* was obtained from analysis of the microtubule marked by the arrowheads in Fig. 4, and the interval shown in Fig. 4 is marked with arrows. Additional representative

examples of microtubule "life histories" (Cassimeris et al., 1988) obtained from PtK<sub>1</sub> cells are shown in Fig. 6, *b-d*. These life history plots further demonstrate that microtubules in epithelial cells undergo frequent conversions between growth, shortening, and pause.

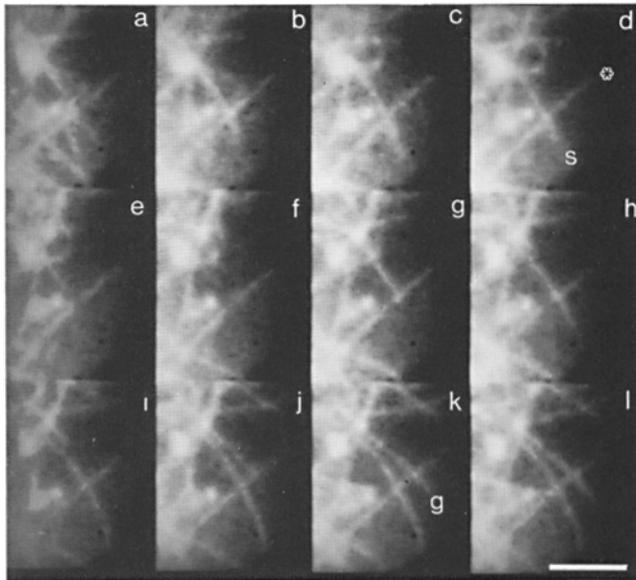
Finally, it should be noted that some microtubule length changes observed here could be due to microtubule movement, rather than growth and depolymerization events. However, previous studies have demonstrated that turnover of lamellar microtubules occurs primarily through microtubule growth and depolymerization rather than microtubule translocation (Sammak et al., 1987). In addition, microtubule fragments are occasionally observed within the lamel-



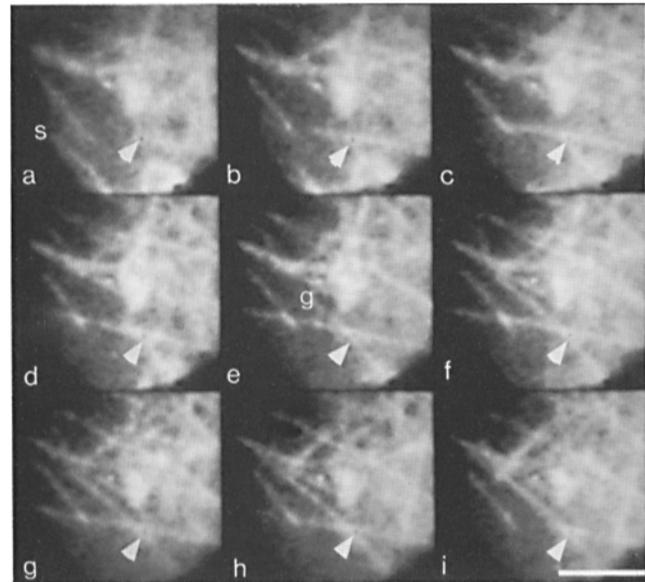
**Figure 6.** Life history plots obtained from individual microtubules in PtK<sub>1</sub> cells further reveal that microtubules in these cells undergo frequent length changes of short duration and extent. A life history plot obtained from the microtubule shown in Fig. 4 is shown in Fig. 6 *a*. The interval shown in Fig. 4 is marked with arrows (*a*). Numerous growth and shrinking events occur during the observation periods shown for this microtubule (*a*) and for other representative microtubules (*b-d*). The axes are marked in microns (*vertical*) and seconds (*horizontal*).

lae of these cells (present study), and these fragments often undergo length changes similar to those observed for intact microtubules (data not shown). In these cases, because both ends of the fragment are visible, we can be certain that the observed length changes result from assembly and disassembly of the microtubule.

Examination of individual microtubules in CHO cells reveals that the behavior of microtubules in CHO cells is strikingly different from the behavior of microtubules observed in PtK<sub>1</sub> cells. In Fig. 7, a single microtubule is clearly observed to grow and then shrink across nearly the entire field of view. This microtubule elongates  $\sim 6.8 \mu\text{m}$  in 20 s, or at a rate of  $20.4 \mu\text{m}/\text{min}$  between *a* and *f*. The growth event seen in *a-f* is immediately followed by an episode of rapid shortening (*f-i*), during which the microtubule depolymerizes to its original length in 12 s, a rate of  $34 \mu\text{m}/\text{min}$ , and subsequently leaves the field of view (not shown). Note that in this particular lamella, many microtubules could not be quantified (upper portion of micrographs). In some cases, this results from insufficient clarity in regions where the cell was particularly thick or microtubules particularly numerous. In other cases, the increased brightness and apparent thickness of fibers suggested that microtubules were laterally associated, or bundled (Sammak and Borisy, 1988*b*) and thus, were not included in these measurements. Finally, a microtubule fragment is clearly observed in this lamella, and remains relatively stable for the observation period. Such free microtubules were observed infrequently in both cell types;



**Figure 7.** Individual microtubules in CHO fibroblasts undergo growth and depolymerization events which result in extensive microtubule length changes. A series of images from a sequence obtained at 2-s intervals is shown; the interval between displayed images is 4 s. An individual microtubule is clearly detected in the lower portion of each image and has been marked with the location of its plus end in the first image. The microtubule grows rapidly across the region of the cell under observation (*a-e*) and then depolymerizes back to its starting location (*f-i*). It subsequently leaves the field of view (not shown). Other microtubules that either grow (*g*) into or shrink (*s*) out of the field of view are also detected. Bar,  $4 \mu\text{m}$ .



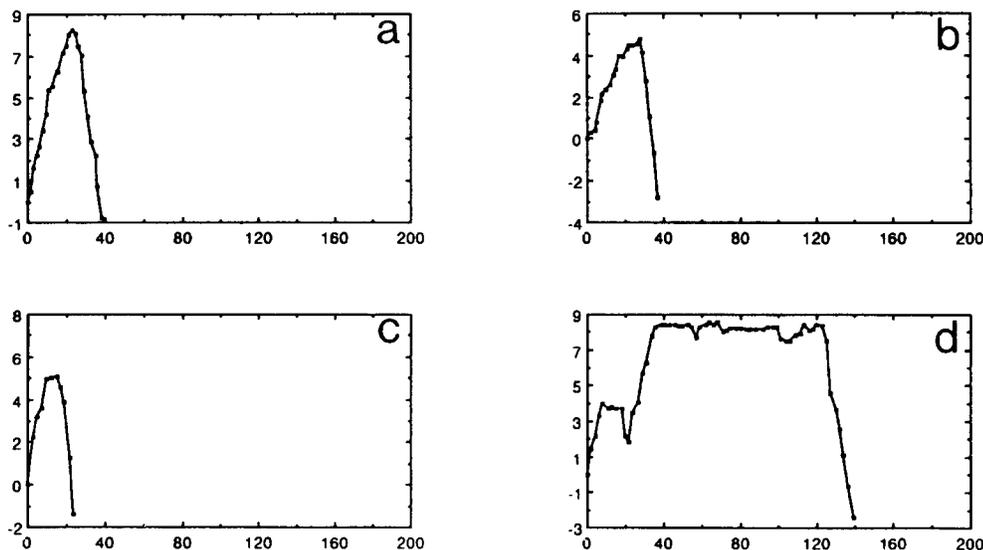
**Figure 8.** Dynamic and stable microtubules are detected in CHO fibroblasts. Images obtained at 2-s intervals are shown; the interval between displayed images is 4 s. Numerous microtubules can be observed to grow (*g*) or shrink (*s*) rapidly across this region of a CHO fibroblast. A single microtubule that does not appear to grow or shrink during the 44-s interval shown is also detected (*asterisk*). Bar,  $4 \mu\text{m}$ .

however, the number of fragments did not increase during long observation periods (data not shown) suggesting that they do not result from cellular damage. Microtubule fragments have also been observed in other cells using different methods of observation (Chen and Schliwa, 1990).

The behavior of individual microtubules in CHO cells in further revealed in Fig. 8. Many microtubules are observed to make long excursions as they polymerize and depolymerize (Fig. 8). In this example, a "stable" microtubule, which does not grow or shrink during this 44-s sequence, is also detected (*asterisk*, Fig. 8). Finally, it is of interest to note that although microtubules are observed to bend and to move laterally in PtK<sub>1</sub> epithelial cells (Fig. 2), CHO lamella are characterized by the straight, almost "stiff" appearance of many microtubules (Fig. 8). At the present time, the basis for the differences in microtubule morphology is not known.

Life history plots of microtubules behavior in CHO cells are shown in Fig. 9. A plot of the behavior of the microtubule examined in Fig. 7 demonstrates that a single growth and a single depolymerization event occurred during the period the microtubule was visible (Fig. 9 *a*). Additional examples, illustrating other typical microtubule behaviors observed in CHO fibroblasts, are shown in Fig. 9, *b-d*). A minority of microtubules in these cells are observed to pause for extended periods of time (Fig. 9 *d*). However, regardless of the pause duration, a single depolymerization event frequently results in the complete disappearance of CHO microtubules from the field of view. Thus, the behavior of microtubules in CHO fibroblasts is qualitatively different from the behavior of microtubules seen in PtK<sub>1</sub> epithelial cells.

The various parameters that characterize microtubule dynamic behavior in CHO fibroblasts and PtK<sub>1</sub> epithelial cells



**Figure 9.** Life history plots of microtubules in CHO fibroblasts demonstrate that most microtubules in these cells undergo long periods of growth and depolymerization with infrequent conversions. (a) A life history plot obtained from the microtubule indicated in Fig. 7 is shown. Only a single growth and depolymerization event occurs during the period this microtubule remains visible. (b and c) Additional examples of typical microtubule behavior in CHO fibroblasts; single growth or depolymerization events result in extensive changes in microtubule length. (d) A minority of microtubules in these cells appear to remain in a paused

state for some period of time. However, these microtubules do undergo growth and depolymerization excursions before and after pausing (d). The axes are marked in microns (vertical) and seconds (horizontal).

have been quantified from images obtained at 2-s intervals (Table I). Microtubules in PtK<sub>1</sub> epithelial cells are characterized by frequent episodes of growth and shrinking which are of short duration and extent. Although the average durations of these events measured in PtK<sub>1</sub> cells are both longer than the 2-s interval between observation (Table I), it is possible that some very brief events were not detected using these methods. The average rates of growth and shrinking events ( $11.9 \pm 6.5$  and  $19.8 \pm 10.8$   $\mu\text{m}/\text{min}$ , respectively) are both substantially higher than rates reported previously for fluorescent microtubules in vivo (Sammak and Borisy, 1988; Schulze and Kirschner, 1988) and are close to the values reported from analysis of microtubule behavior using DIC optics and 0.5-s intervals between observations (Cassimeris et al., 1988). Particularly noteworthy however, is the observation that most microtubules undergoing depolymerization

events in PtK<sub>1</sub> cells were rapidly rescued (Table I) and thus result in little overall changes in microtubule length; only 11% of the depolymerization events in PtK<sub>1</sub> cells resulted in microtubules leaving the field of view.

In contrast, microtubules in CHO fibroblasts are characterized by events of longer average duration and by infrequent conversions between the two states (Table I). The duration of microtubule growth and depolymerization events for the majority of microtubules in these cells ( $9.6 \pm 7.0$  and  $7.4 \pm 4.2$  s, respectively) was significantly greater than the duration of these events in PtK<sub>1</sub> epithelial cells ( $P \leq 0.01$ ). In addition, the rates of microtubule growth and depolymerization for these microtubules ( $19.7 \pm 8.1$  and  $32 \pm 17.7$   $\mu\text{m}/\text{min}$ , respectively) are also significantly higher than rates measured here in PtK<sub>1</sub> cells ( $P \leq 0.01$ ). As a result, the proportion of depolymerization events that resulted in a

**Table I. MT Dynamic Parameters Measured in PtK<sub>1</sub> and CHO Cells at 2-s Intervals**

	PtK (19)	CHO (18)	CHO (stab) (7)
Growth rate*	$11.9 \pm 6.5$	$19.7 \pm 8.1$	$21.2 \pm 11.7$
Growth length†	$1.3 \pm 0.9$	$3.2 \pm 2.5$	$2.6 \pm 2.3$
Growth duration‡	$7.9 \pm 6.6$	$9.6 \pm 7.0$	$7.6 \pm 5.3$
Depolymerization rate*	$19.8 \pm 10.8$	$32.2 \pm 17.7$	$21.1 \pm 11.5$
Depolymerization length†	$1.6 \pm 1.4$	$4.3 \pm 3.3$	$3.2 \pm 3.2$
Depolymerization duration‡	$5.1 \pm 3.8$	$7.4 \pm 4.2$	$7.4 \pm 4.6$
Pause duration‡	$8.5 \pm 5.5$	$8.0 \pm 4.6$	$26.6 \pm 19.9$
Interval between catastrophes‡	$18.5 \pm 15.0$	$16.5 \pm 10.1$	$31.3 \pm 26.5$
Interval between rescues‡	$5.1 \pm 3.8$	$7.7 \pm 3.9$	$7.3 \pm 4.9$
Catastrophe frequency (s <sup>-1</sup> )	0.0541	0.0606	0.0319
Rescue frequency (s <sup>-1</sup> )	0.1961	0.1299	0.1370
Lifespan‡	$97 \pm 50$	$48 \pm 31$	$102 \pm 31$
Events/MT/min	7.3	4.0	4.0

\* In  $\mu\text{m}/\text{min}$ .

† In  $\mu\text{m}$ .

‡ In s.

The values of microtubule dynamic instability parameters measured from images obtained at 2-s intervals. The numbers in parentheses represent the number of individual microtubules analyzed for each group. MT, microtubule.

microtubule leaving the field of view (48%) is much higher in these cells than in PtK<sub>1</sub> cells. In addition, although the frequency of catastrophe was similar in both cell types, microtubules in CHO fibroblasts were rescued less frequently than in PtK<sub>1</sub> epithelial cells ( $P \leq 0.01$ ). Finally, because the duration of microtubule growth, shrinking, and pause events is greater in CHO fibroblasts in PtK<sub>1</sub> epithelial cells, the total frequency of transitions is lower in CHO fibroblasts than in PtK<sub>1</sub> cells (Table I). Taken together, these data reveal that individual microtubules in epithelial cells make frequent transitions of short duration resulting in little overall change in the microtubule network. In contrast, most microtubules in CHO fibroblasts undergo fewer transitions, but microtubule length changes during these events are greater than those seen in epithelial cells, and result in more rapid turnover of the microtubule array.

A small number of more stable microtubules is also detected in CHO fibroblasts (average lifespan,  $102 \pm 31$  s). Although these microtubules represent only 28% of the individual microtubules examined in CHO cells, 72% of the total time microtubules were observed to pause in these cells could be accounted for by these more stable microtubules. In addition, both the rate of microtubule depolymerization and the frequency of catastrophe events for these microtubules are significantly lower than these values measured for the majority of microtubules in these cells (Table I;  $P \leq 0.05$  and  $P \leq 0.01$ , respectively). However, no other significant differences in the value of microtubule dynamic parameters were detected between these microtubules and the majority of microtubules observed in CHO cells (Table I).

Finally, we have analyzed images of microtubules in PtK<sub>1</sub> epithelial cells obtained at 2-s intervals using only every fifth image. The rates of microtubule growth and depolymerization obtained in this manner are significantly lower than rates obtained by analyzing all images (Table II), and are similar to the values obtained by other investigators using images obtained at 10-s intervals (Sammak and Borisy, 1988a; Schulze and Kirschner, 1988). Furthermore, the number of events per microtubule detected using a 10-s observation interval is lower than the number of events detected using 2-s intervals. Finally, the average duration of growth, depolymerization, and pause events is significantly greater when measured at 10-s rather than 2-s intervals (see Discussion).

## Discussion

### Microtubules Dynamics Are Cell-Type Specific

Our observations of individual microtubules in CHO fibroblasts and PtK<sub>1</sub> epithelial cells reveal that on average individual microtubules remain within lamellar regions for longer periods in epithelial cells than in fibroblasts. These observations are consistent with previous studies which demonstrate that the population of interphase microtubules turns over more rapidly in fibroblasts than in epithelial cells (Pepperkok et al., 1990; Wadsworth and McGrail, 1990). To determine which aspects of individual microtubule behavior are involved in generating this cell type-specific difference, we have examined individual microtubules in small regions of interphase cells at 2-s intervals. Quantitative analysis reveals that microtubules in CHO fibroblasts polymerize and depolymerize much more rapidly than those found in PtK<sub>1</sub>

Table II. Comparison of MT Dynamic Parameters in PtK<sub>1</sub> Cells Measured at 2- and 10-s Intervals

	2 s (19)	10 s (10)
Growth rate*	$11.4 \pm 6.2$	$5.15 \pm 2.8$
Growth duration <sup>§</sup>	$6.7 \pm 6.2$	$18.9 \pm 10.8$
Growth length <sup>‡</sup>	$1.0 \pm 0.85$	$1.4 \pm 0.86$
<i>n</i>	113	31
Depolymerization rate*	$17.2 \pm 10.3$	$13.8 \pm 14.1$
Depolymerization duration <sup>§</sup>	$4.4 \pm 3.5$	$11.4 \pm 4.5$
Depolymerization length <sup>‡</sup>	$1.3 \pm 1.3$	$2.0 \pm 1.2$
<i>n</i>	72	21
Catastrophe frequency (s <sup>-1</sup> )	0.054	0.0217
Rescue frequency (s <sup>-1</sup> )	0.225	0.0735
Pause duration <sup>§</sup>	$8.5 \pm 5.4$	$15.8 \pm 9.9$
Percent time paused	46.3	27.6
ps/MT	4.5	2.0
gr/MT	5.9	3.1
sr/MT	3.8	2.1

\* In  $\mu\text{m}/\text{min}$ .

‡ In  $\mu\text{m}$ .

§ In s.

Values for dynamic instability parameters were determined from images acquired at 2-s intervals and analyzed at 2- or 10-s intervals. The numbers in parentheses represent the number of individual microtubules analyzed for each group. gr, growth; sr, shrinking; ps, pause; *n*, number of observed events.

epithelial cells. Surprisingly, this analysis further reveals that the total frequency of microtubule transitions is greater in PtK<sub>1</sub> epithelial cells than in CHO fibroblasts. However, microtubules undergoing depolymerization are rapidly rescued in PtK<sub>1</sub> cells, resulting in little overall change in the length of individual microtubules. In contrast, microtubules in CHO fibroblasts are rescued much less frequently than those found in PtK<sub>1</sub> cells; the catastrophe frequency for the majority of microtubules observed in CHO fibroblasts is slightly but not significantly higher than for microtubules in PtK<sub>1</sub> cells. Thus, although the total frequency of microtubule transitions is lower in CHO fibroblasts than in PtK<sub>1</sub> epithelial cells, individual growth, and depolymerization events in CHO cells result in extensive microtubule length changes and rapid turnover of the microtubule array.

### Microtubule Subpopulations Are Detected in Some Cells

Previous investigation has demonstrated the presence of two kinetically distinct microtubule subpopulations in epithelial cells but not fibroblasts (Pepperkok et al., 1990; Wadsworth and McGrail, 1990), and in both epithelial cells and fibroblasts (Schulze and Kirschner, 1988). In the present study, we detect populations of more stable microtubules in CHO fibroblasts but not in epithelial cells. The reasons for the apparent absence of microtubule subpopulations in the lamellae of epithelial cells in the present study are not clear; however, it is possible that dynamically distinct microtubule subpopulations could be found within lamellar regions of epithelial cells under different culture conditions (Pepperkok et al., 1990). Alternatively, only a small region of the cell periphery could be examined in the present study (see Methods), and less dynamic microtubules are often concentrated near the cell center (Schulze and Kirschner, 1986). Finally,

Table III. Comparison of MT Dynamic Parameters

	PtK,*	CHO*	Newt lung‡	X. neuron§	X. interphase	X. mitotic
Catastrophe rate (/s)	0.054	0.061	0.014	0.012	0.018	0.116
Rescue rate (/s)	0.196	0.130	0.044	0.029	0.011	0.027

\* Present study.

‡ Newt lung epithelial cells; Cassimeris et al., 1988.

§ *Xenopus* neuron; Tanaka and Kirshner, 1991.

|| *Xenopus* extracts; Belmont et al., 1991.

only a small fraction of the total number of cellular microtubules has been examined. It is therefore possible that more stable microtubules are present in these epithelial cells, but are not detected using our methods.

Our observations do reveal the presence of a small percentage of dynamically distinct microtubules in CHO fibroblasts. These microtubules spent longer periods of time in a paused state, during which neither growth nor depolymerization could be detected, than the majority of microtubules observed in these cells. Although the functional significance of these meta-stable microtubules has not yet been determined, quantitative analysis reveals that the frequency of catastrophe events and the rate of microtubule depolymerization are significantly lower than for the majority of microtubules in CHO cells. A similar decrease in the depolymerization rate for meta-stable microtubules has also been reported for individual microtubules in PtK<sub>1</sub> and BSC cells (Schulze and Kirshner, 1988). Finally, recent *in vitro* experiments have shown that both the rate of microtubule polymerization and the frequency of rescue are increased by microtubule-associated protein (MAP)<sup>1</sup> binding (Bre and Karenti, 1990; Pryer, 1989). Importantly, however, our analysis reveals that the more stable CHO microtubules do not differ significantly from the majority of microtubules found in CHO cells with respect to either of these parameters. Our findings, therefore, suggest that the behavior of these less dynamic microtubules in CHO fibroblasts may be regulated by a different mechanism.

### Regulation of Interphase Microtubule Dynamics

Regulation of catastrophe and rescue frequencies has been postulated to play a role in the organization and behavior of diverse microtubule arrays in interphase cells. For example, it has been suggested that reducing the frequency of microtubule transitions is involved in the polarization of cultured epithelial cells (Bre et al., 1990; Pepperkok et al., 1990) and that increasing the number of rescue events may contribute to the formation of stable axonal microtubule arrays during axon outgrowth (Tanaka and Kirshner, 1991). Here, we demonstrate that microtubules in fibroblasts, which are actively ruffling and capable of locomotion, have a lower frequency of rescue than microtubules in stationary epithelia. In addition, although values obtained for rescue and catastrophe frequency from amphibian epithelial and neuronal cells (Table III) are lower than and more similar to each other than values obtained from mammalian tissue culture cells (this report), examination of the reported values reveals

greater differences in the frequency of rescue than catastrophe among interphase cells of both groups (Table III). For example, although the frequency of rescue was not directly measured in *Xenopus* nerve growth-cones, the calculated rescue frequency of microtubules in nerve growth cones (Tanaka and Kirshner, 1991) is lower than the rescue frequency measured in newt lung epithelial cells (Cassimeris et al., 1988) (Table III), whereas the values reported for catastrophe frequency in these two cell types are similar. These results suggest that variation in microtubule behavior among interphase cells involves cell type-specific regulation of microtubule rescue frequencies, regardless of cell origin.

Although the transition from interphase to mitosis also involves changes in microtubule dynamic instability behavior, it is not clear if the frequency of rescue events is regulated during this transition. For example, in *Xenopus* oocyte extracts, the catastrophe frequency in mitotic extracts is approximately sixfold higher than for microtubules in interphase extracts (Belmont et al., 1990); the frequency of rescue was also increased slightly in the mitotic extracts (approximately twofold). However, the measured frequency of rescue events in both types of extracts (Belmont et al., 1990) is much lower than values obtained from living interphase cells (Cassimeris et al., 1988; Tanaka and Kirshner, 1991; present study). The low value of microtubule rescue frequency measured from interphase *Xenopus* oocyte extracts may indicate that interphase conditions present in undifferentiated oocytes are not directly comparable to interphase conditions in nonembryonic cells. Alternatively, the low values for interphase rescue frequency may reflect the difficulties in reproducing *in vivo* conditions in *in vitro* extracts. It should be noted that a decrease in the frequency of rescue events has also been postulated to occur as cells enter mitosis (Cassimeris et al., 1988). Direct observation of the behavior of microtubules in living cells as they progress into mitosis will be necessary to resolve this issue.

### Imaging Techniques Affect the Measurement of Microtubule Dynamic Behavior

Our results directly demonstrate that the interval between successive observations greatly affect the values obtained for the parameters of dynamic instability behavior. For example, the average rates of microtubule growth and shrinking are significantly greater when measured at 2-s rather than 10-s intervals. The rates of microtubule growth and shortening measured at 2-s intervals are also much greater than growth and shortening rates measured *in vitro* (Walker et al., 1988), *in vivo* at longer observation intervals (Sammak and Borisy, 1988a; Schulze and Kirshner, 1988), or from immuno-

1. Abbreviation used in this paper: MAP, microtubule-associated protein.

localization of tubulin analogues after microinjection and fixation (Schulze and Kirschner, 1986; Bre et al., 1990; Wadsworth and McGrail, 1990), indicating that these latter methods may seriously underestimate the *in vivo* rates of microtubule growth and shrinking. In addition, fewer catastrophe and rescue events are detected in PtK<sub>1</sub> epithelial cells from images taken at 10-s rather than 2-s intervals. Thus, our data reveal that both the number of events which can be detected and the rates of growth and shrinking are significantly effected by the interval between observations.

Acquisition of images at 2-s intervals (using the imaging system available for these experiments) requires continuous, although greatly attenuated, illumination of the cell area under observation. Although efforts were made to minimize any adverse effects of illumination on the cells (see Methods), it has been demonstrated previously that fluorescent microtubules *in vitro* can be damaged and may break at certain levels of illumination (Vigers et al., 1988). However, several observations strongly suggest that our experimental conditions do not measurably effect microtubule behavior *in vivo*. First, when sequences collected at 2 s are analyzed at 10 s, the results are similar to values obtained from the analysis of images obtained at 10-s intervals (present study; Schulze and Kirschner, 1988). Second, damage to fluorescent microtubules *in vitro* is accompanied by photobleaching (Vigers et al., 1988), and we do not detect loss of microtubule fluorescence during our observation periods. Third, microtubules were never observed to break during the sequences used for our analysis although microtubule fragments were occasionally detected. Finally, high rates of microtubule growth and depolymerization and high rescue and catastrophe frequencies were observed during our experiments. These observations are inconsistent with the diminished microtubule dynamic behavior that results from photodamage (Schulze and Kirschner, 1988). We therefore conclude that brief exposure to low levels of continuous illumination over small areas of the cell, in the presence of 1 mM ascorbic acid does not result in detectable alterations of microtubule behavior *in vivo*.

Although the observations of microtubules at 2-s intervals represent the greatest temporal resolution thus far reported for images of fluorescent microtubules in living cells, only a limited area of the cell could be examined during these experiments. It is important to consider the effect of this limitation on the quantification of microtubule dynamic instability parameters. For example, microtubule growth and shrinking events resulting in an microtubule entering or leaving the field of view are only partially detected in these experiments, and the measured duration of such an event therefore underestimates its actual duration. Because the duration of growth and depolymerization events is used to calculate the frequency of catastrophe and rescue events, these latter values may in turn be overestimated. In PtK<sub>1</sub> cells, only a small minority of microtubule growth and depolymerization events result in a microtubule entering or leaving the field of view. Thus, the degree to which the behavior of the total population of microtubules in these lamellae could be misrepresented is small. In contrast, half of the observed microtubule depolymerization events and over one third of the observed growth events in CHO fibroblasts result in a microtubule leaving or entering the field of view (see Results). As a result, our analysis may underestimate the ex-

tent and duration of microtubule growth and shortening events in CHO fibroblasts, and overestimate the frequency of rescue and catastrophe events in these cells. Therefore, the differences between microtubule behavior in CHO fibroblasts and PtK<sub>1</sub> epithelial cells may actually be greater than indicated by our results.

### *Microtubule Dynamics and Intracellular Tubulin Concentration May Be Interrelated*

Previous experiments have demonstrated that the behavior of individual microtubules *in vitro* is strongly affected both by the presence of MAPs (Pryer, 1989; Horio and Hotani, 1986) and the concentration of free tubulin subunits (Walker et al., 1988), and it is of interest to consider whether the differences in microtubule dynamic behavior observed in the present study can be explained by these mechanisms. MAPs bound to individual microtubules *in vitro* increase the rate of microtubule polymerization and frequency of rescue events, decrease the frequency of catastrophe, and may decrease the rate of microtubule depolymerization (Bre and Karsenti, 1990; Horio and Hotani, 1986; Pryer, 1989). Further, injection of MAPs into living cells has been shown to increase the total amount of microtubule polymer. Interestingly, the increase in polymer is limited by the available free tubulin (Drubin and Kirschner, 1986), suggesting that addition of MAPs can lower the concentration of free tubulin. These observations suggest that many of the differences in microtubule behavior observed between CHO fibroblasts and PtK<sub>1</sub> epithelial cells can be explained by the cell-type specific expression of MAPs in PtK<sub>1</sub> epithelial cells. The presence of MAPs in PtK<sub>1</sub> epithelial cells may reduce the concentration of free tubulin, thus decreasing the rate of microtubule polymerization as compared with CHO cells, whereas MAPs and the concentration of tubulin may have opposing effects on catastrophe. We therefore predict that PtK<sub>1</sub> epithelial cells contain higher concentrations of MAPs than CHO fibroblasts or a unique type of MAP not found in CHO fibroblasts and that the concentration of free tubulin in PtK<sub>1</sub> epithelial cells is lower than that found in CHO fibroblasts.

Such a model, in which the behavior of microtubules and the intracellular concentration of free tubulin are interdependent, is supported by several lines of evidence. First, the concentration of free tubulin in living cells is known to be autoregulated at the level of tubulin mRNA translation (Yen et al., 1987), suggesting that the concentration of free tubulin is of physiological importance. Second, transient increases in the concentration of free tubulin by microinjection of tubulin subunits have been shown to result in an increased rate of microtubule growth (Schulze and Kirschner, 1986) and the plus end-dependent elongation of kinetochore microtubules in anaphase (Shelden and Wadsworth, 1992). These experiments demonstrate that important aspects of microtubule behavior may be regulated by the concentration of free tubulin *in vivo*.

### *Conclusion*

In summary, the results of this study provide direct evidence of cell type-specific microtubule behavior. The average amount of time individual microtubules under observation

remain within lamellar protrusions is significantly higher in epithelial cells than in fibroblasts. Our observation of microtubules at 2-s intervals reveals that microtubules in the lamellae of epithelial cells are less dynamic than the majority of microtubules found in CHO fibroblasts due to a higher frequency of rescue and a lower rate of microtubule growth and depolymerization; the frequency of catastrophe for these microtubule populations is not significantly different.

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