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Microtubule dynamics *in vitro* are regulated by the tubulin isotype composition

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ABSTRACT **The growing and shortening dynamics of individual bovine brain microtubules at their plus ends at steady state *in vitro*, assembled from isotypically pure $\alpha\beta_{II}$, $\alpha\beta_{III}$, or $\alpha\beta_{IV}$ tubulin dimers, were determined by differential interference contrast video microscopy. Microtubules assembled from the purified $\alpha\beta_{III}$ isotype were considerably more dynamic than microtubules made from the $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ isotypes or from unfractionated phosphocellulose-purified tubulin. Furthermore, increasing the proportion of the $\alpha\beta_{II}$ isotype in a mixture of the $\alpha\beta_{II}$ and $\alpha\beta_{III}$ isotypes suppressed microtubule dynamics, demonstrating that microtubule dynamics can be influenced by the tubulin isotype composition. The data support the hypothesis that cells might determine the dynamic properties and functions of its microtubules in part by altering the relative amounts of the different tubulin isotypes.**

Microtubules (1) are required for structural organization and for many kinds of movements within the eukaryotic cell cytoplasm. They are especially prominent in the central nervous system where they appear to be essential for the organization and function of axonal and dendritic processes of neurons. Although it is unclear how microtubule functions in cells are controlled, a large body of evidence indicates that the dynamic properties of the microtubules play an important role (for reviews, see refs. 2 and 3). For example, microtubule dynamics increase dramatically when cells progress from interphase to mitosis (4–6), and the rapid dynamics of spindle microtubules appear to be important for establishing spindle microtubule organization, for facilitating the linkage of chromosomes to the spindles, and for chromosome movement (2–7).

Little is known about how microtubule dynamics are regulated in cells. *In vitro* and in cells, microtubule ends switch between states of growing and shortening, a process known as "dynamic instability" (8–13), apparently due to the gain and loss of a stabilizing GTP- or GDP-P_i-liganded tubulin cap at the microtubule ends. Also, *in vitro* and in cells, net growing of microtubules can occur at one microtubule end and net shortening can occur at the opposite end, a process termed "treadmilling" or "flux" (9, 14–16). Both dynamic instability and treadmilling are logical targets for control. Biochemical studies *in vitro* have indicated that microtubule dynamics in cells could be regulated at several levels. One possibility is that control of microtubule dynamics involves regulation of the gain and loss of the stabilizing cap. A second possibility is that control of microtubule dynamics occurs through interactions of microtubule-associated proteins (MAPs) with microtubule surfaces and ends.

Another possible mechanism for control of microtubule polymerization dynamics could involve the isotypic composition of the tubulin itself. Tubulin is composed of two 50-kDa polypeptide subunits, α and β, which exist in multiple forms called isotypes. For example, mammalian brain tubulin consists of at least five α- and five β-tubulin isotypes (17, 18). However, despite considerable effort, the functional significance of the multiple isotypes in cells remains obscure (for review, see ref. 19). Recent evidence indicates that some tubulin isotypes may have distinct functions. For example, when Hoyle and Raff (20) expressed the *Drosophila* $\beta 3$ isotype in *Drosophila* male germ cells in place of the testis-specific $\beta 2$ isotype, axoneme formation and meiotic spindle formation did not occur, while other microtubule-dependent processes occurred normally. Similarly, Falconer *et al.* (21) found that the $\alpha\beta_{II}$ isotype was incorporated preferentially into a colchicine-stable subset of microtubules, whereas the $\alpha\beta_{III}$ isotype was preferentially incorporated into a colchicine-labile microtubule subset in cultured neuronal cells. These results indicated that the two isotypes could segregate into different microtubule populations.

Recently, Banarjee *et al.* (22–25) purified the major β-tubulin isotypes from bovine brain by immunoaffinity chromatography with specific antibodies to the isotypes. By analyzing the polymerization properties of the different isotypes in suspension *in vitro*, Banerjee *et al.* (22–25) and Lu and Ludueña (26) found that the polymerization properties of the isotypes differed from one another and that the pattern of the differences varied with the polymerization conditions. Notably, the polymerization properties of the $\alpha\beta_{III}$ isotype were significantly different from those of the $\alpha\beta_{II}$ and $\alpha\beta_{IV}$ isotypes.

While the general polymerization properties of the pure β-tubulin isotypes have been analyzed in suspension *in vitro*, the studies thus far have not provided insight about the growing and shortening events that occur at the ends of the individual microtubules—events closely linked to the functions of individual microtubules within cells. Thus, in the present study we used video microscopy to analyze the dynamic instability parameters of individual microtubules made from different β-tubulin isotypes. Our results indicate that at steady state, microtubules made from the brain-specific $\alpha\beta_{III}$ isotype were considerably more dynamic than microtubules made from unfractionated tubulin or from the purified $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ isotypes. In addition, inclusion of the $\alpha\beta_{II}$ isotype along with the $\alpha\beta_{III}$ isotype modulated the dynamics of the microtubules. The results support the hypotheses that the β-tubulin isotype composition may modulate microtubule polymerization dynamics in cells and that the $\alpha\beta_{III}$ isotype might perform a unique function in neurons.

MATERIALS AND METHODS

Purification of Isotopically Purified Tubulin Dimers and Axonemes. Purified $\alpha\beta_{II}$, $\alpha\beta_{III}$, and $\alpha\beta_{IV}$ isotypes were prepared by immunoaffinity chromatography as described (24,

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Abbreviations: MAP, microtubule-associated protein; PC-tubulin, phosphocellulose-purified tubulin.

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26). The purified isotypes were not detectably contaminated with other β-tubulin isotypes as determined by SDS/polyacrylamide gel electrophoresis and immunoblot analysis (24, 27). [Like β tubulin, α tubulin consists of various isotypes. However, the α-tubulin isotype composition of the isotypically pure β-tubulin dimers used in this work is not known. Subjection of the α subunits to isoelectric focusing indicates that there are relatively minor differences in the isotypic compositions of the α isotypes bound to the different β isotypes (24, 28)]. β-tubulin isotypes were stored frozen in 0.1 M Pipes/1 mM EGTA/1 mM Mg^{2+} (PEM) containing 4 M glycerol and 1 mM GTP at −70°C. Immediately prior to use, the isotypes were transferred to PEM (no glycerol) by passage through a Bio-Gel P-2 (Bio-Rad) column previously equilibrated with PEM plus 0.1 mM GTP. Protein concentrations were determined by the method of Bradford (29) using bovine serum albumin as the standard.

Video Microscopy. Differential interference contrast video microscopy was carried out as described (12). Solutions of individual β-tubulin isotypes (1.6 mg of protein per ml) were incubated with sea urchin flagellar axonemal "seeds" at 30°C in PEM containing 1 mM GTP. The seed concentration was optimized at three to six seeds per microscope field in all experiments. Under these conditions, the purified β-tubulin isotypes only polymerized at the ends of the seeds. Volumes (2 μl) of the seeded microtubules were prepared for video microscopy after 30 min of incubation, at which time the microtubules had reached polymer mass steady state. The growing and shortening dynamics at the plus ends of the individual microtubule were recorded at 30°C and analyzed as described elsewhere (12). We measured the microtubule lengths at 15-s intervals and we considered the change in length at each interval to represent a growing, shortening, or attenuation event. Any growing or shortening event that was too short to be detected during a 15-s interval was scored as an attenuation event. Thus, possible sustained slow growing or shortening that might be detected by measuring the length changes for long periods of time were not detected and, therefore, the method we used to analyze dynamics would give higher percentages of time in the attenuated state than a method in which the changes over a long period of time were averaged. Individual measurements were made two or more times; between 12 and 16 microtubules were used and a minimum of 500 individual measurements were made to analyze the dynamics of microtubules made from phosphocellulose-purified tubulin (PC-tubulin) and each of the β-tubulin isotypes.

RESULTS

Plus-End Dynamics of Isotypically Purified Tubulins. When $\alpha\beta_{II}$, $\alpha\beta_{III}$, or $\alpha\beta_{IV}$ dimers were incubated with axonemal seeds, the isotypically pure microtubules grew primarily at the plus ends of the seeds as determined by the relative lengths of the microtubules at the opposite seed ends (10–12). At steady state, the pure $\alpha\beta_{II}$, $\alpha\beta_{III}$, and $\alpha\beta_{IV}$ microtubules displayed typical dynamic instability behavior, but microtubules made from the $\alpha\beta_{III}$ isotype were much more dynamic than the $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ microtubules (Fig. 1). As shown in Table 1, the dynamic instability parameters of isotypically pure $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ microtubules were quantitatively similar to each other and also similar to the parameters of microtubules made from unfractionated PC-tubulin. For example, the mean growing rates of microtubules made from $\alpha\beta_{II}$, $\alpha\beta_{IV}$, or unfractionated PC-tubulin were 49, 51, and 51 dimers per s, respectively, and the mean shortening rates of the microtubules were 54, 51, and 56 dimers per s, respectively (Table 1). Similarly, the mean growing and shortening durations, the mean percentage of time that the microtubules spent growing or shortening, and the frequency distributions of growing and shortening were not significantly different for microtubules made from isotypically pure $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ dimers or unfractionated PC-tubulin (Table 1).

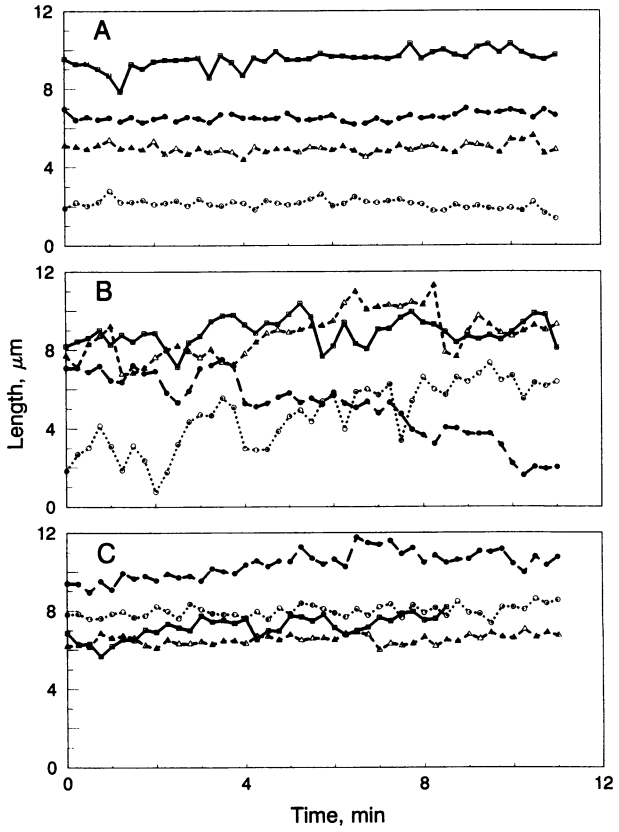

FIG. 1. Growing and shortening dynamics of isotypically pure microtubules at their plus ends. Purified $\alpha\beta_{II}$ (*A*), $\alpha\beta_{III}$ (*B*), or $\alpha\beta_{IV}$ (*C*) tubulin isotypes were polymerized at the plus ends of axonemal seeds and length changes at steady state were measured with time. Each tracing shows the growing and shortening dynamics of an individual microtubule. All of the lengths for the microtubule in *B* denoted by the open circles were displaced downward 2 μm for ease of visualization.

Microtubules made from the $\alpha\beta_{III}$ isotype were significantly more dynamic than $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ microtubules or microtubules made from unfractionated PC-tubulin (Figs. 1 and 2 and Table 1). For example, the mean growing and shortening rates of the isotypically pure $\alpha\beta_{III}$ microtubules were nearly twice as rapid as the growing and shortening rates of microtubules made from the $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ isotypes or from unfractionated PC-tubulin (Table 1). Similarly, microtubules made from the $\alpha\beta_{III}$ isotype (Fig. 2*C*) displayed a much broader distribution of growing and shortening rates than microtubules made from unfractionated PC-tubulin (Fig. 2*A*) or from purified $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ tubulin isotypes (Fig. 2 *B* and *D*).

Microtubules spend a substantial fraction of time neither growing nor shortening detectably, called an attenuated or pause state (10, 12, 13). The attenuated state should be thought of as a state in which microtubules do not grow or shorten or they do so below the resolution of the microscope (≤0.2 μm). As shown in Table 1, $\alpha\beta_{III}$ microtubules were substantially more dynamic than the $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ microtubules by this criterion, spending only 24% of the total time in the attenuated state as compared with 47% and 42% for the $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ microtubules, respectively. The dynamicity, which is calculated from all detectable growing and shortening events including the time spent in the attenuated state, provides a measure of overall dimer exchange at the plus ends (12). As

Table 1. Dynamic instability parameters of microtubules composed of different β-tubulin isotypes and unfractionated PC-tubulin

Parameter	$\alpha\beta_{II}$	$\alpha\beta_{III}$	$\alpha\beta_{IV}$	Unfractionated PC-tubulin
Mean rate, s^{-1}				
Growing	49 ± 6	77 ± 6	51 ± 2	51 ± 2
Shortening	54 ± 4	98 ± 11	51 ± 3	56 ± 3
Mean phase duration, s				
Growing	17 ± 1	22 ± 1	19 ± 1	20 ± 1
Shortening	18 ± 1	21 ± 1	17 ± 1	17 ± 1
Attenuation	37 ± 8	19 ± 1	29 ± 2	28 ± 2
% total time				
Growing	27 ± 2	42 ± 3	32 ± 2	34 ± 3
Shortening	27 ± 2	34 ± 3	26 ± 2	25 ± 3
Attenuation	47 ± 3	24 ± 3	42 ± 3	42 ± 3
Dynamicity, s^{-1}	28 ± 3	67 ± 7	30 ± 2	30 ± 2
Transition frequencies, events per min				
S → G or A	3.3	2.9	3.5	3.5
G or A → S	1.2	1.5	1.2	1.2
A → G or S	1.6	3.2	2.0	2.1
G or S → A	1.4	1.0	1.4	1.5

Experiments were performed as described in Fig. 1. Values are the mean ± SEM. G, growing; S, shortening; A, attenuation. Transition frequencies were calculated by dividing the number of times the transition occurred by the total time that the microtubules spent in the phase or phases from which the transition occurred (12).

shown in Table 1, the dynamicity of the $\alpha\beta_{III}$ microtubules was more than twice that of the $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ microtubules.

The frequency of switching among the growing, shortening, and attenuation states can be calculated from the mean duration of time spent in each state (12). The transition frequencies, calculated from the mean phase durations, indicate that the $\alpha\beta_{III}$ microtubules switched less frequently from the shortening state to growing or attenuation state and switched more frequently from a growing to the shortening or attenuated state than the $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ microtubules. The decreased switching frequencies from shortening to growing or attenuation and the increased frequencies from growing to shortening or attenuation of microtubules made from the $\alpha\beta_{III}$ isotype indicates that microtubules containing this isotype might gain the stabilizing cap less frequently and lose the cap more frequently than microtubules made from the $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ isotypes. In addition, the $\alpha\beta_{III}$ microtubules switched more frequently from attenuation to growing and shortening, and they switched less frequently from growing or shortening to attenuation than $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ microtubules. The transition frequencies from attenuation to growing or shortening and from growing or shortening to attenuation are measures of the overall stability of the microtubules. By these criteria, microtubules made from the $\alpha\beta_{III}$ isotype are significantly less stable than microtubules made from the $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ isotypes.

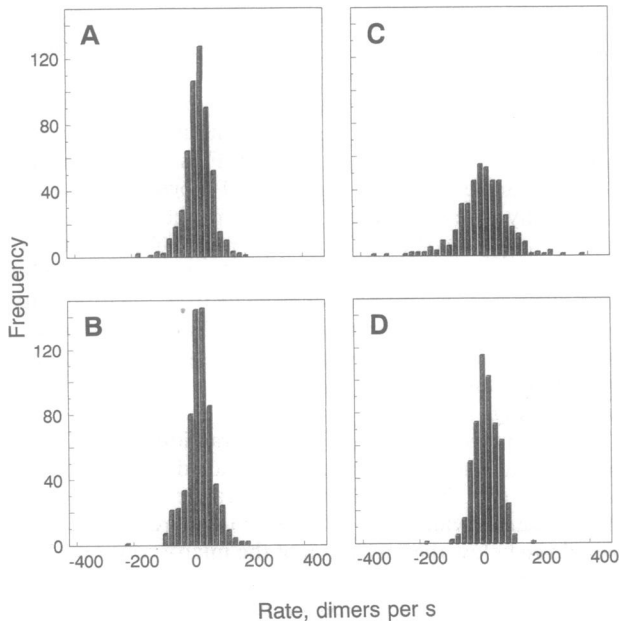

FIG. 2. Frequency distribution histograms of growing and shortening rates at microtubule plus ends made from unfractionated PC-tubulin (*A*) or purified $\alpha\beta_{II}$ (*B*), $\alpha\beta_{III}$ (*C*), or $\alpha\beta_{IV}$ (*D*) tubulin. Positive rates indicate growing and negative rates indicate shortening; rates between +23 and −23 dimer per s were below the resolution of the microscope and are reported as zero.

Microtubules Assembled from Mixtures of the $\alpha\beta_{II}$ and $\alpha\beta_{III}$ Isotypes Display Modulated Dynamics. Because the two major β isotypes in bovine brain tubulin preparations are the $\alpha\beta_{II}$ and $\alpha\beta_{III}$ isotypes, we wanted to determine how inclusion of the $\alpha\beta_{II}$ isotype along with the $\alpha\beta_{III}$ isotype during polymerization might modulate the dynamics of the $\alpha\beta_{III}$ microtubules. We mixed the $\alpha\beta_{II}$ and $\alpha\beta_{III}$ tubulin isotypes at three

Table 2. Dynamic instability parameters of microtubules composed of $\alpha\beta_{II}$ and $\alpha\beta_{III}$ isotypes at different ratios

	$\alpha\beta_{II}/\alpha\beta_{III}$		
Parameter	20%/80%	50%/50%	80%/20%
Mean rate, s^{-1}			
Growing	86 ± 6	50 ± 2	49 ± 3
Shortening	91 ± 6	57 ± 3	43 ± 3
% total time			
Growing	36 ± 10	29 ± 1	19 ± 1
Shortening	33 ± 3	27 ± 1	20 ± 3
Transition frequencies, events per min			
S → G or A	3.1	3.2	3.7
G or A → S	1.6	1.2	0.9
A → G or S	2.5	2.1	1.5
G or S → A	1.1	1.8	2.3

Mixtures of $\alpha\beta_{II}$ and $\alpha\beta_{III}$ tubulin isotypes at the indicated ratios were polymerized to steady state at the ends of axonemal seeds. The total protein concentration in all experiments was 1.6 mg/ml. Values are the mean ± SEM.

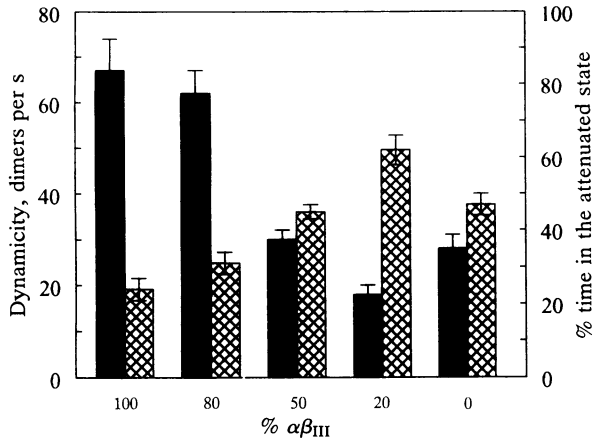

FIG. 3. Effect of different percentages of the $\alpha\beta_{II}$ and $\alpha\beta_{III}$ isotypes on the percentage of total time the microtubules spent in the attenuated state (cross-hatched bars) and dynamicity (solid bars). Experiments were performed by mixing the $\alpha\beta_{II}$ and $\alpha\beta_{III}$ isotypes at different ratios keeping total protein concentration constant at 1.6 mg/ml. The percentage of time in the attenuated state is a measure of the time the microtubules spent neither growing nor shortening detectably. Dynamicity is the mean tubulin dimer exchange rate at microtubule ends calculated from all detectable growing and shortening events including time spent in the attenuated state. Error bars are the SEM.

ratios while keeping the total protein concentration constant (1.6 mg/ml).

The relatively rapid growing and shortening dynamics of microtubules typical of $\alpha\beta_{III}$ microtubules were not appreciably affected when the $\alpha\beta_{II}$ isotype was included along with the $\alpha\beta_{III}$ isotype during polymerization at a ratio of 20% $\alpha\beta_{II}$ and 80% $\alpha\beta_{III}$. For example, the growing and shortening rates (Tables 1 and 2), the percent time the microtubules spent in the attenuated state and the overall dynamicity of the microtubules (Fig. 3) were not significantly different for the microtubules made with pure $\alpha\beta_{III}$ or 20% $\alpha\beta_{II}$ and 80% $\alpha\beta_{III}$. However, when the ratio of $\alpha\beta_{II}$ to $\alpha\beta_{III}$ was increased to 50% $\alpha\beta_{II}$ and 50% $\alpha\beta_{III}$, the dynamics parameters of the resulting microtubules became very similar to those of isotypically pure $\alpha\beta_{II}$ microtubules. For example, the mean growing rates were 49 dimers per s with microtubules made from 100% $\alpha\beta_{II}$, 50 dimers per s with microtubules made from a 50:50 mixture of the $\alpha\beta_{II}$ and $\alpha\beta_{III}$ isotypes, and 77 dimers per s with microtubules made from 100% $\alpha\beta_{III}$ (Tables 1 and 2). Similarly, both the percentage of time that the microtubules spent in the attenuated state and the overall dynamicity (Fig. 3) were not significantly different for the microtubules made from the 50:50 mixture of $\alpha\beta_{II}$ and $\alpha\beta_{III}$ and microtubules made from the pure $\alpha\beta_{II}$ isotype.

Interestingly, when the ratio of $\alpha\beta_{II}$ to $\alpha\beta_{III}$ was increased to 80% $\alpha\beta_{II}$ and 20% $\alpha\beta_{III}$, the dynamics of the resulting microtubules became distinctly slower than the dynamics of the microtubules made from the pure $\alpha\beta_{II}$ isotype. For example, microtubules made from 100% $\alpha\beta_{II}$ spent 47% of the total time in the attenuated state, whereas microtubules made from a mixture of 80% $\alpha\beta_{II}$ and 20% $\alpha\beta_{III}$ spent 62% of the total time in the attenuated state (Fig. 3). Similarly, the dynamicity of the microtubules made from 100% $\alpha\beta_{II}$ was 28 dimers per s, whereas the dynamicity of the microtubules made from a mixture of 80% $\alpha\beta_{II}$ and 20% $\alpha\beta_{III}$ was reduced to 18 dimers per s. Thus, the dynamics of the microtubules polymerized from mixtures of the two isotypes do not reflect the simple averages of the isotypes that are present in solution.

DISCUSSION

We have examined the dynamic instability parameters at steady state at the plus ends of individual microtubules made from three purified β-tubulin isotypes. The purified $\alpha\beta_{II}$, $\alpha\beta_{III}$, and $\alpha\beta_{IV}$ isotypes used in this work account, respectively, for 58%, 25%, and 13% of the total β tubulin in bovine brain tubulin preparations (22). We found that pure $\alpha\beta_{III}$ microtubules were substantially more dynamic than microtubules made from the $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ isotypes or from unfractionated PC-tubulin. In addition, including the $\alpha\beta_{II}$ isotype along with $\alpha\beta_{III}$ during polymerization modulated the dynamics of the microtubules.

Methodological Considerations. The dynamic instability behaviors reported in the present study for microtubules made from the isotypically purified β-tubulin dimers were similar qualitatively to the behaviors observed for microtubules made from unfractionated PC-tubulin (e.g., refs. 10–12). However, the magnitudes of the parameters obtained in the present work were smaller than those reported in other studies and, in general, the magnitudes of the parameters have varied substantially from one study to another. For example, Walker *et al.* (10) observed microtubules *in vitro* to be in a pause (attenuated) state very infrequently, whereas in the present work microtubules made from PC-tubulin spent ≈42% of the total time in the attenuated slate. Such quantitative differences in polymerization dynamics are most likely due to the use of different experimental systems, to the different conditions used, and to the use of different methods of analysis. Important in this work is that microtubule dynamics were analyzed at steady state; most dynamic instability measurements have not been made at steady state (9–11). At steady state, microtubules appear to exhibit smaller length excursions than microtubules undergoing net growing or shortening (16, 30). In addition, the different buffer conditions and temperatures used in the various studies must contribute to the quantitative differences reported. For example, our studies were carried out at 30°C rather than at 37°C, which results in reduced dynamics (D.P. and L.W., unpublished data).

Importantly, values for the growing and shortening rates or for the fraction of total time in a particular phase are highly dependent upon the method of analysis. Because we compared the three β-tubulin isotypes to each other and to PC-tubulin using identical conditions and the same method of analysis, the quantitative differences we observed are valid indicators of differences in dynamic behaviors.

Microtubules Made from Isotypically Pure $\alpha\beta_{III}$ Tubulin Dimers Have Unique Polymerization Dynamics. Microtubules made from the purified $\alpha\beta_{III}$ isotype were significantly more dynamic at their plus ends than microtubules made from the $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ isotypes or PC-tubulin (Fig. 1 and Table 1). For example, the growing and shortening rates and the overall dynamicity of the $\alpha\beta_{III}$ microtubules were more than double those of microtubules made from the $\alpha\beta_{II}$ dimers, $\alpha\beta_{IV}$ dimers, or PC-tubulin (Table 1).

Previous evidence has also indicated that the $\alpha\beta_{III}$ isotype has unique polymerization properties (23, 24, 26). For example, in the presence of 4 M glycerol and 6 mM $MgCl_2$, assembly of the purified $\alpha\beta_{III}$ isotype occurred after a pronounced delay in nucleation while nucleation with the other β-tubulin isotypes occurred quickly (26). Once nucleated, polymerization of the $\alpha\beta_{III}$ isotype proceeded at a slower rate and occurred to a lesser extent than did polymerization of the $\alpha\beta_{II}$ or $\alpha\beta_{IV}$ isotypes. Different rates of polymerization but a similar conclusion regarding the unique properties of $\alpha\beta_{III}$ microtubules were obtained by Banerjee *et al.* (24) who analyzed polymerization of the β-tubulin isotypes in the presence of MAPs.

Our data support the hypothesis that the $\alpha\beta_{III}$ isotype may play a unique role in neurons. Only the neurons in brain and in dorsal root ganglia and Sertoli cells have been shown to express the $\alpha\beta_{III}$ isotype (see ref. 19). The amino acid sequences of the β-tubulin isotypes are substantially different

in their C termini (17–19) and the C terminus appears to play an important role in microtubule assembly (31–33). For example, the ability of MAPs to stabilize microtubule dynamics appears to occur by MAP binding to the C terminus of tubulin (3, 31, 32). All the post-translational modifications known to occur on β tubulin also occur in the C-terminal region, and the $\alpha\beta_{III}$ isotype is uniquely phosphorylated at its C terminus (34). Perhaps the C-terminal domain of the $\alpha\beta_{III}$ isotype interacts more strongly than other β-tubulin isotypes with a neuron-specific MAP, thereby selectively stabilizing neuronal microtubules when the MAP is present and making the microtubules especially dynamic when the MAP is either absent or modified.

The Tubulin Isotype Composition Can Specify the Polymerization Dynamics of a Microtubule. Mixing the $\alpha\beta_{II}$ and $\alpha\beta_{III}$ isotypes resulted in the formation of microtubules with significantly different assembly dynamics depending upon the ratio of the two isotypes present during polymerization. Despite the fact that microtubules made from the pure $\alpha\beta_{III}$ isotype were significantly more dynamic than microtubules made from the pure $\alpha\beta_{II}$ isotype, microtubules formed from a mixture of 20% $\alpha\beta_{III}$ and 80% $\alpha\beta_{II}$ dimers were less dynamic than microtubules made from isotypically pure $\alpha\beta_{II}$ dimers. These data support the idea that presence of a specific isotype in solution or its incorporation into a microtubule may confer unique polymerization properties to that microtubule. Matthews *et al.* (35) observed that mutation and alteration of the normal isotype ratio in *Drosophila* produced severe effects both on mitosis and meiosis and speculated that the isotype composition might control microtubule-dependent cell function. Hoyle and Raff (20) expressed the *Drosophila* β3 isotype in the male germ cell line of *Drosophila* in place of the β2 isotype and observed that altering the isotype ratios allowed certain microtubule-dependent functions to occur normally, but because all such males were sterile, other microtubule functions must have been defective. Replacement of the β2 isotype with the β3 isotype in the experiment of Hoyle and Raff (20) could have resulted in the formation of microtubules with significantly altered dynamics that may have adversely affected the functions of a specific microtubule subset required for spermiogenesis.

In summary, the functional significance of tubulin isotypes remains unclear. Because some isotypes have different tissue distributions and because the sequence differences among the isotypes are very strongly conserved in evolution suggest that isotypes may have a functional significance as first suggested by Fulton and Simpson (36). Due to their amino acid sequence differences, the β-tubulin isotypes may adopt different conformations leading to differences in their tubulin–tubulin interaction sites in the microtubule and to differences in the dynamics. Incorporation of a specific tubulin isotype into a microtubule may modulate the dynamic behavior or function of a microtubule directly, or it may do so less directly by providing specific recognition sites for MAPs or other regulators of microtubule dynamics. One could imagine that cells might regulate the dynamic properties of their microtubules by altering the relative amounts of the different tubulin isotypes that they express.

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