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Removal of β_{III} Isotype Enhances Taxol Induced Microtubule Assembly*

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ABSTRACT. **The interaction of β_{III}-depleted tubulin with taxol was investigated. A monoclonal antibody against the β_{III} tubulin isotype was immobilized on a sepharose 4B column and used to remove the β_{III} tubulin isotype from unfractionated tubulin. The assembly of β_{III}-depleted tubulin in the presence of taxol was enhanced compared to unfractionated tubulin. The critical concentration of unfractionated tubulin in the presence of 10 μM taxol is 0.4 mg/ml, while the critical concentration of β_{III}-depleted tubulin is 0.16 mg/ml. At different concentrations of taxol, the assembly of β_{III}-depleted tubulin is increased relative to that of unfractionated tubulin and reaches the maximum at about a 1 : 1 ratio of tubulin and taxol. The assembly of unfractionated tubulin and β_{III}-depleted tubulin has also been studied by electron microscopy. After 2 minutes at 37°C, unfractionated tubulin assembly in the presence of 10 μM taxol results only in ribbon-like and ring structures; there are no visible microtubules. By 5 minutes, microtubules appear and increase in length. The assembly of β_{III}-depleted tubulin in the presence of 10 μM taxol occurs more quickly. In contrast to the case with unfractionated tubulin, β_{III}-depleted tubulin assembles within 2 minutes into microtubules which increase in length with time. At 30 minutes, microtubules assembled from β_{III}-depleted tubulin are shorter than the microtubules assembled from unfractionated tubulin. There is no visible difference between the microtubules assembled from unfractionated tubulin and β_{III}-depleted tubulin. Taxol-induced β_{III}-depleted tubulin assembly is more resistant to the inhibiting effect of podophyllotoxin and colchicine. It is also less sensitive to the inhibiting effect of cold temperature.**

Microtubules are known to be involved in many cell functions. For example, they are essential for mitosis, cell movement, vesicle transport and maintenance of cell shape (9). The major component of microtubules is tubulin. It is a heterodimer of α and β subunits, each of 50,000 daltons (2). Tubulin can reversibly assemble into microtubules in the presence of GTP, magnesium ion and microtubule associated proteins (MAPs) (2, 9). Although MAPs are essential for microtubule assembly, tubulin depleted of MAPs can assemble into microtubules under certain conditions *in vitro*. These conditions involve the presence of glycerol, dimethyl sulfoxide and high concentrations of magnesium ion (9). Taxol, an antitumor drug derived from the western yew, can also promote microtubule assembly in the absence of MAPs (26). Microtubules assembled in the presence of taxol have very different characteristics from microtubules assembled in the presence of MAPs. They are resistant to depolymerization induced by cold or $CaCl_2$ (26). In contrast to other antimitotic drugs, such as colchicine, podophyllotoxin and vinblastine, which inhibit microtubule assembly (2, 9), taxol seems to exert its antimitotic effect by promoting microtubule assembly. The molecular mechanism by which taxol induces microtubule assembly is unknown.

In mammalian cells, both α and β subunits of tubulin are encoded by multigene families (12, 28). At least 6 α-tubulin genes and 7 β-tubulin genes have been found in mammalian cells (12, 21, 28, 30). Although α and β tubulin gene families are among the most highly conserved in eukaryotic cells (13, 23), significant divergences have been found in the C-terminal sequences of the β-tubulin isotypes (27). Both α and β subunits undergo posttranslational modifications. α-Tubulin is modified by acetylation (15, 22), glutamylation (10) and C-terminal tyrosination and detyrosination (6, 24). In contrast, the class III β-tubulin has been found to be phosphorylated at residue 444 (1, 8, 19) and glutamylated by attachment of one to six glutamic acid residues to the

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The abbreviations used are: MES, 2-[N-morpholino]ethane-sulfonic acid; EGTA, ethyleneglycol-bis-N,N,N′,N′-tetraacetic acid; EDTA, ethylenediamine tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; PC-tubulin, tubulin purified by passage through a phosphocellulose column.

side chain of Glu-438 (1).

The multiplicity of tubulin isotypes has long propelled the argument that different tubulin isotypes may mediate different functions. Recent evidence suggests that this is indeed the case. Removal of the β_{III} isotype from bovine brain tubulin increases microtubule assembly in the presence of MAP2 or tau (4). The β_{III} tubulin isotype seems to be responsible for the slow phase binding of colchicine (5). The removal of the β_{III} tubulin isotype from PC-tubulin changes the binding kinetics of colchicine from biphasic to monophasic and the slow phase of colchicine binding to tubulin is abolished.

Here we have studied the interaction of β_{III}-depleted bovine brain tubulin with taxol. Our results suggest that β_{III}-depleted tubulin assembles more quickly and forms shorter microtubules in the presence of taxol than does phosphocellulose-purified tubulin. Furthermore, the taxol-induced β_{III}-depleted tubulin assembly is more resistant to the inhibiting effect of microtubule-depolymerizing drugs and cold temperatures.

These results have been reported in preliminary form elsewhere (18).

MATERIALS AND METHODS

Materials. Taxol was a gift from the National Cancer Institute and prepared according to the protocol of Schiff *et al.* (26). Podophyllotoxin was from Aldrich Chemical Company, Inc. (Milwaukee, WI); CNBr-activated Sepharose 4B was from Pharmacia LKB (Uppsala, Sweden); Colchicine and protein-A agarose and all other chemicals were from Sigma Chemical Company (St. Louis, MO). Monoclonal anti-β_{III} antibody (SDL.3D10) was prepared and coupled to CNBr-activated Sepharose 4B as described by Banerjee *et al.* (3).

Tubulin preparation. Microtubule protein was prepared by a cycle of assembly and disassembly from bovine cerebra by the procedure of Fellous *et al.* (11) and was stored as pellets at −70°C. Immediately before use, the pellets were resuspended in buffer A (0.1 M Mes, pH 6.4, 1 mM EGTA, 0.1 mM EDTA, 1 mM GTP and 0.5 mM $MgCl_2$) and tubulin was purified by phosphocellulose chromatography by the procedure of Fellous *et al.* (11). β_{III}-depleted tubulin was purified according to the procedure of Banerijee *et al.* (4). Briefly, phosphocellulose-purified tubulin (PC-tubulin) was passed through the anti-β_{III} immunoaffinity column. The unbound fraction was collected and used as β_{III}-depleted tubulin for further experiments within 12 hours after preparation. The bound tubulin contained β_{III} and a small fraction of β_{II} and β_{IV} tubulin. The purity of β_{III}-depleted tubulin was verified later by electrophoresis and immunoblotting.

Electrophoresis and electroblotting. Tubulin samples were reduced and carboxymethylated by the procedure of Crestfield *et al.* (7), and subjected to electrophoresis on 5.5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate (14). Electroblotting was carried out on nitrocellulose membranes in a Biorad transblot apparatus. Electroblotting was done at 30 v overnight at 4°C. The blots were analyzed using the procedure of Banerjee *et al.* (3) with the following modifications: The secondary antibody used was goat-anti-mouse polyclonal antibody conjugated with horse peroxidase obtained from Biorad instead of ^{125}I-labelled antibody. The protein bound on the nitrocellulose membrane was visualized by a colorimetric method using the substrate kit from Biorad instead of autoradiography.

Electron microscopy. Electron microscopy was done according to the procedure of Banerjee *et al.* (4). Tubulin (1 mg /ml) and taxol (10 μM) were incubated at 37°C. At various time points, aliquots were withdrawn and mixed immediately with an equal volume of 1% glutaraldehyde. 10 μl of this mixture was mounted on a collodion and carbon coated copper grid and allowed to absorb for 30 seconds. The grid was washed sequentially with the following solutions (1) 1 mg/ml cytochrome C in water; (2) water; (3) 1% uranyl acetate in water. All solutions were passed through a 0.2 μm filter before use and excess stain was soaked off with blotting paper. The grids were air dried and examined under a Jeol 100cx electron microscope at an accelerating voltage of 60 kv and photographed. The lengths of microtubules on electron micrograph prints at a final magnification of 1450× were measured using a Jandel digitizer connected with the Sigmascan computer program. At least 400 microtubules were measured at each time point.

Microtubule assembly. Aliquots (300 μl) of PC-tubulin or β_{III}-depleted tubulin at various concentrations were incubated with 10 μM taxol at 37°C for 30 min. The samples were centrifuged at 100,000 rpm on a Beckman airfuge for 10 min. The total protein concentration (Y-axis) was plotted against the pellet protein concentration (X-axis) and the intercept with the X-axis was taken as the critical concentration. Tubulin was assembled in the presence of 10 μM taxol and a series of concentrations of either podophyllotoxin or colchicine at 37°C for 30 min. The microtubules were collected by centrifugation and the pellet protein concentration was determined after it was resuspended. The percentage of inhibition was estimated by comparing the pellet protein concentrations at different concentrations of podophyllotoxin or colchicine with controls which only contain tubulin and taxol. the 50% inhibition concentrations of podophyllotoxin and colchicine were defined as the drug concentration at which 50% of microtubule assembly was inhibited.

Other methods. Protein concentrations were determined by the method of Lowry *et al.* (17) as modified by Schacterle and Pollack (25). Bovine serum albumin was used as a standard. Microtubule assembly was carried out at 37°C or other indicated temperatures in a Gilford model 250 spectrophotometer. Cuvette temperature was controlled using a Gilford thermostat. All assembly experiments were done in buffer A (0.1 M Mes, pH 6.4, 1 mM EGTA, 0.1 mM EDTA, 1 mM GTP and 0.5 mM $MgCl_2$).

RESULTS

Assembly of β_{III}-depleted tubulin in the presence of taxol. Phosphocellulose-purified bovine brain tubulin (PC-tubulin) was passed through the anti-β_{III} immunoaffinity column. The flow-through fractions as well as the bound fractions eluted by 0.5 M NaCl were collected. The β_{III}-depleted tubulin was reduced and carboxymethylated and was analyzed by polyacrylamide gel electrophoresis and immunoblot analysis. Fig. 1 shows that there is no detectable β_{III} isotype in the β_{III}-depleted tubulin by immunoblot analysis using anti-β_{III} monoclonal antibody, while the bound fractions of the anti-β_{III} immunoaffinity column were enriched in the β_{III} tubulin isotype.

To study the interaction of taxol with β_{III}-depleted tubulin, both PC-tubulin and β_{III}-depleted tubulin at protein concentrations of 1.0, 1.5, 2.0 and 2.5 mg/ml were assembled in the presence of 10 μM of taxol at 37°C for 30 min. The polymerization was monitored by the change of absorbance at 350 nm. Fig. 2 shows that β_{III}-depleted tubulin assembled much faster and to a greater extent in the presence of taxol than did PC-tubulin at the same protein concentrations. Assembly of β_{III}-depleted tubulin in the presence of taxol was resistant to the depolymerization induced by cold or calcium (data not shown). The same is true for PC-tubulin as demonstrated before (3).

To further characterize the β_{III}-depleted tubulin assembly in the presence of taxol, the critical concentrations of PC-tubulin and β_{III}-depleted tubulin were measured in the presence of 10 μM taxol. Different concentrations of PC-tubulin or β_{III}-depleted tubulin were incubated with 10 μM taxol at 37°C for 30 min. Microtubules were collected by centrifugation. The total protein concentration was plotted against the pellet protein concentration. The intercept with the X-axis (the total tubulin concentration) was the critical concentration. The slope represents the activity of the tubulin preparations (20). The activities of both PC-tubulin and β_{III}-depleted tubulin were estimated about 80%. The critical concentration of PC-tubulin averages about 0.4 mg/ml as measured by six independent experiments, while the critical concentration of β_{III}-depleted tubulin is only

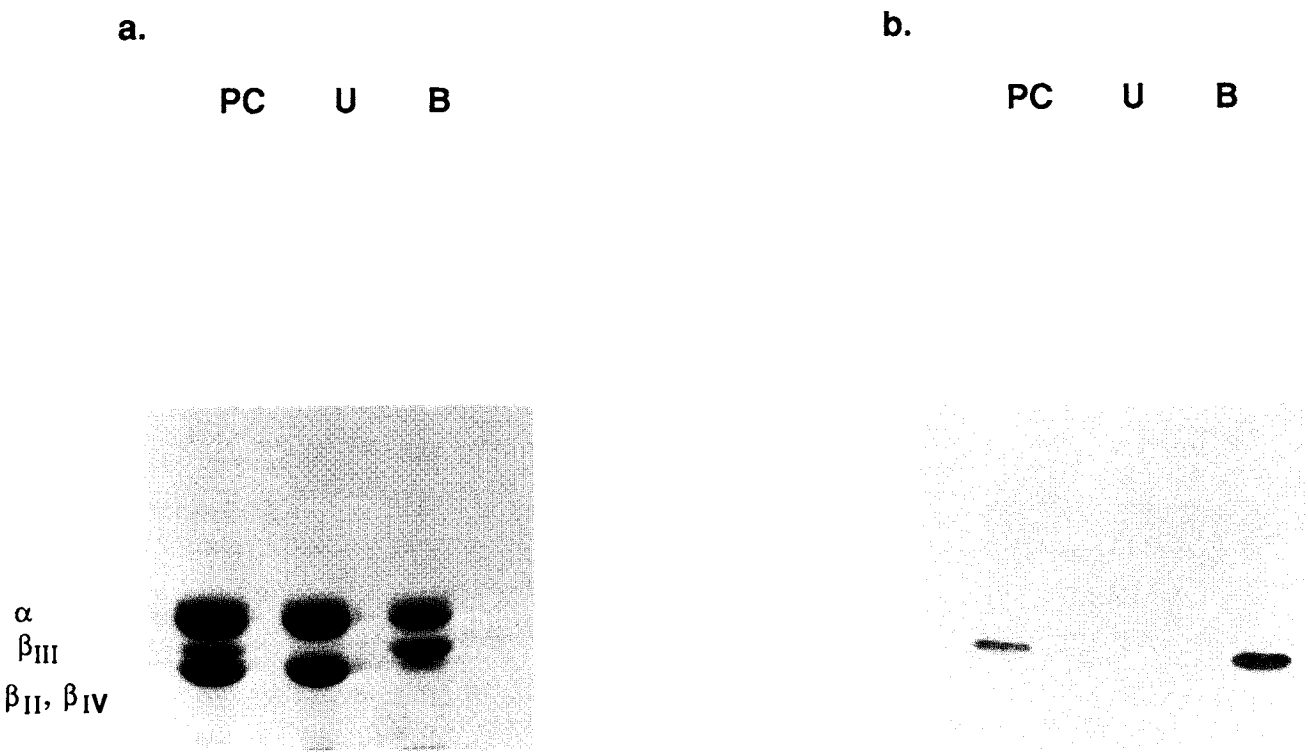

Fig. 1. Immunoblot analysis of β_{III}-depleted tubulin.
Phosphocellulose-purified tubulin (PC-tubulin) was chromatographed on the anti-β_{III} immunoaffinity column (see "Materials and Methods"). The samples: PC-tubulin (PC), unbound fraction (U), or bound fraction (B) which was eluted with 0.5 M NaCl from the immunoaffinity column were reduced and carboxymethylated and subjected to electrophoresis on a 5.5% polyacrylamide gel. Two identical gels were run and approximately 10 μg of each sample were loaded onto the gel. (a). The polyacrylamide gel was stained by Coomassie Blue. (b). The gel was electroblotted on the nitrocellulose membrane first and treated with SDL.3D10, the monoclonal antibody against β_{III} tubulin isotype. The blot was then treated with the secondary goat-antimouse antibody conjugated with horse peroxidase and visualized by the colorimetric method.

about 0.16 mg/ml as measured by eight independent experiments. The difference is significant ($P < 0.05$) (Table I).

The concentration-dependence of the effect of taxol on the assembly of PC-tubulin and β_{III}-depleted tubulin is shown in Fig. 3. Both PC-tubulin and β_{III}-depleted tubulin were assembled in the presence of a series of concentrations of taxol at 37°C for 30 min. Microtubules were collected by centrifugation. The percentage of polymerization was calculated by comparing the pellet protein concentration with the total protein concentration. The assembly of both PC-tubulin and β_{III}-depleted tubulin reached a maximum at a 1 : 1 ratio of the total concentrations of tubulin and taxol, and at all concentrations of taxol, the microtubule mass of β_{III}-depleted tubulin is greater than that of PC-tubulin.

Electron microscopic examination of microtubules assembled from β_{III}-depleted tubulin in the presence of taxol. Samples of PC-tubulin and β_{III}-depleted tubulin (1 mg/ml) were assembled in the presence of 10 μM taxol at 37°C for 30 min. At various time points, aliquots were withdrawn and fixed with 1% glutaraldhyde and prepared for electron microscopic examination (Fig. 4). At 2 min of incubation, no microtubules were visible in PC-tubulin treated with taxol (Fig. 4a); by 5 min, microtubules had appeared (Fig. 4b); they increased with time both in number and length (Fig. 4c, d). Fig. 4 (e-h) shows the microtubules assembled from β_{III}-depleted tubulin in the presence of taxol at 2 min, 5 min, 10 min and 30 min. At 2 min, microtubules had already formed (Fig. 4e), and, with time, they increased in number and length (Fig. 4f-h). In accordance with the spectrophotometer observation, microtubule formation from β_{III}-depleted tubulin in the presence of taxol was faster than that of PC-tubulin when observed by electron microscopy.

Table I. CRITICAL CONCENTRATION OF TUBULIN.

	PC-Tubulin	β_{III}-depleted Tubulin
Critical Concentration (mg/ml)	0.40±0.14 (n=6)	0.16±0.10 (n=8)
t test	P<0.01	

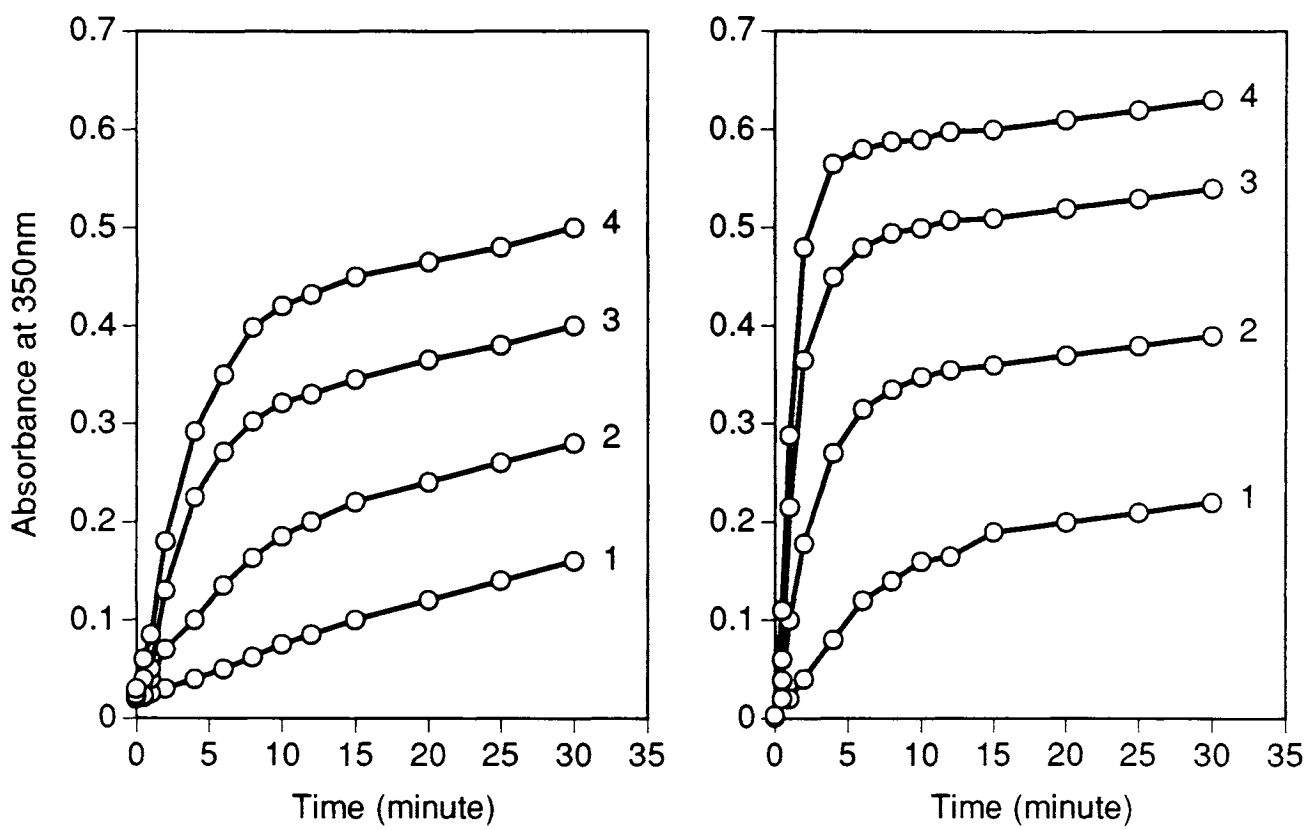

Fig. 2. Effect of protein concentration on microtubule assembly.
Samples of PC-tubulin and β_{III}-depleted tubulin were assembled at different concentrations at 37°C for 30 min in the presence of 10 μM taxol. The left panel is PC-tubulin at 1 mg/ml (curve 1), 1.5 mg/ml (curve 2), 2.0 mg/ml (curve 3) and 2.5 mg/ml (curve 4). The right panel is β_{III}-depleted tubulin at 1 mg/ml (curve 1), 1.5 mg/ml (curve 2), 2.0 mg/ml (curve 3) and 2.5 mg/ml (curve 4).

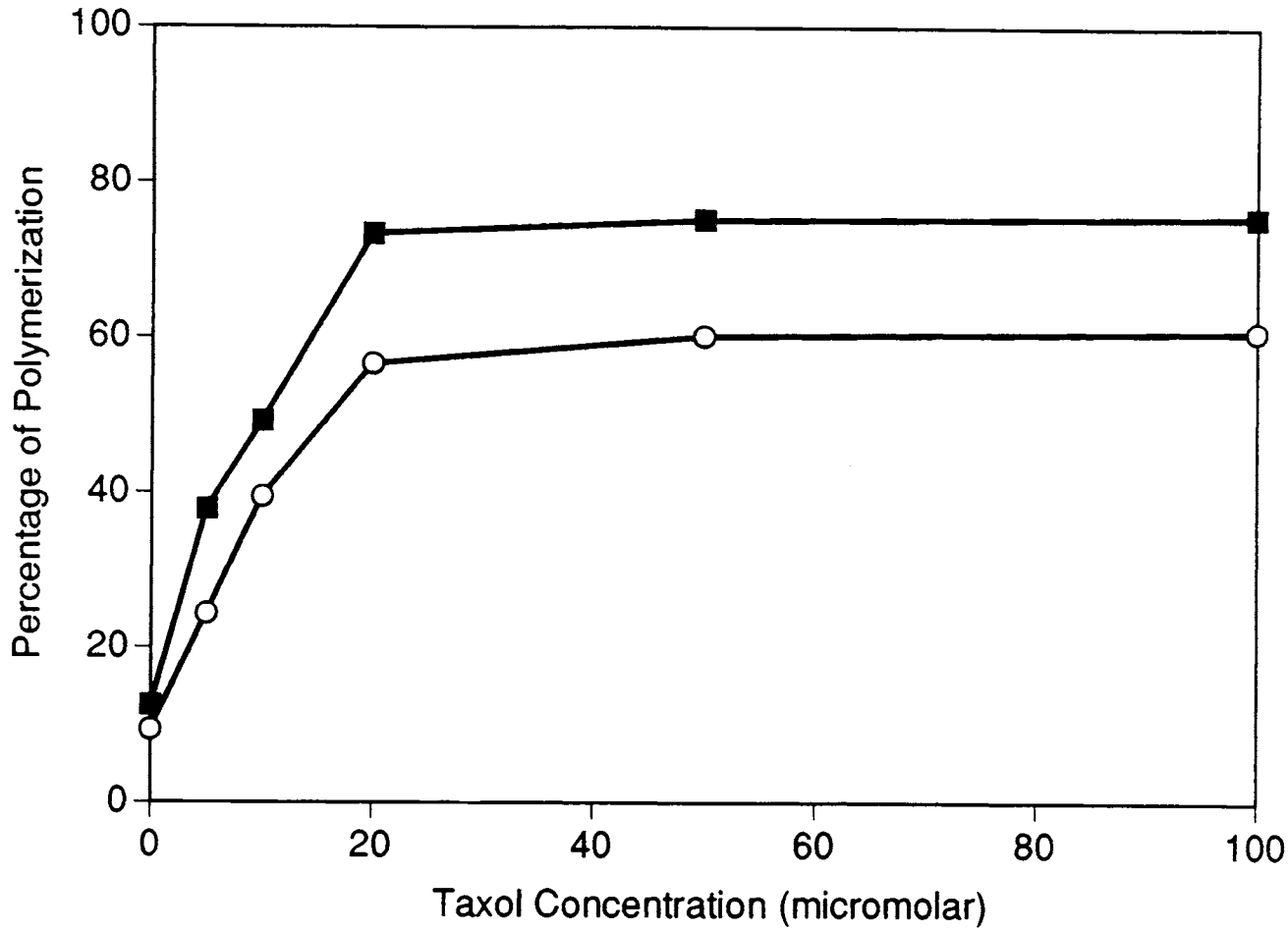

Fig. 3. Effect of taxol concentration on microtubule assembly.
Samples of PC-tubulin (○) and β_{III}-depleted tubulin (●) at 2 mg/ml were assembled in the presence of a series of taxol concentrations at 37°C for 30 min. Microtubules were collected by centrifugation on a Beckman airfuge at 100,000 rpm for 10 min. The total protein concentration and the pellet protein concentration were measured and the percentage of polymerization was calculated.

We have investigated the change in length of microtubules assembled from PC-tubulin and β_{III}-depleted tubulin in the presence of taxol. Fig. 5 (left panel) shows the length distributions of microtubules assembled from PC-tubulin in the presence of taxol at 5 min, 10 min and 30 min. Fig. 5 (right panel) shows the length distributions of microtubules assembled from β_{III}-depleted tubulin in the presence of taxol at 2 min, 5 min, 10 min and 30 min. As microtubule assembly proceeds, the frequency spectrum shifts to the right which indicates net growth of microtubules. The shift of the frequency spectrum in β_{III}-depleted tubulin assembly is less than in that of PC-tubulin. Therefore, microtubule growth in β_{III}-depleted tubulin assembly is slower than in PC-tubulin.

The effect of temperature and microtubule-depolymerizing drugs on the assembly of β_{III}-depleted tubulin in the presence of taxol. To study the effect of temperature on the assembly of β_{III}-depleted tubulin in the presence of taxol, aliquots of PC-tubulin and β_{III}-depleted tubulin (2 mg/ml) were incubated in the presence of 20 μM taxol at either 25°C or 15°C for 30 min. The absorbance change at 350 nm was recorded. The maximum polymerization was assumed as the absorbance change of

Fig. 4. Electron microscopic characterization of microtubules assembled from PC-tubulin and β_{III}-depleted tubulin in the presence of taxol. Samples of PC-tubulin and β_{III}-depleted tubulin (1 mg/ml) prepared as described in "Materials and Methods" were assembled in the presence of 10 μM taxol at 37°C for different amounts of time. At each time point, aliquots were withdrawn and fixed with 1% glutaraldehyde and prepared for electron microscopy. Microtubules assembled from PC-tubulin in the presence of taxol at 2 min, 5 min, 10 min and 30 min (a–d) Microtubules assembled from β_{III}-depleted tubulin in the presence of taxol at 2 min, 5 min, 10 min and 30 min (e–h). Figure a is at a magnification of 98,000×, all other figures are at 78,000×.

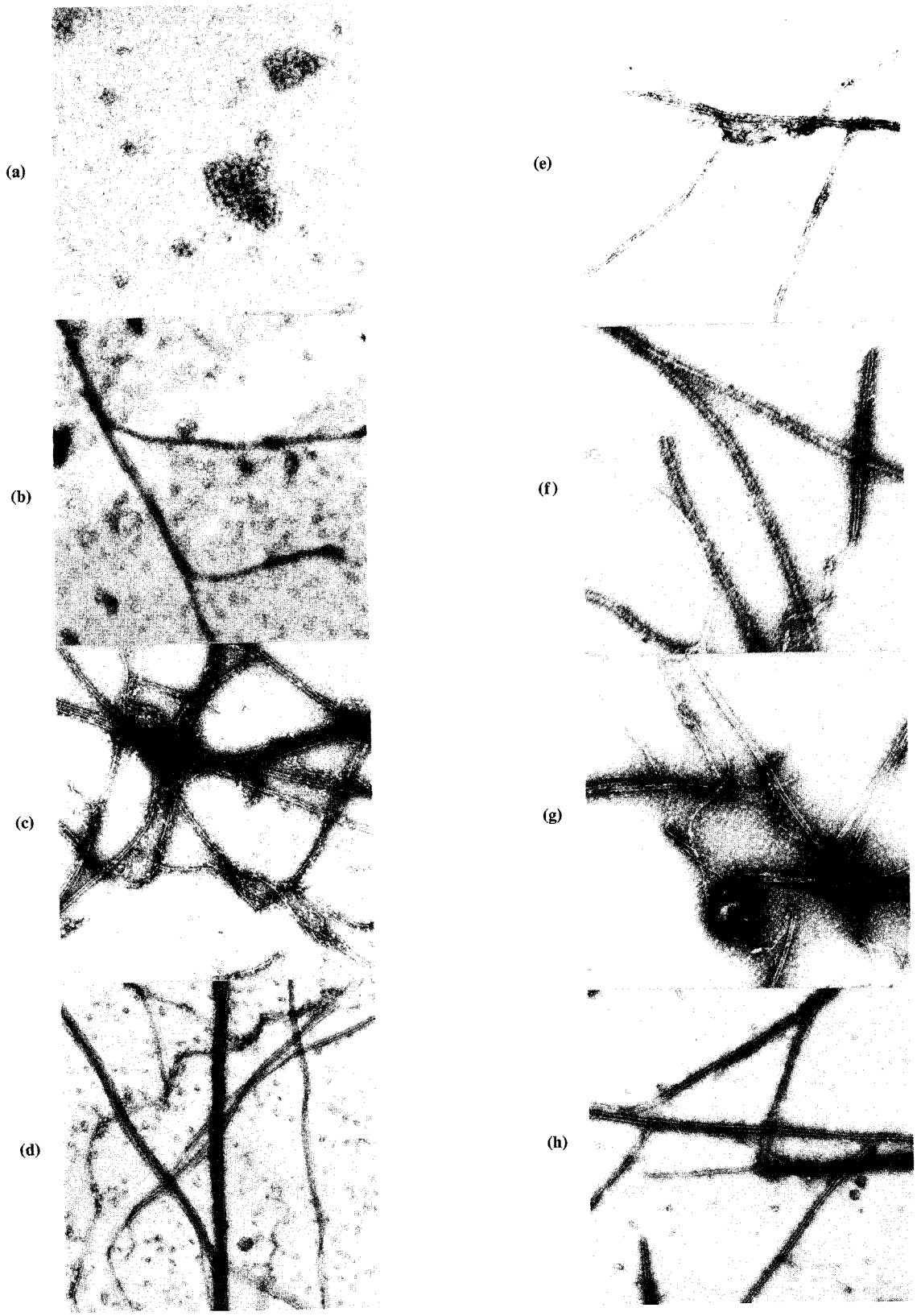

Fig. 4.

either PC-tubulin or β_{III}-depleted tubulin (2 mg/ml) assembled in the presence of 20 μM taxol at 37°C for 30 min. Fig. 6 shows that at 25°C or 15°C, the assembly of β_{III}-depleted tubulin is more resistant to cold temperature than is that of PC-tubulin. At 25°C, the assembly of β_{III}-depleted tubulin is at about 79% of maximum as compared to 49% of maximum for that of PC-tubulin. At 15°C, the assembly of β_{III}-depleted tubulin is at 20% of maximum while that of PC-tubulin is only at 8%.

The effects of microtubule-depolymerizing drugs were studied by incubating PC-tubulin or β_{III}-depleted tubulin (2 mg/ml) and 10 μM taxol in the presence of a series of concentrations of podophyllotoxin or colchicine at 37°C for 30 min. Microtubules were collected by centrifugation and the percentage of polymerization was calculated. The result in Table II shows that the concentration of podophyllotoxin or colchicine required to cause 50% inhibition of the assembly of β_{III}-depleted tubulin in the presence of taxol is almost double that required for PC-tubulin. Therefore, β_{III}-depleted tubulin assembly in the presence of taxol is more resistant to the inhibiting effect of microtubule-depolymerizing drugs such as podophyllotoxin and colchicine.

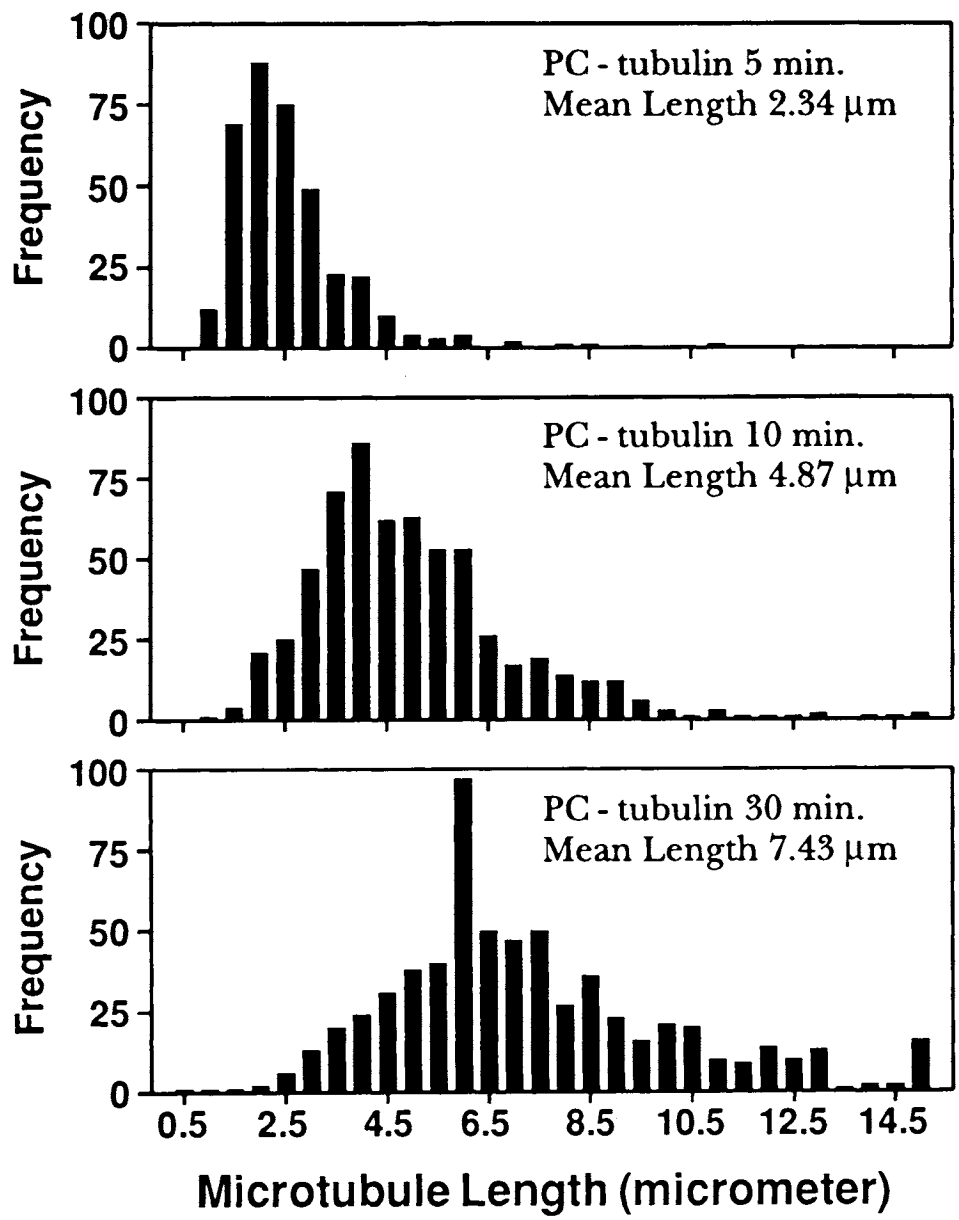

Fig. 5. (to be continued)

DISCUSSION

The β_{III} isotype accounts for about 25% of the total β tubulin in bovine cerebral microtubule preparations (3). β_{III}-tubulin is significantly different from the other β tubulin isotypes in its C-terminal sequence; it appears to be the only β isotype which is phosphorylated (19). β_{III}-tubulin is about 92% homologous to β_I, β_{II}, and β_{IV}. The latter are about 96–98% homologous to each other (16). Therefore, it is not unreasonable to speculate that the β_{III} tubulin isotype may have distinct characteristics which could affect its ability to polymerize into microtubules. The previous report (4) has established that β_{III}-depleted tubulin assembly increases in the presence of either MAP2 or tau compared to that of PC-tubulin. Since taxol has a very different interaction pattern with

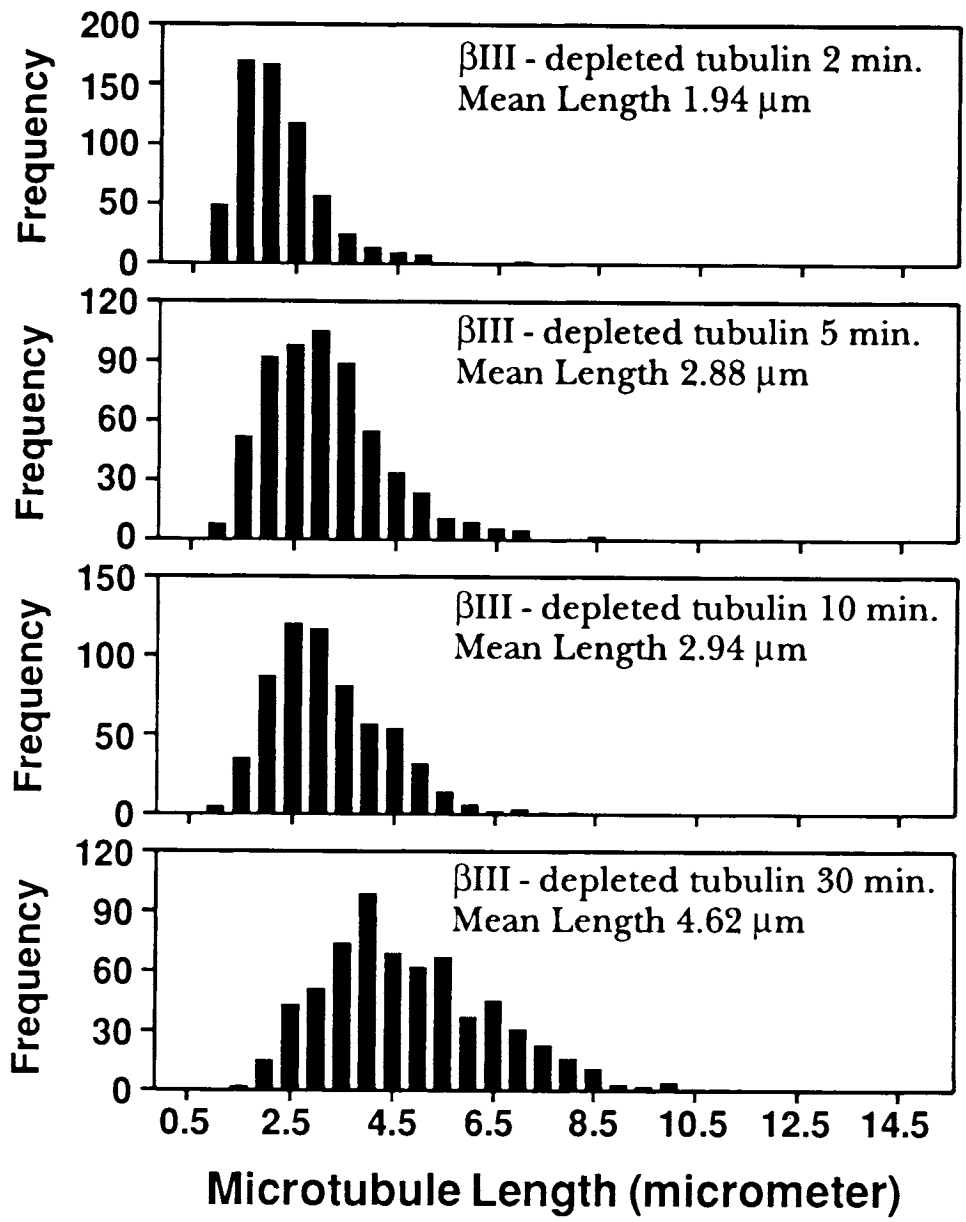

Fig. 5. Microtubule length distributions.
Samples of PC-tubulin and β_{III}-depleted tubulin (1 mg/ml) were assembled in the presence of 10 μM taxol. At different time points, microtubule lengths were determined as described in "Materials and Methods". The left panel shows the length frequency distributions of microtubules assembled from PC-tubulin in the presence of taxol at 5 min, 10 min and 30 min. The right panel shows the length frequency distributions of microtubules assembled from β_{III}-depleted tubulin in the presence of taxol at 2 min, 5 min, 10 min and 30 min.

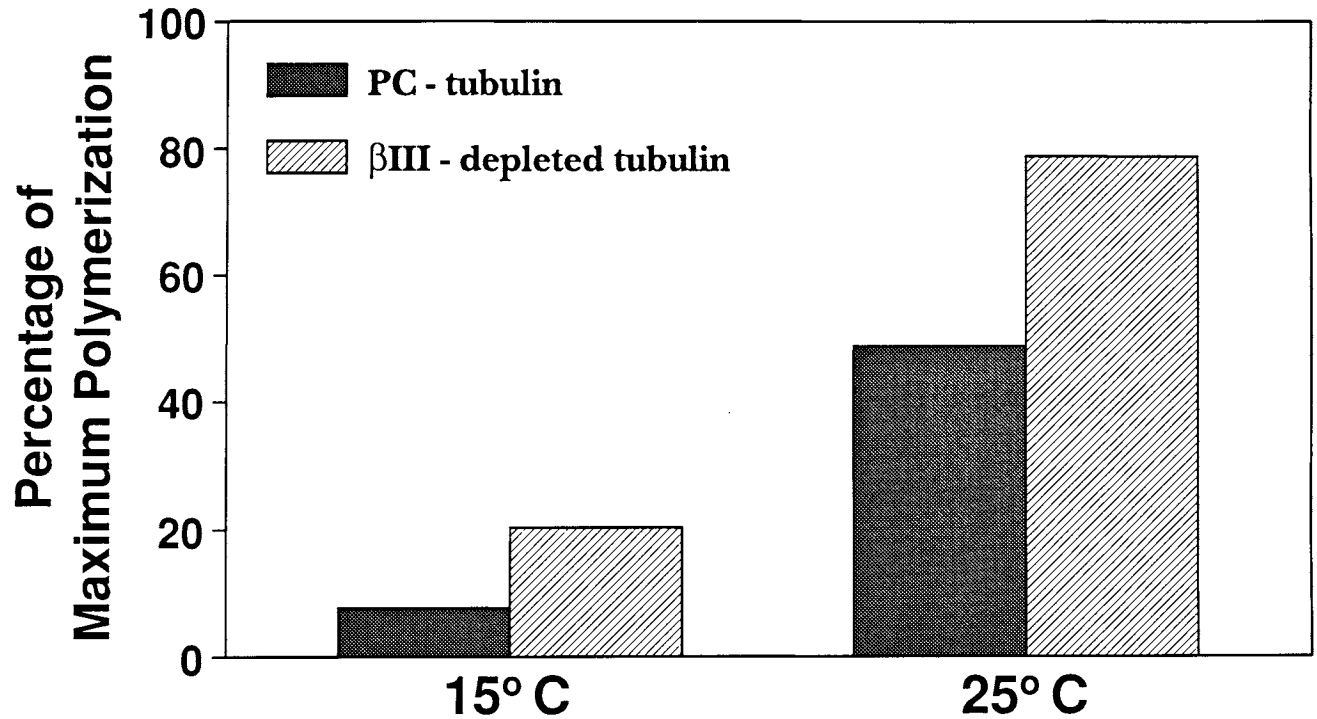

Fig. 6. Effect of temperature on microtubule assembly of β_{III}-depleted tubulin in the presence of taxol.
Samples of PC-tubulin and β_{III}-depleted tubulin (2 mg/ml) were assembled in the presence of 10 μM taxol at the indicated temperatures for 30 min. The polymerization was monitored by turbidimetry at 350 nm. The maximum polymerization was assumed to be the absorbance change after 30 min of assembly at 37°C.

tubulin than do MAP2 or tau (29), we decided to investigate the interaction of β_{III}-depleted tubulin with taxol.

The nucleation of microtubule assembly of β_{III}-depleted tubulin in the presence of taxol is greatly enhanced compared to that of PC-tubulin. This is demonstrated by the increased rate of assembly monitored by the absorbance change at 350 nm, the significant decrease of critical concentration and the early appearance of microtubules as seen by electron microscopic examination of microtubules made from β_{III}-depleted tubulin. The initial increase of absorbance seen in assembly of β_{III}-depleted tubulin is much greater than that seen with PC-tubulin (Fig. 2). The critical concentration of assembly of β_{III}-depleted tubulin is only about 0.16 mg/ml compared to 0.4 mg/ml for PC-tubulin. After 2 min of incubation in the presence of taxol, β_{III}-depleted tubulin formed microtubules visible under electron microscopy while no microtubules formed from PC-tubulin. The mechanism of enhanced assembly of β_{III}-depleted tubulin is not certain. One possible explanation for this is that pure tubulin isotypes may have a greater tendency to form microtubules than a mixture of different isotypes. Even though β_{III}-depleted tubulin is not homogeneous, the β isotypes which it contains resemble each other considerably more than any of them resembles the β_{III} isotype. In agreement with this hypothesis, assembly of β_{III}-depleted tubulin induced by taxol is more resistant to the inhibiting effect of cold temperature or the presence of microtubule-depolymerizing drugs than is that of PC-tubulin.

Table II. THE EFFECT OF MICROTUBULE POISONS ON TUBULIN ISOTYPES ASSEMBLY.

	PC-Tubulin	β_{III}-depleted Tubulin
50% Inhibition Conc. of Podophyllotoxin (μM)	3.3	7.7
50% Inhibition Conc. of Colchicine (μM)	4.2	8.9

Although the nucleation of microtubules made from β_{III}-depleted tubulin is greatly enhanced, these microtubules are slow to elongate. The average length of microtubules formed by β_{III}-depleted tubulin in the presence of taxol at steady state is shorter than that of PC-tubulin. According to the dynamic instability model (20), the microtubule end switches between two phases. In one phase, microtubule ends are relatively stable and

grow slowly, while, in the second phase, the microtubule ends are unstable and depolymerize catastrophically. What mechanism controls the switch is not clear. Because the elongation of microtubules formed from β_{III}-depleted tubulin in the presence of taxol has been somewhat impaired relative to that of PC-tubulin despite the enhancement of nucleation, the β_{III} tubulin isotype may play a role in the stabilization of microtubule ends by switching the shrinking ends to growing ends. Although this is a highly speculative hypothesis, it could be testable when we are able to purify large quantity of pure β tubulin isotypes and study their assembly dynamics.

Our observation is that the dynamics of microtubule assembly-nucleation and elongation rates-are strongly inflenced by the isotypic composition of the tubulin population which forms these microtubules. This result raise the interesting possibility that a cell could modulate the dynamic behavior of its microtubules by altering the relative amounts of the tubulin isotypes that are present in the cell. If such a possibility were true, it would allow very sensitive regulation of the rate and extent of microtubule-related processes *in vivo*.

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REFERENCES

1. Alexander, J.E., Hunt, D.F., Lee, M.K., Shabanowitz, J., Michel, H., Berlin, S.C., MacDonald, T.L., Sundberg, R.J., Rebhun, L.I., and Frankfurter, A. 1991. Characterization of posttranslational modifications in neuron-specific Class III β-tubulin by mass spectrometry. *Proc. Natl. Acad. Sci. USA,* **88**: 4685–4689.
2. Avila, J. 1992. *Microtubule Proteins.* CRC Press Inc., Florida.
3. Banerjee, A., Roach, M.C., Wall, K.A., Lopata, M.A., Cleveland, D.W., and Luduena, R.F. 1988. A monoclonal antibody against the type II isotype of β-tubulin. *J. Biol. Chem.,* **263**: 3029–3034.
4. Banerjee, A., Roach, M.C., Trcka, P., and Luduena, R.F. 1990. Increased microtubule assembly in bovine brain tubulin lacking the type III isotype of β-tubulin. *J. Biol. Chem.,* **265**: 1794–1799.
5. Banerjee, A. and Luduena, R.F. 1991. Distinct colchicine binding kinetics of bovine tubulin lacking the type III isotype of β-tubulin. *J. Biol. Chem.,* **266**: 1689–1691.
6. Barra, H.S., Arce, C.A., Rodriguez, J.A., and Caputto, R. 1974. Some common properties of the protein that incorporates tyrosine as a single unit and the microtubule proteins. *Biochem. Biophys. Res. Commun.,* **60**: 1384–1390.
7. Crestfield, A.M., Moore, S., and Stein, W.H. 1963. The preparation and enzymatic hydrolysis of reduced and S-carborymethylated proteins. *J. Biol. Chem.,* **238**: 622–627.
8. Diaz-Nido, J., Serrano, L., Lopez-Otin, C., Vandekerckhove, J., and Avila, J. 1990. Phosphorylation of a neuronal-specific β-tubulin isotype. *J. Biol. Chem.,* **265**: 13949–13954.
9. Dustin, P. 1984. *Microtubules.* 2nd Ed., Springer-Verlag, Berlin.
10. Edde, B., Rossier, J., Le Caer, J-P., Desbruyeres, E., Gros, F., and Denoulet, P. 1990. Posttranslational glutamylation of α-tubulin. *Science,* **247**: 83–85.
11. Fellous, A., Francon, J., Lennon, A.-M., and Nunez, J. 1977. Microtubule assembly *in vitro*. *Eur. J. Biochem.,* **78**: 167–174.
12. Havercroft, J.C. and Cleveland, D.W. 1984. Programmed expression of β-tubulin genes during development and differentiation of the chicken. *J. Cell Biol.,* **99**: 1927–1935.
13. Krauhs, E., Little, M., Kempf, T., Hofer-Warbinek, R., Ade, W., and Ponstingl, H. 1981. Complete amino acid sequence of β-tubulin from porcine brain. *Proc. Natl. Acad. Sci. USA,* **78**: 4156–4160.
14. Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* **227**: 680–685.
15. L'Hernault, S.W. and Rosenbaum, J.L. 1985. Chlamydomonas α-tubulin is posttranslationally modified by acetylation on the ϵ-amino group of a lysine. *Biochemistry,* **24**: 473–478.
16. Little, M. and Seehaus, T. 1988. Comparative analysis of tubulin sequences. *Comp. Biochem. Physiol.,* **90B**: 655–670.
17. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.,* **193**: 265–275.
18. Lu, Q. and Luduena, R.F. 1991. The interaction of β_{III}-depleted tubulin with taxol. *J. Cell Biol.,* **115**: 38a.
19. Luduena, R.F., Zimmermann, H.P., and Little, M. 1988. Identification of the phosphorylated β-tubulin isotype in differentiated neuroblastoma cells. *FEBS Lett.,* **250**: 142–146.
20. Mitchison, T. and Kirschner, M. 1984. Dynamic instability of microtubule growth. *Nature,* **312**: 237–242.
21. Montero, M.J. and Cleveland, D.W. 1988. Sequence of chicken beta 7 tubulin. Analysis of a complete set of vertebrate β-tubulin isotypes. *J. Mol. Biol.,* **199**: 439–446.
22. Piperno, G. and Fuller, M.T. 1985. Monoclonal antibodies specific for an acetylated form of α-tubulin recognize the antigen in cilia and flagella from a variety of organisms. *J. Cell Biol.,* **101**: 2085–2094.
23. Ponstingl, H., Krauhs, E., Little, M., and Kempf, T. 1981. Complete amino acid sequence of α-tubulin from porcine brain. *Proc. Natl. Acad. Sci. USA,* **78**: 2757–2761.
24. Raybin, D. and Flavin, M. 1975. An enzyme tyrosylating α-tubulin and its role in microtubule assembly. *Biochem. Biophys. Res. Commun.,* **65**: 1088–1095.
25. Schacterle, G.R. and Pollack, R.L. 1973. A simplified method for the quantitative assay of small amounts of protein in biologic material. *Anal. Biochem.,* **51**: 654–655.
26. Schiff, P.B., Fant, J., and Horwitz, S.B. 1979. Promotion of microtubule assembly *in vitro* by taxol. *Nature,* **277**: 665–667.
27. Sullivan, K.F. and Cleveland, D.W. 1986. Identification of conserved isotype-defining variable region sequences for four vertebrate β tubulin polypeptide classes. *Proc. Natl. Acad. Sci. USA,* **83**: 4237–4331.
28. Sullivan, K.F. 1988. Structure and utilization of tubulin isotypes. *Annu. Rev. Cell Biol.,* **4**: 687–716.
29. Vallee, R.B. 1982. A taxol-dependent procedure for the isolation of microtubules and microtubule-associated proteins (MAPs). *J. Cell Biol.,* **92**: 435–442.
30. Villasante, A., Wang, D., Dubner, P., Dolph, P., Lewis, S.A., and Cowan, N.J. 1986. Six mouse alpha-tubulin mRNAs encode five distinct isotypes: testis-specific expression of two sister genes. *Mol. Cell. Biol.,* **6**: 2409–2419.

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