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A Protein Factor Essential for Microtubule Assembly

(tau factor/tubulin/electron microscopy/phosphocellulose)

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ABSTRACT **A heat stable protein essential for microtubule assembly has been isolated. This protein, which we designate tau (τ), is present in association with tubulin purified from porcine brain by repeated cycles of polymerization. Tau is separated from tubulin by ion exchange chromatography on phosphocellulose. In the absence of tau, tubulin exists entirely as a 6S dimer of two polypeptide chains (α and β tubulin) with a molecular weight of 120,000, which will not assemble into microtubules *in vitro*. Addition of tau completely restores tubule-forming capacity. Under nonpolymerizing conditions, tau converts 6S dimers to 36S rings—structures which have been implicated as intermediates in tubule formation. Hence, tau appears to act on the 6S tubulin dimer, activating it for polymerization. The unique ability of tau to restore the normal features of *in vitro* microtubule assembly makes it likely that tau is a major regulator of microtubule formation in cells.**

Microtubules comprise one of the major fibrous systems in eukaryotic cells (1). They consist of long tube-like structures that are 240 Å in diameter with a hollow core. The basic subunit of microtubules is tubulin, a 6S dimer of two polypeptide chains (α and β tubulin) with a molecular weight of 120,000 (2). Microtubules are ubiquitously distributed throughout the animal and plant kingdoms where they are present in a wide variety of intracellular supramolecular structures, generally those which involve cellular or intracellular motion, or the establishment and maintenance of cell shape (3). Both the mitotic and meiotic spindles consist primarily of microtubules, which function in chromosome movement. Microtubules are also a major component of nerve axons, where they are implicated in transport and neurotransmission (4). In the cytoplasm of cells, they radiate from the nucleus toward the plasma membrane. Here, they may be involved in determining both cell shape as well as the lateral mobility of cell surface receptors (5).

In some structures, such as cilia, microtubules are quite stable. By contrast, in others, tubules are ephemeral. For example, in the cell cycle, tubulin is synthesized during interphase, but it is assembled into the mitotic spindle only during the brief metaphase (6). In sea urchin eggs, large amounts of tubulin are synthesized in preparation for rapid cell divisions, yet microtubules do not form until fertilization (7).

The large variety of functions, together with the transitory assembly of many microtubules, implies the existence of regulatory signals which modulate the polymerization of tubulin during differentiation and cell division.

Understanding the mechanism and control of microtubule assembly has been facilitated by the development of conditions for the *in vitro* polymerization of tubulin (8). Microtubules require GTP in order to polymerize and are depolymerized either by Ca^{++} or by temperatures below about 10°. A purification method based on cycles of *in vitro* polymerization and depolymerization permits the preparation from porcine brain of large amounts of relatively pure tubulin capable of assembling into microtubules (9).

In previous studies of *in vitro* microtubule assembly, it was shown that depolymerized tubules contain two components: the 6S tubulin dimer, and a 36S species which contains about 23 tubulin 6S subunits arranged mainly as double rings (10–13). We observed that only the fractions containing 36S rings would repolymerize to form microtubules; the 6S dimer alone would not. Hence, we were faced with the enigma that a major form of tubulin—presumably the one which forms first after biosynthesis of α and β tubulin—was itself unable to form microtubules. Physical chemical studies of the relation between the 6S and 36S species suggested two possible explanations for the difference between them, either modification of the 6S subunit, e.g., by phosphorylation, or the presence of an exchangeable factor (14).

We now report that the ability of 36S rings to assemble into microtubules is due to a salt dissociable factor which resides in the 36S species but is absent in the 6S dimer. We have isolated this factor, a protein which is essential for the assembly of 6S tubulin into 36S rings and microtubules. We propose to call this protein tau (τ) for its ability to induce tubule formation. It is likely that tau operates *in vivo* as a significant regulatory element for microtubule assembly.

MATERIALS AND METHODS

Tubulin was purified from porcine brain by repeated cycles of polymerization according to a modification of the method of Shelanski *et al.* (9) as described previously (14). Immediately prior to use, microtubule protein was repolymerized as already described, pelleted at 100,000 × *g* for 40 min and resuspended in a suitable buffer. For experiments to be done in the absence of Ca^{++}, the protein was resuspended in reassembly buffer. For experiments to be done in the presence of Ca^{++}, morpholinoethanesulfonic acid Mes-Ca^{++} buffer supplemented with 1 mM $CaCl_2$ was used.

Fractionation of tubulin on agarose was described previously (14). Radioactively-labeled microtubule protein from chick brain was prepared by injecting 500 μCi of either [^{3}H]-methionine (11 Ci/mmol) or [^{35}S]methionine (100 Ci/mmol) in 0.2 ml of phosphate-buffered saline directly into the cranial cavity of a 1-day-old chick. After 5 hr, the chick was decapitated, the brain removed, and microtubule protein isolated exactly as described for porcine brain but on a smaller scale. Porcine brain tubulin was added as a carrier. A typical preparation yielded 100–500,000 dpm in purified protein. Autoradiography of sodium dodecyl sulfate-polyacrylamide

Abbreviations: Mes, morpholinoethanesulfonic acid; EGTA, ethyleneglycolbis-(β-aminoethyl ether)N,N'-tetraacetic acid; PC-tubulin, phosphocellulose purified tubulin.

gels of the protein show only the α and β bands of tubulin. Sedimentation analysis of chick brain protein purified in the absence of carrier porcine brain protein shows it to contain 6S and 36S components, in about the same proportions as the protein isolated from porcine brain (14).

For electron microscopy, samples were negatively stained with uranyl acetate either after drop-loading onto parllodion—carbon grids or by the spray drop method described elsewhere (15). Visualization was in a Phillips 300 electron microscope.

Sedimentation analysis of microtubule protein was carried out in a Beckman model E Analytical Ultracentrifuge (14). The rotor speed used in all experiments was 47,660 rpm. Protein concentration was measured by the method of Lowry *et al.* (16) with bovine serum albumin as a standard. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out according to the method of Laemmli (17). Colchicine binding was assayed by gel filtration after the method of Wilson (18).

Biogel A-15m was obtained from Bio Rad Laboratories. Phosphocellulose was purchased from Whatman. Prior to use, phosphocellulose was washed sequentially with 50% ethanol, 0.5 M NaOH, water, 0.5 M HCl, and water. It was equilibrated with Mes-EDTA and stored in this buffer. Pancreatic DNAse was purchased from Worthington. All other enzymes were from Sigma. Radioisotopes were purchased from New England Nuclear Corp.

Buffers used were: Mes-Ca^{++} buffer (0.1 M Mes, 0.5 mM $MgCl_2$, 0.1 mM $CaCl_2$, pH 6.4), Mes-EDTA buffer (25 mM Mes, 0.5 mM $MgCl_2$, 1 mM β-mercaptoethanol, 0.1 mM EDTA, pH 6.4), reassembly buffer (0.1 M Mes, 1 mM EGTA, 0.5 mM $MgCl_2$, pH 6.4).

RESULTS

6S and 36S Tubulin Species Do Not Interconvert During Polymerization. A critical test of whether another factor required for polymerization is present in tubulin preparations would be to see if the two forms of tubulin are capable of interconverting. Only fractions containing 36S rings have the ability to assemble into microtubules. The 6S subunits are inactive alone, although in the presence of 36S rings, they will co-polymerize into microtubules. Consequently, we asked whether the free 6S subunits retain their identity during the polymerization cycle or whether they distribute randomly into 6S and 36S forms upon depolymerization. ^{35}S-Labeled 6S tubulin was mixed with ^{3}H-labeled depolymerized microtubules (a mixture of 6 S and 36 S). After repolymerization the microtubules were collected by centrifugation. Upon depolymerization and analysis by gel permeation chromatography on Biogel A-15m, we found that, in fact, almost all of the [^{35}S]tubulin remained as 6 S, whereas, the [^{3}H]tubulin was again present as both 6S and 36S rings (Fig. 1A). The reciprocal experiment has been performed with the ^{35}S-label in 36S tubulin. In agreement with the above result, 80% of the [^{35}S]tubulin remains as 36 S (results not shown).

These experiments demonstrate that tubulin which enters tubules as the free 6S dimer is released upon depolymerization as the 6S dimer, whereas, tubulin which enters as the 36S ring is released as the 36S ring.

High Salt Concentration Allows 6S and 36S to Interconvert. We had observed that high salt concentration causes complete dissociation of 36S rings to 6S monomers. When the salt was removed by dialysis, the rings reformed (14). We used this observation to test whether tubulin in 6S and 36S forms be-

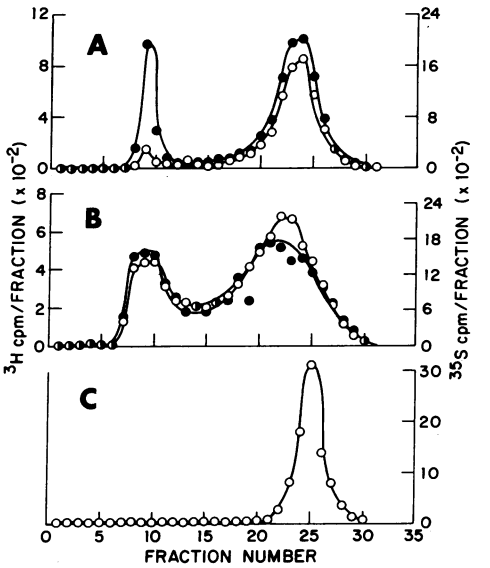

FIG. 1. Exchange of 6S and 36S tubulin species. ^{35}S, (○); ^{3}H(●). (A) Free ^{35}S-labeled 6S dimers were prepared by centrifuging Ca^{++} depolymerized ^{35}S-labeled microtubules at 150,000 × *g* for 2.25 hr at 20° in order to pellet all of the 36S or larger material. The supernatant was mixed with Ca^{++}-depolymerized ^{3}H-labeled microtubule protein, with subsequent addition of 5 mM EGTA and warming to 37° for 20 min to effect polymerization. The microtubules, containing over 60% of the ^{35}S and ^{3}H in the mixture, were collected by centrifugation, resuspended in 1 ml of Mes-Ca^{++} buffer containing 1 mM $CaCl_2$, and fractionated on a column of Biogel A-15m with a 30 ml bed volume. The protein in each fraction was precipitated with 10% trichloroacetic acid, the precipitate collected, washed on glass fiber filters, and radioactivity determined by liquid scintillation counting. (B) Free ^{35}S-labeled 6S dimers were prepared as in (A) and made up to 0.75 M NaCl. They were mixed with a sample of ^{3}H-labeled microtubule protein (originally containing both 6S and 36S tubulin) which had been previously made up to 0.75 M NaCl and centrifuged at 150,000 × *g* for 2.25 hr at 20° to ensure the removal of any remaining large aggregates. The NaCl was removed from the mixture by dialysis against Mes-Ca^{++} buffer at room temperature for 3.5 hr to allow the 36S component to form. The resulting mixture of 6S and 36S tubulin was analyzed by fractionation on Biogel A-15m, as in (A). (C) Elution profile of free ^{35}S-labeled 6S dimers, treated exactly as in (B), but without prior mixing with any ^{3}H-labeled microtubule protein.

comes equivalent with respect to its ability to form rings when both are reduced to the 6S dimer. We mixed free 6S subunits labeled with ^{35}S (themselves unable to form rings or to polymerize) with ^{3}H-labeled protein, initially a mixture of 36S and 6S species, to which salt had been added and which now consisted entirely of 6S subunits. After mixing, the salt was removed by dialysis. The mixture was analyzed by agarose gel filtration. The elution pattern is shown in Fig. 1B. A complete redistribution of label between the 6S and 36S subunits has occurred. Clearly in the presence of high salt, 6S subunits, whether derived from 6S or 36S tubulin, are equally capable of being utilized in formation of 36S rings. The control for this experiment is shown in Fig. 1C: ^{35}S-labeled 6S subunits alone do not form 36S rings when treated with high salt concentrations and then dialyzed; they remain as 6S dimers.

The simplest interpretation of this experiment is that free 6S subunits are identical with those which comprise 36S rings,

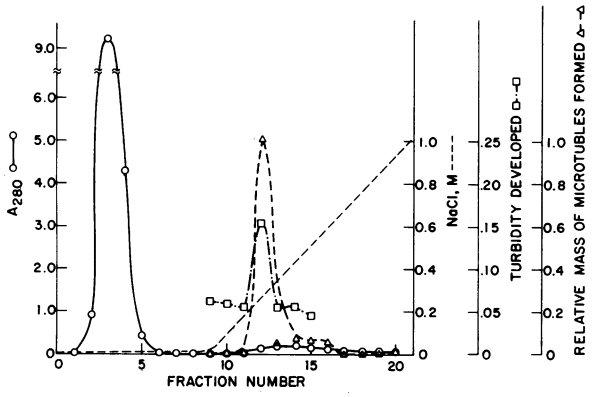

FIG. 2. Separation of tau factor from tubulin on phosphocellulose. Microtubule protein was resuspended to a final concentration of 15 mg/ml in cold Mes-EDTA buffer and applied in 1 ml to a phosphocellulose column with a bed volume of 2.5 ml. The tubulin was eluted with Mes-EDTA buffer, and stored at 0° after being made up to 0.1 M Mes, 2 mM EGTA, and 1 mM GTP. The remaining protein was eluted with a 15 ml gradient between 0 and 1.0 M NaCl in Mes-EDTA buffer. Before being assayed for polymerization-promoting activity, the NaCl in each sample was removed by passage through a Biogel P-6 column pre-equilibrated with Mes-EDTA buffer. For both turbidity measurements and electron microscopic observation, two parts of the fraction to be tested were mixed with one part of a 1:2 dilution of the tubulin in fraction 3, 1 mM GTP was added, and the solution warmed to 37° for 15 min. Turbidity was measured at 380 nm in a Beckman Acta II spectrophotometer. Specimens for the electron microscope were prepared and examined as described in *Materials and Methods*.

but that a salt dissociable factor exists which confers upon an inactive 6S dimer the ability to form the 36S intermediate and polymerize into microtubules.

We have now succeeded in isolating such a factor, a protein whose presence is essential for the assembly of 6S tubulin into microtubules.

Isolation of a Factor Required for Microtubule Assembly. Passage of tubulin through phosphocellulose removes a factor required for polymerization. When a tubulin preparation is passed through phosphocellulose, the tubulin does not itself adsorb to the column (Fig. 2). However, about 5% of the total protein is retained. Gel electrophoresis of the phosphocellulose fractions under denaturing conditions (17), demonstrates that the protein which is unadsorbed to the column (>95% of the total protein) is entirely α and β tubulin; all minor proteins have been removed. Conversely, the adsorbed fraction (which can be removed by 0.6 M NaCl) contains about 15 protein bands, but no tubulin (not shown). Phosphocellulose purified tubulin (PC-tubulin) binds colchicine as well as unfractionated tubulin—2.3 nmol/mg under these assay conditions. No detectable binding (<0.01 nmol/mg) is found in the material adsorbed to phosphocellulose. After phosphocellulose chromatography, tubulin is utterly unable to polymerize into microtubules as judged by electron microscopy (Fig. 3A). The characteristic light scattering of tubulin, due to the presence of 36S rings, has also been lost. No rings are seen by electron microscopy (not shown). Once the tubulin has passed through the column, application of a NaCl gradient (Fig. 2) elutes an activity which, upon incubation with the PC-tubulin, restores its ability to polymerize (Fig. 3B). The morphology of these tubules is identical to those formed by unfractionated tubulin. The factor is removed as a single sharp peak at about 0.25 M NaCl, before most of the adsorbed protein. In keeping with its ability to promote microtubule formation, incubation of the factor with PC-tubulin under non-polymerizing conditions (1 mM $CaCl_2$) allows the tubulin to reform rings as determined by electron microscopy (Fig. 3C). The factor itself is unable to form either rings or tubules (Fig. 3D). Thus, phosphocellulose removes from tubulin a factor which is required for the assembly of microtubules. We call this factor tau (τ), for its ability to induce tubule formation.

TABLE 1. *Properties of tau factor*

Treatment	Microtubule formation
None	+
Trypsin, 10 μg/ml	−
100°, 5 min	+
DNase, 10 μg/ml	+
RNase, 10 μg/ml	+
Phospholipase C, 10 μg/ml	+
Alkaline phosphatase, 10 μg/ml	+
N-Ethyl maleimide, 0.5 mM, 30 min	+

1 ml of microtubule protein (15 mg/ml) in Mes-EDTA buffer was applied to a 2 ml column of phosphocellulose. The tubulin was eluted with Mes-EDTA buffer and made up to 0.1 M Mes, 2 mM EGTA, and 1 mM GTP. The remaining 5% of the protein, containing tau, was eluted with Mes-EDTA buffer containing 0.8 M NaCl. This fraction was desalted into Mes-EDTA buffer by passage through a column of Biogel P-6. Additions, as indicated in the table, were made to the tau factor prior to mixing with PC-tubulin. For the assay of microtubule formation, 25 μl of PC-tubulin, 50 μl of the treated or untreated tau factor, and 1 μl of 0.1 M GTP were mixed, warmed to 37°, and the mixture examined for the presence of microtubules under the electron microscope. All enzyme treatments were for ten minutes at 37°. For the sample treated with trypsin, a tenfold excess of soybean trypsin inhibitor was added prior to addition of the tubulin. Trypsin pre-treated with the same amount of soybean trypsin inhibitor did not abolish the activity of tau. For the sample treated with *N*-ethyl maleimide, 2-mercaptoethanol was omitted from the desalting buffer. The reaction was terminated by addition of a tenfold excess of 2-mercaptoethanol. A "minus" sign in the table indicates that no microtubules whatever were observed. A "plus" sign indicates that qualitatively the control value of polymerization was observed.

Sedimentation Analysis of Tau–Tubulin Interactions. We have confirmed these observations by analytical ultracentrifugation (Fig. 4). In the presence of 1 mM $CaCl_2$, PC-tubulin sediments only as a single 6S boundary (Fig. 4A). The size of the 6S peak is unchanged after attempted polymerization by addition of EGTA and GTP and incubation at 37°. This indicates that no protein has polymerized into microtubules (not shown). Tau factor alone shows no 36S component (Fig. 4B). Addition of tau factor to PC-tubulin results in substantial conversion (41% in this experiment) of 6S tubulin into rings as shown by the appearance of a 36S peak (Fig. 4C). With three times the amount of tau factor, over 80% of the 6S protein can be converted into the 36S form (not shown). Upon polymerization all of the 36S material, together with 32% of the 6S protein, is converted into a heterogeneous, quickly sedimenting species (Fig. 4D) which is seen to contain tubules in the electron microscope.

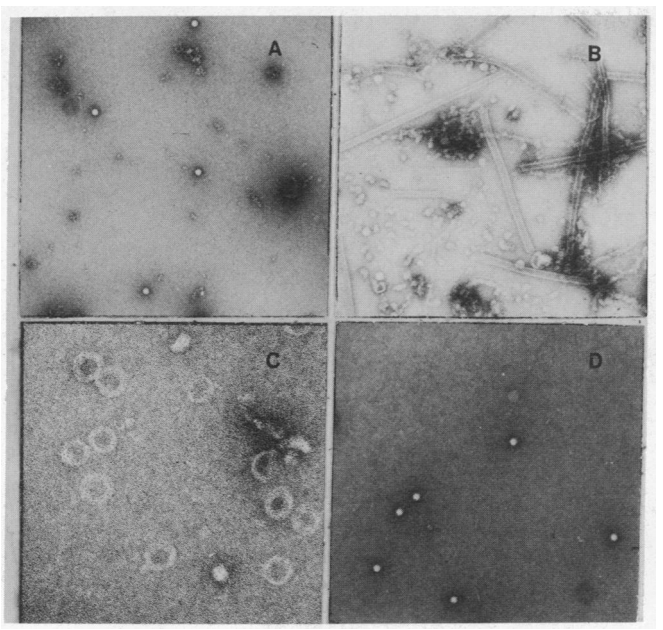

FIG. 3. Electron microscopic analysis of τ-tubulin interaction. (A) Phosphocellulose purified tubulin, ×23,500; (B) phosphocellulose purified tubulin + τ, ×28,500; (C) 1 mg/ml of phosphocellulose-purified tubulin + τ in Mes-EDTA buffer containing 1 mM $CaCl_2$, ×82,000; (D) τ only, ×28,500. Microtubule or ring formation was assayed as described in the legend to Fig. 2. The samples were prepared for electron microscopy by the spray-drop technique (15). The small particles in (A), (B), and (D) are bushy stunt virus which was mixed with the samples immediately before preparation of the grids in order to quantify microtubule formation.

Properties of Tau Factor. The sensitivity of tau factor to trypsin indicates it is a protein. Pre-incubation of tau with trypsin followed by addition of soybean trypsin inhibitor destroys tau's ability to induce tubule formation in PC-tubulin (Table 1). If trypsin and trypsin inhibitor are mixed before addition of tau, tubules form normally. RNase, DNase, phospholipase C, and alkaline phosphatase are without effect (Table 1); sulfhydryl reagents such as *N*-ethyl-maleimide do not inhibit tau function. Somewhat surprisingly, tau is extremely heat stable (100°, 5 min) (Table 1).

Tau also confers upon 6S tubulin prepared from depolymerized tubules by gel filtration (14), the ability to form 36S rings and microtubules. In the absence of tau, these subunits are incapable of assembly into microtubules.

DISCUSSION

Within the cell, microtubules assemble and disassemble at specific times, in defined locations, with unique points of attachment and a particular orientation. For example, during cell division, spindle microtubules assemble around the nucleus only at mitosis; they attach to the kinetochores of chromosomes and are oriented toward the poles. After mitosis the microtubules disassemble.

Clearly such specificity implies a regulatory system. In this paper we have described the isolation of what may be one component of such a regulatory system: a factor, tau, which controls the polymerization of microtubules. Tau is an extremely heat stable protein. It is removed from already quite pure preparations of microtubule protein by chromatography on phosphocellulose. Tubulin passes through the

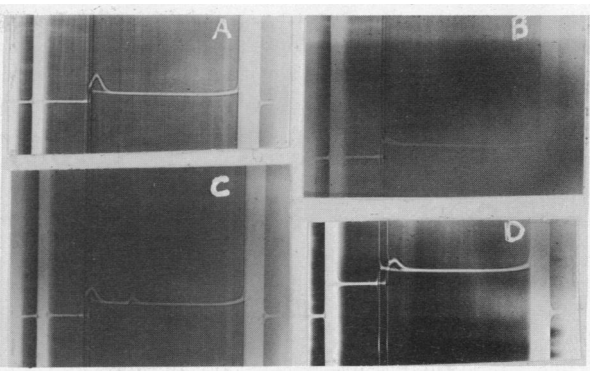

FIG. 4. Sedimentation analysis of τ-tubulin interaction. (A) 5.4 mg/ml of microtubule protein after passage through phosphocellulose; (B) 0.7 mg/ml of the τ-containing 0.8 M NaCl wash of the phosphocellulose column; (C) 3.4 mg/ml of phosphocellulose-purified microtubule protein and 0.7 mg/ml of the 0.8 M NaCl wash; (D) same as (C) with addition of 5 mM EGTA. All samples were analyzed at room temperature in Mes-Ca^{++} buffer containing 1 mM $CaCl_2$ after warming to 37° for 20 min. The photographs were taken 16 min after the rotor reached its operating speed of 47,660 rpm. The phase plate angle used was 80° for (A), and 75° for all other samples.

column without adsorption while tau elutes at about 0.25 M NaCl. That tau co-purifies with tubulin through several cycles of polymerization indicates a specific interaction. After passage through phosphocellulose, tubulin loses its ability to polymerize; it remains as 6S subunits. Addition of tau reactivates tubulin for microtubule formation. The properties of tau clearly distinguish it from tubulin: tau is heat stable, insensitive to sulfhydryl reagents, and does not bind colchicine; tubulin is heat labile, inactivated by *N*-ethyl-maleimide, and does bind colchicine (2, 14). Furthermore, sodium dodecyl sulfate gel electrophoresis reveals that the characteristic tubulin doublet is absent in the tau preparation.

How does tau function in microtubule assembly? Depolymerization of microtubules gives rise to two species: free 6S tubulin subunits and 36S double rings or spirals composed of 6S subunits (11). The 36S rings will assemble into tubules. The free 6S subunits will not form rings or tubules, although they will co-polymerize into tubules when the 36S species is present. Hence, in the sense that they are unable to polymerize alone, free 6S subunits are non-competent. To explain these observations, we previously suggested that the tubulin subunit can exist in two forms: Y tubulin which is competent to form rings and X tubulin which will not (11, 13). We postulated that X and Y tubulin differ either in the possession of a specific co-factor or in the presence of a small chemical difference such as a phosphate substituent. The inactivity of free 6S dimers can now be explained by the absence of tau factor. Under conditions where microtubule formation is prevented, e.g., 1 mM $CaCl_2$, tau will convert 6S subunits to 36S rings. Conversely, removal of tau by phosphocellulose converts all 36S rings to 6S tubulin subunits. Tau appears to act stoichiometrically rather than catalytically. For the same amount of tubulin, the final yield of 36S rings increases as the amount of tau is increased. Preliminary results show that the final yield of microtubules, as judged by quantitative electron microscopy, also increases with the amount of tau.

This being the case, we can attribute the difference between X and Y tubulin to the presence of tau, Y tubulin being the form of the protein associated with tau. Only the 6S·τ

complex is able to form 36S rings. High salt concentration, which dissociates 36S rings, allows tau to redistribute among all 6S dimers whether derived from free 6S or 36S species. In contrast, as shown by the results presented here (Fig. 1A), tau does not normally redistribute among tubulin subunits during disassembly.

Our previous studies on *in vitro* microtubule assembly suggested that 36S rings are true intermediates in microtubule formation. These included the observation in the electron microscope of rings "unrolling" at the ends of tubules, and conversion of rings into a tubule intermediate just prior to tubule formation (11, 15).

Thus, we postulate that the locus of tau function is the 6S subunit and that polymerization proceeds by the pathway:

$$6S + \tau \rightarrow 6S \cdot \tau \rightarrow 36S \text{ rings} \rightarrow \text{microtubules}.$$

Conceivably the $6S \cdot \tau$ complex could represent as many as one molecule of tau per tubulin molecule in the 36S form, although it is also possible that the ratio is far lower, perhaps even as low as one tau per 36S ring.

Are 6S subunits completely nonfunctional in the absence of tau? Their ability to co-polymerize with 36S rings suggests that they may have a role in microtubule formation. Alternatively, the conditions of *in vitro* assembly may be responsible for the co-polymerization observed. In essence, the question is what proportion of tubulin in microtubules is complexed with tau. *In vivo*, there may be one tau per 6S subunit in tubules, or, a certain proportion of uncomplexed 6S might be present. Complete purification of tau will permit determination of the actual stoichiometry of tau to 6S subunit in rings and tubules.

Finally, tau can convert nonpolymerizing, free 6S dimers, obtained by agarose gel filtration of depolymerized tubulin, into rings and tubules. In contrast to 6S tubulin obtained after phosphocellulose, none of these 6S dimers are derived from 36S rings. Hence, we can rule out any additional special property of 6S dimers derived from rings which might be required for assembly.

Are other modifications or proteins important for tubulin assembly? Eipper has reported that tubulin is phosphorylated at a unique residue in the β chain (19). Results from our laboratory indicate that the phosphate resides exclusively on tubulin in the 36S rings; free 6S dimers are not phosphorylated (20). Yet these very dimers will form rings and microtubules in the presence of tau. It would appear that phosphorylation is not necessary for ring formation and tubule assembly. However, we have not yet excluded the possibility that tau phosphorylates tubulin during assembly. The ability to make rings and tubules with tau from initially unphosphorylated subunits should permit elucidation of the role of phosphate in microtubule function.

Borisy and coworkers have reported that tubulin purified under their conditions contains significant amounts of high molecular weight material (21). They have suggested that these components may promote microtubule assembly. Although our tubulin preparations contain very little high-molecular-weight material, the exact relation between such material and tau factor remains to be established.

The discovery of tau is the first example of a protein other than tubulin which has been proven to be essential for the assembly of microtubules. *In vitro*, tubulin assembly can also be regulated by GTP, Ca^{++}, and temperature. It is possible that changes in the *in vivo* levels of calcium and GTP influence microtubule assembly. However, since both calcium and GTP are involved in very many cellular processes, we believe that tubule-specific proteins such as tau are the best candidates for the *in vivo* control of tubule formation.

Tau controls the ability of microtubules to assemble. But tubules not only assemble, they attach to specific structures and are spatially oriented and differ in their stability. The identification of tau suggests a search for other proteins which interact with tubulin and modulate its function.

The actual *in vivo* function of tau is not known, but its *in vitro* ability to induce polymerization encourages an examination of its function in the cell.

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