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# Characterization of Maize Microtubule-Associated Proteins, One of Which Is Immunologically Related to Tau†

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ABSTRACT: Microtubule-associated proteins (MAPs) are identified as proteins that copurify with tubulin, promote tubulin assembly, and bind to microtubules in vitro. Higher plant MAPs remain mostly unknown. One example of non-tubulin carrot proteins, which bind to neural microtubules and induce bundling, has been reported so far [Cyr, R. J., & Palewitz, B. A. (1989) *Planta 177*, 245-260]. Using taxol, we developed an assay where higher plant microtubules were induced to self-assemble in cytosolic extracts of maize cultured cells and were used as the native matrix to isolate putative plant MAPs. Several polypeptides with an apparent molecular masses between 170 and 32 kDa copolymerized with maize microtubules. These putative maize MAPs also coassembled with pig brain tubulin through two cycles of temperature-dependent assembly-disassembly. They were able to initiate and promote MAP-free tubulin assembly under conditions of nonefficient self-assembly and induced bundling of both plant and neural microtubules. One of these proteins, of about 83 kDa, cross-reacted with affinity-purified antibodies against rat brain tau proteins, suggesting the presence of common epitope(s) between neural tau and maize proteins. This homology might concern the tubulin-binding domain, as plant and neural tubulins are highly conserved and the plant polypeptides coassembled with brain tubulin.

Most essential functions of eukaryotic cells, such as chromosome segregation during mitosis, intracellular organelle motility, determination of cell shape, and cell cleavage, are mediated by microtubules. These dynamic polymers are assembled from two major soluble and highly conserved proteins, the $\alpha$- and $\beta$-tubulins. Additional polypeptides, collectively known as microtubule-associated proteins (MAPs), copolymerize with tubulins and bind to the outside surface of the microtubule (Olmsted, 1986). Originally isolated from mammalian brain tissue, MAPs were named MAP 1, MAP 2 (>200 kDa), and tau (35-65 kDa) according to their molecular mass. The presence of MAPs in animal nonneural systems is now well documented (Vallee & Collins, 1986; Aizawa et al., 1989) and suggests that they are universal components of the cytoskeleton. Recently, a wealth of data, primarily based on molecular genetics, has uncovered new properties of MAPs, particularly concerning tau and MAP 2, which highlight their potential role in microtubule function (Mandelkow & Mandelkow, 1990; Cleveland, 1990). It has been demonstrated that multiple tubulin isoforms of higher plant (Hussey et al., 1987) and animal (Lewis et al., 1987) cells or tubulins from various origins (Guens et al., 1989; Vantard et al., 1990) coassemble in functionally distinct microtubules, suggesting that different tubulins might be interchangeable. As a result, much attention is now focused on microtubule-associated proteins as factors that can modulate the assembly of tubulin and microtubule activity in vivo.

In the present debate, higher plant MAPs remain mostly unknown. Only one example of non-tubulin proteins that bind to neural microtubules in vitro and are named microtubule-binding proteins (Cyr & Palewitz, 1989) has been described so far. This lack of information represents a serious handicap in understanding the molecular mechanisms that regulate intracellular plant tubulin assembly and the differential stability and bundling properties of plant microtubules. Higher plant cells represent a peculiar model in that they lack distinct centrosomes, which are known to control microtubule nucleation and spatial distribution in animal cells including neurons. Therefore, the regulation of plant microtubule assembly and dynamics remains poorly documented. Higher plant cells possess peculiar microtubule-organizing domains, such as the nuclear surface (Vantard et al., 1990; Baskin & Cande, 1990; Lambert et al., 1991), whose function seems to involve "MAP"-mediated interactions with cell membranes.

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It is, therefore, probable that higher plant "MAPs" might have particular functions and distribution patterns in the plant cell.

Our aim was to isolate higher plant proteins that could be characterized as MAPs. We developed a method where plant microtubules were induced to self-assemble in maize cytosolic cell extracts and were used as the native matrix to isolate putative plant MAPs. Functional assays that permitted the characterization of the MAP activity of these proteins were defined.

In this report, we present a putative plant MAP-enriched fraction containing proteins that (i) copolymerized with higher plant and also neural tubulins, (ii) were able to initiate and promote MAP-free tubulin assembly under conditions of non-efficient self-assembly, and (iii) as reported for carrot proteins (Cyr & Palewitz, 1989), were capable of inducing bundling of microtubules. These properties are shared with well-characterized MAPs. One of these proteins cross-reacted selectively with antibodies directed specifically against neural tau, suggesting that a putative higher plant MAP shared at least one common epitope with a neural MAP, tau. This result might be relevant to recent reports concerning the potential conservation of the tubulin-binding domain of several MAPs, including tau and MAP 2 (Cleveland, 1990; Maekawa et al., 1990) and also to the striking bundling of microtubules in fibroblasts transfected with neural tau (Kanai et al., 1989), which resembles the peculiar organization of several plant microtubule arrays.

## Experimental Procedures

*Plant Material.* Suspension-cultured cells of maize (*Zea mays*, black Mexican sweet corn) were grown on Murashige and Skoog (1962) medium.

*Preparation of Putative Maize Microtubule-Associated Proteins.* Microtubule proteins were isolated from cells in early growth phase by homogenization in an extraction buffer A (150 mM MES, 15 mM EGTA, 1.5 mM EDTA, 7.5 mM $MgCl_2$, 1.5 M glycerol, 1.5 mM GTP, 15 $\mu$M leupeptin, 15 $\mu$M pepstatin, 1.5 mM DTT, 1.5 mM PMSF, 15 mg/mL aprotinin, pH 6.8), 1 mL/1.5 g of cells. A French press was used for homogenization. All steps were carried out at 0–4 °C. The homogenate was centrifuged at 60000*g* for 20 min and the supernatant recentrifuged at 100000*g* for 30 min. This high-speed supernatant containing soluble tubulin and microtubule-associated proteins, termed the cytosolic extract, was then incubated at 26 °C for 45 min in the presence of 20 $\mu$M taxol (ICSN, CNRS, Gif-sur-Yvette, France) to induce microtubule protein assembly. Microtubules (with their microtubule-associated proteins) were sedimented through a 30% sucrose cushion in buffer B (100 mM MES, 10 mM EGTA, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM GTP, 20 $\mu$M taxol, 1 $\mu$M leupeptin, 1 $\mu$M pepstatin, 1 mM DTT, 1 mM PMSF, 10 mg/mL aprotinin, pH 6.8) at 100000*g* for 45 min at 26 °C. The microtubule pellet was resuspended in buffer B containing 0.15 M NaCl and centrifuged through a sucrose cushion at 100000*g* for 20 min at 26 °C to eliminate nonspecifically bound proteins. The microtubule pellet obtained was then resuspended in buffer B containing 0.7 M NaCl to detach the microtubule-associated proteins from the microtubules and incubated for 5 min at 26 °C. The MAP-free microtubule pellet was collected by centrifugation as described above and stored at −80 °C. The supernatant containing the putative microtubule-associated proteins was applied to gel filtration chromatography (AcA202 Ultrogel, IBF, France) to remove the taxol and salt. Taxol content of the protein samples was assessed by reverse-phase HPLC according to Collins and Vallee (1987). For this analysis, taxol was extracted from the protein samples by addition of methanol to a final concentration of 80% (v/v). Analysis of taxol in the methanol extract was performed on a reverse-phase column ($\mu$Bondapack $C_{18}$, 300 mm × 3.8 mm, Waters Associates) in methanol/water (70:30 v/v) at a flow rate of 0.6 mL/min. Taxol was detected by its absorbance at 214 nm, and the concentration was determined by measurement of the peak areas relative to a taxol standard.

*Preparation of Maize Microtubule-Binding Proteins Using Stabilized Brain Microtubules.* Preformed and taxol-stabilized brain microtubules were used as described by Vallee and Collins (1986). MAP-free neural tubulin, polymerized with taxol, was added to the plant cytosolic extract defined above, to a final concentration of 0.6 mg/mL. After 15 min of incubation at 0–4 °C, the microtubules and bound proteins were centrifuged. The microtubule pellet was resuspended in the buffer B supplemented with 0.5 M NaCl and then warmed for 3 min at 26 °C. After centrifugation, the supernatant containing the microtubule-binding proteins was treated as described above.

*Protein Determination.* The protein concentration was determined by the method of Bradford (1976) using the Bio-Rad dye reagent and bovine serum albumin as a standard.

*Preparation of Tubulins.* Maize tubulin was prepared from the MAP-free taxol-stabilized microtubules obtained as described above. Microtubules were depolymerized at 0–4 °C in the presence of 3 mM $Ca^{2+}$, and soluble tubulin was applied to a gel filtration column (AcA202 Ultrogel, IBF, France) according to Collins and Vallee (1987). Pig brain microtubule proteins were obtained according to Shelanski et al. (1973), and MAP-free neurotubulin was purified by phosphocellulose ion exchange chromatography (Weingarten et al., 1975).

*Tubulin Assembly and Copolymerization Assays.* Plant or neural tubulin polymerization was performed in buffer C (100 mM MES, 5 mM EGTA, 5 mM $MgCl_2$, 1 mM GTP, pH 6.8) at 26 or 30 °C, respectively, depending on the source of tubulin. Microtubule assembly was monitored by measuring the turbidity at 400 nm (UltrospecII, LKB). Purified plant tubulin or neurotubulin was incubated for 45 min in the presence or absence of the plant microtubule-binding protein fraction. In copolymerization assays, the mixture was further centrifuged at 100000 *g* for 20 min through a sucrose cushion. The supernatant and the microtubule pellet, resuspended in the buffer C, were processed for electron microscopy or gel electrophoresis and immunoblotting.

*Electron Microscopy.* The plant microtubule pellets were fixed in 1% glutaraldehyde with 2% tannic acid added in buffer B, for 1 h, washed four times in a phosphate buffer (0.1 M, pH 7), post-fixed with 1% $OsO_4$ at 4 °C for 30 min, and stained with 2% uranyl acetate for 12 h at 4 °C. After ethanol dehydration, the pellet was embedded in Epon-Araldite. Ultrathin sections (ultramicrotome Reichert OMU 2) were again contrasted with 2% uranyl acetate and lead citrate (Reynolds, 1963) and observed with a Philips 410 transmission electron microscope, at 80 kV. During copolymerization assays, the microtubule pellets were resuspended in the buffer B, fixed in 1% glutaraldehyde for 5 min, negatively stained (2% uranyl acetate) on carbon-coated grids, and observed under similar conditions.

*Preparation of Antibodies Directed Against Neural Tau.* Tau proteins were purified from adult rat brains by the procedure described by Lindwall and Cole (1984). Purified tau only contained closely spaced bands that migrated on SDS–polyacrylamide gels with an apparent molecular mass between 55 and 62 kDa. Antiserum to tau was produced by injecting

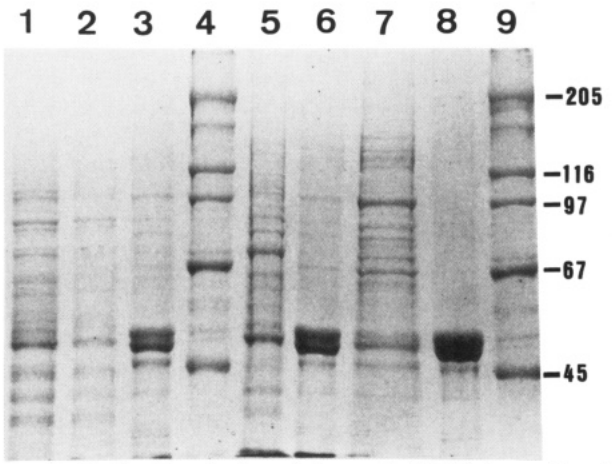


FIGURE 1: Purification of microtubule-protein (MTP) from cultured maize cells by SDS–PAGE (7.5% acrylamide), with Coomassie blue staining. (Lane 1) Cytosolic extract. (Lanes 2 and 3) Supernate and pellet after the first centrifugation of taxol-mediated assembly of MTP proteins; (lanes 5 and 6) supernate and pellet after resuspension of the MTP pellet in 0.15 M NaCl buffer; (lanes 7 and 8) supernate and pellet after resuspension of lane 6 (pellet) in 0.7 M NaCl buffer. The putative maize MAP fraction corresponds to lane 7. MAP-free stabilized microtubules correspond to lane 8 (illustrated in Figure 2). Both $\alpha$- and $\beta$-tubulin subunits are seen in lanes 3, 6, and 8; (lanes 4 and 9) molecular weight standards in kDa $\times 10^3$ as in all figures.

New Zealand white rabbits subcutaneously with 30 $\mu$g of tau protein every two weeks during six weeks. The first injection was performed by mixing tau with complete Freund's adjuvant; the second injection was identical with the first one except that Freund's adjuvant was incomplete; and the third injection was the same as the first one. Monospecific antibodies were then separated from the serum by affinity chromatography using purified tau. The specificity of the tau antibodies was checked by immunoblotting against a crude brain lysate and also against microtubule extracts.

*Electrophoresis and Immunoblotting.* Proteins were analyzed on 7.5% (w/v) SDS–polyacrylamide slab gels according to Laemmli (1970). Gels were stained with Coomassie brilliant blue G or R (Neuhoff et al., 1988). For the immunoblotting procedure, the gels were electrophoretically transferred onto nitrocellulose as described by Towbin et al. (1979). Nonspecific binding sites were saturated with 5% dry milk and 0.3% Tween 20 in TBS buffer for 1 h at 37 °C. Primary antibodies to $\beta$-neurotubulin (N 357, Amersham, 1:2000) or rat brain tau proteins (1:10) were diluted in TBS buffer supplemented with 5% dry milk and 0.03% Tween. Primary antibodies were detected by secondary antibodies conjugated to colloidal gold (1:100) and silver enhanced (Auroprobe BLplus GAM IgG or GAR IgG and Intense II Kit, Janssen Pharmaceutica).

## RESULTS

*Isolation of Microtubules and Putative Microtubule-Associated Proteins from Maize Cultured Cells.* We have isolated putative higher plant microtubule-associated proteins (MAPs) from maize cultured cells using plant microtubules as the native matrix to purify these proteins. This new approach, in higher plant cells, was rendered possible due to the use of taxol-mediated polymerization of microtubules that we obtained directly from the maize cytosolic extract as described under Experimental Procedures. This methodology was derived from the taxol-mediated isolation of neural MAPs (Vallee & Collins, 1986). We used selective conditions during homogenization in order to minimize proteolysis, which is very active in plant material due to the release of the high protease content of the vacuoles. As illustrated on SDS–PAGE (Figure 1), we obtained a net enrichment of tubulin and several other proteins in the first microtubule pellet. Using a step wise procedure that permitted us to eliminate most nonspecifically bound proteins at low ionic strength (see Experimental Procedures), we detached the associated proteins from tubulin polymers during treatment of the microtubule pellet with 0.7 M NaCl. As confirmed by electron microscopy, the last tubulin pellet (lane 8, Figure 1) was composed of purified microtubules (Figure 2), with smooth side-walls and a well-preserved structure with distinct protofilaments. The supernatant, which was considered as the maize MAP-enriched fraction, contained major polypeptides with apparent electrophoretic mobilities within a range of 60–170 kDa, as seen in Figure 1, lane 7. In the hope of characterizing these putative maize MAPs, we studied the competence of this enriched fraction to increase the rate of purified (MAP-free) brain or plant tubulin assembly in vitro. These experiments included the removal of salt and taxol, which would have an artifactual effect. This was obtained by subjecting the supernatant to gel filtration on a desalting column as described under Experimental Procedures. The taxol content was ascertained by reverse-phase chromatography according to Collins and Vallee (1987). After passage over Ultrogel (AcA202), no taxol was detected in the included volume. This protein fraction, containing putative MAPs and depleted of taxol and salt, was named the maize "MAP" fraction.

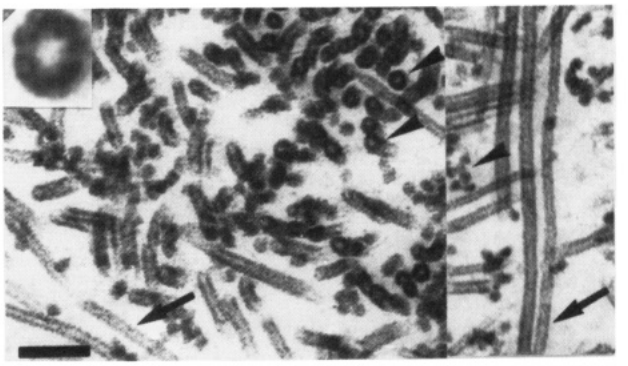

FIGURE 2: Ultrathin sections of the maize purified microtubule pellet (cf. lane 8, Figure 1), observed in electron microscopy. Microtubules are seen in longitudinal (arrows) and transversal (arrow heads) views. (Inset) Higher magnification showing protofilaments. The bar equals 0.1 $\mu$m.

The last tubulin pellet represented an average of 2% of the total amount of proteins contained in the cytosolic extract, while the MAP fraction represented approximately 0.4%. These estimations are the result of the mean of 12 experiments.

*Effects of the Maize "MAP"-Enriched Fraction on Tubulin Assembly.* The effects of the plant "MAP" fraction on plant or neural tubulin assembly in vitro are illustrated in Figure 3. MAP-free maize or pig brain tubulin at a concentration of 10 $\mu$M was polymerized in the presence of 1 mM GTP at 26 or 30 °C, depending on the tubulin source (curves a and b). In these conditions, self-assembly of both purified tubulins was very low or even nonexistent. Addition of the "MAP" fraction either to the plant (curve c) or the brain (curve d) tubulins enhanced the turbidity very significantly. When this "MAP" fraction was incubated with tubulin at a ratio of 1:3 (w/w), the extent of turbidity increased to 5 times that of the control, indicating that some proteins of the maize "MAP" fraction were capable of enhancing the assembly of both neural and plant pure tubulins and/or to induce the cross-linking of microtubules, as both of these phenomena are reflected in turbidity.

Table I: Effects of the Maize "MAP" Fraction on Neurotubulin Assembly

| "MAP" fraction (μg) added to purified neurotubulin (200 μg) | 0 | 40 | 50 | 66 | 100 |
|---|---|---|---|---|---|
| microtubular proteins in the pellets (μg)[a] | 3 ± 0.3 | 20 ± 1.9 | 26 ± 1.3 | 35 ± 1.5 | 38 ± 0.5 |

[a] Corresponds to the value of three experiments.

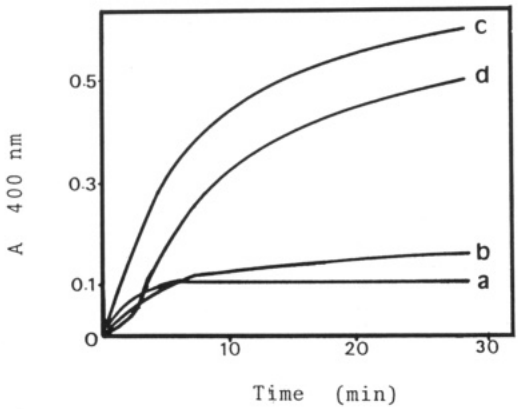


FIGURE 3: Effect of the maize "MAP" fraction (0.3 mg/mL) on maize or pig brain purified tubulin (1 mg/mL) assembly assessed by turbidity. (a and c) Maize tubulin assembly (26 °C) in the absence (a) or the presence (c) of the maize "MAP" fraction. (b and d) Neurotubulin assembly (30 °C), without (b) and with (d) the maize "MAP" fraction.

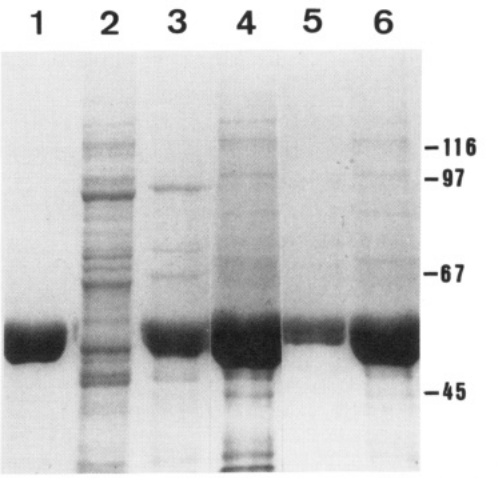


FIGURE 4: Electrophoretic analysis of two cycles of temperature-dependent polymerization/depolymerization of purified neurotubulin (1 mg/mL) in the presence of the maize "MAP" fraction (0.3 mg/mL): 7.5% acrylamide SDS–PAGE with Coomassie blue staining. (Lane 1) Purified neurotubulin (6 μg); (lane 2) maize "MAP" fraction (12 μg); (lanes 3 and 4) supernate and pellet after the first polymerization (12 μg of each); (lanes 5 and 6) supernate (3 μg) and pellet (12 μg) after the second polymerization.

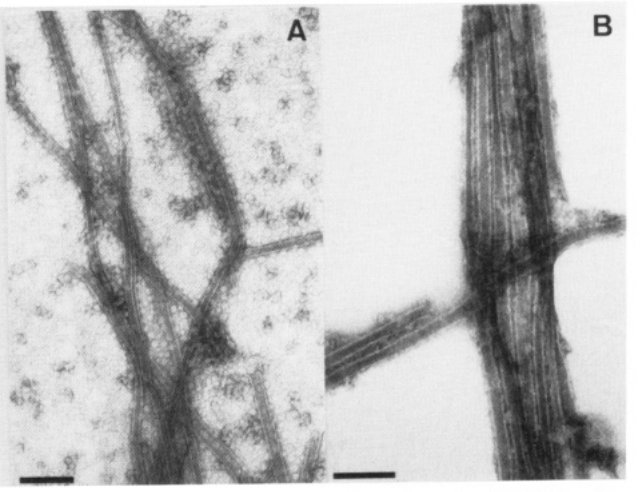


FIGURE 5: Microtubule bundling induced by the maize "MAP" fraction in vitro analyzed in electron microscopy, after negative staining. (A) Control, purified neurotubulin (1 mg/mL) assembly. (B) Assembly of the same tubulin in the presence of the maize "MAP" fraction (0.2 mg/mL), indicating an intense cross-linking of microtubules. The bars equal 0.1 μm.

In the hope of discriminating between the two concomittent effects (microtubule assembly/bundling), we developed cosedimentation assays including quantitative assessment of the mass of polymers and electron microscopy observations to control microtubule polymerization in the presence of the maize MAP fraction. Electrophoretic analysis (SDS–PAGE) was also performed to identify proteins that specifically cosedimented with tubulin. Because neurotubulin was available in large quantities, while this was not the case for plant tubulin, we used mostly pig brain tubulin for these experiments. After polymerization of MAP-free pig brain tubulin (10 μM) at 30 °C for 1 h (as in the turbidity assays) in the presence of different concentrations of the maize "MAP" fraction, microtubules were sedimented, and the pellets were assayed for protein concentration. As seen in Table I, microtubule assembly was enhanced by increasing the concentration of the plant MAP fraction, with a maximum effect occurring at approximately 0.35 mg/mL, i.e., about a 1:3 ratio (w/w). These data are comparable to those obtained in an homologous system (neuro-MAPs and neurotubulin). In the hope of characterizing this putative maize MAP fraction, we carried out rebinding assays of these proteins to neurotubulin during two cycles of temperature-dependent polymerization/depolymerization, as illustrated in SDS–PAGE analysis (Figure 4). The second cycle pellet contained essentially the same polypeptides as in the first cycle pellet, while some low $M_r$ peptides failed to resediment, suggesting that they were not specifically bound to neurotubulin. At least five polypeptides had electrophoretic mobilities similar to those of the maize "MAP" fraction obtained by using taxol-mediated microtubule assembly.

Electron microscopy of the sedimented microtubule pellets, corresponding either to maize or to pig brain tubulin polymerization in the presence of the maize "MAP" fraction, revealed dense microtubule bundles (Figure 5B). Microtubules were associated along their entire length, with an average center-to-center spacing below 300 nm. In control samples (Figure 5A), i.e., pure tubulin assembly without the MAP fraction, randomly oriented single microtubules were observed; although some occasional association could be found, no tight bundles were ever detected. Altogether, these results indicated that at least some polypeptides of the putative maize MAP fraction shared properties of well-characterized MAPs.

*Identification of a Maize Putative MAP Protein Immunologically Related to the Neural Tau.* Affinity-purified polyclonal antibodies directed specifically to neural tau proteins were raised as described under Experimental Procedures. Immunostaining of the maize "MAP" fraction showed that one polypeptide, of an apparent molecular mass of 83 kDa and migrating as a single band, was immunoreactive with these antibodies (Figure 6). In controls, with preimmune serum and secondary antibodies, no reactivity was observed. This tau-reactive polypeptide was enriched in the maize "MAP"

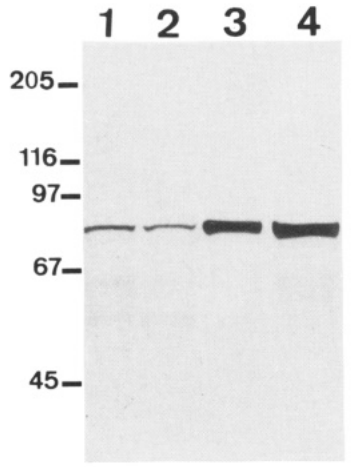


FIGURE 6: Immunoblotting of the maize "MAP" fraction during successive stages of purification, probed with antibodies against rat brain tau proteins. (Lane 1) Cytosolic extract (30 μg). (Lanes 2 and 3) Supernate and pellet after taxol-mediated assembly of microtubule proteins (30 μg of each sample). (Lane 4) Putative maize MAP fraction (30 μg). The anti-tau antibodies react with one single polypeptide of about 83 kDa. Each purification step resulted in further enrichment of this peptide.

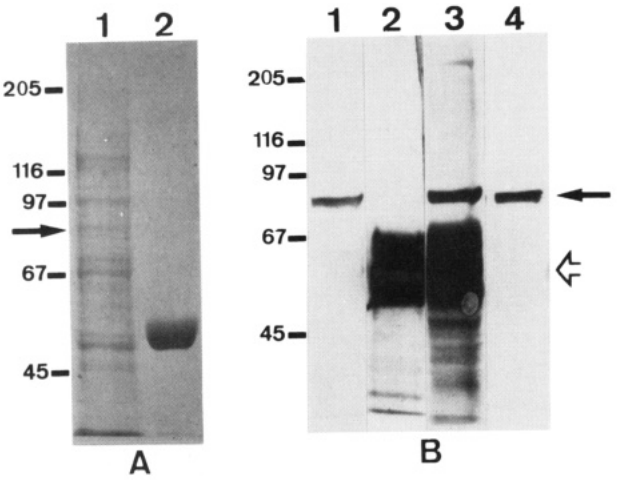


FIGURE 7: Immunodetection of the 83-kDa protein from the maize "MAP" fraction obtained by use of preformed taxol-stabilized brain microtubules. SDS–PAGE (A) transferred to nitrocellulose and (B) immunoblotted against anti-rat brain tau antibodies. (Lane 1) Maize cytosolic extract (20 μg of protein in panel A, and 60 μg in panel B), one single band is immunostained (lane 1, panel B). (Lane 2) Brain taxol-stabilized microtubules (10 μg in panel A; 15 μg in panel B) cross-reacting with tau antibodies (lane 2, panel B). (Lane 3) Maize proteins that copurified with taxol brain microtubules (60 μg protein); the 83-kDa protein is detected while rat brain tau are also labeled with some degraded products (lower molecular mass). (Lane 4) Maize "MAP" fraction obtained by polymerization of maize tubulin as in Figure 1, lane 7 (60 μg of protein), only the 83-kDa band is detected. (Thin arrows in panels A and B) Maize 83-kDa protein; (open arrow) rat brain tau proteins.

fraction if compared with the cytosolic extract. Two-dimensional electrophoresis of the "MAP" fraction showed one major spot located in the acidic part of the gel, indicating its basic character, which is a general rule for all known MAPs (data not shown).

Because we used neurotubulin in the cosedimentation assays with the maize "MAP" fraction, we were interested to know if the plant 83-kDa polypeptide presented an affinity for neurotubulin. We added preformed taxol-stabilized pig brain microtubules to a plant cytosolic extract at 0 °C during 15 min to prevent the plant tubulin assembly. The polymers were then sedimented, and the plant proteins bound to neurotubules were isolated by increasing the ionic strength as described under Experimental Procedures. The 83-kDa protein showed high affinity for neurotubulin as demonstrated by immunostaining (Figure 7). Neural tau proteins that were obviously absent in the plant extract were specifically labeled in the neurotubulin preparation. In the maize "MAP" fraction obtained from polymerization assays of the total maize microtubule proteins, the 83-kDa polypeptide alone cross-reacted, as a single-band, with the anti-tau antibodies. These results indicated the presence of at least one common epitope between neural tau proteins and one of the putative maize MAPs.

## Discussion

MAPs have traditionally been identified as polypeptides that quantitatively copurified with neurotubulin through repeated cycles of temperature-dependent polymerization and depolymerization (Shelanski et al., 1973). Until now such methodology has been inefficient with higher plant extracts as tubulin content remained below the critical concentration required for assembly (Morejohn & Fosket, 1982; Morejohn et al., 1984; Picquot & Lambert, 1988).

In this work we identified maize proteins that shared criteria that have been defined to characterize the original MAPs, i.e., the ability to copurify with microtubules, promote tubulin assembly in a concentration-dependent manner, and induce microtubule bundling. The cross-reactivity between one of these maize proteins and affinity-purified polyclonal antibodies directed to neural tau has brought new insight into recent discoveries of the conservation of particular MAP sequences (Cleveland, 1990; Maekawa et al., 1990).

*Plant Microtubules as the Native Matrix for Plant MAPs Isolation.* We set up a technique that permitted an efficient purification of plant microtubules directly from a maize cell crude extract by using taxol-mediated assembly. We used these polymers as the native matrix to isolate putative plant MAPs. Such a procedure has not been described so far for the purification of plant microtubules. This technique, which appeared to be favorable for the isolation of maize MAPs, has been based on the criteria that were used for the purification of brain (Vallee, 1982) and nonneural (Vallee & Collins, 1986) MAPs. To overcome severe proteolysis problems, we used also appropriate concentrations of chelating agents, which are known to stabilize and inactivate proteolytic enzymes (North, 1989).

Several polypeptides were coenriched with the pellet that contained taxol-stabilized maize microtubules. This permitted us to consider these proteins as putative plant MAPs according to criteria defined for neural MAPs (Vallee, 1982). The apparent molecular mass of these maize proteins extended between 170 and 32 kDa. Available data do not allow us at present to know if this "MAP" fraction might contain degradation products and if higher plants might possess MAPs with a molecular mass greater than 200 kDa.

It is also known that the affinity of MAPs is dependent of their state of phosphorylation. This particular modification needs further investigation in the hope of characterizing properties of the higher plant MAPs.

*Effect of Maize "MAPs" on Plant and Neural Tubulin Assembly and Microtubule Binding in Vitro.* In order to assess to what extent these polypeptides associated specifically to tubulin, we tested their ability to modulate microtubule assembly, a property that characterized neural MAPs. Two main controls were required: (i) conditions where tubulin self-assembly was very low or even did not occur and (ii) removal of taxol from the putative maize MAP fraction. It was, therefore, essential to control the entire elimination of

taxol from the "MAP" fraction before assembly assays.

Promotion of microtubule assembly of both maize and pig brain MAP-free tubulins in the presence of the maize "MAP" fraction in a concentration-dependent manner resulted in a significant increase in the amount of polymers. We concluded that the maize "MAP" fraction contained proteins that promoted microtubule nucleation and assembly.

It was relevant to distinguish the activity of the putative maize MAPs on the assembly of tubulin and/or on microtubule bundling. This latter effect occurred, in our material, as described for carrot "microtubule-bundling proteins" (Cyr & Palewitz, 1989). It is well documented that some MAPs such as tau proteins can promote both assembly and cross-linking of microtubules (Hirokawa et al., 1988) while other proteins cross-link microtubules without effects on tubulin assembly, as glyceraldehyde 3'-phosphatase (Huitorel & Pantaloni, 1985; Launay et al., 1989) or mechano-chemical ATP or GTPases that mediate microtubule-sliding such as dynamin (Sheptner & Vallee, 1989). As we could not, so far, experiment on individual proteins of the maize "MAP" fraction, our present data suggested that at least one or some of these proteins could be competent to associate with tubulins of different (i.e., plant and neural) sources and induce microtubule cross-linking. This might be directly related to the high degree of homology between plant and animal tubulins (Silflow et al., 1987), particularly in their C-terminal regions that include the MAP-binding domains for neurotubulin. Detailed structural comparison of the C-terminal domains of both $\alpha$- (Ludwig et al., 1987) and $\beta$- (Guiltinan et al., 1987) tubulins from different plants and animals indicated more than 90% homology within the 390 to 427 ($\beta$) and 438 ($\alpha$) amino acid sequences. It was shown also that the internal region containing residues 430–441 in $\alpha$-tubulin and 422–434 in the $\beta$-subunits was directly involved in the interactions with MAP 2 and tau (Maccioni et al., 1988; Cross et al., 1991). More recently, dynein was found to interact also within the same tubulin domain (Paschal & Vallee, 1989). These findings suggested that these domains represent common binding sites for different MAPs. Because these conserved domains are found also in higher plant tubulins (Guiltinan et al., 1987), one can postulate that they might function, as well, as the binding region of plant MAPs to plant microtubules.

*Cross-Reactivity with Antibodies Directed Against Neural Tau.* Cross-reactivity between one polypeptide of the maize "MAP" fraction and antibodies that were specifically raised against tau, isolated from adult rat brain, suggested the presence of common epitopes between a neural MAP, tau, and higher plant (maize) proteins. The maize polypeptide that is recognized as a single band of about 83 kDa has an apparent molecular mass above the 35–62-kDa range that characterized tau proteins. The different tau isoforms are derived from the alternative mRNA splicing of a single tau gene (Lee et al., 1988; Himmler et al., 1989; Goedert et al., 1989) and states of phosphorylation (Hagestedt et al., 1989). However, in maize extracts, we did not find multiple bands reacting against the tau antibody, suggesting that either only one or few isoform(s) of comparable electrophoretic properties might be present in this extract. One can hypothesize that the putative homology that was found between tau proteins and the 83-kDa maize protein might concern the region of the tubulin-binding domain, as we have demonstrated that this protein is capable of binding to neurotubules (Figure 7). Recent investigations on tau revealed that this binding domain is composed of three or four tandem repeated sequences of 31–32 amino acids depending on brain development (Lee et al., 1988; Himmler et al., 1989; Goedert et al., 1989). These repeats are involved in promoting tubulin assembly (Ennulat et al., 1989; Goedert et al., 1990). It has been shown also that MAP 2 (Lewis et al., 1988) and MAP U (Aizawa et al., 1990) shared the same tubulin-binding motif as tau proteins. Another example of cross-reactivity between a green algal protein microtubule-associated protein and antibodies raised against bovine adrenal 190-kDa MAP has been reported recently (Maekawa et al., 1990). As we stated above, animal and plant tubulins have a striking homology in their C-terminal domains, which are known, for neurotubulin, to contain the tau and MAP 2 binding domains. Therefore, our present data favor the hypothesis that at least one of the putative maize MAPs (83 kDa) might possess a binding domain to tubulin with an extended homology to tau.

Another striking discovery was the dramatic reorganization of the microtubule cytoskeleton into thick bundles in fibroblasts transfected with a single neural tau cDNA (Kanai et al., 1989). This tau-induced microtubular organization resembles the singular dynamics of plant microtubule arrays in the cell cortex or during premitotic events (Lloyd, 1987; Lambert et al., 1991). In the present context, it will be of great interest to determine if the immunological cross-reactivity that we observed is related to an homology with the particular sequences of the tau tubulin-binding site. Such an approach might now be possible, using molecular probes. This could open the field of investigation on the molecular structure of plant MAPs and their role in the modulation of microtubule functions in relation to growth and development.

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