

# MAP 4: Occurrence in Mouse Tissues

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**ABSTRACT** A polyclonal antiserum to a microtubule-associated protein (MAP) from mouse neuroblastoma cells (MAP 4) was used to examine the distribution of this protein in mouse tissues. Immunoblots of neuroblastoma cell microtubule protein preparations demonstrated that the antiserum reacted with a triplet of proteins at 215,000–240,000 mol wt. Antibodies affinity purified from any of the bands showed cross-reaction with the other bands, indicating these polypeptides were all immunologically related. Antibodies specific to MAP 4 decorated microtubules in cultured murine cells fixed with glutaraldehyde, and diffuse staining was seen following treatment of cells with nocodazole. The antiserum reacted with MAP 4 in extracts of brain, heart, liver, and lung from adult mouse; the triplet in brain was more closely spaced than in the other tissues or neuroblastoma cells. In kidney, spleen, and stomach, only a single band (band 4) was labeled; this band was immunologically related to the triplet and was also present in all tissues positive for the triplet. Skeletal muscle, sperm, and peripheral blood contained no reactive polypeptides. After taxol-induced polymerization, the MAP 4 triplet was preferentially associated with the microtubule pellet whereas band 4 remained in the supernatant. These data indicate that there is tissue specificity in the distribution of MAP 4, and that some tissues contain a polypeptide related to MAP 4 (band 4) that does not bind to microtubules *in vitro*.

The existence of proteins that co-assembled with tubulin (8, 23, 32) and that decorated the surface of microtubules (13, 20, 22, 33, 38, 43) was initially derived from studies with brain tissue. However, most work on the distribution of these microtubule-associated proteins (MAP)<sup>1</sup> has been carried out with cultured cells. Studies in which polyclonal antisera raised against high molecular weight MAP (MAP 1 and MAP 2) (9, 10, 12, 29–31) or low molecular weight MAP (tau) (9–11) were found to label microtubule networks in non-neuronal cells suggested that proteins homologous to the brain MAP were widely distributed among cell types. More recently, monoclonal antibodies to a subspecies of brain MAP 1, MAP 1A, have been shown to react with various continuous cell lines (4), whereas monoclonal antibodies to MAP 2 have been found to have a more restricted distribution (18, 19). While all of these studies indicate that MAP similar to those found in brain exist in continuously growing cultures, little is yet known about the occurrence of any of these proteins in non-neuronal tissues.

MAP distinct from those of brain were identified when

clonal cell lines were used as sources of microtubules. For example, *in vitro* assembly of microtubules demonstrated major MAP of 125,000 and 210,000 mol wt in HeLa cells (5, 39, 40) and 215 mol wt in mouse neuroblastoma cells (26). By using selective solubilization, major MAP species of ~200,000–220,000, 80,000, and 69,000 mol wt were found in a variety of cell types (14). Although the 69,000-mol-wt MAP from cells showed peptides in common with tau proteins from brain (14), the high molecular weight species differed in molecular weight from brain MAP 1 or 2. In addition, the proteins in the 200,000–220,000-mol-wt range showed differences in molecular weight between rodent and human cell lines.

Although the studies cited above have shown that a number of MAP can be identified in cultured cells, the occurrence of any these proteins in primary tissues other than brain has not yet been investigated. Our previous work indicated that the MAP we had identified in mouse neuroblastoma cells was present in very low amounts in cells (27), and we therefore raised antibodies to use as probes for the localization of this protein. As described subsequently, this MAP, originally identified as having a molecular weight of 215,000, is actually a complex of related polypeptides ranging in molecular weight between 215,000 and 240,000, this complex will be referred to as MAP 4. This paper describes the complexity of MAP 4

<sup>1</sup> *Abbreviations used in this paper:* MAP, microtubule-associated proteins; PMSF, phenylmethylsulfonyl fluoride; TAME, Na-*p*-tosyl-L-arginine methyl ester.

in a variety of mouse tissues and demonstrates that this MAP is restricted to a subset of these tissues.

## MATERIALS AND METHODS

### Antibody Preparations

A polyclonal antiserum was produced in a New Zealand White rabbit using microtubule protein fractions (H<sub>4</sub>P; 1 mg total for all injections) obtained from differentiated neuroblastoma cells by four cycles of D<sub>2</sub>O-induced assembly (27). Samples were resuspended in PBS and sonicated with an equal volume of complete Freund's adjuvant and the rabbit was injected subcutaneously with a total of 1.4 ml of immunogen, 0.7 ml in each of two sites on the back. 1 wk later, an additional 1.4 ml of immunogen was injected. The rabbit was bled 2 wk after the second injection, and then every 3–4 d for 12 wk. For some experiments, antibodies to the proteins of interest (see Results) were affinity purified from diazotized paper blots (24), and were used to reprobe blots or for immunostaining. Absorption of the whole antiserum with purified tubulin had no effect on the labeling patterns on blots or cells. Tubulin antisera were prepared as described previously (37).

### Immunoblots

Protein samples were resolved by SDS PAGE (4–6, 7, or 8% acrylamide gels) (21) and then electrophoretically transferred to diazotized paper blotting paper (34) or nitrocellulose (modified from Towbin et al. [35] by inclusion of 0.1% SDS [15] in the blotting buffer). Under the conditions used, proteins of all molecular weights transferred efficiently to the blots. Blots were incubated with antibodies for 10–18 h at room temperature, and antibody binding was detected either with iodinated protein A ( $2 \times 10^5$  or  $10^6$  cpm/ml) and autoradiography, or with goat anti-rabbit serum coupled to peroxidase and reacted with 4-chloro-1-naphthol or *O*-dianisidine. Gels were stained for protein with Coomassie Blue, and blots were stained with amido black.

### Immunofluorescence

For indirect immunofluorescence, cells that had been plated on coverslips were fixed for 10 min at room temperature with 1.0% glutaraldehyde in 0.1 M PIPES, pH 6.9. Cells were rinsed in 0.1 M PIPES, and extracted with 0.5–1% Triton-X 100 in 0.1 M PIPES for 30 min (three changes). Coverslips were rinsed in PBS, reduced with 1 mg/ml NaBH<sub>4</sub> in three parts 0.1 M PIPES/1 part 99% EtOH for 15 min (five changes), rinsed, and then incubated with 1% BSA in PBS or Tris-buffered saline, pH 7.6, for 30 min at room temperature. Coverslips were then split in two, and each half was incubated with blot-purified antibodies to MAP 4 or tubulin antiserum for 30 min at 37°C. After rinsing, coverslips were incubated with fluorochrome-conjugated goat anti-rabbit serum (Miles Laboratories Inc., Elkart, IN), rinsed, and mounted in Gelvatol. Samples were photographed using a Zeiss universal microscope equipped with epifluorescence optics.

### Sample Preparations

**MOUSE TISSUES:** Mice were killed by cervical dislocation, and organs were removed and placed on ice. Organs were immediately homogenized in 0.1 M PIPES, pH 6.9, 0.1 mM MgCl<sub>2</sub>, 1.0 mM EGTA, 2.5 mM GTP, 1.0 mM phenylmethylsulfonyl fluoride (PMSF) (PMEG) (ratio of 1.0 g wet wt/1.5 ml buffer). For portions of this study, 10 mM Na-*p*-tosyl-L-arginine methyl ester (TAME) and 0.1 mg/ml trypsin inhibitor were also included in the PMEG. We prepared extracts by centrifugation for 40 min at 38,000 *g* and by centrifuging the resultant supernatants for 90 min at 40,000 rpm (Beckman type 65 rotor, Beckman Instruments, Inc., Palo Alto, CA). Mouse erythrocytes were washed three times in cold PBS with 1 mM PMSF. Ghosts were prepared by washing the erythrocytes three times with Buffer H (17). The erythrocyte membranes were collected with 10-min spins at 38,000 *g* between each wash.

**MICROTUBULE AND MAP PREPARATIONS:** Extracts were prepared and microtubules were polymerized from neuroblastoma cells using either D<sub>2</sub>O- (27) or taxol- (36) driven assembly. Microtubules from mouse tissues were obtained using the taxol-procedure of Vallee (36), except that 20 μM taxol was used for brain extracts, and 40 μM taxol for all other organs or tissues. After sedimentation of the microtubules, the supernatant (designated HS<sub>T</sub>) was removed, and the pellet (HP<sub>T</sub>) was washed with PMEG containing 20 μM taxol and recentrifuged. Brain microtubules were also prepared by cycles of assembly (28). We prepared thermostable MAP (16) by boiling microtubule fractions resuspended in 0.1 M PIPES, 0.2 mM MgCl<sub>2</sub>, 2 mM EGTA, 2.5 mM GTP, 2.2 mM dithiothreitol, 1 mM PMSF, 0.75 M NaCl, pH 6.9, for 5 min, and centrifuging the boiled sample at 14,000 *g* for 15 min.

## Molecular Weight Determination

7 or 4–6% gradient gels were prepared which contained microtubule preparations from neuroblastoma cells or mouse tissues. The migration of the proteins of interest were compared with those of MAP 2 (270,000 mol wt), alpha-spectrin (245,000 mol wt), beta-spectrin (220,000 mol wt), myosin heavy chain (205,000 mol wt), and β-galactosidase (116,000 mol wt), and the molecular weights were estimated from a semi-log standard plot of denatured molecular weights relative to migrated distance.

## RESULTS

Immunoblotting was used initially to characterize the antiserum raised to four-cycled microtubule protein preparations (27) from mouse neuroblastoma cells. As shown in the Coomassie Blue-stained gel in Fig. 1A, a microtubule pellet (lane 1) prepared by taxol-driven assembly from neuroblastoma cell extracts contained a number of protein bands in addition to tubulin. Immunoblot analysis (Fig. 1B, lane 1) of this sample demonstrated that the MAP 4 antiserum reacted with a triplet of bands; similar results have been obtained with immunoblots of cycled microtubule protein preparations from neuroblastoma cells (26). The presence of all three polypeptides with the microtubule pellet indicated that all of these proteins were MAP. The mobility of the reactive species was between 215,000 and 240,000 mol wt; this is the same range of molecular weight as the dominant neuroblastoma MAP. This MAP, originally designated as 215,000 mol wt (27), has been shown here to be similar in mobility to alpha-spectrin (240,000 mol wt, see Fig. 3 for comparison), and will be called MAP 4. No reaction was observed in the region of tubulin,

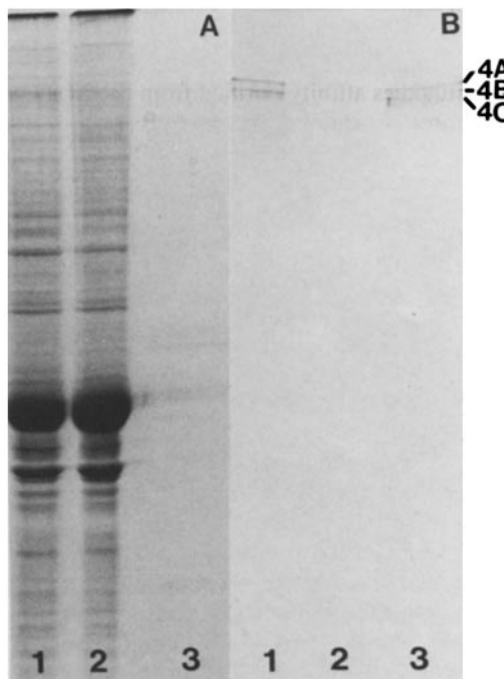


FIGURE 1 Preparation and immunoblotting of thermostable MAP. (A) 8% acrylamide gel stained with Coomassie Blue of microtubule pellet (HP<sub>T</sub>) prepared from neuroblastoma extracts by taxol-driven assembly (lane 1); pellet fraction after boiling and centrifugation of fraction shown in lane 1 (lane 2); supernatant fraction after boiling and centrifugation of fraction shown in lane 1 (lane 3). (B) Immunoblot of fractions shown in A, probed with whole MAP 4 antiserum. Note that the antiserum reacts with a triplet of bands (MAP 4A, 4B, and 4C) and that all of these bands are present in the thermostable fraction (lane 3).

indicating lack of detectable antibodies to this highly conserved protein.

The relationship of the triplet of polypeptides detected by immunoblotting was analyzed in neuroblastoma extracts. 4–6% gradient gels were used to achieve greater separation of the reactive species (e.g., Fig. 3C) and antibodies specific to each of these bands were prepared by affinity purification of the antibodies from individual bands on blots (24). Antibodies eluted from any of the bands reacted with all other bands in this region and indicated that these were immunologically related. These bands were also present in neuroblastoma extracts prepared under conditions where proteolysis would have been favored (no protease inhibitors, incubation of homogenates for 1 or 2 h at 0° or 37°C), as well as under conditions that minimized *in vitro* proteolysis (protease inhibitors, immediate boiling of cells in SDS sample buffer). These data (also see Fig. 4) suggested that all of the polypeptides recognized by the MAP 4 serum were present *in vivo*.

In addition to sharing antigenic determinants and the ability to co-assemble with microtubules, the three polypeptides of the MAP 4 complex were also similar in remaining soluble after boiling. An aliquot of the microtubule preparation shown in Fig. 1A was boiled, and the resultant supernatant and pellet were analyzed by immunoblotting. As shown in Fig. 1B, all three of the polypeptides present in the microtubule fraction (lane 1) were recovered in the supernatant after boiling (lane 3). Thus, like MAP 2 (16), HeLa 210,000-mol-wt MAP (41), and tau (8), the proteins of the MAP 4 complex appear to be thermostable. To remain consistent with the nomenclature recently given to MAP 1 and MAP 2 subspecies (3), we designate the thermostable species reacting with the MAP 4 antisera MAP 4A, MAP 4B, and MAP 4C in order of increasing mobility on SDS gels.

Using antibodies affinity purified from the MAP 4 triplet, immunofluorescent staining was used to assess whether this protein was also associated with microtubules *in situ*. As shown in Fig. 2A, MAP 4-specific antibodies showed a pattern in 3T3 cells similar to that seen with the tubulin antiserum (Fig. 2B). Following treatments that disrupted microtubules, diffuse intracellular staining was observed with either the purified antibodies to MAP 4 (Fig. 2C) or tubulin antiserum (Fig. 2D). Thus, unlike MAP 2, which has been shown to redistribute with intermediate filaments upon dissolution of

microtubules (2), MAP 4 appears to diffuse throughout the cytoplasm.

While the preceding data demonstrated that the MAP 4 antibodies reacted with MAP in cultured murine cells, a primary goal was to establish whether this MAP was present in mouse tissues. Therefore, we prepared immunoblots of extracts from various mouse tissues and probed them with the antiserum. Fig. 3 shows that the antiserum reacts with polypeptides in heart, liver, and lung similar in molecular weight to those in neuroblastoma extracts. Brain also shows a similar pattern, although the triplet is more closely spaced and MAP 4C is not as prominent as in neuroblastoma or other tissues. No triplet is present in kidney, spleen, or stomach. However, these tissues, as well as brain, heart, liver, and lung, contain a lower molecular weight polypeptide (band 4) that reacts with the antiserum (see below). Several preparations contained neither the triplet nor band 4; these included skeletal muscle from thigh, mouse erythrocyte membranes (Fig. 3), whole peripheral blood, and isolated sperm (data not shown). Thus, the complexity and extent of cross-reaction of polypeptides with the MAP 4 antiserum varied depending on tissue origin.

Organ homogenates prepared under various conditions were examined to determine whether the diversity in the patterns seen by immunoblotting might be the result of *in vitro* proteolysis. Samples were prepared from brain homogenized in boiling sample buffer, or from brain homogenates incubated at 0° or 37°C in the presence or absence of PMSF, TAME, and trypsin inhibitor. As shown in Fig. 4, immunoblots demonstrate that the polypeptide patterns are identical under all conditions tested. Similar results were obtained in examining other tissue samples; MAP 4B, MAP 4C, and band 4 were present in tissues homogenized immediately in boiling sample buffer, and showed no change on prolonged incubation, suggesting that these bands exist *in vivo*.

In addition to the MAP 4 triplet, the whole antiserum labeled two other bands in brain extract, one additional band in neuroblastoma extract, and one doublet in heart extract (see Fig. 3). The two bands labeled in brain extract co-migrated with MAP 1 and MAP 2 from mouse brain microtubules obtained by taxol-driven assembly (see Fig. 7). The cross-reactive species in neuroblastoma extract co-migrated with a protein, originally identified as having a molecular

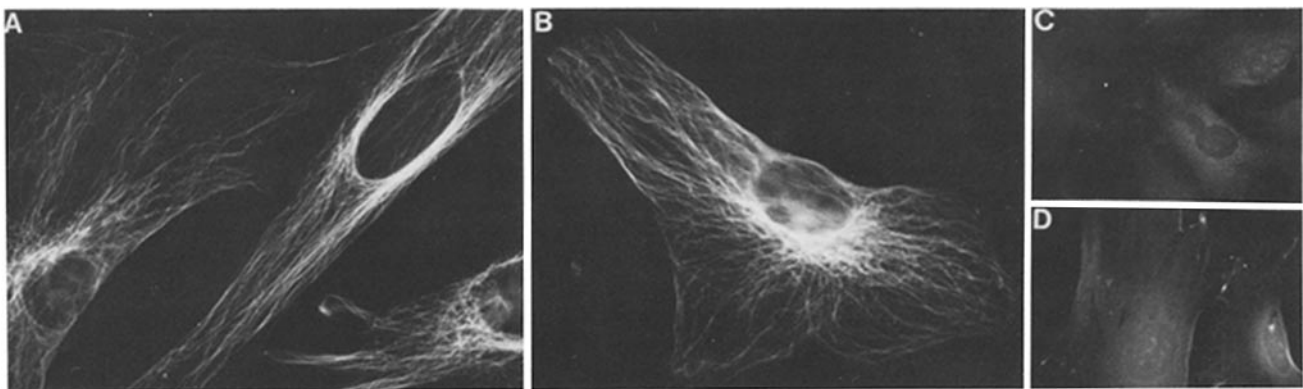


FIGURE 2 Indirect immunofluorescence staining with MAP 4 and tubulin antibodies. 3T3 cells were labeled by indirect immunofluorescence using either antibodies affinity purified from MAP 4 in blots of neuroblastoma HP<sub>T</sub> (A and C) or tubulin antiserum (B and D). 3T3 cells in C and D were treated with 60 ng/ml nocodazole for 3 h before labeling. (A and B)  $\times 500$ ; (C and D)  $\times 214$ .

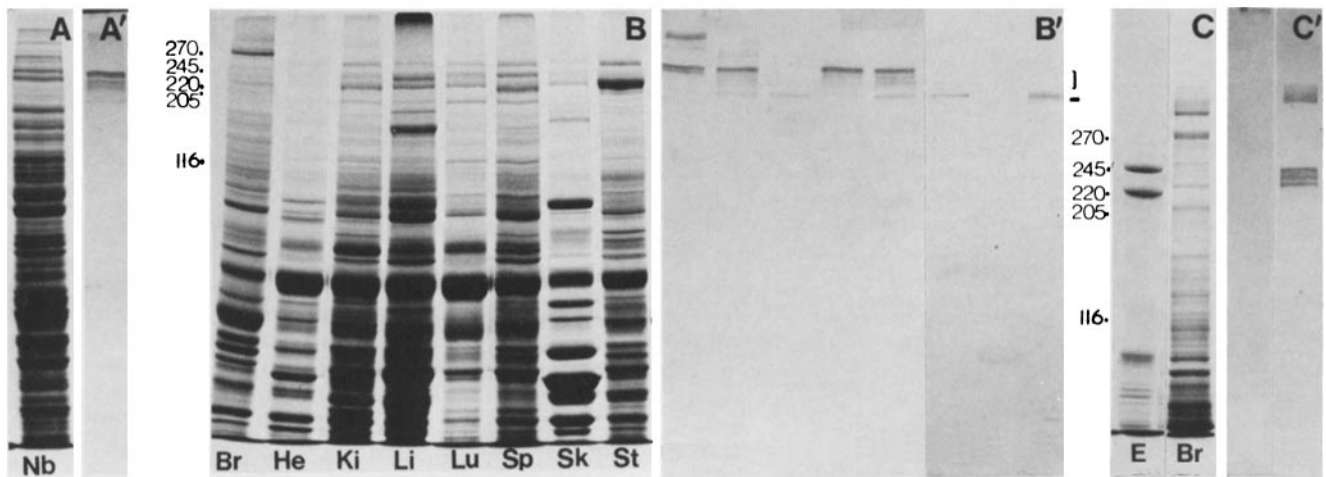


FIGURE 3 Gels and immunoblots of mouse tissue extracts. (A and A') Neuroblastoma extract; 7% gel (A) and corresponding immunoblot (A'). (B and B') Tissue extracts; 7% gel (B) and corresponding immunoblot (B'). (C and C') Erythrocyte ghosts and brain extract; 4-6% gradient gel (C) and corresponding immunoblot (C'). *Nb*, neuroblastoma; *Br*, brain; *He*, heart; *Ki*, kidney; *Li*, liver; *Lu*, lung; *Sp*, spleen; *Sk*, skeletal muscle; *St*, stomach; *E*, erythrocyte ghosts. Whole MAP 4 antiserum reacts with a molecular weight species identical to MAP 4 in only a subset of mouse tissues. Band 4 is common to all organs except skeletal muscle. Note that the antiserum does not react with any polypeptides in mouse erythrocyte membranes, and the position of migration of the triplet is between that of  $\alpha$ - (245,000 mol wt) and  $\beta$ - (220,000 mol wt) spectrin. Molecular weight markers ( $\times 10^{-3}$ ), as defined in Materials and Methods, are indicated. Bracket, MAP 4A, 4B, 4C; dash, band 4.

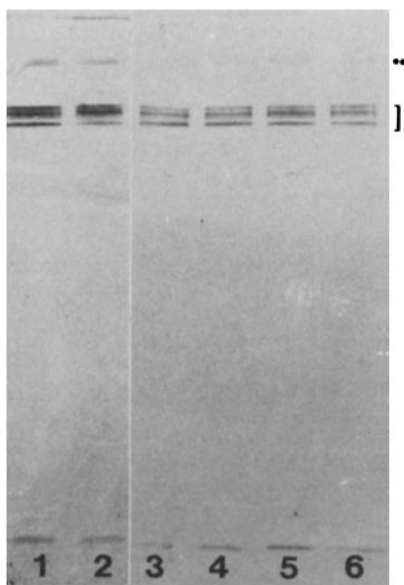


FIGURE 4 Effect of 37°C incubation on MAP 4 in brain homogenates. (lane 1) Whole brain sonicated in boiling sample buffer; (lane 2) whole brain incubated at 37°C, 0.5 h, then sonicated in boiling sample buffer; (lane 3) brain homogenate prepared without inhibitors present, incubated at 0°C, 0.5 h; (lane 4) sample as in lane 3, incubated at 37°C, 0.5 h; (lane 5) brain homogenate prepared in the presence of 1 mM PMSF, 0.1 mg/ml trypsin inhibitor, and 10 mM TAME, incubated at 0°C, 0.5 h; (lane 6) sample as in lane 5, incubated at 37°C, 0.5 h. Samples were run in a 7% gel and immunoblots were incubated with whole MAP 4 antiserum. Preparation of brain homogenates under conditions that would accelerate proteolysis (lanes 2 and 4) does not affect the composition of the MAP 4 complex. Two dots, MAP 1; bracket, MAP 4.

weight of 310,000, that associates with microtubules through three cycles of assembly (27). Although present in blots of extracts, this protein was not consistently detected in immunoblots of microtubule preparations from neuroblastoma cells. Antibodies were affinity purified from the region of

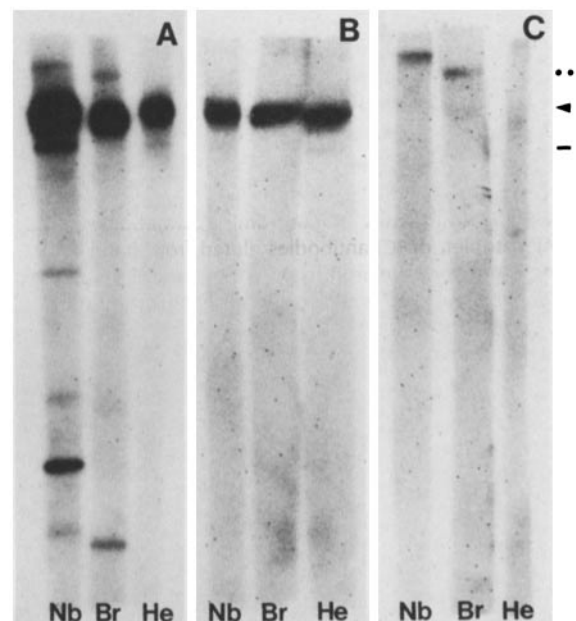


FIGURE 5 Immunoblots probed with antibodies affinity purified from MAP 4 region and MAP 1 region. Neuroblastoma, brain, and heart extract were electrophoresed on a 7% gel, blotted, and then incubated with (A) whole MAP 4 antiserum, (B) antibodies affinity purified from the MAP 4 band, (C) antibodies affinity purified from the MAP 1 region. *Nb*, neuroblastoma; *Br*, brain; *He*, heart. Antibodies eluted from MAP 4 (all three bands) reacted only with MAP 4 in each extract (B). Affinity-purified MAP 1 antibodies reacted with MAP 1 in brain and with a higher molecular weight protein ( $>350,000$  mol wt of MAP 1) in neuroblastoma extract (C). The low molecular weight bands labeled with whole serum in neuroblastoma or brain extract are present in the preimmune serum, and are not related to the immunogen. Two dots, MAP 1; arrowhead, MAP 4; dash, band 4.

MAP 1 and MAP 4 and used to investigate the relationship of these antigenic species on blots of neuroblastoma, heart, and brain extract. Fig. 5 shows that the antibodies eluted from

the MAP 4 complex (seen in this autoradiogram as a fused band) react exclusively with the triplet and band 4 in each extract (Fig. 5B). Antibodies eluted from MAP 1 react with MAP 1 in brain and the high molecular weight polypeptide in neuroblastoma extract (Fig. 5C). These data indicate these proteins are immunologically related, although the neuroblastoma protein has a higher molecular weight than MAP 1.

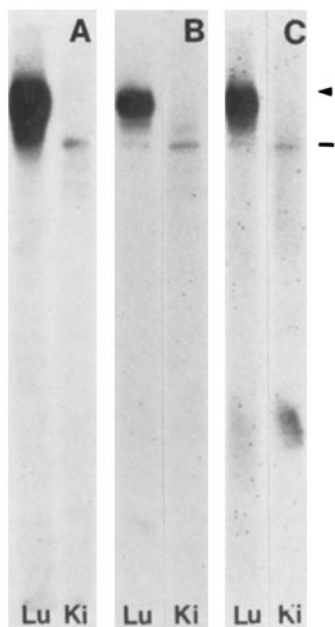


FIGURE 6 Immunoblots of tissue extracts probed with antibodies affinity purified from MAP 4 triplet and from band 4. Each organ extract was electrophoresed on a 7% gel, blotted, and then incubated with (A) whole MAP 4 antiserum, (B) antibodies eluted from the MAP 4 triplet, or (C) antibodies eluted from band 4. Lu, lung, Ki, kidney. Antibodies eluted from MAP 4 label both MAP 4 and band 4 in lung and band 4 in kidney. Conversely, antibodies eluted from band 4 label both MAP 4 and band 4. Arrowhead, MAP 4; dash, band 4.

Sufficient antibodies have not yet been obtained from the area of MAP 2 to assess the relatedness of this protein to other proteins in these extracts. However, as indicated by lack of reaction of affinity-purified antibodies from MAP 1 or MAP 4 with each other or with MAP 2, the three MAP detected in brain extracts all appear to be distinct entities. Antibodies eluted from a control area of diazotized paper blots showed no labeling.

Similar experiments were carried out to determine the relation of band 4 to the MAP 4 triplet. Antibodies from the triplet or band 4 were affinity purified from blots of organ extracts, and were used to probe blots of kidney and lung extracts. Fig. 6 shows that antibodies eluted from the MAP 4 complex will label the triplet in lung as well as band 4 in kidney. Antibodies eluted from band 4 will label only band 4 in blots of kidney extracts but will label the MAP 4 complex and band 4 in organs that possess the MAP 4 triplet. These data indicate that MAP 4A, 4B, and 4C and band 4 all share antigenic determinants.

MAP 4A, B, and C had been identified as MAP from the *in vitro* polymerization studies using neuroblastoma cell extracts, but we also wished to examine whether these MAP and band 4 co-polymerized with microtubules from tissue extracts. As shown in Fig. 7, microtubule pellets ( $HP_T$ ) enriched in tubulin could be obtained from tissue extracts using the assembly promoting drug, taxol; gels of the corresponding supernatants ( $HS_T$ ) were essentially identical in composition to the extracts shown in Fig. 3, except for the diminution of tubulin. As shown in the blots (Fig. 7, A and B), microtubule pellets from brain, heart, liver, and lung extracts contained MAP 4A, and varying amounts of 4B and C, whereas only small amounts of any of these bands remained in the  $HS_T$  fraction (compare immunoblots of  $HP_T$  and  $HS_T$  of brain, heart, liver, and lung in Fig. 7). In contrast, microtubules prepared from kidney and spleen showed no reaction with the MAP 4 antiserum. Band 4, previously identified in extracts of these tissues, was present in the  $HS_T$  fraction. These data suggested that MAP 4A, B, and C can polymerize with

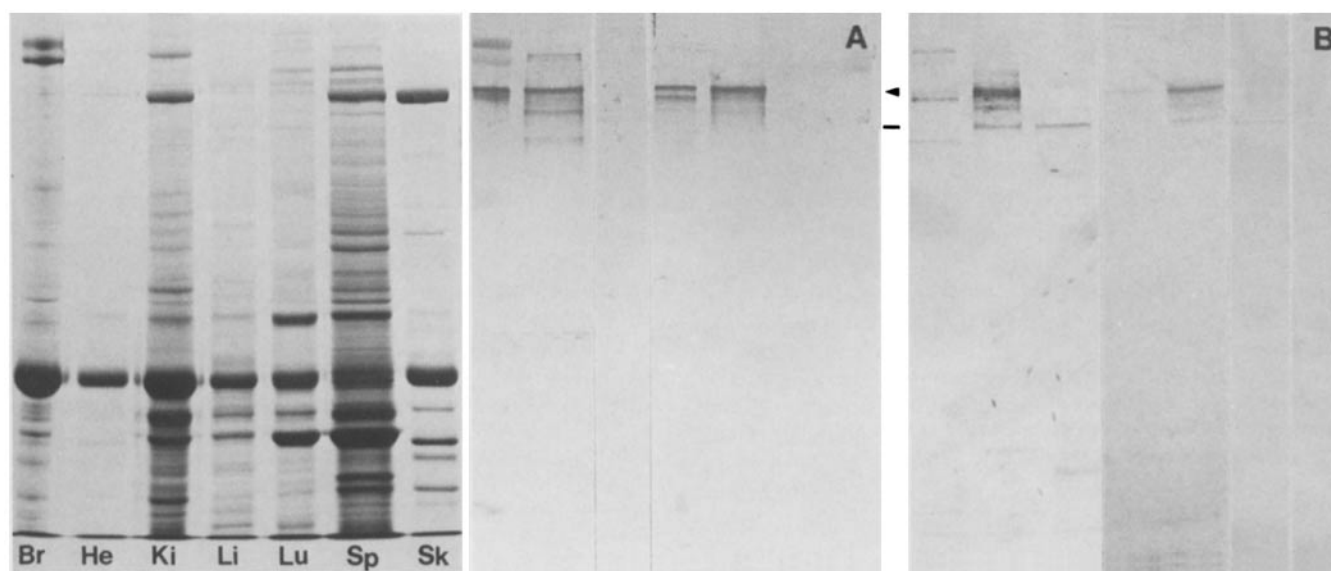


FIGURE 7 Taxol-stabilized microtubules from tissue extracts. (A) Gel (7%) and blot of microtubule pellets ( $HP_T$ ). (B) Blot of supernatants remaining after sedimentation of microtubules ( $HS_T$ ). Comparison of immunoblots of  $HP_T$  and  $HS_T$  probed with whole MAP antiserum indicates that all or portions of MAP 4 are preferentially associated with the microtubule pellet. Small portions of the triplet polypeptides and all of band 4 remain in the  $HS_T$  fraction. Arrowhead, MAP 4; dash, band 4.

microtubules, and therefore are MAP, whereas band 4, although immunologically related to the triplet, does not have the ability to bind to taxol-stabilized microtubules.

## DISCUSSION

Immunoblotting and affinity purification of antibodies have shown that MAP 4 is a complex of related polypeptides; the complex exists both in mouse cell lines (25) and as shown here, in a number of mouse tissues. All three bands of the complex co-assemble with microtubules, and all are thermostable. The 210,000-mol-wt MAP of HeLa is also thermostable (41), and it has been reported this MAP is also a triplet of polypeptides (6), although the antiserum raised to this preparation reacted with only a single band on gels. These properties of thermostability, complexity, and similarity in molecular weight indicate that MAP 4 and the 210,000-mol-wt HeLa MAP may be species-specific homologues. Complexity in both brain MAP 1 (3) and MAP 2 (1, 3) has also recently been described. Low percentage gels resolved each of these brain MAP into discrete species. MAP 2 split into two bands, both of which were thermostable. MAP 1 split into three bands, of which two (MAP 1A and 1B) were more sensitive to proteases than MAP 1C. Monoclonal antibodies raised to MAP 1 reacted only with MAP 1A, suggesting that although the MAP 1 subspecies are functionally related (associate with microtubules *in vitro*), they are structurally different polypeptides. As discussed subsequently, the subtle differences in the polypeptides of the MAP complexes may be important in understanding the cell or tissue-specific functions of these molecules.

The distribution of the MAP 4 complex shows variation among mouse tissues. Certain tissues (brain, heart, liver, lung) contain the MAP 4 triplet, and this complex co-assembles with microtubules derived from extracts of these tissues. In contrast, other tissues (kidney and spleen) contain only band 4, a polypeptide that does not bind to microtubules *in vitro*, but that is antigenically related to MAP 4. Some tissues (skeletal muscle), and microtubules derived from them, show no reaction with the antiserum. Immunoblotting analyses indicated that the proteins reacting with the MAP 4 antiserum, especially MAP 4B, C, and band 4, are present in very low but reproducible amounts in certain tissues. As is shown in the preparations of microtubules obtained from brain, MAP 4 is a very minor component compared with the dominant brain MAP, MAP 1, and MAP 2 (Fig. 7). Immunoblotting has shown that the spacing of MAP 4B, C, and band 4 in brain differs slightly from that in other mouse tissues or neuroblastoma cells. These data suggest that the MAP 4 complex in brain may be unlike that in other tissues, although the MAP 4 polypeptides in all tissues we have examined share the properties of immunologic cross-reaction and thermostability.

Our findings on the heterogeneity of the MAP 4 patterns in tissues contrast with the results on the distribution of the 210,000-mol-wt HeLa MAP. Antisera raised against the 210,000-mol-wt HeLa MAP reacted with a single band in extracts from human brain, or lysates from continuous human or primate cell lines originally derived from liver, kidney, muscle, spleen, bladder, connective tissue, and leukocytes (7). This difference could reflect particular epitope specificities of the two antisera used. Alternatively, the MAP distribution in cultured cells may not mimic the complexity in distribution seen in whole tissues. Differences have also been found in the

distribution of brain MAP 1 and 2 in cultured cells as compared with tissues. For example, although initial studies demonstrated that antibodies raised against high molecular weight MAP labeled microtubule networks in cultured cells (12, 29–31), the organization of the two major brain MAP in brain tissue has recently been demonstrated to be distinguishable. MAP 1 is widespread in both grey and white matter (3), whereas MAP 2 is restricted to neuronal cell bodies and dendrites (2, 3). All of these studies suggest that examining the distribution of MAP in adult tissues may be important in ultimately understanding how various MAP function *in situ*. For example, our recent immunocytologic analyses demonstrate that MAP 4 is very specifically localized to a subset of cells within each tissue type (Parysek, L. M., J. B. Wolosewick, and J. B. Olmsted, *J. Cell Biol.*, in press).

In addition to reacting with the MAP 4 protein in brain, the whole antiserum used in some of these studies labeled MAP 1 and, to a much lesser extent, MAP 2 in brain extracts. Affinity-purified antibodies to MAP 1 and MAP 4 did not cross-react with the nonhomologous antigens, nor with MAP 2. These data indicate that the three MAP in brain are immunologically distinct. Although proteins analogous to brain MAP 1 or MAP 2 were not detected in the original microtubule protein preparations from neuroblastoma cells used for immunogen, the presence of antibodies reacting with these species indicates that antigenically related proteins must be present in these cells. Wiche et al. (42) and Bloom et al. (4) have shown that a high molecular weight protein in neuroblastoma cells cross-reacts with antisera raised to MAP 1 from brain. However, our data demonstrated that the high molecular weight protein in neuroblastoma extracts reacting with affinity-purified antibodies to MAP 1 has a lower electrophoretic mobility than MAP 1 (Fig. 7). Bloom et al. (4) have also recently shown that monoclonal antibodies to MAP 1A from brain react with cultured cell lines derived from a number of species and tissue origins; in some of these cells, this monoclonal antibody appears to react with closely spaced doublets, or with proteins of higher molecular weight than MAP 1A (e.g., Chinese hamster ovary cell line). We found, however, no reaction in the MAP 1 region of blots of tissues other than brain. It remains to be determined whether the MAP 1 species recognized by our antiserum may be different than that reacting with MAP 1A, or whether MAP 1A is restricted in tissues as compared with cell lines. Our data indicate that of the three MAP species in mouse brain recognized by the whole antiserum, only MAP 4 is prevalent in a number of mouse tissues. More detailed analyses of the localization and expression of each of the components of the MAP 4 complex, as well as other MAP, should indicate if discrete structure-function correlations for these proteins in tissues can be made.

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## REFERENCES

1. Binder, L. I., A. Frankfurter, H. Kim, H. Payne, and L. I. Rebhun. 1983. Molecular heterogeneity of MAP2 during rat brain development. *J. Cell Biol.* 97 (5, Pt. 2):201a. (Abstr.)
2. Bloom, G. S., and R. B. Vallee. 1983. Association of microtubule-associated protein 2 (MAP 2) with microtubules and intermediate filaments in brain cells. *J. Cell Biol.* 96:1523-1531.
3. Bloom, G. S., T. A. Schoenfeld, R. B. Vallee. 1984. Widespread distribution of the major polypeptide component of MAP 1 (microtubule-associated protein 1) in the nervous system. *J. Cell Biol.* 98:320-330.
4. Bloom, G. S., F. C. Luca, and R. B. Vallee. 1984. Widespread cellular distribution of MAP 1A (microtubule-associated protein 1A) in the spindle and on interphase microtubules. *J. Cell Biol.* 98:331-340.
5. Bulinski, J. C., and G. G. Borisy. 1979. Self assembly of microtubules in extracts of cultured HeLa cells and the identification of HeLa microtubule associated proteins. *Proc. Natl. Acad. Sci. USA.* 76:293-297.
6. Bulinski, J. C., and G. G. Borisy. 1980a. Immunofluorescence localization of HeLa cell microtubule-associated proteins on microtubules in vitro and in vivo. *J. Cell Biol.* 87:792-801.
7. Bulinski, J. C., and G. G. Borisy. 1980b. Widespread distribution of 210,000-mol-wt microtubule-associated protein in cells and tissues of primates. *J. Cell Biol.* 87:802-808.
8. Cleveland, D. W., S. Y. Hwo, and M. W. Kirschner. 1977. Purification of tau, a microtubule associated protein that induces assembly of microtubules from purified tubulin. *J. Mol. Biol.* 116:207-26.
9. Connolly, J. A., and V. I. Kalnins. 1980. Tau and HMW microtubule-associated proteins have different microtubule binding sites in vivo. *Eur. J. Cell Biol.* 21:296-300.
10. Connolly, J. A., and V. I. Kalnins. 1980. The distribution of tau and HMW microtubule associated proteins in different cell types. *Exp. Cell Res.* 127:341-350.
11. Connolly, J. A., V. I. Kalnins, D. W. Cleveland, and M. W. Kirschner. 1977. Immunofluorescent staining of cytoplasmic and spindle microtubules in mouse fibroblasts with antibody to tau proteins. *Proc. Natl. Acad. Sci. USA.* 74:2437-40.
12. Connolly, J. A., V. I. Kalnins, D. W. Cleveland, and M. W. Kirschner. 1978. Intracellular localization of the high molecular weight microtubule accessory protein by indirect immunofluorescence. *J. Cell Biol.* 76:781-86.
13. Dentler, W. L., S. Granett, and J. L. Rosenbaum. 1975. Ultrastructural localization of the high molecular weight proteins associated with in vitro reassembled brain microtubules. *J. Cell Biol.* 65:237-41.
14. Duerr, A., D. Pallas, and F. Solomon. 1981. Molecular analysis of cytoplasmic microtubules in situ: identification of both widespread and specific proteins. *Cell.* 24:203-212.
15. Erickson, P. F., L. Minier, and R. Lasher. 1982. Quantitative electrophoretic transfer of polypeptides from SDS acrylamide gels to nitrocellulose sheets: a method for their reuse in immunoradiographic detection of antigens. *J. Immunol. Methods.* 51:241-249.
16. Francon, J., A. Fellous, A. Lennon, and J. Nunez. 1978. Requirement for "factors" for tubulin assembly during brain development. *Eur. J. Biochem.* 85:43-53.
17. Granger, B., and E. Lazarides. 1982. Structural associations of synemin and vimentin filaments in avian erythrocytes revealed by immunoelectron microscopy. *Cell.* 30:263-275.
18. Izant, J. G., and J. R. McIntosh. 1980. Microtubule-associated proteins: a monoclonal antibody to MAP 2 binds to differentiated neurons. *Proc. Natl. Acad. Sci. USA.* 77:4741-4745.
19. Izant, J. G., J. A. Weatherbee, and J. R. McIntosh. 1983. A microtubule associated protein antigen unique to mitotic spindle microtubules in PtK<sub>1</sub> cells. *J. Cell Biol.* 96:424-434.
20. Kim, H., L. I. Binder, and J. L. Rosenbaum. 1979. The periodic association of MAP 2 with brain microtubules in vitro. *J. Cell Biol.* 80:266-276.
21. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of phage T4. *Nature (Lond.)* 227:680-685.
22. Murphy, D. B., and G. G. Borisy. 1975. Association of high molecular weight proteins with microtubules and their role in microtubule assembly in vitro. *Proc. Natl. Acad. Sci. USA.* 72:2696-2700.
23. Murphy, D. B., K. A. Johnson, and G. G. Borisy. 1977. Role of tubulin-associated proteins in microtubule nucleation and elongation. *J. Mol. Biol.* 117:33-52.
24. Olmsted, J. B. 1981. Affinity purification of antibodies from diazotized paper blots of heterogeneous protein samples. *J. Biol. Chem.* 256:11955-11957.
25. Olmsted, J. B. 1982. Immunological analyses of a 215,000 dalton murine microtubule associated protein (MAP). *J. Cell Biol.* 95(2, Pt. 2):345a. (Abstr.)
26. Olmsted, J. B., J. V. Cox, C. F. Asnes, L. M. Parysek, and H. D. Lyon. 1984. Cellular regulation of microtubule organization. *J. Cell Biol.* 99(1, Pt. 2):28-32.
27. Olmsted, J. B., and H. D. Lyon. 1981. A microtubule-associated protein (MAP) specific to differentiated neuroblastoma cells. *J. Biol. Chem.* 256:3507-3511.
28. Olmsted, J. B., J. M. Marcum, K. A. Johnson, C. Allen, and G. G. Borisy. 1974. Microtubule assembly: some possible regulatory mechanisms. *J. Supramol. Struct.* 2:429-450.
29. Sherline, P., and K. Schiavone. 1977. Immunofluorescence localization of proteins of high molecular weight along intracellular microtubules. *Science (Wash. DC)* 198:1038-1041.
30. Sherline, P., and K. Schiavone. 1978. High molecular weight MAPs are part of the mitotic spindle. *J. Cell Biol.* 77:R9-R12.
31. Shterline, P. 1978. Localization of the major high molecular weight protein on microtubules in vitro and in cultured cells. *Exp. Cell Res.* 115:460-464.
32. Sloboda, R. D., W. L. Dentler, and J. L. Rosenbaum. 1976. Microtubule-associated proteins and the stimulation of tubulin assembly in vitro. *Biochemistry.* 15:4497-4505.
33. Sloboda, R. D., and J. L. Rosenbaum. 1979. Decoration and stabilization of intact, smooth-walled microtubules with microtubule-associated proteins. *Biochemistry.* 18:48-54.
34. Symington, J., M. Green, and K. Brackmann. 1981. Immunoradiographic detection of proteins after electrophoretic transfer to diazo-paper: analysis of adenovirus encoded proteins. *Proc. Natl. Acad. Sci. USA.* 78:177-181.
35. Towbin, H., T. Staehlin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350-4354.
36. Vallee, R. B. 1982. A taxol-dependent procedure for the isolation of microtubules and microtubule-associated proteins. *J. Cell Biol.* 92:435-442.
37. Van De Water, L., S. D. Guttman, M. A. Gorovsky, and J. B. Olmsted. 1982. Production of antisera and radioimmunoassays for tubulin. *Methods Cell Biol.* 24:79-96.
38. Voter, W. A., and H. P. Erickson. 1982. Electron microscopy of MAP 2 (microtubule-associated protein 2). *J. Ultrastruct. Res.* 80:374-382.
39. Weatherbee, J. A., R. B. Luftig, and R. R. Wehling. 1978. In vitro polymerization of microtubules from HeLa cells. *J. Cell Biol.* 78:47-57.
40. Weatherbee, J. A., R. B. Luftig, and R. R. Wehling. 1980. Purification and reconstitution of HeLa cell microtubules. *Biochemistry.* 19:4116-4123.
41. Weatherbee, J. A., P. Sherline, R. Mascardo, J. Izant, R. B. Luftig, and R. R. Wehling. 1982. Microtubule-associated proteins of HeLa cells: heat stability of the 200,000 mol wt HeLa MAPs and detection of the presence of MAP 2 in HeLa cell extracts and cycled microtubules. *J. Cell Biol.* 92:155-162.
42. Wiche, G., E. Briones, H. Hirt, U. Arleib, and H. Denk. 1983. Differential distribution of microtubule-associated proteins MAP-1 and MAP-2 in neurons of rat brain, and association of MAP-1 with microtubules of neuroblastoma cells (clone N<sub>2</sub>A). *EMBO (Eur. Mol. Biol. Organ.)* 2:1915-1920.
43. Zingsheim, H. P., W. Herzog, and K. Weber. 1979. Differences in surface morphology of microtubules reconstituted from pure brain tubulin using two different microtubule associated proteins—high molecular weight MAP 2 proteins and tau proteins. *Eur. J. Cell Biol.* 19:175-182.