

Association of Microtubule-associated Protein 2 (MAP 2) with Microtubules and Intermediate Filaments in Cultured Brain Cells

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ABSTRACT The classification of MAP 2 as a microtubule-associated protein is based on its affinity for microtubules *in vitro* and its filamentous distribution in cultured cells. We sought to determine whether MAP 2 is also able to bind *in situ* to organelles other than microtubules. For this purpose, primary cultures of rat brain cells were stained for immunofluorescence microscopy with a rabbit anti-MAP 2 antibody prepared in our laboratory, as well as with antibodies to vimentin, an intermediate filament protein, and to tubulin, the major subunit of microtubules. MAP 2 was present on cytoplasmic fibers in neurons and in a subpopulation of the flat cells present in the cultures. Our observations were concentrated on the flat cells because of their suitability for high-resolution immunofluorescence microscopy. Double antibody staining revealed co-localization of MAP 2 with both tubulin and vimentin in the flat cells. Pretreatment of the cultures with vinblastine resulted in the redistribution of MAP 2 into perinuclear cables that contained vimentin. Tubulin paracrystals were not stained by anti-MAP 2. In cells extracted with digitonin, the normal fibrillar distribution of MAP 2 was resistant to several treatments (PIPES buffer plus 10 mM Ca^{++} , phosphate buffer at pH 7 or 9) that induced depolymerization of microtubules, but not intermediate filaments. Staining of the primary brain cells was not observed with preimmune serum nor with immune serum adsorbed prior to use with pure MAP 2. We detected MAP 2 on intermediate filaments not only with anti-MAP 2 serum, but also with affinity purified anti-MAP 2 and with a monoclonal anti-MAP 2 prepared in another laboratory. We conclude from these experiments that material recognized by anti-MAP 2 antibodies associates with both microtubules and intermediate filaments. We propose that one function of MAP 2 is to cross-link the two types of cellular filaments.

Since the introduction of procedures for purifying cytoplasmic microtubules (6, 48), it has become evident that these structures are composed of numerous polypeptide species. Tubulin is the predominant component of cytoplasmic microtubules. In addition, numerous proteins that appear to be associated with tubulin have been discovered. These microtubule-associated proteins, or MAPs (53), have been identified on the basis of their affinity for microtubules *in vitro* (4, 12, 38, 53), and on the basis of immunocytochemical data (10, 11, 13, 14, 27, 31, 41, 50, 51, 52). The MAPs have been implicated in controlling microtubule assembly (37, 63). In addition, at least two MAP species, MAP 2 (25, 30) and MAP 1 (61), represent fine filamentous projections regularly spaced on the microtubule surface. This has given rise to speculation that these proteins may mediate the interaction of microtubules with other organ-

elles and may, therefore, be important in microtubule function as well as microtubule assembly. Evidence for the binding of MAPs *in vitro* to a variety of structures in addition to microtubules has been reported (23, 34, 45, 46, 49, 54), further supporting a role for MAPs in the interaction of microtubules with other cellular components.

MAP 2 has been extensively characterized. It is the most abundant MAP species identified in brain tissue, its major source. This laboratory has shown that the protein may be divided into two structural domains (58). One domain contains the microtubule binding site and promotes microtubule assembly. The other, larger domain represents the portion of MAP 2 observed as a projection on the microtubule surface. The projection domain contains a binding site for the regulatory subunit of a type II cAMP-dependent protein kinase, which

phosphorylates MAP 2 (55, 60). Presumably, the projection domain also contains a site or sites for binding of other cytoplasmic organelles.

Evidence for the binding of MAP 2 *in vitro* to actin filaments, coated vesicles, and intermediate filaments has been reported (1, 24, 34, 40, 45, 46). While these studies have identified structures that are capable of interacting with MAP 2 *in vitro*, they leave open the question of which organelles actually bind MAP 2 in cells. We initiated our study to resolve this question and to further our characterization of the binding domains of this protein. We reasoned that if the cellular microtubule binding site for MAP 2 were dissolved it might be possible to identify other organelles to which the MAP bound. We chose to work with primary cultures of brain cells because these cells contain MAP 2 in apparently high concentration and include a population of well-spread cells that are suitable for high-resolution immunocytochemical techniques (27, 41). We report that, after dissolution of microtubules, MAP 2 is found in association with intermediate filaments, indicating a role for MAP 2 in mediating the interaction of microtubules with this other class of cellular filament.

MATERIALS AND METHODS

Anti-MAP 2 Antibodies: We prepared a rabbit antibody to calf brain MAP 2 in this laboratory, and used it to immunoprecipitate the MAP 2 protein kinase complex (55) and to localize MAP 2 by immunofluorescence in the rat central nervous system (35) (Miller, P., W. E. Theurkauf, R. B. Vallee, and P. De Camilli, manuscript in preparation). The MAP 2 immunogen was purified by a three-step process designed to insure antigen purity. First, calf brain microtubules were purified by four cycles of assembly and disassembly in the absence of glycerol (60). The microtubules were exposed to elevated temperatures (90–100°C) to coagulate tubulin, MAP 1, and a variety of minor polypeptides species (18, 25, 30). The MAP 2-enriched soluble fraction was finally subjected to preparative SDS PAGE. After staining with Coomassie Brilliant Blue R250, the MAP 2 doublet (30) was excised from the gel and homogenized with Freund's complete adjuvant. A rabbit was injected subcutaneously with ~100 µg of MAP 2 on each of three occasions, and was bled 1 wk after the final injection.

Affinity-purified anti-MAP 2 was prepared by passage of the immune serum over a MAP 2-Sepharose 4B column. The MAP 2 used here was prepared from twice-cycled calf brain microtubules by exposure to elevated temperature (see above). The protein was conjugated to CNBr-activated Sepharose 4B, and monospecific anti-MAP 2 was eluted from the column with 200 mM glycine at pH 2.7 (20).

Monoclonal mouse anti-porcine brain MAP 2 (27) was the generous gift of Jonathan Izant and Richard McIntosh (University of Colorado).

Other Antibodies: Goat anti-chick brain tubulin and goat anti-tetanus toxin were kindly provided by Dr. Robert Weising (Worcester Foundation for Experimental Biology) and Dr. William Habig (National Institutes of Health), respectively. Monoclonal mouse anti-vimentin was purchased from Transformation Research, Inc. (Framingham, MA).

Guinea pig antitubulin was prepared in this laboratory. The immunogen was purified by three cycles of assembly and disassembly of calf brain microtubules (60), chromatography on DEAE Sephadex A-50 (59) and preparative SDS PAGE. The tubulin band was homogenized in phosphate-buffered saline (PBS) and emulsified with Freund's Complete Adjuvant. The animal was injected at four subcutaneous sites with ~700 µg of tubulin on each of eight occasions spaced 1–2 wk apart. A terminal bleed was performed 1 wk after the final injection of tubulin.

The following were purchased from Cappel Laboratories (Cochranville, PA): rhodamine-conjugated IgG fractions of sheep and goat anti-rabbit IgG, fluorescein-conjugated IgG fraction of goat anti-guinea pig IgG, and sheep antiserum to goat IgG. The IgG fraction of this antiserum was labeled with fluorescein as described (19) to an average molar ratio of fluorescein:IgG of 2.6. Fluorescein-labeled goat anti-mouse IgG was acquired from Meloy Laboratories (Springfield, VA).

Primary Rat Brain Cultures: Features of several published protocols (8, 66, 67) were adapted to produce cultures with optimal survival of cells found to contain MAP 2. Whole brains from rats varying in age from 16-d fetuses to 1-d postnatal were used. The cultures were grown on glass coverslips in 35-mm petri dishes. The coverslips were coated with a 1:3 mixture of 1 mg/ml poly-L-lysine (Sigma Chemical Co., St. Louis, MO; Type VII-B, 35,000 mol wt): fetal

calf serum. Each brain yielded enough cells for six 22-mm² coverslips.

Freshly dissected brains were rinsed twice in Hanks' balanced salt solution (MA Biologicals, Walkersville, MD) and minced as finely as possible with a scalpel in 2 ml of 0.25% trypsin (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY). An additional 8 ml of trypsin solution was then added, and the minced tissue was triturated gently five times with a 10-ml pipette. This suspension was stirred at 37°C for 15 min, at which time 10 ml of high serum medium was added (43% each F-12 and Dulbecco's MEM, 8% horse serum, 5% fetal calf serum, penicillin, streptomycin; all from Gibco Laboratories). The suspension of single cells and small clumps of tissue was triturated gently another five times, and was centrifuged for 5 min at 1,500 g. The pellet was resuspended in high serum medium by gentle trituration, and 2.5 ml of cell suspension was added to each coverslip. The dishes were then placed in a 37°C-incubator containing an atmosphere of 95% air, 5% CO₂. After 2 h, the medium was aspirated and each dish was fed with 2.5 ml of N2 defined medium (7) supplemented with 2% fetal calf serum, penicillin, and streptomycin. The cultures were ready for use within 3 or 4 d, and could be maintained for several weeks if fed every 3 or 4 d with the N2-based medium.

Indirect Immunofluorescence Microscopy: Cells to be stained for cytoskeletal proteins were processed in the following manner. Coverslips were rinsed twice for 30 s with PBS at 37°C. A 5-min methanol fixation at –20°C was performed either immediately following the PBS rinses, or after a subsequent 2-min extraction of the cells in PEM buffer (100 mM PIPES, 1 mM EGTA, 1 mM MgSO₄) containing 0.005% digitonin (Sigma Chemical Co.). The coverslips were rehydrated with PBS and incubated with 10% nonspecific serum obtained from the same animal species as the fluorescent antibodies. 10% serum was also included in subsequent antibody incubations. All antibodies were diluted in PBS, and were applied to the coverslips for 30 min each at 37°C in humidified chambers. Rabbit anti-MAP 2 serum was used at a final concentration of 1.0–1.3%. Affinity-purified rabbit anti-MAP 2 was used at 8–16 µg/ml. For some experiments, rabbit anti-MAP 2 was used in combination with goat or guinea pig antitubulin, or with monoclonal mouse antibodies to vimentin or MAP 2. Fluorescent anti-IgG antibodies were used alone or in various combinations, as indicated in the figure legends. Thorough PBS washes were performed after each antibody step. After the final PBS wash, the coverslips were rinsed with distilled water and sealed to slides with elvanol (22).

For the tetanus toxin experiment, live cells were incubated for 30-min periods at 37°C in three changes of medium containing, sequentially, 10 µg/ml tetanus toxin, goat anti-tetanus toxin, and fluorescein-sheep anti-goat IgG. A brief PBS rinse followed each step. The cells were then fixed in methanol, and stained with rabbit anti-MAP 2 and rhodamine-sheep anti-rabbit IgG, as described above.

We observed slides on a Zeiss IM-35 inverted microscope equipped with an epifluorescent illuminator, fluorescein and rhodamine filters, and the following Zeiss oil immersion objectives: 40× phase 3 NA 1.0 Apochromat, 63× phase 3 NA 1.4 Planapochromat.

Antibody Staining of Nitrocellulose Transfers: Electrophoresis of protein samples was performed according to the Laemmli (32) method on 1.5-mm thick 7% polyacrylamide gels. Electrophoretic transfer of proteins was performed by the technique of Towbin et al. (56) modified as follows for efficient transfer of high molecular weight polypeptides. After electrophoresis, the gels were soaked for 30 min in 0.125 M Tris, pH 6.8, 0.1% SDS, 1 mM EDTA, 10 mM 2-mercaptoethanol and then transferred to nitrocellulose sheets in 0.0125 M Tris, pH 8.5, 0.096 M glycine, 0.01% SDS, 10 mM 2-mercaptoethanol. The transfer was performed for 16 h at room temperature, with a noncommercial transfer apparatus and a Pharmacia DPS destainer power supply (Pharmacia Fine Chemicals, Piscataway, NJ) at a constant 36 V and a current that varied from 1 to 3 A. This resulted in at least 90% transfer of MAP 2.

Following transfer, the nitrocellulose sheet was incubated for 30 min in staining buffer (0.05 M Tris, pH 7.4, 0.15 M NaCl, 0.05% Nonidet P-40, 0.25% bovine serum albumin–Sigma Fraction V). The paper was then stained for 3 h each with rabbit anti-MAP 2 followed by rhodamine-goat anti-rabbit IgG. Antibodies were diluted in staining buffer, which was used to wash the paper thoroughly after each antibody step. Fully dried nitrocellulose sheets were illuminated in a darkened room with a pair of ultraviolet lamps (Model UVL-21; Ultra-Violet Products, Inc., San Gabriel, CA), and photographs were taken through a yellow Wratten No. 15 gelatin filter (Eastman Kodak) with Polaroid Type 55 P/N film.

RESULTS

Characterization of Anti-MAP 2

Partial characterization of our anti-MAP 2 antibody has been reported elsewhere (35; Miller, P., W. E. Theurkauf, R. B. Vallee, and P. De Camilli, manuscript in preparation). The antibody stained neurons in sections of mature rat brain, and showed particularly bright staining of neuronal dendrites. The

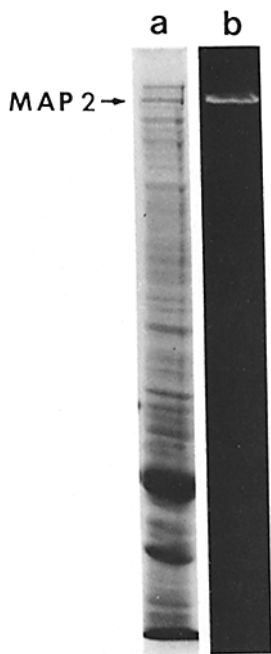


FIGURE 1 Immunoblot detection of MAP 2 in newborn rat brain. A brain from a newborn rat was completely dissolved by homogenization in hot SDS PAGE sample buffer and subjected to electrophoresis. (Lane A) Coomassie Brilliant Blue R250. (Lane B) A nitrocellulose replica of the same sample stained with rabbit anti-MAP 2 followed by rhodamine goat anti-rabbit IgG.

antibody specifically stained a protein of the molecular weight of MAP 2 in whole brain, purified microtubules, and purified preparations of MAP 2. For the present study, we examined newborn rat brain tissue by immunocytochemical means (Fig. 1). It may be observed that only a band at the position of MAP 2 reacts with the anti-MAP 2 antiserum.

Immunofluorescent Detection of MAP 2 in Primary Cultures of Rat Brain Cells

The primary cultures of rat brain cells used in this study contained a variety of morphologically distinct cells. When these cultures were reacted with anti-MAP 2 antibody, only selected cells were stained. Fig. 2*a* and *b* illustrate the most common MAP 2-positive cells. These cells had a neuronal morphology, with small soma (10–25 μm diameter) and numerous fine processes that occasionally extended for $>100 \mu\text{m}$. The processes frequently exhibited periodic varicosities. In favorable images, distinct fluorescent fibrils were visible in large, neuronlike cells (Fig. 2*c*). Almost all MAP 2-positive neuronlike cells were found to possess tetanus toxin receptors (not shown) and were probably neurons, therefore (16, 36). In addition to containing cells of neuronal morphology, the cultures also contained an abundance of flat, well-spread cells, some of which stained with anti-MAP 2 (Fig. 2*d*). The staining of such cells was always found on networks of gently curving cytoplasmic fibrils, whose appearance resembled microtubules or 10-nm filaments. These cells were negative for tetanus toxin receptors (not shown), and their identity is not known at present. We observed both MAP 2-positive cell types whether unfractionated anti-MAP 2 antiserum, affinity-purified anti-MAP 2, or monoclonal anti-MAP 2 was used. Examples of such staining may be observed throughout this paper (Figs. 2–8). No specific staining of either the neurons or flat cells was detected with antiserum preadsorbed with the purified MAP 2 that was used as the immunogen, or with preimmune serum (not shown).

Co-localization of MAP 2 and Tubulin

The MAP 2-positive flat cells provided an excellent experi-

mental system for high-resolution, immunofluorescent location of MAP 2, which was not possible with the more compact neurons. Therefore, our observations focused on the flat subpopulation of cells, as described in the remainder of the paper. To determine whether MAP 2 co-localized with microtubules, the cultures were stained simultaneously with anti-MAP 2 and antitubulin. An example of a flat, MAP 2-positive cell stained also with antitubulin is shown in Fig. 3*a* and *b*. Extensive co-localization of the two antigens on cytoplasmic fibers was observed. Dividing MAP 2-positive cells were occasionally found. We observed co-localization of MAP 2 and tubulin throughout mitosis and cytokinesis (Fig. 3*c–f*).

Uncoupling of MAP 2–Tubulin Co-Localization

The anti-MAP 2 staining patterns shown in Fig. 3 are characteristic of microtubules. To determine the cellular location of MAP 2 in the absence of microtubules, we employed two approaches. First, the cultures were exposed to vinblastine sulfate for several hours prior to fixation and antibody staining. Antitubulin staining (not shown) revealed the absence of microtubules in the flat cells, and the appearance of brightly staining tubulin paracrystals, as is characteristic of cells exposed to this drug. Thin-section electron microscopy of these cultures similarly revealed the complete absence of microtubules (not shown). Fig. 4 shows an example of a flat cell that showed positive staining with anti-MAP 2. The single, striking paracrystal in this cell was readily detected by phase-contrast microscopy (Fig. 4*a*). However, the antibody conspicuously failed to stain the paracrystal. Instead, a sinuous cable of bright fluorescence surrounding the nucleus was observed (Fig. 4*b*). This pattern of staining was clearly not consistent with that found for tubulin. Rather, it was consistent with that expected for certain classes of intermediate filaments. An intermediate filamentlike distribution of MAP 2 was also observed in cells exposed to colchicine (not shown), and was apparent with vinblastine or colchicine incubations ranging from 1 to 24 h. Thus, our results appeared to reveal a second binding site for MAP 2 in cells, one associated with intermediate filaments.

As an independent approach to eliminate microtubules, the primary brain cells were extracted with digitonin under conditions known to result in the disassembly of microtubules. The cells were then fixed and double-stained for tubulin and MAP 2, as shown in Fig. 5. MAP 2 and tubulin co-localized on cytoplasmic fibrils in cells extracted with PIPES buffer (Fig. 5*a* and *b*), which preserves microtubules. Both phosphate buffers (Fig. 5*f* and *h*) and PIPES buffer with 10 mM Ca^{++} (Fig. 5*d*) dissolved microtubules, as judged by antitubulin staining. However, the same cells still exhibited a fibrillar pattern of staining with anti-MAP 2 (Fig. 5*c*, *e*, and *g*), indicating the existence of a nonmicrotubule binding site for MAP 2 in these cells. The fibrillar nature of the staining pattern and its stability in the extracted cells is again consistent with localization of MAP 2 on intermediate filaments.

Co-localization of MAP 2 and Vimentin

To determine directly whether MAP 2 co-localized with intermediate filaments, cells were double-stained with antibodies to vimentin and MAP 2 (Fig. 6). Extensive co-localization of MAP 2 and vimentin on cytoplasmic fibrils was observed. In vinblastine-treated cultures (Fig. 7), we again observed co-localization of vimentin and MAP 2, but now on cables characteristic of intermediate filaments in cells exposed to the drug.

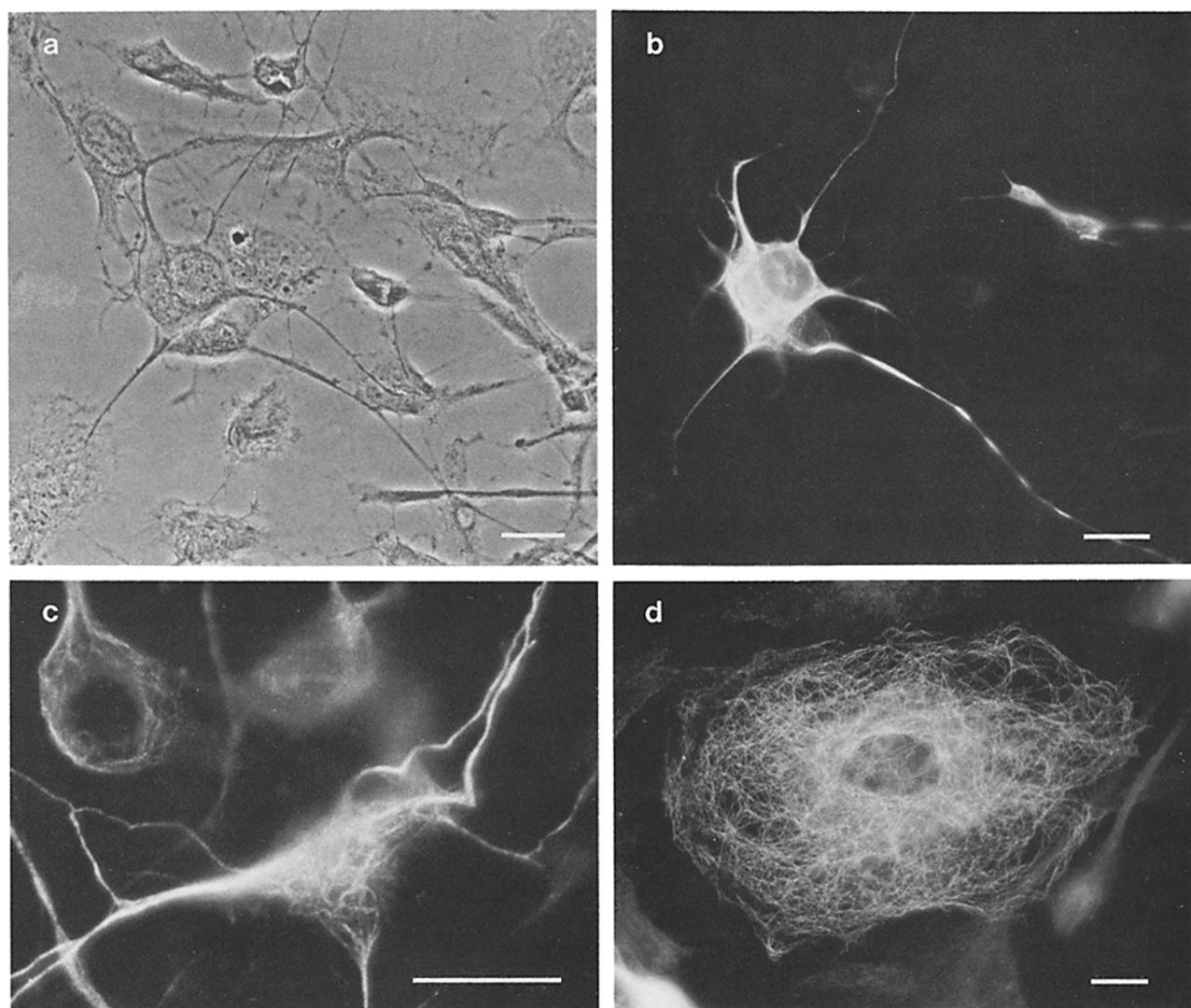


FIGURE 2 Immunofluorescent detection of MAP 2 in two morphological classes of primary newborn rat brain cells. Cells were stained with affinity purified anti-MAP 2 followed by rhodamine-sheep anti-rabbit IgG. (a) Phase-contrast image of a typical field of cells. (b) Corresponding immunofluorescent image revealing presence of MAP 2 in cells of neuronal morphology. (c) Cells of neuronal morphology showing anti-MAP 2 staining of distinct cytoplasmic fibers. (d) Flat cell of nonneuronal morphology showing anti-MAP 2 staining on cytoplasmic fibers. Bars, 10 μm . (a and b) \times 1,000. (c) \times 2,100. (d) \times 800.

Comparison of Polyclonal and Monoclonal Anti-MAP 2

To determine whether the results obtained were unique to our anti-MAP 2 antibody preparation, vinblastine-treated cells were double-labeled with our polyclonal rabbit antibody and a monoclonal antibody prepared by Izant and McIntosh (27). The results of this comparison are shown in Fig. 8. It is clear that both antibody preparations stained intermediate filament cables, but not tubulin paracrystals, in the flat primary brain cells.

DISCUSSION

MAP 2 has been classified as a MAP primarily on the basis of its affinity for microtubules *in vitro* (4, 38, 53). In addition, antibodies to this protein have been found to stain cytoplasmic fibers in a limited number of cultured cell types (27, 31, 41, 57). The conclusion that these fibers were microtubules was based on co-localization of the immunoreactive species with

tubulin (27) and abolition of the fibrous staining pattern by antimicrotubule drugs (27, 31). Here we report that in cultured brain cells an anti-MAP 2 antibody stained fibrils that colocalized with both microtubules (see Fig. 3) and intermediate filaments (see Fig. 6). The intermediate filamentlike staining pattern persisted after dissolution of microtubules in living cells by antimicrotubule drugs (see Figs. 4, 7, and 8), or in cytoskeletal preparations by appropriate buffers (see Fig. 5). These results indicated that material cross-reactive with MAP 2 may be associated with intermediate filaments, as well as with microtubules.

Specificity of the Anti-MAP 2 Antibody

It is possible that these results simply reflect the presence in our anti-MAP 2 preparation of contaminating antibodies specific for an intermediate filament protein. We consider this quite unlikely since both affinity purified anti-MAP 2 (see Fig. 4b) and an independently prepared monoclonal anti-MAP 2 (see Fig. 8b) stained intermediate filaments. In addition, nei-

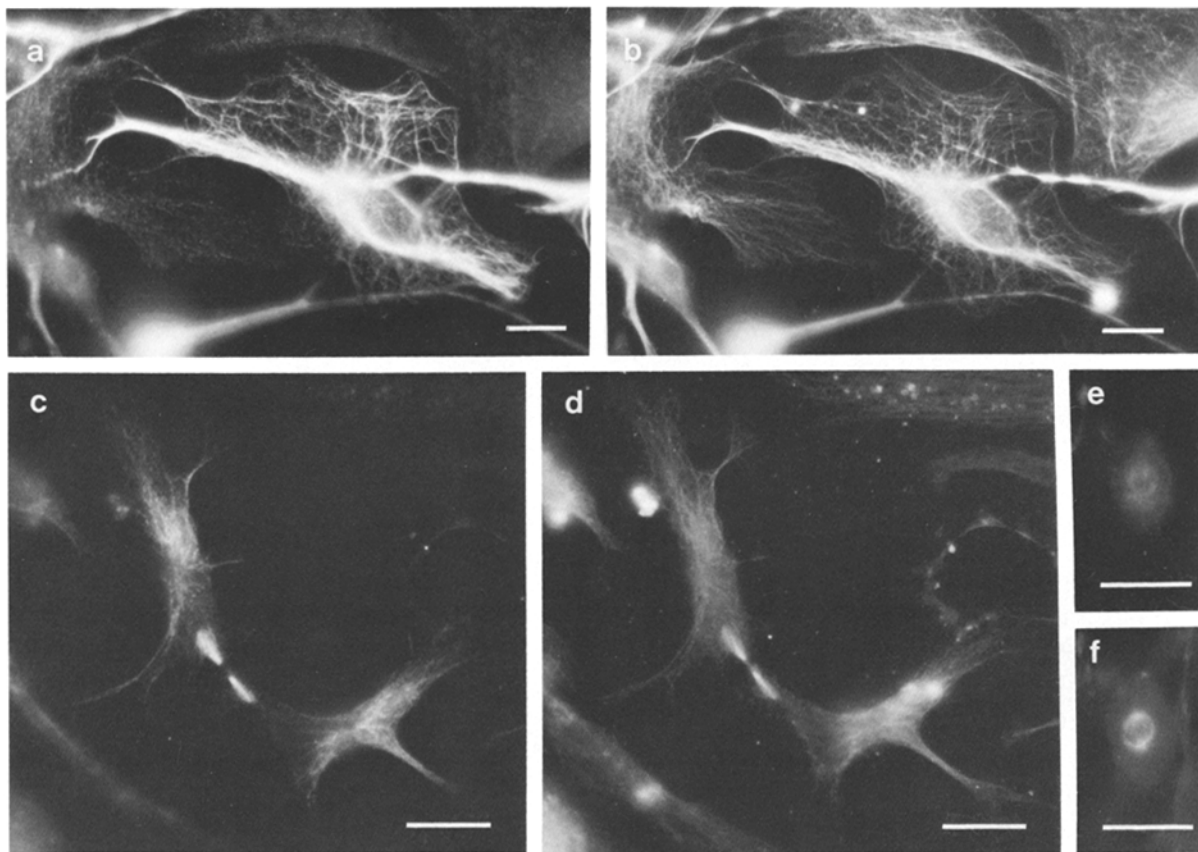


FIGURE 3 Co-localization of MAP 2 and tubulin. Flat, MAP 2-positive cells are shown in interphase (*a* and *b*), telophase (*c* and *d*), and metaphase (*e* and *f*). Cells were incubated with rabbit anti-MAP 2 serum plus either goat (*b* and *f*) or guinea pig (*d*) antiserum to tubulin. Primary antibodies were then stained simultaneously with either rhodamine-sheep anti-rabbit IgG plus fluorescein-sheep anti-goat IgG, or with rhodamine-goat anti-rabbit IgG plus fluorescein-goat anti-guinea pig IgG. (*a*, *c*, and *e*) anti-MAP 2; (*b*, *d*, and *f*) antitubulin. Bars, 10 μm . (*a* and *b*) $\times 800$. (*c* and *d*) $\times 1,100$. (*e* and *f*) $\times 1,200$.

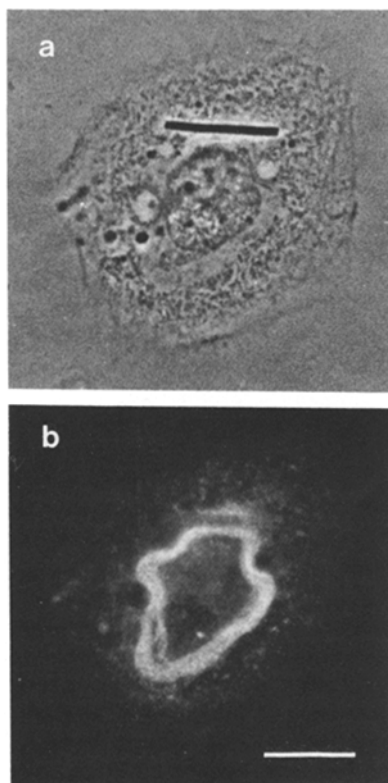


FIGURE 4 Effect of vinblastine on MAP 2 staining. A primary brain cell culture was treated with 10 μM vinblastine sulfate for 15 h before being stained with affinity purified anti-MAP 2 and rhodamine sheep-anti-rabbit IgG. (*a*) Phase contrast. (*b*) Anti-MAP 2. Immediately above the nucleus is a prominent tubulin paracrystal easily visualized by phase contrast, but not stained by the antibody. Bar, 10 μm . $\times 1,300$.

ther immune serum adsorbed prior to use with pure MAP 2, nor preimmune serum-stained cytoplasmic filaments.

This indicates that the intermediate filament-associated antigen recognized by anti-MAP 2 is truly cross-reactive with MAP 2. Nonetheless, it is possible that this antigen represents a protein quite distinct from MAP 2, but which is immunologically cross-reactive due to limited amino acid sequence homology. Several examples of distinct, but immunologically cross-reactive cytoskeletal proteins have been reported (3, 5, 39, 42). Indeed, it has been found that MAP 2 itself reacts with an antispectrin antibody (15).

Thus, it is possible that we have identified an intermediate filament protein which shares one or more antigenic sites with MAP 2, but is otherwise distinct. However, several considerations lead us to believe that MAP 2 itself is the antigen associated with intermediate filaments. First, the only antigenic species detected by our antibody in a mixture of total newborn rat brain proteins has a mobility in SDS PAGE identical to that of MAP 2 (see Fig. 1). Second, the antibody stained only a limited number of the flat cells in these cultures (10% or less). Among these cells, unambiguous staining of microtubules was observed during mitosis, while intermediate filaments were clearly stained after vinblastine treatment. Thus, if two distinct, cross-reactive proteins exist in primary brain cells, both would be present in the same small subpopulation of flat cells. While such a coincidence is conceivable, it seems highly unlikely. Finally, we also observed co-localization of MAP 2 with both

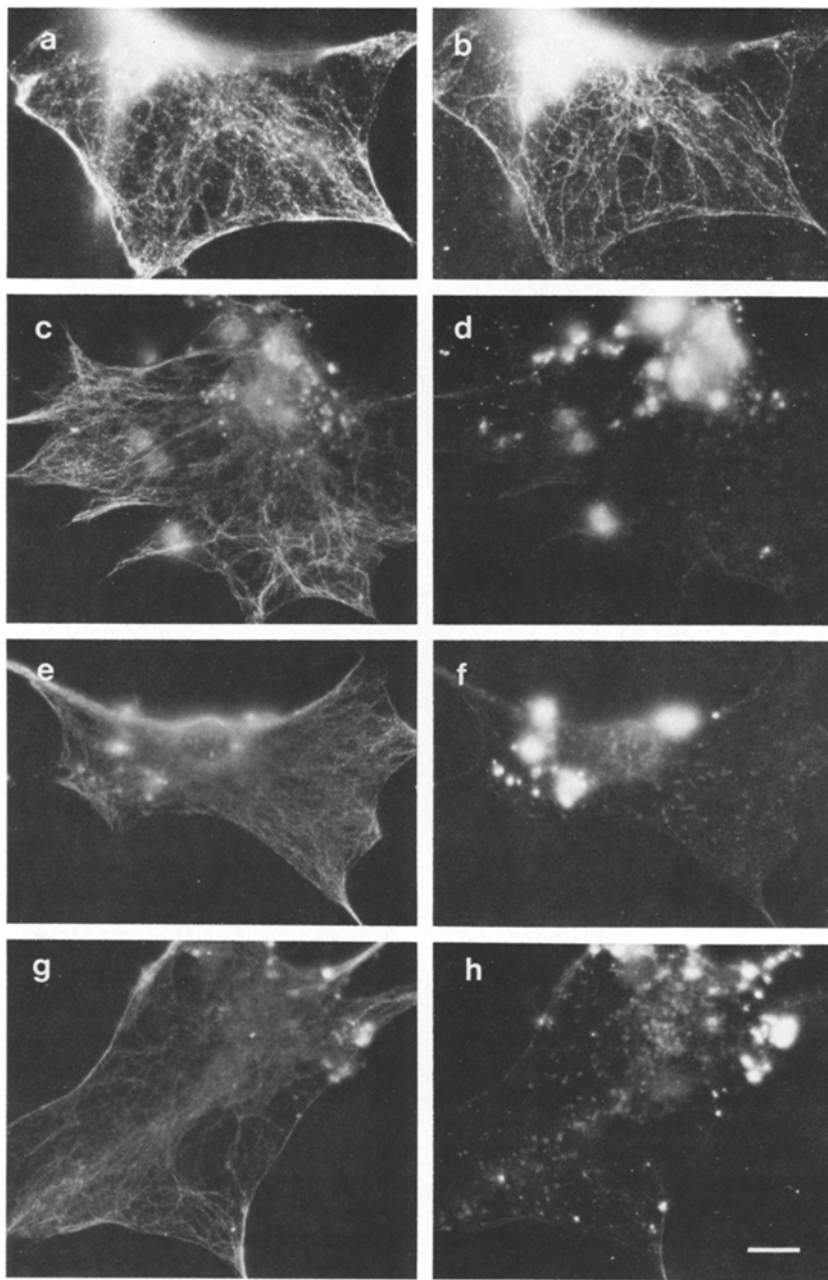


FIGURE 5 Presence of MAP 2 on cytoplasmic fibers in isolated cytoskeletons lacking microtubules. Cells were extracted with digitonin as described in Materials and Methods, incubated for 15 min at room temperature in various buffers, fixed, and treated with rabbit anti-MAP 2 serum and guinea pig antitubulin, followed by rhodamine goat anti-rabbit IgG and fluorescein-goat anti-guinea pig IgG. (a, c, e, and g) Anti-MAP 2 staining; (b, d, f, and h) Antitubulin staining. (a and b) control buffer: 100 mM PIPES, pH 6.6, 1 mM MgSO₄ and 1 mM EGTA; (c and d), the same, plus 10 mM CaCl₂; (e and f) 100 mM phosphate buffer at pH 7; (g and h), 100 mM phosphate buffer at pH 9. Bar, 10 μ m. \times 700.

microtubules and intermediate filaments in the B104 neuroblastoma cell line. In this homogeneous population of cells, only a single protein of *M*, 270,000 reacted with anti-MAP 2, further supporting our contention that a single protein species binds to both microtubules and intermediate filaments (G. S. Bloom and R. B. Vallee, unpublished observations).

Although other laboratories have used anti-MAP 2 antibodies for immunofluorescence microscopy on cultured cells (27, 31, 41, 57), the association of MAP 2 with intermediate filaments has not been reported until now. Detection of this association may depend on the use of an appropriate combination of cultured cell system and experimental protocols. Indeed, we demonstrate here that a monoclonal anti-MAP 2 antibody (27) independently prepared in another laboratory can stain intermediate filaments in our vinblastine-treated primary brain cells (see Fig. 8).

Intracellular Functions of MAP 2

Immunocytochemical, electron microscopic, and physiological evidence (2, 17, 21, 26, 33, 44, 47) have pointed to an association between microtubules and intermediate filaments. In particular, double immunofluorescence microscopy has revealed extensive co-localization of microtubules with desmin filaments in chicken gizzard cells (21), with vimentin filaments in fibroblasts (2) and with glial filaments in primary astrocytes (G. S. Bloom, R. B. Vallee, and R. Liem, unpublished observations). These findings suggest that microtubules and intermediate filaments may be commonly connected to one another in cells, presumably by cross-linking proteins.

Recently, Wiche and co-workers (43, 64) proposed that a high molecular weight MAP-like protein cross-links microtubules and intermediate filaments in numerous varieties of

cultured cells. However, subsequent studies in the same laboratory revealed that the protein, now known as "plectin," has an intracellular distribution that is markedly distinct from microtubules and intermediate filaments (65). Therefore, we feel it is unlikely that plectin acts as a cross-linker. In contrast, our finding that MAP 2 can co-localize with both microtubules and intermediate filaments indicates that this protein, in particular, is responsible for the observed interactions of the two classes of cytoplasmic fibers. Since MAP 2 may not be universally distributed in cells (27, 41, 57), it is possible that other unidentified proteins also serve this function.

Although MAP 2 was found to be co-distributed with vimentin, we do not know whether vimentin is the only intermediate filament protein in the MAP 2-positive, flat brain cells. For this reason, we are not yet certain whether MAP 2 binds directly to vimentin itself, or to another intermediate filament protein with a similar intracellular distribution. Further biochemical work will be required to determine the specific intermediate filament proteins that are able to bind to MAP 2, and to define the relevant binding domains on MAP 2 (cf. reference 58).

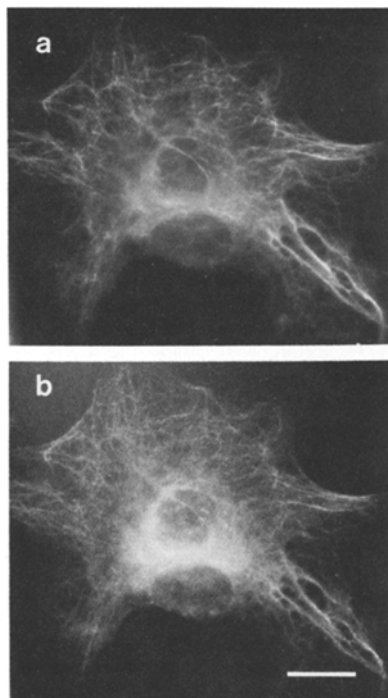


FIGURE 6 Co-localization of MAP 2 and vimentin. Cells were stained with rabbit anti-MAP 2 serum and monoclonal mouse antivimentin, followed by rhodamine goat anti-rabbit IgG and fluorescein-goat anti-mouse IgG. (a) Anti-MAP 2 staining; (b) Antivimentin staining. Bar, 10 μ m. \times 900.

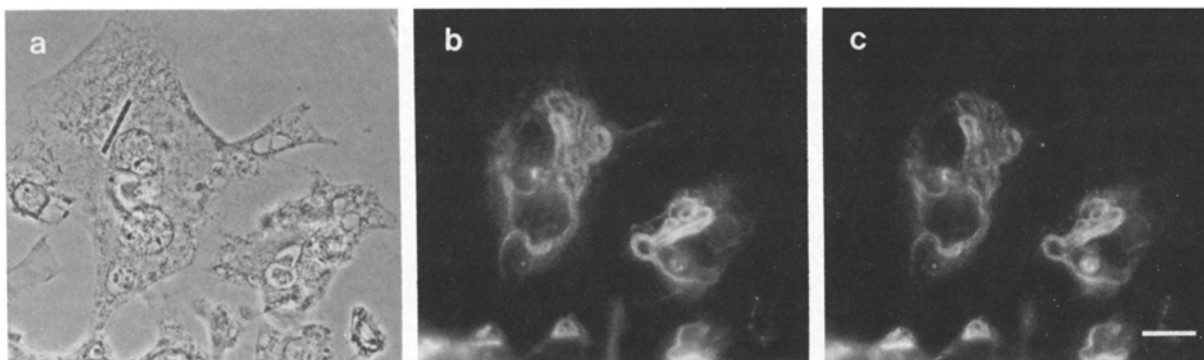


FIGURE 7 Co-localization of MAP 2 and vimentin on perinuclear cables in vinblastine-treated cells. Cells were treated for 17 h with 10 μ M vinblastine sulfate. They were then stained with rabbit antiserum to MAP 2 and monoclonal mouse antivimentin, followed by rhodamine goat anti-rabbit IgG and fluorescein goat anti-mouse IgG. (a) Phase contrast. (b) Anti-MAP 2. (c) Antivimentin. Bar, 10 μ m. \times 800.

Our data represent the first demonstration that one role for MAP 2 in cells is to mediate interactions of microtubules and intermediate filaments. Whether this is the only cellular function of MAP 2 is not clear, since the protein is most abundant *in vivo* in neuronal dendrites (35; Miller, P., W. E. Theurkauf, R. B. Vallee, and P. De Camilli, manuscript in preparation), which contain abundant microtubules, but few intermediate

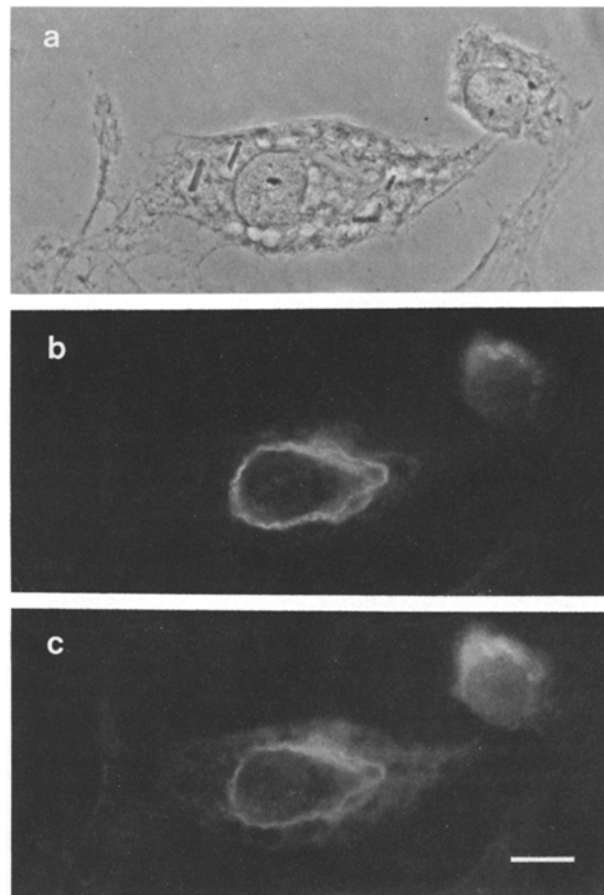


FIGURE 8 Detection of MAP 2 on intermediate-filament cables in vinblastine-treated cells by polyclonal and monoclonal antibodies. Cells were treated for 18 h with 10 μ M vinblastine sulfate before being stained with rabbit anti-MAP 2 serum and monoclonal mouse anti-MAP 2, followed by a combination of goat anti-rabbit IgG and fluorescein goat anti-mouse IgG. (a) Phase contrast; (b) Rabbit anti-MAP 2 serum; (c) Monoclonal mouse anti-MAP 2 antibody. Bar, 10 μ m. \times 900.

filaments. We suggest, therefore, that cross-linking of microtubules and intermediate filaments may be only one role of the protein, and that other cellular functions remain to be defined. Recent work by Izant and co-workers (28, 29) has suggested that the 210,000-dalton MAP identified in HeLa cells (9, 62) may be heterogeneous with regard to cellular distribution and function. Similarly, MAP 2 may be a complex protein species, individual components of which bind to a variety of cellular organelles. Indeed, the size heterogeneity of MAP 2 in SDS PAGE (30) may signify functional heterogeneity as well. It is also possible that the binding specificity of MAP 2 is regulated by posttranslational modification. For example, phosphorylation of MAP 2 by its associated cAMP-dependent protein kinase (55, 60) or by other protein kinases could potentially regulate whether MAP 2 binds to intermediate filaments or to other structures.

We would like to thank Jonathan Izant and Richard McIntosh for the monoclonal mouse anti-MAP 2, Robert Wehling for the goat anti-tubulin, and William Habig for the goat antitetanus toxin. We also express our thanks to Frank Luca for his technical assistance.

This work was supported by National Institutes of Health research grant GM-26701 to Richard B. Vallee.

Received for publication 9 November 1982, and in revised form 4 February 1983.

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