# Immunoelectron Microscopic Localization of the 210,000-Mol Wt Microtubule-associated Protein in Cultured Cells of Primates

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ABSTRACT Results from ultrastructural immunocytochemistry on glutaraldehyde-fixed cells confirmed and extended findings previously obtained with immunofluorescence. A microtubule-associated protein (MAP) of 210,000 molecular weight was shown to be specifically associated with all cytoplasmic and mitotic microtubules along their entire length in primate cells. Specific labeling with the anti-MAP antibody could not be detected on any other subcellular structures, notably the centrosomes, kinetochores, microfilaments, and intermediate filaments. Treatment with the microtubule-disrupting drug, nocodazole, induced diffusion of the MAP throughout the cytoplasm. During repolymerization of microtubules following disassembly by nocodazole, the association of the MAP with the microtubules was immediate and complete. When cells were treated with vinblastine, the tubulin paracrystals formed were heavily stained by the antibody. Neither sodium azide nor taxol affected the association of the MAP with microtubules.

Soon after the introduction of methods enabling the assembly of microtubules in vitro, it became apparent that a number of nontubulin proteins copolymerize with tubulin and alter the kinetics of the reaction. These nontubulin microtubule-associated proteins have been referred to as MAPs.

Two classes of MAPs in particular have been isolated from vertebrate brain tissue and characterized in some detail. One class includes species of high molecular weight and is resolved on SDS polyacrylamide gels as a band of 345,000 molecular weight (MAP 1) and a doublet of 286,000 and 271,000 molecular weight (MAP 2) (19, 26). The other class of MAPs is resolved on gels as four bands with mobilities corresponding to molecular weights of 55,000–62,000, and has been referred to as tau (33).

Recently, two MAPs of molecular weights 125,000 and 210,000 were isolated from HeLa cells and characterized in terms of their molecular properties and interactions with tubulin (1, 2, 31). Both were shown to copurify with HeLa tubulin and to stimulate the assembly of pure HeLa or porcine brain tubulin.

Several groups (5, 16, 29) have suggested that the association of MAPs with microtubules in vitro may be an artifact and not indicative of an association in vivo. However, a number of indirect immunofluorescence studies have identified material immunologically related to the high molecular weight MAPs (7, 9, 15, 21–25, 27) and to tau (6, 9) to be distributed along microtubules in a variety of cell types. Although there are some discrepancies in the distribution patterns reported in the above studies (see references 4, 15 for discussion), nevertheless they do indicate that the association of MAPs with tubulin in vitro is probably not an artifact.

In similar experiments using antisera of well-defined specificity, material immunologically related to the HeLa MAPs (both 125,000 and 210,000) was shown to be associated with Colcemid-sensitive fibers in the cytoplasm and mitotic spindles in HeLa cells. The 210,000 molecular weight species (210,000 MAP) was studied more intensively and cross-reactive material identified in a wide range of primate cell types was shown to be of the same molecular weight (3, 4).

These results have provided highly suggestive evidence for the association of these MAPs with microtubules in living cells. However, the limits of resolution of the light microscope and the inferior preservation of cell structure afforded by the conventional procedures used in immunofluorescence did not permit a definitive demonstration of this association.

We used here ultrastructural immunocytochemical proce-



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dures on glutaraldehyde-fixed cells to localize the 210,000 HeLa MAP at the electron microscopic level. With this approach we hoped to obtain more precise answers to the following questions: (*a*) Is the 210,000 MAP indeed intimately associated with microtubules in adequately fixed cells? (*b*) Do all microtubules contain the MAP or does a population of MAP-negative microtubules exist? (*c*) Is the MAP associated with other structures such as microfilaments or intermediate filaments? (*d*) Is the MAP an integral component of microtubule organizing sites such as centrosomes and kinetochores? (*e*) How is the MAP distributed in cells after treatment with drugs which affect microtubules (nocodazole, vinblastine, sodium azide, taxol)? (*f*) Is the MAP incorporated simultaneously with tubulin in microtubules reassembling in vivo or does it associate with the microtubules after a certain lag period?

## MATERIALS AND METHODS

Human embryonal fibroblasts (NS), HeLa cells, African Green Monkey kidney cells (VERO), C3H mouse 3T3 cells (MO), and PtK-2 cells were cultured in Eagle's minimum essential medium, supplemented with nonessential amino acids and 10% fetal bovine serum, at 36°C in a humidified atmosphere of 5% $CO_2$. For the experiments the cells were trypsinized and seeded onto sterile glass cover slips for light microscopy or in plastic petri dishes for electron microscopy. The cells were used 24–48 h after seeding. The fixation for immunocytochemical staining was done according to a modification of previously published procedures (10, 12). The cells were fixed in 1% glutaraldehyde in PIPES buffer (28) for 10 min at room temperature. Permeabilization was done at room temperature for 30 min in 50% ethanol in PIPES buffer containing 0.5 mg/ml $NaBH_4$ to reduce free aldehyde groups (32). Then the cells were transferred to Tris-buffered saline that was used throughout the antibody labeling procedure. For peroxidase staining, the peroxidase anti-peroxidase (PAP) method of Sternberger (30) was used as described previously (10, 12). Ferritin labeling was done by replacing the PAP complex with an affinity-purified antibody to horse spleen ferritin followed by incubation with ferritin (500 μg/ml, 1 h). After the immunocytochemical staining, the cells were counterstained with toluidine blue and mounted for light microscopy or embedded for electron microscopy as described (10). The ferritin-labeled preparations were viewed in a Philips 300 electron microscope at 40 kV with or without staining with uranyl acetate and lead citrate.

The anti-210,000 mol wt HeLa MAP antibody was characterized previously (3). It was used at dilutions between 1/50–1/10,000. Controls consisted of using 1% solution of normal goat serum, or the 210,000 antibody adsorbed onto the purified 210,000 MAP linked to polyacrylamide gel. The adsorption was done as follows: acrylamide pieces containing 210,000 MAP, tubulin, or no protein were excised from 6.25% SDS polyacrylamide gels which had been stained with Coomassie Brilliant Blue (3). The pieces were crushed with a glass rod and incubated with 210,000 MAP antiserum for 1 h at 37°C. For each 1 ml of a one-fifth dilution of antiserum, pieces containing 20 μg of 210,000 MAP, 100 μg of tubulin or an equal wet weight of acrylamide devoid of protein were used. Acrylamide pieces were removed from the preabsorbed serum by centrifuging at 1,000 *g* for 5 min through a 2-ml surety column (Evergreen Scientific, Los Angeles, Calif.) attached to a 2-ml sample cup (Evergreen Scientific) by a homemade teflon adapter.

Immunoelectron microscope staining was carried out on untreated cultures, cultures treated with sodium azide ($10^{-2}$ M, 30 min–4 h), vinblastine sufate (10 μg/ml, 24 h), taxol ($10^{-5}$ M, 24 h; gift from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute [20]), or nocodazole (1 μg/ml, 24 h), and cultures fixed at various times (10–40 min) after release from nocodazole.

## RESULTS

### *Light Microscopy*

Light microscopic observation of cells stained with the PAP procedure essentially confirmed previous data obtained with indirect immunofluorescence (3, 4). In all cells of primate origin that were investigated, including NS cells, VERO cells, and HeLa cells, a cytoplasmic network focusing on the cell center was stained in interphase cells, and the spindles were stained in mitotic cells. For both interphase and mitotic cells, the patterns observed were indistinguishable from those obtained with antibody to tubulin (10, 11). The staining was optimal with 1/1,200–1/2,500 dilutions of the serum. The exceptionally high concentration of antibodies in the antisera and the documented lack of cross-reactivity of the antibodies with other cellular proteins (3) are probably the main reasons why these antisera produce highly specific staining in combination with the sensitive PAP technique without the necessity of further purification by affinity chromatography. In view of the high sensitivity of the PAP method (30), preabsorption control experiments were performed over a wide range of antiserum dilutions. When the antiserum had been adsorbed onto purified HeLa MAP (see Material and Methods), no staining at all was seen at the 1/600–1/1,200 dilutions. Lower dilutions (1/150–1/300) produced only diffuse background label, probably representing nonspecific adsorption of unrelated immunoglobulins (Fig. 1 *a*). Similar adsorption onto the carrier (polyacrylamide) or onto purified HeLa tubulin did not affect the capacity of the serum to stain microtubule networks even at the higher dilutions (1/1,200).

Fibrous staining could not be obtained in nonprimate cells (C3H mouse 3T3 cells [MO], or rat kangaroo [PtK-2]). Low dilutions (1/50) of the 210,000 MAP antiserum produced only diffuse label. To demonstrate more clearly the lack of cross-reactive material in nonprimate cells, mixtures of primate and nonprimate cells were seeded onto the same cover slips and stained. Fig. 1 *b* shows a mixed culture of NS cells and PtK-2 cells. The primate cells, distinguished by their larger size and definitive morphology, are clearly stained, whereas the PtK-2 cells are not. An advantage of the procedure is that the positive result obtained with the primate cells serves as an internal control for the negative result obtained with the nonprimate cells.

Immunocytochemical staining of drug-treated cells was also examined using the PAP staining technique and light microscopy. Fig. 2 shows the staining patterns observed when NS cells were treated with nocodazole (Fig. 2 *a*) and vinblastine (Fig. 2 *B*). Nocodazole has clearly induced the depolymerization of all the microtubules, while vinblastine has induced the formation of tubulin- and MAP-containing paracrystals.

### *Electron Microscopy*

Fig. 3 shows an electron micrograph of a thin section through an interphase cell incubated with the anti-MAP antibody and stained with the PAP procedure. The interphase microtubules were heavily covered with the peroxidase reaction product, and similar results were obtained with all primate cells examined (NS, HeLa, and VERO). The label coated the microtubules with an irregular, globular layer typical of the PAP complex and obscured to a great extent the underlying structure (Fig. 4). This staining was seen with serum dilutions as high as 1/1,250–1/2,500. When the PAP complex was replaced by an affinity-purified antibody to horse spleen ferritin followed by incubation with ferritin, microtubules were again consistently labeled (Fig. 5). The ferritin bridge procedure does not involve an enzymatic reaction, so there is no accompanying deposition of reaction product on nearby structures. Consequently, the ultrastructural preservation of the labeled microtubules can more accurately be judged. Fig. 5 shows that the ferritin label is associated with the microtubules in a discontinuous manner. Continuous labeling could not be achieved by decreasing the antiserum dilution to 1/50. In contrast, labeling of uniform distribution could be obtained with an antitubulin antibody. The discontinuous labeling may reflect a periodic association

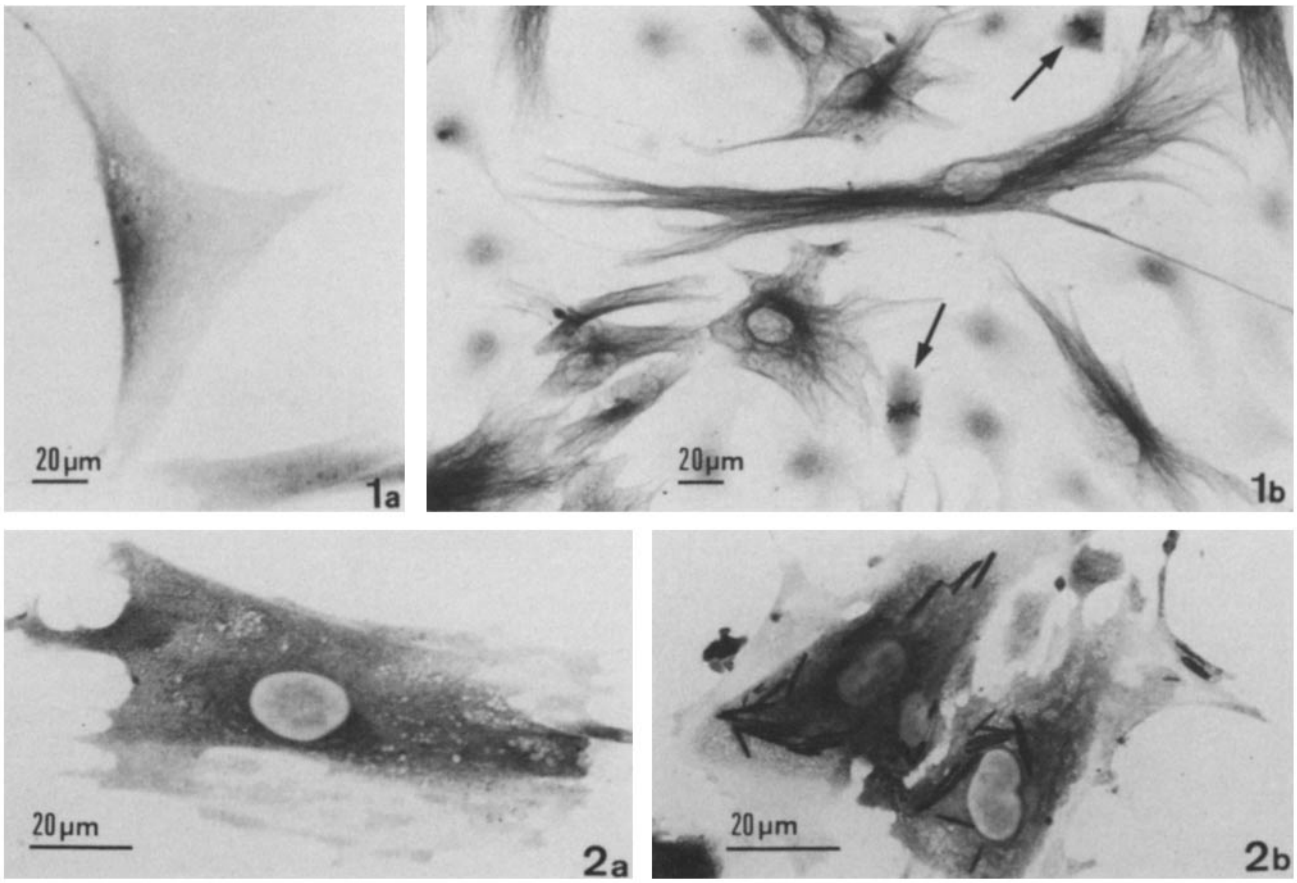
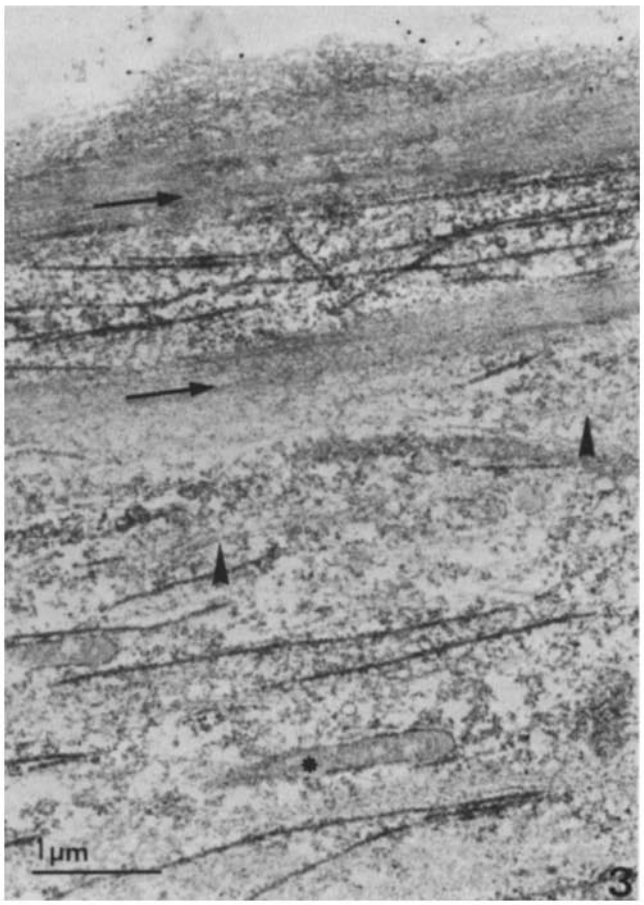



FIGURE 1 Localization of 210,000 MAP at the light microscope level in cultured human fibroblast cells (NS). Nuclei and chromosomes are counterstained with toluidine blue. (*a*) NS cell stained with the 210,000 MAP antiserum after adsorption onto the MAP coupled to polyacrylamide, diluted 300-fold. Even at this low dilution only diffuse staining is seen. × 360. (*b*) Staining of a mixed culture of primate cells (NS) and nonprimate cells ($PtK_2$) with the 210,000 MAP antiserum, diluted 1/600. The NS cells show a dense fiber network. The epithelial marsupial cells ($PtK_2$) are unstained, and the mitotic cells, recognizable by their small number of chromosomes (arrows), are also unstained. × 320.

FIGURE 2 Localization of 210,000 MAP at the light microscope level in NS cells treated with (*a*) nocodazole (1 μg/ml, 24 h), and (*b*) vinblastine sulfate (10 μg/ml, 24 h). In nocodazole-treated cells, the network is replaced by dense diffuse staining. Vinblastine-induced paracrystals are heavily stained. Antiserum dilution 1/200. *a*, × 640. *b*, × 670.

FIGURES 3–11 Electron micrographs of cells stained with the 210,000 MAP antiserum. Fig. 3: interphase NS cell stained by the PAP method and counterstained with uranyl acetate and lead citrate. Microtubules are labeled with PAP complexes. Microfilaments (arrows) and intermediate filaments (arrowheads) are negative. Label is excluded from the interior of mitochondria (asterisk). Antiserum dilution 1/2,500. × 16,800. Fig. 4: high magnification of microtubules stained by the PAP method. The globular aspect of the reaction product is evident. Section counterstained with uranyl acetate and lead citrate. Antiserum dilution 1/2,500. × 28,500. Fig. 5: interphase NS cell stained by the ferritin bridge method. Ferritin is associated in a discontinuous manner with microtubules. Intermediate filaments have very few granules. Stained en bloc with uranyl acetate. Antiserum dilution 1/2,500. × 38,000. Fig. 6: mitotic VERO cell stained by the PAP method. Label is confined to spindle microtubules. Some darkening of the chromosomes is seen in the vicinity of heavily labeled microtubule bundles (arrows). This probably represents diffusion of the reaction product. Antiserum dilution 1/1,250. × 5,950. Fig. 7: midbody region of a telephase VERO cell stained by the PAP method. The ends of the midbody microtubules are labeled, but the center of the midbody is negative. Antiserum dilution 1/1,250. × 13,750. Fig. 8: centrosomal region of an interphase NS cell stained by the PAP method. Label is absent from the centriole, but covers the pericentriolar microtubules. Section stained with uranyl acetate and lead citrate. Antiserum dilution 1/2,500. × 19,600. Fig. 9: spindle pole of an NS cell stained by the PAP method. Spindle microtubules are labeled but the centriole and pericentriolar cloud (arrow) are negative. Antiserum dilution 1/2,500. × 9,100. Fig. 10: Kinetochore region of a NS cell stained by the ferritin bridge method. Unlike the microtubules, the kinetochore (arrow) shows very few ferritin grains. Stained en bloc with uranyl acetate. Antiserum dilution 1/300. × 24,300. Fig. 11: kinetochore region in a NS cell treated with nocodazole (1 μg/ml, 4 h) and stained by the ferritin bridge method. Microtubules are absent. Ferritin grains are diffusely spread through the cytoplasm (e.g., arrowheads). The density of ferritin particles in the vicinity of the kinetochores (arrows) is not higher than in the cytoplasm. Stained en bloc with uranyl acetate. Antiserum dilution 1/1,250. × 31,100.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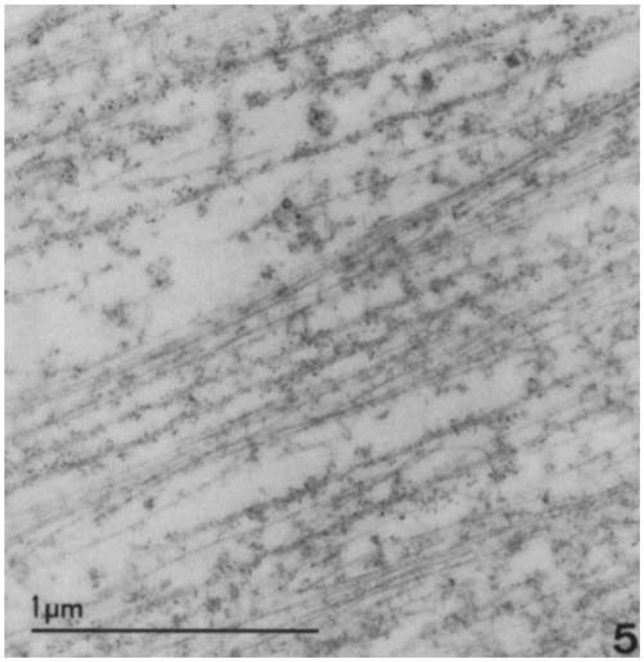
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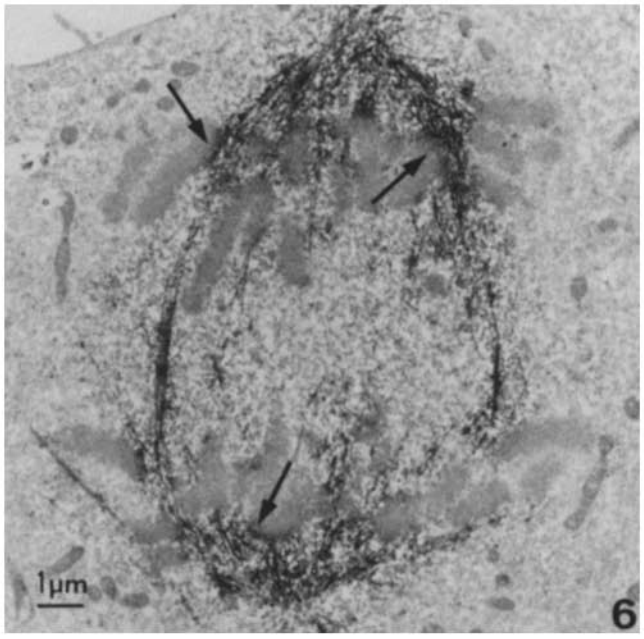
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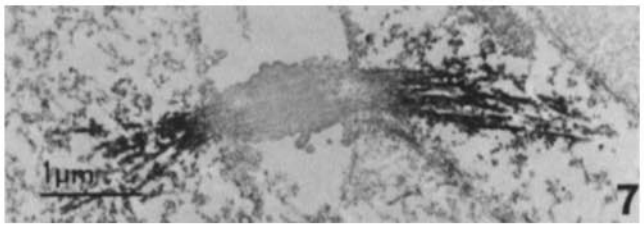
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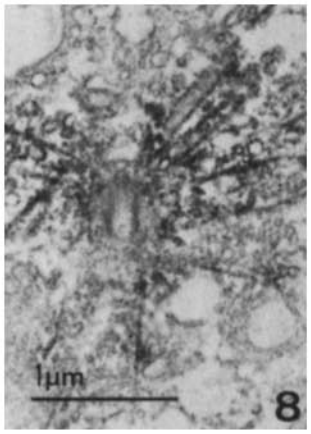
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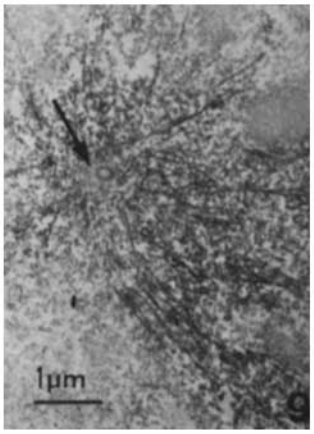
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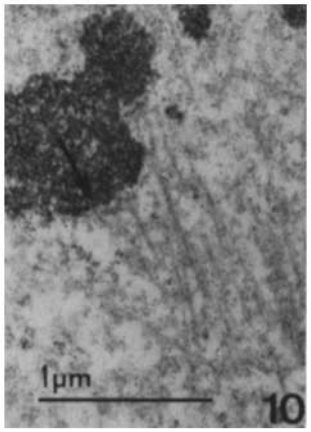
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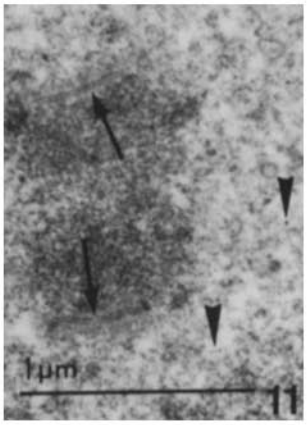
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of the MAP with the microtubules. The large size of the ferritin molecule and the intervening series of antibodies, however, preclude an accurate determination of the periodicity of 210,000 MAP in microtubules in thin sections.

Both PAP and ferritin staining were equivalent in showing that all interphase microtubules were labeled along their entire length. Both methods also demonstrated that microtubules were the only structures to be consistently labeled. Microfilaments and intermediate filaments as well as the various membrane-limited organelles did not show any stain above background levels.

The distribution of the 210,000 MAP in mitotic cells was also examined. Fig. 6 shows the spindle region of an anaphase cell stained with the PAP method. Again, microtubules were stained and the stain was distributed along their entire length. No microtubule was detected which failed to stain. As in interphase cells, no filamentous structure other than microtubules showed stain.

In late telophase cells, staining for the 210,000 MAP was positive for the microtubules of the midbody (Fig. 7), except that staining was not observed in the osmiophilic central region of the midbody. This pattern matches the immunofluorescence pattern seen for 210,000 MAP at the light microscopic level (3, 4).

We carefully examined microtubule-organizing centers for the presence of 210,000 MAP. The centrioles and pericentriolar cloud of interphase (Fig. 8) and mitotic (Fig. 9) cells failed to stain. Likewise, staining at the kinetochore was also negative (Fig. 10). These structures also remained unstained in cells made devoid of microtubules by treatment with nocodazole (Fig. 11).

Besides the microtubule-associated label, a variable amount of diffuse cytoplasmic staining was seen. In particular, the ferritin bridge procedure disclosed a general background staining consisting of individual granules and small clusters. This background label was virtually absent from the interior of all the membrane-limited organelles.

The diffuse cytoplasmic staining was probably largely specific, since it could be adsorbed by the purified MAP (see below). However, it is impossible to conclude to what extent the diffuse staining indicates a pool of MAP not associated with microtubules. At least part of the staining must arise from tangentially cut microtubules or microtubules located just outside the plane of the section.

The control preparations showed that microtubule-associated and cytoplasmic staining could be virtually completely abolished by adsorption with purified 210,000 MAP when the dilution of the antiserum was 1/600 or higher (Fig. 12 and 13). Lower dilutions produced a general background staining without any preference for microtubules (Fig. 14). Similar adsorption onto tubulin or the polyacrylamide carrier did not affect the staining properties of the antiserum.

The observations on normal interphase and mitotic cells showed that almost all of the 210,000 MAP was associated with microtubules. Since certain drugs (nocodazole, vinblastine, and taxol) alter the distribution of tubulin in protomeric and polymeric states, we sought to determine the effects of these drugs on the distribution of 210,000 MAP.

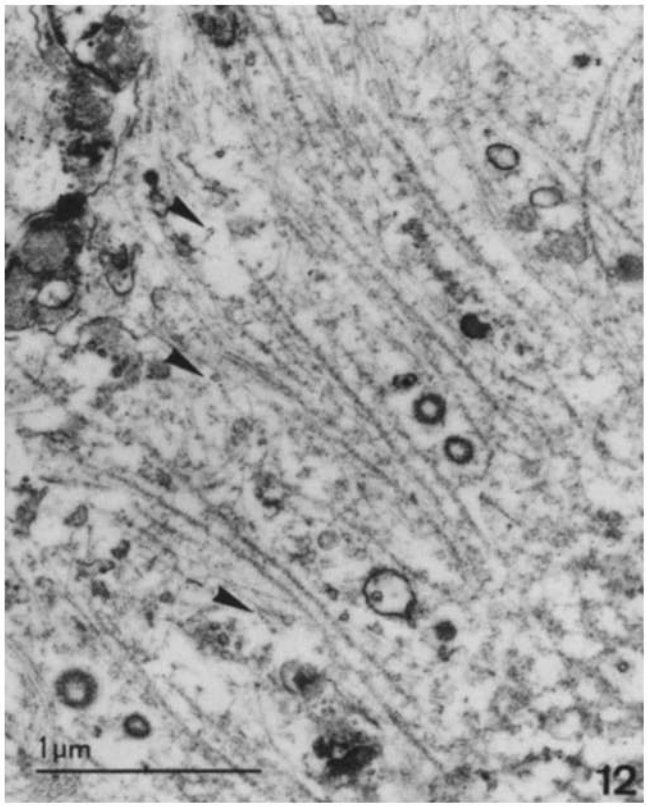
Treatment of the cells with nocodazole (1 μg/ml) for 24 h induced a virtually complete disassembly of cytoplasmic and mitotic microtubules. Figs. 2*a* and 15 show that, as revealed by PAP staining, the 210,000 MAP was diffusely distributed throughout the cytoplasm. During nocodazole treatment, 210,000 MAP stain was absent from the nucleus. Label was also excluded from the intermediate filament coils which were abundant in these nocodazole-treated cells. When nocodazole was washed out of the cells, microtubules reappeared within 10 min in association with the centrosomes and kinetochores in mitotic cells as well as free in the cytoplasm in interphase cells. Within 1–4 h after removal of nocodazole, organized cytoplasmic microtubule systems and mitotic spindles were present in all of the cells. Throughout the recovery period, every microtubule detected was seen to be entirely stained by the antibody (Fig. 16), indicating a virtually immediate association of the MAP with microtubules during in vivo polymerization.

Treatment with high concentrations of vinblastine (10 μg/ml for 24 h) resulted in the formation of tubulin paracrystals that were heavily labeled by the antiserum (Figs. 2*b* and 17). The macrotubules, which are mostly covered with ribosomes, were usually unstained. Identical results were obtained previously with an antitubulin antibody (10).

Treatment of the cells with sodium azide at concentrations which block oxidative phosporylation (10 mM for 30–240 min) did not affect the staining of microtubules by the antiserum (Fig. 2*c*), indicating that the continued association of the MAP with microtubules is largely energy independent.

Treatment of the cells with taxol (20) which lowers the critical concentration for tubulin assembly in vitro induced the formation of multiple short microtubule segments throughout the cytoplasm (M. De Brabander et al., manuscript in preparation). Apparently these microtubules contained normal amounts of the 210,000 MAP (Fig. 2*d*), suggesting that taxol does not compete with and replace the MAP.

FIGURES 12–17 Electron micrographs of cells stained with the 210,000 MAP antiserum. Fig. 12: interphase NS cell stained by the ferritin bridge method after absorption of the antiserum onto the 210,000 MAP. A limited number of ferritin granules is diffusely distributed in the cytoplasm (e.g., arrowheads). No specific association is seen with the microtubules. Stained en bloc with uranyl acetate. Antiserum dilution 1/1,200. × 29,700. Fig. 13: mitotic NS cell stained by the PAP method after absorption of the antiserum onto the 210,000 MAP. Microtubules are only faintly discernible in this unstained section. PAP label is completely absent (compare with Fig. 6). Antiserum dilution 1/1,200. × 6,300. Fig. 14: interphase NS cell stained as in Fig. 13, except with a low dilution of the absorbed antiserum (1/150). All cytoplasmic components are equally contrasted. This is probably due to the nonspecific adsorption of immunoglobulins. × 14,500. Fig. 15: interphase NS cell treated with nocodazole (1 μg/ml; 24 h) and stained by the PAP method. Diffuse PAP label is present in the cytoplasm but excluded from the bundles of intermediate filaments. Antiserum dilution 1/2,500. × 9,300. Fig. 16: interphase NS cell treated with nocodazole (1 μg/ml; 24 h), washed with drug-free medium, fixed 10 min later, and stained by PAP method. Newly formed microtubules are entirely labeled. Antiserum dilution 1/2,500. × 4,000. Fig. 17: interphase NS cell treated with vinblastine sulfate (10 μg/ml; 24 h) and stained by the PAP method. The paracrystals are heavily labeled. The bundles of intermediate filaments are negative. Stained with uranyl acetate and lead citrate. Antibody dilution 1/5,000. × 6,650.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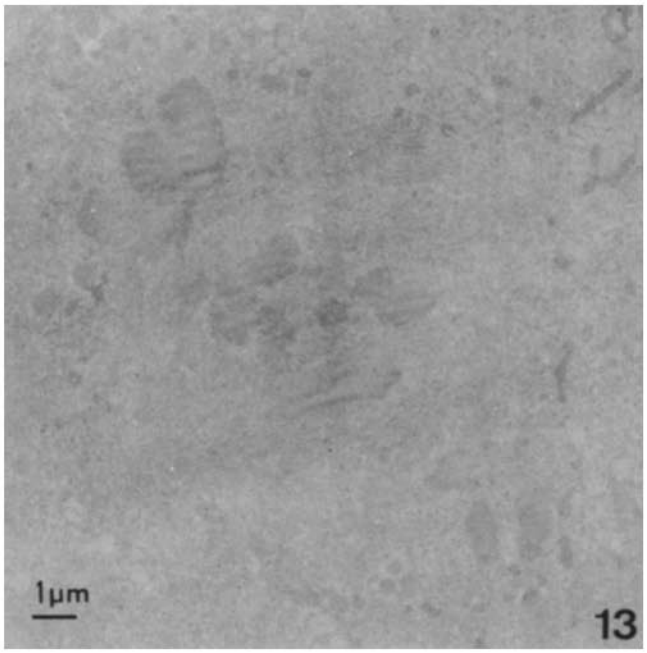
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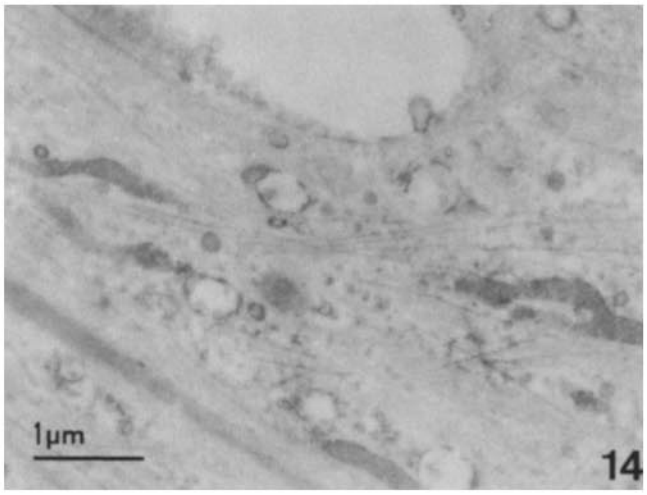
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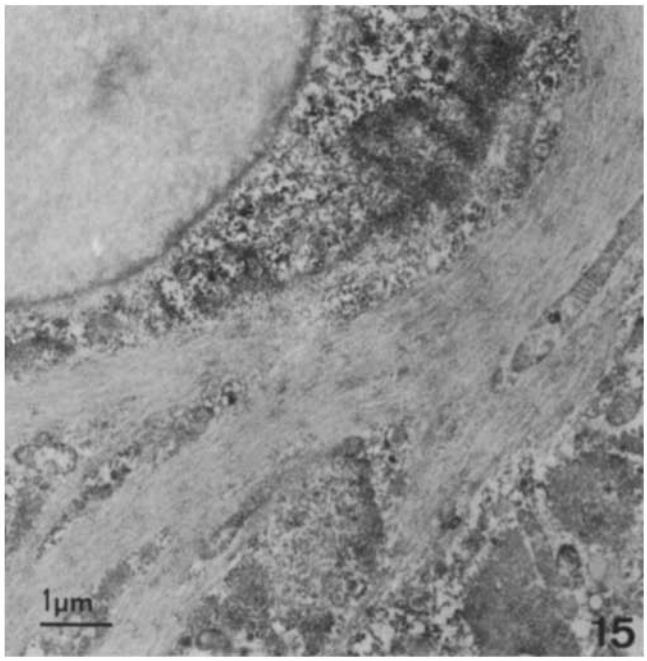
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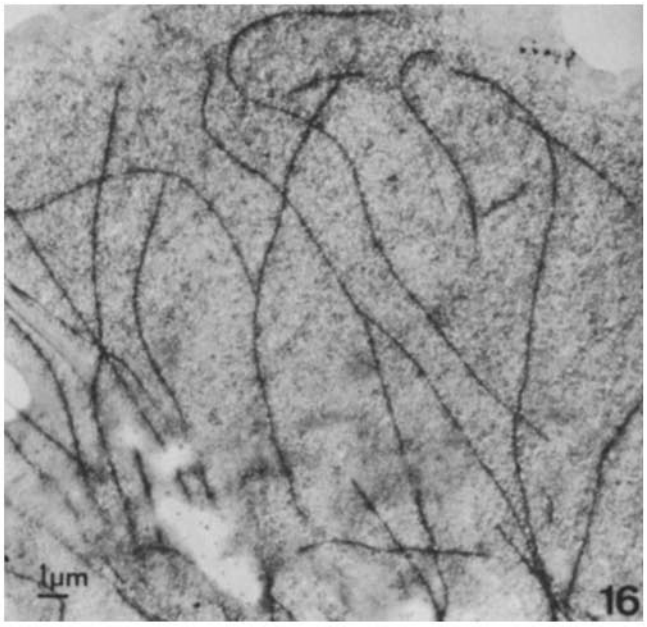
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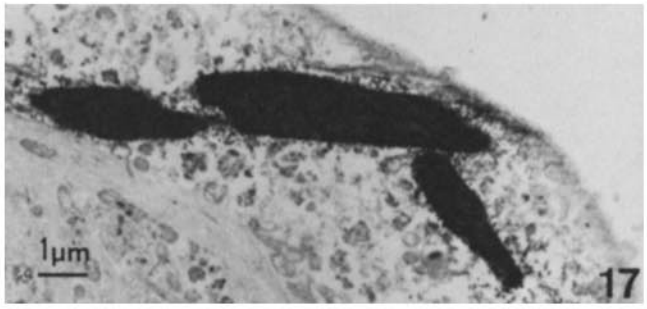
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## DISCUSSION

The validity of positive results obtained with immunocytochemical techniques depends mainly on the following factors: the specificity of the antiserum, the preservation of the cells, and the resolution of the observation technique. The specificity of the 210,000 antiserum has been documented previously (3). Using an immunofluorescent staining technique for polyacrylamide gels, the antiserum was shown not to react with tubulin or other cellular proteins except for the 210,000 species itself and a possibly related MAP of 255,000 mol wt. The control experiments we used further confirm the specificity. Cell preservation by primary fixation with glutaraldehyde is the best procedure to provide optimal preservation of subcellular structure (in particular microtubules) and minimal displacement of proteins. The resolution of ultrastructural observation is two orders of magnitude higher than that provided by light microscopy, and is essential for the validity of some of our conclusions. Since we have fulfilled the three criteria stated above, the following conclusions may be drawn:

(*a*) The data provide the first ultrastructural evidence for the tight association of a MAP with intact microtubules in adequately fixed cells. They lend support to the validity of results obtained with immunofluorescence.

(*b*) The 210,000 MAP is associated with all cytoplasmic and mitotic microtubules along their entire length. The only exceptions are the microtubules of the midbodies and centrioles, which will be discussed below. There is, therefore, an essential difference between the distribution of 210,000 MAP we have shown and the recently described association of calmodulin with specific segments of a defined population of mitotic microtubules (13).

(*c*) The 210,000 MAP is apparently not associated with other structures such as microfilaments or intermediate filaments.

(*d*) The 210,000 MAP is apparently not an integral component of the centrosomes or kinetochores. However, a negative result such as this poses certain problems which can never be completely resolved. Control experiments can only increase the degree of probability but cannot provide absolute certainty. The possibility remains that the absence of antibody binding to a certain site is due to lack of penetration, antigen masking, or denaturation. The negative results we report here are the lack the 210,000 MAP antibody binding to (*i*) microtubules in the dense central region of the midbody, (*ii*) microtubule-organizing centers (centrosomes and kinetochores) and (*iii*) cytoplasmic structures other than microtubules. Problems of antibody penetration or antigen masking, in particular, may account for the lack of staining of the central midbody microtubules. The lack of concentration of the MAP staining in the centrosomes and kinetochores however, probably does not result from penetration, masking, or denaturation problems since these structures can readily be stained using antisera specific for centrosomes and kinetochores (8, 18, and De Brabander et al., unpublished results). Thus, the 210,000 MAP is probably not localized in the centrosomes or kinetochores and is probably not involved in the microtubule nucleating capacity of these organelles.

(*e*) Destruction of the microtubules by nocodazole resulted in a diffuse cytoplasmic distribution of the MAP. No specific association with any organelle system could be detected. One might expect that the MAP proteins which are not associated with microtubules might accumulate on the micro- or intermediate filaments if any affinity between these proteins existed. Therefore, we obtained no evidence that the 210,000 MAP has a role in vivo as a crosslinker between microtubules and microfilaments or intermediate filments, a role which has been suggested for some MAPs from in vitro studies (14).

Vinblastine-induced tubulin paracrystals in vivo contain considerable amounts of the MAP. This is of particular interest in light of a recent report of Ludueña et al. (17). These investigators found that the tau group, but not the high molecular weight group of MAPs derived from mammalian brain tissue promoted the formation of and became incorporated into vinblastine-induced paracrystals of tubulin. It would appear from our results that the primate 210,000 MAP behaves more similarly to tau than to the high molecular weight MAPs with regard to binding to vinblastine paracrystals. The binding of this cultured cell MAP to vinblastine paracrystals in vivo suggests a possible method for the purification of both tubulin and MAPs from cultured cells.

(*f*) The 210,000 MAP apparently associates very rapidly with microtubules during their assembly in living cells. Whether this represents copolymerization or rapid binding of MAP to microtubules can not be discerned by static morphology.

In conclusion, the formal demonstration of the specific and tight association of the 210,000 MAP with all microtubules in primate cells suggests that this and similar proteins may be involved in the regulation of microtubule assembly and stability in living cells.

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*Note Added in Proof*: In addition to the discussion on the presence of 210,000–mol wt MAPs in vinblastine paracrystals, it should be noted that Haskins et al. (K. M. Haskins, J. A. Donoso, and R. H. Himes. 1981. Spiral and paracrystals induced by vinca alkaloid: evidence that microtubule-associated proteins act as polycations. *J. Cell Sci.* 47:237–247.) have recently reported a role for $MAP_2$ in promoting paracrystal formation. Others (F. A. Kuznetsov, E. I. Rodionov, A. D. Bershadfky, V. I. Gelfaud, and V. A. Rosenblat. 1980. High molecular weight protein MAP2 promoting microtubule assembly in vitro is associated with microtubule in cells. *Cell Biology International Reports.* 4:1017–1024.) have also localized $MAP_2$ by immunofluorescence in vinblastine crystals in cultured cells.

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