Widespread Cellular Distribution of MAP-1A (Microtubule-associated Protein 1A) in the Mitotic Spindle and on Interphase Microtubules

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ABSTRACT   In the accompanying paper (Bloom, G. S., T. A. Schoenfeld, and R. B. Vallee, 1983, J. Cell Biol. 98:320–330), we reported that microtubule-associated protein 1 (MAP 1) from brain comprises multiple protein species, and that the principal component, MAP 1A, can be detected in both neuronal and glial cells by immunofluorescence microscopy using a monoclonal antibody. In the present study, we sought to determine the cellular and subcellular distribution of MAP 1A in commonly used cultured cell systems. For this purpose we used immunofluorescence microscopy and immunoblot analysis with anti-MAP 1A to examine 18 types of mammalian cell cultures. MAP 1A was detected in every culture system examined. Included among these were cells of mouse, rat, Chinese hamster, Syrian hamster, Potoroo (marsupial), and human origin derived from a broad variety of tissues and organs. Anti-MAP 1A consistently labeled mitotic spindles and stained cytoplasmic fibers during interphase in most of the cultures. These fibers were identified as microtubules by co-localization with tubulin in double-labeling experiments, by their disappearance in response to colchicine or vinblastine, and by their reorganization in response to taxol. The anti-MAP 1A stained microtubules in a punctate manner, raising the possibility that MAP 1A is located along microtubules at discrete foci that might represent sites of interaction between microtubules and other organelles. Verification that MAP 1A was, indeed, the reactive material in immunofluorescence microscopy was obtained from immunoblots. Anti-MAP 1A stained a band at the position of MAP 1A in all cultures examined. These results establish that MAP 1A, a major MAP from brain, is widely distributed among cultured mammalian cells both within and outside of the nervous system.

Microtubules have been isolated from a variety of tissue and cell sources, and have been found to be composed of tubulin as well as a number of associated proteins (MAP).[1] MAP from brain tissue have been most extensively characterized as a result of the high yield of microtubule protein that can be readily obtained from this source. In brain, the major MAP appear on electrophoretic gels as two groups of high molecular weight (HMW) polypeptides (270,000–350,000), known respectively as MAP 1 and MAP 2 (41). The process of defining these proteins is not yet complete, as indicated by evidence presented in the accompanying paper (3) that MAP 1 consists of several biochemically distinct proteins. In addition to the

HMW MAP, several less prominent MAP that are associated with MAP 1 and MAP 2 have been found in brain microtubule preparations. These include M_r 28,000 and 30,000 light chains of MAP 1 (49), and a type II cAMP-dependent protein kinase, as well as an M_r 70,000 protein associated with MAP 2 (42, 46). In addition, a group of MAP known collectively as tau (M_r 55,000–62,000) are prominent in brain microtubule preparations (7, 52).

A number of investigations have been conducted to determine whether the MAP found in brain tissue are present in cultured cells. The results of these studies have been somewhat contradictory. Microtubules have been isolated and extensively purified from HeLa cells and Neuro-2a neuroblastoma cells (5, 32, 50). Polypeptides found to co-purify to constant stoichiometry with tubulin include HeLa cell peptides of

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~210,000 and ~125,000 mol wt (5, 50), and Neuro-2a cell peptides of 215,000 and 71,000 mol wt (32). HMW MAP corresponding to the brain proteins MAP 1 and MAP 2 were not detected in these purified microtubules or in partially purified microtubules from other cell lines (12, 30, 34). Similarly, Duerr et al. (13) found no evidence for MAP 1 or MAP 2 in a wide variety of cell lines using a procedure designed to extract microtubule proteins selectively from cells.

In contrast to these studies were the results of immunofluorescence microscopy using antibodies to unfractionated HMW MAP from brain tissue (8, 37–39). These antibody preparations reacted with microtubules in a variety of cultured cells, suggesting that some component of the HMW brain MAP was, indeed, present in many cell types. Immunoreactivity was observed both on interphase and mitotic microtubules. In support of a more general distribution for the HMW MAP, there have been two reports that SV40-3T3 cells contain a HMW polypeptide that co-purifies with carrier brain microtubules (7, 25). More recently, Weatherbee et al. (51) identified MAP 2 in purified HeLa cell microtubules with the use of a monoclonal antibody. However, the amount of MAP 2 was very low relative to the other MAP in HeLa cells, and therefore, the functional significance of the HeLa MAP 2 was questioned. MAP 2 has been found in some other cells, but could not be detected in most cell lines examined (23, 26, 33, 43). MAP 2 was also undetectable in non-neuronal cells in sections of brain and spinal cord (11, 29) further supporting the notion that this protein either does not occur universally in cells or is present at very low levels in non-neuronal cells.

This suggests that some component of the MAP 1 complex of HMW polypeptides (3) was detected in those earlier studies that had indicated a widespread occurrence of HMW MAP. One recent study of differentiated PC12 cells identified a polypeptide (termed MAP 1.2) that was in the molecular weight range of MAP 1, and was determined to be a MAP by a combination of microtubule purification and antibody techniques (17). Hill et al. (20a) and Sherline and Moscardo (36a) used polyclonal antibodies to brain MAP 1 to stain cultured cells. The antibodies stained a variety of structures in addition to microtubules, and the precise identities of the immunoreactive proteins were not reported. Aside from those studies and our preliminary identification of MAP 1 in pituitary tissue and cells (47), the distribution of MAP 1 and of the individual polypeptide components of MAP 1 has not been explored.

In addition to the question of its distribution, MAP 1 has been less extensively characterized than MAP 2 with regard to its biochemical properties. Much of the MAP 1 in microtubule preparations can be associated with MAP 2 (49), making an evaluation of its properties difficult. Purified MAP 1 did bind to microtubules in a periodic fashion, indicating a specific binding site for MAP 1 on the microtubule surface (49). However, no direct evidence for an association of MAP 1 with microtubules in cells has been presented.

In the accompanying study (3), we used a monoclonal antibody to MAP 1A, our designation for the major polypeptide in the MAP 1 region on SDS gels, to determine the distribution of this protein in nervous system tissue and cells. We found that, unlike MAP 2, which was largely restricted to the somata and dendritic processes of neuronal cells, MAP 1A was present in axons and glial cells as well. Along with earlier biochemical work comparing the content of MAP 1 and MAP 2 in microtubules from bovine gray and white

matter (44), these results indicated that MAP 1 was, indeed, more widely distributed than MAP 2. In the study described here, we have extended this investigation to a broad spectrum of cultured cell types. We found that MAP 1A is distributed almost universally among cultured mammalian cells, and is particularly prominent in the mitotic spindle of cells undergoing division. It was found in association with cytoplasmic fibers that co-labeled with antitubulin. This indicates that MAP 1A is, indeed, a MAP, and one that is associated with microtubules under a wide range of conditions.

MATERIALS AND METHODS

Cell Cultures: All cell lines and strains were maintained in a 37°C incubator (5% CO_2, 95% air) in 60-mm petri dishes (Falcon Labware, Becton, Dickinson & Co., Oxnard, CA). For immunofluorescence microscopy cells were grown on glass coverslips coated with poly-L-lysine or fetal calf serum. Media components were from Gibco Laboratories (Grand Island Biological Co., Grand Island, NY), and all media contained pen/strep. Most cultures were maintained in Dulbecco's MEM, 10% fetal calf serum. Exceptions were RIN-5F (obtained from Dr. Herbert K. Oie, National Cancer Institute) (RPMI 1640, 10% fetal calf serum), PC12 (Dulbecco's MEM, 10% each fetal calf and horse serum), and B104 (43% each F-12 and Dulbecco's MEM, 8% horse serum, 5% fetal calf serum).

Primary cell cultures from newborn rat lung, heart, kidney, and skeletal muscle tissue were prepared as follows. Freshly dissected tissue was minced with a scalpel and trypsinized for 15 min at 37°C. After brief centrifugation, the single cells and small clumps of tissue were resuspended in the medium used for B104 cells, and plated into 60-mm petri dishes (Falcon Labware) coated with 0.5% gelatin. The cultures were placed in the 37°C incubator for 30 min to permit most fibroblasts to attach. The unattached cells were then replated into new dishes containing gelatin-coated coverslips, and returned to the incubator. These more slowly attaching cells were used for the experiments. Primary cultures of newborn rat brain were prepared as we have described (2).

Immunocytochemistry and Immunochemistry: Single and double immunofluorescence microscopy of cultured cells were performed as described in the preceding paper (3). The primary antibodies were monoclonal mouse anti-MAP 1A (3) and polyclonal goat antitubulin (kindly provided by Dr. Robert Weihing, Worcester Foundation for Experimental Biology). Second antibodies were IgG fractions of sheep anti-mouse IgG and sheep anti-goat IgG, labeled respectively with rhodamine and fluorescein. These conjugates were prepared in our laboratory from crude antisera (Cappel Laboratories, Cochranville, PA) according to the method of Forni (15).

Immunoblot analysis (2, 3) was performed with anti-MAP 1A antibody obtained from ascites fluid. Samples were prepared by dissolving the cells from each confluent 60-mm petri dish in 200 µl of SDS sample buffer. 50 µl of each sample was used for the immunoblots.

RESULTS

Subcellular Localization of MAP 1A in Mitotic, Interphase and Drug-treated Cells

To determine the intracellular structures that were immunoreactive with anti-MAP 1A, we used immunofluorescence microscopy. For these experiments we examined several varieties of cell lines, strains, and primary cultures. The most striking and consistent result of these experiments was the staining of mitotic spindles and spindle remnants in dividing or recently divided cells by anti-MAP 1A. We observed staining in representative spindles from all cultured cell systems examined, except CHO-K1 (see Table I). Examples of MAP 1A localization in mitotic spindles from 12 cultured cell systems are illustrated in Fig. 1.

During interphase, the pattern and intensity of staining by anti-MAP 1A was variable. Staining patterns ranged from diffuse cytoplasmic fluorescence to bright punctate staining of cytoplasmic fibers. Staining of fibers varied in intensity from cell type to cell type, but was observed in most cells examined (Table I). To verify that the cytoplasmic fibers

TABLE I

Distribution of MAP 1A in Cultured Cells

Species	Cell type	Immunofluorescence Microscopy				Immunoblot: MAP 1A labeled?
		Interphase		Mitosis		
		Labeled by anti-MAP 1A?	Micro-tubules visible?	Labeled by anti-MAP 1A?	Spindle visible?	
Mouse	3T3; fibroblast	+	Yes	++	Yes	Yes
"	Neuro-2a; neuroblastoma	+++	Yes	+++	Yes	Yes
"	N1E-115 neuroblastoma	+++	Yes	+++	Yes	Yes
Rat	RIN-5F; pancreatic islet tumor	+++	Yes	+++	Yes	Yes
"	C6; glioma	+++	Yes	+++	Yes	Yes
"	B104; neuroblastoma	+	Yes	++	Yes	Yes
"	PC12; pheochromocytoma	+++	No	+++	Yes	Yes
"	Primary newborn lung	+	Yes	++	Yes	Not tested
"	Primary newborn heart*	++	Yes	+++	Yes	Not tested
"	Primary newborn skeletal muscle*	+	No	++	Yes	Not tested
"	Primary newborn kidney	+	Yes	++	Yes	Not tested
"	Primary newborn brain§	+++	Yes	+++	Yes	Yes
Chinese hamster	CHO-K1; ovary	+	No	+	No	Yes; plus lower molecular weight bands
Syrian hamster	BHK-21; kidney	++	Yes	++	Yes	Yes; plus lower molecular weight bands
Potoroo	PtK1; kidney	−	No	++	Yes	Not tested
Human	WI-38; lung fibroblast	+++	Yes	+++	Yes	Yes
"	HeLa; cervical carcinoma	+	No	+	Yes	Yes
"	MG-63; osteosarcoma	+	Yes	+	Yes	Not tested

Key to symbols: −, never observed; +, rare cells or weak fluorescence; ++, many cells, moderate fluorescence; +++, most cells, moderate-strong fluorescence.
* MAP 1A apparently present in beating cardiac muscle cells.
‡ MAP1A present in fused myotubes and in mononucleate cells.
§ MAP 1A found in neurons, oligodendrocytes and astrocytes (see accompanying paper [3]).

stained by anti-MAP 1A corresponded to microtubules, we performed double labeling experiments with antibodies to MAP 1A and tubulin. Fig. 2 shows primary rat brain cells, mouse neuroblastomas (clones Neuro-2a and N1E-115), human fibroblasts (WI-38), and rat glioma cells (C6) stained in this manner. It is readily apparent that fibers stained almost continuously with antitubulin were also stained discontinuously by anti-MAP 1A. We noted that staining by anti-MAP 1A was punctate in all cell types in which MAP 1A was observed on cytoplasmic fibers. We used a variety of fixation protocols to evaluate their effect on the pattern of MAP 1A immunofluorescence. These included fixation and permeabilization with absolute methanol at −20°C alone or in combination with glutaraldehyde, or fixation of detergent-resistant cytoskeletons with either methanol or glutaraldehyde. All methods resulted in punctate staining of microtubules indistinguishable from those observed in cells permeabilized with methanol, the procedure used for all figures in this paper.

In a recent study (2), we used vinblastine and colchicine treated cells to identify cellular binding sites other than microtubules for MAP 2, the most prominent high molecular weight brain MAP. In the present study, we similarly used drugs that depolymerize or stabilize microtubules to examine the fate of MAP 1A when the normal distribution of tubulin is disrupted. In colchicine-treated (not shown) or vinblastine-treated cells (Fig. 3), fibrous staining by anti-MAP 1A was abolished, consistent with a normal association of MAP 1A with microtubules. Fluorescence was still punctate, with spots of various sizes being distributed randomly throughout the cytoplasm. Comparison of immunofluorescent (Figure 3, a, c, and e) and phase-contrast (Fig. 3, b, d, and f) images for

three cell types treated with 10 μM vinblastine for 18 h illustrates that neither tubulin paracrystals nor intermediate filament bundles were specifically stained by anti-MAP 1A. While anti-MAP 2 also failed to stain vinblastine-induced tubulin paracrystals, it did stain intermediate filament bundles in cells treated with vinblastine or colchicine (2). Therefore, MAP 1A and MAP 2 differ dramatically in their ability to bind to intermediate filaments.

Another drug that alters microtubules is taxol. This compound induces the reorganization of microtubules from a radial distribution emanating from organizing centers near the nucleus, to a randomly distributed system of fascicles, which originate at multiple organizing sites scattered throughout the cytoplasm (10, 35). To test whether the distribution of MAP 1A could be similarly altered by taxol, we exposed cells to 10 μM taxol for 18 h before staining them with anti-MAP 1A. Fig. 4 shows examples from a variety of cultured cells of typical taxol-induced bundles of cytoplasmic fibers that were stained by anti-MAP 1A. Double-labeling of primary rat brain cells with anti-MAP 1A (Fig. 4j) and antitubulin (Fig. 4k) demonstrated that the fiber bundles stained by anti-MAP 1A were, indeed, co-localized with microtubules.

While most staining patterns observed in interphase cells were consistent with localization of MAP 1A on microtubules, one additional pattern of staining was occasionally seen. Numerous patches of fluorescence 1–2 μm across were observed on the surface of the nucleus in rare cells (Fig. 5). More commonly we observed cells with smaller numbers (typically 1–3) of larger nuclear spots, examples of which may be seen elsewhere in this paper (see Fig. 2, c, e, and i).

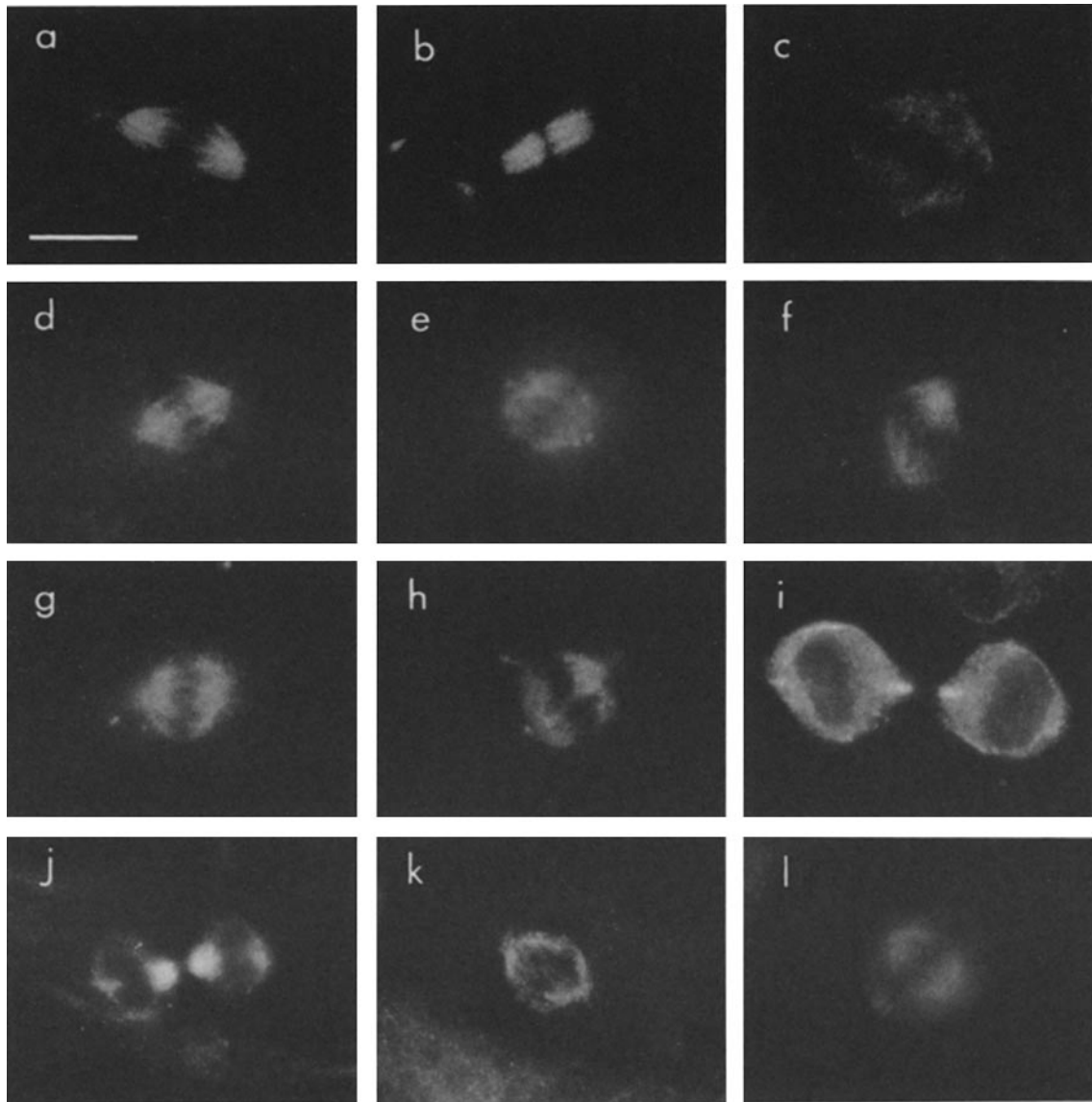None of the staining properties we have described for anti-

FIGURE 1   Localization of MAP 1A in dividing cells. Anti-MAP 1A consistently labeled mitotic spindles and spindle remnants in numerous types of cultured cells. (a) WI-38, human lung fibroblast. (b) BHK-21, baby hamster kidney. (c) Primary newborn rat lung. (d) Primary newborn rat kidney. (e) 3T3, mouse fibroblast. (f) RIN-5F, rat insulin-secreting pancreatic islet tumor. (g) Primary newborn rat skeletal muscle. (h) PtK1, marsupial (Potoroo) kidney. (i) Neuro-2a, mouse neuroblastoma. (j) C6, rat glioma. (k) Primary newborn rat heart. (l) HeLa, human cervical carcinoma. Bar, 10 μm. × 1,500.

MAP 1A were observed in a variety of immunofluorescent control experiments (not shown). No staining was observed when anti-MAP 1A was replaced by unconditioned medium, or by sea urchin-specific monoclonal antibodies (48) present in conditioned medium or ascites fluid. Preadsorption of anti-MAP 1A with excess MAP 1A present in a brain MAP preparation (45) abolished all cytoplasmic staining. However, punctate staining of the outer surface of the nucleus was markedly enhanced with the adsorbed antibody.

Immunochemical Identification of MAP 1A in Cultured Cells

The immunofluorescence results described to this point prove that material cross-reactive with anti-MAP 1A is widely distributed among mammalian cells. To verify that MAP 1A was, indeed, the immunoreactive species, we used immuno-

blot analysis. Cultured cells were washed thoroughly in PBS and dissolved directly in hot SDS gel sample buffer. After SDS PAGE, nitrocellulose replicas of the gels were prepared and stained with anti-MAP 1A. A protein with the electrophoretic mobility of MAP 1A was generally detected (see Table I) and examples from ten different cultured cell systems are shown in Fig. 6. The species and cell types represented here are diverse and include mouse (3T3 fibroblast, Neuro-2a, and N1E-115 neuroblastomas), rat (C6 glioma, B104 neuroblastoma, PC12 pheochromocytoma, and RIN-5F insulin-secreting, pancreatic islet tumor cells [31]), Chinese hamster (CHO-K1 ovary cells), Syrian hamster (BHK-21 kidney cells), and human (WI-38 lung fibroblasts). In many of the cells tested, faintly staining bands with lower molecular weights than MAP 1A could also be detected, and these presumably represented proteolytic fragments of MAP 1A. In two cases, CHO-K1 and BHK-21, we detected prominent
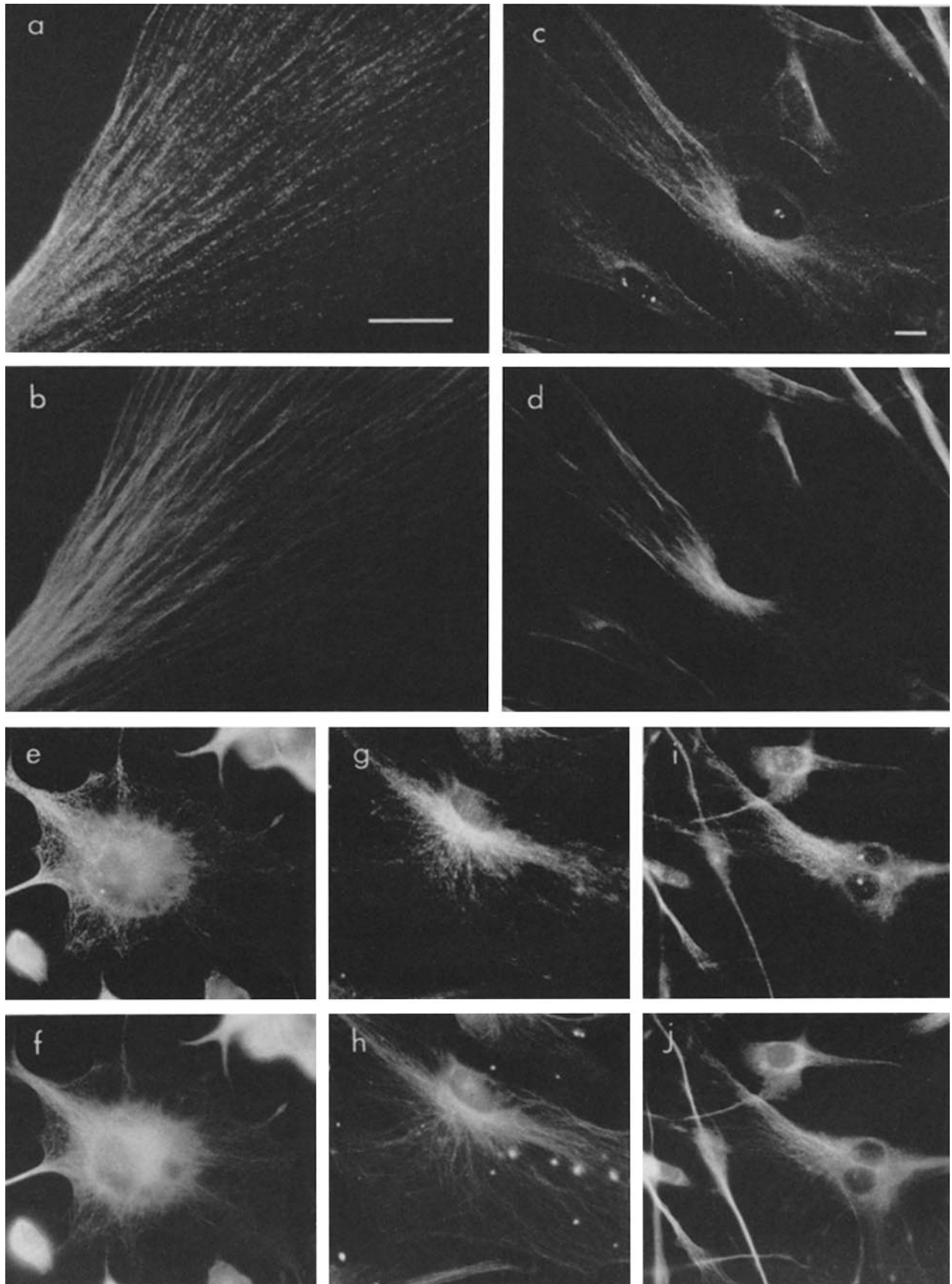
FIGURE 2 Co-localization of MAP 1A and tubulin in nondividing cells. Double-labeling was performed with monoclonal anti-MAP 1A (a, c, e, g, and i) and goat antitubulin (b, d, f, h, and j) followed by rhodamine sheep anti-mouse IgG and fluorescein sheep anti-goat IgG. (a and b) N1E-115, mouse neuroblastoma. These figures illustrate a small portion of the cytoplasm in a giant, multinucleate cell. (c and d) WI-38, human fibroblast. (e and f) Neuro-2a, mouse neuroblastoma. (g and h) Primary newborn rat brain cell, most likely an astrocyte. (i and j) C6, rat glioma. Bars: 10 μm (in a, for a and b; in c for all others). (a and b) × 1,500. (c–j) × 600.
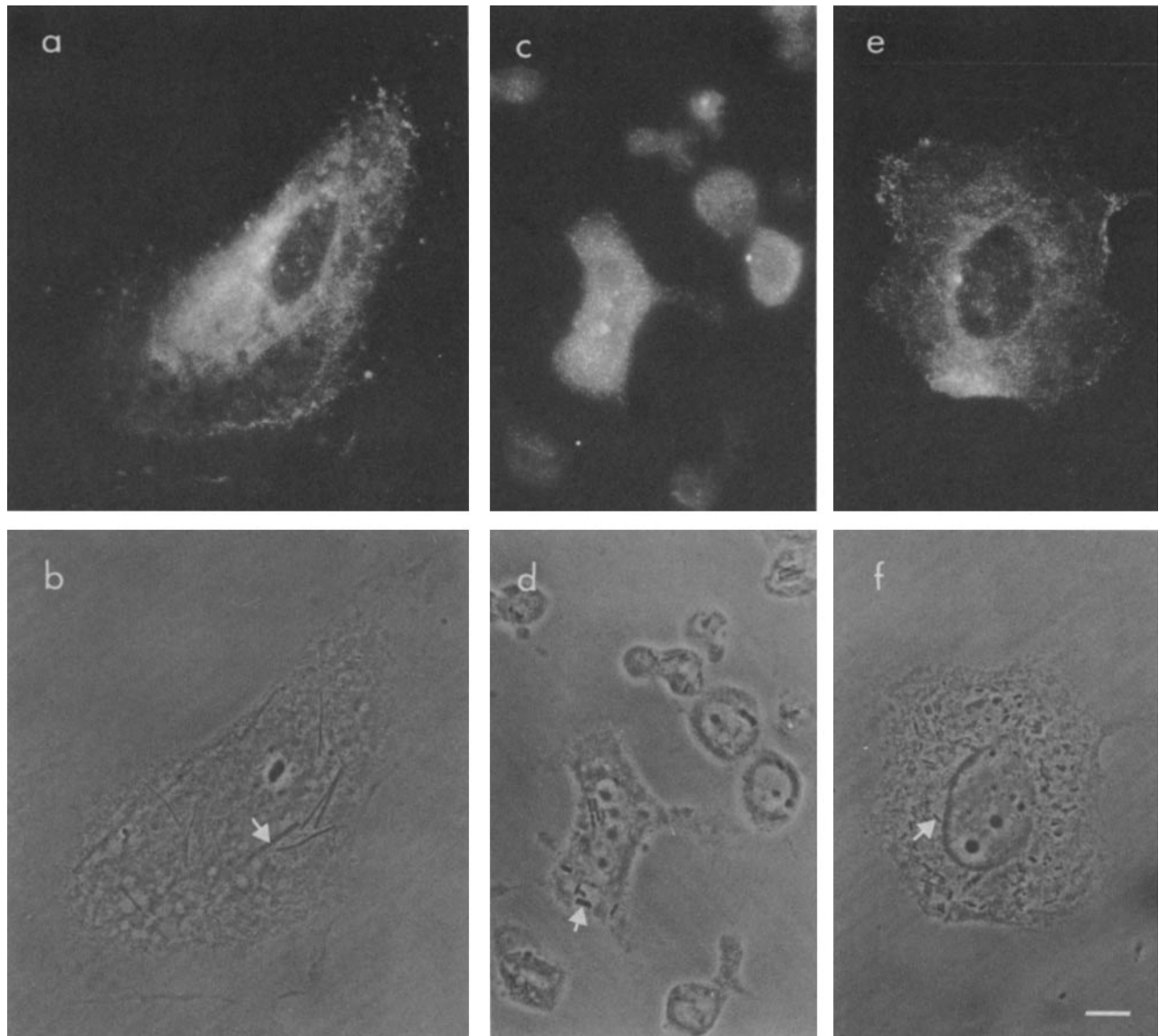
FIGURE 3 Localization of MAP 1A in vinblastine-treated cells. Cells were treated with 10 μM vinblastine sulfate for 18 h before being stained with anti-MAP-1A. Corresponding immunofluorescence (a, c, and e) and phase-contrast (b, d, and f) micrographs are shown. (a and b) WI-38; human fibroblast. (c and d) Neuro-2a; mouse neuroblastoma. (e and f) N1E-115; mouse neuroblastoma. The arrows (b, d, and f) point to vinblastine-induced tubulin paracrystals, several of which are found in each cell. Bar, 10 μm. × 650.

bands of slightly greater electrophoretic mobility than MAP 1A. These may have represented large fragments of MAP 1A. We estimate that we detected between 1 and 10 ng MAP 1A per sample, and that the samples varied from ~10 to 60 μg protein. These figures permit us to estimate that MAP 1A commonly constitutes from 0.01% to 0.04% of the total protein in cultured cells.

DISCUSSION

In the accompanying paper (3), we demonstrated that MAP 1, a prominent HMW component of purified brain microtubules, comprises multiple protein species. The most abundant of these, which we call MAP 1A, was uniquely recognized by a monoclonal antibody which we produced. Immunofluorescence microscopy performed with this antibody indicated that MAP 1A was distributed widely in the nervous system, being present in several types of neuronal and glial cells in vivo, and in primary brain cell cultures. In the present communi-

cation we have demonstrated the presence of MAP 1A by immunoblot analysis and immunofluorescence microscopy in numerous cultured mammalian cell types derived from a broad variety of tissues. The intracellular structures stained most conspicuously by anti-MAP 1A were mitotic spindle microtubules, which were seen in most cell types examined. In interphase cells the antibody stained cytoplasmic fibers identified as microtubules by co-localization with tubulin, and their response to colchicine, vinblastine and taxol. Although staining in interphase cells was generally limited to the cytoplasm, we occasionally observed immunoreactive spots on the surface of the nucleus. A summary of our immunocytochemical and immunochemical analysis of MAP 1A in 18 cultured cell systems is shown in Table I.

Cellular Distribution of MAP 1A

A variety of approaches have been used to identify the MAP in cultured cells. Biochemical approaches have generally

failed to detect appreciable quantities of any of the HMW MAP, i.e., MAP 1 and MAP 2, first described in purified brain microtubules (5, 12, 13, 30, 32, 50). Two notable exceptions are the recent discoveries of MAP 1-like proteins in PC12 cells treated with nerve growth factor (17), and in pituitary tissue and cells (47). The PC12 cell protein identified in the earlier study was evidently not MAP 1A. However, because our anti-MAP 1A reacted with PC12 cells grown in the presence or absence of nerve growth factor on immunoblots and by immunofluorescence microscopy (see lane 7 in

Fig. 6 for an immunoblot of untreated PC12 cells). Therefore, PC12 cells may contain more than one type of MAP 1 protein. Immunological studies have consistently indicated that material cross-reactive with HMW brain MAP is, indeed, found commonly in cultured cells (8, 37–39). The precise identity of the immunoreactive material observed in these studies was not known, though, because the antibodies employed were directed against unfractionated HMW brain MAP, which include several distinct protein species.

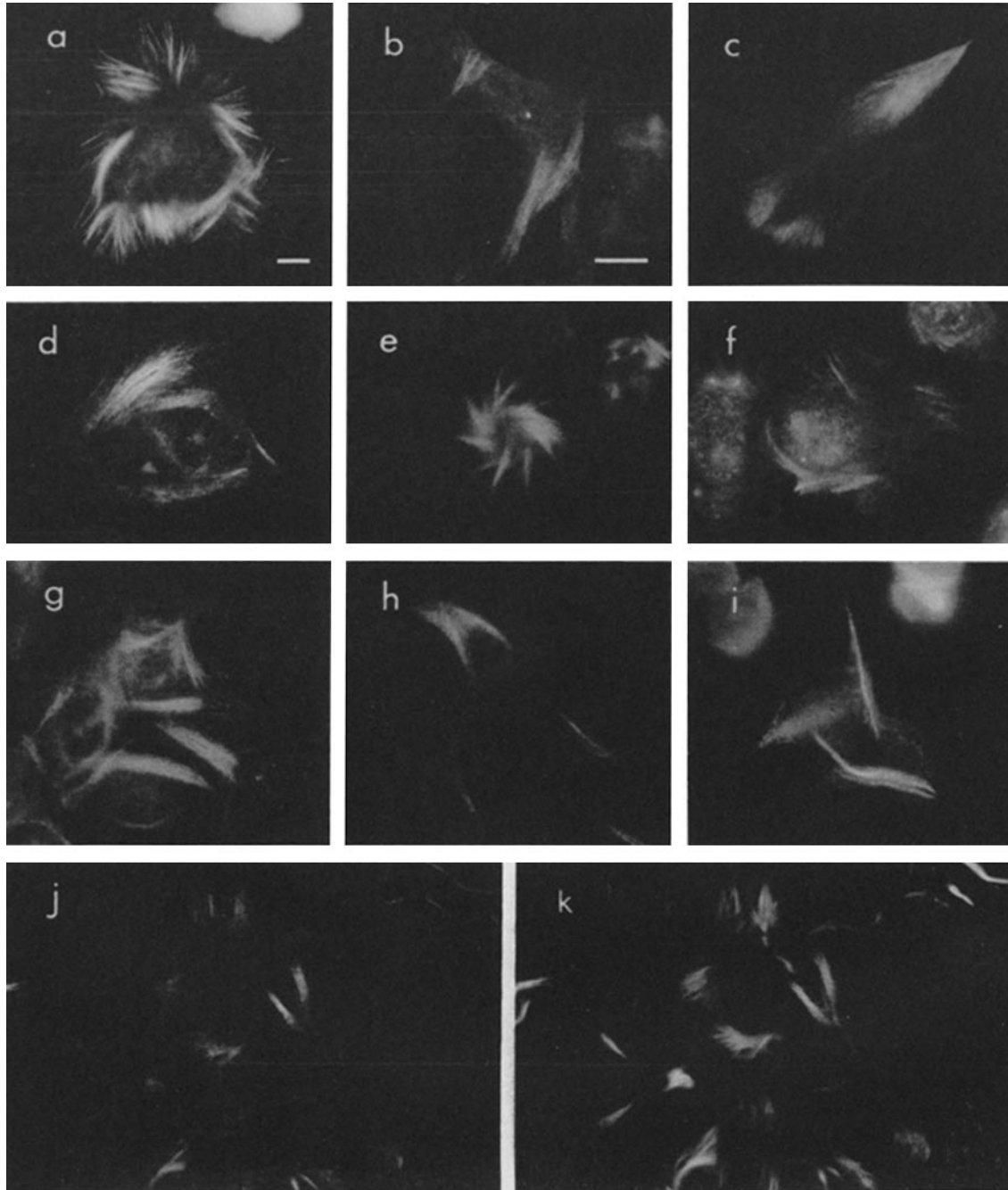Our finding that monoclonal anti-MAP 1A commonly

FIGURE 4   Distribution of MAP 1A in taxol-treated cells. Cultures were treated with 10 μM taxol for 18 h before being labeled with anti-MAP 1A, alone (a–i) or in combination with antitubulin (j and k). (a) N1E-115, mouse neuroblastoma. (b) 3T3, mouse fibroblast. (c) WI-38, human fibroblast. (d) B104, rat neuroblastoma. (e) BHK-21, baby hamster kidney. (f) HeLa, human cervical carcinoma. (g) C6, rat glioma. (h) RIN-5F, rat insulin-secreting pancreatic islet tumor. (i) Neuro-2a, mouse neuroblastoma. (j and k) Primary newborn rat brain cells labeled with monoclonal anti-MAP 1A (j) and goat antitubulin (k) followed by rhodamine sheep anti-mouse IgG and fluorescein sheep anti-goat IgG. Bars: 10 μm (in a, for a, c, d, e, j, and k; in b, for b, f, g, h, and i). (a, c, d, e, j, and k) × 500. (b, f, g, h, and i) × 825.
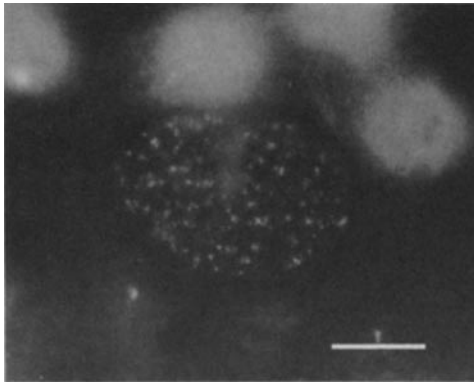
FIGURE 5 Immunoreactivity of the surface of the nucleus with anti-MAP 1A. The nucleus of a HeLa cell stained with anti-MAP 1A is shown. Bar, 10 μm. × 1,300.
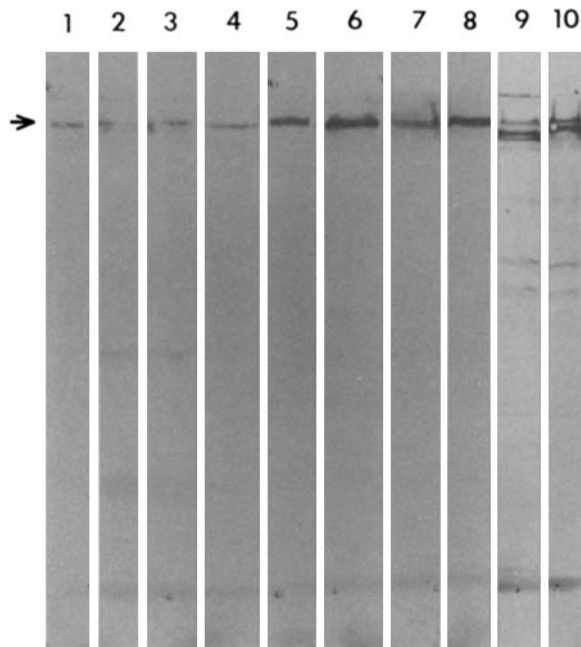

FIGURE 6 Immunochemical detection of MAP 1A in cultured cells. An immunoblot is shown. Cells were dissolved directly in sample buffer, and subjected to SDS PAGE. A nitrocellulose replica of the gel was then stained with anti-MAP 1A followed by peroxidase-sheep anti-mouse IgG, for which 4-chloro-1-naphthol was used as a substrate. Lanes: (1) WI-38, human fibroblast. (2) N1E-115, mouse neuroblastoma. (3) Neuro-2a, mouse neuroblastoma. (4) 3T3, mouse fibroblast. (5) B104, rat neuroblastoma. (6) RIN-5F, rat insulin-secreting pancreatic islet tumor. (7) PC12, rat pheochromocytoma. (8) C6, rat glioma. (9) CHO-K1, Chinese hamster ovary. (10) BHK-21, baby hamster kidney. Arrow at left indicates position of MAP 1A.

stained interphase and mitotic spindle microtubules in cultured mammalian cells derived from numerous tissue sources may explain, at least in part, the earlier immunocytochemical studies using polyclonal antisera of unknown specificities. Immunoblots of the cells we examined suggested that MAP 1A commonly constitutes from 0.01–0.04% of total cellular protein, a range only slightly below that reported for a major HeLa cell MAP of ~210,000 mol wt (6), raising the question of why this protein has not been detected biochemically in microtubule protein preparations obtained from 3T3, Neuro-

2a, C6, PC12, CHO-K1, BHK-21, and HeLa cells (5, 13, 17, 30, 32, 50).

Several considerations may explain why HMW MAP have generally not been detected in cultured cells by biochemical means. First, all of the HMW brain MAP, except MAP 1C, are extremely sensitive to proteases (see, for example, Fig. 1 C in the accompanying paper). Therefore, HMW MAP present in intact cells may be degraded by exposure to cellular proteases during the preparation of microtubule proteins for electrophoretic analysis. We attempted to avoid this potential problem for the study presented here by identifying MAP 1A on immunoblots of cells dissolved directly in hot SDS sample buffer, in which proteolysis was likely to be minimal. Despite our efforts, appreciable fragmentation of MAP 1A may have occurred in samples of CHO-K1 and BHK-21 cells (see Fig. 6, lanes 9 and 10), underscoring the sensitivity of this protein to proteolysis. Another possible explanation is that HMW MAP may associate not only with microtubules, but with other subcellular structures as well. In fact, we have recently presented evidence that MAP 2 associates with intermediate filaments, as well as with microtubules in cultured brain cells (2). In the present study we noted residual anti-MAP 1A immunoreactivity associated with punctate cytoplasmic structures after vinblastine or colchicine treatment, suggesting that MAP 1A may remain bound to particulate structures after dissolution of microtubules (see below). Finally, at least in some cells, MAP 1A appears to be most striking in mitotic spindle microtubules. This suggests that the concentration of this protein could be cell cycle-dependent, and could be low in unsynchronized cultures.

Subcellular Distribution of MAP 1A

MAP 1A has been previously identified as a MAP on the basis of its co-purification with microtubules in vitro. Until now, no evidence had been presented indicating that this association occurs in the cell as well. As we have shown in Fig. 1, anti-MAP 1A stained the spindle in a manner consistent with the localization of MAP 1A on microtubules during mitosis. In addition, anti-MAP 1A stained cytoplasmic fibers in interphase cells, and identification of these fibers as microtubules was determined by three lines of evidence. First, the fibers colocalized in double-labeling experiments with tubulin (see Fig. 2). Next, treatment of cells with anti-microtubule drugs, such as colchicine or vinblastine, abolished the fibrillar distribution of MAP 1A (see Fig. 3). It is noteworthy that MAP 1A was not detected in these experiments on intermediate filament cables, as we have reported for MAP 2 (2), nor in vinblastine-induced tubulin paracrystals, as has been observed for the HeLa cell 210,000-mol-wt MAP (9). Finally, treatment of cells with taxol (see Fig. 4) induced the reorganization of fibers stained by anti-MAP 1A into bundles codistributed with those stained by antitubulin.

Although antibodies to both MAP 1A and tubulin stained cytoplasmic microtubules in interphase cells, the staining was qualitatively different for the two antibodies. Antitubulin produced nearly continuous staining of microtubules in cells fixed and permeabilized by a wide variety of procedures, while anti-MAP 1A stained the microtubules in a punctate manner. It is not clear whether this represents the true distribution of MAP 1A or a fixation artifact. Though we cannot eliminate the latter possibility, we favor the former hypothesis because of the highly reproducible appearance of the punctate pattern observed in numerous cell types fixed and permeabilized by

several protocols. In addition, the punctate appearance of MAP 1A persisted even when the normal microtubule distribution was disrupted by colchicine or vinblastine (see Fig. 3). This suggests that MAP 1A is normally associated with discrete structures along microtubules and that these structures can persist when microtubules are depolymerized. Further work is needed to test this supposition, and to identify what structures, if any, are co-distributed with MAP 1A along microtubules (see further discussion below).

An additional pattern of nonmicrotubular staining was also occasionally observed. Spots of fluorescence were seen on the surface of the nucleus by optically sectioning cells with high magnification objectives (see Fig. 5). This result is reminiscent of a staining pattern observed with an antibody to ankyrin, a protein related immunologically to MAP 1 (1). Our anti-MAP 1A antibody did not cross-react with rat or sheep ankyrin (not shown), however, suggesting that MAP 1A may, indeed, have a binding site on the surface of the nucleus. In support of this possibility we noticed that anti-MAP 1A could be induced to stain a far greater number of nuclear spots than we normally saw by preadsorbing the antibody with a molar excess of MAP 1A present in a MAP preparation (45) from calf brain. Presumably, the antibody was binding indirectly to the nucleus via MAP 1A molecules, whose nuclear binding site was undisturbed by bound antibody, and for which most nuclear binding sites were unoccupied in fixed cells. It remains to be determined what functions, may be attributed to MAP 1A localized at discrete foci on the surface of the nucleus.

Function of MAP 1A

In view of the evidence presented here, we propose that one role for MAP 1A is in mitosis. Electron microscopic studies have indicated the existence of cross-bridges connecting microtubules to one another in mitotic spindles (4, 22, 27, 53), and these cross-bridges have been proposed to be involved in organizing spindle microtubules and in generating the force that drives chromosome movement (28). We have recently purified MAP 1 and found that it has the appearance of fine periodic arms on the surface of the microtubule (49). Thus, MAP 1A could be a component of the microtubule cross-bridges that have been observed in mitotic spindles.

The discontinuous, punctate staining by anti-MAP 1A of microtubules in interphase cells suggests an alternative hypothesis. Ultrastructural studies have consistently supported the idea that individual microtubules can be connected in cells to vesicles, granules, and other membrane-bound organelles (14, 21, 36), and may also be involved in the transport of these structures (16, 19). Further work will be needed to determine whether the discontinuous anti-MAP 1A fluorescence that we regularly observed corresponds to recognizable cellular organelles, thereby implying that MAP 1A links those structures to microtubules. If this turns out to be true, it will raise the further question of how this function of MAP 1A relates to the localization of this protein in neuronal and glial processes in the nervous system. Certainly particle transport is known to occur actively in neuronal processes, and an involvement of MAP 1A in the transport of myelin precursors seems reasonable as well. Perhaps MAP 1A is also involved in mediating the interaction of vesicles with microtubules within the mitotic spindle. The interaction of microtubules and vesicles has been repeatedly observed by electron microscopy (18, 20, 40) and has been proposed to account for localized regulation of calcium concentrations in the spindle

(24, 40, 54). Whether this hypothesis is correct or not, it seems clear that MAP 1A will prove to be involved in more general functions of microtubules than MAP 2.

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