MAP 4: A Microtubule-associated Protein Specific for a Subset of Tissue Microtubules

LINDA M. PARYSEK, JOHN J. WOLOSEWICK,* and J. B. OLMSTED
*Department of Biology, University of Rochester, Rochester, New York 14627; and * Department of Anatomy, University of Illinois, Chicago, Illinois 60612*

ABSTRACT The cytological distribution of microtubule-associated protein 4 (MAP 4) (L. M. Parysek, C. F. Asnes, J. B. Olmsted, 1984, *J. Cell Biol.*, 99:1309–1315) in mouse tissues has been examined. Adjacent 0.5–0.9-μm sections of polyethylene glycol-embedded tissues were incubated with affinity-purified MAP 4 or tubulin antibodies, and the immunofluorescent images were compared. Tubulin antibody labeling showed distinct microtubules in all tissues examined. MAP 4 antibody also labeled microtubule-like patterns, but the extent of MAP 4 reactivity was cell type–specific within each tissue. MAP 4 antibody labeled microtubules in vascular elements of all tissues and in other cells considered to have supportive functions, including Sertoli cells in the testis and glial elements in the nervous system. Microtubule patterns were also observed in cardiac, smooth, and skeletal (eye) muscle, podocytes in kidney, Kuppfer cells in liver, and spermatid manchettes. The only MAP 4-positive cells in which the pattern was not microtubule-like were the principal cells of the collecting ducts in kidney cortex, in which diffuse fluorescence was seen. MAP 4 antibody did not react with microtubule-rich neuronal elements of the central and peripheral nervous system, skeletal muscle from anterior thigh, liver parenchymal cells, columnar epithelial cells of the small intestine, and absorptive cells of the tubular component of the nephron. These observations indicate that MAP 4 may be associated with only certain kinds of cell functions as demonstrated by the preferential distribution with microtubules of defined cell types.

Microtubule associated proteins (MAP)[1] have been isolated from brain tissue and cultured cells, and thus far, analysis of the cytological distribution of these proteins has been confined to studies of these sources. Immunofluorescent and immunoelectron microscopic analysis have shown that MAP are localized on microtubules or in microtubule-like patterns in cultured cells (2, 4, 8–10, 12, 14, 17, 24, 25). These studies have also demonstrated, however, that MAP antibodies reacted to various degrees with cultured cell lines, even when species specificity was not a factor. For example, a monoclonal antibody raised to brain MAP 2 labeled only mouse primary brain cells and differentiated neuroblastoma cells (16). Antibodies to tau and high molecular weight MAP stained a wide variety, but not all, cell types (8). In contrast, antibodies to a 210,000-mol-wt MAP (4) and MAP 1A (2) showed extensive cross-reaction with cells representative of the four major tissue types.

Although the studies have been more limited, distribution of MAP in tissues appears to be more restricted than indicated from the analyses of cultured cells. Immunocytochemical studies have shown association of MAP 2 with the marginal band of nucleated erythrocytes (27) and MAP 1 and MAP 2 in brain structures rich in microtubules (3, 5, 6, 15, 18). Within brain tissue, MAP 1 and MAP 2 are preferentially distributed in neurons (3, 15) and neural components (3, 5, 6, 15, 18); MAP 1A is also seen in oligodendrocytes (3). With the exception of MAP 2 localization in nucleated erythrocytes (27), however, MAP 1 and MAP 2 appear to be restricted to neural tissues (15); no cross-reactive polypeptides have been found in liver and kidney homogenates analyzed with a monoclonal MAP 2 antibody (16), or in liver, spleen, kidney, and muscle homogenates probed with a monoclonal MAP 1 antibody (15). In contrast to the reports on MAP 1 and MAP 2, we have recently shown that antibodies to another high molecular weight MAP, MAP 4, react with proteins of similar molecular weight in extracts and microtubule preparations of mouse brain, heart, liver, and lung (22). To correlate these

[1] *Abbreviations used in this paper:* CNS, central nervous system; MAP, microtubule-associated protein.

THE JOURNAL OF CELL BIOLOGY · VOLUME 99 DECEMBER 1984 2287–2296

biochemical observations with the possible functions of MAP 4, we used affinity-purified antibodies to examine the location of this MAP in semi-thin sections of brain, spinal cord, testis, liver, heart, skeletal muscle, and small intestine. The results demonstrate that MAP 4 is primarily associated with microtubules in situ, but that distribution of this MAP is limited to defined cell types within each tissue.

MATERIALS AND METHODS

Tissue Preparation: BALB/C mice were anesthetized and perfused intracardially with Hank's buffered saline solution followed by 0.4% glutaraldehyde, 1.0% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2. Tissues were excised and subsequently processed as described by Wolosewick and DeMey (28) except that the embedding material was composed of 18% polyethylene glycol 1500 in polyethylene glycol 4000 (J. T. Baker Chemical Co., Phillipsburg, NJ).

Immunofluorescence Microscopy: Sections 0.5–0.75-μm thick were processed for immunofluorescence according to Wolosewick and DeMey (28). Affinity-purified tubulin antibody was the generous gift of Dr. Jan De Mey (Janssen Pharmaceutica, Beerse). Glial fibrillary acidic protein antibody was the gift of Ms. C. delCerro (University of Rochester). MAP 4 antibody was affinity purified using the appropriate region of diazotized paper blots of mouse brain or heart extract (20, 22). Fluorescent and phase-contrast images were recorded on Tri-X film and developed in Diafine.

RESULTS

Nervous System

Representative sections from three areas of the central nervous system (CNS), the spinal cord, the cerebral cortex, and the cerebellar cortex, were labeled with MAP 4 antibody and tubulin antibody, and immunofluorescent labeling patterns of each antibody were compared. As described below, tubulin labeling was prominent in all cell types, but MAP 4 labeling was confined primarily to glial elements within each region of the CNS.

The distribution of MAP 4 was examined in both the white (*Wh*) and gray (*Gr*) matter of the spinal cord. As shown in Fig. 1 *a*, MAP 4 staining was restricted to a thin rim peripheral to the myelin sheaths surrounding the axons in the white matter of the cord (arrows). Electron microscopic observations have demonstrated that cytoplasmic portions of oligodendrocytes, the cells that produce the myelin layers in the CNS, are wrapped around the myelin sheath (11), and it is likely that MAP 4 is localized to these cell regions. No MAP 4 labeling was evident within the axon. In contrast, as shown in Fig. 1 *b*, tubulin antibody labeled the axons (*Ax*) intensely. The oligodendrocyte cytoplasm wrapping was also labeled with tubulin antibody (Fig. 1 *b*, *inset*); in this figure, this wrapping is not as prominent as the axonal labeling. In the gray matter of the spinal cord, MAP 4 antibody labeled only a few cell bodies; these had the morphology of two major glial elements, astrocytes and oligodendrocytes. In Fig. 1 *a*, only flecks of fluorescence, corresponding to portions of glial cell processes, are seen in the gray matter. The bulk of the gray matter, consisting of cell bodies and processes of the neural elements, was not labeled with MAP 4 antibody (Fig. 1 *a*). In contrast, the gray matter was intensely labeled with tubulin antibody (Fig. 1 *b*).

The localization of MAP 4 to astrocytes (not shown) and oligodendrocytes (*O*) was also observed in sections of cerebral cortex (Fig. 1, *c* and *e*), although the full extent of the processes of these cells could not be seen because of the thinness of the sections. Blood vessel walls in all areas of the CNS including the brain stem (Fig. 1 *d*) were also prominently labeled with MAP 4 antibody. As is evident in these figures, MAP 4 was not present in the abundant neural components of the cerebral cortex, although sections incubated with tubulin antibody showed bright labeling of neural and glial cell bodies and processes throughout the cerebrum (not shown), consistent with the unbiquitous presence of microtubules in brain.

The restricted localization of MAP 4 was also seen in cerebellum. Sections of the inner core of white matter (Fig. 1 *f*) showed labeling peripheral to the myelin sheaths similar to that seen in the spinal cord (arrow). The cerebellar cortex, however, contained fibers that were very strongly labeled and spanned the entire molecular layer (Fig. 1 *g*, double arrowheads). These fibers originated from a set of cells surrounding the Purkinje cells in the granular layer. Within the cell bodies, a fibrillar pattern labeled with MAP 4 antibody was visualized. The morphology of these cells is characteristic of the Bergmann glia (*BG*) of the cerebellar cortex; these astrocytes are unusual in the length and specific distribution of the processes (21). We confirmed the astrocytic origin of these MAP 4-positive fibers, as well as cells in other parts of the CNS, by labeling sections with antibodies to glial fibrillary acidic protein, a marker for astrocytes. The parallel Bergmann glial fibers could also be seen in a section of cerebellar cortex labeled with tubulin antibody (Fig. 1 *h*). Short pieces of Purkinje cell dendrites (large arrows) and microtubules in the cell bodies of the Purkinje cells (*Pu*) were also visualized in the 0.5-μm sections stained with tubulin antibody (Fig. 1 *h*).

MAP 4 and tubulin patterns similar to those seen in the CNS were also observed in peripheral nerves in sections of anterior thigh muscle (not shown). MAP 4 antibody labeled connective tissue, as well as a thin rim of cytoplasm surrounding the myelin sheaths. This latter observation suggested that Schwann cell cytoplasm in the peripheral nervous system was labeled by MAP 4 in a manner similar to the oligodendrocytes of the CNS (Fig. 1 *a*). As in the CNS, MAP 4 could not be detected in axons. In contrast, tubulin antibody labeled the axons and bits of connective tissue surrounding the nerve fiber. All of these data suggest that while microtubules are widely distributed in the CNS, MAP 4 is primarily associated with the microtubules of glial elements. The only exception to this distribution was the labeling of one small group of neuron cell bodies in brain stem. A more extensive examination of other regions of the nervous system should reveal whether MAP 4 is a marker for a subset of neurons, as well as having widespread distribution in glia.

Liver

Immunoblots had shown strong reaction of liver extract with MAP 4 antibody (22), and we wished to establish where MAP 4 was localized in this morphologically simple tissue. As shown in Fig. 2 *a*, endothelial cells (*E*) and Kuppfer (*K*) cells lining the hepatic sinusoids reacted with MAP 4 antibody; distinct microtubules could be readily visualized in the cytoplasm of the Kuppfer cells (Fig. 2 *a*, *inset*). The remainder of the sinusoidal lining was labeled in a bright, discontinuous manner (double arrows); this pattern probably arises from the presence of microtubules in Kuppfer cell and endothelial cell processes that line the sinusoid. MAP 4 was also found in the endothelial cells and adventitia of the vein and artery of the hepatic triad, as well as in the cuboidal cells of the bile duct. It is likely that the cells of other portions of the bile duct

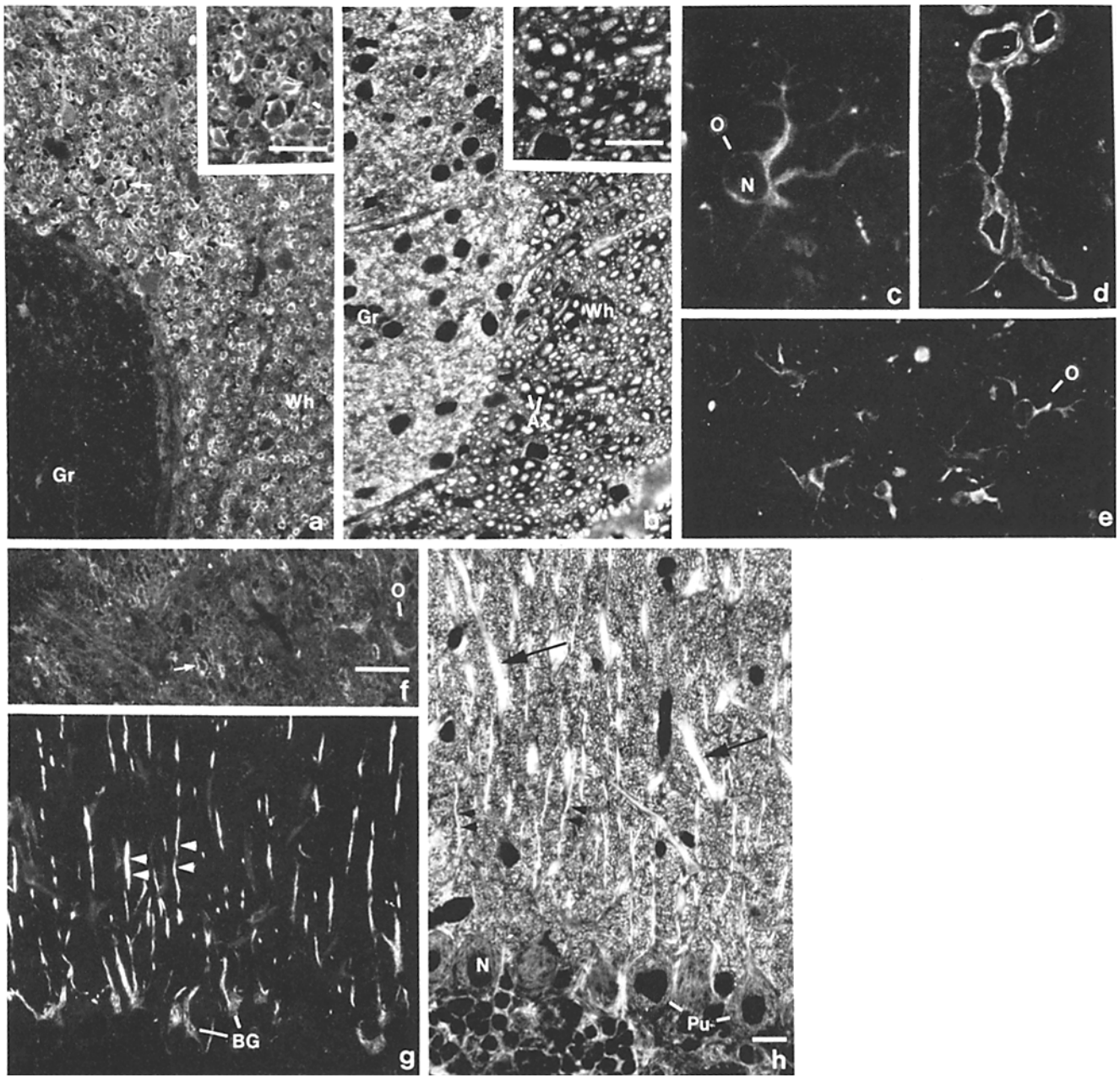

FIGURE 1 Nervous system. Sections of spinal cord (*a* and *b*), cerebral cortex (c and e), brain stem (*d*), and cerebellum (*f–h*) labeled with MAP 4 (a, c, and *d–g*) or tubulin (*b* and *h*) antibody. MAP 4 labeling is restricted to glial elements. MAP 4-labeled oligodendrocyte cytoplasm is seen as a wrapping around myelin sheaths in white matter of spinal cord (*a*, *inset*) and cerebellum (*f*, small arrow). Oligodendrocyte (*O*) cell bodies and processes are more clearly seen in the gray matter of the cerebral cortex (c and e). Blood vessel endothelia and adventitia are labeled in all parts of the CNS as shown in *d*. Astrocytes (Bergmann glia) in the cerebellar cortex (*g*) are intensely labeled with MAP 4 antibody; a fibrillar pattern can be detected in the glial cell bodies (*BG*) and MAP 4 is found throughout their processes (arrowheads). Tubulin antibody (*b*) intensely labels cell bodies (not shown) and processes in the gray area (*Gr*) of the spinal cord and axons (*Ax*) in the white matter (*Wh*); myelin sheaths are negative. Tubulin antibody (*h*) also labels microtubules in Purkinje cell bodies (*Pu*) and their dendritic processes (large arrows) as well as the processes of the Bergmann glia (arrowheads). Nuclei (*N*) (c and *h*) are not labeled with tubulin or MAP 4 antibody. Bars, 10 μm; a–e, *g*, and *h*, × 550; *f* and *insets*, × 900.

system, not easily identified, also contain MAP 4. No immunofluorescence with MAP 4 antibody was detected in the hepatocytes (Fig. 2*a*).

Tubulin labeling demonstrated patterns similar to those obtained with MAP 4 antibody in Kuppfer cells, endothelial cells, the lining of the hepatic sinusoids, and other blood vessels. However, as shown in Fig. 2*b*, parenchymal cells, negative for MAP 4, contained abundant microtubules. These hepatocyte microtubules appeared as curving segments of different lengths, which suggest that the microtubules were not arranged in a highly ordered array. There were areas of liver in which the parenchymal cells contained more microtubules than in adjacent areas. This observation suggests that microtubule-dependent activity of hepatocytes may differ

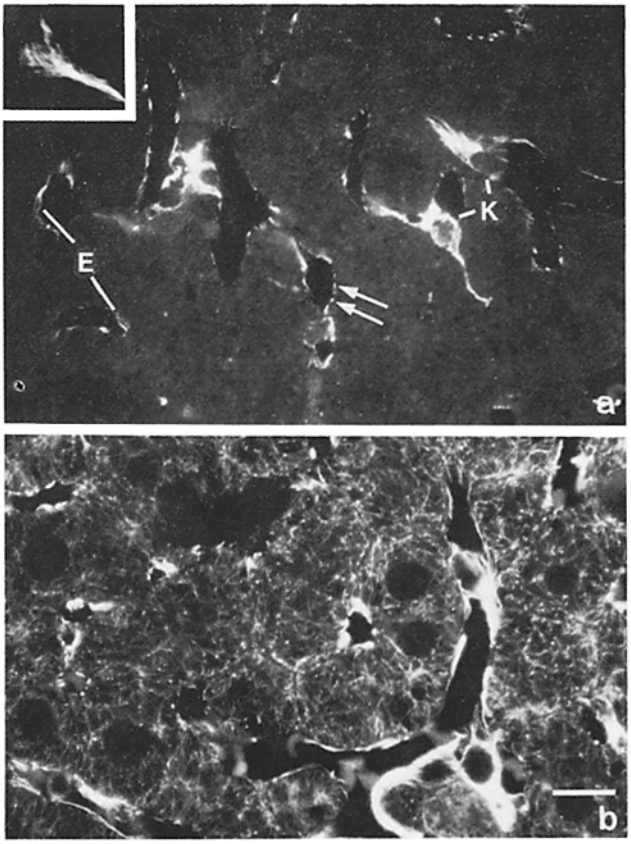

FIGURE 2 Sections of liver labeled with MAP (*a*) or tubulin (*b*) antibody. (a) MAP 4 labels microtubules in both cell types of the sinusoidal lining, Kuppfer cells (*K*), and endothelial cells (*E*). Microtubules in the processes of these cells are seen as discontinous label in other parts of the sinusoid (arrows). (*inset*) A Kuppfer cell labeled with MAP 4 antibody in which microtubules are clearly displayed. (*b*) Note the unordered array of microtubules in liver parenchymal cells labeled with tubulin antibody; these microtubules are not labeled with MAP 4 antibody. Bar, 10 μm × 900.

among portal areas of the liver. In summary, these data indicate that tubulin and MAP 4 do not co-distribute in parenchymal cells but do appear to be associated in other cell types in liver.

Small Intestine

The small intestine consists of highly organized cell layers in which the distribution of microtubules and MAP 4 can be readily compared. As shown in Fig. 3*b*, the columnar epithelial cells of the intestinal villi labeled intensely with tubulin antibody in the apical and Golgi regions; this pattern is consistent with fine structural observations that indicate microtubules are abundant in this epithelium (23). These prominent parallel arrangements of microtubules were not labeled with MAP 4 antibody (Fig. 3*a*). Tubulin and MAP 4 labeling were coincident, however, in the connective tissue elements of the intestinal villus core and base of the villus. MAP 4 was present in the endothelia of capillaries and central lacteals and in smooth muscle cells of the wall of the small intestine (see below and Fig. 4*f*). These results demonstrate that in small intestine, the primary distribution of MAP 4 is in connective tissue cell types, and not in association with microtubules in the absorptive epithelia.

Muscle

Sections of cardiac muscle, smooth muscle from the small intestine, and skeletal muscle from two different sources were examined with tubulin and MAP 4 antibody. In each case, MAP 4 antibody labeling was much weaker than that of tubulin antibody. With the exception of anterior thigh muscle, the immunofluorescent patterns of the two antibody types were coordinate. In each muscle type, presumptive connective tissue cells and blood vessels were also labeled with both tubulin and MAP 4 antibodies.

Skeletal muscle fibers from the orbit and from the anterior thigh were examined. In sections of skeletal muscle from either source, microtubules were clearly delineated with tubulin antibody (Fig. 4*a*). Most microtubules were axially disposed, although some microtubules were arranged perpendicular to the myofibril and spanned several myofibrils before either terminating or becoming lost from the plane of section (Fig. 4*a*). Because of the thinness of the section, it was not possible to assess whether the microtubules were continuous, but it did appear that the perpendicularly arranged microtubules abutted the axially oriented ones in a "T." Some microtubules in skeletal muscle were cut in short segments in a manner that indicated microtubules might also be wrapped helically around the myofibril. Although tubulin antibody labeling indicated microtubules were present in both types of skeletal muscle examined, we could detect MAP 4-labeled

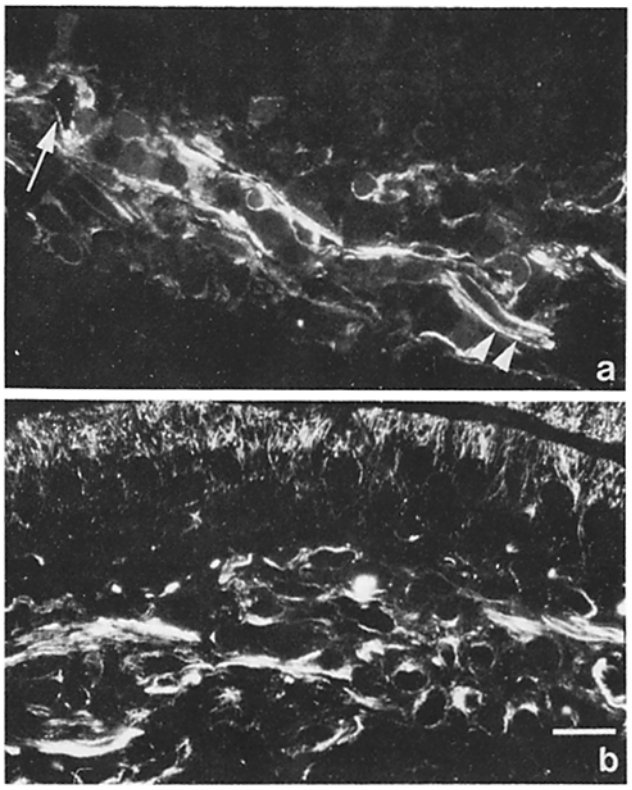

FIGURE 3 Small intestine. A portion of an intestinal villus labeled with MAP 4 (*a*) and tubulin (*b*) antibody. Blood vessel endothelia (arrow), presumed smooth muscle cells (arrowheads), and many other connective tissue elements of the villus core label with MAP 4 antibody in a manner similar to that of tubulin antibody. MAP 4 antibody does not label the microtubule segments in the apical region of the absorptive epithelium (*top* of *a*) that are visualized with tubulin antibody (*top* of *b*). Bar, 10 μm × 850.

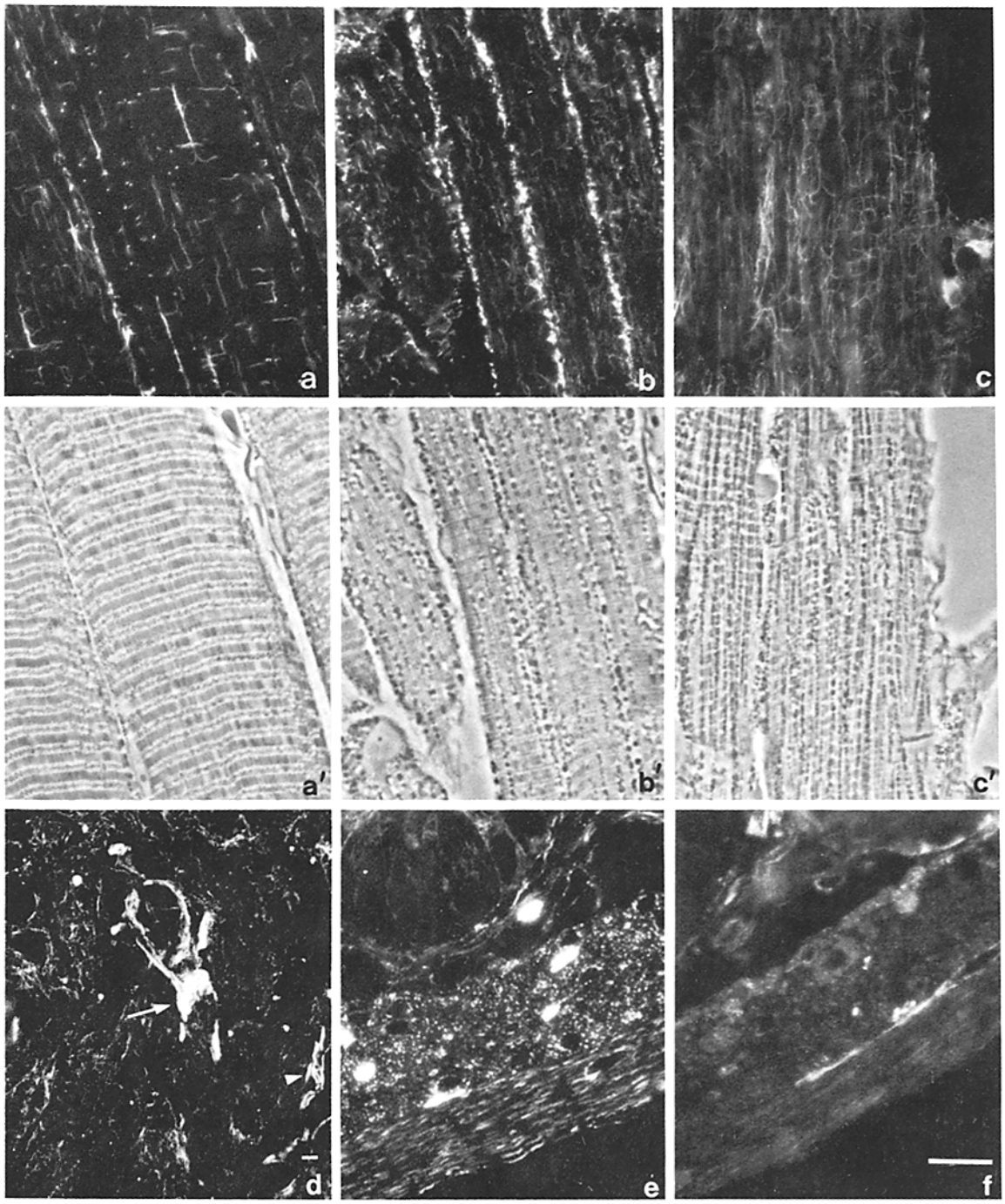

FIGURE 4 Muscle. Sections of skeletal (anterior thigh [*a*, *a'*] and eye [*b*, *b'*]), cardiac (c, c', and *d*), and smooth muscle (e, *f*) labeled with MAP 4 (*b*, *d*, and *f*) or tubulin (*a*, c, and e) antibody. Although weaker in intensity, the MAP 4 antibody labeling patterns are similar to those seen with tubulin antibody. MAP 4 antibody labels microtubules of cardiac muscle (Fig. 4*d*) but not anterior thigh skeletal muscle (not shown; see Results). In Fig. 4*d*, a perivascular cell (arrowhead) and presumptive connective tissue (arrow) are also labeled with MAP 4 antibody. Corresponding phase-contrast images (a', b', and c') are shown for *a*, *b*, and c, respectively, to indicate the size of the myofibril relative to the microtubule pattern. Bar, 10 μm × 1,000.

microtubules only in eye muscle (Fig. 4*b*) and not in anterior thigh muscle (not shown). We have previously used MAP 4 antisera to examine samples of anterior thigh muscle by immunoblot and have found no cross-reactive polypeptides (22). These data suggest that MAP 4 may be distributed only with the microtubules of certain classes of skeletal muscle fiber types.

The microtubule patterns in cardiac muscle sections appeared more confusing than those of skeletal muscle, in part because cardiac muscle fibers branch and are less aligned (Fig. 4*c'*) than skeletal muscle fibers (Fig. 4*a'*). Both MAP 4 and tubulin antibody labeling patterns were similar. As shown in an oblique section of cardiac muscle (Fig. 4*d*), microtubules were labeled with MAP 4 antibody. The antibody can also be seen to react with a perivascular cell (arrowhead) and the presumptive connective tissue surrounding a nerve bundle

(arrow). Helically arranged microtubules were seen more frequently in cardiac muscle than in skeletal muscle; the remainder of heart muscle microtubules had an axial orientation (Fig. 4*c*). These immunocytological observations are consistent with the differences in microtubule distribution seen in electron microscopic studies of skeletal muscle (7) and cardiac muscle (13).

Only short segments of microtubules were seen in the smooth muscle wall of the small intestine labeled with tubulin antibody, and all of these microtubules were arranged in a direction parallel to the length of the smooth muscle fibers (Fig. 4*e*). The microtubules appeared as dots in cross sections of the inner circular layer and as segments of up to 5–7 μm in the longitudinal layer. As in skeletal and cardiac muscle, MAP 4 antibody labeled microtubules in the same pattern as tubulin antibody, but with much decreased intensity (Fig. 4*f*). We identified no area in any of the muscle types from which microtubules appeared to emanate.

Testis

Cross sections of seminiferous tubules from mouse testis were used to examine the occurrence of MAP 4 at various stages of sperm development. As seen in Fig. 5*a*, parallel arrangements of abundant microtubules and bright, V-shaped or semicircular rims of fluorescence around nuclei were labeled with MAP 4 antibody. These two immunofluorescent patterns are indicative of Sertoli cells (*S*) and the manchette (*M*) of spermatids, respectively (28). MAP 4 was also localized as dots of fluorescence around the nuclei of some cells near the lumen of the seminiferous tubule (Fig. 5*b*); this pattern may reflect the initial development of manchette microtubules, which become arranged helically around the spermatid nucleus (19). No labeling of cytoplasmic or axonemal microtubules at any later stage of sperm development was obtained with MAP 4 antibody. Since MAP 4 was associated with microtubules of the manchette, but not with other cytoplasmic microtubules, MAP may play a role in manchette integrity or function during spermatogenesis. In addition to the manchette microtubules, labeling of cytoplasmic microtubules with MAP 4 was found in the supportive Sertoli cells (Fig. 5*a*), the endothelia of the peritubular lymphatics, and in the endothelia and surrounding connective tissue of associated blood vessels (not shown). Mature sperm did not label with MAP 4 antibody (Fig. 5, *b* and *b'*). All of these data are consistent with our previous immunoblot analyses that indicate none of the MAP 4 bands were present in mature sperm (22), whereas whole testis contained significant amounts of MAP 4 (Parysek, L. M., and J. B. Olmsted, unpublished observations). Although cross sections of seminiferous tubules contain many mitotic cells, mitotic spindles, labeled with tubulin antibody, were never labeled with MAP 4 antibody.

The labeling of Sertoli cells and spermatid manchettes with tubulin antibody was identical to that described for MAP 4 antibody. Tubulin antibody also delineated the cytoplasmic microtubules of the spermatogonia and developing spermatocytes and the tails of mature sperm (Fig. 5, *d* and *e*). These labeling patterns on mouse testis are similar to the results previously described for the distribution of tubulin in rat testis (28).

Kidney

We previously determined that kidney extracts contained little if any MAP 4, but that this organ did contain an immunologically related, non-microtubule binding component designated band 4 (22). Kidney cortex was therefore examined for microtubule-like and nonmicrotubule-like labeling pattens with MAP 4 antibody. Initial examination of sections indicated that there were very small amounts of tissue labeled by MAP 4 antibody. This paucity of reactive material is consistent with the lack of reaction in kidney extracts

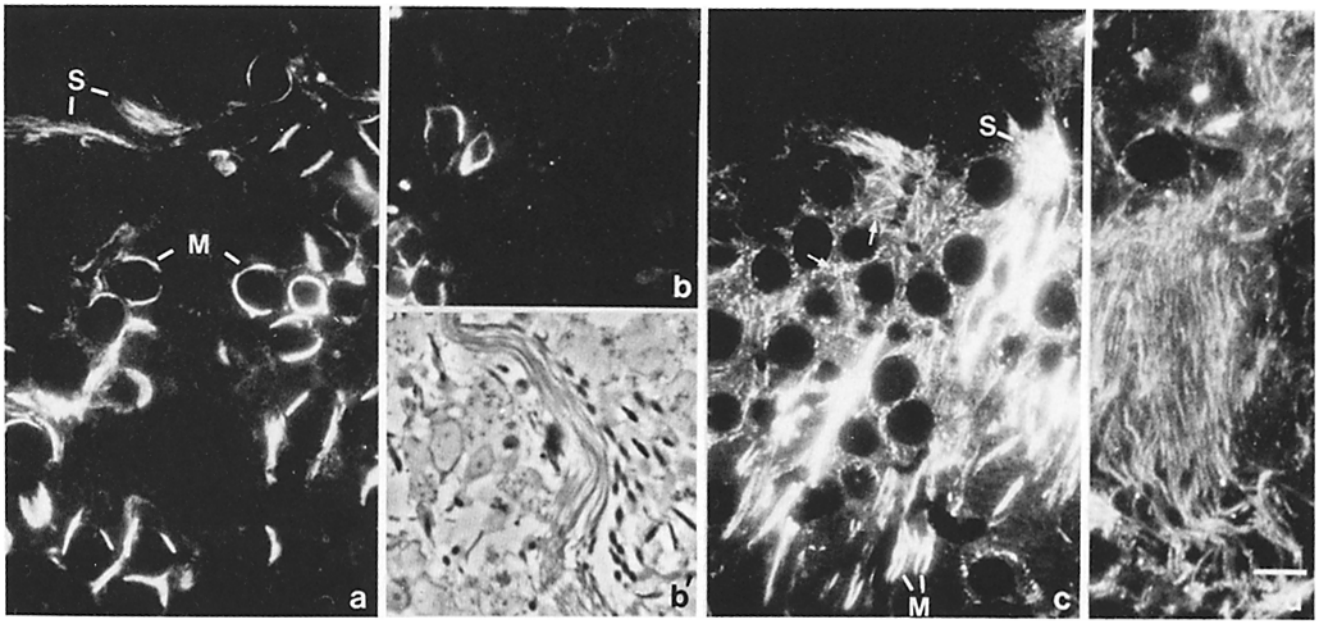

FIGURE 5 Testis. Cross sections of seminiferous tubules labeled with MAP 4 (*a* and *b*) or tubulin (*c* and *d*) antibody. MAP 4 labels the abundant microtubules in Sertoli cell cytoplasm (*S*) and spermatid manchettes (*M*). A section labeled with MAP 4 antibody showing absence of axonemal labeling (*b*) (corresponding phase-contrast image, b') is shown for comparison with axoneme labeling seen with tubulin antibody (*d*). Other microtubules that are visualized with tubulin antibody, but not with MAP 4 antibody, include cytoplasmic microtubules (arrows) of spermatogonia and spermatocytes (*c*). Bar, 10 μm × 750.

analyzed by immunoblotting (22). The major component detected with MAP 4 antibody was connective tissue, visible as a rim of fluorescence surrounding the cross sections of the tubular components of the nephron and blood vessel (*BV*) endothelia and adventitia (Fig. 6*a*). The arterioles of the vascular pole of the glomerulus and the peritubular capillary network and glomerular capillary network were all delineated by MAP 4 antibody labeling.

In addition to labeling connective tissue and vascular elements of the kidney cortex, MAP 4 antibody selectively labeled the podocytes (*P*) in the glomerulus, the epithelial lining of Bowman's capsule, and a subset of cells in the collecting ducts. The cells of the collecting ducts were the only case in which cytoplasmic labeling with MAP 4 antibody was different in distribution than that of tubulin antibody. MAP 4 antibody reacted uniformly in the cytoplasm of these cells (Fig. 6*a*), and we postulate that the pattern that is not microtubule-like may be related to the presence of band 4 in kidney extract. The collecting duct is comprised of two types of cells, light (*L*) (principal) cells and less numerous dark (*D*) cells, and it appears that the light cells alone contain MAP 4. As shown in Fig. 6*a'*, the dark cells are fewer in number but have greater numbers of granules, corresponding to mitochondria, than MAP 4-positive light cells. These two cell types are representative of the two different embryologic origins of the collecting duct, namely, the metanephrogenic blastema and the ureteric bud (11). The restricted distribution of MAP 4 is consistent with the different origins of these two cell types.

Tubulin antibody did not preferentially label light or dark cells of the collecting ducts. Rather, collecting duct cells were dense with a relatively unordered pattern of microtubules. The other portions of the tubular component of the nephron possessed regular, parallel arrangements of microtubules, although microtubule numbers varied. For example, the proximal segment, identified by its brush border in phase-contrast microscopy (*Pr*, Fig. 6, *b'* and *c'*), contained many fewer microtubule profiles (*Pr*, Fig. 6, *b* and *c*) than the distal segment of the convoluted tubules (*Di*, Fig. 6, *b* and *c*). Podocytes (*P*) labeled very intensely with tubulin antibody (Fig. 6*b*), corroborating ultrastructural work showing that cytoplasmic microtubules are abundant in these cells (1). Bits of connective tissue surrounding the kidney tubules, the thin rim of cytoplasm of the capillaries, and other larger blood vessels are all labeled with the tubulin antibody in a manner similar to that seen with MAP 4 antibody.

DISCUSSION

MAP 4 Is Associated with Microtubules In Situ

Immunofluorescence microscopy has enabled investigators to illustrate that MAP distribution has a microtubule-like pattern in cultured cells (2, 4, 8–10, 14, 17, 24, 25). Light microscopic studies of brain tissue have also shown that MAPs are localized to microtubule-containing elements (3, 15). However, largely because of the dense, parallel arrangements of microtubules in neuronal elements, and the thickness of sections used in these studies of tissues, it has not been possible to resolve whether MAP was associated with microtubules in all structures labeled. In the results presented here, greater light microscopic resolution of MAP-labeled structures was achieved by immunolabeling 0.5–0.9-μm sections of polyethylene glycol-embedded tissues. This enabled analysis of the organization of individual microtubules in large areas of tissue. Highly ordered arrays of microtubules, such as those labeled with tubulin antibody in the columnar epithelia of the small intestine, were clearly defined; the uniform density and thickness of the labeling has suggested that individual microtubules are visualized in such sections (28). Microtubules labeled with MAP 4 antiserum were evident in the Kuppfer cells in the liver (Fig. 2*a*), in each of the muscle fiber types (Fig. 4), and in Sertoli cells in testis (Fig. 5*a*). Therefore, although MAP 4 was confined to certain cell types, the labeling patterns indicated that MAP 4 was primarily distributed with microtubules in vivo. The only tissue in which MAP 4 labeling did not appear microtubule-like was in the light (principal) cells of the collecting ducts in kidney (Fig. 6*a*); this pattern contrasted with that obtained with tubulin antibody, in which microtubules were shown to be present in these cells. We have previously found that kidney extracts, which do not contain the MAP 4 triplet, do contain a polypeptide, band 4, that is immunologically related to MAP 4 (22). This protein does not assemble with microtubules in vitro, and it is possible that this non–microtubule binding antigen gives rise to the diffuse labeling in the light cells of the kidney cortex. The function that this polypeptide may have in light cells remains to be determined.

Cytological Distribution of MAP 4

The results on the tissue distribution of MAP 4 are summarized in Table I. Where microtubules could be discerned in the MAP-4-positive cells listed, MAP 4 and microtubule distribution were coincident, suggesting that this polypeptide was a microtubule-associated protein in vivo. MAP 4 was confined, however, to a specific subset of cells in each tissue type. These can be categorized as "supportive" cell types such as Sertoli cells, Kuppfer cells, glia, muscle, endothelia, and other cells of loose connective tissue. Because MAP 4 is so restricted, this MAP may be useful as a marker for the origin of particular cell types in tissues. For example, there are glia in retina and the third ventricle that are thought to be homologues of the Bergmann glia in the cerebellar cortex, and we have identified MAP 4 in all of these cell types (Parysek, L. M., M. del Cerro, and J. B. Olmsted, manuscript submitted). The origin of Kuppfer cells in liver has also been debated, although it has been commonly suggested that these cells are resident macrophages (11). We have examined isolated peritoneal macrophages and bone marrow sections and found no MAP 4 labeling in macrophages (L. M. Parysek, J. Daiss, and J. B. Olmsted, unpublished observations). Thus, if Kuppfer cells are resident macrophages, MAP 4 must be expressed differently in macrophages depending on their tissue location.

The examination of MAP distribution in tissues has, thus far, largely been confined to analyses of brain tissue, and comparison of the results on MAP 1 and MAP 2 with our results on MAP 4 show markedly different patterns. The distribution of MAP 1 and MAP 2 is restricted to neural elements (15). In contrast, MAP 4 is present in glial elements. These data suggest that the functions of each of these MAP may be discrete, and cell type specific. A recent report by Huber and Matus (15) refers to monoclonal antibodies that are specific for both glia and axons. How all of these MAP are integrated in CNS function remains to be determined, and may be approachable by examining animals in which particular developmental or functional defects exist.

The cell type specificity of MAP distribution seen in our

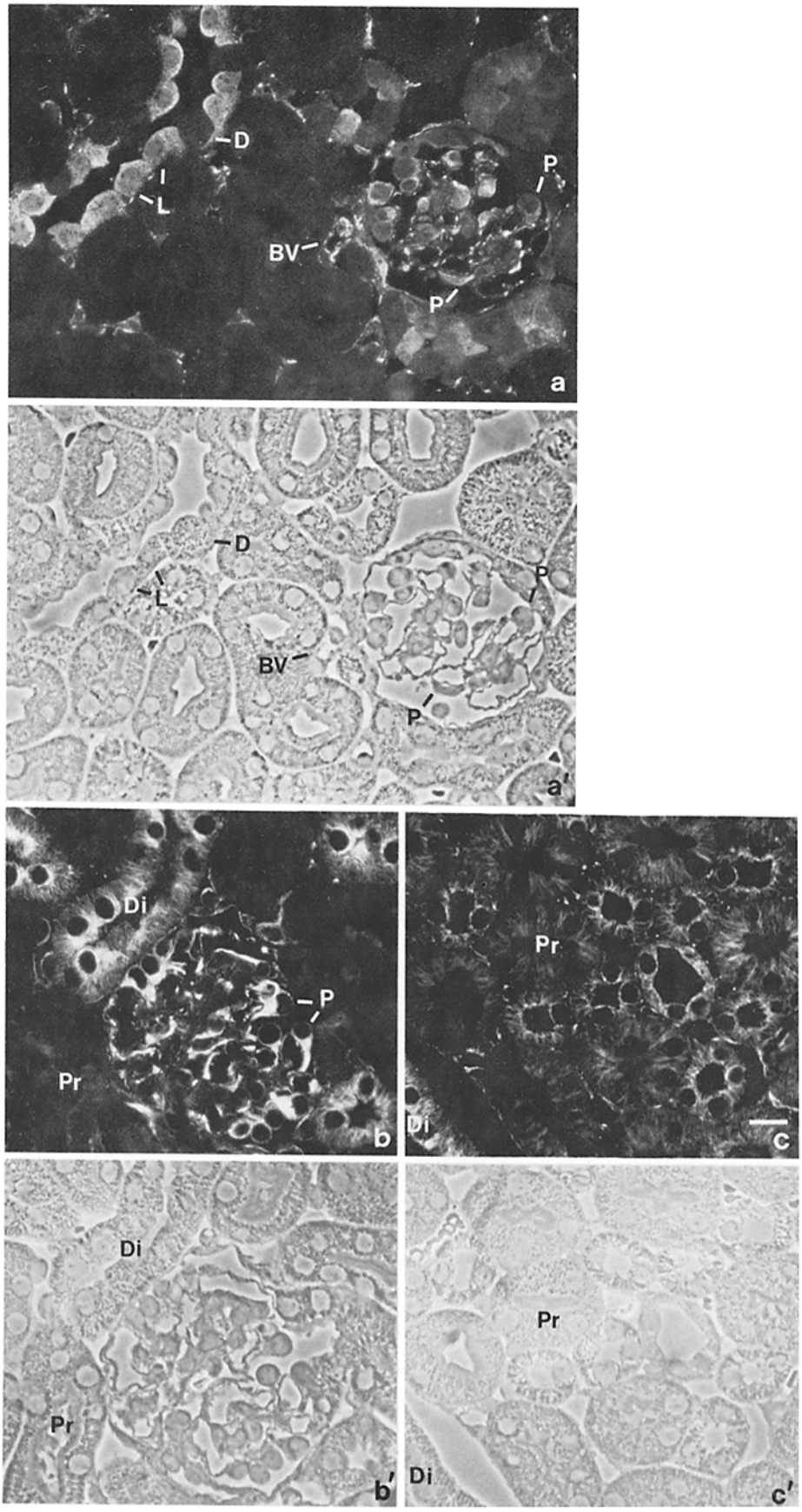
D
L
BV
P
P'
a
D
L
BV
P
P
a'
Di
P
Pr
b
Pr
Di
c
Di
Pr
b'
Pr
Di
c'

TABLE I

Cell Type Distribution of MAP

Tissue	Cell type containing microtubules	MAP 4
CNS	Neurons and processes	−
	Glial elements	+
Testis	Sertoli cells	+
	Spermatogonia, spermatocytes, mature sperm (cytoplasmic microtubules)	−
	Spermatid manchettes	+
Liver	Parenchymal cells	−
	Kuppfer cells, endothelial cells	+
Small intestine	Absorptive cells (columnar epithelia)	−
	Connective tissue in and around villi	+
	Smooth muscle	+
Heart	Muscle fibers	+
Skeletal muscle	Eye muscle fibers	+
	Anterior thigh muscle fibers	−
Kidney	Absorptive cells of tubule	−
	Connective tissue around tubules	+
	Podocytes and glomerular epithelium	+
All tissues	Blood vessel endothelia and adventitia	+
	Lymphatics in testis and small intestine	+

studies and those of Bloom et al. (3) and Huber and Matus (15) is not congruent with the broader distribution of MAP seen in studies of cultured cell lines. For example, antibodies to a 210,000-mol-wt MAP from HeLa (4), or to MAP 1A (2), have shown reactivity with cultured cells derived from each of the four major tissue types. Although various MAP may show different specificities in situ, these data suggest that the occurrence of MAP in continuously growing cultures may differ from that in vivo. For example, we have not seen MAP 4 associated with mitotic spindles in vivo, although antibodies to MAP 1A (2), a 200,000-mol-wt MAP (17), and a 210,000-mol-wt MAP (4) all localized to spindles in cultured cells. By comparing tissue and cell line distributions, it may be possible to establish whether the induction or loss of MAP expression is affected during the transition from in vivo to in vitro conditions.

Functions of MAP 4?

Since microtubule structure is highly conserved, the heterogeneity of MAP distribution may reflect diversity in microtubule functions in cells. The localization of MAPs to defined cell types (reference 3, and 15 and this report) suggests that MAP 1 and 2 play specific roles in neuronal elements and that MAP 4 is associated with microtubule functions common to supportive and connective tissue types of cells. As yet, very little is known about functions of MAP 4-positive cells, such as Sertoli cells and glial cells, and it is therefore difficult to speculate on the role of MAP 4 in these cell types. The localization of MAP 4 to microtubules in muscle appears highly specific, yet little is known about the function microtubules may have in this tissue type. There is a dearth of information on how microtubules may function in many cell types in situ and the possibility that MAP 4 may have a role in stabilizing microtubule arrays or in intracellular transport within supportive cell types cannot be eliminated. However, because we have identified microtubules in cells in each tissue with which MAP 4 is not associated, it is possible to speculate about cellular functions for which this MAP is not required. For example, the absence of MAP 4 in neurons indicates that this MAP may not play a role in axonal transport. The microtubules of hepatocytes are thought to be important in the secretion of albumin and very low density lipoproteins from the liver (26). Since MAP 4 was not associated with these parenchymal cell microtubules, it probably does not function in directed movement of these materials in liver. The studies of Reaven and Reaven (23) have suggested that microtubules play a role in lipid transport in the absorptive epithelial of small intestine, and MAP 4 is also absent from this epithelium. As discussed previously, MAP 4 also does not appear to be associated with the mitotic apparatus in situ, at least in highly proliferative cells of the tissues we examined. By comparing the tissue distribution of MAP 4 with other MAP, it may be possible to establish whether two dissimilar MAP perform the same function in different cell types, or whether MAPs are germ-layer specific and result in defined distributions in the adult organism.

The authors thank Drs. Russell E. Hayes and Manuel del Cerro for aid in interpretation of some of the labeling patterns. We also thank Hiram D. Lyon for his help during certain portions of this work.

L. M. Parysek is a Fellow of the Leukemia Society of America. The work was supported by National Institutes of Health grant GM 22214 awarded to J. B. Olmsted.

Received for publication 13 April 1984, and in revised form 25 June 1984.

REFERENCES

1. Bloom, W., and D. W. Fawcett. 1975. A Textbook of Histology. W. B. Saunders Co., Philadelphia. 10th ed. 698–700 and 770–772.
2. Bloom, G. S., F. C. Lucas, and R. B. Vallee. 1984. Widespread cellular distribution of MAP 1A (microtubule-associated protein 1A) in the mitotic spindle and on interphase microtubules. *J. Cell Biol.* 98:331–340.
3. Bloom, G. S., T. A. Schoenfeld, and R. B. Vallee. 1984. Widespread distribution of the major polypeptide component of MAP 1 (microtubule-associated protein 1) in the nervous system. *J. Cell Biol.* 98:320–330.
4. Bulinski, J. C., and G. G. Borisy. 1980. Widespread distribution of a 210,000 mol wt microtubule-associated protein in cells and tissues of primates. *J. Cell Biol.* 87:802–808.
5. Burgoyne, R. D., and R. Cumming. 1983. Characterization of microtubule associated proteins at the synapse; absence of MAP 2. *Eur. J. Cell Biol.* 30:154–158.
6. Caceres, A., M. R. Payne, L. I. Binder, and O. Steward. 1983. Immunocytochemical localization of actin and microtubule-associated protein MAP 2 in dendritic spines. *Proc. Natl. Acad. Sci. USA.* 80:1738–1742.
7. Cartwright, J., Jr., and M. A. Goldstein. 1982. Microtubules in soleus muscles of the postnatal and adult rat. *J. Ultrastruct. Res.* 79:74–84.
8. Connolly, J. A., and V. I. Kalnins. 1980. The distribution of tau and HMW microtubule-associated proteins in different cell types. *Exp. Cell Res.* 127:341–350.

FIGURE 6 Sections of kidney cortex labeled with MAP 4 (*a*) or tubulin (*b* and *c*) antibody. Podocytes in the glomerulus (*P*), capillaries and other blood vessels (*BV*), and connective tissue surrounding the tubular component of the nephron were labeled with MAP 4 antibody (*a*) in a pattern similar to that of tubulin antibody (*b* and *c*). Diffuse fluorescence fills the cytoplasm of the light (principal) cells (*L*) of the collecting ducts. The dark cells of the collecting ducts, identified by the large numbers of mitochondria (cell labeled *D*) are negative for MAP 4 labeling. MAP 4 does not associate with the microtubules of cells composing the nephronic tubules; these are seen with tubulin antibody. Distal convoluted tubules (*Di*) contain greater amounts of parallel microtubule profiles than proximal convoluted segments (*Pr*) or collecting ducts (not shown) (*b* and *c*). Compare phase-contrast images (*a'*, *b'*, and *c'*) with fluorescence images (*a*, *b*, and *c*) to identify nephron components. Bar, 10 μm × 600.

9. Connolly, J. A., V. I. Kalnins, D. W. Cleveland, and M. W. Kirschner. 1977. Immunofluorescent staining of cytoplasmic and spindle microtubules in mouse fibroblasts with antibody to τ protein. *Proc. Natl. Acad. Sci. USA.* 74:2437–2440.
10. Connolly, J. A., V. I. Kalnins, D. W. Cleveland, and M. W. Kirschner. 1978. Intracellular localization of the high molecular weight microtubule accessory protein by indirect immunofluorescence. *J. Cell Biol.* 76:781–786.
11. Copenhaver, W. M., D. E. Kelly, and R. L. Wood. 1978. Bailey's Textbook of Histology. Williams and Wilkins Co., Baltimore. 17th ed. 308–323 and 593.
12. DeBrabander, M., J. C. Bulinski, G. Guens, J. DeMey, and G. G. Borisy. 1981. Immunoelectron microscopic localization of the 210,000-mol wt microtubule-associated protein in cultured cells of primates. *J. Cell Biol.* 91:438–445.
13. Goldstein, M. A., and M. L. Entman. 1981. Microtubules in mammalian heart muscle. *J. Cell Biol.* 80:183–195.
14. Hill, A., R. Maunoury, and D. Pantaloni. 1981. Cellular distribution of microtubular-associated proteins HMW (350 K, 300 K) by indirect immunofluorescence. *Biol. Cell.* 44:43–50.
15. Huber, G., and A. Matus. 1984. Immunocytochemical localization of microtubule-associated protein 1 in rat cerebellum using monoclonal antibodies. *J. Cell Biol.* 98:777–781.
16. Izant, J. G., and J. R. McIntosh. 1980. Microtubule associated proteins: a monoclonal antibody to MAP 2 binds to differentiated neurons. *Proc. Natl. Acad. Sci. USA.* 77:4741–4745.
17. Izant, J. G., J. A. Weatherbee, and J. R. McIntosh. 1983. A microtubule-associated protein antigen unique to mitotic spindle microtubules in PtK1 cells. *J. Cell Biol.* 96:424–434.
18. Matus, A., R. Bernhardt, and T. Hugh-Jones. 1981. High molecular weight microtubule-associated proteins are preferentially associated with dendritic microtubules in brain. *Proc. Natl. Acad. Sci. USA.* 78:3010–3014.
19. McIntosh, J. R., and K. R. Porter. 1967. Microtubules in the spermatids of domestic fowl. *J. Cell Biol.* 35:153–173.
20. Olmsted, J. B. 1981. Affinity purification of antibodies from diazotized paper blots of heterogenous protein samples. *J. Biol. Chem.* 256:11955–11957.
21. Palay, S. L., and V. Chan-Palay. 1974. Cerebellar Cortex. Springer-Verlag, Berlin. 288–311.
22. Parysek, L. M., C. F. Asnes, and J. B. Olmsted. 1984. MAP 4: occurrence in mouse tissues. *J. Cell Biol.*, 99:1309–1315.
23. Reaven, E. P., and G. M. Reaven. 1977. Distribution and content of microtubules in relation to the transport of lipid. *J. Cell Biol.* 75:559–572.
24. Sherline, P., and K. Schiavone. 1977. Immunofluorescence localization of proteins of hig molecular weight along intracellular microtubules. *Science (Wash. DC).* 198:1038–1040.
25. Sheterline, P. 1978. Localization of the major high-molecular weight protein on microtubules in vitro and in cultured cells. *Exp. Cell Res.* 115:460–464.
26. Singh, A., Y. LeMarchand, L. Orci, and B. Jeanrenaud. 1975. Colchicine administration to mice: a metabolic and ultrastructural study. *Eur. J. Clin. Invest.* 5:495–505.
27. Sloboda, R. D., and K. Dickersin. 1980. Structure and composition of the cytoskeleton of nucleated erythrocytes. I. The presence of microtubule-associated protein 2 in the marginal band. *J. Cell Biol.* 87:170–179.
28. Wolosewick, J., and J. DeMey. 1982. Localization of tubulin and actin in polyethylene glycol embedded rat seminiferous epithelium. *Biol. Cell.* 44:85–88.