

Identification of High Molecular Weight Microtubule-Associated Proteins in Anterior Pituitary Tissue and Cells Using Taxol-Dependent Purification Combined with Microtubule-Associated Protein Specific Antibodies[†]

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ABSTRACT: The composition of microtubule-associated proteins (MAPs) in pituitary tissue and in a cultured pituitary cell line was examined. To isolate MAPs from the tissue, we modified our previously described taxol-dependent method for purifying microtubules and MAPs [Vallee, R. B. (1982) *J. Cell Biol.* 92, 435-442]. Microtubules were assembled from purified calf brain tubulin with the aid of taxol and were then added to a cytosolic extract of pituitary tissue which did not support microtubule assembly from endogenous tubulin. Pituitary MAPs bound to the microtubules and were isolated by centrifugation. Polypeptides corresponding in electrophoretic mobility to the component proteins of MAP 1 were among the major MAPs observed. Immunoblotting of the pituitary MAP fraction revealed the presence of appreciable quantities of MAP 1A, MAP 1B, and MAP 2 polypeptides. Microtubules were also prepared from GH₃ rat pituitary tumor cells by using a slight modification of our previously described taxol procedure. Immunoblotting indicated once again that MAP 1A, MAP 1B, and MAP 2 were included among the MAPs. These results were further confirmed by immunofluorescence microscopy of GH₃ cells and sections of rat anterior pituitary tissue. Thus, we have identified the first system other than brain containing significant levels of several of the high molecular weight MAPs characteristic of brain tissue, suggesting that microtubules in neurons and secretory cells may be involved in at least some related functions. In addition, we now have available preparations of both secretory tissue and secretory cell line microtubules for further in vitro investigation of the interaction of microtubules with secretory granules.

Microtubules have been implicated in a large number of studies in playing a role in hormone secretion. Drugs such as colchicine that disassemble microtubules have been found to inhibit secretion and to lead to the accumulation of secretory granules in the cytoplasm (Lacy et al., 1968; Williams & Wolff, 1970; Gautvik & Tashjian, 1973; Labrie et al., 1973; Poisner & Cooke, 1975; Dustin et al., 1975; Pipeleers et al., 1976). Light microscopic and ultrastructural evidence has also indicated an association of secretory granules with microtubules (Lacy et al., 1968; Smith et al., 1975; Boyd et al., 1982). More recent evidence has indicated that a wide variety of vesicular organelles are associated with microtubules in vivo (Ball & Singer, 1982; Hayden et al., 1983; Herman & Albertini, 1984; Collot et al., 1984; Wehland et al., 1983; Rogalski & Singer, 1984). At least some of these are now thought to be transported through the cell by the microtubules (Hayden et al., 1983; Brady et al., 1983; Herman & Albertini, 1984), though it is not yet known whether this is the case for secretory vesicles.

One important route toward understanding the role of microtubules in secretion is to investigate the properties and interactions of the purified components. Two laboratories have investigated the interaction in vitro of secretory vesicles with microtubules isolated from bovine brain tissue, the richest available source of cytoplasmic microtubules. Sherline et al. (1977) found that secretory granules and secretory granule membranes purified from porcine anterior pituitary tissue cosedimented with microtubules isolated from rat brain.

Suprenant & Dentler (1982) reported an association of pancreatic secretory granules with isolated brain microtubules as monitored in solution by dark-field light microscopy.

Both laboratories obtained evidence that these interactions involved a class of non-tubulin proteins present in microtubule preparations known as the microtubule-associated proteins (MAPs) (Sloboda et al., 1975). The largest of these proteins, known as the high molecular weight (HMW) MAPs, have the appearance of long filamentous projections on the microtubule surface (Murphy & Borisy, 1975; Dentler et al., 1975). Suprenant & Dentler (1982) observed apparent cross-linking of the secretory granules with microtubules mediated by the projections when mixtures of these structures were examined by electron microscopy.

Considerable uncertainty has existed as to whether the MAPs found in brain tissue are characteristic of other tissues and cells. This question is of obvious importance in interpreting the results of in vitro reconstitution studies such as those described above. The HMW brain MAPs have been traditionally classified into two groups known as MAP 1 and MAP 2 (Sloboda et al., 1975), each of which forms armlike projects on microtubules (Herzog & Weber, 1978; Kim et al., 1979; Vallee & Davis, 1983). Brain MAP 1 and MAP 2 are each in turn composed of multiple electrophoretic species (Bloom et al., 1984a) that in the case of MAP 1 represent structurally and immunologically distinct polypeptides (Bloom et al., 1984c, 1985). We have named these MAP 1A, MAP 1B, and MAP 1C.

Biochemical analysis of microtubules purified from non-neuronal cultured cells has shown almost no indication of the presence of HMW MAPs of brain (Wiche & Cole, 1976; Nagle et al., 1977; Doenges et al., 1977; Bulinski et al., 1979; Weatherbee et al., 1978; Olmstead & Lyon, 1981; Duerr et al., 1981). Antibodies to MAP 2 have confirmed that this

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protein is restricted in its distribution. MAP 2 was found in abundance only in neuronal cells (Izant & McIntosh, 1980; Valdivia et al., 1982), and within neuronal cells, only within the cell bodies and dendritic processes (Miller et al., 1982; Vallee, 1982; De Camilli et al., 1984; Huber & Matus, 1984; Caceres et al., 1984). Only trace levels of this protein have been detected in nonneuronal cells (Valdivia et al., 1982; Weatherbee et al., 1982).

In contrast to these results, we have recently found that at least one of the HMW MAPs, MAP 1A, is widely distributed among cell types (Bloom et al., 1984a,b), using a monoclonal antibody specific for this protein. We have also analyzed the composition of microtubules from different regions of the brain and found that outside of gray matter regions, the MAP 1 polypeptides are the most abundant MAP species (Vallee, 1982).

One earlier study has indicated that in partially purified pituitary microtubules, an electrophoretic species in the size range of the HMW MAPs of brain was present, though the precise identification of this species, or its characterization as a MAP, was not reported (Sheterline & Schofield, 1975). In the present study, we have investigated the MAPs of pituitary tissue and cells. We have developed a new procedure for isolating MAPs from sources in which endogenous tubulin assembly was not possible. Using this procedure, we have succeeded in isolating MAPs from bovine anterior pituitary tissue. In addition, we have purified for the first time MAP-containing microtubules from a cultured secreting pituitary cell line. A preliminary account of our findings has been presented in abstract form (Vallee & Bloom, 1982).

MATERIALS AND METHODS

Isolation of MAPs from Calf Anterior Pituitary Tissue. Tubulin (10 mg/mL) was purified from calf brain microtubules by using DEAE-Sephadex as described previously (Vallee & Borisy, 1978). Taxol (Schiff et al., 1979) was added to 200 μ M and GTP to 1 mM. The sample was warmed to 37 °C for 2 min and then chilled on ice before use.

Anterior pituitary tissue (~1 g from six animals) was homogenized by using a Potter-Elvehjem Teflon-in-glass tissue grinder in 1.5 volumes of PEM buffer [0.1 M piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES), pH 6.6, containing 1.0 mM ethylene glycol bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) and 1.0 mM MgSO_4]. This and all subsequent steps were performed at 0–4 °C, unless noted otherwise. The sample was centrifuged at 30000g for 30 min. The supernate was saved and again centrifuged at 135000g for 90 min. The supernate was again saved. Taxol was added to 20 μ M and GTP to 1 mM. The microtubule sample that had been assembled from purified calf brain tubulin was added to the tissue extract to 1 mg/mL. The microtubules were then centrifuged through a cushion of 5% sucrose in PEM containing 20 μ M taxol and 1 mM GTP at 30000g for 30 min. The microtubule pellet was resuspended in one-fourth the extract volume in PEM containing taxol and GTP as above and centrifuged again at 30000g for 30 min. The pellet was resuspended to volume in the same buffer with NaCl added to 0.35 M. The microtubules were warmed to 37 °C for 2 min and centrifuged at 37 °C at 30000g for 30 min. The MAPs were recovered in the supernate. The microtubules were again resuspended to volume in PEM prior to being prepared for electrophoresis.

Purification of Microtubules and MAPs from GH_3 Cells. GH_3 cells were grown in plastic roller bottles in F-12 medium supplemented with 15% horse serum, 2.5% fetal calf serum, penicillin, and streptomycin. Serum (Hy-Clone) was obtained

from Sterile Systems (Logan, UT), and medium and antibiotics were from GIBCO (Grand Island, NY). Microtubules and MAPs were purified with taxol from GH_3 cells following the procedures that were described previously for HeLa cells (Vallee, 1982), with minor modification. All steps were at 0–4 °C unless otherwise noted. The cells were swollen hypotonically in 4 volumes of 1 mM EGTA and 1 mM MgSO_4 and centrifuged. After removal of the supernate, the cells were homogenized with a glass-on-glass Dounce tissue grinder. Immediately thereafter, 0.25 volume of 0.5 M PIPES, pH 6.6, containing 1 mM EGTA and 1 mM MgSO_4 was added to the cell homogenate. Subsequent steps were as described previously (Vallee, 1982), with two modifications. The first was the use of several protease inhibitors throughout the entire procedure [1 mM phenylmethanesulfonyl fluoride (PMSF), 1 μ g/mL leupeptin, 0.1 mg/mL soybean trypsin inhibitor, 10 μ g/mL pepstatin, 0.023 trypsin inhibitory unit (TIU)/mL aprotinin (all from Sigma, St. Louis, MO), and 0.1 mg/mL α_2 -macroglobulin (Boehringer Mannheim, Indianapolis, IN)]. The other change was the centrifugation of taxol-stabilized microtubules at 4 °C instead of 37 °C.

Immunofluorescence Microscopy. GH_3 cells grown on glass coverslips were fixed and permeabilized in methanol at –20 °C and processed for indirect immunofluorescence as described for other types of cultured cells (Bloom et al., 1984b). For some experiments, the cells were extracted with digitonin in a microtubule-stabilizing buffer prior to the methanol step (Bloom & Vallee, 1983).

To analyze pituitary tissue, adult female rats were anesthetized with Nembutal and perfused transcardially with 2% paraformaldehyde and 1.5% picric acid in 0.1 M phosphate at pH 7.3 (Bloom et al., 1984a). Sections of anterior pituitary tissue were cut to a thickness of ~20 μ m on a vibrating microtome (Lancer, Vibratome Series 1000; St. Louis, MO) and analyzed by indirect immunofluorescence microscopy exactly as described previously for brain (Bloom et al., 1984a).

First antibodies included rabbit anti-MAP 2 serum diluted 1:100 or undiluted, conditioned hybridoma medium containing monoclonal antibodies to MAP 1A or MAP 1B. The antibodies to MAP 1A (Bloom et al., 1984a,b) and MAP 2 (Theurkauf & Vallee, 1982; Miller et al., 1982; De Camilli et al., 1984; Bloom & Vallee, 1983) have been described previously. Anti-MAP 1B was obtained from a hybridoma clone which we have named "MAP 1B-4" (Bloom et al., 1984c, 1985). This was one of several hybridoma lines that we produced by fusion of NS1 myeloma cells with lymphocytes isolated from a mouse immunized with electrophoretically purified MAP 1B. Second antibodies (Cappel; Cochranville, PA) were rhodamine-labeled immunoglobulin G (IgG) fractions of sheep anti-rabbit IgG and rabbit anti-mouse IgG, diluted 1:2000 and 1:1000, respectively.

Specimens were observed on a Zeiss IM-35 inverted microscope equipped with 16 \times , 25 \times , 40 \times , and 63 \times objectives. Photomicrographs were taken by using Kodak Tri-X film, which was developed with Microdol-X or D19.

Electrophoresis and Immunoblotting. Electrophoresis with sodium dodecyl sulfate (SDS) was performed by the method of Laemmli (1970) using 7% or 9% polyacrylamide gels. Electrophoretic transfer of proteins from gels to nitrocellulose sheets (Schleicher & Schuell; Keene, NH) was performed under conditions designed for the efficient transfer of high molecular weight proteins (Bloom & Vallee, 1983). Antibody labeling was performed as described previously (Bloom & Vallee, 1983), using the same primary antibodies that were employed for immunofluorescence. The second antibodies

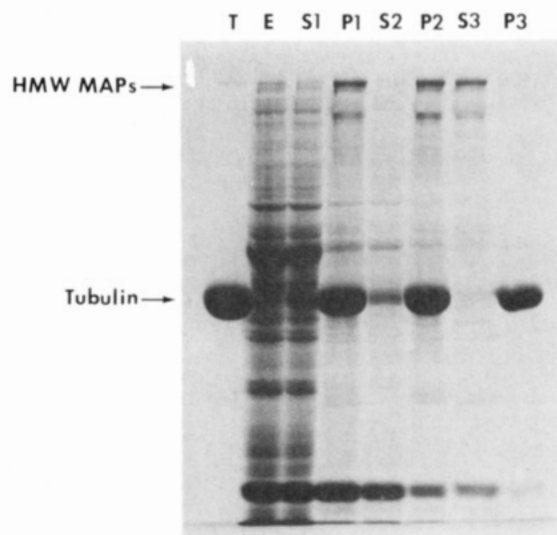


FIGURE 1: Purification of anterior pituitary MAPs. A 9% polyacrylamide-SDS gel stained with Coomassie blue is shown. Microtubules formed from purified calf brain tubulin and taxol (T) were added to a cytosolic extract of calf anterior pituitary tissue (E). The microtubules were then centrifuged, yielding a postmicrotubule supernate (S1) and the first microtubule pellet (P1). The pellet was resuspended in taxol-containing buffer and centrifuged again. This had the effect of removing cytosolic proteins entrapped nonspecifically in the first pellet (S2) and producing a second, washed microtubule pellet (P2). The second pellet was then resuspended as before, and NaCl was added to a final concentration of 0.35 M. A final centrifugation step yielded a soluble MAP fraction (S3) and a microtubule pellet composed largely of tubulin (P3).

were either rhodamine-labeled or peroxidase-labeled IgG fractions of sheep anti-rabbit IgG or sheep anti-mouse IgG (Cappel). The peroxidase-conjugated antibodies were reacted with 4-chloro-1-naphthol.

RESULTS

Biochemical and Immunochemical Characterization of Anterior Pituitary MAPs. To identify MAPs in calf anterior pituitary tissue, we initially attempted to use the taxol-based microtubule purification procedure developed in this laboratory (Vallee, 1982). While this technique has been generally successful with a variety of tissue and cells (Vallee, 1982; Vallee & Bloom, 1982, 1983), only marginal and variable microtubule assembly was achieved in the pituitary tissue extracts. This probably resulted from a combination of factors, including denaturation of endogenous tubulin occurring during transit of the tissue from the slaughterhouse to our laboratory, and a relatively low tubulin level in this tissue compared to brain.

Since a variety of situations may exist in which even the presence of taxol is inadequate to achieve self-assembly, we sought to devise an alternative method for isolating MAPs. To this end, we prepared highly purified tubulin from calf brain and polymerized this protein with taxol. The reassembled microtubules were then added to the tissue extracts, in which they served as a specific adsorbent for endogenous pituitary MAPs. A subsequent centrifugation step pelleted the brain microtubules and the associated anterior pituitary MAPs.

The results of one such experiment are shown in Figure 1. Lane P1 shows the microtubule pellet obtained by mixing taxol-stabilized brain microtubules with a cytosolic extract of anterior pituitary tissue. In addition to tubulin, the pellet contained prominent bands that migrated at the position of the HMW brain MAPs (M_r 270 000–350 000), several bands at M_r 200 000–220 000, and a prominent band near the dye

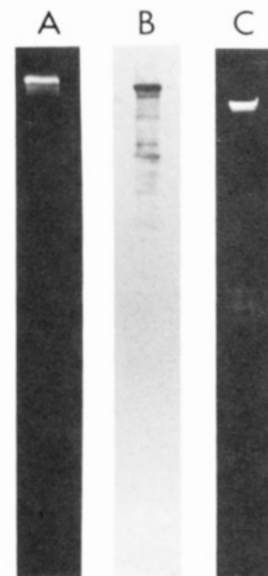


FIGURE 2: Immunoblotting of MAPs obtained from anterior pituitary tissue. The samples shown are of the initial pellets obtained by mixing microtubules made from purified calf brain tubulin and taxol with a tissue extract (same as lane P1 in Figure 1). The proteins were transferred to nitrocellulose from 7% polyacrylamide-SDS gels and stained with anti-MAP 1A (A), anti-MAP 1B clone 4 (B), and anti-MAP 2 (C). Second antibodies in (A) and (C) were labeled with rhodamine, while that in (B) was a peroxidase conjugate.

front. Several less intense bands were also seen throughout the gel. Among the HMW bands, MAP 1 like polypeptides were observed to be the predominant species. The intense band near the dye front was at the approximate position expected for growth hormone and prolactin, which are present at very high concentration in this tissue. When the microtubule pellet was resuspended in taxol-containing buffer and centrifuged again, much of the latter material remained soluble (see lane S2), indicating that these proteins were not stably associated with the microtubules. In contrast, most of the HMW bands and the polypeptides of about 200 000–220 000 daltons did remain associated with the microtubules under these conditions. The same bands were subsequently solubilized by exposure to elevated ionic strength conditions (lane S3), a characteristic feature of MAPs (Vallee, 1982; Vallee & Bloom, 1983). The calf brain tubulin was recovered in the pellet in almost pure form (lane P3).

To test whether the high molecular weight pituitary proteins corresponded to any of the known HMW MAPs of brain, immunoblotting was performed with monoclonal anti-MAP 1A (Figure 2A), monoclonal anti-MAP 1B (Figure 2B), and rabbit anti-MAP 2 (Figure 2C). All three antibodies reacted specifically and strongly with bands at the electrophoretic position expected for each of the MAPs. All of the antibodies also reacted with lower molecular weight bands that we believe represent proteolytic fragments of the three MAP species (see below). Similar blots showed that the purified brain tubulin used for these experiments contained only trace levels of the HMW MAPs (not shown). Therefore, nearly all of the immunoreactive proteins observed in the microtubule pellets were derived from anterior pituitary tissue.

Isolation of MAP-Containing Microtubules from GH₃ Cells. Anterior pituitary tissue contains a complex mixture of endocrine cell types, as well as nonsecretory endothelial cells. We considered it of interest to characterize the MAPs of a homogeneous population of secreting pituitary cells as well. The GH₃ cell line, which secretes growth hormone and prolactin, was chosen for this purpose.

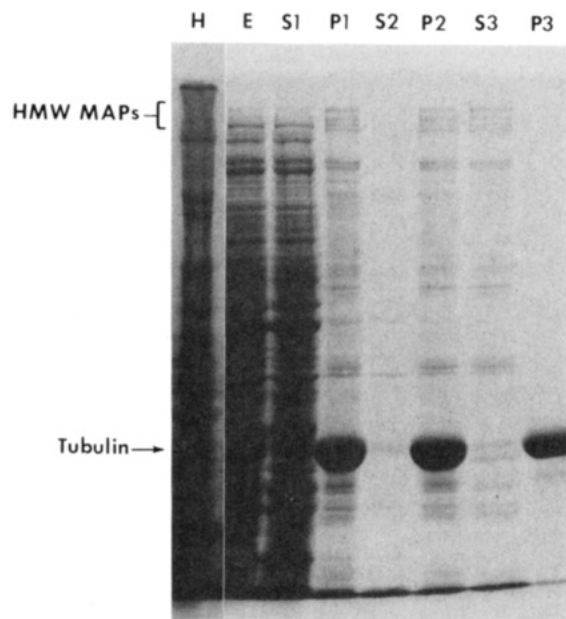


FIGURE 3: Purification of microtubules and MAPs from GH₃ cells. A 7% polyacrylamide-SDS gel stained with Coomassie blue is illustrated. The fractions shown are cells homogenized directly in SDS sample buffer (H), cytosolic extract (E), postmicrotubule supernate (S1), first microtubule pellet (P1), supernate from washed microtubules (S2), second (washed) microtubule pellet (P2), MAP fraction (S3), and final microtubule pellet composed of nearly pure tubulin (P3).

In this case, freshly prepared cell extracts could be prepared, and excellent endogenous assembly of microtubules was obtained with the aid of taxol. Electrophoretic analysis of the purification steps for GH₃ cell microtubules and MAPs is shown in Figure 3. In the first microtubule pellet (lane P1), tubulin was the most abundant protein, but several non-tubulin proteins were present as well. Among the most prominent of these were polypeptides comigrating with known HMW brain MAPs, a group of several bands ranging from M_r 35 000 to about the position of tubulin, a pair of bands at M_r 75 000–80 000, a band at about 120 000 daltons, and a pair of polypeptides at M_r 200 000–220 000. In addition, numerous minor bands were seen, and intense staining of the dye front was observed. The latter material may have once again corresponded to growth hormone and prolactin, because of its electrophoretic mobility and its progressive removal from microtubules throughout subsequent stages of the taxol procedure. Most of the other non-tubulin proteins found in the first pellet remained stably associated with microtubules at low ionic strength (compare lanes S2 and P2), but not in the presence of 0.35 M NaCl (compared lanes S3 and P3), a property common to several known brain, HeLa cell, and sea urchin egg MAPs (Vallee, 1982; Vallee & Bloom, 1983).

In an attempt to identify the protein species comprising the HMW MAP bands, we performed immunoblotting with the anti-MAP antibodies. Figure 4A shows samples taken from each of the various microtubule purification steps stained with anti-MAP 1A. The antibody reacted exclusively with a band at the position of MAP 1A in a sample of GH₃ cells homogenized directly in hot SDS sample buffer (lane H). In the cytosolic extract (lane E), immunoreactivity was observed with a band of the same mobility, as well as with bands of M_r 155 000 and 115 000. Since these latter two bands were not observed in the sample of unfractionated cellular protein, we believe they represent proteolytic fragments of MAP 1A generated during or after the cell lysis step. Immunoreactive material at the position of MAP 1A was specifically diminished

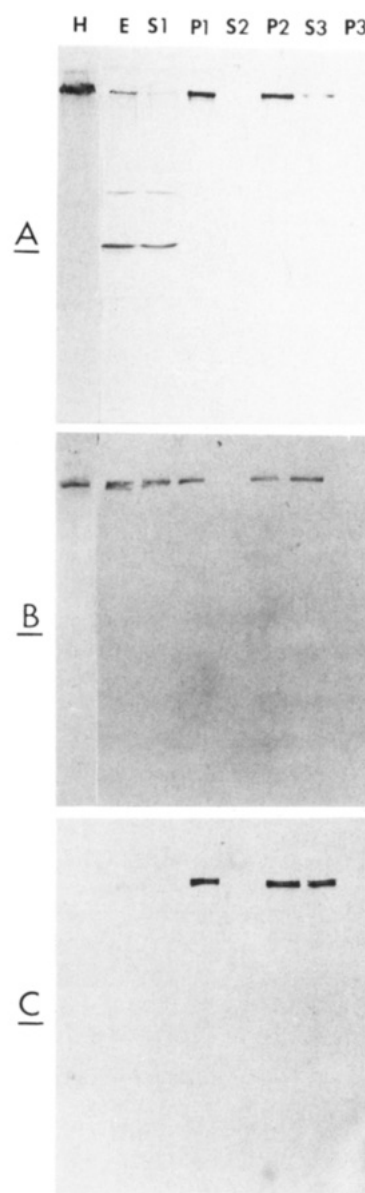


FIGURE 4: Immunoblotting of the purification steps for GH₃ cell microtubules and MAPs. Seven percent polyacrylamide-SDS gels were transferred to nitrocellulose and stained with anti-MAP 1A (A), anti-MAP 1B clone 4 (B), and anti-MAP 2 (C), followed by peroxidase-labeled second antibodies. The lanes shown are the same as in Figure 3. Note the low reactivity of anti-MAP 1A in lane S3 (A) was due to incomplete transfer caused by a bubble between the gel and nitrocellulose sheet.

in the postmicrotubule supernate (lane S1) and enriched in the first two microtubule pellets (lanes P1 and P2) and the purified MAP fraction (lane S3). The presumptive proteolytic fragments of MAP 1A were evidently incapable of binding to microtubules, since they were undetectable in the microtubule fractions and remained totally soluble in the postmicrotubule supernate. These results suggest that MAP 1A is prone to proteolysis in GH₃ cell lysates. Proteolysis was not complete, however, and some intact MAP 1A did copurify with the MAP-enriched fractions.

Immunoblotting of identical samples stained with anti-MAP 1B (clone 4) is shown in Figure 4B. Comparison of the extract, postmicrotubule supernate, and first microtubule pellet (lanes E, S1, and P1, respectively) revealed that only a fraction of the immunoreactive material with the electrophoretic mobility of MAP 1B cosedimented with the microtubules. Nevertheless, the MAP 1B present in the microtubule pellet behaved

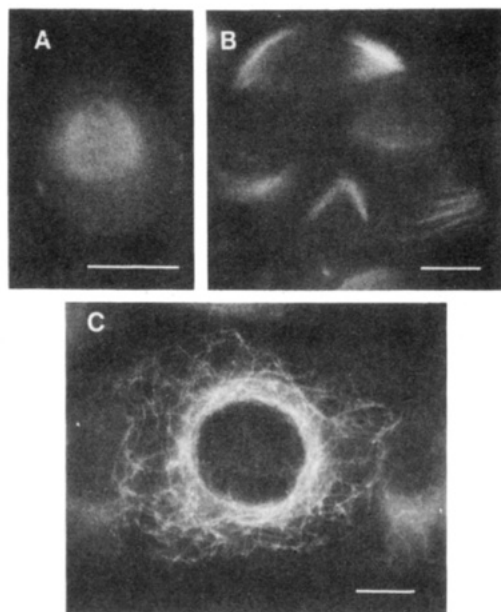


FIGURE 5: Immunofluorescence microscopy of GH₃ cells. (A) Staining of a mitotic spindle in a dividing cell by anti-MAP 1A. (B) Bundles of microtubules stained by anti-MAP 1B clone 4 in a cell treated for 18 h with 10 μ M taxol. (C) Interphase microtubules stained by anti-MAP 2 in a cell not exposed to taxol. The cell in panel C was extracted with digitonin (see Materials and Methods) before fixation with methanol. The digitonin step was omitted for the other panels. Bars equal 5 μ m.

entirely as expected for a MAP in the subsequent purification steps. Obvious proteolytic fragments of MAP 1B were not generally observed.

MAP 2 (Figure 4C) was barely detectable by immunoblotting in the homogenate (lane H) and cytosolic extract (lane E). However, a pair of immunoreactive bands with the electrophoretic mobilities of MAP 2A and MAP 2B were specifically enriched in the first two microtubule pellets (lanes P1 and P2) and in the MAP fraction (lane S3). We did not observe any obvious lower molecular weight fragments of MAP 2 in the cytosolic extract or in subsequent fractions. On the basis of these experiments, we conclude that GH₃ cells contain significant levels of MAP 1A, MAP 1B, and MAP 2, all of which are capable of specifically binding to microtubules in crude cell extracts.

Localization of HMW MAPs in GH₃ Cells. To verify that the immunoreactive HMW MAPs identified biochemically in anterior pituitary tissue and in GH₃ cells were actually localized on microtubules in the cell, we performed immunofluorescence microscopy using monolayer cultures of GH₃ cells. Although these cells are relatively poor specimens because of their compact morphology, we were able to observe specific staining of microtubules with antibodies to all three HMW MAPs.

Figure 5A illustrates the staining of mitotic spindle microtubules with anti-MAP 1A. Bundles of microtubules induced in nondividing cells by taxol and stained with anti-MAP 1B (clone 4) are shown in Figure 5B. An unusually well-spread interphase cell not exposed to taxol, and containing microtubules brightly stained by anti-MAP 2, is illustrated in Figure 5C. Microtubule staining was abolished in cells pretreated with colchicine or vinblastine (not shown).

Cellular Origin of HMW MAPs in the Anterior Pituitary. To determine the cellular distribution of HMW MAPs in the anterior pituitary, we examined tissue sections from adult female rats by immunofluorescence microscopy (Figure 6). For each of the anti-MAP antibodies, strong immunoreactivity

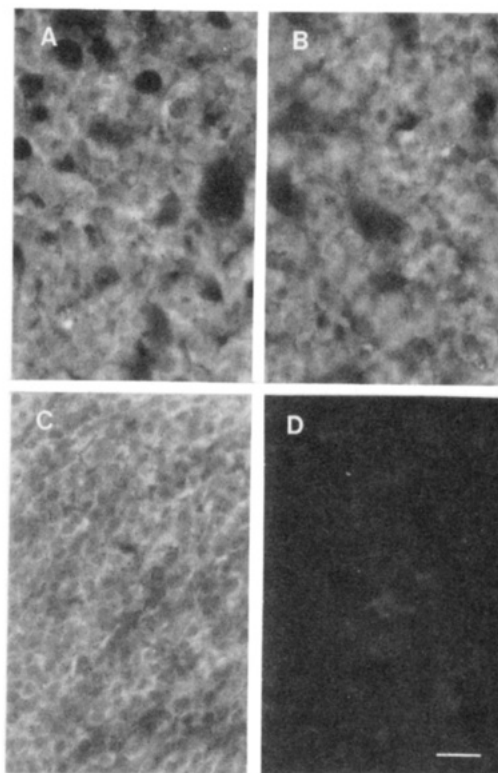


FIGURE 6: Immunofluorescence microscopy of anterior pituitary sections labeled with anti-MAP 1A (A), anti-MAP 1B clone 4 (B), anti-MAP 2 (C), and a monoclonal IgG that does not react with this tissue (D). Bar in (D) equals 20 μ m.

was observed within the cytoplasm of virtually all cells exhibiting the characteristic small, rounded morphology of secretory cells. Sections incubated with unconditioned hybridoma tissue culture medium showed almost no fluorescence relative to antibody-containing medium. We, therefore, conclude that all secretory cells in the anterior pituitary, regardless of which hormone they secrete, contain MAP 1A, MAP 1B, and MAP 2.

DISCUSSION

We have succeeded in isolating MAPs from both anterior pituitary tissue and a hormone-secreting pituitary tumor cell line. A number of polypeptides behaved biochemically as MAPs. Using anti-MAP antibodies, we determined that both the tissue and cultured cells contain three HMW MAP species that were originally described in brain tissue. Thus, those very proteins identified in brain as candidates for mediating the interaction of microtubules with other cellular organelles are present in secreting pituitary cells as well.

In the course of this work, we developed a new modification of the taxol-dependent purification procedure for MAPs.¹ We found that assembly of endogenous tubulin in bovine anterior pituitary extracts was variable and, in general, poor. A number of important situations similar to this may exist. For example, tubulin of some organisms may not bind taxol. In addition, other cases may exist where loss of tubulin self-assembly activity is unavoidable. To overcome this problem in pituitary tissue, we added preformed microtubules assembled from purified brain tubulin to a tissue extract. These microtubules served as specific adsorbents for the anterior pituitary MAPs. Because of the stability of the taxol microtubules, it was not necessary to expose the cellular extract to elevated tempera-

¹ Recently, Bates & Kidder (1984) have described a similar procedure for isolating MAPs from mouse blastocysts.

tures, something that would be necessary without taxol, and which would tend to promote the activity of endogenous proteases.

It seems clear that three HMW proteins, MAP 1A, MAP 1B, and MAP 2, are component proteins of anterior pituitary microtubules. Whether the other polypeptides that copurified with the HMW MAPs were, indeed, microtubule-associated proteins must await further immunocytochemical proof. The species at $M_r \sim 200\,000$ – $220\,000$ may be related to proteins identified in a variety of cultured cells (Bulinski & Borisy, 1979; Weatherbee et al., 1980; Olmsted & Lyon, 1981; Duerr et al., 1981; Izant et al., 1982, 1983; Pepper et al., 1984) and, conceivably, to proteins of similar molecular weight in the sea urchin (Vallee & Bloom, 1983).

The major species at low molecular weight presumably represent polypeptide hormones stockpiled in the anterior pituitary. We do not yet understand the basis for the presence of these species in our microtubule preparations. Possibly, their persistence through the stages of purification is indicative of a specific association of secretory granules with microtubules. Such an association has been suggested by a large body of physiological and morphological work as well as some recent observations of an interaction of secretory granules with brain microtubules in vitro (Sherline et al., 1977; Suprenant & Dentler, 1982). The putative hormonal material in our preparations was lost during MAP isolation, indicating that it does not behave strictly as a MAP. Why this should be the case and whether this observation relates to the function of microtubules and MAPs in the cell cannot be answered at present.

The purification of microtubules from several cultured cell lines has been reported (Wiche & Cole, 1976; Nagle et al., 1977; Doenges et al., 1977; Weatherbee et al., 1978; Bulinski & Borisy, 1979; Olmsted & Lyon, 1981). We have described the purification of microtubules from HeLa cells using taxol (Vallee, 1982), and others (Greene et al., 1983; Wiche et al., 1983, 1984) have used this procedure to promote assembly from a number of other cell lines. Almost no evidence was obtained for the presence of the high molecular weight MAPs in microtubules prepared by procedures not involving taxol. In contrast, both Greene and co-workers and Wiche and co-workers reported the presence of these proteins in their preparations.

We have now found three of the major HMW MAPs of brain tissue in GH₃ cell microtubules. Our results suggest two reasons for the loss of HMW MAPs in the preparation of cultured cell microtubules. First, the HMW MAPs are known to be extremely sensitive to proteolysis (Sloboda et al., 1976; Vallee & Borisy, 1977). With the use of our anti-MAP antibodies, we were able to observe the degradation of MAP 1A directly during the course of microtubule purification. Even with the use of several protease inhibitors in our preparations, significant proteolysis still occurred (Figure 4A).

In addition, we noted that, for MAP 1B, recovery in the first microtubule pellet was inefficient (Figure 4B). We do not know the reason for this but suspect that it may be due either to denaturation of the microtubule binding site or to lack of sufficient binding sites on the assembled microtubules. We have also noted partial copurification of the same protein with brain microtubules (Bloom et al., 1985).

We are not yet able to evaluate fully the remaining polypeptides in the GH₃ microtubule and MAP fractions. Among these were polypeptides of about 35 000–55 000, 75 000–80 000, 120 000, and 200 000–220 000 daltons. Putative MAPs of these sizes have been reported to be widely distributed among

cultured cells (Bulinski & Borisy, 1979; Weatherbee et al., 1980; Olmsted & Lyon, 1981; Duerr et al., 1981), and immunofluorescence microscopy has demonstrated that at least some of these proteins are specifically localized on microtubules in cells, proving that they are genuine MAPs (Izant & McIntosh, 1982; Pepper et al., 1984; Bulinski & Borisy, 1980). While some of the polypeptides could also represent fragments of the HMW MAPs, our immunoblotting data indicated that such fragments tended to be lost during purification. It is also possible that some of these species represent adventitious contaminants present in the preparation. However, all of the polypeptides showed the ionic strength dependent sedimentation properties characteristic of the MAPs (Vallee, 1982; Vallee & Bloom, 1983), suggesting that the presence of these proteins in the preparation is meaningful. We note, finally, that in our preparations of microtubules from sea urchin eggs, we were able to show with the use of monoclonal antibodies that a variety of proteins over a wide range of size and abundance were components of microtubules in the cell (Vallee & Bloom, 1983).

Anterior pituitary tissue and the GH₃ cell line now represent the only sources other than the nervous system known to possess significant levels of several HMW MAPs originally identified in brain. The HMW MAP composition of pituitary cells is apparently quite similar to that found in bovine brain white matter, in which MAP 1A and MAP 1B were found to be more abundant than MAP 2 (Vallee, 1982; Bloom et al., 1984a,b). The detection of MAP 1A in pituitary cells is not surprising in light of our earlier report (Bloom et al., 1984b) that this protein is widely distributed among mammalian cells. We have also obtained evidence that MAP 1B is found in cells of nonneuronal origin, although not as commonly as MAP 1A (Luca et al., 1984; G. S. Bloom et al., unpublished results). MAP 2, in contrast, is a protein generally thought to be restricted to the dendrites and perikarya of neurons (Miller et al., 1982; Vallee, 1982; De Camilli et al., 1984; Huber & Matus, 1984; Caceres et al., 1984), although this protein has been detected at trace levels in some nonneuronal sources (Valdivia et al., 1982; Weatherbee et al., 1982). On the basis of comparative immunoblotting (not shown), we estimate that the level of MAP 2 in anterior pituitary tissue and GH₃ cells is about 10% that of brain, substantially higher than what has been reported for other nonneuronal systems (Valdivia et al., 1982). Pituitary microtubules, therefore, are strikingly similar in their content of HMW MAPs to brain microtubules, particularly those found in white matter regions, such as axons. Perhaps, therefore, pituitary and brain microtubules are also related in function.

As noted above, the precise role of microtubules in the secretory process remains unknown. Hopefully, the availability of pituitary cell microtubules and MAPs as described here will provide a useful avenue for further investigation.

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