Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 5776–5780, September 1985
Cell Biology

M_r 205,000 sulfoglycoprotein in extracellular matrix of mouse fibroblast cells is immunologically related to high molecular weight microtubule-associated proteins

(secretory proteins/cytoskeleton)

ESTHER BRIONES AND GERHARD WICHE

Institute of Biochemistry, University of Vienna, 1090 Vienna, Austria

Communicated by Daniel Mazia, May 20, 1985

ABSTRACT **Rabbit antibodies raised against microtubule-associated protein 1 (MAP-1) from hog brain were found to crossreact with extracellular matrix components of mouse BALB/c 3T3 cell cultures. As shown by immunofluorescence microscopy of confluent cell cultures, the extracellular MAP-related antigen was located on dense fibrillar network arrays underlying and surrounding the cells. The immunoreactive material was sensitive to trypsin but resistant to collagenase. The microtubule-disrupting drug colcemid had no visible effect on the morphology of the anti-MAP-stained network, whereas treatment with cytochalasin B provoked its abolishment. Simian virus 40-transformed BALB/c 3T3 cells expressed considerably less extracellular antigen than did the nontransformed cells. After *in vivo* radiolabeling of BALB/c 3T3 cells, a secreted polypeptide of M_r 205,000 was isolated by immunoprecipitation from culture media as well as from cell-free extracellular matrices. This antigen was identified as a sulfoglycoprotein, noncollageneous in nature, that undergoes intermolecular disulfide bonding. Anti-MAP-1 antibodies affinity-purified on the extracellular M_r 205,000 protein were immunoreactive with MAP-1 and MAP-2 from brain and decorated cytoplasmic microtubules as demonstrated by immunoblotting and immunofluorescence microscopy. Thus, a structural relationship between cytoskeletal and extracellular polypeptides is demonstrated.**

Microtubule-associated proteins 1 and 2 (MAP-1 and MAP-2) from mammalian brain promote the *in vitro* assembly of tubulin into microtubules (1–5) and copolymerize with tubulin to form protrusions along the polymer's surface (1, 6, 7). Their expected association with neuronal microtubules *in vivo* has been demonstrated recently (8–10). Interestingly, MAP-1 and MAP-2 were found to be differentially distributed among axonal and dendritic microtubules (9–11).

Until recently, the question of whether high molecular weight MAPs related to MAP-1 and MAP-2 are also associated with microtubules of nonneuronal cells has been controversial (for a recent review, see ref. 12). However, in a recent comprehensive study using separate polyclonal antisera specific for MAP-1 and MAP-2, the widespread distribution of MAP-1- and MAP-2-related antigens to a variety of microtubule structures present in nonneuronal cultured cells and tissues was clearly demonstrated (13). Recent results of Bloom *et al.* (14), who used a monoclonal antibody to a MAP-1 subcomponent, support this notion (see, however, ref. 11 for a renewed claim that MAP-1 and MAP-2 are neuron-specific).

There is also evidence for the association of MAPs with cellular structures other than microtubules. Regarding neuronal systems, Bernhardt and Matus (15) reported the immunolocalization of high M_r MAPs at distal regions of developing dendrites in neonatal cerebellum before microtubules were detected at this site. MAP-2 was found at postsynaptic densities and in dendritic spines free of microtubules (9, 16). With respect to nonneuronal cells and tissues, high M_r MAPs were found to be enriched at several cell regions including the leading edge of migrating fibroblast cells and Z-line structures of skeletal and cardiac muscle, where no tubulin-specific staining was observed (13, 17).

We report here on a sulfoglycoprotein that occurs in the extracellular matrix of mouse fibroblast cells and is immunologically related to MAP-1 and MAP-2. This is an unexpected example of a structural relationship between cytoskeletal, hence typically intracellular, polypeptides of higher eukaryotes and constituents of extracellular structures.

MATERIALS AND METHODS

Cell Culture. The origin and culture conditions of the various cell lines used have been described (13, 18).

For treatment with drugs, BALB/c 3T3 cell cultures kept on coverslips at confluence were incubated with 1.6 μM colcemid (Sigma) in growth medium for 48 hr at 37°C or with 3–5 μg of cytochalasin B (Sigma) per ml in serum-free medium supplemented with antibiotics and 0.05% bovine serum albumin for 1–3 hr at 37°C (19). Controls were made by using the same medium without drugs.

For enzymatic digestion, confluent BALB/c 3T3 cells grown on coverslips were exposed either to 1–10 μg of L-1-tosylamido-2-phenylmethyl chloromethyl ketone (TPCK)-treated trypsin (Sigma) per ml of 150 mM NaCl/2.7 mM KCl/1.4 mM KH_2PO_4/10 mM Na_2HPO_4 (buffer A) for 10 min at 37°C or to 164 units of bacterial collagenase (form III, Advance Biofactures, Lynbrook, NY) per ml of growth medium containing 330 mM Ca acetate for 12 hr at 37°C and processed for immunofluorescence. Controls (no enzymes) were treated in a similar way.

For *in vivo* radiolabeling, BALB/c 3T3 cell cultures were grown on 33-mm Petri dishes and incubated 24 hr after seeding in (*i*) methionine-free growth medium containing 25 μCi of L-[^{35}S]methionine (1170 Ci/mmol, Amersham; 1 Ci = 37 GBq) per ml for 20 hr; (*ii*) growth medium supplemented with 1% fetal calf serum containing 12.5 μCi of *N*-acetyl-D-[1-^{3}H]glucosamine (2.9 Ci/mmol, Amersham), 12.5 μCi of L-[1-^{3}H]fucose (5.4 Ci/mmol, Amersham), 12.5 μCi of D-[2-^{3}H]mannose (15.8 Ci/mmol, Amersham), and 50 μCi of D-[1-^{3}H]galactose (8.2 Ci/mmol, Amersham) per ml for 2 days; or (*iii*) sulfate-free medium containing 300 μCi of sodium [^{35}S]sulfate (447 mCi/mmol, New England Nuclear) per ml for 20 hr.

Isolation of Extracellular Matrix. After *in vivo* labeling of BALB/c 3T3 cells with [^{35}S]sulfate, cell-free matrices were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: MAP, microtubule-associated protein.

isolated by the method of Hedman *et al.* (20) and finally solubilized in electrophoresis sample buffer (21) containing 8 M urea. Alternatively, preparations of cell-free matrices were suspended in 200 mM sodium borate (pH 9.0) and labeled by reductive methylation (22).

Antisera. The preparation and characterization of an antiserum to hog brain MAP-1 has been described (9, 13, 23). For preadsorption of this antiserum with the immunogen, three-times-cycled microtubule protein preparations from hog brain (24) were electrophoretically fractionated using 5% polyacrylamide gels. Coomassie blue-stained gel bands of MAP-1 were cut out, fixed overnight with 50% (vol/vol) isopropanol/10% (vol/vol) acetic acid, and destained with 2 M sodium salicylate in 10% isopropanol for 6 hr. After washing with buffer A for 2 hr, the gel pieces were incubated with antiserum diluted in buffer A for 24 hr at 37°C, followed by a centrifugation to remove insoluble particles. The supernatant was equivalent in concentration to a 1:20 dilution of the antiserum. The antiserum was adsorbed with electrophoretically purified tubulin in the same way. As assayed by immunoprecipitation (see below), preadsorption of the antiserum with MAP-1, in contrast to that with tubulin, totally abolished the crossreactivity of the antiserum with MAPs (Fig. 1*A*).

Affinity purification of anti-MAP-1 antibodies by adsorption to electrophoretically purified MAP-1 or M_r 205,000 antigens immobilized on nitrocellulose sheets was performed by published procedures (13).

Other antisera—namely, goat anti-human fibronectin, sheep anti-human laminin and sheep-anti-human type IV collagen—were kindly provided by S. I. Katz and G. Stingl (National Institutes of Health and School of Medicine University of Vienna).

Immunoblotting and Immunoprecipitation. Details of immunoblotting experiments have been given elsewhere (13). For immunoprecipitation of *in vivo* radiolabeled extracellular matrix and *in vitro* radiolabeled hog brain microtubule proteins, samples were first boiled in electrophoresis sample buffer (21). Immunoprecipitation was then done following the method of Blose and Meltzer (25) slightly modified (23) using protein A-Sepharose (Pharmacia). Prior to immunoprecipitation of *in vivo* radiolabeled proteins secreted into the culture media, aliquots of the media were adjusted to contain 1% Triton X-100, 1% sodium deoxycholate, 2 mM phenylmethylsulfonyl fluoride, 10 μg of aprotinin per ml, and 2 mM ϵ-aminocaproic acid. In this case immunoprecipitation was performed in the absence of $NaDodSO_4$. In some experiments cell culture media were treated with 30 units of bacterial collagenase per ml of medium for 60 min at 37°C and then were adjusted to contain 2 mM EDTA, 1% Triton X-100, 1% deoxycholate, 2 mM ϵ-aminocaproic acid, and 10 μg of aprotinin per ml for subsequent immunoprecipitation. Electrophoresis of the solubilized immunoprecipitates was carried out on polyacrylamide gels as described by Laemmli (21). Radioactively labeled protein bands were detected by fluorography at −70°C by using preflashed Kodak-X-Omat AR-5 film.

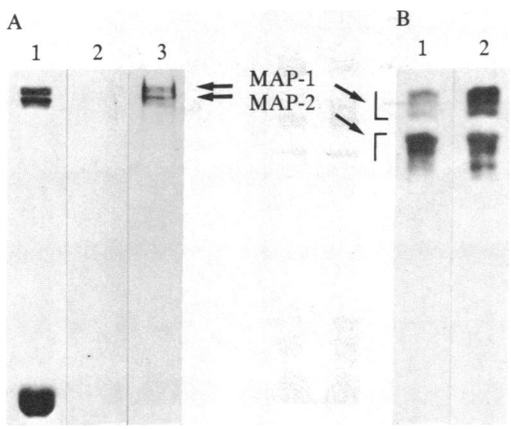

FIG. 1. Characterization of antiserum to MAP-1. (*A*) Immunoprecipitation. Lanes: 1, fluorography of ^{3}H-radiolabeled (22) hog brain microtubule proteins analyzed on 7.5% polyacrylamide gels prior to immunoprecipitation; 2 and 3, fluorography of immunoprecipitates obtained from this preparation with antiserum to MAP-1 preadsorbed with MAP-1 (lane 2) or tubulin (lane 3) gel bands. (*B*) Immunoblotting with affinity-purified antibodies. A microtubule protein preparation (24) was run on a 5% polyacrylamide gel and then transferred to a nitrocellulose sheet. Lanes: 1, anti-MAP-1 antibodies affinity-purified on extracellular M_r 205,000 protein bands; 2, anti-MAP-1 antibodies affinity-purified on MAP-1 protein bands.

Immunofluorescence Microscopy. Cells grown on glass coverslips were fixed (*i*) with 3.7% paraformaldehyde in buffer A for 30 min, or (*ii*) as in method *i* with an additional treatment with acetone for 7 min at −20°C, or (*iii*) with methanol for 5 min at −20°C. Incubations with the primary and secondary antisera each were for 30 min at 37°C. After incubation with antisera, cells were washed with buffer A. Antibodies to MAP-1 were used either undiluted (affinity-purified) or at dilutions of 1:50 or 1:100 in buffer A. The following secondary antibodies were used: fluorescein-conjugated goat anti-rabbit IgG (Nordic, Lausanne, Switzerland) diluted 1:20, rhodamine-conjugated goat anti-rabbit IgG (Nordic) diluted 1:50 or 1:100, and Texas red-conjugated donkey anti-rabbit IgG (Amersham) diluted 1:40 in buffer A. Coverslips were mounted in Gelvatol and viewed in a Leitz Orthoplan fluorescence microscope.

RESULTS

Immunofluorescence microscopy of paraformaldehyde-fixed BALB/c 3T3 cells using antibodies to MAP-1 revealed the staining of extracellular structures. On subconfluent cultures (Fig. 2*A*), we observed the staining of fine fibrillar structures located in parallel arrays on the cell surface and in intercellular spaces. At confluence (Fig. 2*B*), thick filamentous bundles located preferentially between the lower surface of the cell layers and the substratum were brightly stained. In general, the anti-MAP-1 staining resembled that observed after processing similar cultures with antisera to typical extracellular matrix proteins such as fibronectin and laminin. Anti-MAP-1-positive extracellular matrix components were also observed in primary cultures of mouse kidney (Fig. 2*C*), Swiss 3T6, and Swiss 3T3 cells. As reported for other extracellular matrix components (26), the amount of anti-MAP-1 immunoreactive material at extracellular sites was much lower in simian virus 40-transformed fibroblast cells (Fig. 2*D*) than in nontransformed cells.

The microtubule-disrupting agent colcemid had no effect on the distribution of the MAP-related extracellular antigen (Fig. 2*E*). In contrast, after treatment of cells with cytochalasin B, a drug that is known to drastically alter cell morphology and to cause the release of cell surface-associated fibronectin (19, 27), the material immunoreactive with antibodies to MAP-1 was rarely seen in the form of fibrils, but rather in the form of numerous spots on the cell surface (Fig. 2*F*). After removal of this drug from the cell culture, normal cell morphology and reappearance of anti-MAP-1-positive fibrils were observed after 1–2 hr (Fig. 2*G*). Using rabbit nonimmune serum or antiserum to MAP-1 preadsorbed with electrophoretically purified MAP-1 or unrelated antisera, including antiserum to MAP-2 (9), we observed no extracellular staining (Fig. 2*H*; also data not shown).

To identify the extracellular antigen and to establish its provenance, BALB/c 3T3 cell cultures were labeled *in vivo*

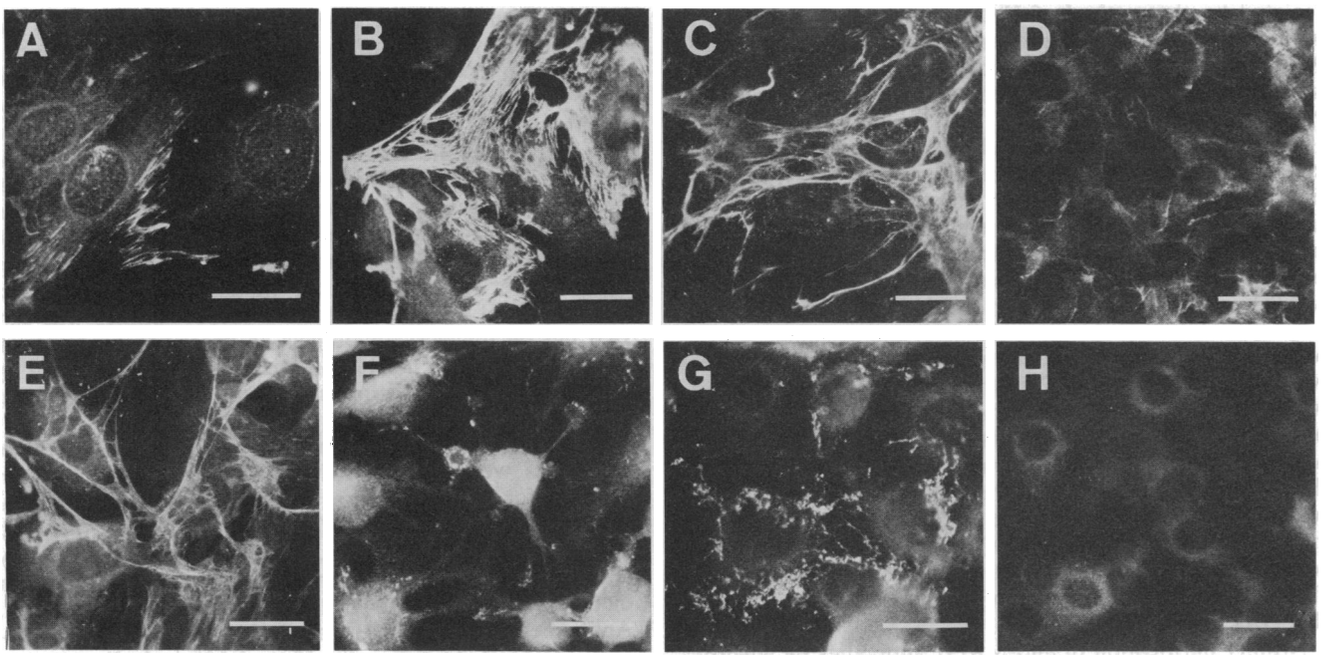

FIG. 2. Immunolocalization of antigens related to MAPs in the extracellular matrix. Cells were fixed by method *i* (*A–D* and *H*) or method *ii* (*E–G*) as described in the text. Indirect immunofluorescence microscopy of BALB/c 3T3 (*A*, *B*, *E–H*), primary cultures of mouse kidney cells (*C*), and SV 101 cells (*D*) are shown. Cells were fixed after incubation with colcemid for 48 hr (*E*), after 2 hr of incubation with cytochalasin B (*F*), and 50 min after removal of cytochalasin B and replenishment with fresh growth medium (*G*). In all cases except for *A*, cell cultures were confluent. (*A–G*) Antiserum to MAP-1 diluted 1:100. (*H*) Antiserum to MAP-1 preadsorbed with MAP-1. [Rhodamine (*A–D*, *F–H*) and fluorescein (*E*); ×472 (*A*), ×390 (*B* and *E*), ×367 (*C* and *H*), ×427 (*D*), ×450 (*F* and *G*).] (Bars = 25 μm.)

with [^{35}S]methionine, and the cell-free medium was subjected to immunoprecipitation with antiserum to MAP-1 (Fig. 3). Out of a great number of cellular proteins released into the medium (Fig. 3, lane 1), the antibodies recognized a single, apparently minor component of M_r 205,000 (Fig. 3, lane 2). In control experiments with antiserum to fibronectin, we observed a band at M_r 240,000 (Fig. 3, lane 3); with antiserum to laminin, we observed bands at M_r 225,000 and 150,000 and minor bands, none of which migrated with the M_r 205,000 polypeptide (Fig. 3, lane 4). However, polypeptides of $M_r \approx$ 205,000 were precipitated with antiserum to collagen type IV (Fig. 3, lane 5). Rabbit nonimmune serum and anti-MAP-1 antiserum preadsorbed with MAP-1 did not crossreact with the M_r 205,000 band (Fig. 3, lane 6; also data not shown). Incubation of [^{35}S]methionine-labeled cell-free medium with bacterial collagenase prior to immunoprecipitation resulted

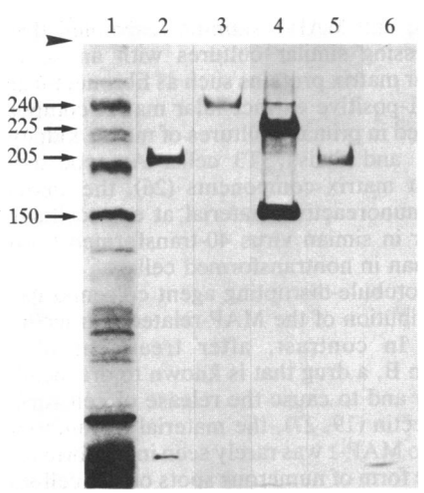

FIG. 3. Identification of MAP-1-related antigen in the culture medium. Samples were analyzed on 7.5% polyacrylamide gels. Lanes: 1, autoradiography of the [^{35}S]methionine-labeled proteins released into the growth medium; 2–6, fluorographies of immunoprecipitates obtained with antiserum to MAP-1 (lane 2), antiserum to fibronectin (lane 3), antiserum to laminin (lane 4), antiserum to collagen type IV (lane 5), and rabbit nonimmune serum (lane 6). Note that the material in lane 2 migrates slightly above a major band in lane 1. Arrowhead, start of running gel. Sizes are shown as $M_r \times 10^{-3}$.

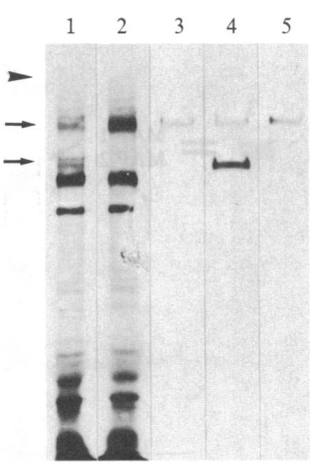

FIG. 4. Collagenase-sensitivity of proteins released into the growth medium. [^{35}S]Methionine-labeled proteins in the growth medium were treated with bacterial collagenase and analyzed on 7.5% polyacrylamide gels, followed by fluorography. Lanes: 1 and 2, total proteins of medium before (lane 1) and after (lane 2) collagenase treatment; 3–5, fluorographies of immunoprecipitates obtained from medium after digestion with collagenase with antiserum to collagen type IV (lane 3), antiserum to MAP-1 (lane 4), and rabbit nonimmune serum (lane 5). The band of M_r 225,000 (upper arrow) present in all lanes was precipitated nonspecifically with the antisera. Note the absence of a double band (lower arrow) at the position around M_r 205,000 in lane 2. Arrowhead, start of running gel.

in the disappearance of two closely spaced bands in the region of M_r 200,000–220,000 (Fig. 4, lanes 1 and 2). Immunoprecipitates obtained after collagenase treatment with antibodies to collagen type IV showed a lack of immunoreactive material (Fig. 4, lane 3), but the proteins immunoreactive with antibodies to MAP-1 resisted such treatment (Fig. 4, lane 4). This insensitivity to collagenase also was demonstrated by immunofluorescence microscopy of 3T3 cell monolayer cultures. However, the anti-MAP-1 immunoreactive material, unlike collagen, was sensitive to mild trypsin treatment (data not shown).

Immunoprecipitation of proteins in the culture medium from BALB/c 3T3 cells labeled *in vivo* with tritiated carbohydrates or [^{35}S]sulfate identified the protein species crossreacting with antiserum to MAP-1 as a sulfated glycoprotein (Fig. 5*A*, lanes 1 and 3). Furthermore, in the absence of 2-mercaptoethanol, the extracellular antigen exhibited a much reduced mobility on polyacrylamide gels (apparent M_r 370,000), suggesting disulfide-dependent dimer formation (Fig. 5*A*, lane 4).

An extracellular anti-MAP-1-positive antigen of M_r 205,000 also was identified as a component of extracellular matrices prepared from [^{35}S]sulfate-labeled BALB/c 3T3 cell cultures (Fig. 5*B*, lane 1). Controls with rabbit nonimmune serum were negative (Fig. 5*B*, lane 2). Similar results were obtained after *in vitro* labeling of cell-free matrix preparations using reductive methylation.

To clarify whether the same antibody population was immunoreactive with MAP-1 and the extracellular M_r 205,000 protein, antibodies of the anti-MAP-1 antiserum were affinity-purified by adsorption to MAP-1 from brain or to M_r 205,000 antigen from BALB/c 3T3 cells, both immobilized on nitrocellulose sheets. In immunoblotting, the antibodies affinity-purified on the M_r 205,000 protein band were reactive with hog brain MAP-1 and, apparently even more extensively, with MAP-2 subcomponents (Fig. 1*B*, lane 1). The same MAP-1 and MAP-2 subcomponents were also immunoreactive with antibodies affinity-purified on hog brain MAP-1 bands (Fig. 1*B*, lane 2). In the latter case, however, the reaction with MAP-1 was stronger than that with MAP-2 subcomponents (see also figure 7 in ref. 13). When analyzed by immunofluorescence microscopy, both affinity-purified antibody preparations stained extracellular material (Fig. 6 *A* and *B*) similar to anti-MAP-1 antiserum. Furthermore, the antibodies affinity-purified on the M_r 205,000 antigen stained intracellular microtubules (Fig. 6*C*). In similar experiments with antibodies to MAP-2 affinity-purified on the M_r 205,000 antigen, neither intra- nor extracellular staining was observed (Fig. 6*D*).

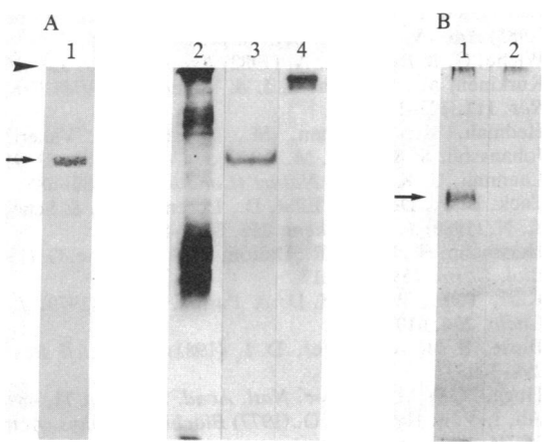

FIG. 5. Identification of M_r 205,000 antigen as sulfoglycoprotein present in growth medium (*A*) and extracellular matrix (*B*). BALB/c 3T3 cell cultures were labeled with tritiated carbohydrates or [^{35}S]sulfate. Samples were analyzed on 7.5% (*A*) or 5% (*B*) polyacrylamide gels. (*A*) Fluorographies of proteins released into the growth medium. Lanes: 1, tritiated carbohydrate-labeled proteins immunoprecipitated with antibodies to MAP-1; 2, total [^{35}S]sulfate-labeled proteins; 3 and 4, [^{35}S]sulfate-labeled proteins immunoprecipitated with antibodies to MAP-1 and electrophoretically analyzed under reducing (lane 3) or nonreducing conditions (lane 4). (*B*) Fluorographies of immunoprecipitated [^{35}S]sulfate-labeled extracellular matrix proteins. Lanes: 1, antibodies to MAP-1, 2, nonimmune serum. Contrary to all other lanes, in lane 4 of *A*, sample buffer without 2-mercaptoethanol was used. Arrowhead, start of running gel; arrows, M_r 205,000 protein.

DISCUSSION

The most intriguing aspect of the findings reported here is that MAP-1 and MAP-2, ubiquitous components of the intracellular cytoskeleton, and an extracellular antigen located in the pericellular matrix of cultured cells are immunologically, hence structurally, related. Thus, MAPs could be secreted from cells after chemical modification, or the extracellular antigen might share common antigenic determinants with MAPs without being derived from them. Further chemical analysis is necessary to distinguish between these possibilities.

The antibodies to MAP-1 used in this study are also reactive with antigenic determinants on MAP-2 subcomponents (9, 13, 23). This is easily explained by the partial structural homology of MAP-1 and MAP-2 revealed by chemical methods (17, 23, 28). Since the anti-MAP-1 antibodies affinity-purified on the extracellular M_r 205,000 protein showed stronger crossreaction with MAP-2 subcomponents than did those affinity-purified on MAP-1 (Fig. 1*B*), it is likely that the major crossreactive determinants of the M_r = 205,000 protein are those that are shared between MAP-1 and MAP-2. Consistent with this interpretation is the observation that our anti-MAP-2 antiserum neither was significantly crossreactive with MAP-1 (9, 13) nor did it bind to extracellular material. Evidently, the antigenic determinants shared by MAP-1 and MAP-2 subcomponents are not recognized by this antiserum.

A series of experiments primarily immunological in nature clearly established that the immunoreactive M_r 205,000 protein present in the cell culture medium and the extracellular matrix was structurally unrelated to fibronectin, laminin, and collagens, including collagen type IV, which possesses a similar molecular weight. Likewise, a M_r 180,000 sulfoglycoprotein, which is synthesized and secreted by various cell lines including mouse 3T3 and 3T6 cells (29) appears to be distinct from this MAP-related protein as it does not undergo disulfide-dependent intermolecular interaction. Furthermore, as judged from gel electrophoresis, the MAP-related antigen appears to be less abundant than most of these other extracellular matrix components. With respect to chemical and functional properties, however, the M_r 205,000 protein to some extent resembles fibronectin. Like fibronectin, the M_r 205,000 protein is highly sensitive to trypsin treatment and undergoes intermolecular disulfide bonding. Also reminiscent of fibronectin is its decreased level in transformed cells, raising the question of whether this protein, too, may be significant in the expression of malignancy and transformation. A further similarity to fibronectin is the protein's response to treatment of cells with drugs that dramatically alter the organization of the cytoskeleton, such as colcemid and cytochalasin B. Based on these results, the anchorage of the M_r 205,000 protein in the matrix, like that of fibronectin, does not seem to depend on the integrity of microtubules (19); whereas its loss from the surface after treatment with cytochalasin B suggests its interaction with cytoplasmic microfilaments. The release of the antigen from the cell surface could also be an indirect effect mediated through a possible association with fibronectin. Different

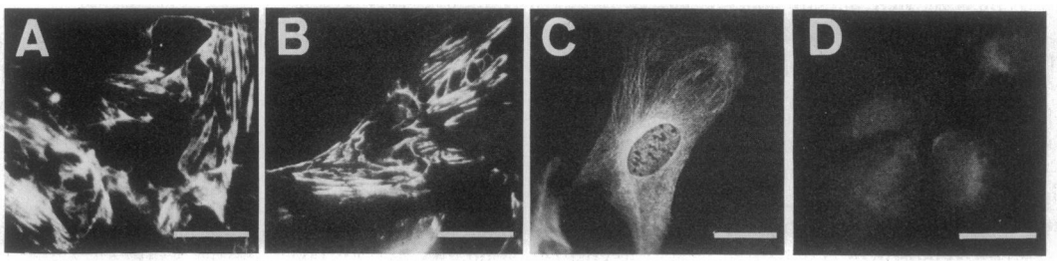

FIG. 6. Immunofluorescence microscopy of BALB/c 3T3 cells using affinity-purified antibodies. Confluent (*A*, *B*, and *D*) or subconfluent (*C*) cell cultures were fixed by method *i* or method *iii*, respectively. (*A*) Anti-MAP-1 antibodies affinity-purified on hog brain MAP-1. (*B* and *C*) Anti-MAP-1 antibodies affinity-purified on the M_r 205,000 extracellular antigen. (*D*) Anti-MAP-2 antibodies affinity-purified on the M_r 205,000 antigen. [Texas red; ×496 (*A*), ×329 (*B*), ×208 (*C*), ×256 (*D*).] [Bars = 20 μm (*A*), 30 μm (*B*), and 40 μm (*C* and *D*).]

functions of the M_r 205,000 protein and fibronectin were indicated, however, by the different responses of monolayer cell cultures when incubated with antibodies to MAP-1 and to fibronectin. Whereas cells incubated with antibodies to fibronectin retracted from the culture dishes as described (30), cells exposed to antiserum to MAP-1 did not alter their morphology (unpublished results). However, this does not rule out a possible role of the MAP-1-related extracellular antigen in cell attachment, since accessibility of the antigens for antibodies might be impaired in living cells.

Among a series of different established cell lines tested by using immunofluorescence microscopy, only mouse 3T3 and 3T6 cells showed a positive immunoreaction of extracellular matrix components with antibodies to MAP-1. No such staining was observed with mouse neuroblastoma N_2A, rat glioma C6, Chinese hamster ovary (CHO), bovine kidney (MDBK), human HeLa, and rat kangaroo PtK_2 cells. Of these, HeLa, PtK_2, and MDBK also showed little intracellular staining, but, similar to 3T3 and 3T6 cells, CHO, N_2A and C6 cells displayed intracellular microtubule structures that were brightly stained (13). Thus, the lack of extracellular staining observed with these latter cell lines indicates either that they do not secrete MAP-related antigens or that they do not produce matrix structures that are capable of incorporating such antigens. An additional, though less likely, possibility is that extracellular antigens are accessible to the antibodies only in mouse fibroblast cells.

Possible functions of the extracellular MAP-related antigen at the present point can only be hypothesized. The experiments with colcemid showed that the anchorage of this protein in the extracellular matrix is independent of the integrity of microtubules. However, as tubulin has been found at the outer surface of cells (31, 32), one could conceive of an interaction between membrane-bound tubulin and the MAP-related extracellular matrix antigen. Such an interaction could play a role in cellular morphogenesis and development.

We are grateful to Professor P. Heistracher for the use of his photolaboratory and to Drs. S. I. Katz and G. Stingl for generous gifts of antisera to laminin, fibronectin, and collagen type IV. We also want to thank our colleagues in the laboratory for comments on the manuscript. This research was supported by Grant 5263 of the Austrian Science Research Fund (Österreichischer Fonds zur Förderung der Wissenschaftlichen Forschung).

1. Murphy, D. B. & Borisy, G. G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2696–2700.
2. Sloboda, R. D., Dentler, W. L. & Rosenbaum, J. L. (1976) *Biochemistry* **15**, 4497–4505.
3. Herzog, W. & Weber, K. (1978) *Eur. J. Biochem.* **92**, 1–8.
4. Kuznetsov, S. A., Rodionov, V. I., Gelfand, V. I. & Rosenblat, V. A. (1981) *FEBS Lett.* **135**, 241–244.
5. Vallee, R. B. & Davis, S. E. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1342–1345.
6. Dentler, W. L., Granett, S. & Rosenbaum, J. L. (1975) *J. Cell Biol.* **65**, 237–241.
7. Amos, L. A. (1977) *J. Cell Biol.* **72**, 642–654.
8. Matus, A., Bernhardt, R. & Hugh-Jones, T. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3010–3014.
9. Wiche, G., Briones, E., Hirt, H., Krepler, R., Artlieb, U. & Denk, H. (1983) *EMBO J.* **2**, 1915–1920.
10. Bloom, G. S., Schoenfeld, T. A. & Vallee, R. B. (1984) *J. Cell Biol.* **98**, 320–330.
11. Huber, G. & Matus, A. (1984) *J. Cell Biol.* **98**, 777–781.
12. Wiche, G. (1985) *Trends Biol. Sci.* **10**, 67–70.
13. Wiche, G., Briones, E., Koszka, C., Artlieb, U. & Krepler, R. (1984) *EMBO J.* **3**, 991–998.
14. Bloom, G. S., Luca, F. C. & Vallee, R. B. (1984) *J. Cell Biol.* **98**, 331–340.
15. Bernhardt, R. & Matus, A. (1982) *J. Cell Biol.* **92**, 589–593.
16. Caceres, A., Payne, M. R., Binder, L. I. & Steward, O. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1738–1742.
17. Wiche, G., Herrmann, H., Dalton, J. M., Foisner, R., Leichtfried, F. E., Lassmann, H., Koszka, C. & Briones, E. (1985) *Ann. N. Y. Acad. Sci.*, in press.
18. Wiche, G. & Baker, M. A. (1982) *Exp. Cell Res.* **138**, 15–29.
19. Kurkinen, M., Wariovaara, J. & Vaheri, A. (1978) *Exp. Cell Res.* **111**, 127–137.
20. Hedman, K., Kurkinen, M., Alitalo, K., Vaheri, A., Johansson, S. & Höök, M. (1979) *J. Cell Biol.* **81**, 83–91.
21. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
22. Tack, B. F., Dean, J., Eilat, D., Lorenz, P. E. & Schechter, A. N. (1980) *J. Biol. Chem.* **255**, 8842–8847.
23. Herrmann, H., Pytela, R., Dalton, J. M. & Wiche, G. (1984) *J. Biol. Chem.* **259**, 612–617.
24. Karr, T. L., White, H. D. & Purich, D. L. (1979) *J. Biol. Chem.* **254**, 6107–6111.
25. Blose, S. H. & Meltzer, D. I. (1981) *Exp. Cell Res.* **135**, 299–309.
26. Hogg, N. W. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 489–492.
27. Ali, I. V. & Hynes, R. O. (1977) *Biochim. Biophys. Acta* **471**, 16–24.
28. Herrmann, H., Dalton, J. M. & Wiche, G. (1985) *J. Biol. Chem.* **260**, 5797–5803.
29. Hogan, B. L. M., Taylor, A., Kurkinen, M. & Couchman, J. R. (1982) *J. Cell Biol.* **95**, 197–204.
30. Yamada, K. M. (1978) *J. Cell Biol.* **78**, 520–541.
31. Estridge, M. (1977) *Nature (London)* **268**, 60–63.
32. Quillen, M., Castello, C., Krishan, A. & Rubin, R. W. (1983) *J. Cell Biol.* **97**, 299a.