A Microtubule-binding Protein Associated with Membranes of the Golgi Apparatus

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Abstract. A monoclonal antibody (M3A5), raised against microtubule-associated protein 2 (MAP-2), recognized an antigen associated with the Golgi complex in a variety of non-neuronal tissue culture cells. In double immunofluorescence studies M3A5 staining was very similar to that of specific Golgi markers, even after disruption of the Golgi apparatus organization with monensin or nocodazole. M3A5 recognized one band of Mr 110,000 in immunoblots of culture cell extracts; this protein, designated 110K, was enriched in Golgi stack fractions prepared from rat liver. The 110K protein has been shown to partition into the aqueous phase by Triton X-114 extraction of a Golgi-enriched fraction and was eluted after pH 11.0 carbonate washing. It is therefore likely to be a peripheral membrane protein. Proteinase K treatment of an isolated Golgi stack fraction resulted in complete digestion of the 110K protein, both in the presence and absence of Triton X-100. As the 110K protein is accessible to protease in intact vesicles in vitro, it is presumably located on the cytoplasmic face of the Golgi membrane in vivo. The 110K protein was able to interact specifically with taxol-polymerized microtubules in vitro. These results suggest that the 110K protein may serve to link the Golgi apparatus to the microtubule network and so may belong to a novel class of proteins: the microtubule-binding proteins.

The Golgi apparatus is the organelle which is the processing and sorting center for proteins en route to the plasma membrane or lysosomes, and also for secretory proteins (for review see Farquhar and Palade, 1981). The Golgi apparatus consists of well-ordered stacks of membrane cisternae which are usually found in the perinuclear region of the cell. Although many of the enzymes involved in the posttranslational modification of proteins passing through the Golgi region are characterized, no proteins have yet been identified that have a role in maintaining Golgi apparatus structure or position within the cell.

There is considerable evidence that the perinuclear location of the Golgi apparatus relies on the presence of the cytoplasmic microtubule network. During mitosis, for example, the cytoplasmic microtubules are depolymerized and the Golgi apparatus becomes scattered and fragmented (e.g., Robbins and Gonatas, 1964; Burke et al., 1982). Drugs which induce depolymerization of microtubules cause similar disruption of the Golgi apparatus' perinuclear organization (Robbins and Gonatas, 1964; Moskalewski et al., 1975; Reaven and Reaven, 1980; Rogalski and Singer, 1984). Microinjection of anti-tubulin antibodies in sufficient quantities to disorganize the microtubule network also results in scattering of the Golgi complex throughout the cytoplasm (Wehland and Willingham, 1983; Wehland et al., 1983).

Consistent with microtubules playing a part in maintaining the position of the Golgi apparatus within the cell, the microtubule-organizing center (MTOC)1 has often been observed in the same perinuclear region by immunofluorescence studies (Kupfer et al., 1982). Electron microscopic data have shown that the MTOC is frequently found in the vicinity of the trans Golgi cisternae (Robbins and Gonatas, 1964; Farquhar and Palade, 1981). In addition, Tassin et al. (1985) observed that during myogenesis the microtubule-initiating sites reorganized to the nuclear membrane and also that the Golgi apparatus redistributed to surround the nuclei completely. It has also been shown in motile cells, such as fibroblasts or macrophages, that mobility results in a relocation of both the MTOC and the Golgi apparatus to the side of the nucleus nearest to the cell's leading edge (Couchman and Rees, 1982; Kupfer et al., 1982; Nemere et al., 1985). This polarization of the Golgi apparatus and the cytoskeleton in the motile cell may direct the transport of new membrane material from the Golgi apparatus to the plasma membrane toward the leading edge of the cell (Bergmann et al., 1983). There is conflicting evidence, however, regarding the importance of the microtubule network in directing polarized transport of plasma membrane components from the Golgi apparatus to the plasma membrane (compare Rogalski et al., 1984, with Salas et al., 1986).

Microtubule-associated proteins (MAPs) are proteins that

1. Abbreviations used in this paper: CEF, chicken embryo fibroblast; MAP, microtubule-associated protein; MTOC, microtubule-organizing center; PB, polymerizing buffer; VSV-G, vesicular stomatitis virus glycoprotein.
Materials and Methods

Cell Culture

Vero cells (African green monkey kidney cells, ATCC CCL 81) were maintained in MEM containing 5% FCS, 1% non-essential amino acids, and 1% L-glutamine. Madin-Darby canine kidney (MDCK) type II cells were grown in MEM containing 10% FCS, 10 mM Hepes, and 1% glutamine. PK13 cells (Potoroo kidney epithelial cells, ATCC CCL 56) were grown in MEM containing 10% FCS, 1% non-essential amino acids, and 1% L-glutamine. All cells were maintained in humidified CO₂ incubators (7% CO₂, 93% air) at 37°C (or 31°C or 39.5°C) in MEM lacking methionine, supplemented with 5% undialyzed FCS. 6 × 10⁷ cells were washed with PBS and then scraped and analyzed by SDS PAGE on 4–15% linear acrylamide gradients. Fluorography with radiolabeled FCS. 6 x 10⁷ cells were washed with ice-cold PBS and then scraped in 1 ml ice-cold PBS. The cells were pelleted by centrifugation at 1,500 × g for 30 s and resuspended in 1 ml 50 mM Tris, 100 mM NaCl, 0.1% (wt/vol) SDS, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), pH 7.5, and sonicated until the DNA was fragmented (15–60 s). The samples were adjusted to 2% (vol/vol) Triton X-100 and 1 mg/ml leupeptin, aprotinin, and pepstatin (Sigma Chemical Co.), and then preincubated with non-immune IgG plus 200 μl of 1:1 slurry of protein A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) in washing buffer (50 mM Tris, 100 mM NaCl, 0.1% (wt/vol) SDS, 0.5% (wt/vol) Triton X-100, pH 7.5) for 30 min at room temperature. The beads were pelleted at 4,300 g for 15 min at room temperature, and the supernatants were incubated with 4°C overnight with either 20 μl M3A5 ascites or 10 μl anti-α-tubulin (YLI2) or 6 μl mouse non-immune IgG. 100 μl protein A-Sepharose in a 1:1 slurry with washing buffer was added per 10 μl antibody, and incubated for 40 min with 4°C overnight. The beads were pelleted by centrifugation at 4,300 g for 30 min, allowed to stand for 10 min at 4°C in washing buffer, then rinsed three times with washing buffer at 4°C, and finally washed with 50 mM Tris, pH 7.5. The beads were boiled in 100 μl gel sample buffer to release bound antigen and analyzed by SDS PAGE on 4–15% linear acrylamide gradients. Fluorography with sodium salicylate as fluor was performed as described (Chamberlain, 1979) using preflashed Kodak X-Omat AR5 film (Laskey and Mills, 1975).

Membrane Extraction and Washing Procedures

A rat liver fraction enriched in Golgi stacks (prepared according to Lee-lavathi et al., 1970) and Vero cells were extracted with Triton X-104 as described by Bordier (1981). The detergent fractions and samples of the initial extract were taken up directly in gel sample buffer; aqueous fractions were first concentrated by precipitation with 10% (wt/vol) TCA. A sample of the Golgi fraction was diluted 30-fold with 100 mM potassium phosphate buffer, pH 6.7, to lower the sucrose concentration and then centrifuged in a Ti45
The pellet and the "IV_A-precipitated supernatant were dissolved in gel sample buffer. The Golgi fraction was also washed with 100 mM sodium carbonate, pH 11.0, as described by Fujiki et al. (1982). After centrifugation at 100,000 g, in a Beckman Airfuge at 4°C for 1.5 h, the carbonate-insoluble material was dissolved directly in gel sample buffer while carbonate-soluble material was dissolved after TCA-precipitation. All samples were analyzed by immunoblotting.

Protease Treatment of Golgi Fractions

Golgi membranes were treated with proteinase K (E. Merck, Darmstadt, FRG) using a modification of the method described by Fleischer (1981). Golgi membranes (230 μg protein in 21 μl of 0.6 M sucrose, 5 mM MgCl₂, 100 mM KH₂PO₄/K₂HPO₄, pH 6.7) were adjusted to a final concentration of 25 mM Hepes, 25 mM KCl, 2.5 mM Mg(CH₃COO)₂, pH 7.9 (final volume of 50 μl) and were incubated for 30 min at 37°C in the presence of 0, 1, 5, or 100 μg/ml proteinase K either with or without 0.4% (vol/vol) Triton X-100. Digestion was halted by placing on ice and addition of PMSF to a final concentration of 4 mM. After digestion, two 7.5-μl aliquots were assayed for galactosyltransferase activity according to the method of Bretz et al. (1980), with ovalbumin as acceptor. The remaining digested samples were resuspended in gel sample buffer and analyzed on a 4-15% linear acrylamide gradient by SDS PAGE followed by immunoblotting, using M3A5 ascites fluid.

Binding of the 110K Protein to Taxol-stabilized Microtubules

A Triton X-114 extract was prepared from five confluent 245 × 245-mm dishes of CEFs according to the method of Bordier (1981), except that the detergent extraction buffer contained microtubule-polymerizing buffer (PB; 100 mM K-Pipes, pH 6.8, 1 mM MgCl₂, 2 mM EGTA, 0.1 mM GTP). The aqueous fraction (13 ml) was removed and clarified an additional two times by centrifuging at 300 g. The microtubule-containing pellets were resuspended in PB and incubated with 3 ml of the CEF Triton X-114 aqueous fraction for 20 min at 25°C. Both supernatants were concentrated by precipitation with 10% (wt/vol) TCA. This concentration of TCA also precipitated the K-Pipes, giving rise to very large pellets. These were resuspended in sample buffer, and in order to dissolve the pellets the bromphenol blue indicator was titrated from yellow to blue with 2 M NaOH. The two supernatant gel samples contained ~220 mM K-Pipes, so the pellet samples were also brought to 220 mM K-Pipes before SDS PAGE. The supernatants and pellets were then analyzed by immunoblotting.

Results

Immunoblotting and Immunoprecipitation of Cell Extracts with M3A5

Microtubule protein prepared from goose brain (the source of the original immunogen) and bovine brain was analyzed by immunoblotting with M3A5. M3A5 detected a high molecular weight doublet in both microtubule protein preparations (Fig. 1, lanes a and e). The same doublet was stained by a polyclonal anti-MAP-2 antibody (a generous gift from G. Scapigliati, Sclavo Institute, Siena, Italy; data not shown). Various non-neuronal cells (Vero, MDCK II, and CEF) were extracted directly into hot sample buffer and then analyzed by immunoblotting. In these extracts M3A5 recognized only a single band of ~110,000 M, (Fig. 1, lanes b-d). Neither M3A5 nor the polyclonal anti-MAP-2 antibodies detected proteins in the 270,000–300,000 M, MAP-2 region of the gel, suggesting that MAP-2 was either present at concent-
Figure 2. M3A5 stains the Golgi apparatus in a variety of tissue culture cell types. Vero cells were double stained with M3A5 (a) and anti-galactosyl transferase (b). CEF cells infected with ts045 VSV and shifted to permissive temperature for 30 min were double stained with M3A5 (c) and anti-VSV-G (d). MDCK cells (e) and Vero cells (f) were stained with M3A5 alone. M3A5 staining of the Golgi was abolished after preabsorption of M3A5 with 7.5 μg of boiled MAPs per μl of ascites fluid (f). Bar, 10 μm.

Concentrations too low to detect in these cell types, or that M3A5 does not recognize the non-neuronal form of MAP-2. To try to ensure that the 110K protein observed in non-neuronal cell extracts was not a degradation product of MAP-2, formed despite the rapid extraction procedure, 235 μg of goose brain microtubule protein was combined with $4 \times 10^6$ Vero cells and then extracted as before. MAP-2 was not degraded during the extraction (Fig. 1, lane f) and so it seems likely that the 110K protein is not an artefactual MAP-2 degradation product. It remains possible, however, that endogenous Vero
MAP-2, if present, may have a different sensitivity to proteolytic enzymes compared to goose brain MAP-2. M3A5 did not bind to the 110K protein on immunoblots of Vero cell extract after preincubation of the antibody with a boiled bovine microtubule protein fraction which was enriched with MAP-2 (Fellous et al., 1977; data not shown). We conclude, therefore, that MAP-2 and the 110K protein share an antigenic determinant.

M3A5 immunoprecipitated a protein of ~110,000 Mₐ (Fig. 1, lane g) from Vero cells labeled with [³⁵S]methionine for 20 h. As a positive control tubulin was immunoprecipitated with YL1/2 (Fig. 1, lane h), a rat monoclonal anti–tubulin antibody (Kilmartin et al., 1982). M3A5 immunoprecipitated the 110K protein from non-neuronal cell extracts and MAP-2 from a bovine brain microtubule protein mixture in either the presence or absence of SDS (data not shown).

**Distribution of the 110K Protein in Tissue Culture Cells**

M3A5 stained a reticular structure in the perinuclear area of the following non-neuronal cells of various species and tissue origins: Vero (Fig. 2 a), CEF (Fig. 2 c), MDCK II (Fig. 2 e), as well as in PtK₂, baby hamster kidney, and HeLa cells (not shown). Double-labeling with an antibody to galactosyltransferase (Roth and Berger, 1982), a marker for the trans Golgi cisternae (Fig. 2 b), revealed that M3A5 stained the Golgi apparatus and a population of vesicles scattered throughout the cytoplasm. In CEF cells infected with ts045 VSV, M3A5, and polyclonal anti-VSV-G gave similar immunofluorescence staining patterns (Fig. 2, c and d), again localizing the M3A5 antigen in the Golgi apparatus. Both M3A5 and anti-VSV-G also stained a number of vesicles in the cytoplasm (arrows, Fig. 2, c and d) which may correspond to transport vesicles carrying VSV-G within the cell, perhaps from the Golgi apparatus to the plasma membrane. The Golgi apparatus staining pattern of M3A5 was abolished after preabsorption of M3A5 (Fig. 2 f) with a boiled microtubule protein fraction. This fraction contained predominantly MAP-2 with some of the Tau proteins, prepared from bovine brain as described by Fellous et al. (1977).

In cultured neuronal cells prepared from chicken brain, M3A5 stained cell processes as well as the cell body. It was not possible, however, to distinguish which structures were labeled within the small, rounded cell bodies. M3A5 and rat anti–tubulin gave similar staining patterns on these cells (Fig. 3, a and b), except that M3A5 labeling was more discontinuous than anti–tubulin labeling. Some filamentous structures were stained with both antibodies; these filaments are probably microtubules or microtubule bundles. The similarity between the M3A5 and anti–tubulin staining patterns is in sharp contrast to the situation in the non-neuronal cells tested, and reflects the fact that the chick neuronal microtubules contain significant amounts of MAP-2.

The co-localization of the M3A5 antigen and galactosyltransferase was confirmed in cells treated with 10 µM monensin or 10 µM nocodazole. Monensin caused vacuolation of the Golgi, giving the dark circular patches seen in Fig. 4, a and b. M3A5 and anti–galactosyltransferase staining patterns were only partially superimposable after monensin treatment, as in some cases M3A5 stained crescents adjacent to vacuoles, whereas the anti–galactosyltransferase staining appeared to extend around the perimeter of the vacuoles (arrows, Fig. 4, a and b). Neither the microtubule network (Fig. 4 c) nor the overall perinuclear location of the Golgi apparatus (Fig. 4, a and b) were affected by 10 µM monensin. Nocodazole, in contrast, caused complete depolymerization of microtubules (Fig. 4 f) and scattering of the Golgi apparatus throughout the cytoplasm (Fig. 4, d and e). M3A5 and anti–galactosyltransferase staining patterns remained virtually coincident after nocodazole treatment. The 110K protein did not redistribute into the nucleus when Vero cells were heat shocked (not shown), and so M3A5 seems not to be specific for the 100/110K protein recognized by the JLI5a monoclonal antibody (Lin and Queally, 1982; Welch et al., 1983). Immunofluorescence staining with M3A5 was successful only with methanol/acetone, paraformaldehyde, or ethylene glycol bis (succinimidyldimaleate) (Mitchinson and Kirschner, 1985) fixation, followed for the latter two methods by permeabilization with 0.1% Triton X-100 and 0.05% SDS. No M3A5 staining was observed when SDS was not present during the permeabilization step. One explanation for this could be that the M3A5 antigenic site is “cryptic” because the 110K protein is involved in a protein–protein interaction which

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**Figure 3.** M3A5 labels cell processes in chicken brain neuronal cells. Chick brain neuronal cells prepared according to Pettmann et al. (1979) were grown on poly-L-lysine-coated coverslips and double-labeled with M3A5 and YLI/2 (a rat anti–tubulin antibody). Both M3A5 and YLI/2 stain filamentous structures within the cell processes. Bar, 10 µm.
Figure 4. Effect of monensin and nocodazole on the distribution of the 110K protein. Vero cells grown on coverslips and treated with 10 μM monensin for 15 min (a–c) or 10 μM nocodazole for 45 min (d–f) were fixed and then the coverslips were divided. Half of each coverslip was double-stained with M3A5 (a and d) and anti-galactosyltransferase (b and e) and each other half was single stained with YL1/2 (c and f), an anti-tubulin antibody. Arrows (a and b) point to a monensin-induced vacuole with associated M3A5 and anti-galactosyltransferase staining. M3A5 and anti-galactosyltransferase both stain the same Golgi elements after nocodazole treatment (arrowheads in d and e). Monensin had no effect on the microtubule network, whereas nocodazole induced complete depolymerization. Bar, 10 μm.

needs to be broken by SDS, for example, before the antigenic site is available. It has therefore so far proved difficult to obtain conclusive results using M3A5 for immunoelectron microscopy on frozen sections. To determine the precise location of the 110K protein within the Golgi apparatus, a polyclonal antibody which can recognize more accessible regions of the protein may be required.
The distribution of the 110K protein in subcellular fractions prepared from rat liver. Rat liver fractionation was performed according to Leelavathi et al. (1970), and fractions were immunoblotted with M3A5. (Lane a) Total homogenate; (lane b) S₁ Golgi stack fraction (from 0.25/0.50 M sucrose interface); (lane c) S₂ Golgi stack fraction (0.50/0.86 M sucrose); (lane d) 0.86 M sucrose fraction; (lane e) 0.86/1.30 M sucrose interface; (lane f) pellet. 75 μg of protein was loaded per lane.

The 110K Protein Is Located on the Cytoplasmic Face of the Golgi Membrane

It was clearly of interest to discover with which face of the Golgi membrane the 110K protein interacted. This was determined by digestion of Golgi vesicles with proteinase K (Fig. 7) or TPCK trypsin (data not shown) in both the presence and absence of Triton X-100. The extent of 110K degradation was established by immunoblotting with M3A5.

The 110K Protein Is a Peripheral Membrane Protein

When the Golgi fraction was centrifuged at 100,000 g₀ for 120 min, all of the 110K protein was found in the pellet (Fig. 6, lane a) suggesting that it was associated with membranes. The same results were obtained with Vero cell homogenates.
Figure 7. Digestion of the 110K protein with proteinase K. Aliquots of rat liver Golgi membranes (230 μg protein) were incubated for 30 min with 0 (a and b), 1 (c and d), 5 (e and f), or 100 (g and h) μg/ml proteinase K either in the absence (a, c, e, and g) or presence (b, d, f, and h) of 0.4% Triton X-100. After incubation and addition of PMSF to block protease activity, duplicate aliquots were assayed for galactosyltransferase activity. The remaining samples were reduced and analyzed by immunoblotting. The 110K protein is accessible to proteinase K in both intact and detergent-disrupted vesicles. It is digested to a 48-kD fragment (48K) which is recognized by M3A5 after treatment with 1 μg/ml of proteinase K (c and d), or completely digested at higher protease concentrations (e–h).

The integrity of the Golgi vesicles was monitored in parallel by assaying galactosyltransferase activity. Galactosyltransferase is a Golgi enzyme which is found exclusively in the lumen of the Golgi cisternae and so should not be accessible for digestion by proteinase K or trypsin unless Triton X-100 is also present in the digestion mixture (Fleischer, 1981). The 110K protein is proteolyzed to give a fragment of ~48 kD by 1 μg/ml proteinase K both in the absence (lane c) and presence (lane d) of Triton X-100. When 5 μg/ml or 100 μg/ml proteinase K was used (lanes e–h), the fragment of the 110K protein carrying the M3A5 epitope was completely degraded. Thus, the ~48-kD fragment observed after treatment with 1 μg/ml proteinase K is an intermediate form, and is not resistant to digestion. A similar intermediate fragment (~51 kD), observed after treatment with 10 μg/ml TPCK-trypsin, disappeared after digestion with 100 μg/ml both with and without Triton X-100 (data not shown). The vesicles were shown to be intact, as digestion with 100 μg/ml proteinase K in the absence of Triton resulted in a decrease in galactosyltransferase activity of only 12%, compared to total loss of activity after incubation with 100 μg/ml proteinase K in the presence of Triton X-100 (Fig. 7, lanes g and h). With 100 μg/ml TPCK-trypsin, galactosyltransferase activity was reduced by 17 or 100%, without and with Triton X-100, respectively (data not shown). Both the ~48-kD and ~51-kD fragments produced by proteinase K and TPCK-trypsin, respectively, remained associated with the membrane, as judged by ultracentrifugation (data not shown). These results show that the 110K protein is accessible for protease digestion when Golgi vesicles are intact, and so must be associated with the cytoplasmic surface of Golgi membranes and not with the luminal face of the vesicle membrane.

The 110K Protein Interacts with Taxol-stabilized Microtubules In Vitro

To find out whether the 110K protein and MAP-2 share any functional properties in addition to being immunologically related, we determined whether the 110K protein could interact with microtubules in vitro. As a source of soluble non-membrane-bound 110K protein, we used the Triton X-114 aqueous fraction from cultured primary CEFs after removal of insoluble material by centrifugation at 105,000 g for 30 min. We then either polymerized microtubules at 37°C from the endogenous tubulin in the extract, in the presence of 10 μM taxol and 1.0 mM GTP, or, we added exogenously taxol-polymerized calf brain tubulin microtubules to the aqueous fraction. The microtubules were then collected by centrifugation through a 4-M glycerol cushion at 100,000 g for 20 min. Immunoblotting was used to analyze the dis...
distribution of the 110K protein and a number of other proteins within the microtubule pellets and the supernatants (Fig. 8). The 110K protein was present in both endogenous and exogenous microtubule pellets (Fig. 8, lanes b and d) as well as in the supernatant fractions (lanes a and c), and so appeared to interact with microtubules. As a control to test whether soluble proteins were becoming nonspecifically pelleted with microtubules, we studied the distribution of IEF 24, a mitochondrial protein (Mose-Larsen et al., 1982) which is located in the lumen of mitochondria (Griffiths, G., and R. Bravo, unpublished results) and so is not normally accessible to microtubules in the cytoplasm. A polyclonal antiserum to IEF 24 (a generous gift from R. Bravo, EMBL, Heidelberg, FRG) revealed that the protein was present in both supernatants (Fig. 8, lanes e and g) but was absent from both microtubule pellets (lanes f and h). The faint band observed above IEF 24 is present in lanes e-h, irrespective of the distribution of IEF 24, and is therefore most likely to be unspecific.

A monoclonal antibody to β-actin (6B1) revealed that actin was present in low amounts in both microtubule pellets (data not shown). Actin was considerably enriched, however, in the supernatant fractions. The 110K protein was clearly enriched in both microtubule pellets compared to actin. Thus it seems unlikely that the presence of the 110K protein in the pellets can be ascribed to its forming a complex with actin. As an additional control, the distribution of vimentin was studied, using another monoclonal antibody (7A3). Vimentin was absent from all pellets and supernatants (data not shown), as would be expected considering that an early step in the Triton X-114 extraction procedure involves the removal of nuclei and insoluble material by centrifugation.

The results described above show that CEF 110K protein can interact specifically with both endogenous CEF microtubules and with calf brain microtubules in vitro. The 110K protein is not present, however, in taxol-polymerized microtubules prepared from CEF according to the method of Vallee (1982) (data not shown). This is to be expected, since digestion by proteinase K in the absence of detergent, the 110K protein must be located on the cytoplasmic face of vesicles derived from a Golgi fraction. So far, only one other monoclonal antibody to cytoplasmic domains of Golgi-specific proteins has been described; the antibody recognizes two peripheral membrane proteins of M, 54,000 and 86,000 (Chicheportiche et al., 1984). Smith et al. (1984) have described two monoclonal antibodies which recognize an M, 103,000–108,000 protein in rat pancreatic acinar cells, but as this protein is an integral membrane protein located on the luminal side of the Golgi membrane, it appears to be distinct from the 110K protein recognized by M3A5. The 110K protein, therefore, is a peripheral, cytoplasmically oriented Golgi protein, which has not been previously described.

In addition to staining the Golgi complex, M3A5 also stained a population of vesicles devoid of galactosyltransferase, which were scattered throughout the cytoplasm. It is not clear to which class of vesicles they belong. The localization of VSV-G and the 110K protein (Fig. 2, c and d) may suggest that they are exocytic vesicles transporting protein from the Golgi apparatus to the cell surface. We cannot exclude, however, that the 110K protein is present in another organelle type, such as endosomes, for example.

MAP-2 and the 110K Golgi protein obviously share an antigenic determinant. Using polyclonal antibodies, several such immunological relationships between MAP-2 and other cytoskeletal proteins, such as the α subunit of erythrocyte spectrin (Davis and Bennett, 1982) and a variety of astrocyte MAPs (Couchie et al., 1985) have been reported. In addition, a relationship between MAP-2 and an extracellular glycoprotein has recently been reported (Briones and Wiche, 1985). Rodionov et al. (1985) have described a monoclonal antibody, RN17, that recognizes MAP-1 and -2 and also a 100-kD protein in various non-neuronal cells, and which stained microtubules and coated vesicles in the same cells. RN17 and M3A5 both appear to recognize distinct proteins, as RN17 did not label the Golgi complex, and M3A5 neither stained microtubules in non-neuronal cells nor detected any 110K protein in a coated vesicle fraction. Whether any of

Discussion

Some of the protein components involved in translocation of vesicles along microtubules in axons have recently been discovered. Kinesin is a translocator molecule which is involved in anterograde movement of vesicles (Vale et al., 1985a), whereas a protein which is immunologically and pharmacologically distinct from kinesin seems to be involved in generating retrograde transport (Vale et al., 1985b). No proteins mediating interactions between microtubules and organelles or vesicles have yet been characterized in non-neuronal cells. MAPs or MAP-related proteins may be good candidates for such interactions. So far several MAPs have been shown to be associated with microtubules in tissues and cultured cells of non-neuronal origin (for reviews see Vallee, 1984; Wiche, 1985). Our aim was to raise monoclonal antibodies against neuronal MAPs and to select for those antibodies which also recognized components of organelles in non-neuronal cells.

With this strategy we obtained M3A5, a monoclonal antibody specific for neuronal MAP-2. In all non-neuronal cells tested, this antibody labeled the Golgi complex but not the microtubule network. Also Wiche et al. (1986) have reported that a polyclonal MAP-2 antibody labeled the perinuclear region of mouse 3T3 cells. In extracts of non-neuronal culture cells and in rat liver Golgi fractions, M3A5 recognized a protein of 100K. This protein was shown to behave like a peripheral membrane protein by two criteria: (a) partitioning into the aqueous phase on extraction with Triton X-114; and (b) removal from the membrane by washing with sodium carbonate at pH 11.0. Furthermore, since it is susceptible to digestion by proteinase K in the absence of detergent, the 110K protein must be located on the cytoplasmic face of vesicles derived from a Golgi fraction. So far, only one other monoclonal antibody to cytoplasmic domains of Golgi-specific proteins has been described; the antibody recognizes two peripheral membrane proteins of M, 54,000 and 86,000 (Chicheportiche et al., 1984). Smith et al. (1984) have described two monoclonal antibodies which recognize an M, 103,000–108,000 protein in rat pancreatic acinar cells, but as this protein is an integral membrane protein located on the luminal side of the Golgi membrane, it appears to be distinct from the 110K protein recognized by M3A5. The 110K protein, therefore, is a peripheral, cytoplasmically oriented Golgi protein, which has not been previously described.

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these observations imply a truly functional, rather than antigenic relationship between MAP-2 and the other proteins, is not clear. When monoclonal antibodies such as M3A5 or RN7 (Rodionov et al., 1985) are used instead of polyclonal antibodies, the likelihood of picking up purely antigenic relationships between two otherwise unrelated proteins must be increased. We have shown, however, that the 110K protein can interact with taxol-stabilized microtubules in a membrane-free system in vitro, so it appears that the 110K protein and MAP-2 are not only immunologically related, but also share the ability to bind to tubulin. This interaction between the 110K protein and microtubules clearly requires further investigation, such as analyzing for co-polymerization with tubulin using a multiple polymerization/depolymerization protocol (without taxol) such as that described by Weatherbee et al. (1980).

Unlike MAP-2, the 110K protein is not located along microtubules in vivo but rather in the Golgi apparatus. The 110K protein therefore should not be termed a MAP. Instead it might act as a microtubule-binding protein which is located on the Golgi membrane and which can also interact with microtubules. In this way, the 110K protein may play a part in linking the Golgi apparatus, and possibly also Golgi-derived transport vesicles, to the microtubule network. A structural link between the Golgi apparatus and microtubules has long been implied, as reviewed above. The 110K protein, being located on the cytoplasmic face of the Golgi apparatus membranes, is clearly in the right position to perform this function. It is also possible that the 110K protein could be involved in linking transmembrane proteins within the Golgi apparatus and transport vesicles, to the cytoskeleton, as has been suggested in a number of other systems (e.g. Ranscht et al., 1984; Bourgignon et al., 1985).

M3A5 recognizes an epitope on the projecting portion of MAP-2 rather than the microtubule-binding domain (data not shown). The projecting arm of MAP-2 has been shown to interact with cyclic AMP-dependent protein kinase (Vallee et al., 1981) and in particular, with its regulatory subunit (RII) (Theurkauf and Vallee, 1982; Lohmann et al., 1984). RII has also been found in association with the Golgi complex and MTOC in epithelial and fibroblastic culture cells (Nigg et al., 1985) and also in a variety of cell types in the nervous system (De Camilli et al., 1986). The 110K protein could be an RII receptor by virtue of its immunological relationship with MAP-2 and its location in the Golgi apparatus. It could not, however, account for the localization of RII at the MTOC, as we have never observed staining of the MTOC with M3A5. Proposing a role for the 110K protein in linking the Golgi apparatus to the microtubular network need not necessarily exclude an interaction with RII, as the presence of RII and the catalytic subunit might provide a mechanism for controlling via phosphorylation the microtubule–Golgi apparatus interaction. Some sort of control mechanism for controlling via phosphorylation the microtubule–Golgi apparatus interaction. Some sort of control system must exist which allows the Golgi apparatus first to interact only at the minus ends of microtubules, and this would involve a passive redistribution of the Golgi complex. Alternatively, the 110K protein could form part of a retrograde translocation complex which would cause movement from the plus to the minus ends of microtubules. The Golgi complex would then be actively transported to the minus ends of microtubules, resulting in a build-up of elements of the Golgi complex around the MTOC. A concentration of actively transported vesicles at the ends of microtubules has indeed been observed in dissociated squid axoplasm (Allen et al., 1985).

Clearly, further experiments are required to enable us to understand the precise function of the 110K protein in the interaction between the Golgi apparatus and the microtubule network, and also in the positioning of the Golgi apparatus within the perinuclear region of the cell.

We would like to thank Jennifer Wellstead for excellent technical assistance, Dr. Kathryn Howell for help with the Golgi stack isolation, and also Dr. Gareth Griffiths for electron microscopic analysis of Golgi membrane fractions. We appreciate the gifts of antibodies from Drs. Eric Berger, Rodrigo Bravo, John Kilminster, Giuseppe Scapigliati, and Kai Simons. We are grateful to Drs. Brian Burke, Wai Chang Ho, and Kathryn Howell for providing helpful comments, and to Annie Steiner for typing the manuscript.

V. J. Allan was supported by a European Scientific Exchange fellowship from the Royal Society of Great Britain.

Received for publication 18 August 1986.

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The Journal of Cell Biology, Volume 103, 1986 2238
Biol. 103:189-203.


Rodionov, V. I., E. S. Nadezhkina, E. V. Leonova, E. A. Vaisberg, S. A.

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