

GMAP-210, A Cis-Golgi Network-associated Protein, Is a Minus End Microtubule-binding Protein

Carlos Infante,* Francisco Ramos-Morales,* Concepción Fedriani,* Michel Bornens,† and Rosa M. Rios*

*Departamento de Microbiología, Facultad de Biología, Universidad de Sevilla Apdo. 1095, 41080-Sevilla, Spain; and †Institut Curie, Section Recherche, UMR144 du CNRS, 75248 Paris, Cedex 05, France

Abstract. We report that a peripheral Golgi protein with a molecular mass of 210 kD localized at the cis-Golgi network (Rios, R.M., A.M. Tassin, C. Celati, C. Antony, M.C. Boissier, J.C. Homberg, and M. Bornens. 1994. *J. Cell Biol.* 125:997–1013) is a microtubule-binding protein that associates in situ with a subpopulation of stable microtubules. Interaction of this protein, now called GMAP-210, for Golgi microtubule-associated protein 210, with microtubules in vitro is direct, tight and nucleotide-independent. Biochemical analysis further suggests that GMAP-210 specifically binds to microtubule ends. The full-length cDNA encoding GMAP-210 predicts a protein of 1,979 amino acids with a very long central coiled-coil domain. Deletion analyses in vitro show that the COOH terminus of GMAP-210 binds to microtubules whereas the NH₂ terminus binds to Golgi membranes. Overexpression of GMAP-

210-encoding cDNA induced a dramatic enlargement of the Golgi apparatus and perturbations in the microtubule network. These effects did not occur when a mutant lacking the COOH-terminal domain was expressed. When transfected in fusion with the green fluorescent protein, the NH₂-terminal domain associated with the cis-Golgi network whereas the COOH-terminal microtubule-binding domain localized at the centrosome. Altogether these data support the view that GMAP-210 serves to link the cis-Golgi network to the minus ends of centrosome-nucleated microtubules. In addition, this interaction appears essential for ensuring the proper morphology and size of the Golgi apparatus.

Key words: autoantigens • Golgi apparatus • microtubule-associated proteins • microtubules • centrosome

INTERPHASE microtubules are believed to play important roles in establishment of cell polarity and organization and distribution of organelles in higher eukaryotic cells. It has become clear that all classes of membrane-bound, cytoplasmic organelles maintain an intimate and dynamic association with the microtubule cytoskeleton (Cole and Lippincott-Schwartz, 1995). The endoplasmic reticulum extends tubular membrane structures towards cell periphery by using microtubule network as a scaffold (Dabora and Sheetz, 1988). The Golgi complex colocalizes with the minus ends of microtubules which are associated with the centrosome (Kreis, 1990). Late endosomes and lysosomes, generally located in the perinuclear area, also display tubular shapes (Matteoni and Kreis, 1987). Recently, involvement of microtubules in the spatial organi-

zation of peroxisomes and in the fast directional movement of a small subpopulation of these organelles has been reported (Wiemer et al., 1997). In each case, the morphology and distribution of the organelles are disrupted by microtubule-depolymerizing drugs.

Microtubules also support the movement of the membranous organelles and their transport intermediates. These intracellular movements that occur in opposite directions along microtubules are thought to be driven by two microtubule motors, kinesin (Vale et al., 1985a) which moves towards the plus ends of microtubules (Vale et al., 1985b), and cytoplasmic dynein which moves towards the minus ends (Paschal and Vallee, 1987). Tubular lysosome extension and anterograde lysosome membrane transport require kinesin activity (Hollenbeck and Swanson, 1990; Nakata and Hirokawa, 1995), and there exist evidences consistent with a role for kinesin in the plus end-directed extension of ER tubules to the cell periphery (Feiguin et al., 1994). Retrograde transport from the Golgi apparatus to the ER is a microtubule-dependent plus end-directed movement also. Consistent with this, kinesin has been shown to be present on membranes that constitutively cy-

This paper is dedicated to the memory of Thomas Kreis.

Address correspondence to Rosa M. Rios, Departamento de Microbiología, Facultad de Biología, Universidad de Sevilla, Apdo. 1095, 41080-Sevilla, Spain. Tel.: 34 954557120. Fax: 34 954557830. E-mail: rrios@cica.es

cle between the ER and the Golgi and microinjection of an anti-kinesin antibody inhibited Golgi to ER but not ER to Golgi membrane transport (Lippincott-Schwartz et al., 1995). In contrast, several studies suggest that movement of membranous organelles towards the centrosome is driven by cytoplasmic dynein. The movement of the Golgi elements towards the minus ends of microtubules has been shown to require cytoplasmic dynein in semi-intact cells (Corthésy-Theulaz et al., 1992). Injection of anti-DHC2 antibodies, a cytoplasmic dynein heavy chain predominantly localized on the ER-Golgi intermediate compartment (Vaisberg, E.A., P.M. Grissom, S.R. Gill, T.A. Schroer, and J.R. McIntosh. 1996. *Mol. Biol. Cell.* 7:403a), induced the dispersion of the Golgi complex (Vaisberg et al., 1996). Overexpression of the dynactin subunit of the dynactin complex led to dispersion of the Golgi complex, endosomes, and lysosomes (Burkhardt et al., 1997) and blocked the ER to Golgi transport (Presley et al., 1997). More recently, cDHC^{-/-} blastocysts have been shown to display a vesiculated Golgi complex, distributed throughout the cytoplasm, although individual Golgi fragments still attached to microtubules (Harada et al., 1998). A similar effect was observed on endosomes and lysosomes distribution. These results indicate that molecules other than cDHC might mediate attachment of the Golgi complex and endosomes and lysosomes to microtubules.

Although the role of motors in these processes is now well established, recent data argue that motors alone are insufficient to mediate interactions of organelles with microtubules. Indeed, in studies where the static binding of organelles to microtubules have been examined, binding have been attributed to nonmotor proteins (Rickard and Kreis, 1996). Comparison of the results obtained in these studies revealed a number of differences suggesting that there are specific nonmotor microtubule-associated proteins for different organelles, that have been named cytoplasmic linker proteins (CLIPs)¹. The only CLIP extensively characterized to date is CLIP-170, a microtubule-binding protein associating transiently with endosomes during early to late endosome transport (Pierre et al., 1992). Recently, it has been found that ch-TOG, the human homologue of XMAP-215, a previously described microtubule-associated protein of *Xenopus* eggs, is associated to ER membranes in interphase human cells (Charasse et al., 1998). In addition, p63, an integral membrane protein of the reticular subdomain of the ER, has been shown to bind microtubules *in vivo* and *in vitro* and it could contribute to the positioning of the ER along microtubules (Klopfenstein et al., 1998). A cytosolic factor of 150 kD responsible for phagosome-microtubule binding has also been described (Blocker et al., 1996).

We have previously described a peripheral Golgi autoantigen of 210 kD whose localization is restricted to the cis-Golgi network (CGN). Interestingly, this protein exhibits an unusual behavior when cells are treated with nocodazole (NZ) or Brefeldin-A. NZ induces a specific and

early segregation of many p210-associated vesicles or tubules from the Golgi apparatus. Upon Brefeldin-A treatment, p210 does not redistribute in the ER but exhibits a vesicular pattern characteristic of proteins residing in the CGN (Rios et al., 1994; Alcalde et al., 1994; Nakamura et al., 1995). In this study we report isolation of the full-length cDNA encoding p210. We show that this protein associates with the Golgi apparatus via its NH₂ terminus and directly interacts with microtubules through its COOH-terminal domain. For these reasons, we have designated it GMAP-210 (Golgi microtubule-associated protein 210). Functional analyses of intact and mutant forms indicate that GMAP-210 is a minus end microtubule-binding protein that plays a role in maintaining the structural integrity of the Golgi apparatus.

Materials and Methods

Cell Culture and Antibodies

HeLa and COS-7 cells were grown under standard conditions. IgG fraction (10 mg/ml final concentration) from RM autoimmune serum (AS) and affinity-purified anti-GMAP-210 antibodies were obtained as described in Rios et al. (1994). CTR433, a mouse monoclonal antibody, is a marker of medial-Golgi (Jasmin et al., 1989). Polyclonal antibody anti- γ -tubulin has been previously characterized (Tassin et al., 1998). Anti-detyrosinated tubulin (α T12 and SG) and anti-p115 antibodies were supplied by Drs. Kreis, Bulinski, and Sztul, respectively.

Molecular Cloning and Sequencing of GMAP-210

10⁶ recombinants of a λ ZAPII human HeLa cell random-primed cDNA expression library (P. Chambon, IGBMC, Université Louis Pasteur, Strasbourg, France) were screened with the autoimmune serum diluted 1:1,500 followed by anti-human IgG alkaline phosphatase conjugated (Promega Corp.), and positive plaques were detected by incubation with BCIP and NBT. Positive clones were plaque purified through several rounds of re-screening. Affinity-purified antibodies against each of the three major autoantigens recognized by the serum (Rios et al., 1994) were obtained and used to group the clones obtained in immunologically related families. pBluescript phagemid containing the cloned cDNA inserts were excised from λ ZAPII by coinfection with R408 helper phage as described in the manufacturer's instructions (Stratagene). The 3' end of GMAP-210 was cloned by RT-PCR. HeLa mRNA was prepared with a Quick prep mRNA purification kit (Pharmacia Biotech). First-strand cDNA synthesis was performed with an oligo-(dT) primer by using a First-strand cDNA synthesis kit (Pharmacia Biotech). The completed first-strand reaction was amplified directly by PCR with specific primers. Clone RT-10, containing the 3' end of GMAP-210 cDNA, was obtained with the primers P1 and P2 as indicated in Fig. 1 A.

A series of overlapping restriction fragments from the clones obtained by immunoscreening were subcloned into pBluescript SK or pTZ19R. Double-stranded cDNA in pBluescript or pTZ19R was sequenced on both strands with an automatic sequencer (Pharmacia) by the dideoxy termination method (Sanger et al., 1977). Sequence data were compiled and analyzed using the University of Wisconsin Genetics Computer Group package version 8.1 for Unix computers and the EGCG extensions to the Wisconsin package version 8.1.0. Comparisons to known sequences were performed using tFASTA and BLAST. Primary and secondary structure analysis was conducted with Motifs, PeptideStructure, PepStats, and Pep-Coil.

The full-length open reading frame of GMAP-210 was assembled in the eukaryotic expression vector pECE (Ellis et al., 1986) using clones R2, R3, RT-7 (obtained by RT-PCR using primers P3 and P4), R6, and RT-10 (see Fig. 1 A). To obtain an HA epitope-tagged GMAP-210 protein, primers P5 and P6 were used to create an EcoRI site in the appropriate frame for cloning into pECE-HA. GMAP-210 cDNA fragments coding for amino acids 1–375 (Δ C375) and 1,778–1,979 (Δ N1778) were cloned in fusion with the green fluorescent protein (GFP) into the eukaryotic expression vectors pEGFPN1 and pEGFPN1, respectively. Full-length or partial GMAP-210 cDNAs were also cloned into the prokaryotic expression plas-

1. *Abbreviations used in this paper:* AS, autoimmune serum; CGN, cis-Golgi network; CLIP, cytoplasmic linker protein; GFP, green fluorescent protein; GMAP, Golgi microtubule-associated protein; GST, glutathione-S-transferase; HSS, high speed supernatant; IB, immunoblotting; IF, immunofluorescence; NZ, nocodazole.

microtubules. The synthesis of the glutathione-*S*-transferase (GST) fusion proteins was induced by addition of 1 mM isopropyl- β -D-thiogalactoside and the fusion proteins were isolated from bacterial lysates by affinity chromatography with glutathione-agarose beads (Sigma).

Northern Blot Analysis

Northern analysis was performed on poly(A)⁺ RNA from human tissues (commercial multiple Northern blot; Clontech). DNA fragments corresponding to nucleotides 418–1,130 and 5,364–5,800 were radiolabeled and used as probes.

Production of Polyclonal Antibodies

After cloning DNA fragments coding for amino acids 375–611 or 618–803 into pGEX vectors, GST-fusion polypeptides were expressed and purified as described above. Fusion proteins were dialyzed in PBS and two rabbit polyclonal antibodies were generated (Agrobio). Sera were collected and Ig fraction purified by ammonium sulfate precipitation. These anti-GMAP-210 polyclonal antibodies were named RM127 and RM130.

Electrophoresis, Immunoblotting and Immunoprecipitation

Gel electrophoresis and immunoblotting (IB) were performed as described (Rios et al., 1994). For immunoprecipitation experiments, HeLa cells were incubated for 30 min on ice in order to depolymerize microtubules and then treated with 50 mM Tris-HCl, 150 mM NaCl, 1% NP-40 containing protease inhibitors. Soluble fraction was clarified at 15,000 *g* for 15 min and preadsorbed with 5 μ l of normal human serum or 5 μ l of each preimmune rabbit sera on 100 μ l of protein A-Sepharose. GMAP-210 was immunoprecipitated from supernatants with 5 μ l AS, 5 μ l RM127, or 5 μ l RM130 on 100 μ l of protein A-Sepharose. After incubation and washing, the presence of GMAP-210 in the bead pellets was detected by IB with RM130 polyclonal antibody.

Preparation of Microtubule-binding Proteins from HeLa Cells

2×10^8 HeLa cells were trypsinized and harvested at 500 *g* for 10 min at room temperature. Subcellular fractionation in order to obtain a high speed supernatant (HSS) was carried out as described by Rickard and Kreis (1990). The protein concentration of the HSSs obtained ranged between 6–8 mg/ml when measured using the Bradford assay. The HSS was adjusted at 3–4 mg/ml with PEM buffer (0.1 M K-Pipes, 2 mM EGTA, 1 mM MgSO₄, pH 6.8), incubated for 30 min on ice and then incubated for 15 min at 37°C in the presence of 10 μ M TaxolTM (Paclitaxel), 1 mM DTT, and 1 mM GTP. Microtubules were centrifuged at 30,000 *g* for 30 min at 30°C through a cushion of 10% sucrose in PEM containing 10 μ M taxol, 1 mM DTT, and 1 mM GTP. Microtubules were washed and resuspended in the same buffer (0.1–0.05 times the original HSS volume). Proteins were eluted from the microtubules by adding 10 mM ATP, 10 mM GTP, 0.5 M NaCl, or 2 M urea, respectively, and incubation at 37°C for 30 min followed by centrifugation.

In Vitro Microtubule Binding Assays with Endogenous GMAP-210 or with GST-Fusion Polypeptides

Tubulin was purified from bovine brain by two cycles of polymerization followed by chromatography on phosphocellulose. 10–20 μ g of pure tubulin/assay were polymerized with 20 μ M taxol, 1 mM GTP, and 1 mM DTT for 15 min at 37°C. Microtubules were centrifuged through 10% sucrose, washed, and finally resuspended in PEM containing GTP and taxol.

After cloning different DNA fragments into pGEX vectors, GST-fusion polypeptides were expressed and isolated from bacterial lysates by affinity chromatography with glutathione-agarose beads. Microtubules were then added to glutathione-agarose beads linked to similar amounts of GST alone, GST-fusion polypeptides or GST-GMAP-210 (<0.5 μ g). After incubation for 2 h at room temperature, beads were low speed sedimented (1,000 *g*) and washed in PEM buffer. The presence of microtubules in the bead pellets was detected with anti- α -tubulin antibodies. Alternatively, GMAP-210 was immunoprecipitated from HeLa cells NP-40 supernatants using 5 μ l AS, 5 μ l RM127, or 5 μ l RM130 coupled to 100 μ l of protein A-Sepharose as described in a previous section. After washing,

beads were incubated for 2 h at room temperature with taxol-polymerized microtubules. Then, beads were low speed sedimented (1,000 *g*) and washed in PEM buffer. The presence of microtubules in the bead pellets was detected with anti- α -tubulin antibodies.

Identification of Microtubule-associated Proteins by Selective Extraction of Cultured Cells

HeLa cells were incubated with 10 μ M NZ or 10 μ M taxol for 4 h at 37°C. Untreated, NZ- or taxol-treated HeLa cells were washed twice with PBS at room temperature and once with a microtubule-stabilizing buffer PM2G (0.1 M K-Pipes, 2 M glycerol, 5 mM MgCl₂, and 2 mM EGTA, pH 6.9). Then, washed cells were extracted for 5 min at room temperature with 0.1–0.3% NP-40 in the same buffer to obtain NP-40 soluble fractions (fraction S). The detergent-extracted preparations, that remained attached to substrate, were washed with PM2G without detergent and incubated for 30 min at 4°C in the same buffer containing 5 mM CaCl₂ to depolymerize microtubules (fraction Ca). The detergent and calcium-insoluble preparations were also harvested (fraction I) and all fractions obtained analyzed by SDS-PAGE and IB. All buffers contained protease inhibitors (1 mM PMSF and 1 μ g/ml each of aprotinin, pepstatin, chymostatin, and leupeptin). In the case of cells pretreated with drugs, each of the washes contained the same concentration of the drug. In parallel, HeLa cells grown on coverslips were treated and successively extracted with NP-40 and calcium as described above. Cell ghosts were then processed for immunofluorescence. The presence of microtubules was revealed by using an anti- α -tubulin antibody.

Binding of GMAP-210 Constructs to Golgi Membranes

Fractions enriched in Golgi membranes were isolated from HeLa cells by flotation in a sucrose gradient as described (Rios et al., 1994). Before further processing, Golgi-enriched fractions were diluted by addition of several volumes of 10 mM Tris-HCl, pH 7.4 and protease inhibitors were added. The suspensions were then centrifuged at 200,000 *g* for 2 h at 4°C. To obtain GMAP-210-depleted Golgi membranes, membrane pellets were incubated with 2 M NaCl for 30 min on ice, high speed centrifuged, and finally resuspended in 10 mM Tris-HCl, pH 7.4, containing 250 mM sucrose. GST-fusion polypeptides were purified on glutathione-agarose beads. Preliminary experiments suggested that the presence of GST in the NH₂ terminus of the fusion proteins could decrease the association of the recombinant proteins to membranes. Therefore, polypeptides were released from the fusion proteins by treatment with 1% thrombin (wt/wt). Full-length GMAP-210 and Δ N1597 construct could not be tested because they were degraded by thrombin treatment. Binding to Golgi membranes was performed using 10 μ g of Golgi membranes and <0.2 μ g of each recombinant polypeptide. Since binding of GMAP-210 to membranes was shown to resist 0.5 M NaCl we performed the binding assay in the presence of this salt concentration. After sedimentation of Golgi membranes, binding of the recombinant polypeptides was detected by IB using the AS. In the absence of Golgi membranes, any recombinant polypeptide sedimented.

Transient Transfection and Immunofluorescence Microscopy

Plasmid DNA used for transfection was purified through two preparative CsCl/ethidium bromide equilibrium gradients, followed by phenol extraction and ethanol precipitation. COS cells were split 24 h before transfection so that they were 60–80% confluent on the day of transfection. $2-5 \times 10^6$ cells/assay were resuspended in 200 μ l of 15 mM HEPES-buffered serum-containing medium, mixed with 50 μ l 210 mM NaCl containing 5–20 μ g plasmid DNA and electroporated using a Bio-Rad Gene Pulser. 6 h after electroporation, medium was replaced by fresh medium and cells were processed for immunofluorescence (IF) staining after 24–48 h. Indirect IF was carried out as described (Rios et al., 1994). For GFP-fluorescence, cells were fixed in –30°C methanol for 3 min. The samples were observed either using a Leica microscope and a Hamamatsu chilled CCD camera or using a TCS4D confocal laser scanning microscope based on a DM microscope interfaced with an Argon/Krypton laser. Simultaneous double fluorescence acquisitions were performed using the 488-nm and the 568-nm laser lines to excite FITC and TRITC dyes using a 63 \times or 100 \times oil immersion PlanApo objectives. Images were transferred to a Macintosh computer for editing. All documents presented except Fig. 8 (CCD images) are two-dimensional projections of images collected at all relevant z-axes.

In Vitro Microtubule Cosedimentation Assay

Microtubules were assembled in vitro from phosphocellulose-purified bovine brain tubulin as described above. To generate more polymer ends while keeping the polymer mass constant, microtubules were sheared by repetitively passing them through a 26-gauge hypodermic needle attached to 1-ml insulin syringe.

HeLa cells were incubated with 20 μ M taxol for 4 h at 37°C and then extracted with 1% NP-40 in PEM buffer. NP-40 soluble fraction from taxol-treated cells (fraction TS) contained a pool of GMAP-210 and γ -tubulin but no α -tubulin as revealed by IB (see Fig. 12, top). Identical amounts of TS fraction were mixed with equal amounts of microtubules before (-) or after (+) shearing, incubated for 30 min at room temperature and then centrifuged through a 10% sucrose cushion at 60,000 *g* for 30 min at 4°C. TS fraction and microtubule pellets were analyzed by IB for the presence of GMAP-210, α -tubulin and γ -tubulin.

Results

cDNA Cloning and Sequence Analysis of GMAP-210

cDNA clones for GMAP-210 (Fig. 1 A) were obtained after immunoscreening of a HeLa cDNA library with the AS (clones R1-R6 and R8) and rescreeing with an R8 fragment as a probe (clone R9). Clones were routinely expressed in fusion with GST and analyzed by IB using affinity-purified anti-GMAP-210 antibodies. Sequence analysis revealed that the 3' end was missing but a sequence database search detected several expressed sequence tags whose 5' ends were identical to the 3' end of R9. Clone RT-10, containing the stop codon and the polyadenylation signal, was obtained by RT-PCR using primers P1 and P2 (from the partial cDNA sequence L40380; Lee et al., 1995). This clone showed a very weak positive reaction in blots revealed with affinity-purified anti-GMAP-210 antibodies, explaining why the 3' end of the protein was not obtained by screening of the cDNA library with the AS.

The combined sequence derived from the overlapping clones R2, R3, R5, R6, and RT-10 comprises a total of 6452 bp and contains an open reading frame of 5,937 bp. The predicted protein sequence consists of 1,979 amino acid residues (Fig. 1 B) and has a calculated molecular mass of 223,902 and a predicted pI of 5.1, consistent with the molecular weight and pI of GMAP-210 determined by one- and two-dimensional gel electrophoresis (Rios et al., 1994). The PROSITE Dictionary of Proteins Sites and Patterns revealed multiple consensus motifs for phosphorylation and two leucine zipper patterns between amino acids 413-448 and 1,440-1,461. No known consensus nucleotide-binding motifs were found consistent with the lack of a direct effect of ATP or GTP on the binding of GMAP-210 to microtubules.

Analysis of GMAP-210 amino acid sequence with Pep-Coil program showed that a significant portion of the protein had a high probability of forming an extended coil (Fig. 1, B and C). The secondary structure and charge display of GMAP-210 was predicted using the programs PeptideStructure and PepStats. According to this analysis, GMAP-210 appears as a very long coil encompassing most of the sequence although interrupted by a 100-amino acid stretch poorly structured and slightly basic. The NH₂ terminus is highly acidic and contains a small coiled-coil domain whereas the COOH terminus is a highly basic proline and glycine-rich domain (Fig. 1, B and C). The two longest coils are interrupted by two short segments that

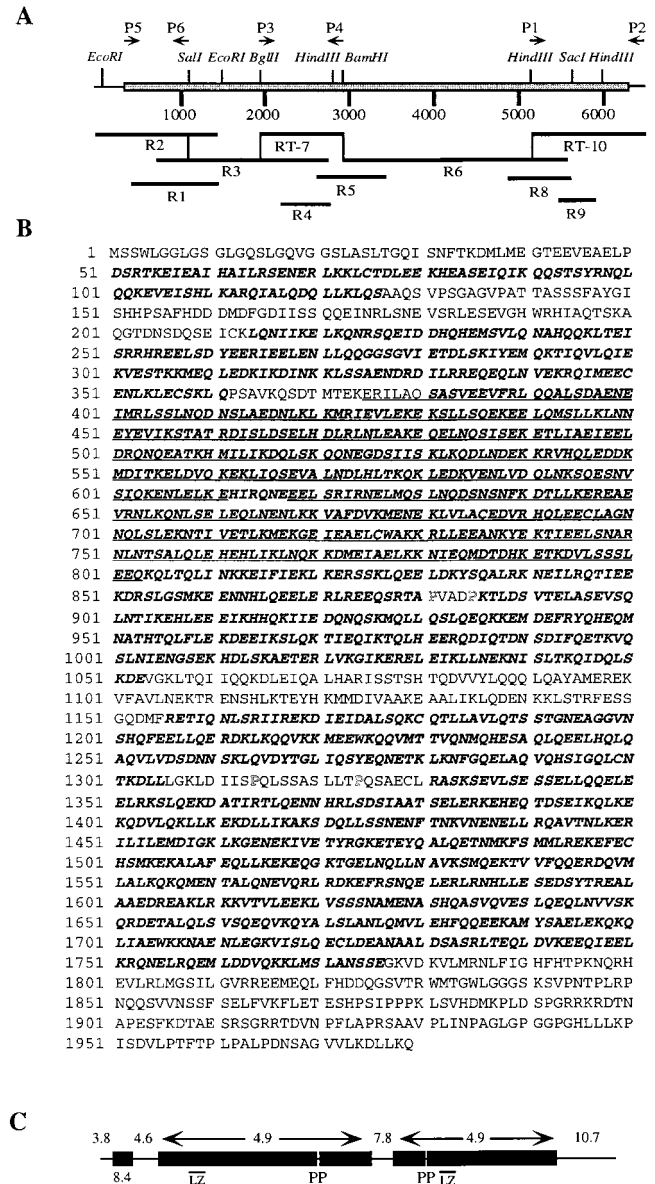


Figure 1. (A) Restriction map of the full-length GMAP-210 cDNA clone. Schematic representation of the cDNA clones obtained from a HeLa random-primed cDNA library (R1-R6, R8, and R9) and RT-7 and RT-10 clones obtained by RT-PCR. The full-length construct was assembled using clones R2, R3, R6, RT-7, and RT-10. The restriction map of GMAP-210 cDNA is represented at the top; the stippled region corresponds to the open reading frame. (B) Deduced amino acid sequence of GMAP-210. Regions predicted to form coiled-coil structures are in italics and boldface. Fragments used to generate polyclonal antibodies appear underlined. Proline residues disrupting coiled-coil are outlined. These sequence data are available from EMBL/GenBank under accession number Y12490. (C) Schematic representation of the secondary structure of GMAP-210. The data suggest an extended coiled-coil domain (boxes) interrupted by a non-coiled-coil region of 100 amino acids and surrounded by a highly acidic NH₂-terminal region containing a small coiled-coil domain and a basic proline/glycine-rich COOH terminus. Isoelectric points of the different regions are indicated at the top. LZ, leucine zipper; PP, proline-containing sequence.

contain proline residues. Since proline usually disrupts an α -helix, GMAP-210 seems to be organized into five coiled-coil segments that are separated by non-helical linkers (Fig. 1, B and C). The presence of two leucine-zippers each localized in a long coiled-coil domain, further supports the possibility of an oligomeric form for GMAP-210 *in vivo*. Finally, the high content in proline and glycine (19%) in the basic COOH-terminal domain suggests a globular structure for this portion of the protein.

Potential protein sequence similarities between GMAP-210 and known proteins were analyzed by searching the GenBank and SwissProt data libraries using tFASTA and BLAST. 20% identity of amino acid sequence was found between GMAP-210 sequence predicted to be coiled-coil and several structural and motor proteins of the cytoskeleton that contain coiled-coil domains such as CENP-E and CENP-F, NuMA, CLIP-170, myosin heavy chains, kinesins and dyneins, and the yeast proteins Nuf1, Uso1p, and Cut14. Comparison of GMAP-210 sequence with those of previously reported Golgi autoantigens such as p230 (golgin 245), GM130 (golgin 95), or GCP170 (golgin 160) revealed no significant similarity (18% through coiled-coil regions).

The tissue expression pattern of GMAP-210 mRNA was studied by Northern blot analysis using two clones as probes, corresponding to 5' and 3' ends. Both probes revealed a single transcript of >7 kb that was more abundant in testis, heart, placenta, skeletal muscle and pancreas although it could be also detected in other tissues (not shown). The size of revealed mRNA easily exceeds that required to encode a protein of 223 kD, supporting that the isolated cDNA codes for GMAP-210.

To definitively demonstrate that the cDNA sequence corresponded to that of the p210 autoantigen, we have generated two polyclonal antibodies against two GST-fusion proteins containing distinct regions of GMAP-210 (amino acids 375–611 and 618–803; Fig. 1 B). These polyclonal antibodies, named RM127 and RM130, revealed by IB a band of identical electrophoretic mobility as that recognized by the AS (Fig. 2 A) and decorated the Golgi apparatus by IF (see Figs. 7–11). Most importantly, GMAP-210 was immunoprecipitated from PNS fractions of HeLa cells using the AS, RM130, and RM127 antibodies and immunoprecipitates were further analyzed by IB using RM130 polyclonal antibody. As expected GMAP-210 was detected in all cases (Fig. 2 B), clearly demonstrating that the isolated cDNA encodes GMAP-210.

GMAP-210 Is a Microtubule-binding Protein

The first indication that GMAP-210 could be a microtubule-binding protein came from its behavior during cell treatment with microtubule-active drugs (Fig. 3 A). In untreated HeLa cells, GMAP-210 distributed in both Triton X-100 soluble and insoluble fractions matching the distribution of tubulin in these fractions. In taxol-treated cells, all tubulin became insoluble whereas it was still possible to detect part of GMAP-210 in the soluble fraction. Finally, prolonged treatment of HeLa cells with NZ induced complete depolymerization of tubulin and concomitantly complete solubilization of GMAP-210. As a control, distribu-

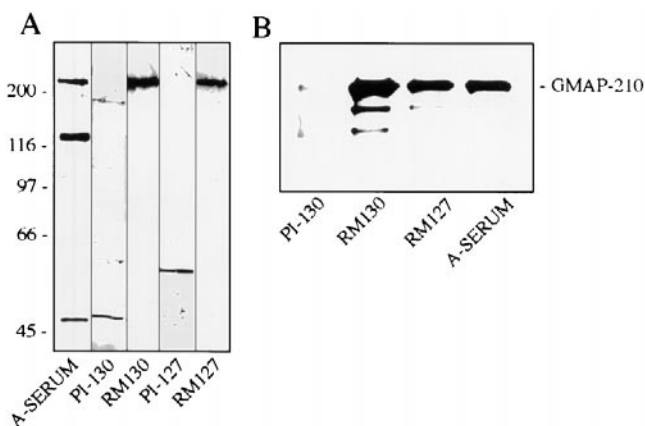


Figure 2. Isolated cDNA codes for GMAP-210. Two polyclonal antibodies, named RM127 and RM130, were generated by immunizing rabbits with GST- Δ 375-611 and GST- Δ 618-803 fusion proteins. (A) These antibodies, the preimmune sera, and AS were assayed on blots of HeLa fractions. RM127 and RM130 revealed a band of identical electrophoretic mobility as that recognized by the AS that was absent from blots incubated with preimmune sera. (B) Endogenous GMAP-210 was immunoprecipitated from HeLa cells extracts using AS, RM127 or RM130 polyclonal antibodies linked to protein A-Sepharose beads. Immunoprecipitates were then analyzed by SDS-PAGE and IB with RM130 polyclonal antibody. Preimmune serum was included as a control.

tion of actin was analyzed and as expected, remained unchanged under all of these treatments.

The association of GMAP-210 with microtubules was further analyzed *in vitro*. We investigated whether GMAP-210 cosedimented with microtubules purified from HeLa cells using taxol. A post-nuclear supernatant was prepared and centrifuged at high speed in order to obtain a supernatant depleted of most of membrane organelles. This high speed supernatant (HSS), that contained a pool of GMAP-210 (Fig. 3 B), was incubated with taxol to polymerize endogenous tubulin. Microtubules were further purified by sedimentation through a sucrose cushion. GMAP-210 cosedimented with microtubules as revealed by IB using the AS (Fig. 3 B). When HeLa cytosol was incubated instead with NZ to prevent tubulin polymerization, neither tubulin nor GMAP-210 sedimented through the sucrose cushion, indicating that GMAP-210 sedimentation was microtubule-dependent (not shown). To investigate the microtubule-binding characteristics of GMAP-210, taxol-polymerized microtubules were incubated with 0.5 M NaCl, 10 mM ATP, or 10 mM GTP, centrifuged through sucrose, and then the supernatants and pellets analyzed for the presence of GMAP-210 by IB (Fig. 3 C). Under all these conditions GMAP-210 was detected in the microtubule-containing pellet. Similar results were obtained when these agents were added to cytosol before incubation with taxol in order to prevent the association (not shown). Only when 2 M urea was added to taxol-polymerized microtubules, could GMAP-210 be detected in the supernatant but, in these conditions, approximately half of tubulin was unpolymerized (Fig. 3

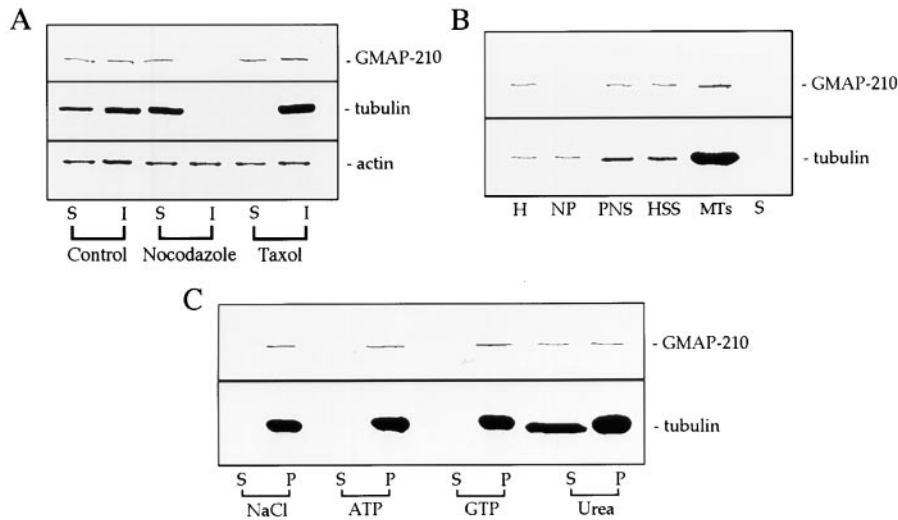


Figure 3. (A) Effects of microtubule-active drugs on the distribution of GMAP-210. HeLa cells were incubated with culture medium alone (Control), or with culture medium containing 10 μ M nocodazole or 10 μ M taxol for 4 h at 37°C. Cells were then extracted with 0.5% Triton in PHEM buffer for 5 min. Soluble (S) and insoluble (I) fractions were immunoblotted and blots probed with the AS, anti- α -tubulin antibody and anti-actin antibody as a control. (B) GMAP-210 binds to taxol-polymerized microtubules. HeLa cells were mechanically homogenized and the homogenates (H) were centrifuged at low speed to obtain a nuclear pellet (NP) and a post-nuclear supernatant (PNS). PNS was centrifuged at high speed and the supernatant (HSS) was incubated at

37°C for 15 min in the presence of 10 μ M taxol. Microtubules (MTs) were sedimented through a cushion of 10% sucrose and all these subcellular fractions together with the microtubule-depleted supernatant (S) were then analyzed by SDS-PAGE and IB using AS and an anti- α -tubulin antibody. (C) Taxol-stabilized microtubules from HeLa cells were incubated with 0.5 M NaCl, 10 mM ATP, 10 mM GTP, and 2 M urea and equivalent amounts of supernatants (S) and pellets (P) analyzed by SDS-PAGE and IB for GMAP-210 and tubulin.

C). From these experiments, we conclude that GMAP-210 binds tightly to microtubules *in vitro*, in a nucleotide-insensitive manner.

GMAP-210 Binds Directly to Microtubules via Its COOH-terminal Domain

To determine whether GMAP-210 binding to microtubules is direct and to decide which domain(s) is responsible for this interaction, a panel of GST-fusion proteins containing different portions of GMAP-210 or the complete protein was prepared (Fig. 4 A, top) and immobilized on glutathione-agarose beads. The immobilized proteins were allowed to interact with microtubules assembled from bovine brain phosphocellulose-tubulin with taxol. The presence of microtubules bound to wild-type or mutant proteins was detected by IB using an anti- α -tubulin antibody (Fig. 4 A, bottom). These experiments demonstrated a direct binding of GMAP-210 to microtubules and further established that the microtubule binding site(s) corresponded to the region containing amino acids 1,712–1,979, the basic COOH-terminal domain.

A similar experiment was then carried out but endogenous GMAP-210 was used instead of recombinant GST-GMAP-210 fusion protein. GMAP-210 was immunoprecipitated from HeLa cell lysates using AS, RM127, or RM130 antibodies precoupled to Sepharose beads. Beads were then incubated with microtubules assembled from bovine phosphocellulose-tubulin with taxol. Fig. 4 B shows that endogenous GMAP-210 efficiently interacted with microtubules. In the absence of added microtubules, no tubulin was detected (RM130-Mts) indicating that GMAP-210 did not bind soluble tubulin present in the cell lysates.

GMAP-210 Associates Preferentially with a Subpopulation of Stable Microtubules

To further characterize the association of GMAP-210 with microtubules *in situ*, we analyzed the microtubular cytoskeleton by selective extraction of cells (Solomon, 1986; Fig. 5). To do so, untreated (Control), 10 μ M NZ-, or 10 μ M taxol-treated cells were permeabilized with a non-ionic detergent in order to extract cytoplasmic proteins (fraction S). Then, microtubules were depolymerized and extracted with a cold-calcium-containing buffer (fraction Ca). All fractions obtained together with the detergent- and calcium-insoluble fractions (fraction I) were processed by IB to analyze the distribution of tubulin and GMAP-210. Fig. 5 A shows that, unexpectedly, tubulin was present in detergent- and calcium-insoluble fractions corresponding to control and taxol-treated cells. This result suggested that a subpopulation of microtubules could resist the depolymerization induced by cold-calcium. To confirm this assumption, untreated-, NZ-, and taxol-treated cells were extracted with NP-40 and cold-calcium. Cell ghosts were then processed for IF and labeled with an anti- α -tubulin monoclonal antibody (Fig. 5 B). According to biochemical data, some microtubules growing from the centrosome could be observed in untreated cells and no microtubules were present in NZ-treated cells whereas many microtubules resisted extraction in taxol-treated cells. GMAP-210 was absent from all Ca-soluble fractions but present in insoluble fractions corresponding to control and taxol-treated cells (Fig. 5 A). This result indicates that GMAP-210 does not bind to all assembled microtubules *in situ* but interacts preferentially with a subset of cold-calcium-resistant microtubules. GMAP-210 was also detected in the soluble fractions under all conditions suggesting that bind-

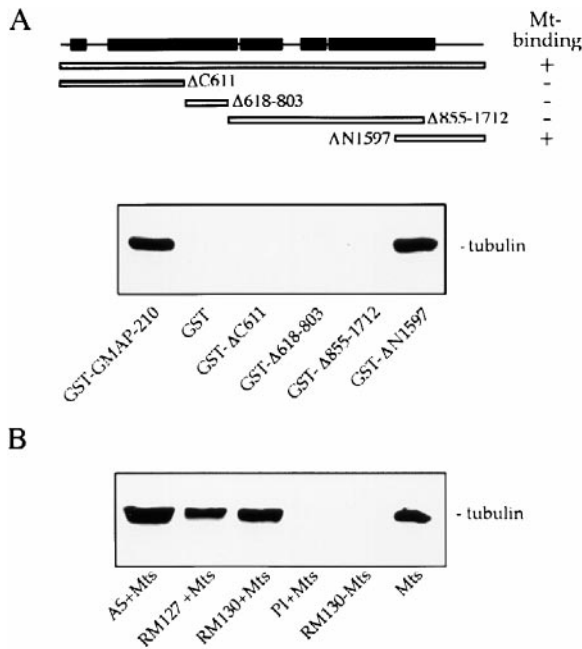


Figure 4. GMAP-210 binds directly to microtubules via its COOH-terminal domain. (A) Different portions of GMAP-210 or the complete protein were cloned in fusion with GST and immobilized on glutathione-agarose beads. Pure bovine brain tubulin was polymerized using taxol, microtubules were centrifuged through sucrose, washed and resuspended in PEM buffer. Polymerized microtubules were added to beads linked to GST alone, GST-fusion polypeptides, or GST-GMAP-210. After incubation and washing, the presence of microtubules in the bead pellets was revealed by IB using an anti- α -tubulin antibody. A summary of results obtained is shown at the top. The clones expressed as GST-fusions are represented as boxes. (B) Taxol-polymerized microtubules (lane Mts) were added to AS (lane AS+Mts), RM127 (lane RM127+Mts), and RM130 (lane RM130+Mts) immunoprecipitates from HeLa extracts, incubated and finally analyzed by SDS-PAGE and IB with anti- α -tubulin antibodies. As controls, preimmune sera (lane PI+Mts) or microtubules were not added (lane RM130-Mts).

ing of GMAP-210 to microtubules in vivo must be regulated since all GMAP-210 did not bind to microtubules even when all tubulin became polymerized with taxol (see also Fig. 3 A).

To characterize this subpopulation of stable microtubules, we quantitatively analyzed the distribution of detyrosinated α -tubulin (glu-tubulin) with respect to total tubulin (α -tubulin) in the three fractions obtained from control cells, using the same subfractionation procedure (S, Ca, and I; Fig. 5 C). In untreated cells, tubulin was found to distribute as follows: 35% was soluble, 52% was polymerized in microtubules that were sensible to cold-calcium (corresponding to 80% of total microtubules), and 13% in microtubules resistant to these conditions (corresponding to 20% of microtubules). This subpopulation of more stable microtubules contained 29% of the total detyrosinated α -tubulin whereas 80% of more labile microtubules contained 67% of glu-tubulin. These results indicate

that resistant microtubules contain 1.8 times more glutubulin than the rest. Thus, GMAP-210 appears preferentially associated in situ to a subpopulation of stable microtubules that are enriched in detyrosinated α -tubulin.

GMAP-210 Associates with Golgi Membranes via Its Acidic NH₂ Terminus

The binding site for Golgi membranes on GMAP-210 was also mapped by deletion analysis. A series of NH₂-terminal and COOH-terminal truncation mutants were constructed and expressed as GST-fusion proteins (Fig. 6, top). They were immobilized on glutathione-agarose beads and further treated with thrombin to release recombinant proteins from GST. Golgi membranes were purified by flotation in a sucrose gradient, washed with 2 M NaCl to remove endogenous GMAP-210 and centrifuged at high speed. 10 μ g of washed Golgi membranes were incubated with <0.2 μ g of different fragments of GMAP-210 in the presence of 0.5 M NaCl. After centrifugation, both supernatants and pellets were analyzed by IB using the AS (Fig. 6). The constructs Δ C611 and Δ C375 appeared enriched in Golgi membranes containing pellets, whereas fragments lacking NH₂-terminal domain mostly remained in supernatants. Binding of GMAP-210 NH₂ terminus to Golgi membranes was further confirmed in vivo by transfection experiments (see below).

Functional Analysis of GMAP-210 In Vivo

To approach the function of GMAP-210 in the organization of the Golgi apparatus, we transiently expressed intact or mutant proteins in COS cells. Full-length cDNA coding for GMAP-210 with or without an NH₂-terminal HA-epitope was introduced in cells by electroporation. Cells were incubated, fixed, double labeled and finally observed in a confocal laser microscope. An effect on the structure of the Golgi apparatus and microtubule network could be observed depending on the expression level of the transfected protein. At low expression level, the full-length GMAP-210 localized to a compact juxtannuclear reticulum characteristic of the Golgi apparatus as revealed by an anti-HA antibody (not shown). The labeling pattern overlapped exactly with that exhibited by the AS that recognized both exogenous and endogenous proteins, indicating that the expressed protein is targeted to the same place as the endogenous GMAP-210, namely the CGN. No significant changes in the structure of the Golgi complex or the microtubule network could be appreciated (not shown).

By contrast, overexpression of GMAP-210 dramatically perturbed the organization of the Golgi apparatus. Under these conditions, transfected cells could be easily distinguished from the surrounding nontransfected cells by IF, due to the high amount of exogenous GMAP-210. Several features could be observed. First, a dramatic enlargement of the Golgi apparatus that could remain as a single pericentrosomal structure (Fig. 7 a) or as multiple fragments centrosomally localized but also distributed throughout the cytoplasm (Fig. 7 d). Double labeling for GMAP-210 and the medial Golgi marker CTR433 (Jasmin et al., 1989; Fig. 7, a-c) or the cis-Golgi marker p115 (Barroso et al., 1995; Fig. 7, d-f) revealed that not only the CGN but also

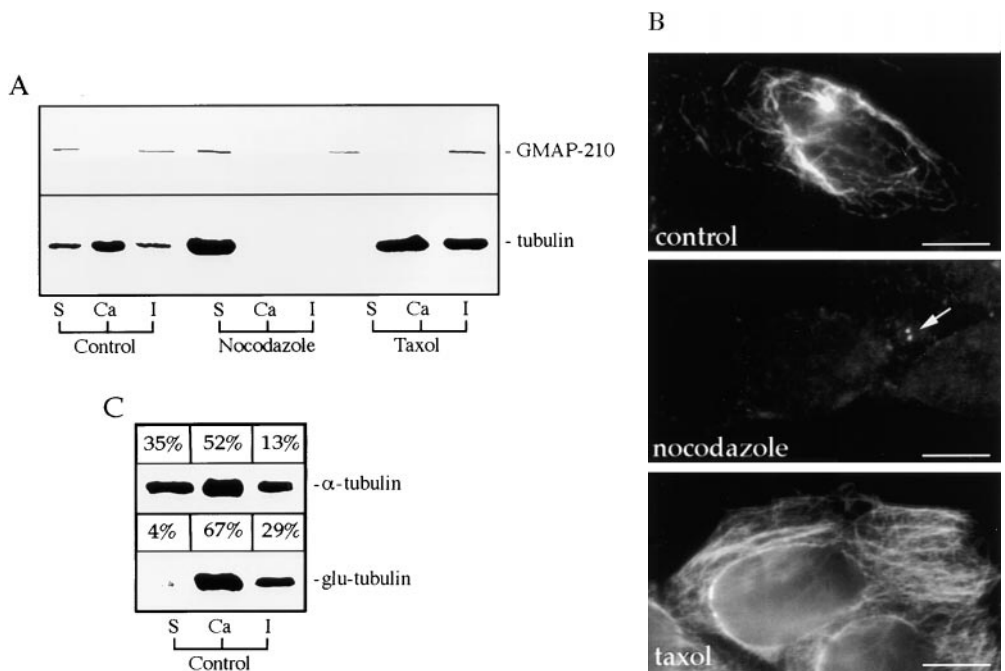


Figure 5. GMAP-210 selectively interacts with a subpopulation of stable microtubules. (A) Untreated-, nocodazole- or taxol-treated HeLa cells were extracted for 5 min with 0.3% NP-40 in a microtubule-stabilizing buffer and the detergent-soluble fractions obtained were harvested (S fractions). Extracted cells were then incubated for 30 min on ice in the same buffer containing 5 mM CaCl₂ in order to depolymerize microtubules (Ca fractions). All fractions together with the detergent- and calcium-insoluble fractions (I) were also analyzed by SDS-PAGE and IB using the AS and anti-α-tubulin antibodies. (B) HeLa cells grown on coverslips were treated and successively extracted with NP-40 and calcium as described in A. Cell ghosts were then processed for IF. The presence of microtubules was revealed by using an anti-α-tubulin antibody. Bars, 10 μm. (C) NP-40-soluble, CaCl₂-soluble, and insoluble fractions from untreated HeLa cells were resolved by SDS-PAGE and blotted. Two identical blots were incubated with anti-α-tubulin antibody or with SG, a polyclonal antibody against detyrosinated tubulin (glu-tubulin). The same number of cells were used in each experiment, the different extractions were made in the same volume and identical volumes of samples analyzed in each line of gels. Blots were quantitatively analyzed using a Bio-Rad densitometer. Values are means of three independent experiments.

described in A. Cell ghosts were then processed for IF. The presence of microtubules was revealed by using an anti-α-tubulin antibody. Bars, 10 μm. (C) NP-40-soluble, CaCl₂-soluble, and insoluble fractions from untreated HeLa cells were resolved by SDS-PAGE and blotted. Two identical blots were incubated with anti-α-tubulin antibody or with SG, a polyclonal antibody against detyrosinated tubulin (glu-tubulin). The same number of cells were used in each experiment, the different extractions were made in the same volume and identical volumes of samples analyzed in each line of gels. Blots were quantitatively analyzed using a Bio-Rad densitometer. Values are means of three independent experiments.

the other compartments of the Golgi apparatus were distorted by overexpression of GMAP-210. Remarkably, the spatial distribution of GMAP-210 and the Golgi markers was largely coincident, although they demonstrated a

graded inverse distribution: GMAP-210 labeling increased towards the periphery whereas the intensity of CTR433 and p115 decreased. A careful analysis of optical cuts suggested an hypertrophy of the CGN extending towards the cell periphery whereas the remnant of the Golgi stacks remained close to the nucleus (Fig. 7, c and f). Second, the array of microtubules in the centrosomal area appeared modified in overexpressing cells. The microtubule aster became undefined and many microtubules appeared to emanate from the enlarged Golgi area rather than from the centrosome (Fig. 7, g-i). In overexpressing cells in which the Golgi complex was fragmented, perturbation was more pronounced and microtubules appeared randomly distributed.

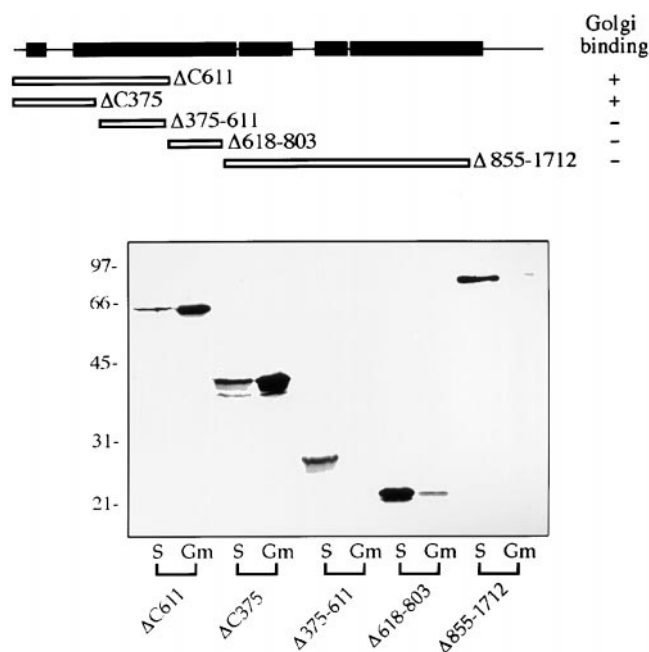


Figure 6. NH₂-terminal domain of GMAP-210 mediates its association to Golgi membranes. Different regions of GMAP-210 were expressed in fusion with GST and purified on glutathione-

agarose beads. Beads were treated with thrombin to release recombinant polypeptides. Golgi membranes were purified by flotation in a sucrose gradient, washed with 2 M NaCl and centrifuged. 10 μg of Golgi membranes was incubated with <0.2 μg of recombinant polypeptides, centrifuged, and then supernatants and pellets were analyzed by IB using the AS. It must be noted that not all fragments were recognized with identical affinity by the AS. A summary of results obtained is shown at the top. The clones tested in the binding assay are represented as boxes.

agarose beads. Beads were treated with thrombin to release recombinant polypeptides. Golgi membranes were purified by flotation in a sucrose gradient, washed with 2 M NaCl and centrifuged. 10 μg of Golgi membranes was incubated with <0.2 μg of recombinant polypeptides, centrifuged, and then supernatants and pellets were analyzed by IB using the AS. It must be noted that not all fragments were recognized with identical affinity by the AS. A summary of results obtained is shown at the top. The clones tested in the binding assay are represented as boxes.

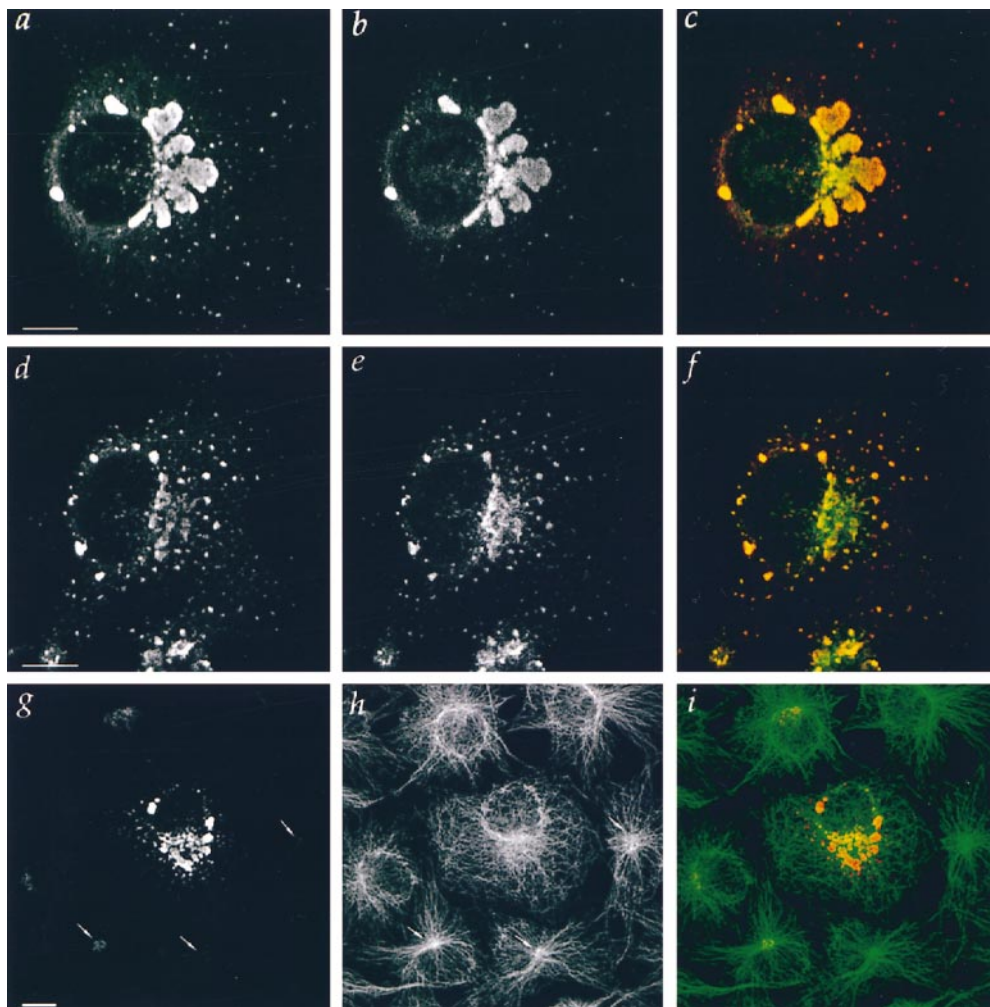


Figure 7. Localization and effect of GMAP-210 overexpression on the Golgi apparatus and the microtubule network structure. Double IF was performed with RM130 (a, red) and CTR433 (b, green), or the AS (d, red) and anti-p115 (e, green). Separate red and green images were collected and overlaid (c and f). Yellowish staining indicates colocalization of GMAP-210 enriched membranes with the Golgi apparatus. Cells were also stained with RM130 (g, red) and an anti-tubulin antibody (h, green). Red and green images were collected separately and superposition of the two labelings is shown in i. Arrows in g and h indicate the Golgi apparatus and the microtubule aster in nontransfected cells. Bars, 10 μ m.

homogeneous cytoplasmic staining was also observed (Fig. 8, a–c). Comparison between a Δ C1778 overexpressing cell (Fig. 8, d, f, and h) and a nontransfected cell (Fig. 8, e, g, and i) revealed no significant differences in the Golgi apparatus or the microtubule network structure. It must be noted that overexpression of this mutant had no detectable effect on the distribution of endogenous GMAP-210.

We also studied the effect of microtubule-active drugs on the phenotype displayed by GMAP-210 overexpressing cells. When transfected cells were incubated with NZ, the Golgi apparatus became fragmented and scattered throughout the cytoplasm in all overexpressing cells and complete colocalization with CTR433 and p115 was observed (not shown). Transfected cells were also incubated with taxol to promote the formation of microtubule bundles. In the presence of taxol, GMAP-210-enriched membranes preferentially redistributed at the periphery of the cells, whereas the rest of the Golgi complex remained juxtanuclear (Fig. 9, a–c). GMAP-210-enriched membranes were found to be always located at one end of microtubule bundles (Fig. 9, d–f). Higher magnifications at the cellular periphery allowed to distinguish individual microtubules and GMAP-210-enriched membranes could be seen associated with them (Fig. 9, g–i). Thus, taxol-induced micro-

tubules were able to tear the GMAP-210-enriched elements away from the rest of the Golgi apparatus.

To further characterize the functional domains of GMAP-210, both NH₂-terminal and COOH-terminal domains were cloned in fusion with the green fluorescent protein and transiently expressed in COS cells. Δ C375-GFP mutant appeared mostly cytosolic but also localized in punctate structures clustered in the Golgi region (Fig. 10 a). Labeling with an antibody that specifically recognized endogenous GMAP-210 revealed partial colocalization (Fig. 10, a–c). This result indicated that the truncated chimera was able to bind to Golgi membranes. At higher expression level, endogenous GMAP-210 was hardly visible consistent with the truncated mutant displacing the endogenous protein from membranes (Fig. 10, d–f). Under these conditions, the medial Golgi marker CTR433 also showed an altered distribution (Fig. 10, g–i). Instead of the normal compact Golgi seen in the untransfected cell in this field, CTR433 appeared in some punctate structures in the Golgi area and the labeling intensity had considerably decreased. Therefore, Golgi compartments other than the CGN appeared morphologically perturbed in the absence of membrane-bound GMAP-210. These data together with those presented in Fig. 6 demonstrate that the NH₂

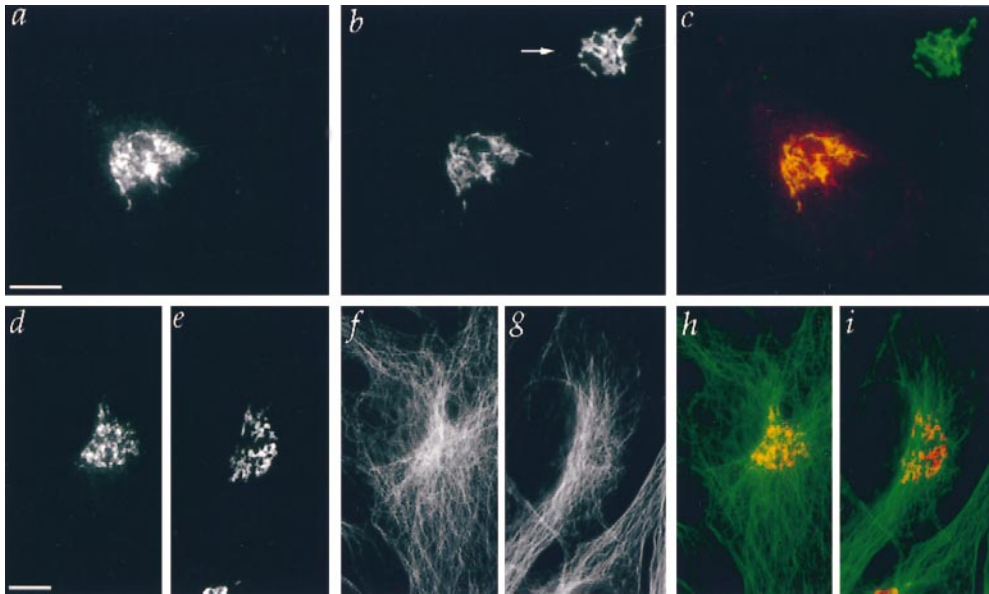


Figure 8. Expression of a GMAP-210 mutant form lacking the microtubule-binding site. After transfection, cells were fixed and double labeled for GMAP-210 (a) and the medial Golgi marker CTR433 (b). Red and green image pair is shown in c. Arrow in b indicates a nontransfected cell. Alternatively, cells were stained for GMAP-210 (d, e) and α -tubulin (f, g) and image pairs are shown in h and i, respectively. In d, f, and h, a transfected cell is shown. In e, g, and i, a nontransfected cell is presented for comparison. Bars, 10 μ m.

terminus of GMAP-210 is involved in targeting the protein to Golgi membranes and strongly suggest that GMAP-210 is required for processes involved in maintaining the integrity of the Golgi apparatus.

Similar experiments were carried out with the microtubule-binding domain for which GFP was placed in the NH₂ terminus of the mutant protein. Contrary to our expectations, the GFP- Δ N1778 mutant protein did not

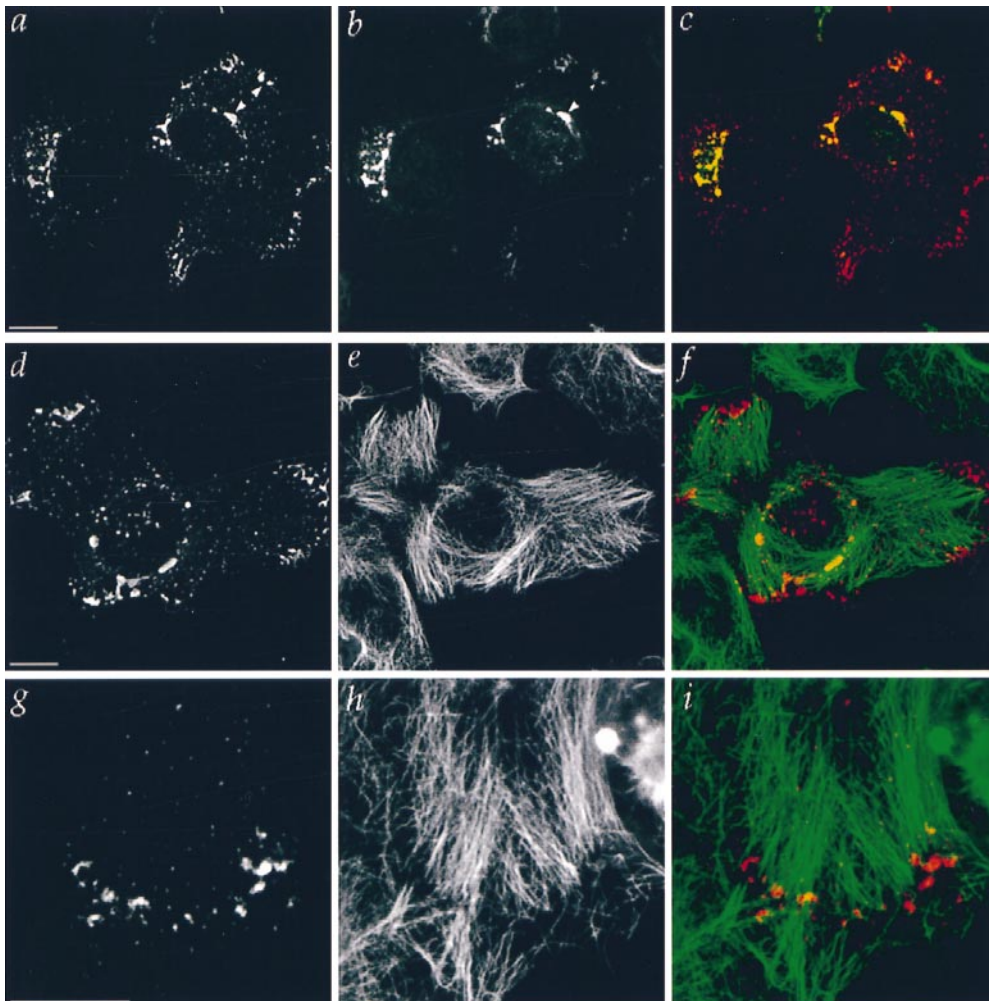


Figure 9. Effect of taxol on GMAP-210 overexpressing cells. Transfected cells were incubated with 10 μ M taxol for 4 h and double stained for GMAP-210 (a, d, and g, red) and CTR433 (b, green) or tubulin (e and h, green). Superpositions of labelings are shown in c, f, and i. Preferential redistribution of GMAP-210-enriched membranes was observed at the cell periphery where the staining for GMAP-210 was equivalent to that observed in a juxtannuclear location (two opposed arrowheads in a). In b, the two unequal arrowheads indicate unequal partitioning of a Golgi marker that mostly remains in a juxtannuclear location. Note in f and i that GMAP-210-enriched Golgi elements are invariably associated with one end of microtubule bundles. Bars, 10 μ m.

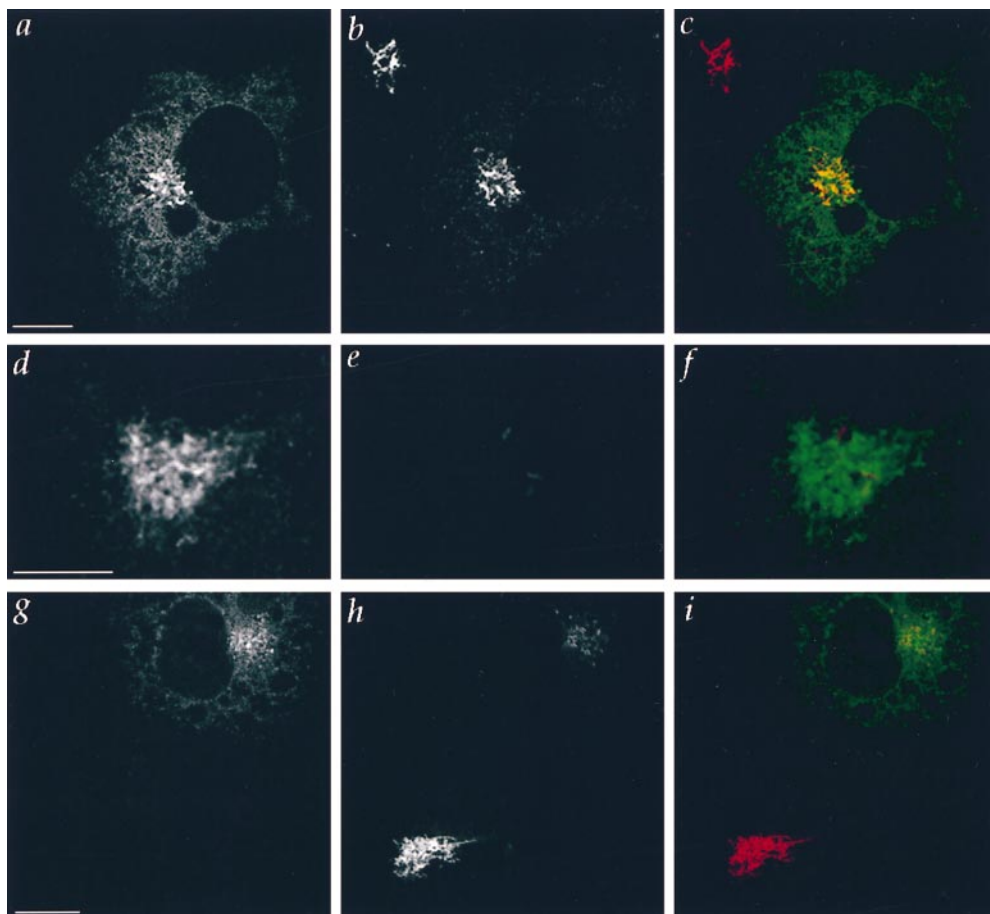


Figure 10. Transient expression of GFP-tagged NH₂-terminal domain of GMAP-210. Cells were transfected with Δ C375-GFP (a, d, and g, green), processed and labeled with RM130 (b and e, red) or CTR433 (h, red). A nontransfected cell is shown in b and h for comparison. In c, f, and i superpositions of labelings are shown. Bars, 10 μ m.

decorate microtubules. At low expression level, one or two spots that colocalized with the centrosomal marker CTR453 (Bailly et al., 1989) could be observed (Fig. 11, a–c). With increasing expression levels of the mutant, increasing accumulation of GFP at the centrosome occurred together with the dispersion of the pericentriolar material that became almost undetectable with the monoclonal antibody CTR453 (Fig. 11, a_{1–3}, b_{1–3}, and c_{1–3}). Centrioles were present in the center of the GFP-labeled centrosomes, as revealed by anti-detyrosinated tubulin antibodies (not shown). After longer periods of expression, GFP- Δ N1778 accumulated massively at the centrosome (Fig. 11, d–f) although heterogeneous sized bright foci of GFP scattered throughout the cytoplasm were also observed. The Golgi apparatus was displaced to the periphery of the massive centrosomal aggregate (Fig. 11, e and f) that appeared surrounded by a dense network of detyrosinated microtubules (Fig. 11, g–i).

GMAP-210 Binds to Microtubule Ends

The finding that the COOH-terminal microtubule-binding domain of GMAP-210 localizes *in vivo* to centrosome strongly suggested that GMAP-210 might interact only with the minus ends of microtubules. One prediction of this hypothesis is that for constant polymer mass, GMAP-210 binding to microtubules *in vitro* will increase when more ends are present. To test this, we sheared a constant

mass of microtubules (polymerized from phosphocellulose-bovine brain tubulin using taxol) by passing them through a hypodermic needle attached to 1-ml syringe. In parallel, we prepared a NP-40 soluble fraction from HeLa cells that had been treated with taxol for 4 h in order to completely polymerize the endogenous tubulin. This tubulin-free fraction (TS) contained a pool of GMAP-210 and γ -tubulin, as revealed by IB (Fig. 12, top). Equal amounts of fraction TS were mixed with equal amounts of microtubules before (–) or after (+) shearing. After incubation, microtubules were centrifuged through a sucrose cushion and microtubule-pellets analyzed by IB. A typical experiment is shown in Fig. 12 (top) and the mean of values of three independent experiments is represented in Fig. 12 (bottom). Shearing increased the number of microtubules twice as determined by quantification of bound γ -tubulin before and after shearing. By using the same procedure Li and Joshi (1995) obtained an increase in the microtubules number of 1.5 times, fitting well with our results. The amount of GMAP-210 bound to microtubules also increased 1.8 times indicating that GMAP-210 interacts only with the ends of microtubules.

Discussion

Significant progress has been made towards understanding the molecular mechanisms of microtubule-dependent mo-

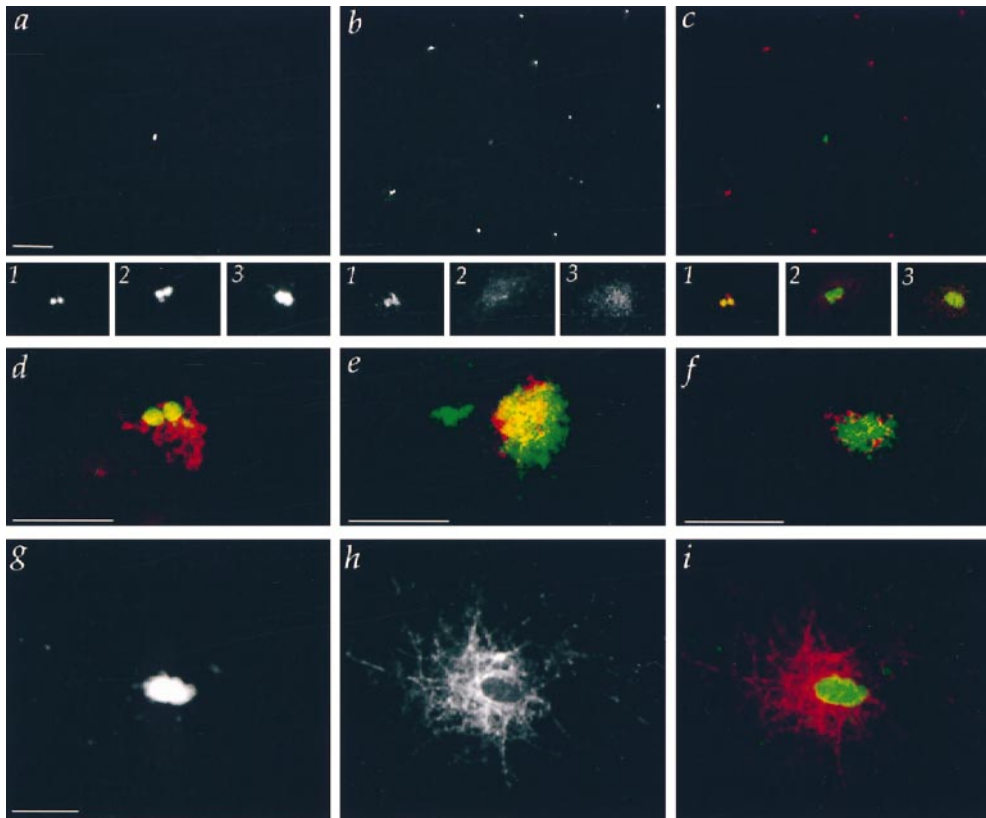


Figure 11. Transient expression of GFP-tagged COOH-terminal domain of GMAP-210. Cells were transfected with GFP- Δ N1778, processed and labeled with different markers. (a–c) A low magnification image showing a GFP- Δ N1778 expressing cell surrounded by several nontransfected cells (a, green). After transfection, cells were labeled with the centrosomal marker CTR453 (b, red). In c, superposition of both labelings is shown. Note that no staining with CTR453 could be detected in the GFP-COOH-terminal domain expressing cell. a_{1–3} (green) show increasing expression levels of GFP- Δ N1778; b_{1–3} (red) show staining with CTR453 and in c_{1–3} superpositions of both labelings are shown. (d–f) Effect of overexpression of GFP-COOH-terminal domain on the Golgi apparatus. Image pairs of GFP- Δ N1778 (green) and RM130 (d and e, red) or CTR433 (f, red) are presented. (g–i) GFP- Δ N1778 overexpressing cell (g, green) labeled for deetyrosinated tubulin (h, red). Image pair is shown in i. Bars, 10 μ m.

tors and their roles in membrane traffic. By contrast, the possible role of other microtubule-binding proteins lacking motor activity in the organization of the central membrane system has remained elusive. We report here the cloning and functional characterization of a previously described CGN-associated protein, p210.

GMAP-210 Is a Large Coiled-Coil Protein that Binds to Microtubule Ends via Its COOH Terminus and to Membranes through Its NH₂-terminal Domain

Several pieces of evidence argue for GMAP-210 being a microtubule-binding protein. First, the presence of GMAP-210 in Triton-extracted cell ghosts is always dependent on the existence of polymerized microtubules. Second, GMAP-210 cosediments with taxol-polymerized microtubules from HeLa cells and remains associated in situ with stable microtubules. Finally, both endogenous GMAP-210 and purified bacterially expressed GMAP-210 interact with purified bovine brain microtubules. Interestingly, GMAP-210 binding to microtubules increases with the number of free ends in a given mass of microtubule polymers. This was demonstrated by shearing microtubules into short fragments, hence keeping the polymer mass

constant. Interaction of GMAP-210 with polymerized tubulin appears to be a tight nucleotide-insensitive binding as it is not released from the microtubule pellet by salt conditions that are known to dissociate most MAPs, or by ATP or GTP addition. These binding characteristics correlate well with those reported for interaction between the Golgi complex and microtubules (Karecla and Kreis, 1992) and differ from those exhibited by CLIP-170, motors, or other MAPs.

The complete sequence of GMAP-210 has been obtained (accession number Y12490). Recently, an almost identical sequence, submitted to data banks after submission of our sequence, has been reported under the accession number AF007217 (Chang et al., 1997). This protein was reported to be a thyroid hormone receptor coactivator, negatively regulated by the retinoblastoma protein and named Trip230. We have no explanation for such a functional discrepancy but we are confident that the sequence we have cloned corresponds to the p210 autoantigen associated to the Golgi apparatus described previously (Rios et al., 1994) by all the experimental data presented in this paper. In addition, a partial cDNA sequence of a novel gene named CEV-14 (these data are available from GenBank/EMBL/DDBJ under accession number

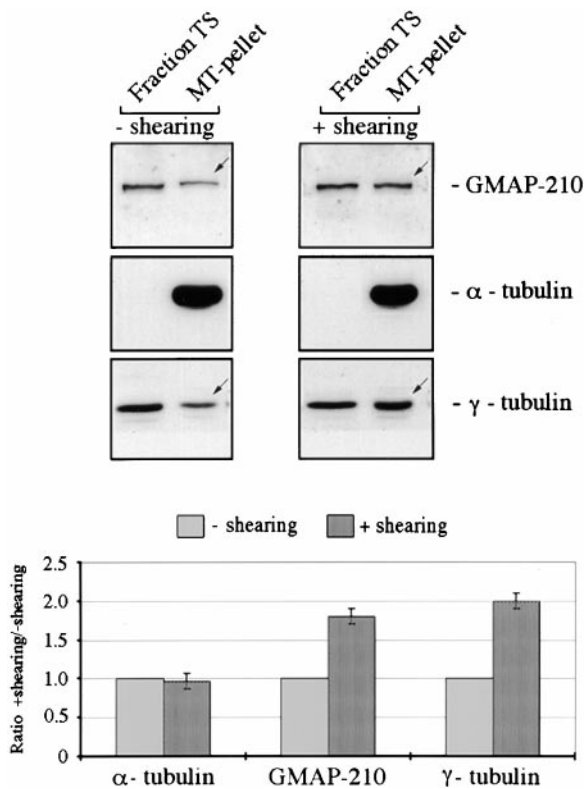


Figure 12. Binding of GMAP-210 to microtubules depends on the number rather than on the mass of microtubules. (Top) A NP-40-soluble and tubulin-free fraction was obtained from HeLa cells treated during 4 h with 20 μ M taxol (fraction TS). This fraction contained a pool of GMAP-210 and γ -tubulin. Equal amounts of fraction TS were mixed with equal amounts of a suspension of taxol-induced microtubules from phosphocellulose-tubulin, before (-) or after (+) shearing through a 26-gauge hypodermic needle, and centrifuged through a sucrose cushion. Microtubule pellets were analyzed by IB. Shearing microtubules resulted in increased binding of γ -tubulin, indicating that more microtubule minus ends had been generated. Increased binding of GMAP-210 was also observed. (Bottom) Blots corresponding to three independent experiments were quantitatively analyzed using a Bio-Rad densitometer and NIH Image software. Quantification analysis indicates a twofold increase of bound γ -tubulin to sheared microtubules. In parallel, bound GMAP-210 increased 1.8 times.

AF011368) that corresponds to amino acids 1,188–1,753 of GMAP-210 has been reported. This partial sequence has been identified as the NH₂-terminal half of a fusion gene in which the COOH-terminal half is a fragment of the platelet-derived growth factor receptor containing transmembrane and tyrosine kinase domains. CEV14-PDGFR fusion gene is generated by a chromosomal translocation that is associated with acute myelogenous leukemia (Abe et al., 1997). Our finding that this fragment corresponds to the second large coiled-coil domain of GMAP-210 supports the idea proposed by the authors that the mechanism of transformation is the ligand-independent dimerization of PDGFR leading to ectopic constitutive tyrosine kinase activation.

Structural features predicted by the molecular characterization of GMAP-210 indicate a long α -helical domain

with high potential to form a coiled-coil structure. Such a long coiled-coil domain has been found in other autoantigens associated with the cytoplasmic face of the Golgi membrane including giantin or GCP372 (Seelig et al., 1994; Sohda et al., 1994), golgin-245 or p230 (Fritzler et al., 1995; Erlich et al., 1996), golgin-95 or GM130 (Fritzler et al., 1993; Nakamura et al., 1995), and golgin 160 or GCP170 (Misumi et al., 1997).

GMAP-210 is distinct from previously described microtubule-interacting proteins. GMAP-210 is predicted to possess a three domain structure consisting of the long α -helical central domain and the nonhelical end domains, similar to other proteins that interact with the cytoskeleton. The organization of charged regions of GMAP-210 is also reminiscent of that of several other MAPs. The NH₂-terminal end is acidic whereas the COOH-terminal domain has a high pI like MAP2 (Lewis et al., 1988), MAP4 (West et al., 1991), tau (Lee et al., 1988), or NuMA (Compton et al., 1992). However, it is inverse to CLIP-170 that contains a basic NH₂-terminal domain, a central coiled-coil domain, and an acidic COOH-terminal domain. The highly basic COOH-terminal region of GMAP-210 was shown to mediate direct interaction with microtubules. Neither the acidic NH₂-end, nor the long α -helical domain associate with microtubules *in vitro*. Thus, GMAP-210 behaves like most MAPs characterized so far, in which basic regions play a significant part in conferring ability to bind microtubules. The microtubule-binding domain of GMAP-210 shows no overall homology to any of previously characterized sites. It does not contain internal repeats like MAP2, tau, or CLIP-170, suggesting that only one binding site is present in each GMAP-210 molecule.

The binding domain of GMAP-210 to the cytoplasmic side of CGN membranes has been localized in the NH₂ terminus of GMAP-210. This region consists of two acidic nonhelical regions interrupted by a small coiled-coil domain and followed by a large coil. The acidic COOH-terminal domain of CLIP-170 has also been postulated to interact with other proteins on the surface of endocytic organelles. A molecular design consisting of an elongated molecule containing a conserved microtubule-binding region at one end and a variable domain at the other end involved in specifying interactions with organelles has been proposed for nonmotor proteins implicated in linking cytoplasmic organelles to microtubules such as CLIP-170 (Pierre et al., 1992). Although no significant sequence homology with CLIP-170 out of the coiled-coil region has been found, the molecular design of GMAP-210 fits well with this model.

GMAP-210 Is Required for Processes Maintaining the Structural Integrity of the Golgi Apparatus

The biochemical data provide strong evidence that GMAP-210 is a Golgi-associated protein that directly interacts with microtubule ends. Consistent with this, overexpression of GMAP-210 induced perturbations in both the Golgi apparatus and the microtubule network, and these effects increased with the expression level of the protein. Exogenous GMAP-210 was correctly addressed to the Golgi apparatus that appeared dramatically enlarged and sometimes fragmented in structures scattered throughout

the cytoplasm. This hypertrophy of the Golgi apparatus occurred together with a modification of the microtubule network that mainly affected the Golgi-centrosome area. In fact, transfected cells could be easily identified by looking at the microtubule network, due to the absence of a distinct microtubule aster at the centrosome. Instead, a dense network of short microtubules could be appreciated colocalizing with the enlarged Golgi apparatus, and cytoplasmic microtubules seemed to emanate from the periphery of this perturbed region. Deletion of the COOH-terminal domain completely abolished both effects, strongly suggesting that the basic COOH terminus is responsible for binding of GMAP-210 to microtubules *in vivo* as well as *in vitro*. These data also indicate that the perturbation of the Golgi apparatus produced by GMAP-210 overexpression is primarily due to an excess of binding to microtubules.

It was intriguing that truncated GMAP-210 lacking microtubule-binding domain ($\Delta C1778$) accumulated mostly in the cytosol whereas intact GMAP-210 associated almost completely with Golgi membranes. The $\Delta C1778$ mutant partially associated with Golgi membranes without apparently displacing endogenous GMAP-210, suggesting that endogenous GMAP-210 is present at subsaturating levels in cells and that interaction of GMAP-210 with membranes is saturable. This view is also consistent with the results obtained *in vitro* in which purified GMAP-210-containing membranes were able to bind a limited amount of $\Delta C611$ or $\Delta C375$ mutants. But how to understand that GMAP-210 can steadily accumulate on CGN membranes in cells overexpressing the full-length protein if the interaction of GMAP-210 with CGN membranes is saturable? An amplification mechanism could reasonably explain this paradoxical observation: the progressive accumulation of GMAP-210 to the CGN would induce the stabilization of microtubules in this area that would allow the progressive enlargement of CGN elements. Such an increase in membrane surface would in turn allow that more GMAP-210 accumulates.

Numerous observations indicate that the size of the Golgi apparatus is maintained by a fine tuning of the membrane flux in and out of the Golgi complex. Overexpression of GMAP-210 produces a significant increase in the Golgi apparatus size. On the contrary, when endogenous GMAP-210 is displaced from Golgi membranes by overexpression of a truncated mutant containing only the membrane binding domain, the size of the Golgi complex decreased ($\Delta C375$; see Fig. 10). Thus, GMAP-210 seems to play a role in determining the size of the Golgi apparatus, probably by regulating the number of microtubule-binding sites on the surface of the CGN. A tighter association of the CGN with microtubules could prevent its maturation into Golgi cisternae (Bannykh and Balch, 1997) or impede anterograde vesicular transport from the CGN to the medial Golgi (Orci et al., 1997), producing the enlargement of the CGN by accumulation of newly synthesized or recycling proteins. This could explain the partial colocalization of *cis*- and medial-Golgi markers with GMAP-210 in the enlarged CGN. At higher expression levels, translocation of pre-Golgi elements from peripheral ER sites to the Golgi area could be also affected and fragmentation of the enlarged Golgi apparatus would oc-

cur. Consistent with this, microtubules appeared randomly nucleated in cells in which the Golgi apparatus was fragmented.

The relationship between CGN and microtubules thus appears as a finely regulated interaction in order to ensure not only the localization but also the steady state structure and size of the Golgi apparatus. Many recent studies have emphasized the role of microtubule motors not only in membrane traffic but also in determining the localization and morphology of the Golgi apparatus. It has been proposed that in the steady state the Golgi complex is in dynamic equilibrium between the anterograde force mediated by kinesin family motors and the retrograde force mediated by cytoplasmic dyneins rather than simply being associated with microtubules near their minus end (Harada et al., 1998). Our results indicate that association of CGN membranes with the minus end of microtubules is also necessary for the proper localization, morphology and size of the Golgi apparatus. They are consistent with a model in which dynein is responsible for moving Golgi elements towards the centrosome whereas GMAP-210 is regulating the static binding of the Golgi membranes to microtubules.

GMAP-210 Links the CGN to the Minus End of Microtubules

We present here several lines of evidence showing that GMAP-210 is a minus end microtubule-binding protein. First, GMAP-210 binding to microtubules *in vitro* is related to the number rather than the mass of microtubule polymers. Second, the COOH-terminal domain of GMAP-210, responsible for the microtubule-binding property of the protein *in vitro*, localizes *in vivo* to the centrosome, where the minus ends of microtubules accumulate. Moreover, it is able to disorganize the pericentriolar material suggesting that it could displace other centrosomal proteins from the minus ends of microtubules. In the presence of NZ, GMAP-210 does not remain associated with the centrosome (Rios et al., 1994). Taxol treatment of GMAP-210 overexpressing cells induced specific redistribution of GMAP-210 enriched elements to the cell periphery where they associated with one end of microtubule bundles. Finally, in GMAP-210 overexpressing cells, a dense network of short microtubules appears in the Golgi area from which cytoplasmic microtubules seem to emanate, consistent with the notion that binding or capping of the minus ends of microtubules by proteins result in their stabilization.

All data presented here support a model in which GMAP-210 serves to maintain the structural integrity of the Golgi apparatus by interacting with the minus end of microtubules. The NH₂-terminal domain binds to CGN membranes whereas the COOH-terminal domain interacts with the minus end of microtubules at the centrosome. Remarkably, a low expression level of the COOH-terminal domain leads to the centrosomal localization of GFP in a manner identical to the centrosomal decoration obtained with an antibody directed against γ -tubulin (Moudjou et al., 1996), or against a γ -tubulin binding protein (Tassin et al., 1998), i.e., the centriole pair and a domain surrounding it. From the study of γ -tubulin distribution in epithelial

cells, Mogensen et al. (1997) have proposed that peripheral docking elements distinct from nucleating elements could be closely associated with centrosomes. In their model, microtubule minus ends would be capped and stabilized before being released from nucleating elements, which would remain in the juxtacentriolar vicinity to nucleate new microtubules. Control mechanisms would ensure that a microtubule is anchored to the centrosome periphery or released. Several reports have indeed suggested that centrosomally nucleated microtubules can escape to other locations, particularly in neurons (Ahmad and Baas, 1995) but also in other cell types (Vorobjev and Chentsov, 1983; Belmont et al., 1990; Keating et al., 1997).

Based on this idea, one could propose that free microtubule ends could be captured by GMAP-210 via its COOH-terminal domain. This might also account for the existence of a subpopulation of short, stable microtubules that colocalizes with the Golgi apparatus (Schulze and Kirschner, 1987). This subpopulation is rich in deetyrosinated microtubules (Skoufias et al., 1990) and we have shown that GMAP-210 is specifically enriched in a microtubule fraction with similar characteristics. In this view, CGN membranes, rather than the centrosome itself, would be the anchoring sites for microtubules participating in Golgi dynamics and stability.

A direct homologue of GMAP-210 has not been found in the yeast genome database. This is consistent with the fact that in lower eukaryotic cells no spatial segregation between the ER and the Golgi apparatus exists. In such cells, the Golgi complex is often found next to the ER sites and pre-Golgi elements form the first cisternae of the Golgi stack. However, in many eukaryotic cells the Golgi apparatus is centered around the centrosome and actively maintained there. Our data uncover a critical role for the CGN in this process and identify GMAP-210 as an important factor in mediating both the localization and the integrity of this Golgi compartment. During mitosis, the microtubule dynamics changes and the centrosomal localization of the Golgi apparatus is lost. One can speculate that GMAP-210 could be a target for the mitotic regulation of the CGN. Since GMAP-210 remains bound to Golgi membranes during mitosis (unpublished results), the obvious candidate for this mitotic regulation is the microtubule-binding domain. It is interesting to note in this regard that the COOH-terminal domain has two putative cyclin-dependent kinase phosphorylation sites. Experiments are now in progress to test this hypothesis.

We thank B. Goud, C. Antony, and J. Salamero for helpful discussions and J. Salamero for assistance with laser confocal microscopy. We are indebted to Drs. Sztul, Bulinski, and Kreis for kindly providing us with antibodies and Dr. P. Chambon for the generous gift of random and oligo-(dT) primed λ ZAP-II libraries. We are grateful to Dr. M. Tortolero for her support during the progress of this work.

This work was supported by grants from the Junta de Andalucía, Spain and by CNRS (grant Biologie Cellulaire: du Normal au Pathologique, no. 96070) and Institut Curie, France. F. Ramos-Morales was supported by a return grant of the Training and Mobility of Researchers program from the European Union. C. Infante was supported by a Fundación Camara fellowship from the University of Seville.

Received for publication 2 November 1998 and in revised form 25 February 1999.

References

- Abe, A., N.N. Emi, M. Tanimoto, H. Terasaki, T. Marunouchi, and H. Saito. 1997. Fusion of the platelet-derived growth factor receptor β to a novel gene CEV14 in acute myelogenous leukemia after clonal evolution. *Blood* 90: 4271-4277.
- Ahmad, F.J., and P.W. Baas. 1995. Microtubules released from the neuronal centrosome are transported into the axon. *J. Cell Sci.* 108:2761-2769.
- Alcalde, J., G. Egea, and I.V. Sandoval. 1994. gp74, a membrane glycoprotein of the cis-Golgi network that cycles through the endoplasmic reticulum and intermediate compartment. *J. Cell Biol.* 124:649-665.
- Bailly, E., M. Doree, P. Nurse, and M. Bornens. 1989. p34cdc2 is located in both nucleus and cytoplasm; part is centrosomally associated at G2/M and enters vesicles at anaphase. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:3985-3995.
- Bannykh, S.I., and W.E. Balch. 1997. Membrane dynamics at the endoplasmic reticulum-Golgi interface. *J. Cell Biol.* 138:1-4.
- Barroso, M., D.S. Nelson, and E. Sztul. 1995. Transcytosis-associated protein (TAP)/p115 is a general fusion factor required for binding of vesicles to acceptor membranes. *Proc. Natl. Acad. Sci. USA.* 92:527-531.
- Belmont, L.D., A.A. Hyman, K.E. Sawin, and T.J. Mitchinson. 1990. Real-time visualization of cell cycle-dependent changes in microtubule dynamics in cytoplasmic extracts. *Cell.* 62:579-589.
- Blocker, A., F.F. Severin, A. Habermann, A.A. Hyman, G. Griffiths, and J.K. Burkhart. 1996. Microtubule-associated protein-dependent binding of phagosomes to microtubules. *J. Biol. Chem.* 271:3803-3811.
- Burkhardt, J.K., C.J. Echeverri, T. Nilsson, and R.B. Vallee. 1997. Overexpression of the dynamin (p50) subunit of the dynactin complex disrupts dynein-dependent maintenance of membrane organelle distribution. *J. Cell Biol.* 139:469-484.
- Chang, K., Y. Chen, T. Chen, W. Chou, P. Chen, Y. Ma, T.L. Yang-Fen, X.B. Leng, W. O'Malley, and W. Lee. 1997. A thyroid hormone receptor coactivator negatively regulated by the retinoblastoma protein. *Proc. Natl. Acad. Sci. USA.* 94:9040-9045.
- Charasse, S., M. Schroeder, C. Gautier-Rouviere, F. Anjo, L. Cassimeris, D.L. Gard, and C. Larroque. 1998. The TOGp protein is a new human microtubule-associated protein homologous to the *Xenopus* XMAP215. *J. Cell Sci.* 111:1371-1383.
- Cole, N.B., and J. Lippincott-Schwartz. 1995. Organization of organelles and membrane traffic by microtubules. *Curr. Opin. Cell Biol.* 7:55-64.
- Compton, D.A., I. Szilak, and D.W. Cleveland. 1992. Primary structure of NuMA, an intranuclear protein that defines a novel pathway for the segregation of proteins at mitosis. *J. Cell Biol.* 116:1395-1408.
- Corthésy-Theulaz, I., A. Pauloin, and S.R. Pfeffer. 1992. Cytoplasmic dynein participates in the centrosomal localization of the Golgi complex. *J. Cell Biol.* 118:1333-1345.
- Dabora, S.L., and M.P. Sheetz. 1988. Microtubule-dependent formation of a tubulovesicular network with characteristics of the endoplasmic reticulum from cultured cell extracts. *Cell.* 54:27-35.
- Ellis, L., E. Clauser, D.O. Morgan, M. Ederly, R.A. Roth, and W.J. Rutter. 1986. Replacement of insulin receptor tyrosine residues 1162 and 1163 compromises insulin-stimulated kinase activity and uptake of 2-deoxyglucose. *Cell.* 45:721-732.
- Erlich, R., P.A. Gleeson, P. Campbell, E. Dietzsch, and B.H. Toh. 1996. Molecular characterization of trans-Golgi p230. *J. Biol. Chem.* 271:8328-8337.
- Feiguin, F., A. Ferreira, K.S. Kosi, and A. Caceres. 1994. Kinesin-mediated organelle translocation revealed by specific cellular manipulations. *J. Cell Biol.* 127:1021-1039.
- Fritzler, M.J., J.C. Hamel, R.L. Och, and E.K. Chan. 1993. Molecular characterization of two human autoantigens: unique cDNAs encoding 95- and 160-kD proteins of a putative family in the Golgi complex. *J. Exp. Med.* 178:49-62.
- Fritzler, M.J., C. Lung, J.C. Hamel, K.J. Griffith, and E.K. Chan. 1995. Molecular characterization of golgin-245, a novel Golgi complex protein containing a granin signature. *J. Biol. Chem.* 270:31262-31268.
- Harada, A., Y. Takei, Y. Kanai, Y. Tanaka, S. Nonaka, and N. Hirokawa. 1998. Golgi vesiculation and lysosome dispersion in cells lacking cytoplasmic dynein. *J. Cell Biol.* 141:51-59.
- Hollenbeck, P.J., and J.A. Swanson. 1990. Radial extension of macrophage tubular lysosomes supported by kinesin. *Nature.* 346:864-866.
- Jasmin, B.J., J. Cartaud, M. Bornens, and J.P. Changeux. 1989. Golgi apparatus in chick skeletal muscle: changes in its distribution during end plate development after denervation. *Proc. Natl. Acad. Sci. USA.* 86:7218-7222.
- Karecla, P.I., and T.E. Kreis. 1992. Interaction of membranes of the Golgi complex with microtubules in vitro. *Eur. J. Cell Biol.* 57:139-146.
- Keating, T.J., J.G. Peloquin, V.I. Rodionov, D. Momcilovic, and G.G. Borisov. 1997. Microtubule release from the centrosome. *Proc. Natl. Acad. Sci. USA.* 94:5078-5083.
- Klopfenstein, D.R., F. Kappeler, and H.P. Hauri. 1998. A novel direct interaction of endoplasmic reticulum with microtubules. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:6168-6177.
- Kreis, T. 1990. Role of microtubules in the organization of the Golgi apparatus. *Cell Motil. Cytoskeleton.* 15:67-70.
- Lee, G., N. Cowan, and M. Kirschner. 1988. The primary structure and heterogeneity of tau protein from mouse brain. *Science.* 239:285-288.
- Lee, J.W., H. Choi, J. Gyuris, R. Brent, and D.D. Moore. 1995. Two classes of proteins dependent on either the presence or absence of thyroid hormone

- for interaction with the thyroid hormone receptor. *Mol. Endocrinol.* 9:243–254.
- Lewis, S.A., D. Wang, and N.J. Cowan. 1988. Microtubule-associated protein 2 (MAP2) shares a microtubule binding motif with tau protein. *Science.* 242: 936–939.
- Li, Q., and H.C. Joshi. 1995. γ -tubulin is a minus end-specific microtubule-binding protein. *J. Cell Biol.* 131:207–214.
- Lippincott-Schwartz, J., N.B. Cole, A. Marotta, P.A. Conrad, and G.S. Bloom. 1995. Kinesin is the motor for microtubule-mediated Golgi-to-ER membrane traffic. *J. Cell Biol.* 128:293–306.
- Matteoni, R., and T.E. Kreis. 1987. Translocation and clustering of endosomes and lysosomes depends on microtubules. *J. Cell Biol.* 105:1253–1265.
- Misumi, Y., M. Sohda, A. Yano, T. Fujiwara, and Y. Ikehara. 1997. Molecular characterization of GCP170, a 170-kD protein associated with the cytoplasmic face of the Golgi membrane. *J. Biol. Chem.* 272:23851–23858.
- Mogensen, M.M., J.B. Mackie, S.J. Doxsey, T. Stearns, and J.B. Tucker. 1997. Centrosomal deployment of γ -tubulin and pericentrin: Evidence for a microtubule-nucleating domain and a minus end docking domain in certain mouse epithelial cells. *Cell Motil. Cytoskeleton.* 36:276–290.
- Moudjou, M., N. Bordes, M. Paintrand, and M. Bornens. 1996. γ -tubulin in mammalian cells: the centrosomal and the cytosolic forms. *J. Cell Sci.* 109: 875–887.
- Nakamura, N., C. Rabouille, R. Watson, T. Nilsson, N. Hui, P. Slusarewicz, T.E. Kreis, and G. Warren. 1995. Characterization of a cis-Golgi matrix protein, GM130. *J. Cell Biol.* 131:1715–1726.
- Nakata, T., and N. Hirokawa. 1995. Point mutation of adenosine triphosphate-binding motif generated rigor kinesin that selectively blocks anterograde lysosome membrane transport. *J. Cell Biol.* 131:1039–1053.
- Orci, L., M. Stammes, M. Ravazzola, M. Amherdt, A. Perrelet, T.H. Sollner, and J. Rothman. 1997. Bidirectional transport by distinct populations of COP-I coated vesicles. *Cell.* 90:335–349.
- Paschal, B.M., and R.B. Vallee. 1987. Retrograde transport by the microtubule-associated protein MAP1C. *Nature.* 330:181–183.
- Pierre, P., J. Scheel, J.E. Rickard, and T.E. Kreis. 1992. CLIP-170 links endocytic vesicles to microtubules. *Cell.* 70:887–890.
- Presley, J.F., N.B. Cole, T.A. Schroer, K. Hirschberg, K.J.M. Zall, and J. Lippincott-Schwartz. 1997. ER-to-Golgi transport visualized in living cells. *Nature.* 389:81–85.
- Rickard, J.E., and T.E. Kreis. 1990. Identification of a novel nucleotide-sensitive microtubule-binding protein in HeLa cells. *J. Cell Biol.* 110:1623–1633.
- Rickard, J.E., and T.E. Kreis. 1996. CLIPs for organelle-microtubule interactions. *Trends Cell Biol.* 6:178–183.
- Rios, R.M., A.M. Tassin, C. Celati, C. Antony, M.C. Boissier, J.C. Homberg, and M. Bornens. 1994. A peripheral protein associated with the cis-Golgi network redistributes in the intermediate compartment upon brefeldin A treatment. *J. Cell Biol.* 125:997–1013.
- Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.
- Schulze, E., and M. Kirschner. 1987. Dynamic and stable populations of microtubules in cells. *J. Cell Biol.* 104:277–288.
- Seelig, H.P., P. Schranz, H. Schroter, C. Weimann, and M. Renz. 1994. Macrogolgin—a new 376 kD Golgi complex outer membrane protein as target of antibodies in patients with rheumatic diseases and HIV infections. *J. Autoimmun.* 7:67–91.
- Skoufias, D.A., T.L. Burgess, and L. Wilson. 1990. Spatial and temporal colocalization of the Golgi apparatus and microtubules rich in detyrosinated tubulin. *J. Cell Biol.* 111:1929–1937.
- Sohda, M., Y. Misumi, T. Fujiwara, M. Nishioka, and Y. Ikehara. 1994. Molecular cloning and sequence analysis of a human 372 kD protein localized in the Golgi complex. *Biochem. Biophys. Res. Commun.* 205:1399–1408.
- Solomon, F. 1986. Direct identification of microtubule-associated proteins by selective extraction of cultured cells. *Methods Enzymol.* 134:139–147.
- Tassin, A.M., C. Celati, M. Moudjou, and M. Bornens. 1998. Characterization of the human homologue of the yeast Spc98p and its association with γ -tubulin. *J. Cell Biol.* 141:689–701.
- Vaisberg, E.A., P.M. Grissom, and J.R. McIntosh. 1996. Mammalian cells express three distinct dynein heavy chains that are localized to different cytoplasmic organelles. *J. Cell Biol.* 133:831–842.
- Vale, R.D., T.S. Reese, and M.S. Sheetz. 1985a. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell.* 42: 39–50.
- Vale, R.D., B.J. Schnapp, T. Mitchison, E. Steuer, T.S. Reese, and M.P. Sheetz. 1985b. Different axoplasmic proteins generate movement in opposite directions along microtubules in vitro. *Cell.* 43:623–632.
- Vorobjev, I.A., and Y.S. Chentsov. 1983. The centrosome and its role in the organization of microtubules. *Int. Rev. Cytol.* 106:227–293.
- West, R.R., K.M. Tenbarge, and J.B. Olmsted. 1991. A model for microtubule-associated protein 4 structure. Domains defined by comparisons of human, mouse, and bovine sequences. *J. Biol. Chem.* 266:21886–21896.
- Wiemer, E.A.C., T. Wenzel, T.J. Deerinck, M.H. Ellisman, and S. Subramani. 1997. Visualization of the peroxisomal compartment in living mammalian cells: dynamic behavior and association with microtubules. *J. Cell Biol.* 136: 71–80.