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Sequence and Overexpression of GPP130/GIMPc: Evidence for Saturable pH-sensitive Targeting of a Type II Early Golgi Membrane Protein

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It is thought that residents of the Golgi stack are localized by a retention mechanism that prevents their forward progress. Nevertheless, some early Golgi proteins acquire late Golgi modifications. Herein, we describe GPP130 (Golgi phosphoprotein of 130 kDa), a 130-kDa phosphorylated and glycosylated integral membrane protein localized to the cis/medial Golgi. GPP130 appears to be the human counterpart of rat Golgi integral membrane protein, cis (GIMPc), a previously identified early Golgi antigen that acquires late Golgi carbohydrate modifications. The sequence of cDNAs encoding GPP130 indicate that it is a type II membrane protein with a predicted molecular weight of 81,880 and an unusually acidic lumenal domain. On the basis of the alignment with several rod-shaped proteins and the presence of multiple predicted coiled-coil regions, GPP130 may form a flexible rod in the Golgi lumen. In contrast to the behavior of previously studied type II Golgi proteins, overexpression of GPP130 led to a pronounced accumulation in endocytotic vesicles, and endogenous GPP130 reversibly redistributed to endocytotic vesicles after chloroquine treatment. Thus, localization of GPP130 to the early Golgi involves steps that are saturable and sensitive to lumenal pH, and GPP130 contains targeting information that specifies its return to the Golgi after chloroquine washout. Given that GIMPc acquires late Golgi modifications in untreated cells, it seems likely that GPP130/GIMPc continuously cycles between the early Golgi and distal compartments and that an unidentified retrieval mechanism is important for its targeting.

INTRODUCTION

The Golgi complex modifies and sorts proteins and lipids in the secretory pathway (Farquhar and Palade, 1981; Mellman and Simons, 1992; Rothman and Orci, 1992). Secreted proteins typically transit the mammalian Golgi complex in several minutes, whereas the resident Golgi proteins that function to modify these secretory molecules are localized to the Golgi for periods extending to several days. The mechanism that localizes resident Golgi proteins has been the subject of extensive investigation, but the theories derived from these studies remain controversial.

One theory states that resident Golgi proteins form oligomers too large to enter transport vesicles and that these oligomers stay behind in the Golgi as secretory molecules travel forward (Nilsson *et al.*, 1991; Swift and Machamer, 1991). Oligomer-based retention can explain why overexpression of Golgi proteins leads to backup in the endoplasmic reticulum (ER) rather than mislocalization to post-Golgi compartments (Munro, 1991; Nilsson *et al.*, 1991; Swift and Machamer, 1991). Indeed, versions of a chimeric viral protein that are Golgi-localized are recovered in cell extracts as large SDS-resistant oligomers, whereas mutant versions

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that are not retained in the Golgi fail to form oligomers (Weisz *et al.*, 1993). Also, mislocalization of the medial Golgi enzyme *N*-acetylglucosaminyltransferase I to the ER caused ER accumulation of mannosidase II, another medial Golgi enzyme, suggesting that heterooligomers form in vivo and are important for targeting (Nilsson *et al.*, 1994). On the other hand, large oligomers of Golgi proteins would be expected to have low mobilities in the membrane, and recent work indicated that several chimeric Golgi-localized proteins actually had very high mobilities (Cole *et al.*, 1996).

An alternative theory states that the length of the transmembrane segment excludes resident Golgi proteins from transport vesicles (Bretscher and Munro, 1993; Masibay *et al.*, 1993). It has been demonstrated in numerous studies that the transmembrane domains of resident Golgi proteins are required for proper localization (reviewed in Machamer, 1993; Nilsson *et al.*, 1993). Golgi-resident enzymes are mostly type II membrane proteins with membrane-spanning domains that are an average of five amino acids shorter than those of type II plasma membrane proteins (Bretscher and Munro, 1993; Masibay *et al.*, 1993). Increasing the length of the transmembrane segments led to the mislocalization of several Golgi proteins to the plasma membrane (Munro, 1991; Masibay *et al.*, 1993). How might transmembrane domain length exclude a protein from transport vesicles? One possibility is that a higher cholesterol content in the transport vesicle membrane makes this membrane too thick to accommodate a short transmembrane segment (Bretscher and Munro, 1993). Nevertheless, under in vitro conditions, it has been shown that Golgi membrane proteins are in fact incorporated into Golgi-derived transport vesicles (Sönnichsen *et al.*, 1996).

In addition to these postulated retention mechanisms, retrieval plays a role in localizing proteins to the Golgi. Retrieval mechanisms are characterized in part by their saturability. Trans-Golgi network protein 38 kDa (TGN38) and furin are localized to the trans-Golgi network, but they cycle through post-Golgi compartments (Reaves *et al.*, 1993; Wong and Hong, 1993). Overexpression of either protein leads to increased accumulation in endosomes (Bos *et al.*, 1993; Humphrey *et al.*, 1993; Chapman and Munro, 1994; Reaves and Banting, 1994). In contrast to the resident proteins of earlier Golgi compartments, TGN-localized proteins may have either a type I or a type II topology, and the cytoplasmic tails of these proteins are critical for their localization to the Golgi (Bos *et al.*, 1993; Humphrey *et al.*, 1993; Nothwehr *et al.*, 1993; Wong and Hong, 1993). As in other recycling pathways, the cytoplasmic tails of TGN-localized proteins are thought to contain signals that promote incorporation into recycling vesicles (Voorhees *et al.*, 1995). Thus, a general scheme emerges in which targeting of type II proteins to the Golgi stack involves retention, whereas targeting of TGN proteins involves retrieval (Machamer, 1993; Nilsson and Warren, 1994). However, there is evidence that at least some type II proteins may cycle within the Golgi stack (Johnston *et al.*, 1994; Hoe *et al.*, 1995; Harris and Waters, 1996). For example, Och1p, a type II early Golgi protein from *Saccharomyces cerevisiae*, acquires late Golgi modifications with a half-time of 5 min, even though Och1p has a half-time for residence in the Golgi of 60 min (Harris and Waters, 1996). These data suggest that Och1p is repeatedly retrieved from a late Golgi compartment.

Obviously, it is unlikely that a single process explains the targeting of all resident Golgi proteins. Different Golgi proteins probably use different localization mechanisms, and more than one mechanism may operate in the localization of a given protein. A comprehensive understanding of Golgi localization will require analysis of a diverse array of Golgi proteins. This article describes the sequence and biochemical characterization of GPP130 (Golgi phosphoprotein of 130 kDa), a type II protein that resides in the cis/medial Golgi. On the basis of shared properties, including antibody cross-reactivity, GPP130 appears to be the human homologue of a rat Golgi-localized antigen known as Golgi integral membrane protein, cis (GIMPc). Despite its localization to the early Golgi, GIMPc acquires late Golgi modifications (Yuan *et al.*, 1987). Unlike other mammalian type II Golgi proteins, GPP130/GIMPc redistributes to post-Golgi compartments when the cells are treated with chloroquine, suggesting that GPP130/GIMPc is targeted to the early Golgi by a saturable pH-sensitive retrieval pathway.

MATERIALS AND METHODS

Preparation of Monoclonal Antibodies

The mouse monoclonal antibody A1/118, an IgG1, was obtained by the hybridoma technique (Hauri *et al.*, 1985) using PAI myeloma cells as the fusion partner. A membrane fraction enriched in ERGIC-53 was isolated from Vero cells (Schweizer *et al.*, 1991) and injected into mice. The first injection (intracisternal, day 0) was 500 μg of protein in complete Freund's adjuvant; the second injection (subcutaneous, day 48) was 610 μg of protein in incomplete Freund's adjuvant; the third injection (50% intravenous, 50% introperitoneal, day 110) was 700 μg of protein without adjuvant. Cell fusion was performed on day 113. Culture supernatants positive by dot blot were tested for reactivity in Vero cells by immunofluorescence microscopy and immunoprecipitation and then concentrated 10-fold by ammonium sulfate precipitation.

Immunofluorescence and Immunoelectron Microscopy

Immunofluorescence was performed as described previously (Linstedt and Hauri, 1993) except that the cells were grown on 12-mm circular glass coverslips and digital images were acquired in the program Photoshop (Adobe Systems, Mountain View, CA) using a fluorescence microscope (Nikon, Melville, NY) equipped with a

Hamamatsu black-and-white cooled charge-coupled device camera (Hamamatsu Photonics K.K., Hamamatsu City, Japan). Antibody dilutions were as follows: A1/118 at 1:100, anti-giantin (Linstedt *et al.*, 1995) at 1:1000, anti-galactosyltransferase (Berger *et al.*, 1986) at 1:200, and anti-mannose-6-phosphate receptor at 1:500. Fixable fluorescein isothiocyanate (FITC)-dextran (M_r, 10,000, Molecular Probes, Eugene, OR) was added to DMEM at 1 mg/ml and the cells were cultured for 1 h and then fixed for 12 h. Antibody uptake was performed by incubation of cells with 1 μg/ml A1/118 or anti-giantin for 3 h in DMEM. The cells were then fixed and processed as indicated above except that primary antibodies were omitted. For immunoelectron microscopy, Vero cells were fixed with 2% formaldehyde containing 0.01% glutaraldehyde and processed as described previously (Reggio *et al.*, 1983). Alternatively, COS-7 cells cultured on 35-mm plates were fixed with 3% paraformaldehyde for 24 h, washed twice with phosphate-buffered saline (PBS) and twice with PBS containing 20 mM glycine, and then permeabilized for 20 min with PBS-glycine containing 0.1% saponin. Antibody incubations were in 1 ml of PBS-saponin for 1 h and were followed by five washes with PBS-saponin. A1/118 and horseradish peroxidase-labeled goat anti-mouse (Cappel, West Chester, PA) were used at a 1:100 dilution. Controls omitted the primary antibody. The samples were further processed as follows: three PBS washes, 30 min in 1% OsO_4 buffered with PBS, three water washes, dehydration in an ethanol series (50%, 70%, 80%, 90%, and 100%), infiltration in a 1:1 mixture of 100% ethanol:Epon-araldite for 1 h, 100% Epon-araldite for 18 h in a desiccator, 100% Epon-araldite for 48 h in a desiccator, 35°C for 48 h, 50°C for 24 h, 60°C for 12 h. Pieces of the polymerized Epon-araldite disk were sectioned and viewed on a Hitachi 7100 transmission electron microscope at 50 keV. Digital images were recorded with an AMT Advantage 10 image acquisition system (AMT, Danvers, MA), using a Kodak Megaplus 1.6I charge-coupled device camera and National Institutes of Health Image acquisition software.

Metabolic Labeling and Immunoprecipitation

In vivo phosphorylation was as follows. COS-7 cells were grown to near confluency in a six-well dish in DMEM containing 10% calf serum. The cells were washed and then incubated for 30 min with phosphate-free DMEM. Labeling was carried out for 3 h in 0.5 ml of phosphate-free DMEM containing 0.25 mCi/ml [^{32}P]orthophosphate (Dupont NEN, Wilmington, DE). Cell lysates were prepared by addition of RIPA buffer containing inhibitors (1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 2 mM EDTA, 20 mM triethanolamine pH 7.5, 50 mM NaF, 0.2 mM Na_3VO_4, 1 μM microcystin, 1 μg/ml pepstatin, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride). Methionine labeling was as follows. COS-7 cells in a six-well dish were washed and incubated for 30 min with methionine- and cysteine-free DMEM. Labeling was carried out for 30 min in 0.5 ml of the same medium containing 0.1 mCi/ml ^{35}S-labeling mixture (Dupont NEN). Chase incubations were carried out for the indicated times in normal growth medium. Lysates were prepared as described above except that phosphatase inhibitors were not present. Immunoprecipitation of the lysates was as follows. After incubation for 30 min on ice, the lysate was centrifuged for 15 min in a microcentrifuge at 4°C, and the resulting supernatant was precleared and then rotated for 90 min at 4°C with 12 μl of A1/118-coated protein A-Sepharose (Pharmacia, Piscataway, NJ) or control beads. The Sepharose beads were collected and washed five times with 1 ml of RIPA buffer, boiled in sample buffer, and analyzed by SDS-PAGE (7% gels). Each 12 μl of protein A-Sepharose were coated by sequential incubations with 2 μl of rabbit anti-mouse IgG (Cappel) and 10 μl of A1/118. Control beads were incubated only with the rabbit anti-mouse IgG.

Subcellular Fractionation

Gradient fractionation was exactly as described (Linstedt *et al.*, 1995). HepG2 cells grown to confluence on three 15-cm plates were homogenized by 10 passages through a ball-bearing homogenizer with a clearance of 20 μm in 3 ml of 120 mM NaCl, 5 mM KCl, and 25 mM $NaHCO_3$ (pH 7.4) containing protease inhibitors. After centrifugation at 750 rpm for 10 min in an SS-34 rotor (Sorvall, Newtown, CT), the resulting postnuclear supernatant was applied to the top of a 12-ml linear Nycodenz gradient [13–29% (wt/vol) with a 1-ml 35% cushion; all solutions contained 10 mM triethanolamine, pH 7.4, 1 mM EDTA, and protease inhibitors]. After centrifugation at 25,500 rpm for 3 h in an TST-28.17 rotor (Kontron, Zürich, Switzerland), the gradients were fractionated from the bottom, and each fraction was assayed for density, content of total protein, glucose 6-phosphatase, GalNAc transferase, and galactosyltransferase. The amount of GPP130 was determined by immunoblotting with A1/118 used at a 1:200 dilution. The F1 membrane fraction used in protease protection and extraction experiments was prepared as follows. HeLa cells grown to confluency were rinsed twice with ice-cold PBS, scraped into PBS, and collected by centrifugation. The pellet was resuspended and centrifuged in homogenization buffer (250 mM sucrose, 1 mM EDTA, 10 mM triethanolamine, pH 7.4, and protease inhibitors). The washed pellet was resuspended and homogenized in homogenization buffer (1 ml/10-cm plate) by 20 passages through a 25-gauge needle. The postnuclear supernatant, obtained by centrifugation of the homogenate at 1000 × g for 5 min, was adjusted to 50% sucrose (6-ml volume), and placed in the bottom of a centrifuge tube. Then, 45% (4 ml) and 10% (1.5 ml) sucrose steps were carefully overlayed. The resulting step gradient was centrifuged for 3 h at 30,000 rpm in a Kontron 41.14 swinging bucket rotor. The F1 fraction was collected from the 45%/10% interface with a pipette.

Cloning and Transfection

A unizap human intestinal cDNA library (Stratagene, La Jolla, CA) was first screened with A1/118 as described for immunoscreening (Ausubel *et al.*, 1995). Screening of 2.7 × 10^5 plaques yielded two positive clones that were plaque-purified, excised as inserts in Bluescript (Stratagene), and analyzed by restriction mapping and sequencing. The positive plaques were found to be derived from the same gene. A 1.1-kb *Xba*I fragment derived from the 5′ end of one of the clones was then used as a probe to rescreen the library as described for cDNA screening (Ausubel *et al.*, 1995). This resulted in the isolation of a 4.0-kb clone that was sequenced by using exonuclease III nested deletions. By Northern blot analysis, the cDNA clone was lacking approximately 0.5 kb. A 5′-rapid amplification of cDNA ends kit (Life Technologies, Gaithersburg, MD) was used to isolate DNA encompassing the remaining 0.5 kb. Shared restriction sites were then used to piece together the entire open reading frame, which was then cloned into the *Eco*RI site of the expression vector pECE. The COS transfection protocol was as follows. COS-7 cells grown overnight to 75% confluence on a 60-mm plate were rinsed twice with PBS, incubated with 0.3 ml of PBS containing 2 μg of DNA and 10 μg/ml DEAE for 30 min at 37°C, and then incubated with 2 ml of DMEM containing 10% NuSerum (Life Technologies) and 10 mM chloroquine for 3.5 h. This was followed by a 2-min incubation with DMEM containing 10% dimethyl sulfoxide and an overnight incubation in normal growth medium. The next day the cells were passaged onto plates or coverslips in preparation for analysis on the following day. CHO and HeLa, 1 × 10^6 cells in 0.1 ml of PBS, were transfected by electroporation in 0.4-cm cuvettes with a Gene Pulser (Bio-Rad, Richmond, CA) set at 0.3 kV and 250 μF. For transfection of HeLa TetON (Clontech, Palo Alto, CA) the cDNA was cloned into the pTRE vector (Clontech). Doxycycline (Sigma, St. Louis, MO) at 0.001 mg/ml was used for induction.

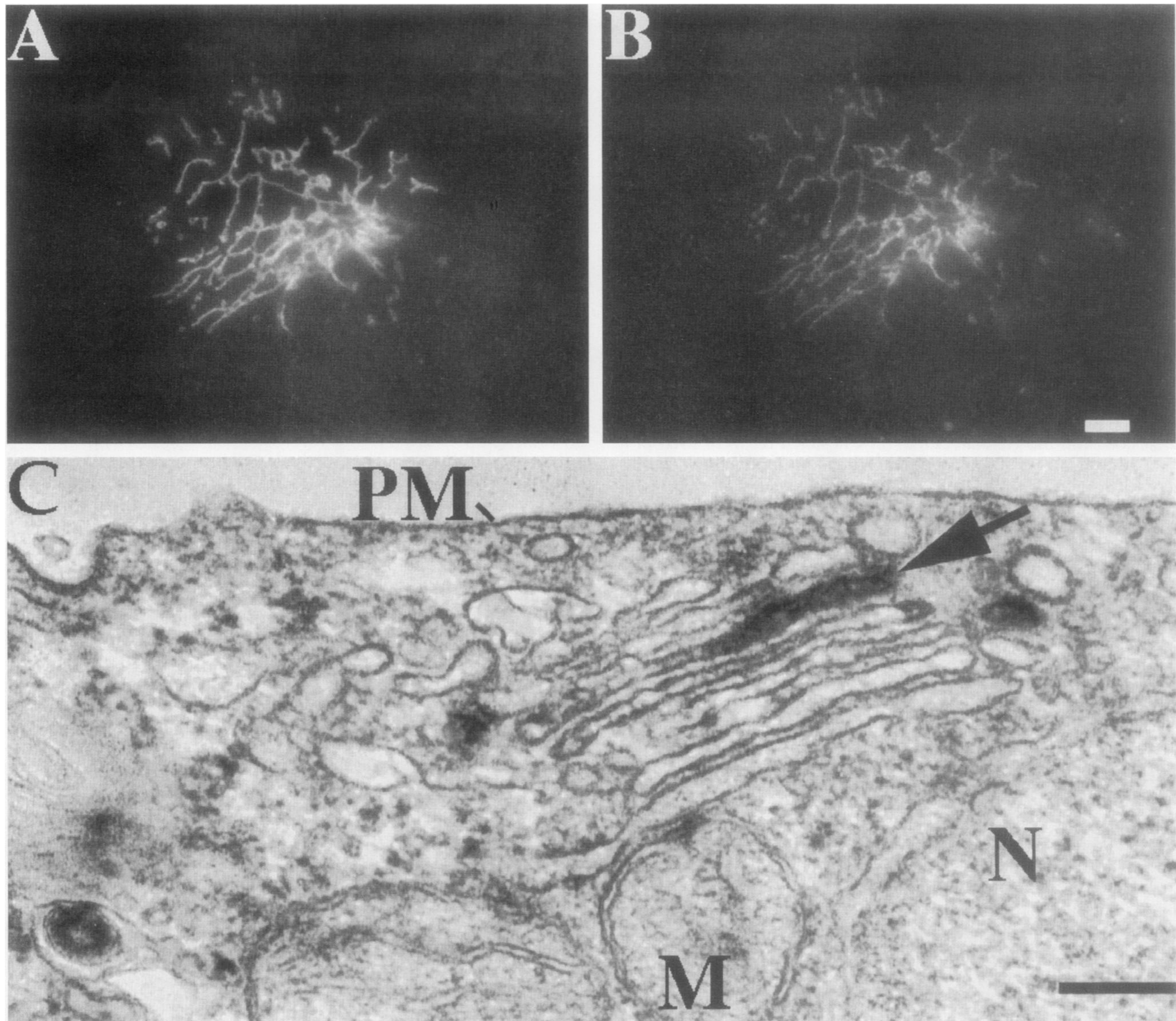

Figure 1. Localization of GPP130 by immunofluorescence and immunoelectron microscopy. (A) GPP130 immunofluorescent staining pattern in the perinuclear region of a COS-7 cell. The ribbon-like perinuclear staining is characteristic for proteins localized to the Golgi apparatus. (B) Giantin staining in the same cell. Despite the intricate staining pattern no significant differences between GPP130 and giantin were observed. Bar, 8 μm. (C) Immunoperoxidase localization of GPP130 in Vero cells. Reaction products were observed in the lumen of a single Golgi cisterna (arrow). Nucleus (N), mitochondria (M), and plasma membrane (PM) are marked.

RESULTS

GPP130 Is a cis/Medial Golgi Phosphoprotein of 130 kDa

In an attempt to identify novel residents of the Golgi complex, we characterized an antigen recognized by monoclonal antibody A1/118, a mouse IgG1 that was generated by immunizing with a membrane fraction enriched for the protein ERGIC-53 (Schweizer *et al.*, 1991). Indirect immunofluorescence analysis of COS cells indicated that A1/118 recognized a Golgi-localized epitope (Figure 1A). This pattern was indistinguishable from the pattern of the Golgi protein giantin in the same cells (Figure 1B). Specific Golgi staining was observed in several human and monkey cell lines (Caco-2, HeLa, Vero, and COS), but the antibody did not react with cell lines derived from hamster, rat, or *Xenopus*. Treatment of COS-7 cells with cycloheximide for 3 h did not alter the staining pattern, suggesting that A1/118 antigen is a resident of the Golgi complex. The localization of the antigen was also determined in

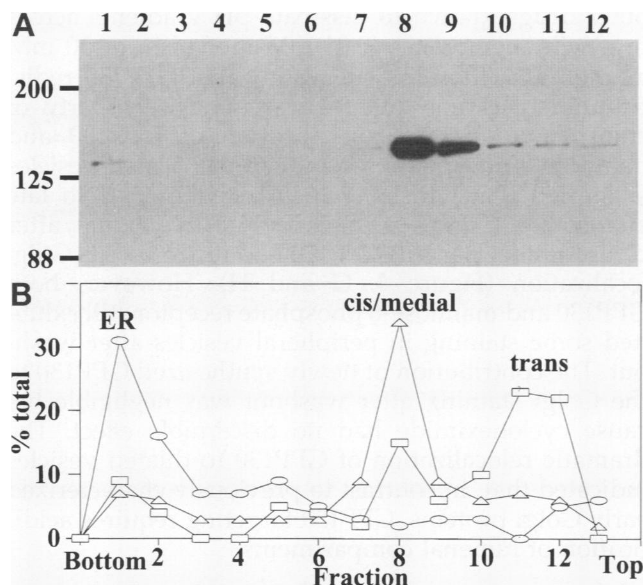

Figure 2. Distribution of GPP130-containing membranes after gradient fractionation. A postnuclear supernatant was separated on a 13–29% Nycodenz gradient containing a 35% cushion. Each fraction was assayed for GPP130 by immunoblotting (A) and for several markers (B). The markers were glucose 6-phosphatase (ER), GalNAc transferase (cis-Golgi), and galactosyltransferase (trans-Golgi) as previously reported (Linstedt *et al.*, 1995).

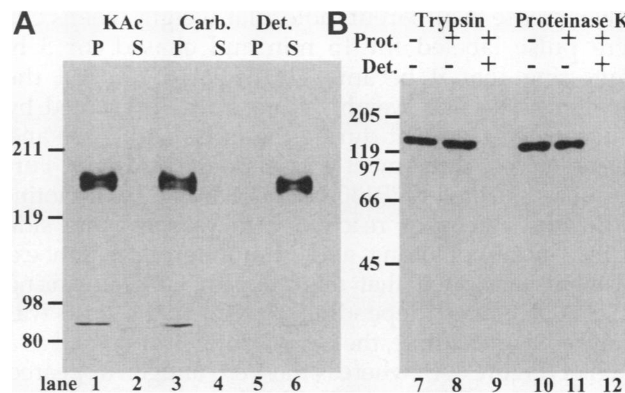

Figure 3. Membrane association and topology of GPP130. (A) Membrane extraction. Aliquots of a membrane fraction (0.1 mg of protein) were incubated on ice for 30 min in various extraction buffers and then collected by centrifugation (100,000 × *g* for 30 min). The recovery of GPP130 in the pellet (P) and in trichloroacetic acid-precipitated supernatant fractions (S) was assayed by immunoblotting. The extraction buffers were 1 M potassium acetate (KAc); 100 mM sodium carbonate, pH 11.5 (Carb.); or 2% SDS (Det.). (B) Protease protection. Membrane vesicles (1 mg/ml protein) were incubated on ice for 60 min with additions of trypsin (50 μg/ml), proteinase K (20 μg/ml), and Triton X-100 (1%) where indicated. Each sample was then adjusted to equal amounts of protease inhibitors, protease, and detergent and precipitated with trichloroacetic acid. GPP130 recovery was assayed by immunoblotting.

cells treated with nocodazole, brefeldin A, or okadaic acid prior to immunofluorescence staining. In each case, the change in staining pattern mirrored that of integral membrane markers of the Golgi complex such as giantin and galactosyltransferase. Immunoelectron microscopic staining of Vero cells also clearly revealed the Golgi localization of the antigen. The staining was restricted to perinuclear membranes that were stacked in appearance (Figure 1B). The electron-dense reaction product accumulated within the Golgi lumen, suggesting that the antibody bound to a lumenal epitope. Staining was asymmetrically distributed on one side of a stack in Vero (Figure 1B), COS-7, and HeLa cells. To determine whether the antigen is localized on the cis or trans side of the stack, we used a gradient fractionation protocol that separates the cis-Golgi marker GalNAc transferase from the trans-Golgi marker galactosyltransferase (Linstedt *et al.*, 1995). Immunoblotting of the gradient fractions revealed a single protein of approximately 130 kDa that cosedimented with the cis-Golgi marker GalNAc transferase and was separated from the trans-Golgi marker galactosyltransferase (Figure 2). A1/118 specifically immunoprecipitated a 130-kDa protein from cells labeled with [^{35}S]methionine or [^{32}P]orthophosphate. The phosphorylation of the 130-kDa protein was readily detected after a 3-h labeling of a single 35-mm plate of COS-7 cells with 0.25 mCi/ml [^{32}P]orthophosphate. From these results we conclude that the A1/118 antigen is a 130-kDa phosphoprotein that resides primarily in the cis and/or medial Golgi. This protein is provisionally named GPP130 (for Golgi phosphoprotein of 130 kDa).

GPP130 Is a Glycosylated Integral Membrane Protein with a Large Lumenal Domain

To determine whether GPP130 is an integral component of Golgi membranes, we extracted isolated vesicles with 0.1 M sodium carbonate at pH 11.5. The membranes were also treated with low salt buffer or detergent-containing buffer. As expected for an integral membrane protein, the 130-kDa antigen remained membrane-associated after extraction unless detergent was added (Figure 3A). The protein was also not extracted by either 1 M NaCl or 5 M urea. Protease protection was used to determine the topology of GPP130 in the membrane. Isolated vesicles were treated with trypsin or proteinase K in the presence or absence of a membrane-solubilizing detergent. Reactivity of A1/118 with the 130-kDa antigen was protected from protease digestion in the absence of detergent but was completely lost in the presence of detergent (Figure 3B). This result indicates that the antigenic epitope and most or all of the GPP130 molecule resides in the Golgi lumen. GPP130 exhibited a

slow increase in apparent molecular weight when cells were pulse labeled for 15 min and chased for 3 h, suggesting that it became glycosylated. Indeed the apparent molecular weight increase was prevented by tunicamycin treatment during the pulse and chase and was reversed by treatment with endoglycosidase F. Furthermore, purified GPP130 became labeled with biotin-hydrazide, a reagent reactive with carbohydrate side chains. Immunoblotting and immunoprecipitation experiments indicated that migration of GPP130 during SDS-PAGE varied depending on which cell type was analyzed. For example, the Caco-2 antigen appeared as a doublet (Figure 3A), whereas the Vero antigen appeared as a single band (Figure 3B). This behavior may reflect differences in glycosylation and/or phosphorylation between different cell types.

Chloroquine Treatment Reversibly Alters the Intracellular Distribution of GPP130

TGN38, furin, and mannose-6-phosphate receptor continually recycle between the TGN and the cell surface (Griffiths *et al.*, 1988; Reaves *et al.*, 1993; Wong and Hong, 1993; Molloy *et al.*, 1994). Treatment of cells with weak bases such as chloroquine, which block the acidification of lumenal compartments, prevents the return of TGN proteins to the Golgi by blocking transport out of the late endosome (Brown *et al.*, 1986; Chapman and Munro, 1994). Chloroquine treatment also causes a dramatic enlargement of endosomal vesicles, resulting in a "Swiss cheese" appearance of the cytoplasm. Therefore, chloroquine treatment results in a redistribution of TGN proteins from the Golgi to dilated endosomes. In contrast, type II Golgi proteins such as *N*-acetylglucosaminyltransferase I, mannosidase II, sialyltransferase, and galactosyltransferase remain associated with the Golgi stack in chloroquine-treated cells (Berger *et al.*, 1993; Chapman and Munro, 1994). Because the behavior of these various proteins in response to chloroquine treatment is probably related to their targeting mechanisms, we examined the distribution of GPP130, giantin, and mannose-6-phosphate receptor in COS-7 cells treated with 0.1 mM chloroquine for 3 h. In untreated cells the giantin and GPP130 staining patterns were coincident (Figure 4, A and B). Surprisingly, chloroquine treatment led to the accumulation of GPP130 in dilated peripheral vesicles, but giantin staining remained in a characteristic perinuclear pattern (Figure 4, C and D). The slight compaction of the giantin pattern has been observed for other Golgi proteins after chloroquine treatment (Chapman and Munro, 1994) and may reflect the swelling of neighboring acidic vesicles. Significant GPP130 redistribution occurred within 30 min of treatment, and redistribution appeared to be maximal within 2 h. GPP130 also redistributed to peripheral vesicles after treatment of cells with either of two other drugs known to dissipate pH gradients across organelle membranes (0.01 mM monensin or 50 mM ammonium chloride), suggesting that GPP130 redistribution was not due to a secondary property of chloroquine. Partial colocalization of GPP130 and mannose-6-phosphate receptor in the dilated vesicles suggested that GPP130 redistributed in part to late endosomes (Figure 4, E and F). Three hours after chloroquine washout, GPP130 had recovered its Golgi localization (Figure 4, G and H). However, both GPP130 and mannose-6 phosphate receptor still exhibited some staining in peripheral vesicles after washout. The contribution of newly synthesized GPP130 to the Golgi staining after washout was negligible because cycloheximide had no discernible effect. The dramatic relocalization of GPP130 to dilated vesicles indicated that, in contrast to previously characterized early Golgi proteins, GPP130 targeting requires acidification of lumenal compartments.

GPP130 Is a Type II Membrane Protein with an Unusually Acidic Lumenal Domain

Expression screening of a human epithelial cDNA library with the A1/118 antibody resulted in the isolation of several overlapping clones. Subsequent cDNA screening identified a 4.0-kb cDNA that contained a poly(A) addition site at its 3′ end and a large open reading frame at its 5′ end. Northern blotting indicated that the corresponding HeLa mRNA was 4.5 kb. 5′ rapid amplification of cDNA ends (Frohman *et al.*, 1988) produced a PCR fragment containing an additional 0.5 kb of N-terminal coding sequence, flanked by a start codon with surrounding nucleotides that match the derived consensus for translational initiation (Kozak, 1996). The DNA sequence encompassing the open reading frame and the predicted protein sequence are presented in Figure 5.

Surprisingly, the calculated molecular weight for GPP130 of 81,880 is substantially smaller than the apparent molecular weight derived from SDS-PAGE migration. Several observations indicate that this discrepancy is due to inherent properties of the GPP130 polypeptide rather than to posttranslational modifications such as glycosylation: tunicamycin treatment only slightly altered the migration of GPP130 on SDS-PAGE, a coupled in vitro transcription/translation reaction programmed with the GPP130 cDNA and performed in the absence of membranes produced a protein migrating at approxi-

Figure 4 (on facing page). Comparison of the distribution of GPP130 and marker proteins in chloroquine-treated COS-7 cells. Untreated cells were double-stained for GPP130 (A) and giantin (B). Cells treated with chloroquine (3 h, 0.1 mM) were double-stained for GPP130 (C and E) and either giantin (D) or mannose-6-phosphate receptor (F). Chloroquine-treated cells were washed and recultured in the absence of chloroquine for 3 h and then stained for GPP130 (G) and giantin (H). Bar, 30 μm.

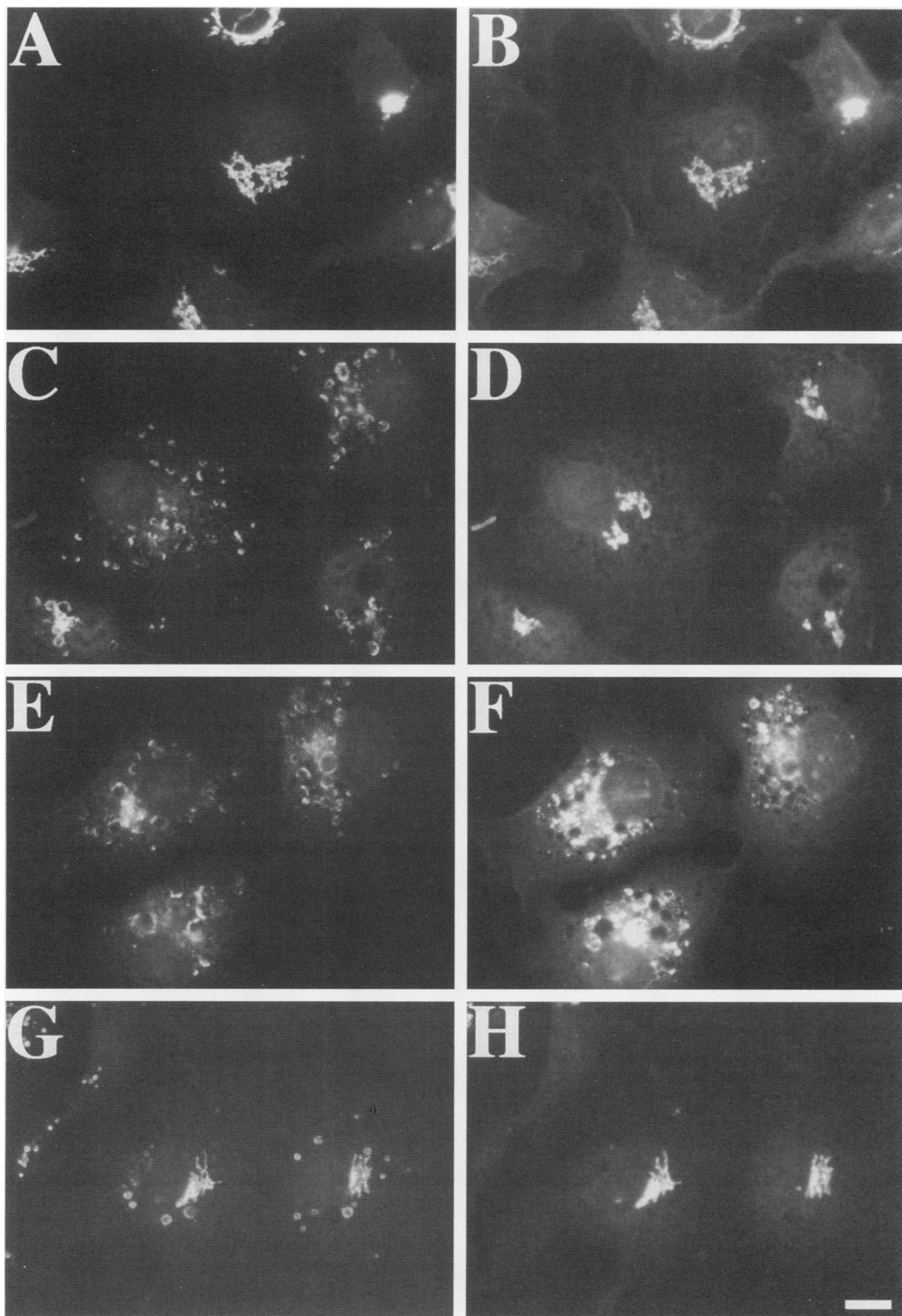

Figure 4.

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 -13                                                        TCCAGGCGGGACT

   1  ATGGGAAACGGGATGTGCTCCCGAAAGCAGAAGCGGATTTTCCAGACGCTGCTGCTGCTG
   1   M  G  N  G  M  C  S  R  K  Q  K  R  I  F  Q  T  L  L  L  L

  61  ACCGTCGTGTTCGGCTTTCTCTACGGCGCGATGCTCTACTACGAGCTGCAGACGCAGCTG
  21   T  V  V  F  G  F  L  Y  G  A  M  L  Y  Y  E  L  Q  T  Q  L

 121  CGGAAAGCCGAGGCGGTGGCGCTCAAGTACCAGCAGCACCAGGAGTCCCTCTCCGCCCAG
  41   R  K  A  E  A  V  A  L  K  Y  Q  Q  H  Q  E  S  L  S  A  Q

 181  TTACAAGTTGTATATGAACACAGATCAAGATTAGAGAAATCCTTGCAAAAAGAAAGACTT
  61   L  Q  V  V  Y  E  H  R  S  R  L  E  K  S  L  Q  K  E  R  L

 241  GAACATAAAAAAGCAAAGGAAGATTTTCTTGTTTATAAGTTAGAAGCACAAGAAACATTA
  81   E  H  K  K  A  K  E  D  F  L  V  Y  K  L  E  A  Q  E  T  L

 301  AATAAAGGAAGGCAAGATTCCAATAGCAGATACAGTGCACTGAATGTCCAACATCAGATG
 101   N  K  G  R  Q  D  S  N  S  R  Y  S  A  L  N  V  Q  H  Q  M

 361  TTGAAAAGCCAACACGAGGAGCTAAAGAAACAGCACAGTGACTTGGAAGAGGAACATCGC
 121   L  K  S  Q  H  E  E  L  K  K  Q  H  S  D  L  E  E  E  H  R

 421  AAACAAGGGGAAGACTTCAGTAGAACATTTAATGACCATAAGCAAAAATACTTGCAGCTC
 141   K  Q  G  E  D  F  S  R  T  F  N  D  H  K  Q  K  Y  L  Q  L

 481  CAGCAAGAAAAAGAACAAGAACTTTCTAAGCTAAAAGAGACTGTATACAATTTGAGAGAA
 161   Q  Q  E  K  E  Q  E  L  S  K  L  K  E  T  V  Y  N  L  R  E

 541  GAGAATAGACAACTAAGGAAAGCACACCAAGACATACATACACAGCTTCAAGATGTCAAG
 181   E  N  R  Q  L  R  K  A  H  Q  D  I  H  T  Q  L  Q  D  V  K

 601  CAACAGCATAAGAATTTACTCTCCGAGCATGAACAACTTGTAGTGACTTTGGAAGACCAC
 201   Q  Q  H  K  N  L  L  S  E  H  E  Q  L  V  V  T  L  E  D  H

 661  AAGAGTGCACTAGCTGCTGCACAGACTCAAGTTGCAGAATATAAACAACTGAAAGATACT
 221   K  S  A  L  A  A  A  Q  T  Q  V  A  E  Y  K  Q  L  K  D  T

 721  CTGAATAGGATTCCAAGCCTTCGAAAACCTGATCCAGCAGAACAGCAAAATGTGACCCAG
 241   L  N  R  I  P  S  L  R  K  P  D  P  A  E  Q  Q  N  V  T  Q

 781  GTGGCACATTCTCCACAAGGTTACAACACAGCAAGGGAGAAGCCAACCCGAGAGGTGCAG
 261   V  A  H  S  P  Q  G  Y  N  T  A  R  E  K  P  T  R  E  V  Q

 841  GAGGTGTCTCGAAATAATGATGTGTGGCAGAACCATGAAGCAGTTCCTGGAAGAGCAGAA
 281   E  V  S  R  N  N  D  V  W  Q  N  H  E  A  V  P  G  R  A  E

 901  GACACAAAACTCTATGCTCCCACCCATAAGGAGGCAGAATTTCAGGCTCCCCCAGAGCCA
 301   D  T  K  L  Y  A  P  T  H  K  E  A  E  F  Q  A  P  P  E  P

 961  ATCCAACAAGAAGTGGAACGCAGAGAACCTGAGGAGCATCAGGTGGAAGAGGAGCACAGA
 321   I  Q  Q  E  V  E  R  R  E  P  E  E  H  Q  V  E  E  E  H  R

1021  AAGGCCCTGGAGGAGGAAGAAATGGAGCAGGTCGGGCAAGCAGAACATCTTGAGGAGGAA
 341   K  A  L  E  E  E  E  M  E  Q  V  G  Q  A  E  H  L  E  E  E

1081  CACGATCCATCACCAGAGGAGCAGGATCGGGAGTGGAAAGAGCAGCATGAGCAACGAGAA
 361   H  D  P  S  P  E  E  Q  D  R  E  W  K  E  Q  H  E  Q  R  E

1141  GCAGCCAACCTCCTGGAAGGGCACGCGCGTGCTGAGGTGTACCCTTCAGCCAAGCCAATG
 381   A  A  N  L  L  E  G  H  A  R  A  E  V  Y  P  S  A  K  P  M

1201  ATCAAATTCCAATCACCCTATGAGGAACAGTTGGAACAGCAGAGACTGGCAGTGCAGCAG
 401   I  K  F  Q  S  P  Y  E  E  Q  L  E  Q  Q  R  L  A  V  Q  Q

1261  GTGGAGGAGGCCCAGCAGCTGCGGGAACACCAGGAAGCTTTGCACCAGCAGAGGCTGCAG
 421   V  E  E  A  Q  Q  L  R  E  H  Q  E  A  L  H  Q  Q  R  L  Q

1321  GGGCACTTACTACGGCAGCAGGAACAGCAGCAGCAGCAGGTGGCAAGAGAGATGGCCCTG
 441   G  H  L  L  R  Q  Q  E  Q  Q  Q  Q  Q  V  A  R  E  M  A  L

1380  CAGAGGCAGGCTGAGCTTGAGGAGGGCCGGCCGCAGCACCAGGAGCAGCTCCGGCAGCAA
 461   Q  R  Q  A  E  L  E  E  G  R  P  Q  H  Q  E  Q  L  R  Q  Q

1441  GCTCATTATGATGCTATGGATAATGATATCGTTCAGGGAGCAGAGGACCAGGGAATCCAA
 481   A  H  Y  D  A  M  D  N  D  I  V  Q  G  A  E  D  Q  G  I  Q

1501  GGAGAGGAAGGAGCCTATGAAAGAGACAACCAGCACCAAGATGAAGCAGAAGGAGATCCA
 501   G  E  E  G  A  Y  E  R  D  N  Q  H  Q  D  E  A  E  G  D  P

1561  GGTAATAGACATGAGCCTCGTGAACAAGGACCCCGAGAAGCCGACCCAGAATCTGAGGCA
 521   G  N  R  H  E  P  R  E  Q  G  P  R  E  A  D  P  E  S  E  A

1621  GATAGGGCAGCTGTAGAAGATATAAACCCAGCAGATGACCCTAATAATCAAGGTGAGGAT
 541   D  R  A  A  V  E  D  I  N  P  A  D  D  P  N  N  Q  G  E  D

1681  GAATTTGAAGAAGCCGAGCAAGTGAGAGAAGAAAATTTGCCAGATGAAAATGAAGAGCAA
 561   E  F  E  E  A  E  Q  V  R  E  E  N  L  P  D  E  N  E  E  Q

1741  AAACAAAGTAATCAAAAGCAAGAGAATACAGAAGTGGAGGAACATTTGGTGATGGCAGGA
 581   K  Q  S  N  Q  K  Q  E  N  T  E  V  E  E  H  L  V  M  A  G

1801  AATCCAGACCAGCAGGAGGACAATGTTGATGAACAGTACCAGGAAGAGGCAGAAGAGGAG
 601   N  P  D  Q  Q  E  D  N  V  D  E  Q  Y  Q  E  E  A  E  E  E

1861  GTTCAGGAAGATTTGACTGAAGAGAAAAAAAGGGAACTGGAGCATAATGCTGAAGAGACC
 621   V  Q  E  D  L  T  E  E  K  K  R  E  L  E  H  N  A  E  E  T

1921  TATGGTGAAAATGATGAAAATACTGATGATAAAAATAATGATGGAGAAGAGCAAGAAGTT
 641   Y  G  E  N  D  E  N  T  D  D  K  N  N  D  G  E  E  Q  E  V

1981  CGAGATGACAACCGCCCCAAAGGCCGAGAGGAACACTACGAGGAGGAAGAAGAGGAGGAA
 661   R  D  D  N  R  P  K  G  R  E  E  H  Y  E  E  E  E  E  E  E

2041  GAAGACGGGGCTGCAGTTGCTGAGAAATCACATCGAAGAGCTGAAATGTAGCAGCACCCA
 681   E  D  G  A  A  V  A  E  K  S  H  R  R  A  E  M

2101  ATTTCTAGACAACGCTCAGCCAACGGATTCTTTTCAAGCTGCTCAAACATAAATCTGCCT
2161  ACTGAACTCTAGGATATTTAATTACAAAAATTAAGAACTTAGACTTTTTTAAAACTTTGT
2221  ATTAGAAATGCGCATACATTTATATGAATATATTTTGATAACATAGGTCTAGAGCTTCTT
2281  TTATATTCAAGCTAAACATGAAAAAGAAGAAAAACAATAAAGTAAACCTGAGCCCCCACG
2341  TCCCAATTTTTTTAATAGATTATGTGATGTTGGAAAGCTCATTGATTTGTATATGTTTCA
2401  GTGTGTTACCTTTCTGGCTTCCAGTTCCCAGGTGTTCTTTGTTTGCCTTTGATAAAATAC
2461  AGGATTTAAGAACAGAAAGTAGCTGCAAAATGC
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Figure 5. DNA and deduced protein sequence of GPP130. The predicted transmembrane domain is underlined, and the potential glycosylation sites are double underlined. The GenBank accession number is U55853.

mately 130 kDa on SDS-PAGE, and bacteria transformed with the GPP130 cDNA also produced a 130-kDa protein. Therefore, it seems clear that the primary sequence of GPP130 leads to aberrant migration on SDS-PAGE. Retarded mobility on SDS-PAGE has been observed for many calcium-binding proteins. High-affinity calcium-binding proteins typically yield strong signals in the $^{45}Ca^{2+}$ overlay assay (Maruyama *et al.*, 1984) and many exhibit altered mobility in SDS-PAGE if calcium chloride is present in the sample buffer. In contrast, calcium binding by GPP130 in the overlay assay was barely detectable, and calcium chloride did not alter the mobility of GPP130 on SDS-PAGE. However, GPP130 and many other proteins that exhibit retarded mobility on SDS-PAGE share the property of being acidic. Acidic residues may interfere with SDS binding and thus reduce mobility. GPP130 has a calculated isoelectric point of 4.7. Strikingly, the GPP130 sequence is 19% glutamic acid. GPP130 contains a higher percentage of acidic residues than 99% of the proteins in the database (Brendel *et al.*, 1992). The distribution of glutamic acid (Figure 6C) and aspartic acid (Figure 6D) demonstrates that the C-terminal region of GPP130 contains a particularly large number of acidic residues.

The GPP130 sequence contains a single predicted transmembrane domain of 20 amino acids (residues 13–32) near the N terminus (underlined in Figure 5). The output of the TMPred program (Hofmann and Stoffel, 1993) is shown in Figure 6A. Several other algorithms yielded a similar prediction. The large C-

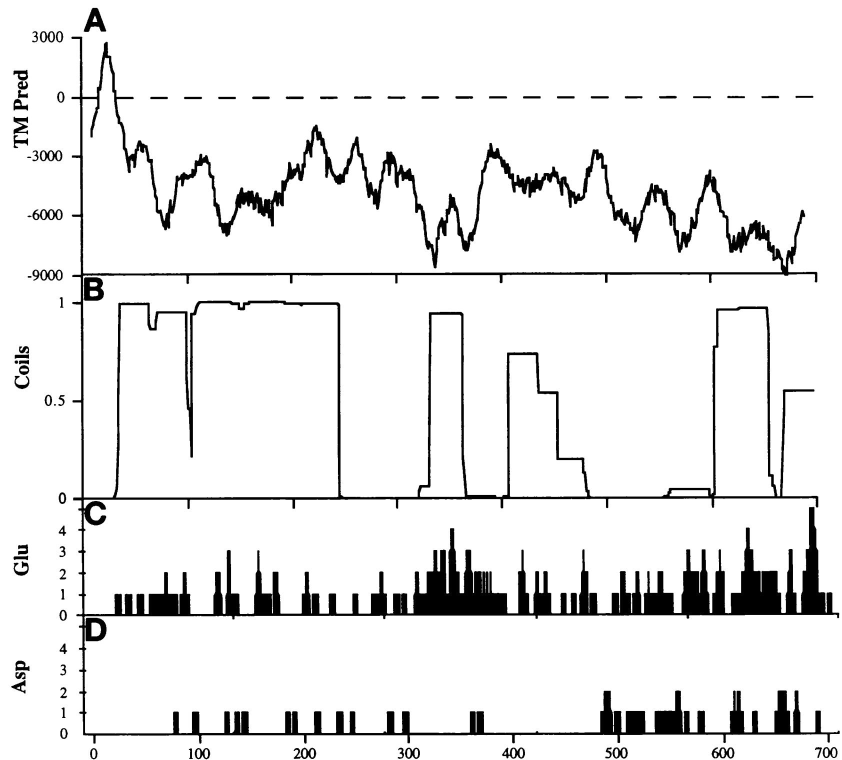

Figure 6. GPP130 sequence features. (A) The GPP130 amino acid sequence was analyzed by the program TMPred using the default parameters. A plot of the output against the GPP130 amino acid sequence is shown. Scores above the dashed line indicate possible transmembrane domains. (B) The output from the program COILS using a window size of 28 and default parameters. Predicted domains of coiled-coil structure are indicated by the positive scores throughout GPP130. (C) The GPP130 amino acid sequence was analyzed for the number of glutamic acid residues in a window size of 5. The result is plotted against the GPP130 amino acid sequence. (D) The distribution of aspartic acids is also plotted.

terminal domain is hydrophilic and, based on motif searches (Appel *et al.*, 1994; Hansen *et al.*, 1995; Hobohm and Sander, 1995), contains the single potential sites for N-glycosylation (N at 257) and O-glycosylation (T at 259). The C-terminal domain also contains the binding site for monoclonal antibody A1/118 as proteins produced in bacteria containing GPP130 amino acids between residues 278 and 433 reacted with A1/118, whereas those lacking this region did not. Because this antibody binds the lumenal domain of GPP130 and because GPP130 was found to be glycosylated and was protected from protease digestion by the Golgi membrane, we conclude that GPP130 is a type II integral membrane protein with a 12-residue cytoplasmic tail and a 665-residue lumenal domain. A serine at position 7 matches a protein kinase C consensus site and represents the sole potential cytoplasmic phosphorylation site. The lumenal domain contains multiple elements matching phosphorylation consensus sites, including 13 casein kinase II sites. Of unknown relevance is a match between the cytoplasmic N-terminal tail of GPP130 (amino acids 2–7) and the consensus sequence for myristylation (Hobohm and Sander, 1995).

Comparison searches with sequences in the data banks did not reveal obvious GPP130 homologues. However, a long alignment was found between GPP130 and trichohyalin, a cytoplasmic calcium-binding protein predicted to form an elongated flexible rod that participates in keratin intermediate filament assembly (Lee *et al.*, 1993). Human trichohyalin is 28% identical and 50% similar in a stretch encompassing 95% of GPP130. The region of trichohyalin that aligns with GPP130 is largely composed of a variable length peptide repeat rich in glutamic acid and glutamine. Glutamic acid (19%) and glutamine (13%) are the most abundant amino acids in GPP130, and GPP130 does not contain a substantial repeating element. Nevertheless, the alignment between GPP130 and trichohyalin may reflect a structural similarity, because long alignments above 25% identity were also found between GPP130 and other filamentous proteins, including neurofilament subunit NF-180, involucrin, and myosin heavy chain. Interestingly, analysis of the GPP130 sequence with the COILS program (Lupas *et al.*, 1991) indicated that GPP130 contains large noncontiguous regions predicted to participate in coiled-coil dimer formation (Figure 6B). A putative leucine zipper is also present (amino acids 114–135). Thus, the analysis indicates that the GPP130 protein sequence has not been previously identified in any organism and that GPP130 may form a flexible dimeric rod-shaped structure in the Golgi lumen.

High-Level Overexpression of GPP130 Causes Its Mislocalization to Endocytotic Vesicles and Apparent Vesiculation of the Golgi

Transient transfection of COS-7 resulted in the production of a protein that comigrated during SDS-

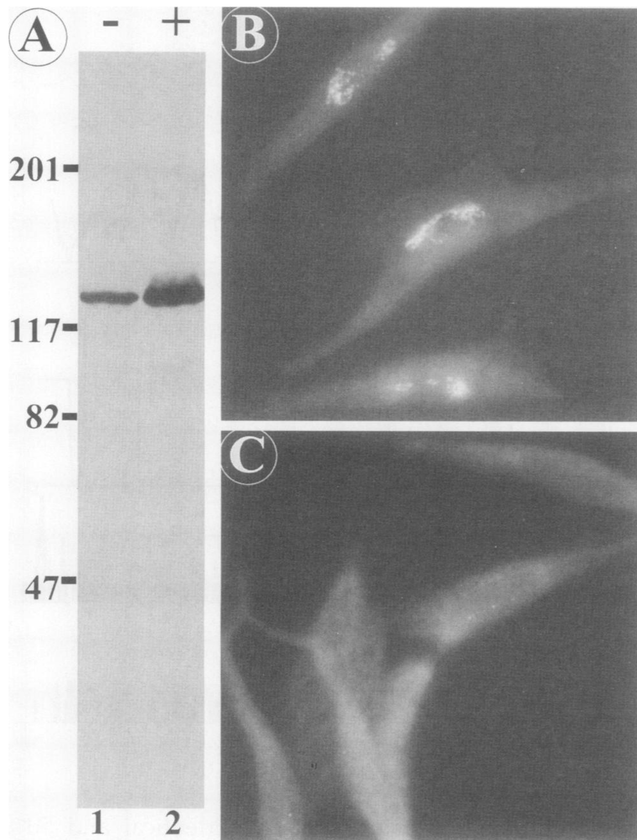

Figure 7. Detection of GPP130 expression in transfected cells. (A) GPP130 levels were determined by immunoblotting cell extracts from COS-7 cells transfected with a control DNA (−) or GPP130 cDNA (+) in the pECE expression vector. The transfected protein was the same size as endogenous GPP130 but more abundant. (B) Anti-GPP130 (A1/118) staining pattern of CHO cells 2 d after transfection with the pECE-GPP130 expression vector. (C) Anti-GPP130 staining pattern of nontransfected CHO cells.

PAGE with endogenous GPP130 (Figure 7A). Similar results were obtained with transfected HeLa cells (see below). Indirect immunofluorescence microscopy of transfected cells was used to confirm the Golgi localization of the transfected protein. We first analyzed CHO cells because the A1/118 antibody does not react with these cells (Figure 7C). Most transfected cells exhibited a characteristic Golgi pattern typified by a perinuclear ribbon-like network (Figure 7B). Therefore, transfection of CHO cells with the cloned GPP130 cDNA primarily resulted in proper Golgi targeting of the expressed protein.

However, a few percent of the transfected CHO cells exhibited strong staining and a distinct and unexpected pattern, typified by a marked accumulation of punctate staining in a perinuclear position and distributed punctate staining in the cell periphery. To further investigate this pattern, we examined transfected COS-7 cells, which produce high levels of expressed protein because of their capacity to replicate the expression vector. Perinuclear and peripheral GPP130-positive vesicles were prominent in transfected COS-7 cells 2–3 d after transfection (Figure 8, A, C, and E). This GPP130 overexpression pattern was not restricted to transiently transfected CHO or COS cells because a stably transfected HeLa cell line expressing GPP130 under the control of an inducible promoter (Gossen and Bujard, 1992) also showed a punctate GPP130 pattern after induction. Double-label immunofluorescence microscopy of COS-7 cells was used to better understand the abundant overexpression pattern. The overexpressed GPP130 only partially overlapped with the giantin pattern in the same cells (Figure 8, A and B). Many GPP130-positive punctate structures, particularly those in the cell periphery, were devoid of giantin immunoreactivity but did partially colocalize with endosomal vesicles marked by mannose-6-phosphate receptor staining (Figure 8, C and D) or by uptake of fluorescent dextran (Figure 8, E and F). To confirm that transfected cells accumulated GPP130 in endocytotic vesicles, we allowed the cells to endocytose A1/118 or control antibodies. After 3 h of antibody uptake, prominent A1/118 accumulation in peripheral vesicles was found in transfected cells (Figure 8G) but not in nontransfected cells (Figure 8H). The control anti-giantin antibody was not detectably endocytosed by either transfected or nontransfected cells. On the basis of these observations, we conclude that abundant overexpression of GPP130 led to partial mislocalization of GPP130 to endocytotic vesicles. Because previous reports have indicated that overexpression of type II Golgi proteins resulted in mislocalization to the ER rather than to post-Golgi compartments, we performed control transfections with human galactosyltransferase. In parallel transfections of COS-7 with either GPP130 or galactosyltransferase cloned in the same expression vector, we observed dramatically different patterns in cells expressing high levels of the transfected proteins. As previously reported, abundant overexpression of galactosyltransferase yielded prominent ER staining, whereas the punctate GPP130 pattern was completely distinct from an ER network pattern.

Figure 8 (on facing page). Double-label immunofluorescence of COS-7 cells overexpressing GPP130. The GPP130 pattern is shown in A, C, and E. Note the accumulation of punctate perinuclear staining and the peripheral punctate staining. Patterns of giantin (B), mannose-6-phosphate receptor (D), and FITC-dextran (F) after a 60-min uptake are shown for comparison. The peripheral GPP130-positive vesicles were devoid of giantin staining but many overlapped with mannose-6-phosphate receptor– and FITC-dextran–stained structures. Representative double-stained structures are indicated by arrowheads. The typical staining pattern after anti-GPP130 antibody uptake by transfected (G) or nontransfected (H) cells is shown. Bar, 30 μm.

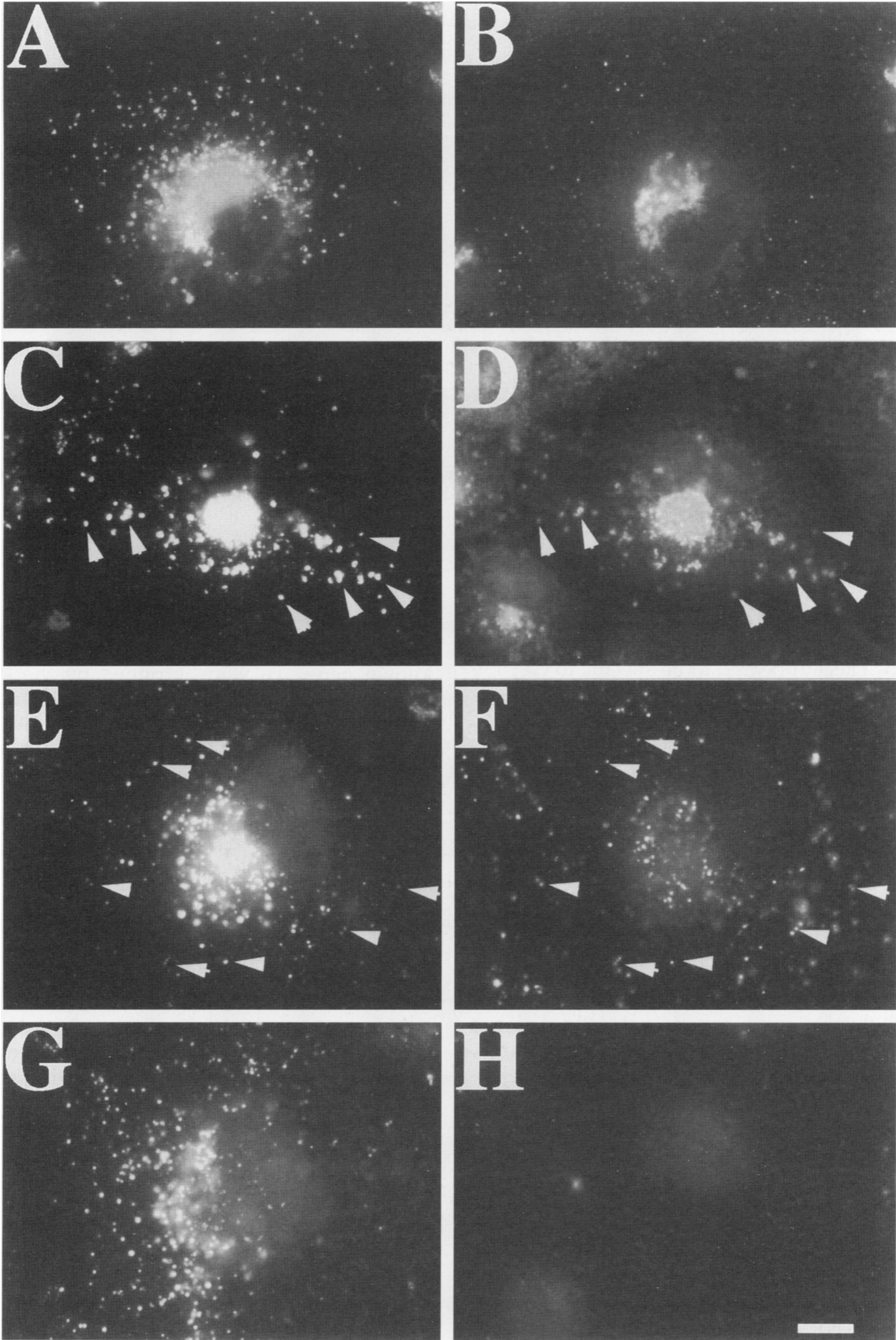

Figure 8.

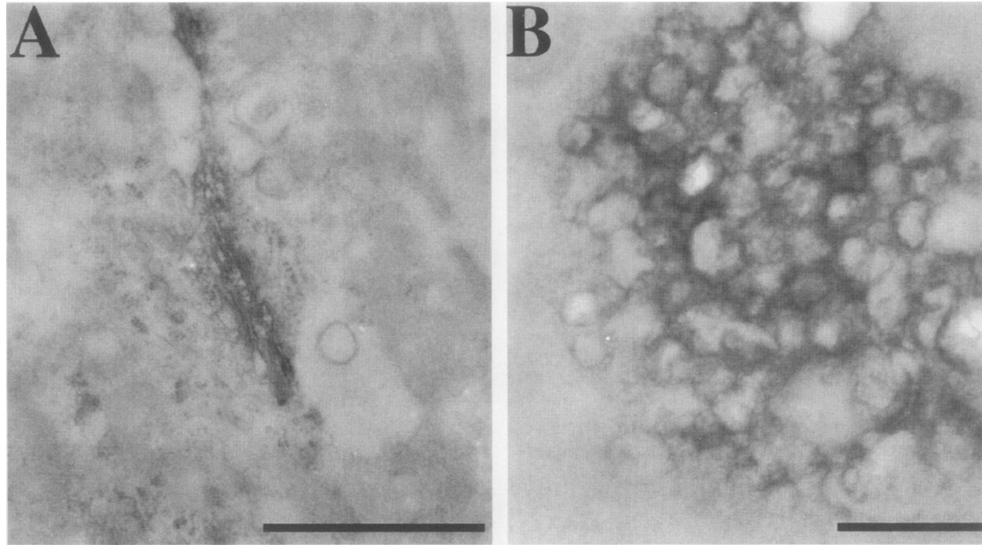

Figure 9. Immunoperoxidase staining of GPP130 in the perinuclear region of COS-7 cells overexpressing GPP130. The typical GPP130 staining pattern in nontransfected cells (A) and GPP130 transfected cells (B). Note that overexpression of GPP130 led to a dramatic accumulation of stained vesicles in the perinuclear region. Bars, 1 μm.

Interestingly, overexpression of GPP130 appeared to convert the giantin pattern from the typical perinuclear ribbon into a perinuclear punctate pattern (Figure 8B). This effect appeared to be specific to GPP130 overexpression as cells overexpressing galactosyltransferase did not exhibit an altered giantin pattern. A reduction in giantin staining intensity was also observed in GPP130 overexpressors, but giantin abundance, as determined by immunoblotting, was not significantly reduced. This suggests that giantin was diluted in the membrane of overexpressors. Electron microscopy was used to examine the basis of the staining change in GPP130-overexpressing cells. Cells overexpressing GPP130 (Figure 9B) were readily distinguished from nontransfected cells (Figure 9A) by a dramatic accumulation of clusters of GPP130-positive vesicles near their nuclei. In contrast to the control cells, GPP130 staining in transfected cells was not associated with flattened or stacked membranes. GPP130-positive vesicles were also observed at peripheral sites, although these vesicles were not usually clustered. To test whether the accumulation of vesicles in the perinuclear region of transfected cells was due to a GPP130-induced transport block, we measured the appearance of endogenous secreted proteins in the medium. Even though immunofluorescence analysis demonstrated that about 50% of the GPP130-transfected cells had accumulated the vesicles characteristic of abundant GPP130 overexpression, no transport defect was observed for any secreted protein (our unpublished results).

GPP130 Is Apparently Identical to GIMPc, a Sialylated cis/Medial Golgi Antigen

Although the GPP130 cDNA did not correspond to a known gene, we were intrigued by the similarities between GPP130 and GIMPc, a 130-kDa cis-Golgi-localized phosphoprotein (Yuan *et al.*, 1987). To test whether GPP130 is related to GIMPc, we obtained a rabbit antibody raised against GIMPc purified from rat (kindly contributed by Dr. I. Sandoval, Universidad Autónoma de Madrid, Madrid, Spain). This antibody and the A1/118 anti-GPP130 antibody were used in immunoblot analyses to examine extracts from control and GPP130-transfected HeLa cells, as well as extracts from control bacteria and bacteria producing a glutathione *S*-transferase (GST)-GPP130 fusion protein (Figure 10). Both cell types strongly overproduced their respective GPP130 constructs (lanes 2 and 6), and these proteins were specifically recognized by the anti-GIMPc antibody (lanes 4 and 8). Significant degradation was also apparent, particularly in the bacterial extracts, and many of these degraded forms were detected by both antibodies. Longer exposures indicated that the anti-GIMPc antibody also detected a band in nontransfected HeLa cells that comigrated with endogenous or overexpressed GPP130. The purified GST-GPP130 fusion protein was also strongly reactive with both antibodies. On the basis of their antibody cross-reactivity, their similar biochemical characteristics, and their similar cellular localization, we conclude that GIMPc and GPP130 are homologous proteins.

DISCUSSION

GPP130/GIMPc is a phosphoprotein specifically localized to the Golgi apparatus, where it appears to be enriched in the cis and/or medial subcompartment. Like most previously identified Golgi-localized integral membrane proteins, GPP130/GIMPc has a short N-terminal cytoplasmic tail, a single membrane spanning segment, and large C-terminal lumenal domain. This topology was elucidated by protease protection experiments, topology prediction computer programs, and the location of glycosylation and antibody binding sites. Although the previously identified type II Golgi proteins are predominantly oligosaccharide-modifying enzymes (Paulson and Colley, 1989; Shaper and Shaper, 1992), we did not find any significant alignment between the GPP130/GIMPc sequence and known glycosylation enzymes. Furthermore, the lumenal domain of GPP130/GIMPc is rich in acidic amino acids and contains a large number of heptad repeats predicted to participate in coiled-coil dimer formation, suggesting that GPP130/GIMPc may have a novel structural or organizational role in the Golgi lumen (see below).

The identity of cloned human GPP130 and the previously reported rat protein GIMPc (Yuan *et al.*, 1987) was strongly suggested by several observations: 1) An anti-GIMPc antibody reacts with human GPP130 that had been overproduced in HeLa cells or expressed in bacteria as a fusion protein, and this antibody recognizes a protein that exactly comigrates with endogenous HeLa GPP130. 2) GPP130 and GIMPc are both phosphoproteins localized to cis/medial Golgi cisternae. 3) Both GPP130 and GIMPc are integral membrane proteins that are protected from protease digestion by the Golgi membrane and that exhibit only a small shift in SDS-PAGE mobility as the result of carbohydrate processing. On the basis of the presence of GPP130/GIMPc in rodent and primate cells, it seems likely that GPP130/GIMPc resides in the Golgi of most or all mammalian species.

The phosphorylation of GPP130/GIMPc is on serine and is likely to be lumenal because it resists digestion in a protease protection assay (Yuan *et al.*, 1987). However, GIMPc phosphorylation was stimulated by dibutyryl cAMP treatment (Yuan *et al.*, 1987), and the cytosolic face of Golgi membranes contains a high-affinity binding site for the regulatory subunit of cyclic AMP-dependent kinase (Rios *et al.*, 1992). Because the short cytoplasmic tail of GPP130/GIMPc contains a serine and could be protease-resistant, we are currently testing whether this serine is required for GPP130/GIMPc phosphorylation. On the other hand, a number of proteins are phosphorylated in lumenal compartments, and GPP130/GIMPc has several potential casein kinase II sites in its lumenal domain. One of these matches a consensus sequence (SXE) recognized by a Golgi-localized class of casein kinase (Lasa-Benito *et al.*, 1996). The identity and location of the kinase that phosphorylates GPP130/GIMPc remains to be determined.

Even though it resides in the early Golgi and has a 20-h half-life, GPP130/GIMPc is quantitatively modified by sialyltransferase, which is located in the trans-Golgi/trans-Golgi network (Yuan *et al.*, 1987). How does GPP130/GIMPc become sialylated? One possibility is that GPP130/GIMPc is modified by newly synthesized sialyltransferase that is transiting through the early Golgi. This explanation would be consistent with retention models for Golgi targeting, because GPP130/GIMPc would not have to leave the early Golgi to acquire late Golgi modifications. An alternative possibility is suggested by our finding that elevating the pH of lumenal compartments with chloroquine caused a redistribution of GPP130/GIMPc to peripheral dilated vesicles, which appear to be endosomes. These data indicate that retention of GPP130/GIMPc in the early Golgi is weak or nonexistent. Thus, GPP130/GIMPc may continually leak out of the early Golgi into the late Golgi where it encounters sialyltransferase. The transmembrane domain of GPP130/GIMPc is 20 residues, 5 residues longer than the average for Golgi-localized type II proteins (Bretscher and Munro, 1993); this difference might allow GPP130/GIMPc to escape retention in the early Golgi. If GPP130/GIMPc reaches the late Golgi, a retrieval mechanism must operate to return it to the early Golgi (Johnston *et al.*, 1994; Hoe *et al.*, 1995; Harris and Waters, 1996). This retrieval mechanism appears to be saturable, because we found that overexpression of

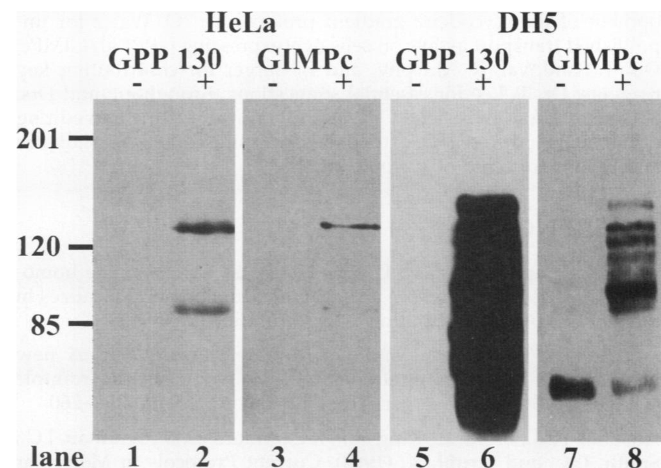

Figure 10. Anti-GPP130 and anti-GIMPc blot of cells expressing GPP130. Extracts from induced (+) and noninduced (−) transiently transfected TetON HeLa cells or extracts from DH5 bacteria transformed with a GST-GPP130 expression vector (+) or a control vector (−) were subjected to immunoblotting with either A1/118 or a anti-rat GIMPc polyclonal antibody.

GPP130/GIMPc led to its mislocalization. In contrast, previously studied type II Golgi proteins are localized by a nonsaturable chloroquine-insensitive mechanism. Interestingly, mislocalized GPP130/GIMPc accumulated in endosomes rather than at the plasma membrane, suggesting that this protein contains targeting information that functions in the TGN/endosomal system.

A great deal of descriptive information is available regarding the structure of the Golgi apparatus (Rambourg and Clermont, 1990), but the proteins that establish and regulate Golgi structure have not been identified. The lumenal domain of GPP130/GIMPc contains features that point to a structural rather than enzymatic role. Our analysis revealed that GPP130/GIMPc overexpression led to vesiculation and/or dilation of the Golgi. The mechanistic basis for this effect is unclear. GPP130/GIMPc overexpression was not accompanied by substantial defects in secretion. GPP130/GIMPc appears to be structurally similar to cytoskeletal proteins, possibly indicating a related function for GPP130/GIMPc in the Golgi lumen. Alternatively, the high concentration of acidic residues in the GPP130/GIMPc lumenal domain may mean that this protein functions in maintaining the ionic balance within the Golgi, perhaps by providing low-affinity high-capacity calcium binding (Antebi and Fink, 1992; Scherer *et al.*, 1996). Further work should uncover the function of GPP130/GIMPc and shed light on the intriguing question of how proteins are localized to the Golgi.

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