

# Characterization of a *cis*-Golgi Matrix Protein, GM130

Nobuhiro Nakamura, Catherine Rabouille, Rose Watson, Tommy Nilsson, Norman Hui, Paul Slusarewicz, Thomas E. Kreis,\* and Graham Warren

Cell Biology Laboratory, Imperial Cancer Research Fund, London WC2A, 3PX, UK.; and \*Département de Biologie Cellulaire, Université de Genève, Sciences III, CH-1211 Genève 4, Switzerland

**Abstract.** Antisera raised to a detergent- and salt-resistant matrix fraction from rat liver Golgi stacks were used to screen an expression library from rat liver cDNA. A full-length clone was obtained encoding a protein of 130 kD (termed GM130), the COOH-terminal domain of which was highly homologous to a Golgi human auto-antigen, golgin-95 (Fritzler et al., 1993). Biochemical data showed that GM130 is a peripheral cytoplasmic protein that is tightly bound to Golgi membranes and part of a larger oligomeric complex. Predictions from the protein sequence suggest that GM130 is an extended rod-like protein with coiled-coil domains. Immunofluorescence microscopy showed partial over-

lap with *medial*- and *trans*-Golgi markers but almost complete overlap with the *cis*-Golgi network (CGN) marker, syntaxin5. Immunoelectron microscopy confirmed this location showing that most of the GM130 was located in the CGN and in one or two cisternae on the *cis*-side of the Golgi stack. GM130 was not re-distributed to the ER in the presence of brefeldin A but maintained its overlap with syntaxin5 and a partial overlap with the ER-Golgi intermediate compartment marker, p53. Together these results suggest that GM130 is part of a *cis*-Golgi matrix and has a role in maintaining *cis*-Golgi structure.

THE Golgi apparatus receives the entire output of newly-synthesized proteins from the ER. They enter at the *cis* or entry face, in a compartment termed the *cis*-Golgi network (CGN)<sup>1</sup> which can be thought of as the last quality control step on the pathway (Huttner and Tooze, 1989; Pelham, 1991). Only properly folded proteins are allowed to proceed on to the stacked cisternae (Hurtley and Helenius, 1989) where posttranslational modifications are carried out, most notably to the bound oligosaccharides (Roth, 1987). When they reach the TGN, the proteins are sorted to their correct destination (Griffiths and Simons, 1986).

Considerable progress has been made in identifying the proteins involved in the vesicle machinery responsible for conveying cargo from one compartment to the next (Pryer

et al., 1992; Rothman, 1994). The same is also true for those enzymes that carry out the posttranslational modifications. Many have now been sequenced and the signals that determine their precise location within the Golgi apparatus are being investigated (see Nilsson and Warren, 1994).

Much less progress has been made in identifying structural proteins responsible for the unique architecture of the Golgi apparatus. Morphological studies have shown that there are structures in the space between the cisternae that might be involved in cisternal stacking (Franke et al., 1972; Mollenhauer, 1965; Mollenhauer et al., 1973; Cluett and Brown, 1992). Each Golgi apparatus is also embedded in a "zone of exclusion" containing many fibrillar structures (Mollenhauer and Morré, 1978). It is not, however, clear to what extent these structures can be matched to the increasing number of cytoplasmic proteins that are now known to be associated with the Golgi apparatus.

These proteins are of several types. There are those that interact with the classical cytoskeleton or are homologues of it. Comitín, an actin-binding protein, has been localized to the periphery of the Golgi apparatus and may be involved in tethering the organelle to the actin cytoskeleton (Weiner et al., 1993). A homologue of the erythrocyte  $\beta$ -spectrin has been localized to the Golgi apparatus (Beck et al., 1994b) as well as a homologue of ankyrin (Beck et al., 1994a). Given the role of the parental proteins in maintaining the structure of the erythrocyte, these homologues might be involved in maintaining the structural integrity of the Golgi apparatus.

Address all correspondence to G. Warren, Cell Biology Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A, 3PX, UK. (Ph.): (44) 171 269 3561. Fax: (44) 171 269 3417. e.mail: warren@europa.lif.icnet.uk.

T. Nilsson's present address is European Molecular Biology Laboratory, 69012 Heidelberg, Germany.

P. Slusarewicz's present address is Department of Biochemistry, Dartmouth Medical School, 7200 Vail Building, Hanover, NH 03755-3844.

1. *Abbreviations used in this paper:* BFA, Brefeldin A; CGN, *cis*-Golgi network; immuno-EM, immunoelectron microscopy; Man II,  $\alpha$ 1,3-1,6-mannosidase II; NAGT I,  $\beta$ -1,2-*N*-acetylglucosaminyltransferase I; NRK cells, normal rat kidney cells; RACE, Rapid Amplification of cDNA End; SialylT,  $\alpha$ 2,6-sialyltransferase; TGN, *trans*-Golgi network; VSV-G, vesicular stomatitis virus glycoprotein.

The second type of protein belongs to a group of human autoantigens. Most are autoantigens found in Sjögren's syndrome, and comprise a 230-kD protein found on the *trans* side of the Golgi apparatus (Kooy et al., 1992), a 210-kD protein found on the *cis* side (Rios et al., 1994) and two other proteins (golgin-95 and golgin-160) which are associated with the Golgi apparatus though the precise location is still unclear (Fritzler et al., 1993). The sequences of both golgin-95 and golgin-160 have been determined and reveal a high content of heptad repeats suggesting a rodlike structure (Fritzler et al., 1993).

Giantin/GCP372 is also an autoantigen found in rheumatoid arthritis. It has a high content of heptad repeats and a molecular weight in excess of 350 kD (Linstedt and Hauri, 1993; Seelig et al., 1994; Sohda et al., 1994). Unlike the other autoantigens found so far it is a membrane protein that is anchored by a COOH-terminal spanning domain. It has been suggested that this protein might be involved in cisternal stacking, but immunoelectron microscopy (immuno-EM) indicates that most of the protein surrounds the Golgi stacks (Seelig et al., 1994). It is, therefore, more likely to act as a tethering or positioning device.

Our approach to identifying structural proteins of the Golgi has been to prepare a detergent- and salt-resistant matrix from highly purified rat liver Golgi stacks (Slusarewicz et al., 1994b). One property of this matrix is the capacity to bind *medial-trans*-Golgi enzymes with high affinity. Since these enzymes are present throughout individual Golgi cisternae and the binding is sensitive to prior treatment with proteases, this suggests that at least part of this matrix is present within the intercisternal space.

Here we have used an antiserum to this matrix to screen an expression library and describe the properties of a 130-kD protein (termed GM130 for Golgi matrix protein of 130 kD) which is related to golgin-95 and may be a new family member.

## Materials and Methods

### Materials

All reagents were of analytical grade or higher and purchased from either Sigma Chemical Co. (St. Louis, MO) or BDH Ltd. (Poole, UK) unless otherwise stated.

### Isolation of the Golgi Matrix Fraction

The Golgi matrix fraction was prepared from purified rat liver Golgi membranes as described previously (Slusarewicz et al., 1994a,b). Briefly, purified rat liver Golgi membranes were suspended in Triton buffer (2% [wt/vol] Triton X-100, 50 mM MOPS-NaOH, pH 7.0, 0.1 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 10% [wt/vol] sucrose) to 1 mg/ml protein, incubated on ice for 30 min, and then centrifuged at 15,000 *g* for 30 min at 4°C. The Triton pellet was isolated, suspended in the same volume of Triton buffer containing 150 mM NaCl, incubated on ice for 30 min, and then again centrifuged at 15,000 *g* for 30 min at 4°C to yield the Golgi matrix fraction.

### Antisera

Antisera to the Golgi matrix fraction were raised in rabbits by immunizing and boosting with 80–160 µg protein in Freund's adjuvant as described previously (Kreis, 1986). 25 ml of blood was collected from immunized animals 12–14 d after boosting and repeated up to six times at weekly intervals.

Rabbit antisera to recombinant GM130 were raised against two overlapping regions of the protein. The first was a 17-kD fragment (amino acids 322 to 471; nucleotides 1200 to 1651) fused with β-galactosidase in the pUEX plasmid. Inclusion bodies were isolated and used for immuniza-

tion. The second was a 97-kD fragment (amino acids 194 to 912; nucleotides 817 to 2977) expressed using the QIA express system (QIAGEN Inc., Chatsworth, CA) following the manufacturer's protocol. The NH<sub>2</sub>-terminal His<sub>6</sub> tag was used to purify the recombinant protein which was then used for immunization. Affinity purification of the antibody was performed using the same recombinant proteins after coupling to Thiopropyl Sepharose 6B (Pharmacia Biotech, Uppsala, Sweden) following the manufacturer's protocol as described by Harlow and Lane (1988).

### cDNA Cloning

Anti-Golgi matrix serum was used to screen a rat liver cDNA library that was constructed in the expression plasmid, pUEX1 (Luzio et al., 1990). Expression of clones, antibody screening and purification of the fusion proteins was performed as previously described (Brake et al., 1990; Stanley and Luzio, 1984). A 452-bp cDNA fragment (17-kD fragment, nucleotide 1200 to 1651; Fig. 3 A) and a 3,464-bp cDNA fragment (the 3.5-kbp fragment or 97-kD fragment; Figs. 2 A and 3 A) were obtained by screening. Poly A<sup>+</sup> RNA was isolated from rat (Sprague-Dawley) liver and used for 5'-rapid amplification of cDNA end (RACE) using the 5'-AMPLIFINDER RACE Kit (CLONTECH, Palo Alto, CA) following the manufacturer's protocol. Gene specific primers used were 5'-GGAGCT-GTTTCAGTGACTCCATCAGCTGG-3' (Fig. 3 A, #1) for first strand synthesis and 5'-CCATGGCCTGTGCAACTGCTCATTACTGC-3' (Fig. 3 A, #2) for polymerase chain reaction.

### In Vitro Transcription/translation

A full-length plasmid was constructed by combining the EcoRI-BsrGI fragment from the 5'-RACE product and the BsrGI-BamHI fragment from the 3.5-kbp cDNA fragment and cloning into Bluescript II KS+ (Stratagene, La Jolla, CA). Coupled in vitro transcription and translation was performed using the TNT T3 coupled Reticulocyte Lysate System (Promega Corp., Madison, WI), a sample was solubilized in SDS-PAGE sample buffer and analysed by Western blotting using antiserum against the 17-kD fragment of GM130.

### DNA Sequence Analysis

The cDNA sequence was determined in both directions by appropriately synthesized oligonucleotide primers. The hydrophathy plot was performed according to the method of Kyte and Doolittle (1982). Coiled-coil prediction was performed according to the method of Lupas et al. (1991).

### Western Blotting

This was performed as described previously (Misteli and Warren, 1994). Cells were lysed using SDS-PAGE sample buffer without dye and dithiothreitol. Other proteins were precipitated before fractionation by SDS-PAGE using a 6% or 6–15% gradient gel. After transfer to a nitrocellulose membrane (Hybond-C super; Amersham International plc, UK) the blots were probed with antibodies to the Golgi matrix, GM130 (17- and 97-kD fragment) and α1,3,6-mannosidase II (Man II) (Moremen and Touster, 1985) at a dilution of 1:1,000. Goat anti-rabbit antibody conjugated with HRP (Biosource International, Camarillo, CA) was used as a secondary antibody at 1:1,000 dilution and this was visualized using the ECL system (Amersham). Protein was assayed using the BCA protein assay reagent (Pierce, Rockford, IL).

### Quantitation of GM130

Samples were fractionated by SDS-PAGE in parallel with serially diluted, total Golgi membrane lysates to provide an internal standard curve. The x-ray film was scanned by an Imagerstore 5000 system (Ultra Violet Products Ltd., Cambridge, UK) and the intensity of each band was extracted using the NIH image program, version 1.56 (Wayne Rasband, NIH, Bethesda, MD).

### Extraction of Golgi Membranes

All extraction buffers contained protease inhibitors (1 µg/ml aprotinin, 1 µg/ml leupeptin, 1 µg/ml pepstatin, 1 µg/ml antipain, 1 µg/ml chymostatin, 1 mM benzamidin, and 1 mM PMSF). Purified Golgi membranes (100 µg) were suspended in 100 µl of phosphate buffer (100 mM potassium phosphate buffer, pH 6.7, 5 mM MgCl<sub>2</sub>, and 0.25 M sucrose), 1 M KCl in phosphate buffer, 0.2 M sodium carbonate, or 6 M urea in phosphate

buffer. After incubation on ice for 30 min, the samples were centrifuged at 4°C, 100,000 g for 10 min to pellet the membranes. Triton X-114 extraction was performed as described by Bordier (1991) with slight modifications. Briefly, purified Golgi membranes (100 µg) were suspended in 100 µl of buffer containing 1% (wt/vol) Triton X-114, 100 mM potassium phosphate buffer, pH 6.7 and 5 mM MgCl<sub>2</sub>, incubated on ice for 10 min, and centrifuged at 4°C, 1,700 g for 5 min to remove insoluble aggregates. The supernatant was recovered and condensed at 37°C for 3 min and centrifuged at 1,700 g for 5 min to separate the aqueous and detergent phases. Protein was precipitated by the method of Wessel and Flugge (1984), and analyzed by Western blotting after SDS-PAGE.

### Chymotrypsin Treatment of Golgi Membranes

Purified Golgi membranes (100 µg) were suspended in 90 µl of phosphate buffer (100 mM potassium phosphate buffer, pH 6.7, 5 mM MgCl<sub>2</sub>, and 0.25 M sucrose), 10 µl of chymotrypsin (0, 2.5, 5, 10 mg/ml) was added, and incubated on ice for 30 min. Then, 1 µl of 0.2 M PMSF was added to neutralize the chymotrypsin and membranes were pelleted at 100,000 g for 10 min at 4°C. Protein was precipitated and analyzed by Western blotting after SDS-PAGE as described above.

### Man II Assay

Man II was assayed spectrophotometrically as described by Bischoff and Kornfeld (1984) except that the concentration of the substrate, *p*-nitrophenyl- $\alpha$ -D-mannopyranoside, was 2 mM in 100 mM sodium phosphate, pH 6.0. The assay was carried out at 37°C for 1 h.

### Gel Filtration

The Triton pellet, isolated from 10 mg of purified Golgi membranes as described above, was incubated with 500 µl of Triton buffer containing 500 mM NaCl for 30 min on ice, then centrifuged at 15,000 g for 30 min at 4°C. The supernatant was applied onto a Superose 6 column (1-cm diam, 30-cm height; Pharmacia Biotech) equilibrated with Triton buffer containing 500 mM NaCl and 0.1% (wt/vol) Triton X-100, and 1-ml fractions were collected. Protein was precipitated and analyzed by Western blotting after SDS-PAGE as described above.

### Cell Culture and BFA Treatment

HeLa and NRK cells were grown in Dulbecco's modified eagle medium (Life Technologies Inc., Gaithersburg, MD) supplemented with 10% FCS (Life Technologies Inc.) whereas HeLa cells expressing myc-tagged  $\beta$ -1,2-*N*-acetylglucosaminyltransferase I (NAGT I) (Nilsson et al., 1993) and vesicular stomatitis virus glycoprotein (VSV-G) tagged  $\alpha$ 2,6-sialyltransferase (SialylT) (Rabouille et al., 1995) were grown in Dulbecco's modified eagle medium supplemented with 10% FCS and 200 µg/ml geneticin (Life Technologies Inc.).

The cDNA encoding rat syntaxin 5 (GenBank/EMBL/BBJ accession No. L20822) with a myc epitope engineered into the NH<sub>2</sub> terminus (Banfield et al., 1994) was cloned into the pSR $\alpha$  vector and stable lines generated and maintained in HeLa cells as described (Nilsson et al., 1993; Rabouille et al., 1995).

Cells were plated on coverslip for 24 h before treatment with 200 µg/ml BFA for 30 min (Epicentre, Madison, WI).

### Immunofluorescence Microscopy

Cells were fixed with 3% paraformaldehyde and permeabilized with 0.1% Triton X-100 as described previously (Louvard et al., 1982; Nilsson et al., 1994). Affinity purified rabbit antibody for GM130 (17-kD fragment) was used for double labeling the cells together with mouse monoclonal antibodies for the myc epitope (9E10; Evan et al., 1985), the VSV-G epitope (P5D4; Kreis and Lodish, 1986; Soldati and Perriard, 1991), or p53 (Schweizer et al., 1988). Goat anti-rabbit antibodies and goat anti-mouse antibodies conjugated to Texas red or FITC (Biosource International, Camarillo, CA) were used to visualize the primary antibodies. Cells were visualized using a Bio-Rad MRC-1000 Laser scanning confocal Imaging system (Bio-Rad Laboratories, Richmond, CA).

### Immuno-EM

Cells were fixed in a mixture of 2% paraformaldehyde and 0.2% glutaraldehyde in 0.2 M phosphate buffer, pH 7.4, and cut as described previously

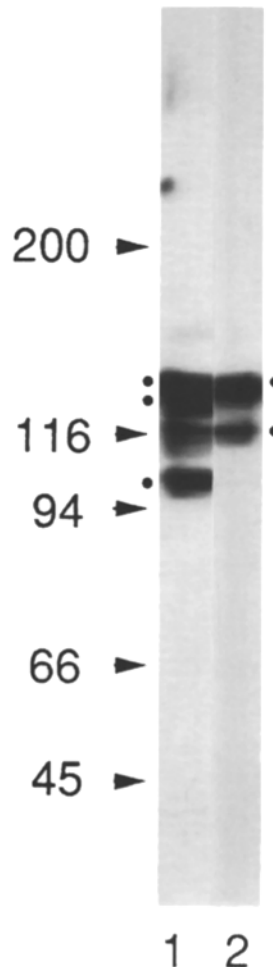
(Rabouille et al., 1995). Sections of NRK cells were incubated with affinity purified polyclonal antibodies to the 97-kD fragment of GM130 followed by goat anti-rabbit antibodies coupled to 10 nm gold particles. Sections of the HeLa cells expressing VSV-G-tagged SialylT (Rabouille et al., 1995) were incubated with a mixture of rabbit anti-97-kD fragment antibodies and a mouse monoclonal antibody (P5D4) to the VSV-G epitope on the SialylT. This was followed by goat anti-rabbit antibodies coupled to 10 nm gold particles and then by goat anti-mouse antibodies coupled to 5 nm gold particles. Samples were examined at 80 kV using a Philips CM10 electron microscope.

The labeling for SialylT was used to determine the polarity and GM130 was assigned to the *cis* or *trans* side according to an imaginary line that bisected each stack of cisternae.

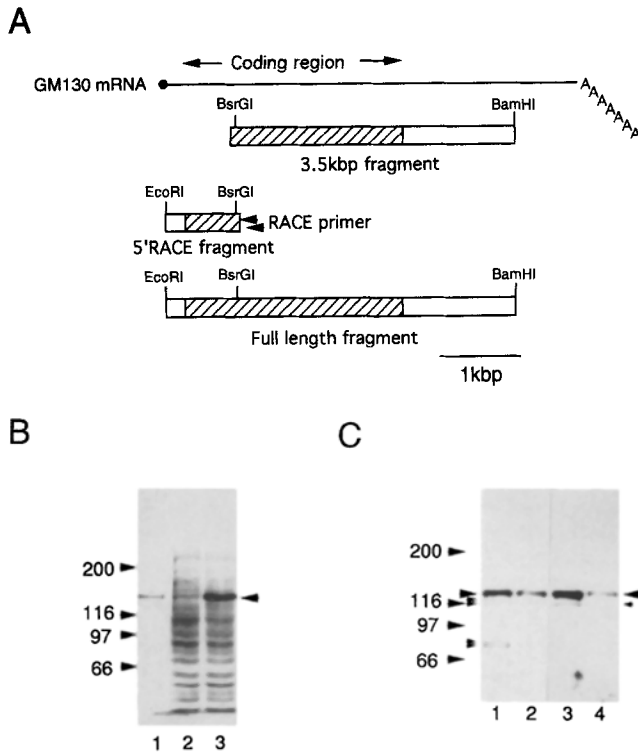
## Results

### Cloning of GM130

Rat liver Golgi membranes were purified 150-fold over homogenate (using  $\beta$ 1,4-galactosyltransferase as the marker) and then extracted sequentially with 2% Triton X-100 and 150 mM NaCl. The insoluble pellet, the Golgi matrix fraction, was used to raise polyclonal antisera which were screened by immunofluorescence microscopy. One antiserum, which gave a particularly striking Golgi staining pattern was chosen for further analysis by Western blotting after fractionation of Golgi membranes by SDS-PAGE and the results are shown in Fig. 1. This antiserum only detected a few proteins in purified rat liver Golgi membranes,



**Figure 1.** Western blotting using anti-matrix and anti-GM130 antisera. Purified rat liver Golgi membranes (17.4 µg/lane) were fractionated on a 6% SDS-polyacrylamide gel and Western blotted using antibodies either to the Golgi matrix fraction (lane 1) or GM130 (lane 2). The anti-matrix antiserum recognized proteins of 130, 120 and 100 kD (lane 1, dots on the left) whereas the anti-GM130 antiserum recognized a major band at 130kD and a likely proteolytic fragment at 116kD (lane 2, dots on the right). Molecular weight markers are shown on the left.



**Figure 2.** Construction and transcription/translation of the full-length cDNA encoding GM130. (A) The longest clone encoding GM130 was 3.5 kbp in length and comprised an incomplete coding region (2,161 bp, shaded area) followed by 1,303 bp of noncoding 3' sequence. The missing 5' sequence was obtained using the 5'-RACE protocol. Both fragments were linked using the BsrGI site to construct the full length fragment (see Material and Methods). (B) Coupled transcription/translation in the absence (lane 2) or presence (lane 3) of the full-length cDNA encoding GM130. Samples were fractionated by 6% SDS-polyacrylamide gel and Western blotted using antiserum to a 17-kD fragment of GM130. Note that the translated product (lane 3) had exactly the same molecular weight as the GM130 in rat liver Golgi membranes (lane 1). Molecular weight markers are shown on the left. (C) Lysates (100  $\mu$ g) of NRK (lanes 1 and 3) and HeLa (lanes 2 and 4) cells were fractionated by 6% SDS-polyacrylamide gel and Western blotted using affinity-purified polyclonal antibodies to a 17-kD (lanes 1 and 2) or a 97-kD fragment (lanes 3 and 4) of GM130. Both antibodies recognized a 130-kD protein (large arrowheads) and minor lower molecular weight proteins with molecular weight of 120, 116, 83, and 80 kD (small arrowheads). Molecular weight markers are shown on the left.

the most prominent having molecular weights of 130, 120, and 100 kD (Fig. 1, lane 1).

This antiserum was then used to screen a rat liver cDNA library in pUEX1 expression plasmid (Luzio et al., 1990).  $5 \times 10^5$  clones were screened and ten positives were iso-

lated. These were grouped into three classes on the basis of their sequence and a recombinant protein from each class was used to raise polyclonal antisera. Antisera to a 17-kD fragment in one of these classes recognized the uppermost 130-kD protein identified using the original antiserum and a likely proteolytic fragment of 116 kD (Fig. 1, lane 2). This protein, GM130, was chosen for further study.

The longest cDNA clone encoding GM130 was 3.5 kbp in length. This was significantly shorter than the 5-kb mRNA encoding GM130 characterized by Northern blotting (data not shown) showing that sequence at the 5' and/or 3' end was missing. Sequence analysis predicted an open reading frame of 2,161 bp (720 amino acids giving a calculated molecular weight of 82 kD), followed by 1,303 bp of noncoding sequence (Figs. 2 A and 3 A). Expression of this open reading frame in bacteria showed that it only encoded a protein of 97 kD indicating that sequence was missing at the 5' end.

The missing sequence was obtained using the 5'-rapid amplification of cDNA end (RACE) protocol (see Materials and Methods). An open reading frame calculated to be 104 kD (912 amino acids) was obtained when the 5'-RACE fragment was combined with 3.5-kbp fragment (Fig. 2 A, Full length fragment). This was checked by coupled transcription/translation. As shown in Fig. 2 B, the molecular weight of the translated product (lane 3) was identical to that of authentic GM130 (lane 1) showing that this cDNA encoded full length GM130.

Antibody was also raised to a 97-kD recombinant fragment of GM130 and was shown to recognize the same protein as the antibody to the 17-kD fragment (Fig. 2 C, cf. lanes 1 and 3). Both antibodies recognized the same protein in human (lanes 2 and 4) as well as rat cell extracts (lanes 1 and 3). Minor bands seen at 120, 116, 83, and 80 kD were either degradation products or homologues of GM130 (see Discussion).

### The Sequence and Structural Analysis of GM130

The complete sequence of the cDNA and deduced amino acid sequence are shown in Fig. 3 A. A plot of hydrophilicity showed that there was no classical signal sequence nor was there a stretch of hydrophobic and neutral amino acids sufficient in length to span the lipid bilayer (Fig. 3 B). Strikingly, the entire central part of the protein had a high probability of assuming a coiled-coil structure (Fig. 3 C). There were, however, several interruptions suggesting that GM130 is a rod-like protein with several joints. Coiled-coil structures imply oligomerization of the protein, a prediction borne out by biochemical experiments (see below).

The PROSITE Dictionary of Protein Sites and Patterns (Release 12.2) revealed 16 casein kinase II phosphorylation sites, 7 protein kinase C phosphorylation sites, and 2 tyrosine kinase phosphorylation sites. There were also two

**Figure 3.** Sequence and structural analysis of GM130. (A) Nucleotide and deduced amino acid sequence of GM130. Note the two gene specific primers used for 5'-RACE, the 5' and 3' end of the 17-kD fragment, the 5' end of the 97-kD fragment and the BsrGI site used to construct the full-length fragment. These sequence data are available from GenBank/EMBL/DDBJ under accession number U35022. (B) Hydrophilicity plot of GM130. Note the absence of any hydrophobic regions that could function as a signal or a transmembrane sequence. (C) The predicted coiled-coil structure shows that the central part of GM130 is likely to be a coiled-coil. (D) Comparison of the amino acid sequence of GM130 and golgin-95. The COOH-terminal region of GM130 is 76.3% identical and 85.5% similar to golgin-95.



predicted N-glycosylation sites but these would not be used since the protein is cytoplasmic (see below).

A search of the protein sequence Database (GenBank: Release 89.0; EMBL: Release 42.0) revealed homology to a human protein, golgin-95 which was identified as the antigen in an autoimmune disease, Sjögren's syndrome (Fritzer et al., 1993). As shown in Fig. 3 D, the homology was restricted to the COOH-terminal 616 residues of GM130. Sequence identity was 76.3% and the similarity was 85.5%. Golgin-95 lacked the NH<sub>2</sub>-terminal 296 amino acids of GM130. In addition, GM130 has one short insertion and two short deletions when compared to golgin-95.

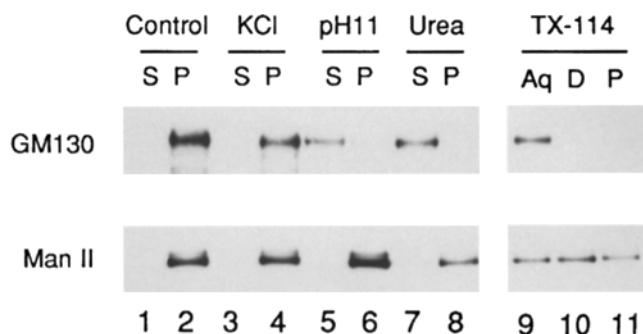
No other proteins were found to be highly homologous to GM130. Many proteins with predicted coiled-coil domains were found to be weakly similar to GM130 (~20% identity, ~50% similarity), but these similarities were marginal and varied depending on the search algorithm used (FASTA, BLAST, and SHARQ: Pearson and Lipman, 1988; Altschul et al., 1990; Collins and Coulson, 1990). The regions of similarity were restricted to the coiled-coil domain of GM130 and were scattered throughout the molecule. The most similar proteins included giantin/GCP372, which is also a Golgi resident protein (Seelig et al., 1994; Sohda et al., 1994); involucrins, which are part of the plasma membrane skeleton in epidermis (Eckert and Green, 1986); Uso1p, a yeast protein essential for ER to Golgi transport (Nakajima et al., 1991); various myosin heavy chains (Sun and Chantler, 1992); p150<sup>Glued</sup>, a subunit of the dynactin complex (Holzbaur et al., 1991); NuMA, a protein involved in nuclear structure, spindle assembly, and nuclear re-formation (Maekawa and Kuriyama, 1993; Yang et al., 1992); and CENP-E, a putative centromeric motor protein (Yen et al., 1992). Though some of these might suggest that GM130 is involved in performing mechanical work, there were no predicted binding sites for either ATP or GTP.

### Topology of GM130

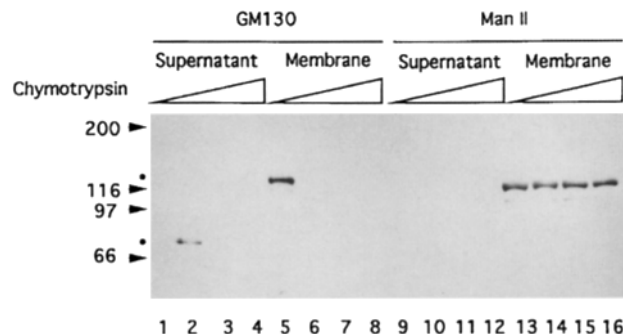
GM130 is a peripheral protein strongly associated with Golgi membranes. As shown in Fig. 4 (top), all of the GM130 sediments with Golgi membranes (lanes 1 and 2) and it cannot be removed with 1 M KCl (lanes 3 and 4). The protein could only be removed by treatment with high pH (lanes 5 and 6), 6 M urea (lanes 7 and 8), or treatment with Triton X-114 (lanes 9–11). GM130 was entirely extracted into the aqueous phase (lane 9) after phase partitioning of Triton X-114.  $\alpha$ 1,3-1,6-mannosidase II (Man II), a resident membrane enzyme was used as a positive control in all of these experiments. As shown in Fig. 4 (bottom), it resisted all extraction conditions except Triton X-114. The presence of about 30% of Man II in the aqueous phase after phase partitioning probably reflects the large luminal domain of this protein (lane 9, bottom; Moremen et al., 1991). In addition, ~10% of Man II remained in an insoluble precipitate (lane 11, bottom).

GM130 is located on the cytoplasmic side of Golgi membranes (Fig. 5). When Golgi membranes were treated with increasing concentrations of chymotrypsin, GM130 was degraded at the lowest concentration used (0.25 mg/ml). A doublet of about 82-kD appeared and disappeared at higher concentrations of chymotrypsin. In contrast, concentrations of chymotrypsin up to 1.0 mg/ml had no effect on Man II which only has 5 amino acids exposed to the cytoplasm (Moremen and Robbins, 1991).

GM130 was present in the Golgi matrix fraction prepared by treating Golgi membranes with 2% Triton X-100, followed by 150 mM NaCl. A protease inhibitor cocktail had to be added to prevent degradation of GM130 and this had the effect of preventing complete removal of Man II from the final matrix fraction that was observed previously (Slusarewicz et al., 1994b). As shown in Table I, >80% of Man II and GM130 remained in the Triton pellet but whereas only ~20% of the Man II remained in the matrix



**Figure 4.** Association of GM130 with Golgi membranes. Purified Golgi membranes were treated with phosphate buffer (lanes 1 and 2), 1 M KCl (lanes 3 and 4), 0.2 M sodium carbonate, pH 11 (lanes 5 and 6), or 6 M urea (lanes 7 and 8) for 30 min on ice, and then separated into a supernatant (S) or a pellet (P) fraction by centrifugation at 4°C, 100,000 g for 10 min. Golgi membranes were also treated with Triton X-114 for 10 min on ice, centrifuged at 4°C to remove insoluble aggregates (P, lane 11) and then phase separated at 37°C yielding an aqueous (Aq, lane 9) or detergent (D, lane 10) phase. Samples were fractionated on a 6–15% gradient SDS–polyacrylamide gel and Western blotted using antiserum to Man II (bottom) or a 17-kD fragment of GM130 (top).



**Figure 5.** Sensitivity of GM130 to chymotrypsin. Rat liver Golgi membranes were treated with 0 (lanes 1, 5, 9 and 13), 0.25 (lanes 2, 6, 10, and 14), 0.5 (lanes 3, 7, 11, and 15), or 1.0 (lanes 4, 8, 12, and 16) mg/ml chymotrypsin for 30 min on ice. After quenching with 2 mM PMSF (final concentration), the membranes were re-isolated by high speed centrifugation. The pellets (lanes 5–8 and 13–16) and supernatants (lanes 1–4 and 9–12) were fractionated on a 6–15% SDS–polyacrylamide gradient gel and Western blotted using polyclonal antisera either to Man II (lanes 9–16; dot on the right) or the 17-kD fragment of GM130 (lanes 1–8; upper dot on the left). Note the degradation product of GM130 in the supernatant at 0.25 mg/ml chymotrypsin (lane 2, lower dot on the left).

**Table I. Detergent and Salt Extractability**

Fraction	Man II	GM130
Triton pellet	87.9 ± 0.4%	80.2 ± 2.1%
Golgi matrix	20.5 ± 2.4%	36.7 ± 0.9%

Purified rat liver Golgi membranes were extracted sequentially with 2% Triton and 150 mM NaCl yielding a Triton pellet and then a Golgi matrix fraction. The percentage yield of Man II and GM130 in each fraction was determined by enzyme activity or Western blotting respectively. Results are presented as the mean of three experiments ± SEM.

fraction, ~37% of the GM130 was found there (Table I). This shows that GM130 is enriched in the matrix fraction when compared to resident Golgi enzymes such as Man II but that a considerable fraction was solubilized by treatment with 150 mM salt.

The size of GM130 after complete solubilization of the matrix in 500 mM NaCl was determined by gel filtration on a Superose 6 column. Man II was also completely solubilized and ran, as expected, as a homodimer of about 250 kD (Fig. 6, lower panel, peaks in lanes 9 and 10; Moremen et al., 1991). GM130, in contrast, ran at the position of a protein with a molecular weight of about 1,000 kD (upper panel, lanes 4 and 5). This is most easily explained by the formation of homo-oligomers or hetero-oligomers with those proteins that appear to co-elute (data not shown).

### Cellular Localization

The localization of GM130 was mapped by immunofluorescence microscopy using a series of HeLa cell lines each stably expressing a Golgi marker restricted to one part of the organelle. The cell lines expressing epitope-tagged versions of the medial-/trans-Golgi enzyme,  $\beta$ -1,2-N-acetylglucosaminyltransferase I (NAGT I), and the trans-Golgi/TGN enzyme,  $\alpha$ 2,6-sialyltransferase (SialylT), have been described previously (Nilsson et al., 1993; Rabouille et al., 1995). A HeLa cell line stably expressing the t-SNARE, syntaxin5 (Bennett et al., 1993), was generated in the same way using the myc-tagged protein (Banfield et al., 1994) and characterized by immuno-EM. Most of the syntaxin5 was found in the CGN, comprising an extensive tubulovesicular network abutting the cis-face of the stack, the rest being present in the stacked cisternae, most often the cis-most one. This is almost exactly the same distribution as was found for the transiently expressed protein (Ban-

field et al., 1994).

These cell lines were double labeled using monoclonal antibodies to the tagged Golgi markers and affinity-purified polyclonal antibodies to the 17-kD fragment of GM130. As shown in Fig. 7, GM130 partially co-localized with SialylT (cf. Fig. 7, A and B) and even more so with NAGT I (cf. Fig. 7, C and D). However, the best co-localization was obtained with the CGN marker, syntaxin5 (cf. Fig. 7, E and F). Antibody to p53 was also used. This labels those compartments involved in transport of cargo between the ER and the Golgi stack (Schweizer et al., 1990). As shown in Fig. 7, G and H, GM130 almost always co-localized with that part of the p53 resident in the CGN but not with the peripheral punctate structures (arrowheads) that are likely involved in cargo transport.

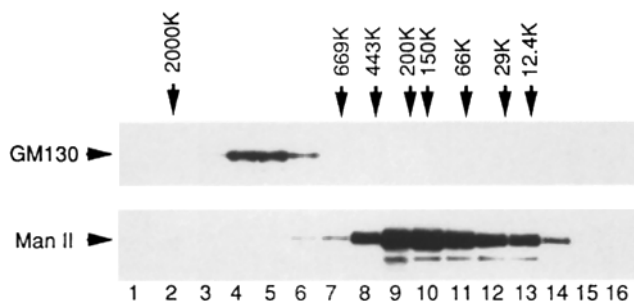
Experiments with BFA provided additional evidence for the location of GM130. Both NAGT I and SialylT were redistributed to the ER in the presence of BFA (data not shown) but syntaxin5 and p53 were found in dispersed, punctate structures (Fig. 7, J and L). GM130 was also found in these structures, co-localizing almost completely with syntaxin5 (Fig. 7, I and J) and substantially with p53 (Fig. 7, K and L). These effects were reversed within 30 min of removing the drug (data not shown).

The location of GM130 was also examined by immuno-EM. In NRK cells, labeling for GM130 was found over one or two cisternae on one side of the stack and over an associated network (Fig. 8, A-C). Approximately one third of the labeling was found over the network, and two-thirds over the stacked cisternae. This labeling was mostly opposite the side that had the unique morphology of the TGN comprising extensive networks of tubules and many budding profiles, some coated with clathrin. This was confirmed by double-labeling using HeLa cells expressing epitope tagged SialylT. As shown in Fig. 8 (D-F), most of the labeling for GM130 was found on the side opposite to that labeled for SialylT. The level of labeling was low because of poor cross-reactivity of the antibody but quantification confirmed the location of GM130 showing that 79 ± 2% (SEM) of the gold particles (total = 66) were present on the cis-side of the Golgi apparatus.

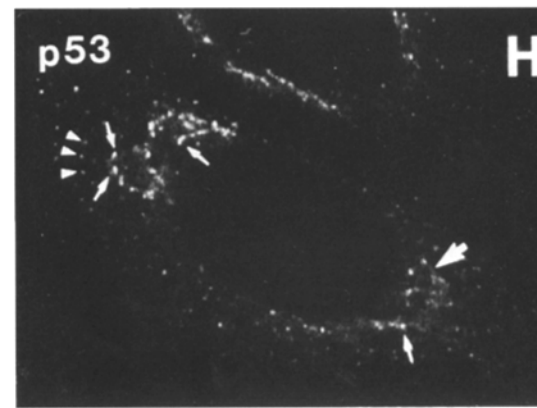
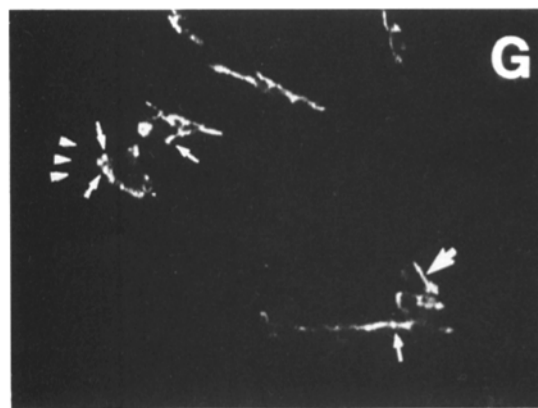
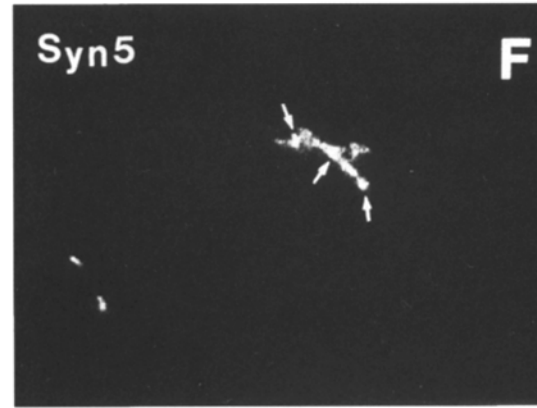
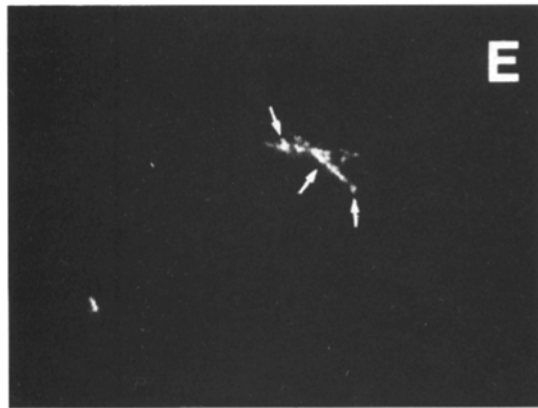
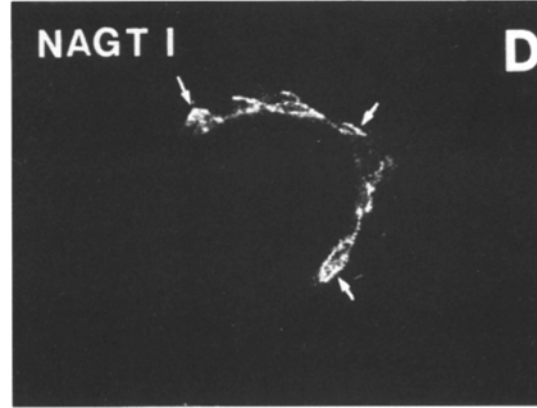
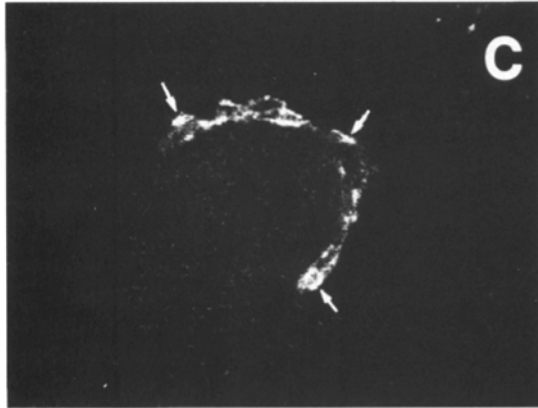
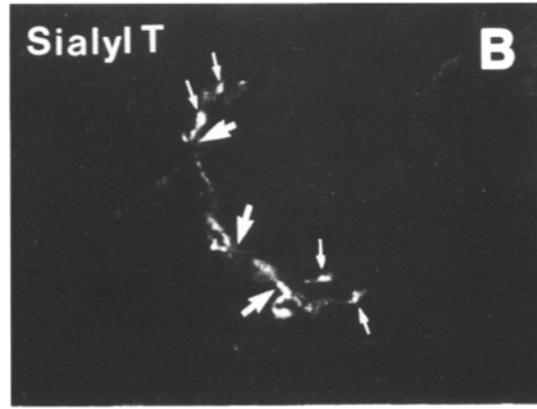
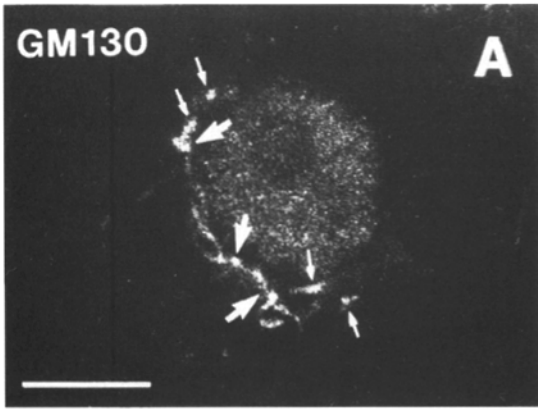
Much of the cisternal labeling for GM130 was found on the cis-face of the cis-most cisterna (Fig. 8, A-C, arrowheads) and some was found between the cis-most cisternae (Fig. 8, A-C, arrows). Since GM130 is a peripheral, cytoplasmic protein by biochemical criteria, this shows that at least some of this protein is located in the intercisternal space.

### Discussion

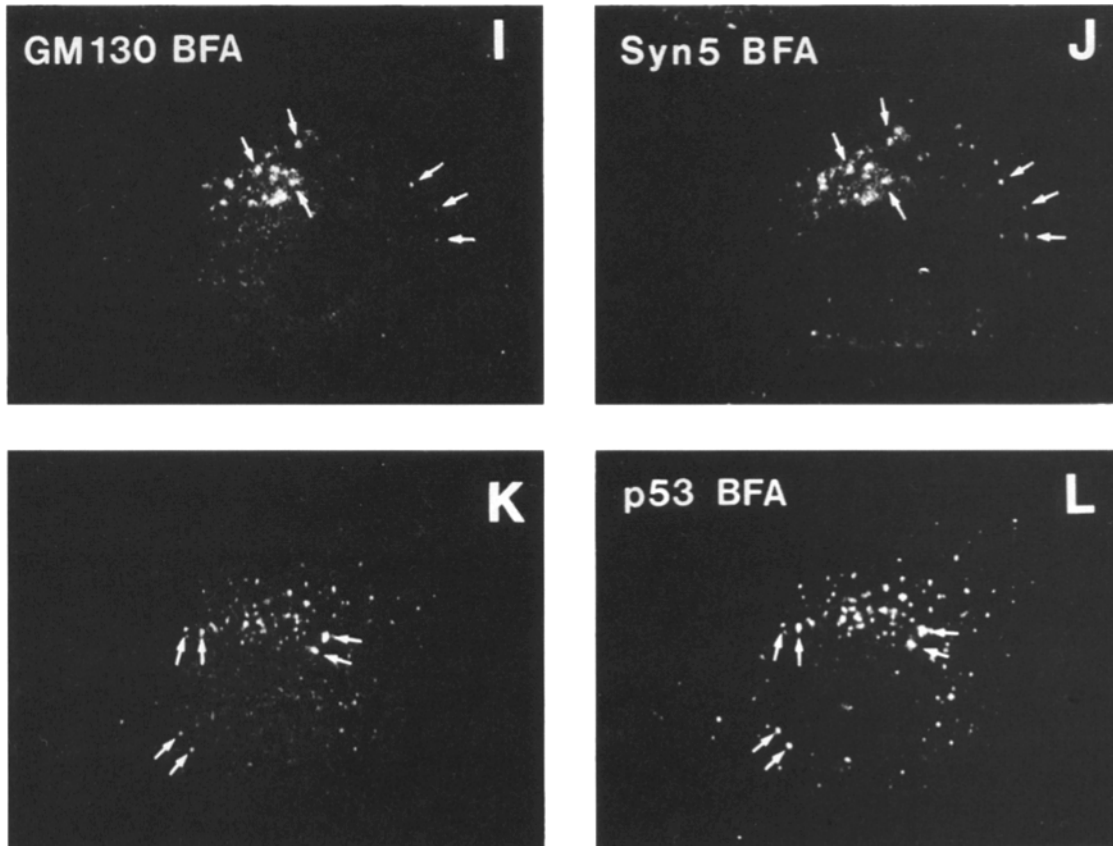
GM130 has many of the properties expected of a structural protein of the Golgi apparatus. It is a tightly bound peripheral membrane protein that can be released by high pH, urea and Triton but not by high salt (Fig. 4). In the presence of both detergent and high salt it runs as a large oligomer that is likely to contain other structural proteins (Fig. 6). It is present on the cytoplasmic side of the membrane because it is accessible to digestion by proteases under conditions where Man II is not (Fig. 5). Sequence analysis corroborated these results showing that there was no putative signal sequence nor membrane-spanning domain (Fig. 3 B). Structural analysis predicted a jointed, rod-like



**Figure 6.** Gel filtration of GM130. The Golgi matrix, solubilized in 500 mM NaCl, was fractionated by gel filtration and the fractions subjected to SDS-PAGE followed by Western blotting using antisera either to Man II (bottom) or the 17-kD fragment of GM130 (top). Positions of the size markers are shown at the top.







**Figure 7.** Localization of GM130 by immunofluorescence microscopy. HeLa cells stably transfected with epitope-tagged SialylT (A and B), NAGT I (C, D, G, H, K, and L), or Syntaxin5 (E, F, I, and J) were fixed, permeabilized, and double labeled with antibodies to the 17-kD fragment of GM130 (A, C, E, G, I, and K) and either antibodies to the VSV-G tag (P5D4: B), the myc tag (9E10: D, F, and J), or p53 (H and L). Secondary antibodies were coupled to FITC (A, C, F, G, J and K) or Texas red (B, D, E, H, I, and L). In I–L, cells were pre-treated with BFA for 30 min before fixation. Small arrows indicate areas where both markers co-localize, whereas large arrows (A, B, G, and H) and arrowheads (G and H) indicate differences in the staining patterns. Bar, 10  $\mu$ m.

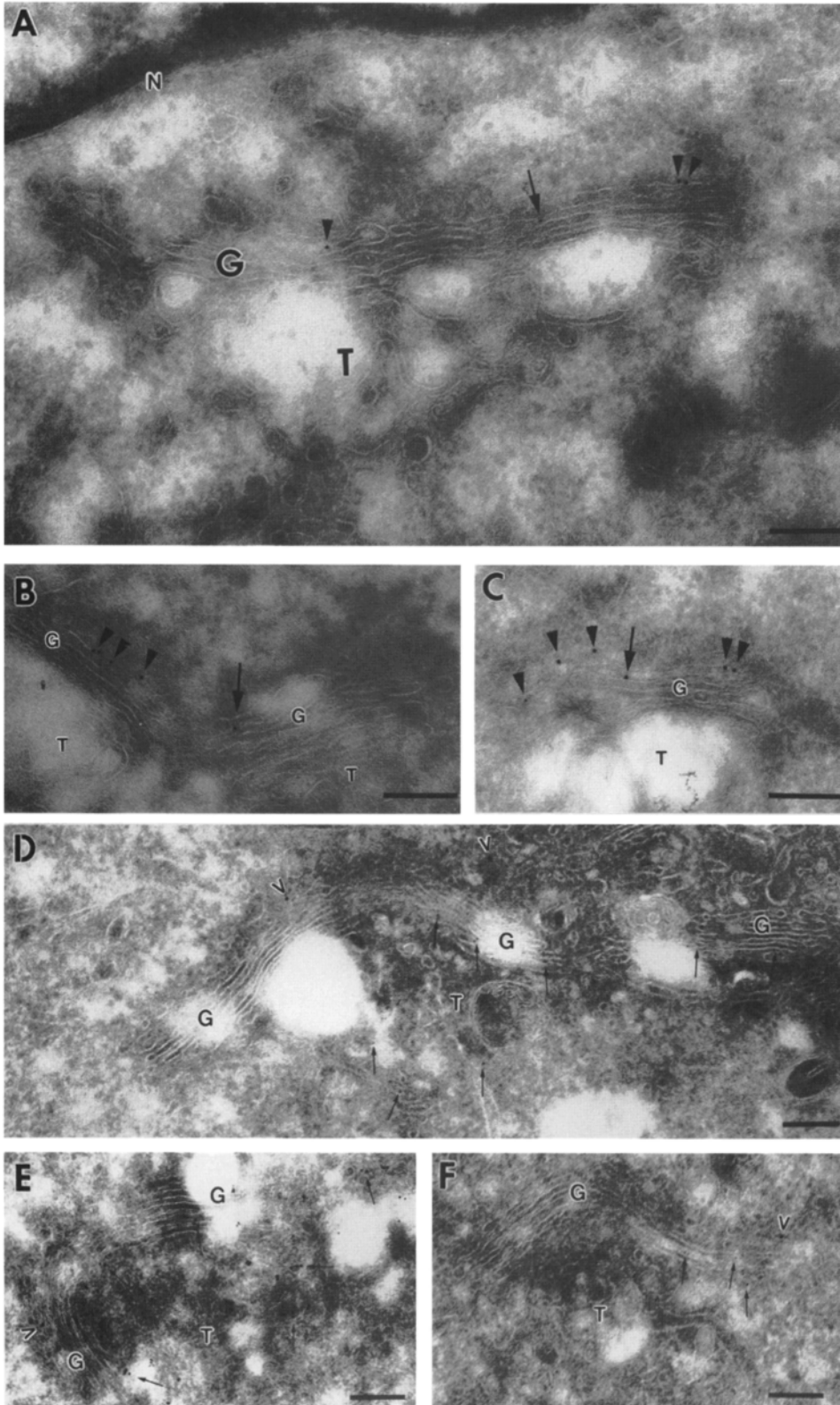
structure throughout the central region of the molecule (Fig. 3 C). Coiled-coils are characteristic of many structural proteins with which GM130 shares weak homology. The presence of so many different types of phosphorylation site suggests that its structure might be regulated in vivo though at present we have no evidence that this is the case. GM130 appears to be a permanent resident of Golgi membranes since preliminary experiments show that there is little if any of this protein in HeLa cytosols at any stage of the cell cycle. It is, therefore, more likely to act as a membrane scaffold than an active participant in the formation or fusion of transport vesicles (Rothman, 1994).

The COOH-terminal part of rat GM130 was highly homologous to a 95-kD human Golgi auto-antigen, golgin-95 (Fritzler et al., 1993). One possible explanation is that GM130 and golgin-95 are the products of alternative splicing or initiation of translation from different start sites. The two antibodies used in these experiments were raised to regions that would be common to both proteins. The antibody to rat GM130 recognizes a protein of the same molecular weight in HeLa cells. It does not, however, recognize a protein of 95 kD in either NRK or HeLa cells. This could be explained by the fact that Fritzler and colleagues (1993) did not use HeLa cells for their analyses and the molecular weight of golgin-95 might depend on the cell

line. Minor proteins with molecular weights other than 95 kD were found in HeLa cells and one of these might be the HeLa cell isoform of golgin-95. However, exactly the same pattern of minor bands was seen using NRK cells arguing that these minor bands are degradation products of GM130 and not cell type specific variants of golgin-95. The alternative and more interesting explanation for the homology between GM130 and golgin-95 is that they might be different members of a family of proteins involved in structural maintenance of the Golgi apparatus. This is supported by the fact that several deletions had to be introduced to align the rat and human sequences. Experiments are in progress to look for other family members.

The location of GM130, on the *cis*-side of the Golgi apparatus was initially somewhat surprising. The detergent and salt resistant matrix had been characterized by its binding of *medial-trans*-Golgi resident enzymes (Slusarewicz et al., 1994b). However, re-examination of the original binding data showed that whereas *trans*-Golgi/TGN markers were only present at 10–15% of their original level, CGN markers were present at higher levels (20–35%). This suggests that elements of a *cis*-matrix are also present in the isolated fraction and this would help explain why antibodies were raised to GM130.

These antibodies were used to localize GM130 using



**Figure 8.** Localization of GM130 by immuno-EM. (A–C) Frozen sections of NRK cells were labeled with antibodies to the 97-kD fragment of GM130 followed by goat anti-rabbit antibodies coupled to 10 nm gold particles. Labeling was found over intercisternal regions (arrows), cisternae and tubular structures (arrowheads) on the *cis*-side of the Golgi stack. (D–F) Frozen sections of HeLa cells stably expressing a VSV-G-tagged SialylT were double labeled with polyclonal antibodies to the 97-kD fragment of GM130 (10 nm gold) and P5D4 monoclonal antibodies to the VSV-G tag (5 nm gold). Labeling for GM130 (open arrowheads) was restricted to the side of the Golgi stack opposite to that which was labeled for the *trans*-Golgi/TGN marker, SialylT (arrows). G, Golgi stack; N, nuclear envelope; T, TGN. Bars, 200 nm.

both immunofluorescence microscopy and immuno-EM. GM130 co-localized only partially with the *trans*-Golgi/TGN marker, SialylT, and more so with the *medial/trans*-Golgi marker, NAGT I, but the best overlap was obtained with the CGN marker, syntaxin5. It also co-localized with that portion of p53 that is thought to reside in the CGN.

Localization to the CGN was supported by the action of BFA. This drug redistributes most Golgi enzymes to the ER but not those markers present in the CGN (Lippincott-Schwartz et al., 1990; Saraste and Svensson, 1991; Tang et al., 1993; Alcade et al., 1994; Rios et al., 1994; Subramaniam et al., 1995). In the presence of BFA, GM130

fragmented but was not redistributed to the ER. Instead it co-localized almost completely with syntaxin5 and partially with p53. Immuno-EM confirmed the location of GM130. It was found in the CGN and also in one or two cisternae on the *cis*-side of the Golgi apparatus.

Of the putative Golgi structural proteins, GM130 most closely resembles, in location and behavior, p210, a Golgi auto-antigen (Rios et al., 1994). It will be interesting to see whether this protein is present in the same complex as GM130. The other large Golgi auto-antigen, with a molecular weight of 230 kD, has been localized to the opposite side of the Golgi apparatus, the TGN (Kooy et al., 1992). This raises the interesting possibility of a family of structural proteins that might be involved in establishing the polarity of the Golgi stack. The sequence of these two proteins should help establish the validity of this suggestion.

In conclusion we have identified a structural protein of the Golgi matrix that is likely involved in the organization of the *cis*-Golgi apparatus. Further analysis of the hetero-oligomer generated by detergent and high salt extraction should help us to identify other proteins needed to maintain the architecture of this organelle.

We thank Dr. Francis Barr, Dr. Birte Sönnichsen, and Dr. Dorothy Mundy (ICRF, London, UK) for critical reading of the manuscript; Dr. Mark Craighead (Sloan-Kettering Institute, New York, NY) for the coiled-coil analysis; Dr. George Banting (University of Bristol, Bristol, UK) for rat liver cDNA library; Dr. Hugh R. B. Pelham (MRC Laboratory for Molecular Biology, Cambridge, UK) for rat syntaxin5 cDNA; Dr. Kelley W. Moremen (Massachusetts Institute of Technology, Cambridge, MA) for anti-Man II antiserum; Dr. Hans-Peter Hauri (Biocenter of the University of Basel, Basel, Switzerland) for the anti-p53 antibody.

This work was partly supported by a Network Grant (No. ERB4050-PL932029) from the European Union (to G. Warren and T. E. Kreis), a grant from the Fonds National Suisse (31-33546.92) and the Canton de Genève (to T. E. Kreis), and a Postdoctoral Fellowship for Research Abroad from the Japan Society for the Promotion of Science (to N. Nakamura).

Received for publication 26 July 1995 and in revised form 28 September 1995.

## References

- Alcade, 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.
- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403-410.
- Banfield, D. K., M. J. Lewis, C. Rabouille, G. Warren, and H. R. B. Pelham. 1994. Localization of sed5, a putative vesicle targeting molecule, to the *cis*-Golgi network involves both its transmembrane and cytoplasmic domains. *J. Cell Biol.* 127:357-371.
- Beck, K. A., J. Buchanan, and W. J. Nelson. 1994a. Golgi ankyrin: association of an ankyrin isoform with the Golgi complex. *Mol. Biol. Cell.* 5 Suppl.:240a.
- Beck, K. A., J. A. Buchanan, V. Malhotra, and W. J. Nelson. 1994b. Golgi spectrin: identification of an erythroid  $\beta$ -spectrin homolog associated with the Golgi complex. *J. Cell Biol.* 127:707-723.
- Bennett, M. K., A. J. Garcia, L. A. Elferink, K. Peterson, A. M. Fleming, C. D. Hazuka, and R. H. Scheller. 1993. The syntaxin family of vesicular transport receptors. *Cell.* 74:863-873.
- Bischoff, J., and R. Kornfeld. 1984. The effect of 1-deoxymannojirimycin on rat liver  $\alpha$ -mannosidases. *Biochem. Biophys. Res. Commun.* 125:324-331.
- Bordier, C. 1991. Phase separation of integral membrane proteins in Triton X-114 solution. *J. Biol. Chem.* 256:1604-1607.
- Brake, B., P. Braghetta, G. Banting, G. Bressan, J. P. Luzio, and K. K. Stanley. 1990. A new recombinant DNA strategy for the molecular cloning of rare membrane proteins. *Biochem. J.* 267:631-637.
- Cluett, E. B., and W. J. Brown. 1992. Adhesion of Golgi cisternae by proteinaceous interactions: intercisternal bridges as putative adhesive structures. *J. Cell Sci.* 103:773-784.
- Collins, J. F., and A. F. W. Coulson. 1990. Significance of protein sequence similarities. In *Methods in Enzymology*, Vol. 183, Doolittle, R. F., editor, Academic Press. 474-487.
- Eckert, R. L., and H. Green. 1986. Structure and evolution of the human involucrin gene. *Cell.* 46:583-589.
- Evan, G. I., G. K. Lewis, G. Ramsay, and J. M. Bishop. 1985. Isolation of monoclonal antibodies specific for human c-myc proto-oncogene product. *Mol. Cell. Biol.* 5:3610-3616.
- Franke, W. W., J. Kartenbeck, S. Krien, W. J. Van der Woude, U. Scheer, and D. J. Morré. 1972. Inter- and intracisternal elements of the Golgi apparatus. A system of membrane-to-membrane cross-links. *Z. Zellforsch. Mikrosk. Anat.* 132:365-380.
- Fritzler, M. J., J. C. Hamel, R. L. Ochs, 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.
- Griffiths, G., and K. Simons. 1986. The *trans* Golgi network: sorting at the exit site of the Golgi complex. *Science.* 234:438-443.
- Harlow, E., and D. Lane. 1988. Immunoaffinity purification of antibodies on an antigen column. In *Antibodies: a laboratory manual*. Cold Spring Harbor Laboratory. 313-315.
- Holzbaun, E. L. F., J. A. Hammarback, B. M. Paschal, N. G. Kravitz, K. K. Pfister, and R. B. Vallee. 1991. Homology of a 150K cytoplasmic dynein-associated polypeptide with the *Drosophila* gene *Glued*. *Nature.* 351:579-583.
- Hurtley, S. M., and A. Helenius. 1989. Protein oligomerization in the endoplasmic reticulum. *Annu. Rev. Cell Biol.* 5:277-307.
- Huttner, W. B., and S. A. Tooze. 1989. Biosynthetic protein transport in the secretory pathway. *Curr. Opin. Cell Biol.* 1:648-654.
- Kooy, J., B.-H. Toh, J. M. Pettitt, R. Erlich, and P. A. Gleeson. 1992. Human autoantibodies as reagents to conserved Golgi components: Characterization of a peripheral, 230-kDa compartment-specific Golgi protein. *J. Biol. Chem.* 267:20255-20263.
- Kreis, T. E. 1986. Microinjected antibodies against the cytoplasmic domain of vesicular stomatitis virus glycoprotein block its transport to the cell surface. *EMBO. J.* 5:931-941.
- Kreis, T. E., and H. F. Lodish. 1986. Oligomerisation is essential for transport of Vesicular Stomatitis Viral Glycoprotein to the cell surface. *Cell.* 46:929-937.
- Kyte, J., and R. F. Doolittle. 1982. A simple method for displaying the hydrophobic character of a protein. *J. Mol. Biol.* 157:105-132.
- Linstedt, A. D., and H.-P. Hauri. 1993. Giantin, a novel conserved Golgi membrane protein containing a cytoplasmic domain of at least 350 kDa. *Mol. Biol. Cell.* 4:679-693.
- Lippincott-Schwartz, J., J. G. Donaldson, A. Schweizer, E. G. Berger, H.-P. Hauri, L. C. Yuan, and R. D. Klausner. 1990. Microtubule-dependent retrograde transport of proteins into the ER in the presence of brefeldin A suggests an ER recycling pathway. *Cell.* 60:821-836.
- Louvard, D., H. Reggio, and G. Warren. 1982. Antibodies to the Golgi complex and the rough endoplasmic reticulum. *J. Cell Biol.* 92:92-107.
- Lupas, A., M. V. Dyke, and J. Stock. 1991. Predicting coiled coil from protein sequences. *Science.* 252:1162-1164.
- Luzio, J. P., B. Brake, G. Banting, K. E. Howell, P. Braghetta, and K. K. Stanley. 1990. Identification, sequencing and expression of an integral membrane protein of the *trans*-Golgi network (TGN38). *Biochem. J.* 270:97-102.
- Maekawa, T., and R. Kuriyama. 1993. Primary structure and microtubule-interacting domain of the SP-H antigen: a mitotic MAP located at the spindle pole and characterized as a homologous protein to NuMA. *J. Cell. Sci.* 105:589-600.
- Misteli, T., and G. Warren. 1994. COP-coated vesicles are involved in the mitotic fragmentation of Golgi stacks in a cell-free system. *J. Cell Biol.* 125:269-282.
- Mollenhauer, H. H. 1965. An intercisternal structure in the Golgi apparatus. *J. Cell Biol.* 24:504-511.
- Mollenhauer, H. H., and D. J. Morré. 1978. Structural compartmentation of the cytosol: zones of exclusion, zones of adhesion, cytoskeletal and intercisternal elements. *Subcell. Biochem.* 5:327-359.
- Mollenhauer, H. H., D. J. Morré, and C. Totten. 1973. Intercisternal substances of the Golgi apparatus. Unstacking of plant dictyosomes using chaotropic agents. *Protoplasm.* 78:443-459.
- Moremen, K. W., and P. W. Robbins. 1991. Isolation, characterization, and expression of cDNAs encoding murine  $\alpha$ -mannosidase II, a Golgi enzyme that controls conversion of high mannose to complex N-glycans. *J. Cell Biol.* 115:1521-1534.
- Moremen, K. W., and O. Touster. 1985. Biosynthesis and modification of Golgi mannosidase II in HeLa and 3T3 cells. *J. Biol. Chem.* 260:6654-6662.
- Moremen, K. W., O. Touster, and P. W. Robbins. 1991. Novel purification of the catalytic domain of Golgi  $\alpha$ -mannosidase II. *J. Biol. Chem.* 266:16876-16885.
- Nakajima, H., A. Hirata, Y. Ogawa, T. Yonehara, K. Yoda, and M. Yamasaki. 1991. A cytoskeleton-related gene, *USO1*, is required for intracellular protein transport in *Saccharomyces cerevisiae*. *J. Cell Biol.* 113:245-260.
- Nilsson, T., M. H. Hoe, P. Slusarewicz, C. Rabouille, R. Watson, F. Hunte, G. Watzel, E. G. Berger, and G. Warren. 1994. Kin recognition between medial Golgi enzymes in HeLa cells. *EMBO. J.* 13:562-574.
- Nilsson, T., M. Pypaert, M. H. Hoe, P. Slusarewicz, E. G. Berger, and G. Warren. 1993. Overlapping distribution of two glycosyltransferases in the Golgi apparatus of HeLa cells. *J. Cell Biol.* 120:5-13.
- Nilsson, T., and G. Warren. 1994. Retention and retrieval in the endoplasmic reticulum and the Golgi apparatus. *Curr. Opin. Cell Biol.* 6:517-521.
- Pearson, W. R., and D. J. Lipman. 1988. Improved tools for biological sequence

- comparison. *Proc. Natl. Acad. Sci. USA.* 85:2444–2448.
- Pelham, H. R. B. 1991. Recycling of proteins between the endoplasmic reticulum and Golgi complex. *Curr. Opin. Cell Biol.* 3:585–591.
- Pryer, N. K., L. J. Wuestehube, and R. Schekman. 1992. Vesicle-mediated protein sorting. *Annu. Rev. Biochem.* 61:471–516.
- Rabouille, C., N. Hui, F. Hunte, R. Kieckbusch, E. Berger, G. Warren, and T. Nilsson. 1995. Mapping the distribution of Golgi enzymes involved in the construction of complex oligosaccharides. *J. Cell Sci.* 108:1617–1627.
- 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.
- Roth, J. 1987. Subcellular organisation of glycosylation in mammalian cells. *Biochim. Biophys. Acta.* 906:405–436.
- Rothman, J. E. 1994. Mechanisms of intracellular protein transport. *Nature.* 372:55–63.
- Saraste, J., and K. Svensson. 1991. Distribution of the intermediate elements operating in ER to Golgi transport. *J. Cell Sci.* 100:415–430.
- Schweizer, A., J. A. Fransen, T. Bachi, L. Ginsel, and H.-P. Hauri. 1988. Identification, by a monoclonal antibody, of a 53-kD protein associated with a tubulo-vesicular compartment at the *cis*-side of the Golgi apparatus. *J. Cell Biol.* 107:1643–1653.
- Schweizer, A., J. A. M. Fransen, K. Matter, T. E. Kreis, L. Ginsel, and H.-P. Hauri. 1990. Identification of an intermediate compartment involved in protein transport from endoplasmic reticulum to Golgi apparatus. *Eur. J. Cell Biol.* 53:185–196.
- Seelig, H. P., P. Schranz, H. Schroter, C. Wiemann, G. Griffiths, and M. Renz. 1994. Molecular genetic analyses of a 376-kilodalton Golgi complex membrane protein giantin. *Mol. Cell. Biol.* 14:2564–2576.
- Slusarewicz, P., N. Hui, and G. Warren. 1994a. Purification of rat liver Golgi stacks. In *Cell Biology: A Laboratory Handbook*. Vol. 1. J. E. Celis, editor. Academic Press Inc., Orlando, Florida. 509–516.
- Slusarewicz, P., T. Nilsson, N. Hui, R. Watson, and G. Warren. 1994b. Isolation of a matrix that binds *medial* Golgi enzymes. *J. Cell Biol.* 124:405–413.
- Sohda, M., Y. Misumi, T. Fujiwara, M. Nishida, and Y. Ikehara. 1994. Molecular cloning and sequence analysis of a human 372-kDa protein localized in the Golgi complex. *Biochem. Biophys. Res. Commun.* 205:1399–1408.
- Soldati, T., and J. C. Perriard. 1991. Intracompartamental sorting of essential myosin light chains: molecular dissection and in vivo monitoring by epitope tagging. *Cell.* 66:277–289.
- Stanley, K. K., and J. P. Luzio. 1984. Construction of a new family of high efficiency bacterial expression vectors: identification of cDNA clones coding for human liver proteins. *EMBO J.* 3:1429–1434.
- Subramaniam, V. N., J. Krijnse-Locker, B. L. Tang, M. Ericsson, A. R. b. M. Yusoff, G. Griffith, and W. Hong. 1995. Monoclonal antibody HFD9 identifies a novel 28 kDa integral membrane protein on the *cis*-Golgi. *J. Cell Sci.* 108:2405–2414.
- Sun, W., and P. D. Chantler. 1992. Cloning of the cDNA encoding a neuronal myosin heavy chain from mammalian brain and its differential expression within the central nervous system. *J. Mol. Biol.* 224:1185–1193.
- Tang, B. L., S. H. Wong, X. L. Qi, S. H. Low, and W. Hong. 1993. Molecular cloning, characterization, subcellular localization and dynamics of p23, the mammalian KDEL receptor. *J. Cell Biol.* 120:325–338.
- Weiner, O. H., J. Murphy, G. Griffiths, M. Schleicher, and A. A. Noegel. 1993. The actin-binding protein comitin (p24) is a component of the Golgi apparatus. *J. Cell Biol.* 123:23–34.
- Wessel, D., and U. I. Flugge. 1984. A Method for the Quantitative Recovery of Protein in Dilute Solution in the Presence of Detergents and Lipids. *Anal. Biochem.* 139:141–143.
- Yang, C. H., E. J. Lambie, and M. Snyder. 1992. NuMA: an unusually long coiled-coil related protein in the mammalian nucleus. *J. Cell Biol.* 116:1303–1317.
- Yen, T. J., G. Li, B. T. Schaar, I. Szilak, and D. W. Cleveland. 1992. CENP-E is a putative kinetochore motor that accumulates just before mitosis. *Nature.* 359:536–539.