Identification and Characterization of Golgin-84, a Novel Golgi Integral Membrane Protein with a Cytoplasmic Coiled-coil Domain*

(Received for publication, September 24, 1998, and in revised form, November 12, 1998)

Roger A. Bascom, Sudha Srinivasan, and Robert L. Nussbaum‡

From the Genetic Disease Research Branch, NHGRI, National Institutes of Health, Bethesda, Maryland 20892

The cytoplasmic face of the Golgi contains a variety of proteins with coiled-coil domains. We identified one such protein in a yeast two-hybrid screen, using as bait the peripheral Golgi phosphatidylinositol(4,5)P₂ 5-phosphatase OCRL1 that is implicated in a human disease, the oculocerebrorenal syndrome. The ~2.8-kilobase mRNA is ubiquitously expressed and abundant in testis; it encodes a 731-amino acid protein with a predicted mass of 83 kDa. Antibodies against the sequence detect a novel ~84-kDa Golgi protein we termed golgin-84. Golgin-84 is an integral membrane protein with a single transmembrane domain close to its C terminus. In vitro, the protein inserts post-translationally into microsomal membranes with an N-cytoplasmic and C-lumen orientation. Cross-linking indicates that golgin-84 forms dimers, consistent with the prediction that it, forming the RET tyrosine kinase domain had the ability to activate the RET-II oncogene. Data base searching also indicates golgin-84 is similar in structure and sequence to OCRL1 that is implicated in a human disease, the oculoce
cbrorenal syndrome. The cytoplasmic face of the Golgi contains a variety of proteins of unknown function have been identified as antigens in autoimmune diseases (6) and include golgins 95, 160, and 245. One autoantigen, giantin-376, (macrogolgin) has recently been shown to tether COPI vesicles to the Golgi, thereby facilitating vesicle transport within the Golgi stack (7). Here we report the cloning and characterization of another, novel cytoplasmic-associated coiled-coil integral membrane Golgi protein termed golgin-84.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Screen—The yeast two-hybrid screen was performed on a human brain cDNA library (CLONTECH) using a Matchmaker kit (CLONTECH) and a ~4.3-kb human OCRL1 cDNA (full open reading frame) as bait. The human OCRL1 cDNA was subcloned into vector pGBT9 (GAL4 DNA binding domain) and the brain cDNA library into vector pGAD424 (GAL4 activating domain). The integrity of the OCRL1 construct was verified by in vitro transcription and translation. Approximately 1.5 million brain clones were screened. Screening and elimination of false positives were performed as described by the manufacturer.

Screening Human Testis cDNA Library—Plaque screening in a Xgt10 human testis 5'-Stretch Plus cDNA library (CLONTECH) was performed using double lifts onto nitrocellulose filters (DuPont) from 12 Petri dishes (150-mm diameter) containing ~37,000 plaques on an NMS/Escherichia coli lawn. A golgin-84 expressed sequence tag probe 134426 (828 bp) was [32P]dCTP-labeled (Amersham Pharmacia Biotech) using a Prime-itTM TMT labeling kit (Stratagene) and NuClampTM probe purification columns (Stratagene). After a 4-h prehybridization, the filters were hybridized with the labeled golgin-84 probe under high stringency (50% formamide, 6× saline/sodium phosphate/EDTA, 5× Denhardt’s solution, 1% SDS, 5× dextran sulfate, 0.1 mg/ml sheared salmon sperm DNA) at 42 °C overnight. Blots were washed twice at room temperature with 2× SSC, 0.1% SDS for 15 min each and then at 65 °C for the same time. The blots were finally washed with 0.2× SSC and 0.1% SDS at 65 °C for 15–30 min. Seventeen plaques were positive through the tertiary screen. Inserts were then amplified from the purified phage with xgt10 arm primers (CLONTECH) and confirmed as golgin-84. DNA was purified (Qiagen) from three phage (numbers 3, 12, and 16) with inserts closest in size to full-length golgin-84 mRNA (~2.8 kb), and the inserts were subcloned into EcoRI-digested pUC18 (Amersham Pharmacia Biotech). The largest subclone, number 16 (2.6 kbp), was missing ~200 bp from its 3'-end.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) AF085199.

‡ To whom correspondence should be addressed: GDRB/NHGRI, 49 Convent Dr., Bethesda, MD 20892-4472; Tel.: 301-402-2039; Fax: 301-402-2170; E-mail: rlnuss@nhgri.nih.gov.

This paper is available on line at http://www.jbc.org

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

† The abbreviations used are: COPI and COPII, coatamer complex I vesicles to the Golgi.
Golgin-84, a Novel Golgi Membrane Protein

Fixed samples were immunolabeled with the affinity-purified polyclonal golgin-84 antibody (1:1000) as well as with commercial monoclonal antibodies mannosidase II (clone 53FC3, Babco), protein-disulfide isomerase (clone RL90, ABR) and β-tubulin (clone Z023, Zymed Laboratories Inc.). The commercial antibodies were used at dilutions recommended by their manufacturers. Primary antibodies were detected with fluorochrome and horseradish peroxidase-labeled secondary antibodies.

For fluorochrome labeling, cells were fixed, blocked with 10% serum, and then probed with primary and secondary antibodies in PBS. Fluorescein isothiocyanate- and Texas Red-labeled secondary antibodies were washed and their working dilutions (Jackson Laboratories).

After immunostaining, cells were PBS-washed and mounted with Immunofluor mounting media (ICN).

Horseradish peroxidase immunostaining was accomplished with Vectastain® Elite ABC (peroxidase) and VECTOR® VIP substrate kits (Vector Laboratories). The protocols were carried out essentially as described in the kits with the exception that additional blocking was performed with avidin and biotin (Vector Laboratories). Immunostained testis sections were also counterstained with methyl green (Vector Laboratories) prior to mounting with Permount®.

Immunostained samples were observed by fluorescence epillumination and bright field microscopy on a Leica DMRBE microscope. Images were recorded on Kodak Ektachrome Slides (400 ASA) and then scanned and stored as cleared digital images.

In Vitro Transcription and Translation—Golgin-84 number 16 cDNA was subcloned into Bluescript II SK+ with its 5'-end oriented toward the T7 promoter. The construct DNA was digested with Spel and phenol chloroform-extracted. Golgin-84 mRNA was then transcribed from the T7 promoter and capped using an mRNA capping kit (Stratagene). Capped golgin-84 mRNA was translated (0.2 μg/25 μl) for 2 h at 30 °C in a rabbit reticulocyte lysate (Promega) containing [35S]methionine (Amersham Pharmacia Biotech) label and canine pancreatic microsomes (Promega). After translation, the microsomes were collected by centrifugation (10 min, 12,000 × g), washed three times with PBS, and incubated in 0.1 M Na2CO3, pH 11.0, at 4 °C for 30 min to remove peripheral membrane proteins. The stripped microsomes were centrifuged at 4 °C and resuspended in 1 × PBS.

In the proteinase protection study, CaCl2 and Tris (pH 8.0) were added to the translation mix and put on ice for 15 min. Before the proteinase K was added. The final concentrations were 10 mM CaCl2, 10 mM Tris (pH 8.0), and 0.2 μg/ml proteinase K. Proteinase K digestion was performed for 1 h at 4 °C, after which the microsomes were pelleted (4 °C, 10 min) and resuspended in a protease stop buffer (15 μl of PBS, 5 μl of phenylmethylsulfonyl fluoride, 5 μl of solid phase renatured protein, and 0.2 μg/ml proteinase K). The resuspended microsomes were immediately boiled for 5 min. As a positive translocation control, β-lactamase was used to demonstrate the microsome membranes were functional and intact.

To analyze the mechanism of golgin-84 insertion into microsomal membranes, translation was terminated with a final concentration of 25 μg/ml cycloheximide. Microsomes were added to the terminated translation reactions and incubated for 2 h at 30 °C before being PBS-washed and alkaline carbonate-stripped.

In cross-linking studies, PBS-washed (two times) golgin-84-containing microsomes were exposed to 0.01 and 0.10 mM dithiobis(sulfosuccinimidylpropionate) (DTSSP) in PBS (1.8 μl of Promega microsome stock/50 μl of DTSSP) for 30 min at room temperature. The cross-linking reactions were quenched with 50 μl of Tris, pH 7.5 (final concentration). Cross-linked protein was analyzed by SDS-PAGE under nonreducing conditions, since the DTSSP cross-linker is thiol-cleavable.

After separation by SDS-PAGE, the in vitro translated products were electrophoresed to polyvinylidene difluoride membrane and sprayed with Enhance spray (DuPont) before being exposed to Kodak X-Omat film.

Enhanced Green Fluorescent Protein (EGFP)-tagged Golgin-84 Deletion Constructs, Plasmid Construction, and Expression—EGFP-tagged fusion proteins were expressed in cultured cells from cytomegaloavirus promoters. Golgin-84 CDNA restriction fragments encoding the C-terminal 51 (BglII, XbaI) and 184 (BglII, XbaI) amino acid residues were subcloned into BglII, XbaI-digested pEGFP-C2 DNA (CLONTECH). Enhanced GFP constructs were made with nonmethylated DNA and screened for in a methylease mimics E. coli strain C5110 (Stratagene). Once made, the constructs were transformed into E. coli DH5α. DNA was then prepared for transfection using Qiagen kits. Twenty-four hours prior to transfection, normal rat kidney (NRK) cells were seeded (~1.6 × 10^5 cells/mL) into chambered (four or eight chambers) slides (Lab-Tek) coated with poly-L-lysine (Sigma). Cells were transfected by exposing them to serum-free DMEM containing DNA and liposomes (1)

2 Additional analyses, including data base searching, were performed at various World Wide Web sites, listed at http://www-biol.univ-mrs. fr/english/loglign.html.
Golgin-84, a Novel Golgi Membrane Protein

RESULTS

Yeast Two-hybrid Screen

An adult human brain cDNA library was screened with a 4.3-kbp human OCL1 cDNA (8) using a Matchmaker kit. Of ~1.5 million clones screened, two positives were identified. One clone was a novel 781-bp partial cDNA termed 13d. Based on its size and subcellular localization (see below) we term the 13d protein golgin-84. A search of the Unigene database indicated that the golgin-84 gene maps to distal human chromosome 14q.

mRNA Is Ubiquitously Expressed and Abundant in Testis—To analyze golgin-84 expression a mouse multiple tissue poly(A) + mRNA blot was probed with a golgin-84 cDNA (Fig. 1). A single ~2.9-kb message was detected that was ubiquitously expressed and abundant in testis. Similar results were obtained with human poly(A) + mRNA blots (message ~2.8 kb, data not shown). A 2.63-kbp golgin-84 cDNA (number 16) was then isolated from a human testis library with a 828-bp exon.

To study golgin-84 expression further, mouse testis cryosections were histochemically immunostained with the affinity-purified golgin-84 antibody. Immunostained sections indicated that golgin-84 was abundant in the seminiferous tubules and Leydig cells (Fig. 3B). Not all seminiferous cells immunostained with the antibody; staining was greatly reduced or absent in the spermatozoa. The immunostaining pattern differed between seminiferous tubules, probably reflecting differences in the stage of spermatogenesis occurring in different tubules (12). The juxtanuclear immunostaining pattern and its absence/reduction from mature spermatozoa suggested that golgin-84 localized to the Golgi.

A Resident Golgi Protein—To determine if golgin-84 was sublocalized to the Golgi, NRK cells were double-labeled with the affinity-purified golgin-84 antibody and an antibody against the Golgi enzyme mannosidase II (medial-trans cisterna). Golgin-84 was detected by fluorescein isothiocyanate and mannosidase II with Texas Red-labeled secondary antibodies. The fluorescent signals from the two proteins overlapped considerably, indicating that golgin-84 is indeed a Golgi protein (Fig. 4A). Consistent with golgin-84 being a Golgi protein, anti-golgin-84 antibody labeled fragmented structures in mitotically dividing NRK cells (13); the mitotic status of the cells was established by double labeling with a β-tubulin antibody (data not shown).

To establish the resident time of golgin-84 in the Golgi, NRK cells were treated with 50 μg of cycloheximide/μl of culture media for 1, 2, 4, and 7 h. Cycloheximide inhibits protein synthesis without affecting protein traffic across the Golgi compartments, thus clearing the Golgi of proteins in transit. At 7 h, golgin-84 was still in the Golgi, implying that it is resident there (data not shown).

BFA-induced Redistribution Kinetics Is Similar to Known Golgi Integral Membrane Proteins—To confirm golgin-84's sublocalization as well as to examine its redistribution kinetics on Golgi perturbation, NRK cells were exposed to 10 μg/ml brefel-
din A (BFA) in tissue culture media (14). After a 1-h incubation with BFA, golgin-84 was transported to and distributed throughout the endoplasmic reticulum (Fig. 4B). Several cells still showed an intense staining immediately adjacent to their nuclei, suggesting that golgin-84 may be present in the trans-Golgi in addition to the earlier disassembling cis/middle cisternae (15). Shorter BFA exposure times of 2, 5, 15, and 30 min showed that golgin-84 began to redistribute to the endoplasmic reticulum (ER) at between 2 and 5 min of BFA treatment (data not shown), similar to integral membrane Golgi proteins such as mannosidase II (data not shown), but much slower than rapid on-off proteins involved in coat formation such as β-COP (16, 17). Upon drug removal, the Golgi reformed, and golgin-84 returned to it. Cells recovered at different rates. By 1 h, many cells appeared almost fully recovered, with small amounts of golgin-84 remaining in the ER (Fig. 4B).

A C-terminally Anchored Type II Integral Membrane Protein—Golgin-84 was predicted to be a transmembrane protein with type II topology. Hydropathy profile analysis (18) predicted a single transmembrane domain (TMD) close to its C terminus at amino acid residues 699–717 (Fig. 5A) with a slight hydrophobic moment on an Eisenberg hydrophobic moment plot (19). The hydrophobic moment suggests the golgin-84 TMD associates with one or more other TMDs in the membrane (19). Based on the charge flanking the golgin-84 TMD, the N-terminal 698 residues of the protein were predicted to be cytoplasmic, and the short 14-residue C terminus was predicted to be luminal (type II membrane protein). No signal sequence was detected with a von Heijne weighted matrix algorithm (20), suggesting that golgin-84 is a type II integral membrane protein.

Fig. 2. Human golgin-84 cDNA sequence and its encoded protein (GenBank™ accession number AF086199). The first 2.63 kb of sequence was derived from a testis cDNA; the remaining 0.2 kb of 3′-UTR sequence, shown after the dashed line, was taken from expressed sequence tag AA405331. The largest open reading frame is predicted to have a 92% chance of coding (11); it encodes a 731-amino acid protein of 83 kDa. The sequence flanking the ATG initiation codon conforms with the Kozak consensus and is preceded by an in frame stop codon 21 nucleotides upstream. A polyadenylation signal, AATAAA, is located immediately upstream of the poly(A) tail.

Golgin-84, a Novel Golgi Membrane Protein

2956
that the protein inserts post-translationally into membranes.

To determine if golgin-84 is an integral membrane protein, its mRNA was translated into [35S]methionine-labeled protein in the presence of canine pancreatic microsomes. After translation, the microsomes were washed, stripped to remove peripheral membrane proteins, and collected by centrifugation. The microsomes contained golgin-84, indicating that the protein had been translocated into them (Fig. 5B).

The membrane location and orientation of golgin-84 was then established by a protease protection assay. Microsomes containing the translocated protein were treated with proteinase K. Golgin-84 was digested by the protease to below 66.2 kDa, suggesting that the 698-amino acid N terminus was exposed on the surface of the microsomes, consistent with it being a type II membrane protein (Fig. 5, C and D). A control secretory protein β-lactamase was translocated into the microsome lumen and protected from digestion, indicating that the microsomes were functional and intact (Fig. 5C).

Golgin-84 lacks a predicted signal sequence and is thus expected to undergo post-translational insertion into membranes (21). To test this hypothesis, microsomes were added either prior to the start of translation or after translation had been terminated by the addition of cycloheximide (Fig. 5E). Microsomes that were exposed to translating or full-length golgin-84 protein (translation terminated by cycloheximide) for a period of 2 h at 30 °C were washed with PBS, alkaline-extracted, and analyzed by SDS-PAGE. As expected, translating golgin-84 was translocated into the microsomes. Full-length golgin-84 protein, exposed to microsomes after termination of translation, was also translocated into the microsomal membranes, implying a post-translational mechanism of insertion.

The C-terminal 51 Residues Contain the Membrane Insertion Sequence and Golgi Retention Signal—In vitro transcription and translation studies indicated golgin-84 to be a C-terminally anchored membrane protein. To confirm that the protein is C-terminally anchored as well as to determine if its C terminus contains membrane insertion and Golgi retention sequences, the C-terminal 51-amino acid region of golgin-84 was tagged at its N terminus with EGFP and expressed in cultured NRK cells (Fig. 6).

EGFP, when expressed by itself, was present throughout the cells. In contrast, the tagged 51-residue C terminus (fusion protein I) was targeted to the Golgi. In low expressing cells, the fusion protein (I) was predominantly in the Golgi, while in high expressing cells it accumulated in the ER as well. The ER and Golgi were distinguished by their morphology as well as by immunostaining with antibodies against mannosidase II (Golgi) and protein-disulfide isomerase (ER). Once in the Golgi, fusion protein I was effectively retained there, and no fluorescent signal could be detected on the plasma membrane. The presence of fusion protein I in the Golgi and its absence from the plasma membrane indicate that the C-terminal 51 residues of golgin-84 contain membrane insertion and Golgi retention sequences. In addition, the absence of coiled-coil sequence in the C terminus implies that coiled-coil interactions are not required for Golgi retention, as suggested by an oligomerization-based model of Golgi retention (3). Finally, backup of fusion protein I in the ER of high expressing cells suggests that (a) the protein inserts into the ER membrane and is then transported to the Golgi, as has been reported for giantin, a Golgi protein of similar structure (22) and (b) export from the ER is inefficient because the fusion protein (I) lacks an ER export signal (23, 24) or is detained by the ER quality control system (25, 26).

Since the 51-residue C terminus was inefficiently transported to the Golgi, a larger tagged construct encoding the last 184 residues of golgin-84 (fusion protein II) was made and expressed in NRK cells (Fig. 6). The larger fusion protein was transported efficiently to the Golgi in both low and high expressing cells. Endogenous golgin-84 could not be co-immunoprecipitated with fusion protein II, suggesting that it did not interact with the fusion protein and is not involved in transporting it to the Golgi (data not shown). A putative ER export signal present in fusion protein II but absent in I may be responsible for its more efficient transport to the Golgi. The sequence of amino acid residues DTE, located 37 residues N-
Golgin-84 was detected with fluorescein isothiocyanate-labeled secondary antibodies and mannosidase II with Texas Red-labeled secondary antibodies (bar, 2.5 μm). Golgin-84 was redistributed to the ER in BFA-treated NRK cells. NRK cells were double-labeled with antibodies against golgin-84 and a Golgi marker protein mannosidase II. Golgin-84 was predicted by a multicoil program (29) to dimerize via its coiled-coil domain (Fig. 7A). To investigate if golgin-84 dimerizes, PBS-washed microsomes containing 35S-labeled golgin-84 were incubated at room temperature in 0.01 and 0.10 mM DTSSP, a membrane-impermeable cross-linker. As predicted, cross-linked golgin-84 migrated by SDS-PAGE as dimers. The amount of cross-linked dimers increased with increasing cross-linker concentrations. At 0.01 mM DTSSP, monomers and dimers were present, while at 0.10 mM no monomers remained (Fig. 7B).

Golgin-84 contains a putative ~400-amino acid coiled-coil domain in its cytoplasmic N terminus. Two leucine zippers were identified in the golgin-84 sequence (residues 227–248 and 301–322) by a prosite pattern search. Since leucine zippers are a specialized type of coiled-coil, a coils program (version 2.1) was used to analyze the extent of the coiled-coil domain (27, 28). The coiled-coil domain, containing discontinuities within it, spans more than half the length of the protein; its boundaries were predicted at amino acid residues ~217 and 632 using a window of 28 and an arbitrary cut-off probability of >90%. The coiled-coil domain was also visible as internal heptad repeats on a dot matrix plot of golgin-84 against itself generated with a moving window of 30 and cut-off of 35% (data not shown).

Golgin-84 was predicted by a multi-coil program (29) to dimerize via its coiled-coil domain (Fig. 7A). To investigate if golgin-84 dimerizes, PBS-washed microsomes containing 35S-labeled golgin-84 were incubated at room temperature in 0.01 and 0.10 mM DTSSP, a membrane-impermeable cross-linker. As predicted, cross-linked golgin-84 migrated by SDS-PAGE as dimers. The amount of cross-linked dimers increased with increasing cross-linker concentrations. At 0.01 mM DTSSP, monomers and dimers were present, while at 0.10 mM no monomers remained (Fig. 7B).

Golgin-84 is similar to coiled-coil-containing Golgi proteins—To gain insight into golgin-84 function homology, searches were performed against available World Wide Web data bases such as GenBank™. Golgin-84 shows significant sequence similarity to several coiled-coil-containing proteins, including Golgi proteins. The strongest similarity is to the RET-II oncogene (BLAST p value = 6.2e−305), which it turns out consists of the first 497 amino acid residues of golgin-84 fused to residues 713–1114 of the RET protein (30). RET residues 713–1114 contain a tyrosine kinase domain that is activated by dimerization (31, 32). Activation of the kinase domain in RET-II is probably achieved by constitutive dimerization induced by the golgin-84 coiled-coil sequence. An earlier RET-II report presented the partial golgin-84 protein sequence but neither characterized it nor suggested a mechanism by which it activates RET-II (33).

Golgin-84 shows sequence similarity to the coiled-coil-containing myosin family (BLAST p value <5.8e−16) and to several coiled-coil Golgi proteins (range of BLAST p values was 2.2e−14 to 4.6e−9). Golgin-84 is similar to six known Golgi proteins: giantin (macrogolgin), golgin-160, Golgi complex autoantigen-97, golgin-245, trans-Golgi p230, and cis-Golgi matrix protein GM130. Sequence similarities are associated primarily with the ~400-residue coiled-coil domain. The functions of most of these Golgi proteins are currently unknown, many having been identified as autoantigens. Golgin-84 is structurally similar to one of the autoantigens, known as giantin, a C-terminally anchored integral membrane protein with an extensive cytoplasmic coiled-coil domain (34, 35). The golgin-84 coiled-coil domain and its immediate flanking sequence (residues 173–642) are 45% similar (22% identical) to the giantin coiled-coil sequence (residues 1322–1806). Recently, giantin was shown to tether COPI vesicles to the Golgi and has been proposed to play a role in stacking Golgi disks (7).

FIG. 4. Golgin-84 is sublocalized to the Golgi. A, NRK cells were double-labeled with antibodies against golgin-84 and a Golgi marker protein mannosidase II. Golgin-84 was detected with fluorescein isothiocyanate-labeled secondary antibodies and mannosidase II with Texas Red-labeled secondary antibodies (bar, 2.5 μm). B, golgin-84 is redistributed to the ER in BFA-treated NRK cells. NRK cells were exposed to 10 μg/ml BFA in culture media for 1 h, after which time the BFA was removed and the cells were allowed to recover for an additional 1 h. After 1 h of BFA treatment, golgin-84 had been reabsorbed into and distributed throughout the endoplasmic reticulum. An intense juxtanuclear signal was also present in some cells, indicating that some of the protein may be associated with the trans-Golgi network. One hour after the removal of BFA, golgin-84 had regained its Golgi type distribution, although the recovery process was still incomplete (bar, 2.5 μm).
The hydropathy profile and its inability to be extracted in vitro from microsomal membranes with a high pH buffer. Protease protection studies confirmed a predicted type II topology. The post-translational mode of golgin-84 insertion into membranes is consistent with its lack of a signal peptide and the presence of its TMD within the last 50 residues of the protein (21, 36). Golgin-84 targeting sequence(s) were shown by EGFP fusion studies to be in the last 51 residues of the golgin-84 protein, suggesting its transmembrane domain may be involved in Golgi retention. A sequence comparison of Golgi and plasma membrane TMDs indicates that Golgi TMDs are on average 5 residues shorter than plasma membrane TMDs (37). Consistent with this observation, synthetic TMDs 17 leucines in length are retained in the Golgi, while those 23 leucines in length are transported to the plasma membrane (38). The Golgin-84 TMD is predicted to be 19 residues. A lipid-sorting model has been proposed to explain TMD retention by length. The model suggests post-Golgi membranes are thicker (sterols and sphingolipids), thereby preventing the shorter Golgi TMDs from moving forward (38). Oligomerization (kin selection) has also been suggested to play a role in the retention of some resident Golgi proteins (39).

The golgin-84 coiled-coil domain spans more than half the length of the protein, suggesting that the molecule is rod-like in shape. Discontinuities within the coiled-coil domain may create fixed bends or provide flexibility to the structure (40). The coiled-coil domain further suggests that, like myosin II, golgin-84 could form dimers, and we confirmed this by in vitro cross-linking studies. Golgin-84 dimerization may explain its ability to constitutively activate the RET-II oncogene. Included
in RET-II are the first ~274 residues of the golgin-84 coiled-coil domain with its two leucine zippers, which may activate RET-II by constitutive homodimerization. Constitutive leucine zipper-mediated homodimerization has been reported to activate another RET oncogene termed PTC1 in which a fragment of a coiled-coil protein H4 is fused to the RET tyrosine kinase domain (41, 42). Unlike PTC1, which is rearranged with RET in 11–25% of papillary thyroid carcinomas, the RET-II rearrangement appears to be an artifact created during transfection of NIH3T3 cells with human colon cancer DNA, since the rearrangement was not detected in the original tumor DNA by Southern blot analysis (30).

The function of golgin-84 is unknown, but some clues are provided by its ubiquitous expression, Golgi sublocalization, and structure and sequence similarity to Golgi proteins of known function. Abundant expression in the testis indicates that it may serve a specialized role there such as in the formation of the acrosome. Alternatively, or in addition, its abundant testis expression may reflect the secretory potential of that organ.

Coiled-coil integral membrane proteins with structures similar to golgin-84 have been implicated in specific docking or in tethering (Velcro factor) of transport vesicles (5). Specific docking proteins termed SNARES ensure that transport vesicles fuse with their appropriate target membranes. A vesicle SNARE (v-SNARE) interacts with a specific target SNARE (t-SNARE) on the acceptor membrane. At first glance, golgin-84 does not appear to be a SNARE, since a prosite profile search indicates it lacks the t-SNARE homology domain reported previously (43). Golgin-84 does, however, show struc-
Golgin-84, a Novel Golgi Membrane Protein

Golgin-84, a Novel Golgi Membrane Protein

FIG. 7. Golgin-84 forms dimers. A, golgin-84 is predicted to dimerize via its coiled-coil domain (29). B, in the presence of DTSSP, golgin-84 is cross-linked to form dimers. The membrane-impermeable cross-linker DTSSP was added at different concentrations to microsomes containing 35S-labeled golgin-84. Golgin-84 dimers are indicated by the arrow.

T 1. Mellman, I., and Simons, K. (1992) Cell 68, 829–840
2. Farquhar, M. G., and Palade, G. E. (1998) Trends Cell Biol. 8, 2–10
3. Munro, S. (1998) Trends Cell Biol. 8, 11–15
4. Lupas, A. (1998) Trends Biochem. Sci. 21, 375–382
5. Pinzani, R. (1996) Annu. Rev. Cell Dev. Biol. 12, 441–461
6. Dolh, J. G., Lupas, A., and Carison, M. (1993) Biochem. Biophys. Res. Commun. 195, 686–696
7. Sonnichsen, B., Lowe, M., Levine, T., Jamsa, E., Dirac-Svejstrup, B., and Warren, G. (1998) J. Cell Biol. 140, 1013–1021
8. Attree, O., Olivi, I. M., Okake, I., Bailey, L. C., Nelson, D. L., Lewis, R. A., McInnes, R. R., and Nussbaum, R. L. (1992) Nature 358, 239–242
9. Kozak, M. (1994) Nucleic Acids Res. 12, 867–872
10. Kozak, M. (1986) Cell 44, 283–292
11. Fickett, J. W. (1982) Nucleic Acids Res. 10, 5303–5318
12. Desjardins, C., and Ewing, L. L. (1993) Cell and Molecular Biology of the Testis, Oxford University Press, New York
13. Lowe, M., Nakamura, N., and Warren, G. (1998) Trends Cell Biol. 8, 40–44
14. Fujitama, T., Oda, K., Yokota, S., Takatsuki, A., and Ikehara, Y. (1988) J. Biol. Chem. 263, 18545–18552
15. Alcalde, J., Bonay, P., Roa, A., Vilario, S., and Sandoval, I. V. (1992) J. Cell Biol. 116, 69–83
16. Donaldson, J. G., Lippinsco-Schwart, J., Bloom, G. S., Kreis, T. E., and Klukner, R. D. (1990) J. Cell Biol. 111, 2295–2306
17. Serafino, T., Stenbeck, G., Brecht, A., Lottspeich, F., Orci, L., Rothman, J. E., and Wieland, F. T. (1991) Nature 349, 215–220
18. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
19. Eisenberg, D., Schwartz, E., Komaryy, M., and Wall, R. (1984) J. Mol. Biol. 179, 125–142
20. Von Hejne, G. (1986) Nucleic Acids Res. 14, 4683–4690
21. Kutay, U., Hartmann, E., and Rapport, T. A. (1993) Trends Cell Biol. 3, 72–75
22. Linstedt, A. D., and Hauri, H.-P. (1993) Trends Cell Biol. 3, 72–75
23. Nishimura, N., and Balch, W. E. (1997) Science 277, 556–558
24. Bannykh, S. I., Nishimura, N., and Balch, W. E. (1998) Trends Cell Biol. 8, 21–25
25. Riezman, H. (1997) Science 278, 1728–1729
26. Biederer, T., Volkein, C., and Sommer, T. (1997) Science 278, 1806–1809
27. Lupas, A., Van Dyke, M., and Stock, J. (1991) Science 252, 1162–1164
28. Lupas, A. (1997) Curr. Opin. Struct. Biol. 7, 388–393
29. Wolf, E., Kim, P. S., and Berger, B. (1997) Protein Sci. 6, 1179–1189
30. Ishizaka, Y., Tahira, T., Ochiai, M., Ikeda, I., Sugimura, T., and Nagao, M. (1988) Oncogene 3, 193–197
31. Nemegege, A. M., Schmidt, A., Ullrich, A., and Schlessinger, J. (1990) Mol. Cell. Biol. 10, 4035–4044
32. Ullrich, A., and Schlessinger, J. (1990) Cell 61, 203–212
33. Ishizaka, Y., Ochiai, M., Tahira, T., Sugimura, T., and Nagao, M. (1989) Oncogene 4, 789–794
34. Linstedt, A. D., and Hauri, H.-P. (1993) Mol. Biol. Cell 4, 679–693
35. Schlegel, P., Schranz, P., Schrater, H., Weimann, C., and Renz, M. (1994) J. Autoimmunity 7, 67–91
36. Anderson, D. J., Mostov, K. E., and Bladel, G. (1983) Proc. Natl. Acad. Sci.

R. A. Bascom, S. Srinivasan, and R. L. Nussbaum, unpublished results.
U. S. A. 80, 7249–7253
37. Munro, S. (1995) Biochem. Soc. Trans. 23, 527–530
38. Munro, S. (1995) EMBO J. 14, 4695–4704
39. Nilsson, T., Slusarewicz, P., Hoe, M. H., and Warren, G. (1993) FEBS Lett. 330, 1–4
40. Oas, T. G., and Endow, S. A. (1994) Trends Biochem. Sci. 19, 51–54
41. Tong, Q., Xing, S., and Jhiang, S. (1997) J. Biol. Chem. 272, 9043–9047
42. Grieco, M., Santoro, M., Berlingieri, M. T., Melillo, R. M., Denuchi, R., Bongarzone, I., Pierotti, M. A., Della Porta, G., Fusco, A., and Vecchio, G. (1990) Cell 60, 557–563
43. Weimbs, T., Low, S-H., Chapin, S. J., Mostov, K. E., Bucher, P., and Hofmann, K. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3046–3051
44. Nakamura, N., Lowe, M., Levine, T. P., Rabouille, C., and Warren, G. (1997) Cell 89, 445–455
45. Barr, F. A., Puype, M., Vandekerckhove, J., and Warren, G. (1997) Cell 91, 253–262
46. Orci, L., Perrelet, A., and Rothman, J. E. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2279–2283