Molecular Characterization of GCP170, a 170-kDa Protein Associated with the Cytoplasmic Face of the Golgi Membrane

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We have isolated a cDNA clone encoding a protein (designated GCP170) of 1530 amino acid residues with a calculated molecular mass of 170 kDa that is localized to the Golgi complex. Hydrophathy analysis shows that GCP170 contains no NH₂-terminal signal sequence nor a hydrophobic domain sufficient for participating in membrane localization. It is also predicted that GCP170 has characteristic secondary structures including an extremely long α-helical domain that likely forms a coiled-coil between non-coil domains at the NH₂ and COOH termini, suggesting that the protein is organized as a globular head, a stalk, and a tail. Immunocytochemical observations revealed that GCP170 was localized to the Golgi complex and the cytoplasm, consistent with biochemical data indicating that the protein exits as a membrane-associated form and a soluble form. GCP170 was dissociated from the Golgi membrane in response to brefeldin A as rapidly as a coat protein complex of non-membrane-associated form and a soluble form. GCP170 has characteristic secondary structures including an extremely long α-helical domain that likely forms a coiled-coil between non-coil domains at the NH₂ and COOH termini, suggesting that the protein is organized as a globular head, a stalk, and a tail. Immunocytochemical observations revealed that GCP170 was localized to the Golgi complex and the cytoplasm, consistent with biochemical data indicating that the protein exits as a membrane-associated form and a soluble form. GCP170 was dissociated from the Golgi membrane in response to brefeldin A as rapidly as a coat protein complex of non-clathrin-coated vesicles (β-COP, a subunit of coatomer), but did not co-localize with β-COP on the Golgi membrane when examined by immunoelectron microscopy. The protein was detected as phosphorylated and unphosphorylated forms, of which the unphosphorylated form was more tightly associated with the Golgi membrane. When cells were extracted with 1% Triton X-100 under microtubule-stabilizing conditions, GCP170 remained in the cells in association with the Golgi complex. These results indicate that GCP170 is a peripheral membrane protein with a long coiled-coil domain that may be involved in the structural organization or stabilization of the Golgi complex. The Golgi complex is a highly organized organelle comprised of the cis-Golgi network, Golgi stack, and trans-Golgi network and involved in transport, processing, and sorting of newly synthesized proteins including secretory, plasma membrane and lysosomal proteins (1, 2). Various enzymes involved in proteolytic and oligosaccharide processing of the transported proteins have been shown to be localized to subcompartments of the Golgi complex (3–5). These Golgi-resident enzymes are all transmembrane proteins with their major domains disposed to the lumen. In contrast, there are many cytosolic proteins and cytoplasmically disposed membrane proteins that are involved in the vesicular transport through the Golgi complex (2, 6). Coatomer (COP) and ADP-ribosylation factor are required for budding of transport vesicles from the Golgi membrane (7–9). Other cytosolic proteins such as N-ethylmaleimide-sensitive fusion protein and soluble N-ethylmaleimide-sensitive fusion protein attachment proteins (SNAPs) and membrane-associated SNAP receptors (SNAREs) are required for attachment and/or fusion of the vesicles to target membranes (10–12), leading to the proposal of the SNARE hypothesis (6, 12).

The Golgi complex has morphological characteristics that include ordered (from cis to trans) stacking, close apposition, and constant spacing of stacked cisternae. The characteristic stacked structure of the Golgi complex is rapidly disrupted by treatment of cells with brefeldin A (BFA) (13–16). The evidence that BFA primarily blocks the budding of transport vesicles (6, 17, 18) indicates that the maintenance of the Golgi structure is supported by the vesicular transport system. In addition, it has been suggested that the stacking of Golgi cisternae can be explained by an extension of the SNARE hypothesis (19); stacking is most simply viewed as an extension of the docking process with the heterotypic v-SNAREs in each cisterna interacting with the cognate t-SNAREs in the next. This would require a mechanism preventing the fusion that leads to vesicular transport, postulating the existence of "fusion clamps" (19). Whether or not the SNARE system is involved in this process, there may be cytosolic and/or cytoplasmically disposed membrane proteins responsible for the structural maintenance of the Golgi complex, although little is known about them. Recently several new Golgi-associated proteins have been identified by antibodies from patients with autoimmune diseases and by antibodies prepared in animals. These include giantin or GCP372 (20–22), golgin-245 or p230 (23, 24), GCP230 (25), p210 (26), and GM130 or golgin-95 (27, 28). These proteins have several characteristic features. Their molecular masses are quite large, ranging from 370 kDa for the largest giantin/GCP372 to 130 kDa for the smallest GM130/golgin-95. All of them are associated with the cytoplasmic face of the Golgi membrane. Although giantin/GCP372 is an integral membrane protein anchored to the membrane by the COOH-terminal hydrophobic domain (21, 22), all the other proteins have no hydrophobic domain that could function as a signal sequence or participate in membrane localization. The most characteristic feature is that all of the proteins have extensively large domains, enabling the formation of coiled-coil structures analogous to the myosin family (21–28). Several other proteins in-

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volved in membrane targeting and fusion also contain long coiled-coil domains, which are implicated in interactions leading to vesicle targeting and/or fusion (29–31). However, the physiological function of the above mentioned proteins remains to be determined.

Golgin-160 is another Golgi-associated protein that was also identified by human autoantibodies (28), but its structure and properties have not been well characterized. In the present study, we report the use of a partial cDNA sequence from golgin-160 to isolate a full-length cDNA clone encoding a 170-kDa protein (termed GCP170) and describe the properties of this protein in comparison with those of other Golgi-associated proteins.

EXPERIMENTAL PROCEDURES

Materials—Western blotting detection kit was purchased from Amersham Corp. (Tokyo, Japan). Anti-α-tubulin IgG was from Funakoshi (Tokyo, Japan); rhodamine-conjugated goat anti-rabbit IgG was from DAKO (Tokyo, Japan); and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG was from Cappel Laboratories (West Chester, PA). Guinea pig anti-human GCP372 and rabbit anti-β-COP antibodies were prepared as described previously (21, 25). Rabbit anti-α-tubulin II (Man II) was supplied by Dr. K. W. Moremen (University of Georgia).

Cell Culture—HepG2 (human hepatocarcinoma), QGP-1 (human pancreas carcinoma), HeLa (human cervix carcinoma), COS-1 (monkey kidney), and baby hamster kidney cells were cultured as described (21, 25).

Isolation and Sequencing of cDNA Clones—Poly(A)+ RNA prepared from HepG2 cells was used for construction of a cDNA library in XZAPII bacteriophage. A golgin-160 cDNA fragment (28) was prepared by reverse transcription-PCR from QGP-1 RNA to obtain a probe for screening and Northern blot analysis. Primers used in the PCR reaction were 5′-GGATCCTGCAGCAGGAGAC-3′ and 5′-GCTTGGAACTGTGCTATGCTGATATC-3′. Screening of 1 × 10^6 independent clones yielded 11 positive clones. These clones were subcloned into pBluescript SK plasmid vector by automatic excision process (32). A cDNA clone with the longest insert (3.5 kilobase pairs, named QSY103) was subcloned and subjected to nucleotide sequence determination by the dideoxynucleotide chain termination method (33). To obtain a full-length cDNA, we constructed another cDNA library using a poly(A)+ RNA fraction that was enriched with 7-kb RNA hybridized with a fragment of QSY103 (34). Screening of 5 × 10^6 independent clones of the new cDNA library yielded 12 positive clones. A clone with the longest insert obtained (FQSY1024; 8.27–990 and 1136–1231 in Fig. 2) prepared in pT7-blue were digested at the BamHI and EcoRI site of pGEX3X vector. The glutathione S-transferase fusion protein with the green fluorescent protein (GFP). For metabolic labeling experiments, the resultant postnuclear supernatant was separated by centrifugation at 105,000 × g for 1 h into a pellet (membrane fraction) and a supernatant (cytosol fraction). A Golgi fraction was prepared by flotation of the postnuclear supernatant in a sucrose gradient as described by Balch et al. (21). The Golgi membranes (Golgi fraction) were used for immunoprecipitation at 105,000 × g for 30 min and resuspended in the homogenizing buffer containing either 0–0.5 M KCl, 1 mM NaCl, or 0.1 mM Na_2CO_3. After being incubated on ice for 30 min, the suspensions were centrifuged at 105,000 × g for 30 min to obtain supernatants and membrane pellets. Phase separation of the indicated samples in a Triton X-114 solution was carried out by the method of Bordier (38).

Accessibility of GCP170 to Proteolytic Digestion—A Golgi fraction (1 mg protein/ml) was incubated at 0 °C for 30 min with a mixture of chymotrypsin and trypsin (50 μg each/ml) in 10 mM Tris-HCl (pH 8.0) and 150 mM NaCl with or without 1% Triton X-100. 35S-labeled cells were suspended in 50 mM Tris-HCl (pH 7.5), and 2 mM EDTA (homogenizing buffer) with a nitrogen bombardment apparatus (Parr Instrument Co.), followed by centrifugation at 1,000 × g for 10 min. The resultant postnuclear supernatant was separated by centrifugation at 105,000 × g for 1 h into a pellet (membrane fraction) and a supernatant (cytosol fraction). A Golgi fraction was prepared by flotation of the postnuclear supernatant in a sucrose gradient as described by Balch et al. (21). The Golgi membranes (Golgi fraction) were used for immunoprecipitation at 105,000 × g for 30 min and resuspended in the homogenizing buffer containing either 0–0.5 M KCl, 1 mM NaCl, or 0.1 mM Na_2CO_3. After being incubated on ice for 30 min, the suspensions were centrifuged at 105,000 × g for 30 min to obtain supernatants and membrane pellets. Phase separation of the indicated samples in a Triton X-114 solution was carried out by the method of Bordier (38).

RESULTS
cDNA Cloning and Predicted Structure of GCP170—Based on the available SY2 sequence (28), a cDNA fragment was obtained by the reverse transcription-PCR method using QGP-1 RNA as a template (PCR probe in Fig. 1A). Screening of 1 × 10^6 independent clones of the QGP-1 cDNA library yielded the probe yielded 11 positive clones, of which clone QSY103 had the longest insert of 3.3 kb. Because Northern blot analysis showed the presence of a single 7-kb RNA (Fig. 1B), we further screened another cDNA library constructed from a 7-kb RNA-enriched fraction and finally isolated a clone, FQSY1024, with a longer insert of 6.6 kb containing an open reading frame (Fig. 1A).

The open reading frame encodes a protein of 1550 amino acid residues with a calculated mass of 170 kDa (Fig. 2A). We
designated the protein GCP170 (for the Golgi complex-associated protein of 170 kDa). The protein does not contain a sequence characteristic of a signal sequence at the NH2 terminus nor a hydrophobic domain sufficient to span a membrane in the entire sequence, as shown by a hydropathy analysis (Fig. 2B).

Search of the protein data base shows that GCP170 has no overall homology to known proteins nor particular motifs except for phosphorylation sites. Secondary structure analysis for coiled-coil probabilities (40) predicts that GCP170 consists of three major domains: an NH2-terminal non-coil (positions 1–400), a long α-helical domain with strong coiled-coil forming potential (positions 400–1400), and a COOH-terminal non-coil domain (1400–1530), although the coiled-coil domain has an interruption (about 60 residues) (Fig. 2C). The NH2-terminal non-coil domain contains a proline-rich region and a serine-rich region (Fig. 2A). Thus, it is likely that GCP170 has a domain structure with a globular head, long stalk, and short tail, similar to kinesin (41) and CLIP-170 (42), although they have no significant homology to each other.

**Intracellular Distribution and Membrane Interaction of GCP170—**Immunofluorescence microscopy with anti-recombinant GCP170 showed that GCP170 was concentrated at juxtanuclear regions corresponding to the Golgi complex, although its presence in the cytoplasm was also suggested by faint and diffuse staining of whole cells (Fig. 3a). The same perinuclear regions were co-stained with antibodies to GCP372, a Golgi-membrane-anchored protein (21) (Fig. 3b). The general staining pattern of GCP170 was most similar to that of β-COP, a subunit of coatomer (Fig. 3c). Both GCP170 and β-COP displayed heavy staining of the Golgi region and faint staining throughout the cytoplasm, in contrast to the staining profile of GCP372, which was confined to the Golgi region. A more detailed localization of GCP170 was attained by immunoelectron microscopy (Fig. 3d). The immunogold particles were detected at the rims of cisternal structures and related elements characteristic of the Golgi complex. The gold particles were not significantly detected in other organelles including nuclei, mitochondria, and lysosomes, although some were found in the cytoplasm free from membrane association.

A postnuclear fraction of HepG2 cells was subfractionated into cytosol, total membrane, and Golgi fractions, which were analyzed by Western blotting. Anti-GCP170 antibodies recognized a 170-kDa protein not only in the Golgi and total membrane fractions but also in the cytosol (Fig. 4A). GCP170 was found to be more abundant in the cytosol than in the total membranes, although it was much enriched in the Golgi fraction. The membrane topology of GCP170 was examined using protease treatment. When the intact Golgi fraction was incubated with trypsin and chymotrypsin, GCP170 was completely digested even in the absence of a detergent (Fig. 4B, upper panel), whereas Man II, which has a cytoplasmic tail of only 5 amino acids, was degraded only when incubated with the detergent (Fig. 4B, lower panel). In addition, GCP170 was completely released from the membrane with sodium carbonate, although not completely extracted with 1 M NaCl, and entirely partitioned into an aqueous phase when subjected to Triton X-114 phase separation (Fig. 4C). Taken together, these results indicate that GCP-170 is a peripheral protein associated with the cytoplasmic face of the Golgi membrane.

**Expression of GCP170 cDNA—**The product encoded by the GCP170 cDNA was examined by transfection experiments in baby hamster kidney cells. Because the anti-GCP170 antibodies were found to cross-react with cells derived from other species and could not distinguish exogenously introduced GCP170 from the endogenous protein, we transfected the plasmid pGFP/C1/GCP170 into cells, which expressed a fusion protein containing the GFP as a reporter. Fluorescence microscopy shows essentially the same profile between the immunostaining and the GFP image (Fig. 5A, a and b), demonstrating that the fusion protein expressed is concentrated in the Golgi complex. Immunoprecipitation experiments were also carried out, for which cells were transfected with the plasmid pSG5 carrying the GCP170 cDNA and metabolically labeled with [35S]methionine. GCP170 expressed in the transfected cells was very heavily labeled and had the same molecular mass as that from HepG2 cells (Fig. 5B, lanes 1 and 3). In this experiment, we used 5% gels for SDS-PAGE, under the conditions of which GCP170 was resolved into two closely migrating forms, although they migrated as an apparently single form when analyzed by SDS-PAGE on 7% gels (Fig. 4). Smaller bands detected in the transfected cells (Fig. 5B, lane 1) may be mostly degradation products of GCP170.

**Phosphorylation of GCP170—**The presence of the doublet GCP170 (Fig. 5B) may be explained by the expression of independent molecules with similar epitopes reactive with the anti-GCP170 or by modifications such as phosphorylation and limited proteolysis of the single molecule. Because the protein contains many potential phosphorylation sites that are substrates for protein kinase C and casein kinase II, we examined a possible phosphorylation of GCP170. An immunoprecipitate prepared from cells that had been metabolically labeled with [35S]methionine contained a single [35S]-labeled protein (Fig. 6, lane 2), which comigrated on SDS-PAGE (5% gel) with the slow-migrating form of the two [35S]methionine-labeled polypeptides (Fig. 6, lane 1). When the [35S]-labeled sample was treated with alkaline phosphatase, the doublet was converted into a single form that co-migrated with the faster one of the original sample (Fig. 6, lanes 3 and 4). These results indicate that the slower molecule is a phosphorylated form of GCP170.

The two forms with or without phosphorylation were found...
FIG. 2. Sequence and structural analysis of GCP170. A, amino acid sequence of GCP170 predicted by the cDNA sequence. A proline-rich region (double underline), a serine-rich region (dotted underline), and heptad-repeat domains (single underline) are shown. B, hydropathy plot analysis of GCP170 using a window of 9 amino acids for scanning. Line segments above and below the horizontal axis indicate hydrophobic and hydrophilic portions, respectively. C, predicted coiled-coil structure of GCP170 analyzed by the method of Lupas et al. (40).
in both the membrane and cytosol fractions. When the Golgi membrane was treated with various concentrations of KCl, the membrane and cytosol fractions (lanes 5 and 6) or detergent (D) fractions (lanes 5 and 6). The samples were immunoblotted with anti-GCP170.

Rapid Response of GCP170 to Brefeldin A—When cells were incubated with BFA, GCP170 localized in the Golgi (Fig. 7a) was rapidly dispersed into the cytoplasm, detected already at 2 min after the treatment (Fig. 7b). This is in contrast to the response of GCP372, an integral membrane protein, which was still retained in the Golgi region at 2 min (Fig. 7f) and gradually distributed to the endoplasmic reticulum-like structures (Fig. 7g). The rapid response of GCP170 was quite similar to that of β-COP (Fig. 7, i–k). Removal of BFA from the culture medium allowed GCP170 to recover the normal Golgi distribution (Fig. 7d), as observed for the other proteins (Fig. 7, h and l).

No Interaction of GCP170 with COP Coatomer—GCP170 is present both as a soluble form in the cytosol and as a membrane-associated one in the Golgi. In addition, GCP170 is dissociated from the Golgi as rapidly as β-COP in response to BFA and has a molecular mass close to that estimated for α-COP (2, 7). These properties raise the possibility that GCP170 is a subunit of the COP coatomer. To test this possibility, we examined whether GCP170 was coprecipitated with anti-β-COP. The immunoprecipitate was found to contain four major components with apparent molecular masses of 150, 100, 95, and 60 kDa and several minor components (Fig. 8a, lane 1), which may correspond to those identified in the coatomer complex (7).

Effect of Microtubule Disruption on GCP170 Distribution—It
FIG. 7. Response of GCP170 to BFA treatment. HeLa cells were incubated with BFA (1 μg/ml) for 0 (a, e, and i), 2 (b, f, and j), or 20 min (c, g, and k) at 37 °C, fixed, and double-immunostained for GCP170 (a–c) and GCP372 (e–g) or stained for β-COP alone (i–k). Cells were treated with BFA (1 μg/ml) for 30 min and then incubated for 60 min in a fresh medium without BFA (d, h, and l). After being fixed, the cells were double-immunostained for GCP-170 (d) and GCP372 (h) or stained for β-COP alone (l). Scale bar, 10 μm.

Fig. 8. Analysis of interaction of GCP170 with COPs. A, HepG2 cells were labeled with [35S]methionine for 5 h, lysed, and immunoprecipitated with anti-β-COP under low stringent washing conditions (lane 1). The remaining supernatant was immunoprecipitated with anti-GCP170 (lane 2). The immunoprecipitates were analyzed by SDS-PAGE (7% gels) and fluorography. An arrow indicates the position of GCP170. B, cryosections of HepG2 cells were incubated with anti-GCP170 and then with 12 nm gold-conjugated protein A. After being washed, the sections were incubated with anti-β-COP and then with 6 nm gold-conjugated protein A. Large particles are those stained for GCP170, whereas smaller ones indicated by arrowheads are those for β-COP. The arrows indicate the stack structure. GC, Golgi complex. Scale bar, 0.2 μm.

is known that microtubules and associated proteins are not extracted with 1% Triton X-100 under some conditions (20, 43). When cells were treated with 1% Triton X-100 under microtubule-stabilizing conditions (Fig. 9f), GCP170 and the integral membrane protein GCP372 were retained in the juxtanuclear region (Fig. 9d and e), although the diffusely stained cytoplasmic GCP170 in control cells (Fig. 9a) was no longer seen in the treated cells. Under microtubule-destabilizing conditions, however, GCP170 and GCP372 were completely extracted from the cells with the detergent and were undetectable in the Golgi (data not shown). The results indicate that GCP170 is very tightly associated with the Golgi membrane, being resistant even to the detergent extraction, as long as the Golgi structure is stabilized.

We further examined the effect of the microtubule-depolym-erizing drug nocodazole on the localization of GCP170. Incubation of cells at 20 °C in the presence of nocodazole resulted in complete depolymerization of microtubules (Fig. 9i) but caused little effect on the distribution of GCP170 as well as GCP372 (Fig. 9, g and h). In contrast, when cells were incubated at 37 °C with the drug, the Golgi complex was disrupted into fragments where GCP170 was co-localized with GCP372 (Fig. 9, j and k). It is known that the drug causes the fragmentation and dispersion of the Golgi in an energy- and temperature-dependent manner (36) and that the fragmented Golgi is still functional in transport and modification of proteins, although the transport rate is not the same as in the intact Golgi (44). Taken together, these results suggest that GCP170 is not directly associated with microtubules but intimately associated with the functional unit of the Golgi.

DISCUSSION

Golgin-160 was initially identified as a Golgi-associated protein by Fritzler et al. (28) using autoantibodies from a patient with systemic lupus erythematosus. They also isolated two overlapping cDNA clones (SY2 and SY10) for golgin-160, of which SY10 had a slightly longer extension (54 nucleotides) at the 5′-end. The combined sequence was found to encode only a partial sequence (579 amino acid residues) of the protein. In the present study, based on the available sequence of the golgin-160 cDNA (SY2), we have isolated a full-length cDNA clone that encodes a 1530-residue protein of 170 kDa. The sequence encoded by SY2 was found between positions 788 and 1348 from the NH2 terminus of the entire sequence we determined. The sequence of the 5′-extension of SY10, however, was very different from our data, resulting in 53.7 and 44.4% identity in the corresponding nucleotide and amino acid sequences, respectively, between the two. Based on such differences in the amino acid sequence and the predicted molecular mass, we designated our protein GCP170.

GCP170 was found to be phosphorylated. The phosphorylated form, when analyzed by SDS-PAGE (5% gels), migrated to a position with an apparent molecular mass slightly (at least 5 kDa) larger than the unphosphorylated one. Such a difference in the mobility reflects a hyperphosphorylation of the protein.
In fact, the protein contains many phosphorylation sites by protein kinase C (26 sites) and casein kinase II (31 sites) and was heavily labeled with $^{32}$P in cells. The two forms with or without phosphorylation were found both in the Golgi membrane and in the cytosol at a similar proportion, suggesting that phosphorylation of the protein does not affect its binding to the membrane. Of the two forms in the Golgi membrane, however, the phosphorylated form was more easily dissociated from the membrane when treated with KCl. Thus, it is likely that the tightness of its association with the membrane is influenced by the phosphorylation.

The properties of GCP170 are quite similar to those of p200. p200 is a phosphoprotein that is also found in the Golgi membrane and in the cytosol and is very rapidly dissociated from the membrane in the presence of BFA (45), as observed for GCP170 and $\beta$-COP. The binding of p200 to the membrane is regulated by activation of heteromeric GTP-binding proteins with GTPyS, AlF$_4^-$, or mastoparan in vitro (46). Activation of GTP-binding proteins induces budding and accumulation of small vesicles from the trans-Golgi network where p200 is localized, suggesting the involvement of p200 in the formation of transport vesicles from the trans-Golgi network (47). In contrast, our preliminary experiments showed that incubation of the Golgi membrane with cytosol and GTPyS did not increase the binding of GCP170 to the membrane (data not shown). A structural comparison of the two proteins cannot be made, because the primary structure of p200 is still not available. However, the difference in the molecular mass and response to GTPyS could rule out the possibility that GCP170 and p200 are the same protein.

One of the structural features predicted is that GCP170 contains a long $\alpha$-helical domain with high potential to form a coiled-coil structure. It is of interest to note that such a long coiled-coil domain is found in other Golgi-associated proteins including giantin (22) or GCP372 (21), golgin-245 (23) or p230 (20), and golgin-95 (28) or GM130 (27). All these proteins are associated with the cytoplasmic face of the Golgi membrane. Giantin/GCP372 is an integral membrane protein anchored by the COOH-terminal domain and is almost entirely occupied by the coiled-coil structure (21, 22). Golgin-245/p230 is a peripheral protein localized to trans-Golgi elements. GM130/golgin-95 is also a peripheral protein but is tightly associated with the cis-Golgi cisternae. Because these proteins exist as dimers (20, 27), as expected by their coiled-coil structures, it is likely that GCP170 is also present as a dimer in cells. Although the functions of these proteins in cells remain to be determined, a possible function of GCP170 may be to stabilize the Golgi structure by interacting with other Golgi-associated proteins including the above mentioned proteins. In fact, GCP170 and giantin (20) are not extracted from the Golgi complex with 1% Triton X-100 under microtubule-stabilizing conditions, whereas the other integral membrane proteins Man II and galactosyl transferase are completely extracted under the same conditions. The possibility that GCP170 is involved in the stabilization of the Golgi complex may be closely related with the SNARE hypothesis (19) in which one of the proposals is to postulate the presence of clamps for preventing the membrane fusion and maintaining the Golgi stack structure. GCP170 may be considered a candidate for the clamps.

GCP170 is predicted to have a head-stalk-tail structure. A similar domain structure is found in p115 (31), CLIP-170 (42), and kinesin (41), which are involved in vesicular transport. p115 was initially identified as a factor required for cis to medial Golgi transport (31) and found to be identical to the transcytosis-associated protein TAP (48). p115/TAP is a mammalian homolog of the yeast Uso1p that is involved in vesicular transport from the endoplasmic reticulum to the Golgi (49). In contrast, CLIP-170, which has strong similarity in secondary structure with GCP170, is involved in endocytosis; it may act as a linker by binding to microtubules at the head domain and to endocytic carrier vesicles at the COOH-terminal domain. The binding to microtubules is regulated by phosphorylation of
the head domain (42). In addition to the secondary structure, CLIP-170 and GCP170 have similar properties in that each head domain is basic and contains a serine-rich region. These observations raise the possibility that GCP170 is also involved in vesicular transport, although there is no direct evidence at present.

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