Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 4319–4323, May 1992
Cell Biology

Golgi retention of a trans-Golgi membrane protein, galactosyltransferase, requires cysteine and histidine residues within the membrane-anchoring domain

(glycosyltransferase/glycosylation/mutagenesis/intracellular targeting transport/oligomerization)

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Communicated by Stuart Kornfeld, January 23, 1992

ABSTRACT **Galactosyltransferase (GT; UDPgalactose:β-D-*N*-acetylglucosaminide β-1,4-galactosyltransferase, EC 2.4.1.22) is a type II membrane-anchored protein composed of a short N-terminal cytoplasmic tail, a signal/membrane-anchoring domain, and a stem region followed by a large catalytic domain including the C terminus. To identify the peptide segment and key amino acid residues that are critical for Golgi localization of GT, the expression vector pGT·hCG was designed to encode the entire GT molecule fused to the C-terminal region of human chorionic gonadotropin α subunit (hCGα) as a reporter. COS-1 cells transfected with pGT·hCG expressed the chimera in the Golgi region, as detected by immunofluorescence microscopy using anti-hCG antibodies. Two deletion mutants, Δ tail and Δ stem, which are lacking most of the N-terminal cytoplasmic tail or 10 amino acids immediately after the membrane-anchoring domain, were localized in the Golgi. Replacement mutations of the membrane-anchoring domain of GT showed that the second quarter of the transmembrane domain or Cys^{29}-Ala^{30}-Leu^{31}-His^{32}-Leu^{33} is necessary for GT to be retained in the Golgi. Furthermore, the point mutants $Cys^{29} \rightarrow Ser^{29}$ and $His^{32} \rightarrow Leu^{32}$ were partially transported to the plasma membrane, whereas an Ala^{30}-$Leu^{31} \rightarrow Phe^{30}$-$Gly^{31}$ mutant was localized in the Golgi. Finally, a double mutant, $Cys^{29}/His^{32} \rightarrow Ser^{29}/Leu^{32}$, was found to be transported efficiently to the plasma membrane. The signal-anchoring domain of the transferrin receptor, a type II plasma membrane protein, was then replaced by portions of the GT transmembrane domain. Although the Cys-Xaa-Xaa-His sequence by itself cannot retain the transferrin receptor in the Golgi, the cytoplasmic half of the transmembrane domain of GT was partially capable of retaining the transferrin receptor in the Golgi. These results suggest that the cytoplasmic (or N-terminal) half of the transmembrane domain of GT contributes to the Golgi retention signal and that particularly Cys^{29} and His^{32} in this region are critical for GT to be retained in the Golgi.**

Eukaryotic cells contain subcellular organelles in which specific proteins reside. Defining the mechanisms that cause targeting and sorting of particular proteins to the organelles is essential to understand how subcellular organelles are formed. Although studies on resident endoplasmic reticulum (ER) and lysosome proteins have progressed recently (1, 2), information on the characteristic localization of Golgi resident proteins has been quite limited. Glycosyltransferases, as indicated by their cDNA sequences, are all type II membrane proteins (3). Thus, each of these proteins is composed of a short N-terminal cytoplasmic tail followed by an uncleavable signal membrane anchor, a presumptive stem region, and a large catalytic domain including the C terminus. Each of these glycosyltransferases is localized specifically in one of the cis-, medial-, or trans-Golgi compartments or in the trans-Golgi network (4).

Subcellular localization of galactosyltransferase (GT; UDPgalactose:β-D-*N*-acetylglucosaminide β-1,4-galactosyltransferase, EC 2.4.1.22) has been examined by immunoelectron microscopy, and the localization of this enzyme in the trans-Golgi cisternae is well documented (5). The soluble form of GT is derived from the membrane-bound form by proteolytic cleavage at the stem region (6, 7) and subsequent release of the catalytic domain (12). Because the catalytic domain by itself does not stay in the Golgi and is secreted from the cells, it is likely that the Golgi retention signal lies somewhere in the region composed of the tail, membrane-anchoring domain, and stem region.

In the present study, we have introduced a series of deletion mutations and replacement mutations into the tail, membrane anchor, and stem region of the GT and found that Cys^{29} and His^{32} within the membrane-anchoring domain are the most critical amino acids for GT to be retained in the Golgi.

MATERIALS AND METHODS

Expression Vector and cDNAs. The eukaryotic expression vector pcDNAI, which is a modified form of the pCDM8 vector (8), was obtained from Invitrogen (La Jolla, CA). The cDNAs for human GT were isolated as reported (6). The cDNA for human chorionic gonadotropin α chain (hCGα) (9) was kindly provided by J. Rose (Yale University, New Haven, CT). The cDNA for human transferrin receptor (10) and monoclonal anti-transferrin receptor antibodies (11) were kindly provided by I. Trowbridge (The Salk Institute, La Jolla, CA).

Plasmid Construction and Mutagenesis. GT cDNA encoding the full-length coding region for GT was fused to the *Xba* I/*Bam*HI hCGα fragment (350 base pairs) encoding the C-terminal half of hCGα in-frame. The cDNA encoding full-length GT and the hCG chimera was ligated in pcDNAI vector (8) at the *Eco*RI and *Xho* I sites. The resulting pcDNAI plasmid is designated as pGT·hCG. Site-specific mutagenesis for all deletion mutants, replacement mutants, and point mutants was performed as described (12).

The cDNA for human transferrin receptor was inserted at *Hin*dIII and *Eco*RI sites of pcDNAI. Deletion of the cyto-

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Abbreviations: GT, galactosyltransferase; hCG, human chorionic gonadotropin; FITC, fluorescein isothiocyanate; ER, endoplasmic reticulum.

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plasmic tail from the transferrin receptor was achieved by PCR (13) using oligonucleotides $TR^{GTm\Delta tail}$ and TR^{Rev} as primer and TR^{GTm} mutant cDNA as template.

Transfection of COS-1 Cells and Immunofluorescence Microscopy. Transfection of COS-1 cells was carried out by the DEAE-dextran method (14). After culturing for 48 hr, the cells on coverslips were fixed with 1% paraformaldehyde for 15 min and then treated with phosphate-buffered saline (PBS) containing 1% bovine serum albumin with or without 0.1% saponin (15). The cells were incubated with anti-hCG antiserum at room temperature for 1 hr. After washing with PBS, cells were stained with fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG $(Fab')_2$ and examined by immunofluorescence microscopy. Immunofluorescence for COS-1 cells expressing transferrin receptor was performed by using a monoclonal anti-transferrin receptor antibody and FITC-labeled rabbit anti-mouse IgG $(Fab')_2$ fragment.

Flow Cytometry. The COS-1 cells were harvested from the tissue culture plate by incubation in enzyme-free cell dissociation solution (Specialty Media, Lavallette, NJ). The cells were incubated with rabbit anti-hCG antibodies on ice for 20 min, followed by incubation with FITC-conjugated goat anti-rabbit IgG $(Fab')_2$ fragment on ice for 20 min. The cells were suspended in the cell dissociation solution containing propidium iodide (10 μg/ml) before being subjected to analysis with a FACScan (Becton Dickinson).

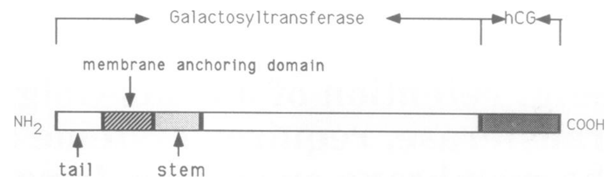
Immunoprecipitation. The COS-1 cells transfected with expression vector plasmid were metabolically labeled with $[^{35}S]$methionine plus $[^{35}S]$cysteine in methionine-free RPMI medium, supplemented with $Tran^{35}S$-label (ICN Radiochemicals) (100 μCi/ml; 1 Ci = 37 GBq) for 1 hr, and chased with complete medium for 2 hr. Cell lysates were prepared with 1% Nonidet P-40. Immunoprecipitation was performed with anti-hCG antibodies (Cappel Laboratories) as described (16), except protein G Sepharose (Pharmacia) was used in place of *Staphylococcus aureus* cells.

Assay of GT Activity. The COS-1 cells transfected with pcDNAI vector alone or pGT·hCG plasmid were harvested with rubber policemen. The crude homogenates were prepared as described (17). Enzyme activity of GT was assayed as described (12) with $[^3H]$UDPgalactose and di-*N*-acetylchitobiose.

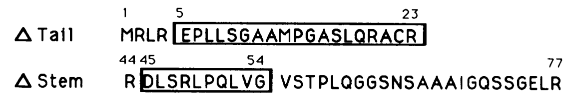
RESULTS

Expression of GT·hCG Chimera in COS-1 Cells. GT is expressed in all mammalian cells. In our studies, it is important to be able to distinguish between exogenous human GT and the endogenous monkey GT expressed in COS-1 cells. We therefore chose to tag GT with hCG peptide. The full-length human GT peptide was fused to the 59 C-terminal amino acids of the hCGα peptide (Fig. 1). The fusion protein can readily be detected by anti-hCG antibodies, since hCG, a peptide hormone, is not expressed naturally in COS-1 cells. The cDNA coding for this fusion protein was inserted into the eukaryotic expression vector pcDNAI. COS-1 cells were transfected with this plasmid DNA and expression of the fusion protein was examined as follows.

Crude homogenates of COS-1 cells were assayed for GT activity. The homogenate prepared from the transfected COS-1 cells with pGT·hCG exhibited a GT activity of 35.9×10^{-3} units per mg of protein, while the homogenate prepared from the transfected COS-1 cells with vector alone showed a GT activity of 9.9×10^{-3} units per mg of protein. As the transfection efficiency in our experiments was 20–30%, this degree (3.6-fold) of overexpression is similar to the previously reported activity levels seen for COS cells transfected with GT cDNA (18, 19). The transfected COS-1 cells were metabolically labeled and the immunoprecipitates with anti-hCG antibodies from the total cell lysate were examined by



Deletion Mutants (tail and stem region)



Replacement Mutants and Point Mutants (membrane anchoring domain)

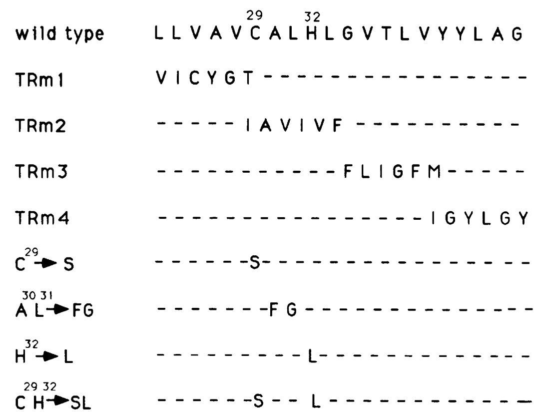


FIG. 1. Schematic diagram of wild-type GT·hCG chimeric protein and various mutants of GT·hCG used in this study. The tail and stem region was examined by deletion mutations. ΔTail mutant lacks 19 amino acids (residues 5–23; boxed) in the 23-amino acid N-terminal cytoplasmic tail. ΔStem mutant lacks 10 amino acids (residues 45–54; boxed) immediately after the membrane-anchoring domain. The membrane-anchoring domain was examined by replacement mutations and point mutations. In TRm1, -2, -3, and -4, all five amino acids in the membrane-anchoring domain of GT were replaced with the corresponding six amino acids of the membrane-anchoring domain of the transferrin receptor.

SDS/PAGE followed by fluorography (Fig. 2). Two bands with apparent molecular masses of 63 and 56 kDa were detected as specific immunoprecipitates. Earlier studies on native GT in various types of cells also showed two bands, and the upper and lower bands were characterized as a mature form GT with both *N*- and *O*-glycans and a precursor form GT with *N*- but not *O*-glycans, respectively (20). These results indicate that upon transfection of pGT·hCG plasmid, COS-1 cells express enzymatically active and immunologically recognizable GT·hCG chimera molecules.

Subcellular localization of the GT·hCG chimera was examined by immunofluorescence microscopy. In COS-1 cells, GT localization was restricted to a crescent-shaped reticular pattern adjacent to the nucleus (Fig. 3*A*). This pattern of immunofluorescence is characteristic for the Golgi complex and is seen with native GT (5, 21). Mock transfections, or transfections with vector alone, resulted in no detectable immunofluorescence signal (data not shown).

About 20% of transfected COS-1 cells apparently expressed a high level of GT·hCG, and such cells exhibited an immunofluorescence signal not only in the Golgi region but throughout the cytoplasm in a reticular structure characteristic of the ER. Similar results have been reported (17, 22, 23) for expression of Golgi proteins. These observations suggest

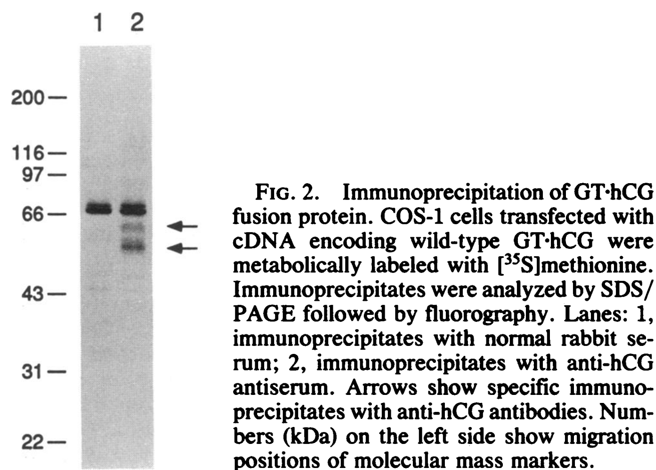


FIG. 2. Immunoprecipitation of GT·hCG fusion protein. COS-1 cells transfected with cDNA encoding wild-type GT·hCG were metabolically labeled with [^{35}S]methionine. Immunoprecipitates were analyzed by SDS/PAGE followed by fluorography. Lanes: 1, immunoprecipitates with normal rabbit serum; 2, immunoprecipitates with anti-hCG antiserum. Arrows show specific immunoprecipitates with anti-hCG antibodies. Numbers (kDa) on the left side show migration positions of molecular mass markers.

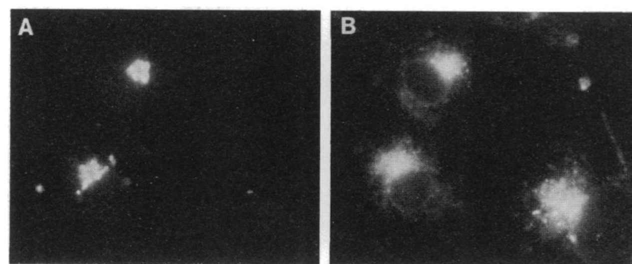


FIG. 3. Immunofluorescence of COS-1 cells expressing wild-type GT·hCG chimera. (*A*) COS-1 cells were transfected with pGT·hCG vector. The cells were fixed and permeabilized with saponin. The GT·hCG chimeric protein was detected with anti-hCG antibodies and FITC-conjugated goat anti-rabbit IgG. (*B*) Immunofluorescence of COS-1 cells with rhodamine-conjugated wheat germ agglutinin. The same field of COS-1 cells as in *A* is shown.

that the high level of expression of these Golgi proteins results in their detection in the ER.

As shown in Fig. 3, immunofluorescence of the fusion protein GT·hCG in COS-1 cells partially overlaps with the area stained with wheat germ agglutinin. This lectin, which binds to the glycoconjugates having *N*-acetylglucosamine and sialic acid, has often been used as a Golgi marker reagent, but this lectin can also detect lysosomes and plasma membrane (24). Immunofluorescence with the GT·hCG, however, did not reveal staining of the lysosomes and plasma membrane (Fig. 3). These results indicate that recombinant GT·hCG expressed in COS-1 cells is mostly localized in the Golgi, where the GT naturally resides.

Identification of Peptide Segment Necessary for Golgi Retention. As the first step toward finding the peptide segment involved in the Golgi retention of GT, two deletion mutants, Δ tail and Δ stem, were made (see Fig. 1). The Δ tail mutant has a deletion from Glu^{5} to Arg^{23} and thus lacks most of the cytoplasmic tail except for Met^{1}-Arg^{2}-Leu^{3}-Arg^{4}. The Δ stem mutant has a deletion from Asp^{45} to Gly^{54} and thus lacks 10 amino acids adjacent to the membrane-anchoring domain in the stem region. The subcellular localization of these mutant proteins in the COS-1 cells was examined by immunofluorescence microscopy. As shown in Fig. 4, both Δ tail and Δ stem mutants localized in the Golgi (Fig. 4*I A* and *B*). The results suggest that Golgi retention of GT is not critically affected by these deleted peptide segments.

The membrane-anchoring domain of GT was then examined by replacement mutations (see Fig. 1). This domain, which consists of 20 amino acids, was divided into four blocks. Each block of the GT membrane anchor was replaced with 6 amino acids from the corresponding membrane-anchoring domain of the transferrin receptor. The transferrin receptor was chosen because it is also a type II membrane-anchoring protein (10) and is transported to the plasma membrane. Thus, the transferrin receptor is assumed not to have a Golgi retention signal.

Immunofluorescence of the TRm1 mutant showed tight juxtanuclear staining (Fig. 4*I C*) but never showed crescent-shaped reticular distribution, a pattern that was characteristically observed in the immunofluorescence staining of wild-type GT·hCG. This suggests that the TRm1 mutant localizes in a restricted region of the Golgi but not trans-Golgi. It is also possible that the TRm1 mutant is accumulated in the so-called Russell bodies, organelles accumulating truncated proteins that can neither exit from, nor be degraded in, the ER (25).

Localization of the TRm2 mutant showed that this mutant was transported to the plasma membrane (Fig. 4*I D*). Strong to moderate surface expression of the TRm2 mutant was detected by flow-cytometry analysis (Fig. 5*B*). On the other hand, both the TRm3 and TRm4 mutants localized in the Golgi (Fig. 4*I E* and *F*). These results suggest that the second block of the GT membrane-anchoring domain composed of Cys^{29}-Ala^{30}-Leu^{31}-His^{32}-Leu^{33} plays a critical role in Golgi retention of GT.

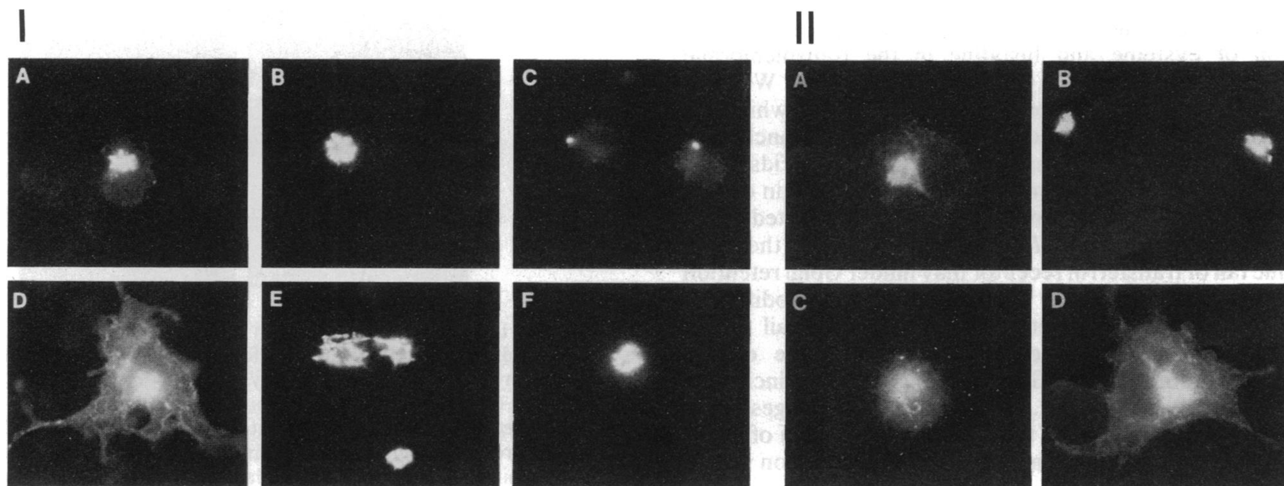


FIG. 4. Immunofluorescence of COS-1 cells transfected with pGT·hCG mutant DNAs. (*I*) Localization of each deletion mutant and replacement mutant protein. (*A*) Δ tail. (*B*) Δ stem. (*C*) TRm1. (*D*) TRm2. (*E*) TRm3. (*F*) TRm4. (*II*) Localization of each point-mutant protein. (*A*) Cys^{29} → Ser^{29}. (*B*) Ala^{30}-Leu^{31} → Phe^{30}-Gly^{31}. (*C*) His^{32} → Leu^{32}. (*D*) Cys^{29}/His^{32} → Ser^{29}/Leu^{32}. In both *I* and *II*, the cells were permeabilized before being subjected to immunostaining.

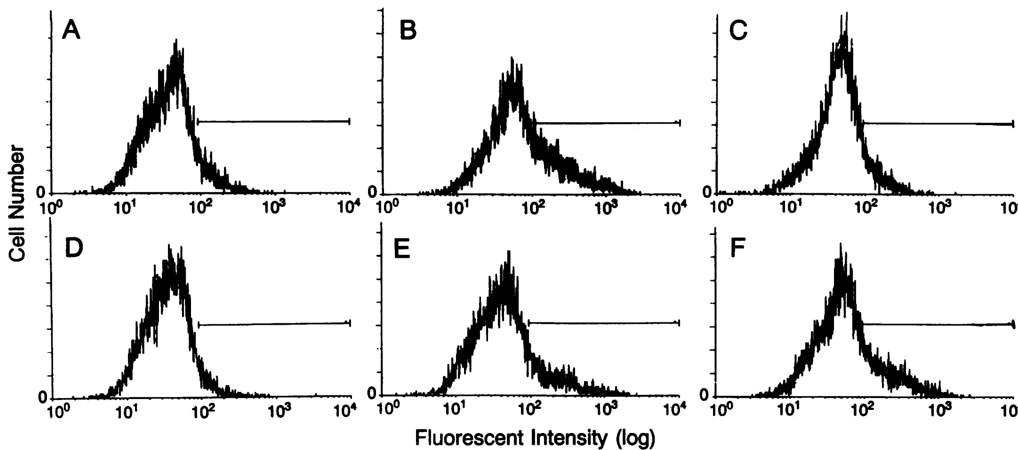


FIG. 5. Flow cytometry analysis of cell-surface expression of GT·hCG mutant proteins on COS-1 cells. (*A*) Wild-type GT·hCG. (*B*) TRm2 mutant. (*C*) $Cys^{29} \rightarrow Ser^{29}$ mutant. (*D*) Ala^{30}-$Leu^{31} \rightarrow Phe^{30}$-$Gly^{31}$ mutant. (*E*) $His^{32} \rightarrow Leu^{32}$ mutant. (*F*) $C^{29}/H^{32} \rightarrow Ser^{29}/Leu^{32}$ double mutant. The proportion of cells gated as positive for each cell population (horizontal bars) was 9% in *A*, 33% in *B*, 12% in *C*, 6% in *D*, 17% in *E*, and 24% in *F*.

Identification of Amino Acids Necessary for Golgi Retention. To define further which particular residues in the region from Cys^{29} to Leu^{32} are important for Golgi retention, point mutants were constructed (see Fig. 1). As shown in Fig. 4, a double point mutation, Ala^{30}-$Leu^{31} \rightarrow Phe^{30}$-$Gly^{31}$, had no effect on Golgi localization (Fig. 4*II B*). Significantly, however, the point mutants $Cys^{29} \rightarrow Ser^{29}$ and $His^{32} \rightarrow Leu^{32}$ showed diffuse Golgi staining (Fig. 4*II A* and *C*). Weak but definite surface expression was detected by immunofluorescence microscopy (data not shown) as well as by flow cytometry (Fig. 5 *C* and *E*). However, the $Cys^{29} \rightarrow Ser^{29}$ and $His^{32} \rightarrow Leu^{32}$ mutations still permitted some of the mutant protein to be retained in the Golgi (Fig. 4*II A* and *C*). Finally, a double-mutant $Cys^{29}/His^{32} \rightarrow Ser^{29}/Leu^{32}$ was constructed. The staining pattern of this mutant was quite similar to that observed with the TRm2 mutant (Fig. 4*II E*) and was transported efficiently to the cell surface. These results indicate that efficient retention of GT in the Golgi depends on the presence of both Cys^{29} and His^{32} residues.

A Potential Golgi Retention Signal of the Transmembrane Domain Containing Cys^{29} and His^{32}. As described above, our results indicated that the cytoplasmic (or N-terminal) half of the transmembrane domain of GT is necessary for Golgi targeting and retention. We therefore tested whether this region is capable of retaining any type II protein, such as transferrin receptor, in the Golgi.

The COS-1 cells expressing cDNA encoding wild-type transferrin receptor gave a strong surface staining (Fig. 6*II A*). A transferrin receptor mutant, TR^{CH}, in which Val^{73} and Phe^{76} were replaced by Cys^{73} and His^{76}, was transported to the cell surface (Fig. 6*II B*), indicating that the mere introduction of cysteine and histidine in the transmembrane domain does not create a Golgi retention signal. We constructed a transferrin receptor mutant, TR^{GTm}, in which the cytoplasmic 10 amino acids of the membrane anchor of transferrin receptor were replaced with 10 amino acids of the cytoplasmic half of the membrane-anchoring domain of GT. The TR^{GTm} mutant was, however, largely transported to the plasma membranes (Fig. 6*II C*). Considering that the cytoplasmic tail of transferrin receptor may hinder Golgi retention in this particular case, the TR^{GTm} mutant was modified to $TR^{GTm\Delta tail}$ mutant by deleting the cytoplasmic tail of the transferrin receptor. The immunofluorescence of the $TR^{GTm\Delta tail}$ mutant in the Golgi was significantly increased compared to wild type (Fig. 6*II D*). This result suggests that the cytoplasmic half of the transmembrane domain of GT is partially capable of functioning as the Golgi retention signal.

DISCUSSION

The present study was aimed at identifying the peptide segment and the key amino acid residues responsible for Golgi retention of GT. The results obtained by deletion, replacement, and point-mutation experiments (Figs. 1, 4, and 5) indicate that the cytoplasmic side of the membrane-anchoring domain, particularly Cys^{29}-Xaa^{30}-Xaa^{31}-His^{32}, plays a critical role for GT to be retained in the Golgi. Observations of TRm1 and TRm2 mutants (Figs. 4 and 5) led us to focus on the cytoplasmic (or N-terminal) half of the transmembrane domain. A dramatic effect of the TRm2 replacement mutation and point mutations within the second quarter of the transmembrane domain indicates that this region is most important for Golgi retention of GT. It may

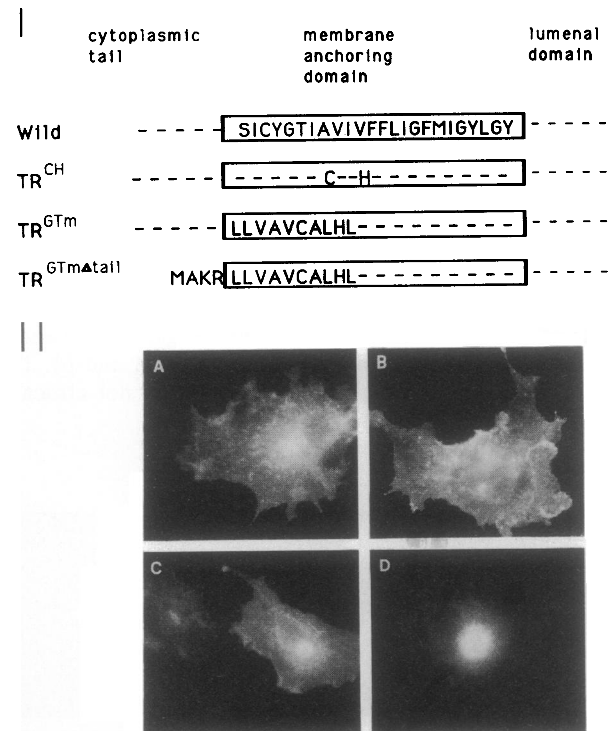


FIG. 6. Localization of transferrin receptor and its mutants. (*I*) Schematic diagram of wild-type transferrin receptor and mutants of transferrin receptor used in this study. In TR^{CH} mutant, Val^{73} and Phe^{76} in the transmembrane domain have been changed to Cys^{73} and His^{76}, respectively. In TR^{GTm} mutant, the cytoplasmic half of the transferrin receptor membrane-anchoring domain has been replaced with 10 amino acids from the cytoplasmic half of the membrane-anchoring domain of GT. In the $TR^{GTm\Delta tail}$ mutant, 60 amino acids of the cytoplasmic tail have been deleted from the TR^{GTm} mutant. (*II*) Immunofluorescence of COS-1 cells transfected with cDNAs encoding transferrin receptor and its mutant. (*A*) Wild type. (*B*) TR^{CH} mutant. (*C*) TR^{GTm} mutant. (*D*) $TR^{GTm\Delta tail}$ mutant.

also be possible that replacement mutations such as TRm2 changed the conformation of the other part of the transmembrane domain, resulting in release of GT from the Golgi. However, mutation of Cys^{29} to Ser^{29} should cause a minimal change of peptide conformation; yet, it clearly resulted in release of GT from the Golgi. We have also constructed the $His^{32} \rightarrow Phe^{32}$ and the $His^{32} \rightarrow Arg^{32}$ mutant. These mutants were not retained in the Golgi and gave a staining pattern similar to that of the $His^{32} \rightarrow Leu^{32}$ mutant (data not shown). These observations together with the results obtained with $Cys^{29} \rightarrow Ser^{29}$, $His^{32} \rightarrow Leu^{32}$, and $Cys^{29}/His^{32} \rightarrow Ser^{29}/Leu^{32}$ mutants favor the conclusion that Cys^{29} and His^{32} residues are directly involved in Golgi retention of GT. The unusual localization of the TRm1 mutant (Fig. 4*C*) suggests that this region is also necessary for proper targeting of GT to the Golgi apparatus. Further analysis is needed to define the role of this region.

In contrast to TRm1 and TRm2 mutations, we did not detect obvious effects of mutating the lumenal (or C-terminal) half of the transmembrane domain (see TRm3 and TRm4 mutations; Fig. 4). We focused on the tyrosine residues present in this region because tyrosine is known to play a critical role in endocytosis (2) and because in other glycosyltransferases the aromatic amino acids are present in the lumenal side of the transmembrane domain. Point mutations such as $Tyr^{39} \rightarrow Ala^{39}$ and Tyr^{39}-$Tyr^{40} \rightarrow Ala^{39}$-$Ile^{40}$, however, did not alter Golgi localization of GT (data not shown).

While this manuscript was in preparation, Nilsson *et al.* (22) reported the Golgi retention signal of GT. They showed that the lumenal half of the membrane-anchoring domain of GT can retain the GT-invariant chain chimera in Golgi (22). On the other hand, the present study indicates that the cytoplasmic half of the transmembrane domain of GT also can function as a Golgi retention signal in a dominant manner (Fig. 6). In addition, the presence of the Golgi retention signal within a membrane-anchoring domain has been shown for avian coronavirus El glycoprotein, which is retained in the cis-Golgi. An important feature of the Golgi retention signal described was the uncharged polar residues that line one face of an α-helix (26). As the predicted conformation of the cytoplasmic half of the transmembrane domain of GT is α-helix, Cys^{29} and His^{32} are likely to face the same side of the helix. This structure could be recognized by other protein(s) in the membranes, and such protein–protein interactions could be responsible for targeting and retention of GT in the Golgi.

The drastic effect of the mutation from Cys^{29} to Ser^{29} raised the question of whether Cys^{29} is involved in disulfide cross-linking. Earlier studies on native GT in HeLa cells and hepatoma cells provided no sign of di- or polymerization of GT in the Golgi membranes (20). The molecular mass of wild-type GT·hCG and TRm2 mutant proteins in nonreducing gels showed that the monomeric form is the major species for both wild-type GT·hCG and TRm2 mutant proteins (unpublished results). Therefore, intermolecular disulfide cross-linking, if it occurs at Cys^{29}, is not a necessary factor for Golgi retention.

The role of Cys^{29} and His^{32} for Golgi retention might be dependent on their unique structures. It is noteworthy that cysteine and histidine are often involved in metal binding sites. In zinc finger proteins, cysteines and histidines compose the zinc binding peptide motif (27). In cytochrome oxidases, these two amino acids bind iron and copper (28). A recently identified yeast zinc finger protein, Vps18p, plays a role in protein sorting in the late Golgi (29). Furthermore, the possibility for a zinc binding cation activator in the Golgi vesicle was predicted by kinetic analysis of GT in the Golgi (30). It is therefore an interesting possibility that GT molecules could interact with each other or bind other molecule(s) at Cys^{29} and His^{32} through metal ions such as zinc.

A possible mechanism for Golgi retention would be if the transmembrane regions of GTs recognize each other to form oligomers, which become large enough to immobilize themselves, as suggested for ER proteins (31). Oligomerization must be facilitated in the specific environments of the trans-Golgi, where some endogenous protein and/or lipid could promote oligomerization of the GT under appropriate pH and/or ionic strength conditions. It also seems possible that the GT dimer results in creation of an epitope that is recognized by an endogenous protein, which functions as a recycling receptor, the mechanism analogous to ER resident proteins retained by the Lys-Asp-Glu-Leu receptor (1, 32). The expression vector for the GT·hCG fusion protein and a series of mutants obtained in this study will be useful for testing these possible Golgi retention mechanisms.

We would like to thank Dr. Mark Williams for his helpful suggestions and critical discussions. We thank Dr. Donald Newmeyer for his pertinent advice on the manuscript, Allison Hughes for her excellent technical help, and Henny Bierhuizen for secretarial assistance. This work has been supported by a grant (R01-DK37016) from the National Institutes of Health. N.Y. is a recipient of a Uehara Memorial Foundation research fellowship.

1. Pelham, H. R. B. (1989) *Annu. Rev. Cell Biol.* **5**, 1–24.
2. Kornfeld, S. & Mellman, I. (1989) *Annu. Rev. Cell Biol.* **5**, 483–525.
3. Paulson, J. C. & Colley, K. J. (1989) *J. Biol. Chem.* **264**, 17615–17618.
4. Kornfeld, R. & Kornfeld, S. (1985) *Annu. Rev. Biochem.* **54**, 631–664.
5. Roth, J. & Berger, E. G. (1982) *J. Cell Biol.* **92**, 223–229.
6. Masri, K. A., Appert, H. E. & Fukuda, M. N. (1988) *Biochem. Biophys. Res. Commun.* **157**, 657–663.
7. Appert, H. E., Rutherford, T. J., Tarr, G. E., Thomford, N. R. & McCorquodale, D. J. (1986) *Biochem. Biophys. Res. Commun.* **138**, 224–229.
8. Aruffo, A. & Seed, B. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8573–8577.
9. Fiddes, J. C. & Goodman, H. M. (1979) *Nature (London)* **281**, 351–356.
10. Schneider, C., Owen, M. J., Banville, D. & Williams, J. G. (1984) *Nature (London)* **311**, 675–678.
11. Trowbridge, I. S. & Omary, M. B. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3039–3043.
12. Aoki, D., Appert, H. E., Johnson, D., Wong, S. S. & Fukuda, M. N. (1990) *EMBO J.* **9**, 3171–3178.
13. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
14. Danielsen, M., Northrop, J. P. & Ringold, G. M. (1986) *EMBO J.* **5**, 2513–2522.
15. Williams, M. A. & Fukuda, M. (1990) *J. Cell Biol.* **111**, 955–966.
16. Carlsson, S. R. & Fukuda, M. (1986) *J. Biol. Chem.* **261**, 12779–12786.
17. Moremen, K. W. & Robbins, P. W. (1991) *J. Cell Biol.* **115**, 1521–1534.
18. Masibay, A. S. & Qasba, P. K. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5733–5737.
19. Nakazawa, K., Furukawa, K., Kobata, A. & Narimatsu, H. (1991) *Eur. J. Biochem.* **196**, 363–368.
20. Strous, G. J. (1986) *CRC Crit. Rev. Biochem.* **21**, 119–151.
21. Berger, E. G. & Hesford, F. J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4736–4739.
22. Nilsson, T., Lucocq, J. M., Mackay, D. & Warren, G. (1991) *EMBO J.* **10**, 3567–3575.
23. Munro, S. (1991) *EMBO J.* **10**, 3577–3588.
24. Rose, J. K. & Bergmann, J. E. (1983) *Cell* **34**, 513–524.
25. Valetti, C., Grossi, C. E., Milstein, C. & Sitia, R. (1991) *J. Cell Biol.* **115**, 983–994.
26. Swift, A. M. & Machamer, C. E. (1991) *J. Cell Biol.* **115**, 19–30.
27. Beng, J. M. (1990) *J. Biol. Chem.* **265**, 6513–6516.
28. Holm, L., Saraste, M. & Wikström, M. (1987) *EMBO J.* **6**, 2819–2823.
29. Robinson, J. S., Graham, T. R. & Emr, S. D. (1991) *Mol. Cell. Biol.* **12**, 5813–5824.
30. Witsell, D. L., Casey, C. E. & Neville, M. C. (1990) *J. Biol. Chem.* **256**, 15731–15737.
31. Hurtley, S. M. & Helenius, A. (1989) *Annu. Rev. Cell Biol.* **5**, 277–307.
32. Lewis, M. J. & Pelham, H. R. B. (1992) *Cell* **68**, 353–364.