

# The 17-Residue Transmembrane Domain of $\beta$ -Galactoside $\alpha$ 2,6-Sialyltransferase Is Sufficient for Golgi Retention

Siew Heng Wong, Seng Hui Low, and Wanjin Hong

Membrane Biology Laboratory, Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511, Singapore

**Abstract.**  $\beta$ -Galactoside  $\alpha$ 2,6-sialyltransferase (ST) is a type II integral membrane protein of the Golgi apparatus involved in the sialylation of N-linked glycans. A series of experiments has shown that the 17-residue transmembrane domain of ST is sufficient to confer localization to the Golgi apparatus when transferred to the corresponding region of a cell surface type II in-

tegral membrane protein. Lectin affinity chromatography of chimeric proteins bearing this 17-residue sequence suggests that these chimeric proteins are localized in the *trans*-Golgi cisternae and/or *trans*-Golgi network. Further experiments suggest that this 17-residue sequence functions as a retention signal for the Golgi apparatus.

**T**HE Golgi apparatus is a highly organized organelle composed of networks of membrane-bound tubules, cisternae, and vesicles. Recent studies have shown that the Golgi apparatus is further differentiated into distinct subcompartments, each having a distinct protein composition and performing distinct functions (Chege and Pfeffer, 1990; Farquhar and Palade, 1981; Klausner, 1989; Pelham, 1991; Pfeffer and Rothman, 1987). Generally, the Golgi apparatus can be further divided into the *cis*-Golgi network (Duden et al., 1991; Hsu et al., 1991; Pelham, 1991), the *cis*-, *medial*-, and *trans*-Golgi cisternae, as well as the *trans*-Golgi network (TGN)<sup>1</sup> (Griffiths and Simons, 1986). Proteins in transport vesicles derived from the ER enter the Golgi apparatus from the *cis*-Golgi network, are transported along the *cis*-, *medial*-, and *trans*-Golgi cisternae through a series of vesicular intermediates, and exit the Golgi apparatus from the TGN. The Golgi apparatus performs a crucial role in protein sorting or targeting in both the exocytotic pathways (Dahms et al., 1989; Hsu et al., 1991; Kornfeld and Mellman, 1989; Orci et al., 1987; Pelham, 1988, 1991; Rodriguez-Boulant and Nelson, 1989; Simons and Wandinger-Ness, 1990; Tooze et al., 1987; Vaux et al., 1990) and the endocytotic pathways (Green and Kelly, 1990; Snider and Rogers, 1985; Woods et al., 1986). Several proteins have been localized to distinct subcompartments of the Golgi apparatus (Kornfeld and Kornfeld, 1985; Kornfeld and Mellman, 1989; Lewis and Pelham, 1990; Pelham, 1991; Saraste et al., 1987; Schweizer et al., 1988). *N*-Acetylglucosaminyl-

transferase I (NTI),  $\beta$ -1,4-galactosyltransferase (GT), and  $\alpha$ 2,6-sialyltransferase (ST) have been localized to the *medial*-, *trans*-cisternae, and the *trans*-cisternae/TGN, respectively (Dunphy et al., 1985; Roth and Berger, 1982; Roth et al., 1985; Chege and Pfeffer, 1990). Despite our current knowledge about the morphology, organization, function, and composition of the Golgi apparatus, the mechanism for establishing and maintaining the highly ordered Golgi structure is poorly understood, and very little is known about the signals that mediate the Golgi localization of its resident proteins.

Previous studies have shown that a catalytically active fragment of ST is secreted (Lammers and Jamieson, 1988, 1989; Paulson and Colley, 1989). Further experiments also showed that a 41–43 kD fragment of ST could be released from Golgi membranes by endogenous cathepsin D-like protease (Lammers and Jamieson, 1988; Weinstein et al., 1987). This 41–43-kD fragment is not anchored in the membrane but is still enzymatically active. Protein sequencing analysis has established that this fragment is released by cleavage between residues 62 and 63 (Weinstein et al., 1987). It seems that the secreted form of ST is also the result of cleavage around this site (Paulson and Colley, 1989). When fused to a cleavable signal sequence, an enzymatically active ST fragment from residue 58 to the COOH terminus is secreted in transfected cells (Colley et al., 1989). These observations suggest that the NH<sub>2</sub>-terminal region of ST is necessary for membrane anchorage and Golgi localization (Colley et al., 1989; Paulson and Colley, 1989).

In this report, we have fused different regions of the NH<sub>2</sub>-terminal ST sequence to the ectodomain of dipeptidyl peptidase IV (DPPIV), a type II surface membrane protein (Hong and Doyle, 1990; Ogato et al., 1989) to study their abilities in conferring Golgi localization of the chimeric proteins. We found that the 17-residue transmembrane domain of ST is sufficient for Golgi localization and that this se-

1. **Abbreviations used in this paper:** DPPIV, dipeptidyl peptidase IV; ECA, *Erythrina cristagalli*; endo H, Endoglycosidase H; GT,  $\beta$ -1,4-galactosyltransferase; MAA, *Maackia amurensis*; NTI, N-acetylglucosaminyltransferase I; PBSCM, PBS with 1 mM CaCl<sub>2</sub> and 1 mM MgCl<sub>2</sub>; PCR, polymerase chain reaction; SNA, *Sambucus nigra*; ST,  $\alpha$ 2,6-sialyltransferase; sWGA, succinylated wheat germ agglutinin; TGN, *trans*-Golgi network; WGA, wheat germ agglutinin.

quence may function as a retention signal for the *trans*-Golgi cisternae and/or the TGN.

## Materials and Methods

### Materials

Cell culture media, FBS, dialyzed FBS, and geneticin (G418) were purchased from Gibco Laboratories (Grand Island, NY). [<sup>35</sup>S]Methionine (>1,000 Ci/mmol) was from Amersham Corp. (Arlington Heights, IL). Goat anti-mouse IgG and its FITC-conjugated derivative were from Calbiochem. Corp. (La Jolla, CA). Wheat germ agglutinin (WGA)-rhodamine was obtained from Vector Labs., Inc. (Burlingame, CA). s-NHS-ss-biotin and streptavidin-agarose were purchased from Pierce Chem. Co. (Rockford, IL). All the lectin agaroses were purchased from E. Y. Laboratories, Inc. (San Mateo, CA). Neuraminidase and endoglycosidase H (endo H) were from Boehringer Mannheim Corp. (Indianapolis, IN). Transwells were from Costar Corp. (Cambridge, MA). All other reagents were from Sigma Chemical Co. (St. Louis, MO). mAbs against rat DPPIV have been described previously (Hong et al., 1989) and were generous gifts from Dr. D. L. Mendrick (Harvard Medical School).

### Oligonucleotides

The sequences of the oligonucleotides used are listed and all are read from 5' to 3'.

No. 1: CGGAATCTCGAGTCTGGACCATTCAATTATG;  
No. 2: GCCTCGAGCCTGGCTAGGTAACAACAACG;  
No. 3: CAAGCGCCGCGCTGTTTGAGAACAACAAC;  
No. 4: CTAGCGGCCGCCACTTCTCCTGGCTC;  
No. 5: CTAGCGGCCGCTTGGGCATCTGGAACCTC;  
No. 6: GAAGCGCCGCGCTTGGCTGTCAGTGTAAAG;  
No. 7: CTAGCGGCCGCTCATAGTCGCTCCCTTTC;  
No. 8: CTCATAGCGGCCGCTTCTTCCAAACACAGATG;  
No. 9: GTGCGCCGCTTCATTTTCCAAACACAGATGACTGCG;  
No. 10: GTGAATTCTCGAGATTATGATTCATACCAACTTGAA-  
GAAAAAGTCTTCTGGGACTGCTTGG;  
No. 11: GTTCTCGAGTGTGCTCTTGAGTAAAGCTG;  
No. 12: CTGAATTCTCGAGACCATGAAGACACCGTGGAAAGTT-  
CAGCCTCTTCACTCTGGTC;  
No. 13: CAGCGCCGCTCATAGTCGCTCCCTTCTGTTC-  
GCAGAACCACTGGCAC;  
No. 14: GTGAATTCTCGAGGAGCCGCCCAACCATG;  
No. 15: AAGCTCTAGCCCCGGGGTC.

### Native and Chimeric cDNA

**DPPIV.** A 3.2-kbp EcoRI cDNA fragment encoding for DPPIV in the pGEM-4Z vector (its 5' end facing the Sp6 promoter) has been described (Hong and Doyle, 1988). To facilitate the construction of chimeric cDNA, the recombinant plasmid was digested with the restriction enzymes XhoI and BamHI, blunt ended, and then self-ligated. This resulted in the deletion of most of the 3'-untranslated region of DPPIV cDNA. The resulting construct was referred to as pGEM/D4S. This process resulted in the creation of a unique EcoRI site at the 5' end and unique XhoI, XbaI, SalI, and HindIII sites at the 3' end of the DPPIV coding region. There is a unique NotI site in the codons for residues 33–35 in the DPPIV cDNA. The 5' unique EcoRI site and this NotI site were used to replace the DNA sequence encoding for the NH<sub>2</sub>-terminal 32-residue sequence of DPPIV with various DNA sequences encoding for different NH<sub>2</sub>-terminal ST sequences.

**ST.** A cDNA clone of unknown identity was obtained by screening a rat kidney Agt11 cDNA library with polyclonal antibodies against rat Golgi WGA-binding proteins. Limited sequence analysis established that it encodes for ST. To facilitate the analysis, oligonucleotides 1 and 2 were used to retrieve the ST coding region by polymerase chain reaction (PCR). The PCR product was digested with XhoI and inserted into the XhoI site of pGEM-11Z(+) with its 5' end facing the Sp6 promoter.

**S64D.** Oligonucleotides 1 and 3 were used to retrieve ST cDNA encoding for the NH<sub>2</sub>-terminal 64-residue sequence by PCR. The PCR product was digested with EcoRI and NotI and used to replace the 5' EcoRI-NotI sequence of DPPIV in pGEM/D4S. The resulting chimeric cDNA thus encodes for the fusion protein S64D.

**S51D.** Oligonucleotides 1 and 4 were used to create chimeric cDNA for fusion protein S51D as described above for S64D.

**S46D.** Chimeric cDNA coding for S46D was similarly constructed by using oligonucleotides 1 and 5.

**S40D.** This was constructed as above using oligonucleotides 1 and 6.

**S33D.** Oligonucleotides 1 and 7 were used to construct chimeric cDNA for S33D the same way as described above for S64D.

**S27D.** This was created as above using oligonucleotides 1 and 8.

**S26D.** Oligonucleotides 1 and 9 were used to construct the chimeric cDNA for S26D.

**S9D.** Oligonucleotides 10 and 11 were used to modify DPPIV cDNA by PCR (DPPIV cDNA as template). The PCR product was digested with XhoI and inserted into the XhoI site of pGEM-11Z(+). This process resulted in the substitution of DPPIV cytoplasmic domain by that of ST.

**S10–33D.** Oligonucleotides 7 and 9 were used by PCR (ST cDNA as template) to create a cDNA fragment that encodes a protein fragment in which the NH<sub>2</sub>-terminal 6-residue cytoplasmic domain of DPPIV was fused to an ST sequence from residue 10 to 33. The resulting PCR fragment was digested with EcoRI and NotI and used to replace the 5' EcoRI-NotI region of DPPIV cDNA.

**S10–26D.** Oligonucleotides 9 and 12 were used by PCR (ST cDNA as template) to create a cDNA fragment. After digesting with EcoRI and NotI, this PCR fragment was used to replace the EcoRI-NotI region of DPPIV cDNA. This created the chimeric cDNA encoding for S10–26D.

**S27–33D.** Oligonucleotides 13 and 14 were used by PCR (DPPIV cDNA as template) to create a DNA fragment. The PCR product was digested with EcoRI and NotI and used to replace the EcoRI-NotI region in pGEM/D4S. The resulting chimeric cDNA encodes for S27–33D.

Chimeric cDNAs (mainly the ST and fusion regions) were all confirmed by DNA sequencing in pGEM vectors. The resulting chimeric cDNAs all have a unique XhoI site in both 5' and 3' ends. For insertion into pRSN, they were digested with XhoI to retrieve the chimeric cDNA and ligated into the XhoI site of pRSN. The right orientation was verified by restriction enzyme digestion and direct DNA sequencing of the 5' region (200–300-bp sequence) with oligonucleotide No. 15, which hybridizes to the sequence before the XhoI site of the pRSN vector. Insertion of DPPIV cDNA into pRSN has been described previously (Low et al., 1991a,b).

### Cell Culture

MDCK (strain II) cells were a generous gift from Dr. K. Simons (European Molecular Biology Laboratory). MDCK and the transfected cells were cultured as before (Low et al., 1991b).

### Transfection of Cells

This was performed as described previously (Low et al., 1991b).

### Immunofluorescence Microscopy

Cells grown on coverslips were washed twice with PBS containing 1 mM CaCl<sub>2</sub> and 1 mM MgCl<sub>2</sub> (PBSCM), and then fixed with 2.7% paraformaldehyde in PBSCM at 4°C for 60 min. The fixed cells were washed once with PBSCM, twice with PBSCM containing 50 mM NH<sub>2</sub>Cl, and then three times with PBSCM (5–10 min each). The cells were either not permeabilized for surface staining or permeabilized with 0.1% saponin in PBSCM for 15 min for total staining (surface plus intracellular). mAbs (ascitic fluid), diluted 1:1,000 in fluorescence dilution buffer (PBSCM with 5% normal goat serum, 5% FBS, and 2% BSA, pH 7.6) were added to the cells and incubated at room temperature for 60–120 min. After washing six times with PBSCM (5–10 min each), the cells were then incubated with goat anti-mouse IgG conjugated with FITC (10 μg/ml) for 60–120 min. After washing, the cells were mounted in 90% glycerol in PBS, pH 8.0, containing 1 mg/ml *p*-phenylenediamine, observed using the Axiophot microscope (Carl Zeiss, Inc., Thornwood, NY) equipped with epifluorescence optics and photographed with Kodak Tri-X 400 film. Diluted mAbs and goat anti-mouse IgG conjugated with FITC were spun down at 4°C for 10 min (14 krpm in a microfuge) before use.

For colocalization with WGA, cells were first incubated with 0.5 mg/ml WGA for 60 min on ice to block surface WGA binding sites. After a brief rinse with PBSCM, the cells were fixed and permeabilized as above. Cells were then incubated with rhodamine-WGA (2.5 mg/ml) in PBSCM for 60 min. After washing, the cells were fixed again to immobilize the bound rhodamine-WGA. After WGA staining, the cells were sequentially incubated with monoclonal antibodies against DPPIV and goat anti-mouse IgG-FITC. The coverslips were then processed and observed as above.

## Metabolic Labeling of Cells

Cells were washed twice with PBSCM and then incubated for 45 min at 37°C in methionine-free medium supplemented with 10% dialyzed FBS (labeling buffer). The cells were then pulse-labeled with [<sup>35</sup>S]methionine (1 mCi/ml in labeling buffer), washed, and chased in medium containing excess cold methionine (100 mg/liter) for various times as detailed in each figure. For assessing the surface expression, cells were grown on Transwell filters so that the entire surface was maximally exposed for biotinylation. Cells grown on Transwell filters were similarly labeled with [<sup>35</sup>S]methionine.

## Cell Surface Biotinylation

This was performed as described (Le Bivic et al., 1990a,b; Low et al., 1991a,b). Briefly, cells grown on Transwell filters were washed four times with PBSCM (5–10 min each) on ice. The entire surface was biotinylated by adding to both the upper and lower chambers 1 ml of PBSCM containing s-NHS-biotin (0.5 mg/ml diluted from 200 mg/ml stock in DMSO). The biotinylation was performed twice on ice (15–20 min each) and stopped by repeated washing with PBSCM containing 50 mM NH<sub>4</sub>Cl and medium with 10% FBS.

## Immunoprecipitation and Recovery of Biotinylated Proteins

These were performed exactly as described previously (Low et al., 1991a,b).

## Endo H Treatment

The immunocomplex on Sepharose beads was resuspended in 300 μl of 0.1 M sodium citrate, pH 5.5, 0.1% Triton X-100, 0.1% β-mercaptoethanol, 100 mg/ml aprotinin, and 1 mM CaCl<sub>2</sub>. 10 mU of endo H was then added. After overnight incubation at 37°C, the beads were recovered and lysed in 80 μl SDS sample buffer followed by SDS-PAGE.

## Lectin Affinity Chromatography

Immunoprecipitated proteins were eluted from beads by boiling in 80 μl SDS sample buffer (without β-mercaptoethanol) followed by dilution in several milliliters of lectin buffer (40 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, and 1 mM MnCl<sub>2</sub>). An aliquot of this was incubated with the various lectin-agaroses (120 μl slurry) for 60–120 min. After extensive washing, the bound materials were eluted by boiling in SDS sample buffer and analyzed by SDS-PAGE.

## In Vitro Translation and Sodium Bicarbonate Treatment

These were performed as described previously (Hong and Doyle, 1990).

## SDS-PAGE and Analysis of Fluorography

These were carried out exactly as described before (Low et al., 1991a).

## Results

### Localization of S33D and S64D to the Golgi Apparatus

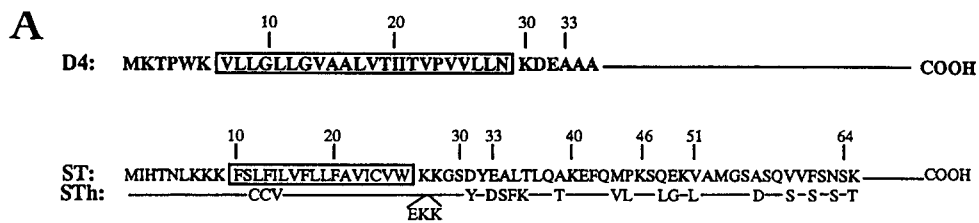
ST is a 47–48-kD integral membrane protein of type II orientation (single transmembrane domain with its NH<sub>2</sub> terminus in the cytosol and the COOH terminus in the lumen of the Golgi apparatus) (Paulson and Colley, 1989; Weinstein et al., 1987; our unpublished observations). The NH<sub>2</sub>-terminal 65-residue sequence of rat ST is shown in Fig. 1 A (ST). The small cytoplasmic domain consists of the NH<sub>2</sub>-terminal 9-residue sequence. The following 17-residue hydrophobic sequence functions as the transmembrane domain. Residue 27 to the COOH terminus comprises the luminal domain. The sequence from residue 27 to 62 has been defined as the stem region which links the catalytic domain of ST

(from residue 63 to the COOH terminus) to the transmembrane domain (Paulson and Colley, 1989).

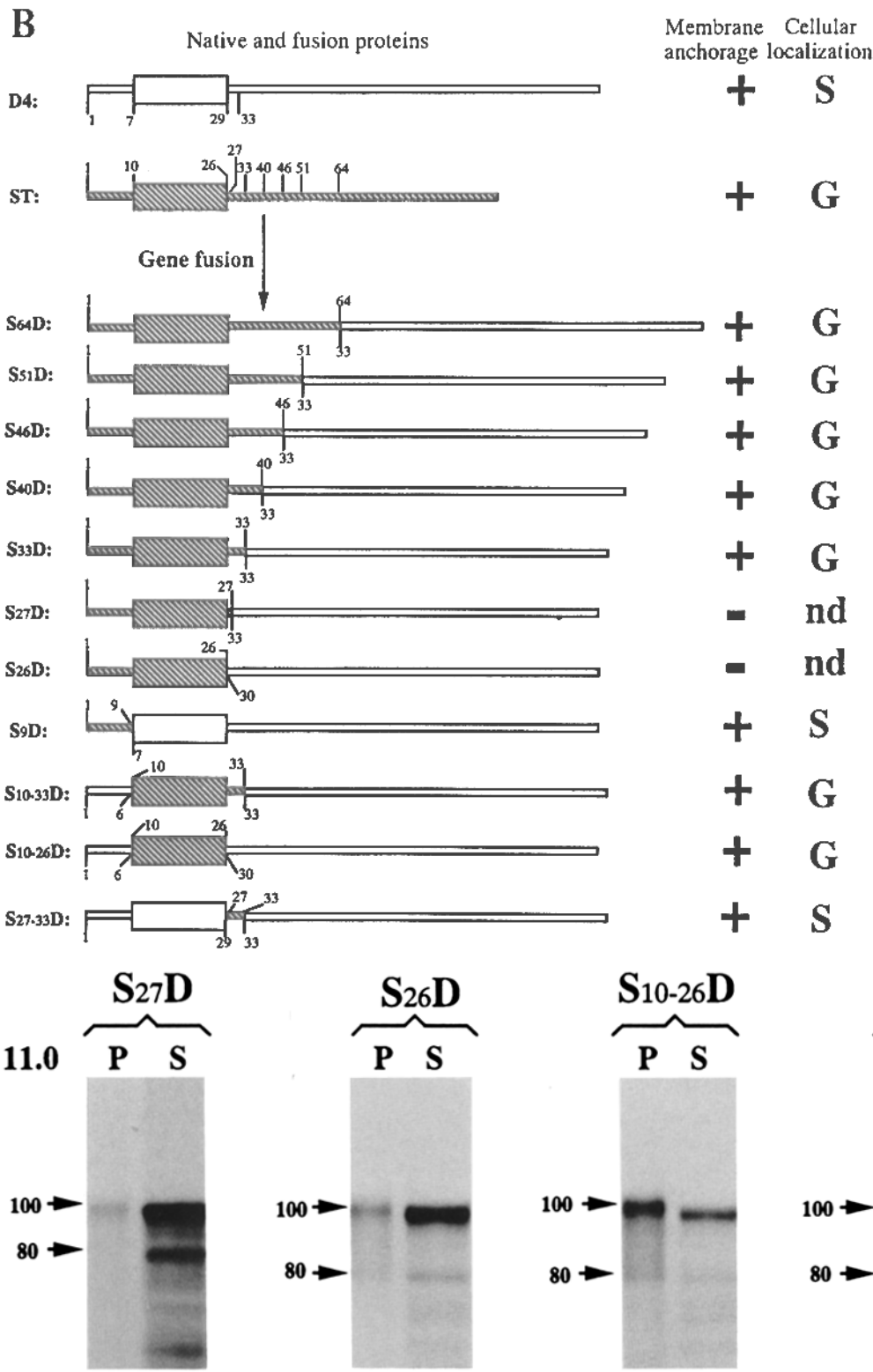
To determine the minimum sequence of the NH<sub>2</sub>-terminal region of ST that is sufficient for membrane anchorage and Golgi localization, we have constructed a series of chimeric cDNAs which encode different fusion proteins (Fig. 1 B), with decreasing lengths of the NH<sub>2</sub>-terminal ST sequence fused to the ectodomain of DPPIV. The NH<sub>2</sub>-terminal 35-residue sequence of DPPIV is also shown in Fig. 1 A (D4). Previous studies have shown that the NH<sub>2</sub>-terminal 34-residue sequence of DPPIV is the signal/anchor sequence (Hong and Doyle, 1990). The first set of experiments resulted in the construction of chimeric cDNAs encoding for fusion proteins S64D, S51D, S46D, S40D, S33D, and S27D in which the same ectodomain of DPPIV (from residue 33 to the COOH-terminus) was fused to the NH<sub>2</sub>-terminal 64-, 51-, 46-, 40-, 33-, and 27-residue sequence of ST (Fig. 1 B). In vitro translation studies demonstrated that all of them, except for S27D, were efficiently anchored in the membrane. The results for S27D and S33D are shown in Fig. 2. Results similar to S33D were obtained for S64D, S51D, S46D, and S40D (data not shown). These results demonstrated that the NH<sub>2</sub>-terminal 33- but not 27-residue sequence of ST is sufficient for membrane anchorage. Since S27D was not anchored in the membrane, it was not pursued further.

To examine the role in Golgi localization of the ST sequence in the remaining five fusion proteins, the respective chimeric cDNAs were inserted into the eukaryotic expression vector pRSN (Low et al., 1991a,b) and transfected into MDCK cells. Stably transfected cells were found to express the desired fusion proteins. Since similar results were obtained for all the five fusion proteins, only the results obtained for fusion proteins S64D and S33D are shown.

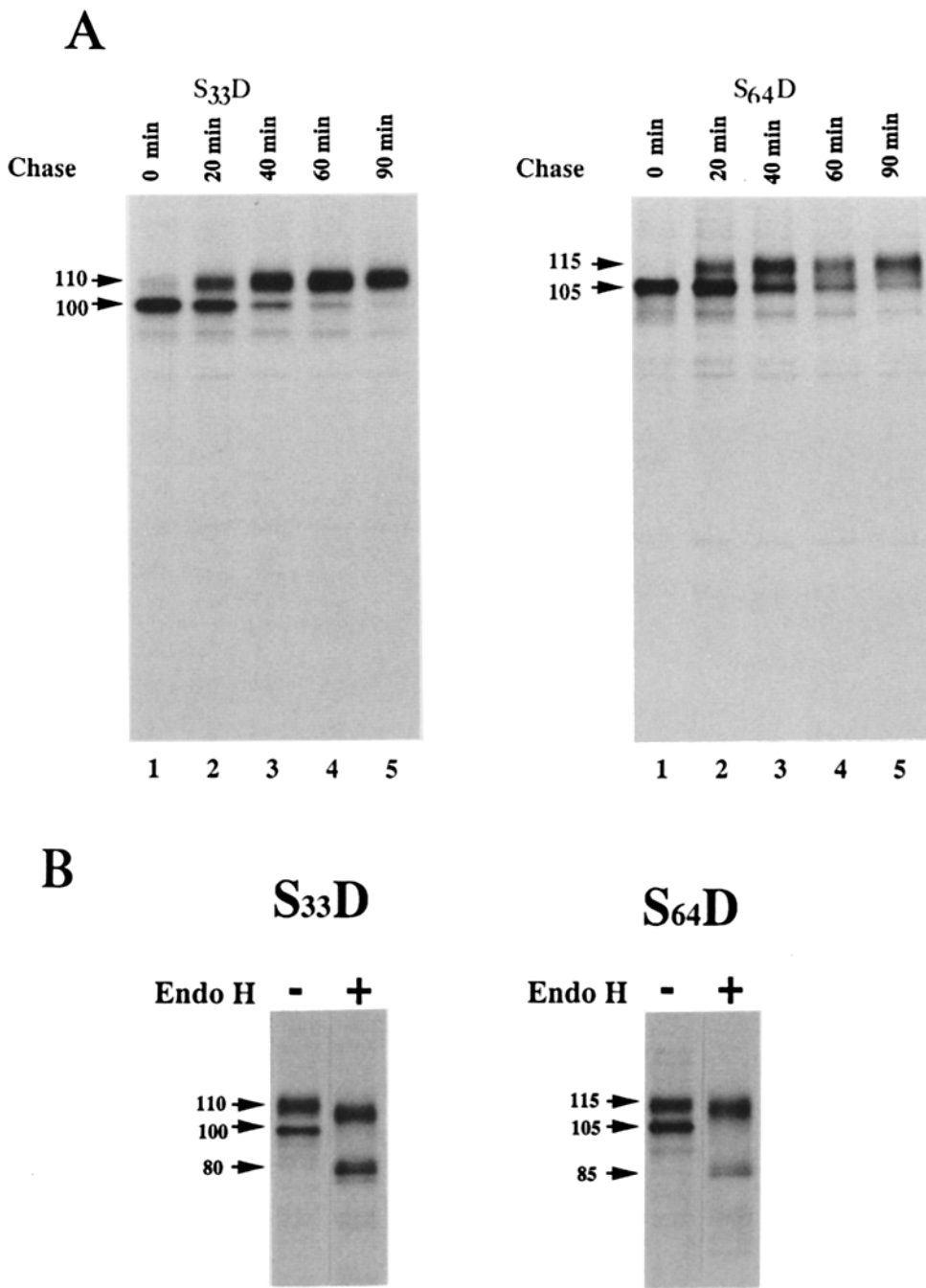
Pulse-chase experiments were performed to follow the intracellular transport of S64D and S33D (Fig. 3 A). As shown, a 100-kD protein was specifically detected in the pulse-labeled cells expressing S33D. As chase proceeded, this 100-kD form was converted into a 110-kD form. This conversion was very efficient, as all newly made 100-kD molecules were converted into the 110-kD form within 90 min of chase. In cells expressing S64D, a 105-kD form was initially detected in pulse-labeled cells and this form was converted into a 115-kD form during the chase period. A majority of the 105-kD form was converted into the 115-kD form after 90 min of chase, although a small amount of the 105-kD form was still observed. Endo H treatment of immunoprecipitated proteins (Fig. 3 B) showed that the 100-kD form of S33D and the 105-kD form of S64D were mainly converted into 80- and 85-kD polypeptides, respectively, which were of the same size as the respective polypeptides without any glycosylation (produced by in vitro translation without microsomes). This result suggests that all the N-linked glycans (about 7–8 N-linked glycans, but no detectable O-linked glycans, were present in the ectodomain of DPPIV [Hong and Doyle, 1988; Hong et al., 1989]) attached to the 100-kD form of S33D and the 105-kD form of S64D were essentially sensitive to endo H and that they represent the newly made proteins in the ER bearing N-linked glycans of the high-mannose type. The size of the 110-kD form of S33D was reduced by 4–5-kD after endo H treatment, suggesting that about two of the N-linked glycans in the polypeptide were of the high-mannose type while the remaining N-linked glycans (~5–6) were resistant to endo H. For the 115-kD form



**Figure 1.** (A) Amino acid sequence of the NH<sub>2</sub>-terminal region of DPPIV (D4), rat  $\beta$ -galactoside  $\alpha$ 2,6-sialyltransferase (ST), and human ST (Sth). For human ST, only the residues that differ from rat ST are indicated. The transmembrane domains are boxed. Residue numbers from the NH<sub>2</sub> terminus are indicated above the sequence. (B) Summary of fusion proteins used in this study and their properties in membrane anchorage and cellular localization. Residue numbers corresponding to ST are indicated above while those to D4 are indicated below each fusion protein. For fusion proteins, they were named SxD (in which the NH<sub>2</sub>-terminal X-residue sequence of ST was fused to the ectodomain of DPPIV) or Sx-yD (in which the ST sequence from residue x to y was used to replace the corresponding region of DPPIV). For membrane anchorage, + and - indicates that the majority of the protein is membrane anchored or not, respectively, as assessed by in vitro translation experiments. For cellular localization, S and G represent surface or Golgi localization, respectively, as examined by indirect immunofluorescence microscopy, while nd indicates "not determined" because of their nonanchorage in the membrane.



**Figure 2.** Membrane anchorage of selected fusion proteins. The mRNA for the fusion protein was translated in rabbit reticulocyte lysate supplemented with microsomes as described previously (Hong and Doyle, 1988, 1990). 10  $\mu$ l of total translation reaction was diluted with 100  $\mu$ l of sodium bicarbonate, pH 11.0, and the membrane-associated proteins (P) were separated from those extractable by this treatment (S). Samples equivalent to 4  $\mu$ l of original translation were analyzed for both P and S fractions. The 80-kD polypeptide is the untranslocated form, while the 100-kD polypeptide is the translocated N-glycosylated form for the respective fusion proteins.

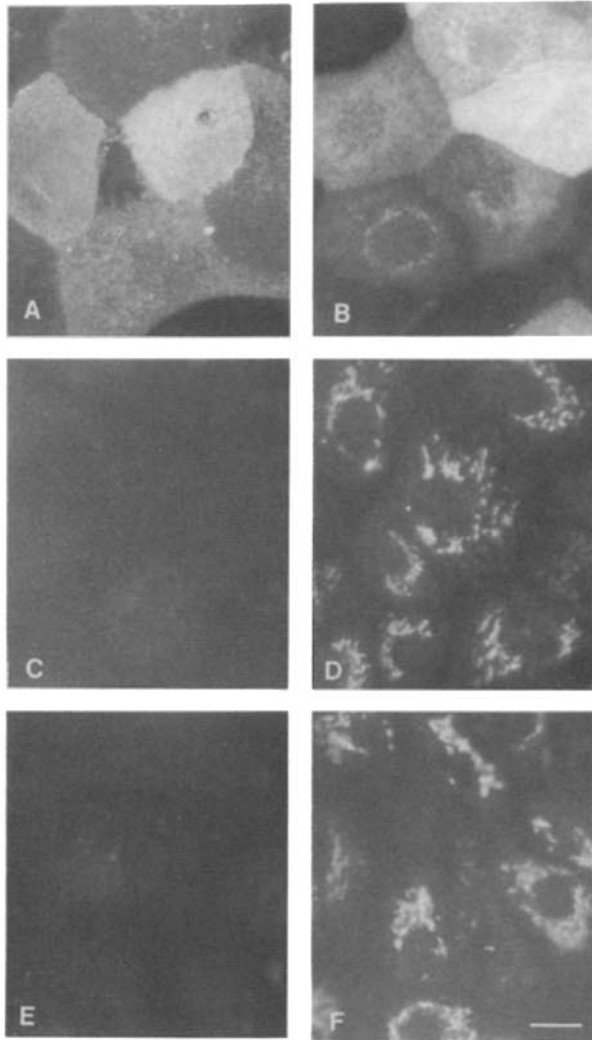
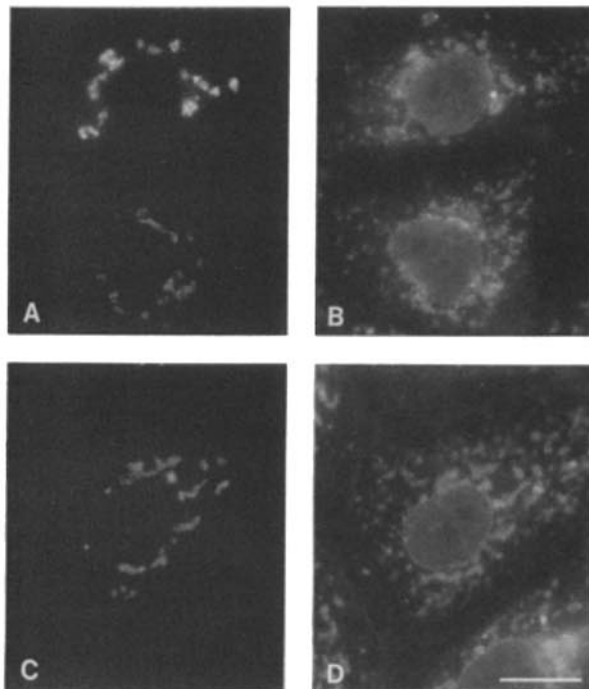


**Figure 3.** (A) Efficient transport from the ER to the Golgi of S33D and S64D. Stably transfected MDCK cells expressing the respective fusion proteins were pulse-labeled with [<sup>35</sup>S]methionine for 30 min followed by a series of chase intervals as indicated. Cell lysates were immunoprecipitated with monoclonal antibodies to DPPIV and the immunocomplexes were analyzed by SDS-PAGE and fluorography. The smaller- and the larger-sized bands represent the ER and Golgi forms, respectively. (B) Endo H treatment of fusion proteins S33D and S64D. Cells expressing the respective fusion proteins were labeled with [<sup>35</sup>S]methionine for 30 min followed by 30 min of chase. After being immunoprecipitated, half of the sample was treated with endo H (+) while the remaining half was processed in an identical way except that no endo H was included (-).

of S64D, a 2–3 kD size decrease was observed after endo H treatment, suggesting that one of the *N*-linked glycans was a high-mannose type while the rest (~6–7) of the *N*-linked glycans were endo H resistant. These results demonstrate that the majority of the *N*-linked glycans in the 110-kD form of S33D and the 115-kD form of S64D had been modified by enzymes in the *medial*-Golgi cisternae. These results therefore show that both fusion proteins were transported efficiently from the ER to the Golgi apparatus and that they were localized in the *medial*- and/or post-*medial*-Golgi compartment.

Indirect immunofluorescence microscopy was used to determine the cellular localization of these fusion proteins

(Fig. 4 A). An intense staining signal was detected on the surface of MDCK cells expressing normal DPPIV (A). A similar staining pattern was observed for normal DPPIV in permeabilized cells (B) except that some perinuclear signal was also detected. The perinuclear staining most likely represents molecules in transit through the Golgi apparatus. In contrast, only background staining was observed on the surface of cells expressing S33D (C) and S64D (E). When the cells were permeabilized, intense perinuclear vesicular-tubular staining was detected in the cells expressing S33D (D) and S64D (F). This pattern of intracellular staining is typical for the Golgi apparatus (Lipsky and Pagano, 1985; Louvard et al., 1982; Saraste et al., 1987), suggesting that

**A****B**

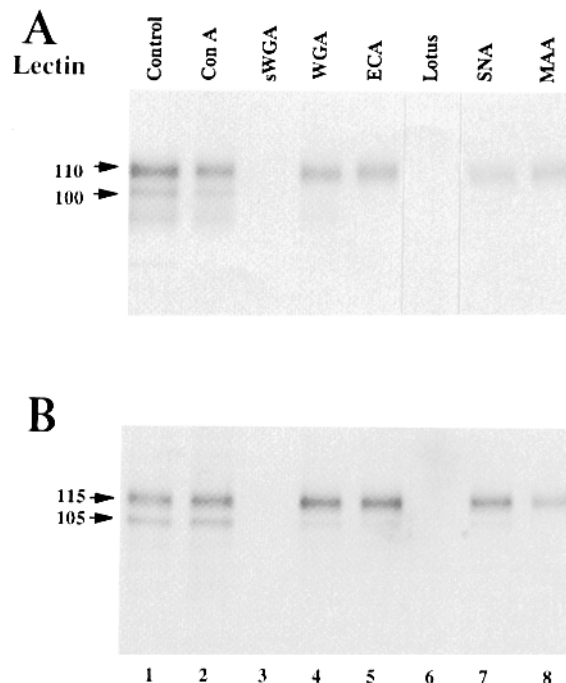
**Figure 4.** (A) Indirect immunofluorescence microscopy of stably transfected MDCK cells. Cells expressing native DPPIV (A and B), S33D (C and D), and S64D (E and F) were processed for surface staining (A, C, and E) and total (surface plus intracellular) staining as detailed in Materials and Methods. (B) Colocalization of S33D and S64D with intracellular WGA binding sites. Cells expressing S33D (A and B) and S64D (C and D) were processed for intracellular WGA binding sites (blocking surface binding sites with WGA followed by incubation with WGA-rhodamine after cell permeabilization) as well as fusion protein staining (subsequent incubation with mAbs and anti-mouse IgG-FITC). The cells were photographed for FITC (A and C) or rhodamine (B and D) signals to detect the fusion proteins and WGA binding sites, respectively. Bar, 15  $\mu$ M.

S33D and S64D are predominantly localized to the Golgi apparatus.

To further confirm the Golgi localization of S33D and S64D, colocalization of S33D or S64D with intracellular WGA binding sites was carried out. Intracellular WGA binding sites are present predominantly in the Golgi apparatus (Lipsky and Pagano, 1985; Tartakoff and Vassalli, 1983). As shown in Fig. 4 B, the staining for S33D (A) and S64D (C) colocalized well with that for WGA binding sites (B and D). These results demonstrated that S33D and S64D were indeed localized to the Golgi apparatus.

#### Localization of S33D and S64D to the Trans-Golgi Cisternae and/or TGN

The results obtained from pulse-chase and endo H treatment experiments suggest that S33D and S64D were present in the medial- and/or post-medial-Golgi cisternae. The indirect fluorescence microscopy demonstrated that they were localized in the Golgi apparatus. To further determine the compartment of their localization, their N-linked carbohydrate structures were examined by lectin affinity chromatography (Haselbeck et al., 1990; Lis and Sharon, 1986) (Fig. 5). Cells expressing S33D were pulse labeled with [<sup>35</sup>S]methionine followed by 30 min of chase such that both the 100-kD ER form and the 110-kD Golgi form of S33D were present in the cells. Similar amounts of labeled S33D were incubated with various lectin-agarose beads. The material bound to the beads was analyzed. As shown in Fig. 5 A, both the 100- and 110-kD forms could be quantitatively recovered with Con A-agarose (mannose-binding lectin) (lane 2), consistent with the presence of N-linked glycans of high-mannose type in both 100- and 110-kD forms of S33D as concluded from the endo H experiment. The 110- but not the 100-kD form was also recovered from WGA-, *Erythrina cristagalli* (ECA)-, *Sambucus nigra* (SNA)-, and *Maackia amurensis* (MAA)-agarose beads. Since ECA binds specifically to the Gal-β1,4-GlcNAc structure in N-linked glycans, this result demonstrates that the 110-kD form has been modified by *trans*-Golgi GT. SNA and MAA bind α-2,6- and α-2,3-linked sialic acids, respectively. Thus the 110-kD form of S33D has been modified by both α-2,6- and α-2,3-sialyltransferases, both of which are localized to the *trans*-Golgi cisternae and/or TGN. These results demonstrate that S33D had been modified by glycosyltransferases of the *trans*-Golgi cisternae and the TGN and suggest that it is localized to the *trans*-Golgi cisternae and/or TGN. WGA binds both sialic acid and *N*-acetylglucosamine while succinylated WGA (sWGA) binds only *N*-acetylglucosamine (Monsigny et al., 1979, 1980). The binding of 110-kD S33D to WGA but not sWGA not only confirmed the presence of sialic acid on the N-linked glycans but also demonstrated that no *N*-acetylglucosamine residues were exposed on the glycans. Both the 100- and the 110-kD forms of S33D failed to bind Lotus-agarose, a fucose-specific lectin. This result suggests that no significant fucose residues were added to the N-linked glycans of S33D. Similar experiments performed with cells expressing S64D (Fig. 5 B) revealed that the 115-kD Golgi form of S64D binds Con A-, WGA-, ECA-, SNA-, and MAA-, but not sWGA- or Lotus-agarose (identical pattern as the 110-kD form of S33D). The 115-kD form of S64D has thus been modified by both GT and sialyltransferases. The 105-kD form of S64D was quantitatively recovered from Con



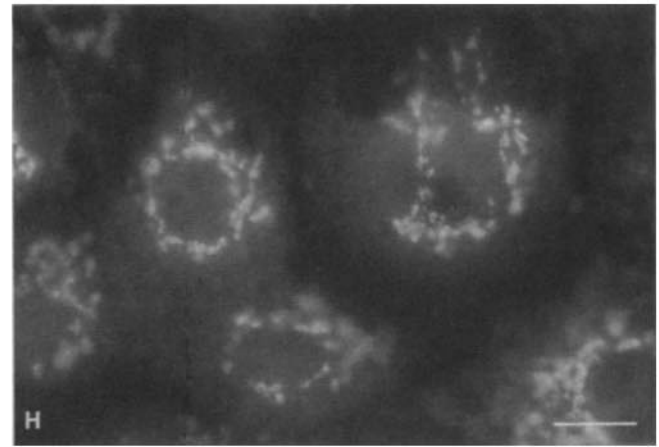
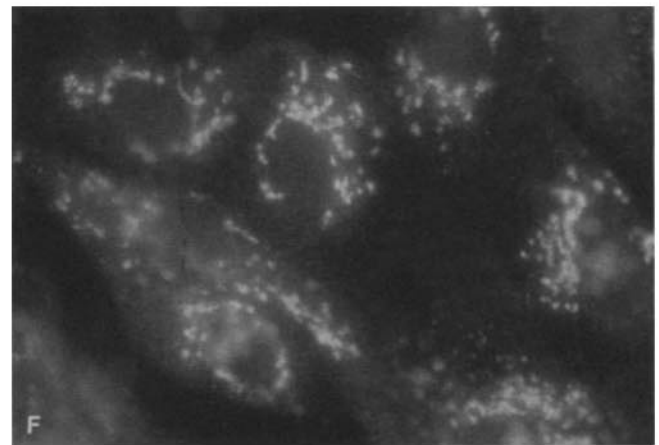
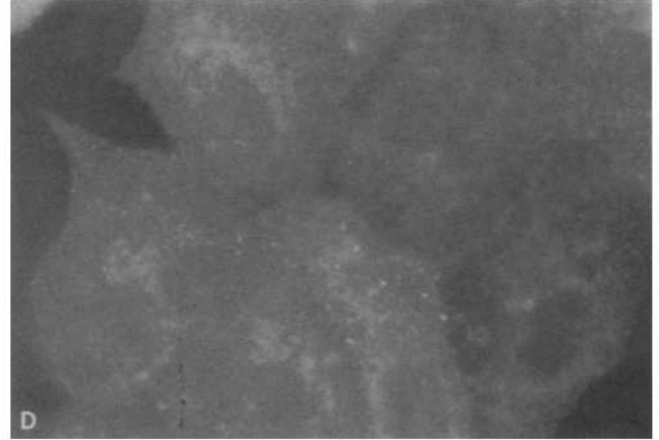
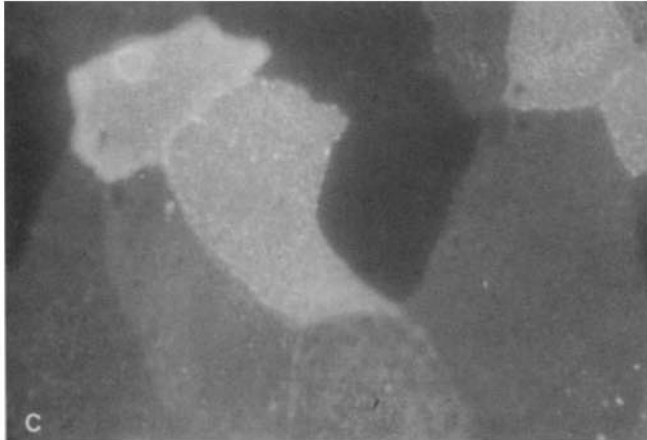
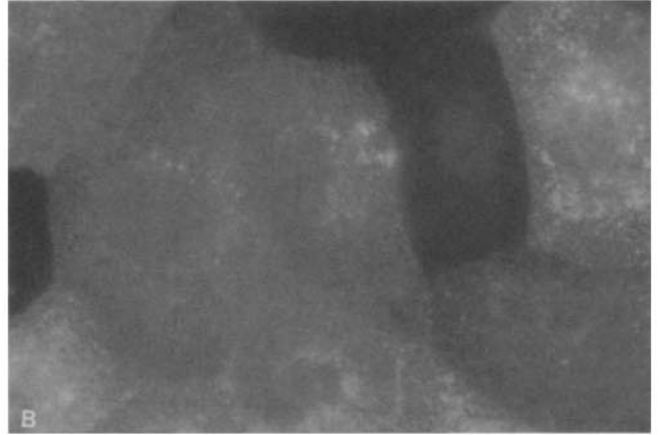
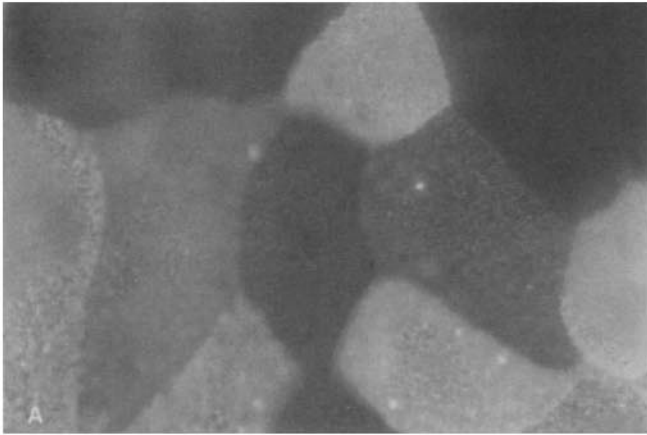
**Figure 5.** Characterization of the N-linked glycans in the fusion proteins. (A) Lectin-binding properties of S33D. Cells were pulse labeled with [<sup>35</sup>S]methionine for 30 min followed by 30 min of chase so that both the ER (100-kD) and Golgi (110-kD) forms were present. Similar amounts of the immunocomplex were analyzed directly (Control) or incubated with various lectin-agaroses and the bound fraction was then analyzed. (B) Lectin-binding properties of S64D. Cells expressing S64D were analyzed as described for S33D. Con A, concanavalin A; sWGA, succinylated wheat germ agglutinin; WGA, wheat germ agglutinin; ECA, *Erythrina cristagalli*; Lotus, *Lotus tetragonolobus*; SNA, *Sambucus nigra*; MAA, *Maackia amurensis*.

A-agarose beads. Interestingly, a small but detectable portion of the 105-kD form of S64D was also capable of binding WGA, ECA, and SNA (but not sWGA, Lotus, or MAA). This observation suggests that a small fraction of the 105-kD form might have undergone modifications by β-GT and ST, although the possibility that the observed binding of the 105-kD form of S64D to WGA, ECA, and SNA was due to nonspecific background binding could not be excluded yet. The small amount of 105-kD form present in pulse-labeled cells after 90 min of chase (Fig. 3 A, lane 5) may therefore have been transported to the Golgi apparatus. These results, taken together, suggest strongly that both S33D and S64D are localized in the *trans*-Golgi cisternae and/or TGN.

#### The 17-Residue Transmembrane Domain of ST Is Sufficient for Golgi Localization

We have thus demonstrated that both S33D and S64D are predominantly present in the Golgi apparatus. Similar results were obtained for S51D, S46D, and S40D (data not shown). These results demonstrate that the NH<sub>2</sub>-terminal 33-residue sequence of ST is sufficient for Golgi localization and that the stem region of ST (sequence from residue 34 to 63) is apparently not essential for Golgi localization. The sequence in this stem region is in fact the most divergent be-







tween rat and human ST. The NH<sub>2</sub>-terminal 64-residue sequence of human ST (Grundmann et al., 1990) is also shown in Fig. 1 A (*STh*). In contrast to the stem region, the sequence of the cytoplasmic domain is totally conserved between the rat and human ST. The transmembrane domain is also highly conserved. The sequence conservation of the cytoplasmic and transmembrane domains but not the stem region is consistent with our finding that the NH<sub>2</sub>-terminal 33-residue sequence of ST is sufficient for Golgi localization. This 33-residue sequence can be further divided into three regions: the cytoplasmic domain, the transmembrane domain, and the 7-residue sequence flanking the COOH-terminal side of the transmembrane domain. To further narrow down the sequence required for Golgi localization, an additional five chimeric cDNAs were constructed which encode fusion proteins S26D, S9D, S10-33D, S10-26D, and S27-33D (Fig. 1 B). In vitro translation studies demonstrated that S26D, like S27D, was not anchored in the microsomal membrane (Fig. 2). Interestingly, S10-26D, which is identical to S26D except that the ST cytoplasmic domain was replaced by that of DPPIV, was mainly (~70%) anchored in the membrane (Fig. 2). For S26D, S27D, and S10-26D, the translocated form in the supernatant was of a smaller size than the translocated form in the membrane, implying that the nonanchorage in the membrane of S26D, S27D, and, to a much lesser extent, S10-26D might be due to a cleavage of (or part of) the signal/anchor sequence of ST by the signal peptidase, although this explanation will have to be verified by future experiments. Replacing the cytoplasmic domain of ST by that of DPPIV in S10-26D significantly enhanced the membrane anchorage. In connection with the results obtained with S27D and S33D (Fig. 2), it can be concluded that the signal/anchor sequence of ST is within its NH<sub>2</sub>-terminal 33-residue sequence, comprising the 17-residue hydrophobic core and flanking hydrophilic sequences on both sides. The nature of the hydrophilic sequences on either side of the hydrophobic core significantly affect its efficiency in acting as a signal/anchor sequence. Results similar to S33D (Fig. 3) were obtained for S9D, S10-33D, and S27-33D (data not shown). Based on these in vitro translation results, chimeric cDNAs encoding S9D, S10-33D, S10-26D, and S27-33D (S26D was not pursued further) were inserted into the pRSN expression vector and transfected into MDCK cells. The cellular localization of the fusion proteins was examined by indirect fluorescence microscopy. As shown in Fig. 6, intense surface staining was detected for S9D (A) and S27-33D (C), but not for S10-33D (E) or S10-26D (G). When the cells were permeabilized, intense intracellular staining of perinuclear vesicular-tubular network was observed for S10-33D (F) and S10-26D (H), typical of the Golgi apparatus. This was further confirmed by their colocalization with intracellular WGA binding sites (data not shown). S10-26D and S10-33D have lectin-binding properties similar to those of S33D and S64D, demonstrating the modification of their N-linked glycans by GT and sialyltransferases (data not shown). S10-

26D and S10-33D thus reside in the *trans*-Golgi cisternae and/or TGN.

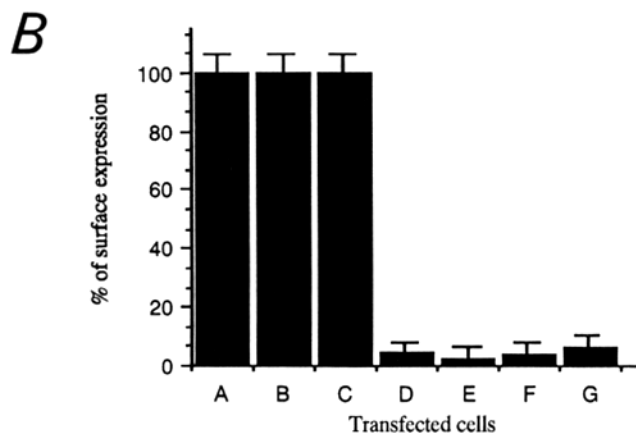
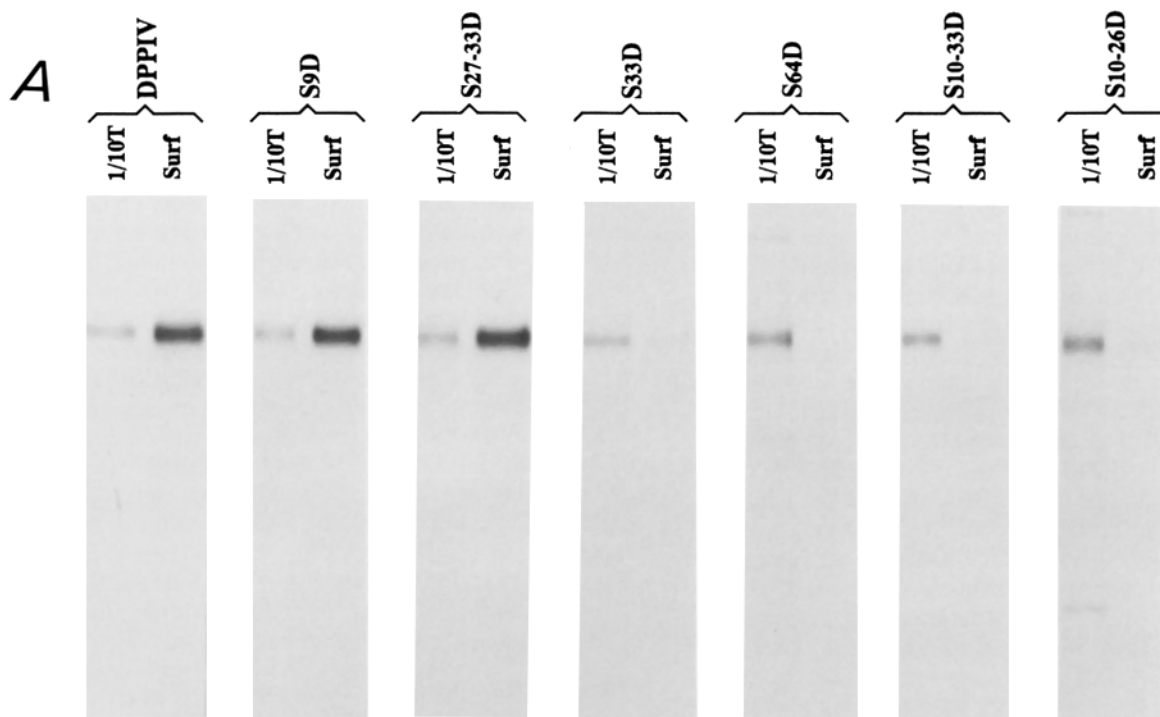
### Quantitation of the Efficiency of Golgi Localization

To obtain quantitative data on the intracellular Golgi localization of the various fusion proteins, we used a biochemical approach to determine the levels of surface expression of respective proteins (Fig. 7) (Bretscher and Lutter, 1988; Le Bivic et al., 1990a,b; Low et al., 1991a,b). For convenience, we arbitrarily defined the surface expression of DPPIV as 100%. The extent of surface expression for other proteins was normalized with that of DPPIV and defined as a percentage of surface expression. As shown, the surface expression of S33D, S64D, S10-33D, and S10-26D was between 2 and 6% as compared with DPPIV. The loss of surface expression demonstrates that the Golgi localization signal of ST is highly effective in targeting the fusion proteins to the Golgi apparatus. In contrast, high levels of surface expression, comparable to DPPIV, were observed for S9D and S27-33D. These biochemical results are consistent with and further extend the indirect immunofluorescence data.

### The Golgi Localization Signal Functions as a Retention Signal

Newly made molecules of S33D, S64D, S10-33D, and S10-26D were hardly detectable on the cell surface after 3 h of chase (Fig. 7). In connection with the predominant Golgi staining of these proteins, these results suggest that they are specifically and efficiently retained in the Golgi apparatus. However, the possibility that they may be initially transported to the cell surface and then rapidly and effectively endocytosed and sorted to the *trans*-Golgi cisternae and/or TGN could not be ruled out because only a single chase point was examined. If endocytosis followed by sorting is the mechanism for their Golgi localization, a peak in surface expression should be expected during a series of chase intervals after a single pulse-labeling. Cells expressing S33D, S10-26D, and DPPIV were selected for further examination. Cells were pulse-labeled with [<sup>35</sup>S]methionine for 30 min. After chasing for 15 min, 45 min, 90 min, 2 h, 6 h, and 22 h, cells were surface biotinylated with NHS-biotin and cell lysates were immunoprecipitated. One tenth of the immunoprecipitate served as a measure of the total amount of labeled fusion protein (*1/10 Total*), while nine tenths was absorbed with streptavidin-agarose to recover the biotinylated surface proteins (*Surface*) (Fig. 8). All were initially made as the 100-kD form followed by rapid conversion into the 110-kD form, demonstrating that they were efficiently transported from the ER to the Golgi apparatus. Strong surface expression was detected for DPPIV, and an almost maximal level of surface expression was detected after 90 min of chase. Since S33D and S10-26D were transported to the Golgi apparatus as efficiently as DPPIV, a peak expression on the surface at ~90 min of chase should be ex-

**Figure 6.** Cellular localization of fusion proteins as revealed by indirect immunofluorescence microscopy. Stably transfected cells expressing S9D (A and B), S27-33D (C and D), S10-33D (E and F), and S10-26D (G and H) were processed for surface staining (not permeabilized) (A, C, E, and G) or total staining (surface plus intracellular) (B, D, F, and H) by sequentially incubating with monoclonal antibodies to DPPIV and anti-mouse IgG-FITC. Bar, 15 μM.



**Figure 7.** Quantitation of the efficiency of Golgi localization. (A) Cells expressing DPPIV or the respective fusion proteins were pulse-labeled with [<sup>35</sup>S]methionine for 30 min followed by 3 h of chase. Proteins exposed on the cell surface were biotinylated with NHS-biotin. Cell lysates were immunoprecipitated with monoclonal antibodies against DPPIV. One tenth of the immunocomplex was analyzed directly and served as a measure of the total amount of the respective protein (1/10T). The remaining nine tenths was absorbed to streptavidin-agarose to recover the biotinylated proteins and analyzed (Surf). (B) Quantitation of the results in A. The cell surface expression of each fusion protein was normalized to that of DPPIV (arbitrarily defined as 100%). Transfected cells A, B, C, D, E, F, and G represent cells expressing DPPIV, S9D, S27-33D, S33D, S64D, S10-33D, and S10-26D, respectively.

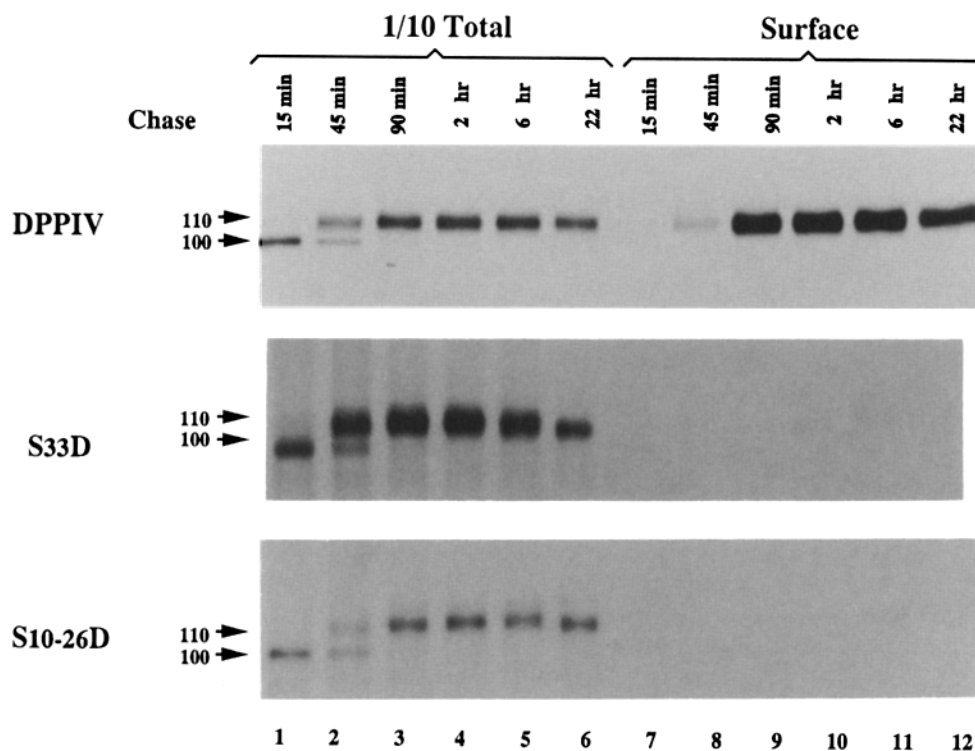
pected if their Golgi localization was mediated by initial surface expression followed by specific retrieval and endocytosis. This was not observed for either S33D or S10-26D. The surface expression for S33D and S10-26D was undetectable after 90 min of chase and only ~1-2% (normalized to DPPIV surface expression) was visible after 2 h of chase. Longer chase up to 6 h revealed a slight increase (up to 4-6%) in surface expression for S33D and S10-26D. This was most likely due to the slight leakiness in their Golgi retention. Interestingly, surface expression was undetectable after 22 h of chase, which could be explained by surface deg-

radation and/or slow internalization with the bulk membrane endocytosis. These results suggest that both S33D and S10-26D are specifically retained intracellularly in the Golgi apparatus.

### Discussion

#### The 17-Residue Transmembrane Domain of ST Is Sufficient for Golgi Localization

Previous observations have shown that the NH<sub>2</sub>-terminal re-



**Figure 8.** S33D and S10-26D are predominantly retained intracellularly. Cells expressing DPPIV, S33D, or S10-26D were pulse-labeled with [<sup>35</sup>S]methionine for 30 min followed by a series of chase intervals as indicated. The cell surface proteins were biotinylated at each chase point. Cell lysates prepared from each chase interval were immunoprecipitated. One tenth of the immunocomplex was analyzed as a measure of total newly made protein (*1/10 Total*). The remaining nine tenths was absorbed to streptavidin-agarose to recover the surface biotinylated proteins as a measure of surface expression (*Surface*). Intense surface expression was detected for DPPIV while the surface expression of the two fusion proteins was hardly detectable.

gion of ST is required for membrane anchorage and Golgi localization (Lammers and Jamieson, 1988, 1989; Paulson and Colley, 1989; Colley et al., 1989; Weinstein et al., 1987). We thus constructed a series of chimeric cDNAs encoding fusion proteins in which the NH<sub>2</sub>-terminal ST sequence of different sizes were fused to the ectodomain of a cell surface protein DPPIV (Hong and Doyle, 1990; Ogato et al., 1989; Low et al., 1991a,b). When expressed in transfected MDCK cells, fusion proteins bearing NH<sub>2</sub>-terminal 64-, 51-, 46-, 40-, and 33-residue ST sequences were all localized to the Golgi apparatus as assessed by indirect fluorescence microscopy and other biochemical methods. The results with two fusion proteins, S33D and S64D, are presented in this report. Pulse-chase experiments demonstrated that both S33D and S64D were efficiently transported from the ER to the Golgi apparatus and have passed beyond the *medial*-Golgi cisternae. Affinity chromatography with several lectin-agaroses demonstrated that both S33D and S64D were extensively modified by GT and ST. Since GT has been previously localized to the *trans*-Golgi cisternae (Roth and Berger, 1982) and ST has been localized to the *trans*-Golgi cisternae and TGN (Roth et al., 1985), these results suggest that S33D and S64D are localized in the *trans*-Golgi cisternae and/or the TGN. Because the actual localization of ST in MDCK cells has not been established, our conclusion for the *trans*-Golgi cisternae and/or TGN localization of S33D and S64D is based on the assumption that ST is localized in the same compartments in MDCK cells. Our results, nevertheless, demonstrated that S33D and S64D are localized in the Golgi subcompartments in which they have extensive interaction with endogenous GT and ST. Since S33D and S64D (as well as S51D, S46D, and S40D) were localized to the Golgi apparatus with comparable efficiencies, it was concluded that the NH<sub>2</sub>-terminal 33-

residue ST sequence is sufficient for Golgi localization and that the stem region from residues 34 to 64 is not required for Golgi localization.

The NH<sub>2</sub>-terminal 33-residue sequence was further divided into three regions: the 9-residue cytoplasmic domain, the 17-residue transmembrane domain, and the 7-residue sequence flanking the COOH-terminal side of the transmembrane domain. More chimeric cDNAs were thus constructed encoding additional fusion proteins. The transmembrane domain alone (in S10-26D) or in combination with its COOH-terminal flanking sequence (in S10-33D) could confer Golgi localization, while the cytoplasmic domain alone (in S9D) or the COOH-terminal flanking sequence alone (in S27-33D) was not capable of conferring Golgi localization. These results thus demonstrate that the 17-residue transmembrane domain of ST is sufficient for Golgi localization. Further characterization of the fusion protein S10-26D by lectin affinity chromatography demonstrated that the 17-residue sequence can confer the same Golgi subcompartment localization as the NH<sub>2</sub>-terminal 33- and 64-residue sequence of ST.

Recently, a common peptide stretch was described for Golgi-localized glycosyltransferases (Bendiak, 1990). This stretch (Ser-Gln-Glu-Lys) is located between residues 46 and 51 (residue 47-50) in ST. Our results clearly demonstrate that this stretch is not critical for Golgi localization. Furthermore, the sequence of this stretch is not conserved in the human ST (Ser-Leu-Gly-Lys) (Grundmann et al., 1990). Since glycosyltransferases are localized to distinct subcompartments of the Golgi apparatus, it is hard to envision a common sequence for distinct subcompartment localization. Further studies with other glycosyltransferases will reveal the structural features required for localization to other subcompartments of the Golgi apparatus.

Since our study involved extensive "Cut-and-Paste" ex-

periments for the fusion of domains derived from two different proteins, one may argue that conformational changes might have contributed to the results observed in our system. This seems highly unlikely because the chimeric proteins were transported from the ER to the Golgi apparatus as efficiently as normal DPPIV and were modified by enzymes in all subcompartments of the Golgi apparatus. If there was any conformational abnormality, the fusion proteins would have been retained in the ER and/or pre-Golgi compartments. Many misfolded chimeric proteins have been described and all failed to be transported beyond the *cis*-face of the Golgi apparatus (Rose and Doms, 1988). Furthermore, preliminary experiments have shown that all the fusion proteins possessed similar enzymatic activity as the normal DPPIV, suggesting no global conformational change in the ectodomain of DPPIV in the chimeric proteins. Additional experiments demonstrated that the Golgi localization of the chimeric proteins by the ST transmembrane domain is a specific event, because the replacement of the transmembrane domain of DPPIV by that of another type II surface protein resulted in a chimeric protein that was transported to the cell surface (data not shown).

### ***The 17-Residue Transmembrane Domain Functions as a Retention Signal***

The Golgi localization of the fusion proteins could be mediated by their specific retention in the Golgi subcompartment (in this case, the Golgi localization signal functions as a retention signal). Alternatively, they could be initially transported to the cell surface followed by their efficient retrieval and transcytosis back to the final Golgi localization. To distinguish between these two possibilities, we used the membrane-impermeable reagent NHS-biotin to selectively tag the proteins present on the cell surface after a single pulse and several chase intervals. The biotinylated proteins were specifically recovered by absorption to streptavidin-agarose and served as a measure of surface expression. Efficient surface expression of DPPIV was detected. Studies with S33D and S10-26D demonstrated that they were hardly detectable on the cell surface. These results established that the majority of S33D and S10-26D were specifically retained in the Golgi apparatus and that the 17-residue transmembrane domain of ST functions as a retention signal for the Golgi subcompartment.

### ***Transmembrane Domain-mediated Subcellular Targeting***

Our studies demonstrated that the membrane-spanning region of ST, in addition to its passive role in membrane anchorage, plays a dominant role in Golgi retention. Our observations raised the question of whether the transmembrane domains of other glycosyltransferases play similar roles in specifying their subcompartment Golgi localization. Comparison of the primary sequences of GT revealed that the entire transmembrane domain is identical in human and bovine and that 19 of the 20 residues in the transmembrane domain are also conserved in mouse (D'Agostaro et al., 1989; Masri et al., 1988; Russo et al., 1990; Shaper et al., 1988). Furthermore, the sequence of the transmembrane domain of NTI is totally conserved between human and rabbit (Kumar et al., 1990; Sarkar et al., 1991). The high degree of conser-

vation in the transmembrane domain sequence of GT and NTI suggests that they might also be involved in other functions in addition to their role in membrane anchorage. Whether the transmembrane domain of GT and/or NTI is similarly involved in Golgi localization needs further investigation. Our recent studies with NTI suggest that its transmembrane domain is indeed involved in Golgi localization (Tang et al., 1992). The sequence of the transmembrane domain varies greatly between ST, GT, and NTI, and this sequence divergence of the transmembrane domain among different glycosyltransferases might be related to their distinct subcompartment localization in the Golgi apparatus. Further studies will shed more light on this issue.

The Golgi localization specified by the transmembrane domain of ST could potentially be mediated by two different but related mechanisms. The membrane spanning domain may interact with specific lipid components (or specific lipid microenvironment) in the Golgi membrane. This interaction may immobilize the proteins in a particular microdomain in the membrane. Proteins in this microdomain may be specifically prevented from entry into the budding vesicles for subsequent transport. Alternatively, the transmembrane domain may interact with a specific membrane-spanning region of other proteins that are already immobilized in the Golgi membrane through unknown mechanisms. Furthermore, interaction of the transmembrane domains among different molecules of ST (self interaction) induced by the Golgi environment may also be involved. At present, we are unable to propose a detailed molecular mechanism to explain the Golgi localization mediated by the ST transmembrane domain. Future studies with point mutations in the ST transmembrane domain and other glycosyltransferases will enable us to gain more understanding about the cellular targeting mediated by the transmembrane domain.

Cellular localization determined by a membrane anchoring region has been described for other proteins. Recent studies have established that the GPI lipid anchor functions as a targeting signal for the apical plasma membrane domain in epithelial cells (Brown et al., 1989; Lisanti et al., 1989). Furthermore, the transmembrane domain of the T cell antigen receptor  $\alpha$ -subunit has been shown to be sufficient for ER degradation and subunit assembly of the T cell antigen receptor (Bonifacino et al., 1990). Studies with the E1 protein of avian coronavirus infectious bronchitis virus, a *cis*-Golgi protein (Machamer et al., 1990), demonstrated that the first of its three transmembrane domains is necessary and sufficient for Golgi localization (Machamer and Rose, 1987; Swift and Machamer, 1991), although different results were obtained with the E1 protein of another coronavirus (mouse hepatitis virus A59) (Armstrong et al., 1990; Armstrong and Patel, 1991). These observations suggest that the subcellular destination of some integral membrane proteins is signaled by their membrane-spanning regions.

During the review of this manuscript, two papers were published which demonstrated that the membrane-spanning domain of GT (Nilsson et al., 1991) and the transmembrane domain and its flanking sequences of ST (Munro, 1991) could specify Golgi localization. Although similar conclusions were reached by Munro in his study, there were a few significant differences in the approach and results presented from our study. (a) Munro used a transient expression system in COS cells while our study was performed in stably

transfected MDCK cells. (b) Our results clearly demonstrate that the 17-residue transmembrane domain itself was sufficient for Golgi localization of the chimeric protein and its flanking sequences did not contribute much to the efficiency of Golgi localization. This difference could be due to the different expression systems used and the fact that our chimeric proteins were derived from two well-defined cellular proteins of the same membrane topology while those in Munro's study involved a tripartite fusion among three proteins. (c) In addition to confirming biochemically the Golgi localization of the chimeric proteins, we also used surface biotinylation to demonstrate that the Golgi-localized chimeric proteins were not initially transported to the cell surface followed by recycling back to the Golgi, providing the first evidence that they were selectively retained in the Golgi complex.

We thank Mr. B. L. Tang for his continuous interest and useful discussions during the course of this project and for performing some of the transfection and indirect immunofluorescence microscopy experiments. We also thank Mr. B. L. Tang and Drs. William Chia, Catherine J. Pallen, Paramjeet Singh, and V. N. Subramaniam for their critical reading of the manuscript; Dr. K. Simons for MDCK (II) cells; Dr. D. L. Mendrick for monoclonal antibodies to DPPIV; and Dr. Y. H. Tan and Ms. Png Hong Lan for their support. Mr. Siew Heng Wong and Ms. Seng Hui Low contributed equally to this research.

This work was supported by a research fund from the Institute of Molecular and Cell Biology, National University of Singapore, to W. Hong.

Received for publication 2 November 1991 and in revised form 7 January 1992.

## References

- Armstrong, J., and S. Patel. 1991. The Golgi sorting domain of coronavirus E1 protein. *J. Cell Sci.* 98:567-575.
- Armstrong, J., S. Patel, and P. Riddle. 1990. Lysosomal sorting mutants of coronavirus E1 protein, a Golgi membrane protein. *J. Cell Sci.* 95:191-197.
- Bendiak, B. 1990. A common peptide stretch among enzymes localized to the Golgi apparatus: structural similarity of Golgi-associated glycosyltransferase. *Biochem. Biophys. Res. Commun.* 173:879-882.
- Bonifacino, J. S., P. Cosson, and R. D. Klausner. 1990. Colocalized transmembrane determinants for ER degradation and subunit assembly explain the intracellular fate of TCR chains. *Cell.* 63:503-513.
- Bretscher, M. S., and R. Lutter. 1988. A new method for detecting endocytosed proteins. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:4087-4092.
- Brown, D. A., B. Crise, and J. K. Rose. 1989. Mechanism of membrane anchoring affects polarized expression of two proteins in MDCK cells. *Science (Wash. DC)*. 245:1499-1501.
- Chege, N. W., and S. R. Pfeffer. 1990. Compartmentation of the Golgi complex: brefeldin-A distinguishes *trans*-Golgi cisternae from the *trans*-Golgi network. *J. Cell Biol.* 111:893-899.
- Colley, K. J., E. U. Lee, B. Adler, J. K. Brown, and J. C. Paulson. 1989. Conversion of a Golgi apparatus sialyltransferase to a secretory protein by replacement of the NH<sub>2</sub>-terminal signal anchor with a signal peptide. *J. Biol. Chem.* 264:17619-17622.
- D'Agostaro, G., B. Bendiak, and M. Tropolak. 1989. Cloning of cDNA encoding the membrane-bound form of bovine  $\beta$ 1,4-galactosyltransferase. *Eur. J. Biochem.* 183:211-217.
- Dahms, N. M., P. Lobel, and S. Kornfeld. 1989. Mannose-6-phosphate receptors and lysosomal enzyme targeting. *J. Biol. Chem.* 264:12115-12118.
- Duden, R., V. Allan, and T. Kreis. 1991. Involvement of  $\beta$ -COP in membrane traffic through the Golgi complex. *Trends in Cell Biology.* 1:14-19.
- Dunphy, W. G., R. Brands, and J. E. Rothman. 1985. Attachment of terminal *N*-acetylglucosamine to asparagine-linked oligosaccharides occurs in central cisternae of the Golgi stack. *Cell.* 40:463-472.
- Farquhar, M. G., and G. Palade. 1981. The Golgi apparatus (complex)—(1954-1981)—from artifact to center stage. *J. Cell Biol.* 91(3, Pt. 2): 77s-103s.
- Green, S. A., and R. B. Kelly. 1990. Endocytotic membrane traffic to the Golgi apparatus in a regulated secretory cell line. *J. Biol. Chem.* 265:21269-21278.
- Griffiths, G., and K. Simons. 1986. The *trans*-Golgi network: sorting at the exit site of the Golgi complex. *Science (Wash. DC)*. 234:438-443.
- Grundmann, U., C. Nerlich, T. Rein, and G. Zettlmeissl. 1990. Complete cDNA sequence encoding human  $\beta$ -galactoside  $\alpha$ -2,6-sialyltransferase. *Nucleic Acids Res.* 18:667.
- Haselbeck, A., E. Schickanedar, H. von der Eilt, and W. Hosel. 1990. Structural characterization of glycoprotein carbohydrate chains by using digoxigenin-labeled lectins on blots. *Anal. Biochem.* 191:25-30.
- Hong, W., and D. Doyle. 1988. Membrane orientation of rat gp110 as studied by *in vitro* translation. *J. Biol. Chem.* 263:16892-16898.
- Hong, W., and D. Doyle. 1990. Molecular dissection of the NH<sub>2</sub>-terminal signal/anchor sequence of rat dipeptidyl peptidase IV. *J. Cell Biol.* 111:323-328.
- Hong, W., G. A. Piazza, D. C. Hixson, and D. Doyle. 1989. Expression of enzymatically active rat dipeptidyl peptidase IV in Chinese hamster ovary cells after transfection. *Biochemistry.* 28:8474-8479.
- Hsu, V. W., L. C. Yuan, J. G. Nuchtern, J. Lippincott-Schwartz, G. J. Hammerling, and R. D. Klausner. 1991. A recycling pathway between the endoplasmic reticulum and the Golgi apparatus for retention of unassembled MHC class I molecules. *Nature (Lond.)*. 352:441-444.
- Klausner, R. D. 1989. Sorting and traffic in the central vacuolar system. *Cell.* 57:703-706.
- Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* 54:631-664.
- Kornfeld, S., and I. Mellman. 1989. The biogenesis of lysosomes. *Annu. Rev. Cell Biol.* 5:483-525.
- Kumar, R., J. Yang, R. D. Larsen, and P. Stanley. 1990. Cloning and expression of *N*-acetylglucosaminyltransferase I, the medial Golgi transferase that initiates complex N-linked carbohydrate formation. *Proc. Natl. Acad. Sci. USA.* 87:9948-9952.
- Lammers, G., and J. C. Jamieson. 1988. The role of a cathepsin D-like activity in the release of Gal  $\beta$ 1-4GlcNac  $\alpha$ 2-6sialyltransferase from rat liver Golgi membranes during the acute-phase response. *Biochem. J.* 256:623-631.
- Lammers, G., and J. C. Jamieson. 1989. Studies on the effect of lysosomotropic agents on the release of Gal  $\beta$ 1-4GlcNac  $\alpha$ 2-6sialyltransferase from rat liver slices during the acute-phase response. *Biochem. J.* 261:389-393.
- Le Bivic, A., A. Quaroni, B. Nichols, and E. Rodriguez-Boulan. 1990a. Biogenetic pathways of plasma membrane proteins in Caco-2, a human intestinal epithelial cell line. *J. Cell Biol.* 111:1351-1361.
- Le Bivic, A., Y. Sambuy, K. Mostov, and E. Rodriguez-Boulan. 1990b. Vectorial targeting of an endogenous apical membrane sialoglycoprotein and ovomorulin in MDCK cells. *J. Cell Biol.* 110:1533-1539.
- Lewis, M. J., and H. R. B. Pelham. 1990. A human homologue of the yeast HDEL receptor. *Nature (Lond.)*. 348:162-163.
- Lipsky, N. G., and R. E. Pagano. 1985. A vital stain for the Golgi apparatus. *Science (Wash. DC)*. 228:745-747.
- Lis, H., and N. Sharon. 1986. Lectins as molecules and as tools. *Annu. Rev. Biochem.* 55:35-67.
- Lisanti, M. P., I. W. Caras, M. A. Davitz, and E. Rodriguez-Boulan. 1989. A glycosphospholipid membrane anchor acts as an apical targeting signal in polarized epithelial cells. *J. Cell Biol.* 109:2145-2156.
- Louvard, D., H. Reggio, and G. Warren. 1982. Antibodies to the Golgi complex and the rough endoplasmic reticulum. *J. Cell Biol.* 92:92-107.
- Low, S. H., S. H. Wong, B. L. Tang, and W. Hong. 1991a. Involvement of both vectorial and transcytotic pathways in the preferential apical cell surface localization of rat dipeptidyl peptidase IV. *J. Biol. Chem.* 266:19710-19716.
- Low, S. H., S. H. Wong, B. L. Tang, V. N. Subramaniam, and W. Hong. 1991b. Apical cell surface expression of rat dipeptidyl peptidase IV in transfected Madin-Darby canine kidney cells. *J. Biol. Chem.* 266:13391-13396.
- Machamer, C. E., and J. K. Rose. 1987. A specific transmembrane domain of a coronavirus E1 glycoprotein is required for its retention in the Golgi region. *J. Cell Biol.* 105:1205-1214.
- Machamer, C. E., S. A. Mentone, J. K. Rose, and M. G. Farquhar. 1990. The E1 glycoprotein of an avian coronavirus is targeted to the cis Golgi complex. *Proc. Natl. Acad. Sci. USA.* 87:6944-6948.
- Masri, K. A., H. E. Appert, and M. N. Fukada. 1988. Identification of the full-length coding sequence for human galactosyltransferase ( $\beta$ -*N*-acetylglucosamine:  $\beta$ 1,4-galactosyltransferase). *Biochem. Biophys. Res. Commun.* 157: 657-663.
- Monsigny, M., C. Sene, A. Obrenovitch, A.-C. Roche, F. Delmotte, and E. Boschetti. 1979. Properties of succinylated wheat germ agglutinin. *Eur. J. Biochem.* 98:39-45.
- Monsigny, M., A.-C. Roche, C. Sene, R. Maget-Dana, and F. Delmotte. 1980. Sugar-lectin interactions: how does wheat germ agglutinin bind sialoglycoconjugates. *Eur. J. Biochem.* 104:147-153.
- Munro, S. 1991. Sequences within and adjacent to the transmembrane segment of  $\alpha$ -2,6-sialyltransferase specify Golgi retention. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:3577-3588.
- Nilsson, T., J. M. Lucocq, D. Mackay, and G. Warren. 1991. The membrane spanning domain of  $\beta$ -1,4-galactosyltransferase specifies *trans* Golgi localization. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:3567-3575.
- Ogata, S., Y. Misumi, and Y. Ikehara. 1989. Primary structure of rat liver dipeptidyl peptidase IV deduced from its cDNA and identification of the NH<sub>2</sub>-terminal signal sequence as the membrane-anchoring domain. *J. Biol. Chem.* 264:3596-3601.
- Orci, L., M. Ravazzola, M. Amherdt, A. Perrelet, S. K. Powell, D. L. Quinn, and H.-P. H. Moore. 1987. The *trans*-most cisternae of the Golgi complex: a compartment for sorting of secretory and plasma membrane proteins. *Cell.* 51:1039-1051.
- Paulson, J. C., and K. J. Colley. 1989. Glycosyltransferase: structure, localiza-

- tion, and control of cell type-specific glycosylation. *J. Biol. Chem.* 264:17615-17618.
- Pelham, H. R. B. 1988. Evidence that luminal ER proteins are sorted from secreted proteins in a post-ER compartment. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:913-918.
- Pelham, H. R. B. 1991. Recycling of proteins between the endoplasmic reticulum and Golgi complex. *Current Opinion in Cell Biology.* 3:585-591.
- Pfeffer, S. R., and J. E. Rothman. 1987. Biosynthetic protein transport and sorting by the endoplasmic reticulum and Golgi. *Annu. Rev. Biochem.* 56:829-852.
- Rodriguez-Boulan, E., and W. J. Nelson. 1989. Morphogenesis of the polarized epithelial cell phenotype. *Science (Wash. DC).* 245:718-725.
- Rose, J. K., and R. W. Doms. 1988. Regulation of protein export from the endoplasmic reticulum. *Annu. Rev. Cell Biol.* 4:257-288.
- Roth, J., and E. G. Berger. 1982. Immunocytochemical localization of galactosyltransferase in HeLa cells: codistribution with thiamine pyrophosphatase in trans-Golgi cisternae. *J. Cell Biol.* 92:223-229.
- Roth, J., D. J. Taatjes, J. M. Lucocq, J. Weinstein, and J. C. Paulson. 1985. Demonstration of an extensive trans-tubular network continuous with the Golgi apparatus that may function in glycosylation. *Cell.* 43:287-295.
- Russo, R. N., N. L. Shaper, and J. H. Shaper. 1990. Bovine  $\beta$ 1-4-galactosyltransferase: two sets of mRNA transcripts encode two forms of the protein with different amino-terminal domains: in vitro translation experiments demonstrate that both the short and the long forms of the enzyme are type II membrane-bound glycoproteins. *J. Biol. Chem.* 265:3324-3331.
- Saraste, J., G. E. Palade, and M. G. Farquhar. 1987. Antibodies to rat pancreas Golgi subfractions: identification of a 58-kD cis-Golgi protein. *J. Cell Biol.* 105:2021-2029.
- Sarkar, M., E. Hull, Y. Nishikawa, R. J. Simpson, R. L. Moritz, R. Dunn, and H. Schachter. 1991. Molecular cloning and expression of cDNA encoding the enzyme that controls conversion of high-mannose to hybrid and complex N-glycans: UDP-N-acetylglucosamine:  $\alpha$ -3-D-mannoside  $\beta$ -1,2-N-acetylglucosaminyltransferase I. *Proc. Natl. Acad. Sci. USA.* 88:234-238.
- Schweizer, A., J. Fransen, T. Baechli, L. Ginsel, and H. P. Hauri. 1988. Identification, by a monoclonal antibody, of 53-kD protein associated with a tubulovesicular compartment at the cis-side of the Golgi apparatus. *J. Cell Biol.* 107:1643-1653.
- Shaper, N. L., G. H. Hollis, J. G. Douglas, I. R. Kirsch, and J. H. Shaper. 1988. Characterization of the full length cDNA for murine  $\beta$ -1,4-galactosyltransferase: novel features at the 5'-end predict two translational start sites at two in-frame AUGs. *J. Biol. Chem.* 263:10420-10428.
- Simons, K., and A. Wandering-Ness. 1990. Polarized sorting in epithelia. *Cell.* 62:207-210.
- Snider, M. D., and O. C. Rogers. 1985. Intracellular movement of cell surface receptors after endocytosis: resialylation of asialo-transferrin receptor in human erythroleukemia cells. *J. Cell Biol.* 100:826-834.
- Swift, A. M., and C. E. Machamer. 1991. A Golgi retention signal in a membrane-spanning domain of coronavirus E1 protein. *J. Cell Biol.* 115:19-30.
- Tang, B. L., S. H. Wong, S. H. Low, and W. Hong. 1992. The transmembrane domain of N-acetylglucosaminyl-transferase I contains a Golgi retention signal. *J. Biol. Chem.* In press.
- Tartakoff, A. M., and P. Vassalli. 1983. Lectin-binding sites as markers of Golgi subcompartments: proximal-to-distal maturation of oligosaccharides. *J. Cell Biol.* 97:1243-1248.
- Tooze, J., S. A. Tooze, and S. D. Fuller. 1987. Sorting of progeny coronavirus from condensed secretory proteins at the exit from the trans-Golgi network of AtT20 cells. *J. Cell Biol.* 105:1215-1226.
- Vaux, D., J. Tooze, and S. Fuller. 1990. Identification by anti-idiotypic antibodies of an intracellular membrane protein that recognizes a mammalian endoplasmic reticulum retention signal. *Nature (Lond.).* 345:485-502.
- Weinstein, J., E. U. Lee, K. McEntee, P.-H. Lai, and J. C. Paulson. 1987. Primary structure of  $\beta$ -galactoside  $\alpha$ 2,6-sialyltransferase: conversion of membrane-bound enzyme to soluble forms by cleavage of the NH<sub>2</sub>-terminal signal anchor. *J. Biol. Chem.* 262:17735-17743.
- Woods, J. W., M. Doriaux, and M. G. Farquhar. 1986. Transferrin receptors recycle to cis- and middle as well as trans-Golgi cisternae in Ig-secreting myeloma cells. *J. Cell Biol.* 103:277-286.