Dipeptidyl peptidase IV (DDD) is a type II plasma membrane protein. Replacement of its transmembrane domain with that of another surface protein, aminopeptidase N, resulted in accumulation in the Golgi apparatus of Madin-Darby canine kidney cells and a delayed Golgi to surface transport in Chinese hamster ovary (CHO) cells. The compartment of retardation was identified as post medial-Golgi, most likely to be the trans-Golgi/trans Golgi network (TGN). Compared to native DDD, the rate of endoplasmic reticulum to Golgi transport in CHO cells resulted in a significant accumulation in the Golgi apparatus of the chimera was largely unchanged in both cell types. On the other hand, Golgi to surface transport was delayed by more than 2 h in CHO cells and essentially undetectable up to 22 h of chase in Madin-Darby canine kidney cells. The decrease in the rate of Golgi to surface transport in CHO cells resulted in a significant accumulation of the fusion protein in the trans-Golgi/TGN. This phenomenon is very unlikely to be due to any drastic conformational changes, as neither the enzyme activity nor the dimerization of the constructed molecule was affected. The findings of this study indicate that the transmembrane domain, in the context of its flanking sequences, is important for efficient Golgi to cell surface transport.
protein resulted in its accumulation in the Golgi in MDCK cells and a Golgi to surface transport retardation in CHO cells. The precise compartment of transport blockade in these two cell types is defined by immunocytochemical localization and biochemical characterization. The significance of this finding with regard to the understanding of Golgi retention and Golgi to surface transport is discussed.

EXPERIMENTAL PROCEDURES

Materials—DNA modification and restriction enzymes were purchased from Life Technologies, Inc. Amersham Corp. Cell culture media, fetal bovine serum (FBS), and dialyzed FBS and geneticin (G418) were from Life Technologies, Inc. Brefeldin A (BFA) was from Epicentre Technologies (Madison, WI). [35S]Met (>1000 Ci/mmol) was from Amer sham Corp. FITC-conjugated goat anti-mouse IgG, endoglyc osidase H, and neuraminidase were from Boehringer Mannheim. All the lectin-agaroses were purchased from E. Y. Labs., Inc. (San Mateo, CA). Streptavidin-agarose and a-NHS-biotin were from Pierce Chemical Co. All other reagents were from Sigma. Monoclonal antibodies against rat DPP IV have been described previously (Low et al., 1991b) and were a generous gift from Dr. D. L. Mendrick (Harvard Medical School, Cambridge, MA).

Construction of the DAD Chimera—Standard procedures of DNA manipulation, polymerase chain reaction, and transfection were followed (Sambrook et al., 1989). The transmembrane domain of DPP IV was replaced by that of ApN to generate the chimera DAD (Fig. 1A) by oligonucleotide-primed polymerase chain reaction. The primers used were: Sense primer, 5'-CTGAAATTCGGAGACCATGAACACCGTG-GAACGTGGCATCTGGCCTTGGAAGCCGCTGCCTGTT-GACA-3'; Antisense primer, 5'-CAGGGGCGGCTACTCGTCTTACCCACACA-TGTAAGCTTCCAGGCGCATCTCAGTGTAGGACGCGTCTGC-3'. Its sequence was confirmed by DNA sequencing, and the chimera was inserted into the mammalian expression vector pRSN (Low et al., 1991a, 1991b) under the control of a Rous sarcoma virus-SV 40 promoter-enhancer system.

Cell Culture and Transfection—MDCK (strain IB) cells were kindly provided by Dr. K. Simons (European Molecular Biology Laboratory, Heidelberg, Germany). CHO cells were from the American Type Culture Collection (Rockville, MD). They were maintained in Dulbecco's essential medium supplemented with 10% FBS. Stable transfection was achieved by the calcium phosphate precipitation method. Transfected cells were selected and maintained in Dulbecco's essential medium with 750 mg/ml or 2500 pg/ml G418 for MDCK and CHO cells, respectively (Low et al., 1991b).

Immunofluorescence Microscopy—This was performed as previously described (Wong et al., 1992). Cells were cultured as a monolayer on glass coverslips and fixed with 2.7% paraformaldehyde and not permeabilized for surface staining or permeabilized with 0.1% saponin for total staining. Cell labeling was visualized using a Zeiss Axioskop microscope with epifluorescence optics and photographed with Kodak Tri-X 400 film.

Metabolic Labeling of Cells—Cells were washed twice with Hank's balanced salt solution 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 [35S]Met (0.5 mCi/ml in labeling buffer) for 30 min, washed, and chased in medium containing excess unlabeled methionine (100 mg/ml) for various times as indicated.

Cell Surface Biotinylation and Immunoprecipitation—This was performed as previously described (Low et al., 1991b).

Lectin Affinity Chromatography—Immunoprecipitated proteins were eluted from the protein A-Sepharose beads by boiling in 80 ml of SDS sample buffer (without β-mercaptoethanol) followed by dilution in luc- tbin buffer (40 ml Tris-HCl, pH 7.5, 150 mm NaCl, 0.5% Triton X-100, 1 mm CaCl2, 1 mm MgCl2, and 1 mm MnCl2). Equal aliquots were then incubated with the various lectin agarooses (100 pg of slurry) for 2-2 h. The agarose beads were washed four times in buffer A (25 mm Tris-HCl, pH 7.5, 500 mm NaCl, 0.5% Triton X-100, and 1 mm phenylmethylsulfonyl fluoride) and three times with buffer B (30 mm Tris-HCl, pH 7.8, and 150 mm NaCl). After washing, the bound material was eluted by boiling in SDS sample buffer and analyzed by SDS-PAGE.

SDS-PAGE and Analysis of Fluorography—This was performed as described (Low et al., 1991a).

Sucrose Gradient Centrifugation—The protocol of Jascier et al. (1991) was closely adhered to. Cells were labeled with [32P]Met for 20 min and not chased or chased for 3 h. The cells were solubilized in 400 ml of 100 mm Na2HPO4, 1% Triton X-100, and 10 mm phenylmethylsulfonyl fluoride, pH 7.0. This was loaded onto a 9-mi 5-25% (w/v) sucrose gradient in 100 mm Na2HPO4, 1% Triton X-100, and 10 mm phenylmethylsulfonyl fluoride, pH 7.0. The gradients were centrifuged in an SW 41 rotor ( Beckman) at 41,000 revolutions/min (205,000 gav) using the Beckman L8-R ultracentrifuge for 20 h at 6 °C. Each gradient was divided into 18 fractions of 0.5 ml each and analyzed by immunoprecipitation and SDS-PAGE.

Enzyme Assay—This procedure was carried out exactly as previously described (Tang et al., 1992). Briefly, DDD and DAD were immunoprecipitated and incubated with 0.5 mg/ml substrate, glycyl-glycyl-proline-p-nitroanilide; in a 75 mM glycine reaction buffer, pH 8.5, at 37 °C for 30 min. The reaction was terminated with 1 m sodium acetate, pH 4.2. The p-nitroanilide released in the reaction was determined by measuring absorbance at 385 nm.

RESULTS

ER to Golgi Transport of DAD Is Unaffected in CHO and MDCK Cells—In the construct DAD, the transmembrane domain of DDD was replaced by that of ApN (Fig. 1A). In order to study the kinetics of biogenesis of DAD, pulse-chase experiments were performed. Transfected cells were pulse-labeled with [35S]Met for 30 min and chased for varying lengths of time as indicated in Fig. 1. B and C. Labeled DDD and DAD molecules were immunoprecipitated from cell lysates and analyzed by SDS-PAGE and fluorography.

As previously reported (Low et al., 1991a), DDD in MDCK cells is synthesized as a 100-kDa precursor in the ER, which is endo H-sensitive. This precursor is then further processed into a 110-kDa endo H-resistant form, due to modification of its N-glycans by the Golgi enzyme, mannosidase II, which has been localized to the cis/medial-Golgi (Dunphy and Rothman, 1983; Farquhar, 1985; Kornfeld and Kornfeld, 1985). As shown in Fig. 1B, approximately 80% of the 100 kDa form was converted into 110 kDa within 40 min of chase (lanes 3 and 7) and by 60 min (lane 4), virtually all the DDD had been converted into the 110 kDa form. The results were confirmed in the presence of endo H, where the molecule was seen to become endo H-resistant after a chase time of 60 min (lane 1). In CHO cells (Fig. 1C), the ER to Golgi rate of transport for DDD was approximately the same, the molecule also acquiring endo H resistance in 60 min.

When the biogenesis of DAD in CHO and MDCK cells was studied, results virtually identical to those for DDD were obtained. From this, it can be concluded that the chimera was able to exit the ER to become modified by enzymes in the medial-Golgi and that the ER to Golgi rate of transport was essentially not affected by the transmembrane domain replacement.

Accumulation of DAD in the Golgi Apparatus—The localization of the protein in the two cell types was determined by indirect immunofluorescence. Transfected cells were cultured on coverslips and either permeabilized or not, for total or cell surface staining, respectively. Visualization of the proteins was by sequential incubation with monoclonal antibodies and goat anti-mouse IgG conjugated with FITC. The coverslips were then processed for microscopy and photography.

MDCK and CHO cells expressing DDD exhibited staining over the entire cell surface in both permeabilized and unpermeabilized cells (Fig. 2), typical of a cell surface protein. DAD was undetectable on the surface of non-permeabilized MDCK cells, but in permeabilized cells showed intense perinuclear staining, characteristic of the Golgi apparatus (Louvard et al., 1982; Lipsky and Pagano, 1985). This clearly indicates that in MDCK cells, the DAD molecule is not transported to the surface but retarded in the Golgi. When CHO cells were stained for DAD, intact cells displayed distinct surface staining, while permeabilized cells possessed the intense Golgi ring (which colocalized with an endogenous Golgi marker, mannosidase II, data
Fig. 1. A, amino acid sequence of the transmembrane domain of DDD and DAD. Only sequences in the transmembrane domain of DAD have been altered, and the residues are shown. The cytoplasmic and ectoplasmic residues remain unchanged as DPPIV.

B, effect of the trans-transport in MDCK cells. 'bansfected membrane substitution on ER to Golgi cells were pulsed with [35S]Met for 30 min and chased for various periods as indicated. DDD or DAD were immunoprecipitated from the cell lysates with monoclonal antibodies (bound to protein A-Sepharose beads) directed against the ectoplasmic domain of DPPIV. Half of the sample was treated with endo H (lanes 8-14), while the other half, processed identically without endo H, served as a control (lanes 1-7). The proteins were resolved on a 7% SDS-PAGE and [35S]-labeled proteins were detected by fluorography. The 100-kDa polypeptide is the endo H-sensitive ER form, while the 110 kDa band represents the endo H-resistant Golgi form. The product of endo H digestion is indicated by arrowheads (~88 kDa).

C, effect of the transmembrane substitution on ER to Golgi transport in CHO cells. The experiment was performed identically as mentioned above, except that CHO cells were studied. Since DAD was found to acquire endo H resistance, the molecule is deduced to reside in a post-medial-Golgi compartment. To confirm this, MDCK and CHO cells were treated with 50 and 10 µg/ml of BFA, respectively, 1 h prior to immunofluorescence processing. BFA treatment caused DAD to be redistributed from a Golgi staining pattern into diffuse reticular punctate staining (including the nuclear envelope) present throughout the cytoplasm (Fig. 3), typical of the ER (Louvard et al., 1982; Terasaki et al., 1984; Tang et al., 1992). This confirms that DAD is retained in the Golgi system (Lippincott-Schwartz et al., 1990; Tang et al., 1992b).

The Compartment of Retardation Is Defined as the trans-Golgi/TGN—In order to more precisely determine the nature of the Golgi subcompartment in which DAD collects, its N-linked carbohydrate structures were examined by lectin affinity chromatography. CHO cells were labeled for 30 min and chased for 3 h to allow sufficient time for DAD to accumulate in the Golgi. The cells were lysed and DAD was immunoprecipitated and further subjected to capture by various lectin-agarose beads. The material bound to the beads was analyzed by SDS-PAGE. Only the results in CHO cells are presented, although similar results were obtained for both cell types.

As seen in Fig. 4A, only the mature 110 kDa form of the molecule was recovered indicating that the chase time was more than sufficient to allow ER to Golgi transport. DDD and DAD were recovered from ConA- and WGA-agarose which bind mannose and both sialic acid and N-acetylglucosamine, respectively. ECA binds specifically to the Gal-β1,4-GlcNac moieties in N-linked glycans and sialic acid substitution on this structure prevents its binding. Since DDD was seen to bind strongly not shown), in addition to the surface staining, implying that in CHO cells, retardation in the Golgi is incomplete, with detectable surface expression. The intensity of the surface staining varied proportionately with the level of expression in the cell.
to ECA, whereas binding of DAD was negligible, it can be surmised that there is a transport block of DAD through the Golgi, where it accumulates in the ST compartment to become extensively sialylated and prevented from its binding to ECA. Transport of DDD, however, is unchecked, enabling it to continue to the surface and remain incompletely sialylated. SNA and MAA bind α-2,6- and α-2,3-linked sialic acids, respectively, signifying modification by α-2,6- and α-2,3-ST, both of which have been localized to the trans-Golgi cisternae and/or the TGN. CHO cells do not have the capacity for α-2,6-sialylation (Lee et al., 1989), thus no binding was observed with SNA-agarose beads. In contrast to DDD, DAD was seen to bind strongly to MAA-agarose, indicating that it is retarded in the α-2,3-ST compartment, allowing ample opportunity for modification. The DDD molecule enroute to the surface may have a very transient stay in this compartment and thus escaped extensive modification. Binding to Lotus- and Jacalin-agarose was not observed, suggesting that no significant fucose residues or O-glycosidically linked oligosaccharide side chains were added.

The results of a pulse-chase time course followed by MAA-agarose capture is shown in Fig. 4B. Only the mature 110 kDa form that has been processed by α-2,3-ST was recovered by MAA binding. A faint band is visible after only 20 min chase, implying that DAD was able to travel to the trans-Golgi to be sialylated efficiently and quickly, thus preventing its binding to ECA agarose, in agreement with earlier data. By 2 h, essentially maximal levels of DAD had reached the MAA-binding compartment.

For neuraminidase treatment, the cells were pulsed for 30 min with [35S]Met and chased for various times as indicated in Fig. 5. The cell lysate was analyzed by immunoprecipitation followed by digestion with or without neuraminidase. This enzyme removes sialic acid residues added in the trans-Golgi/TGN (Kornfeld and Kornfeld, 1985), resulting in a reduction in size of the 110 kDa form, which first appears after 20 min of chase, while the 100-kDa precursor remains resistant. This serves as a further confirmation that the DAD molecule has been modified by α-2,3-ST and has travelled as far as the trans-Golgi/TGN compartment.

FIG. 3. Effect of BFA on the subcellular localization of DAD by immunofluorescence. Cells grown on coverslips were incubated with (B and D) or without (A and C) BFA at 50 μg/ml for MDCK cells (A and B) and 10 μg/ml for CHO cells (C and D), 1 h prior to processing for immunofluorescence as detailed in Fig. 2 (bar, 10 μm).

Transport to the Cell Surface Is Essentially Abolished in MDCK Cells, But Significantly Retarded in CHO Cells—The transport of DDD and DAD to the cell surface was compared using the technique of pulse-chase and surface biotinylation. The cells were pulse-labeled for 30 min and chased for different times, followed by cell surface biotinylation. Proteins arriving at the cell surface were quantitatively determined by immunoprecipitation and streptavidin-aided recovery of biotinylated proteins.

Less than 1 h was required for the appearance of DDD on the surface of both cell types and maximal surface expression was achieved by 2 h (Figs. 6 and 7, lane 3). After 1 h of chase in MDCK cells, approximately 50% of the labeled DDD had reached the surface (Fig. 6B); however in CHO cells almost all the molecules had been transported to the surface within the equivalent chase period (Fig. 7B). This implies that the rate of transport to the surface is more efficient in CHO cells. In contrast, DAD in MDCK cells was not detected on the surface even after 22 h (Fig. 6A, lane 7), but remained inside the cell (lane 14), in compliance with the immunofluorescence data. However, prolonged exposure revealed faint bands, increasing in
intensity for the 7–22-h chase periods (data not shown), suggesting that the accumulation of DAD in the Golgi of MDCK cells is due to severe retardation of its Golgi to surface transport. In CHO cells, DAD was only detected on the cell surface at the 2-h chase period (Fig. 7A, lane 3) and only after 4 h was the maximal level reached (Fig. 7B). If the more efficient surface transport in CHO cells is taken into consideration, transport of DAD to the cell surface in CHO cells is significantly delayed by more than 2 h in comparison to DDD, with a large portion of the protein staying intracellularly. This is consistent with the enhanced Golgi staining data from the immunofluorescence studies.

Dimerization of DDD and DAD Occurs Normally in Both Cell Types—Many small intestinal brush border enzymes exist as noncovalently linked homodimers (Kenny and Maroux, 1982) and DDD is no exception. A previous study has demonstrated that the ER form of DDD exists as a monomer and dimerization occurs in the Golgi apparatus (Jascur et al., 1991). To examine whether the block in surface transport of the DAD molecule is the result of its failure to dimerize, its sedimentation pattern was compared to DDD in sucrose gradient rate-zonal centrifugation experiments. The cells were pulse-labeled for 30 min and either not chased or chased for 3 h. Cell extracts were subjected to sucrose gradient centrifugation, and the gradients were divided into 18 equal aliquots (fraction 1 corresponds to the top of the gradient) and analyzed by immunoprecipitation and SDS-PAGE (Fig. 8).

In MDCK cells after 30 min of labeling and no chase, DDD was present as a high-mannose 100-kDa ER form, mainly in fractions 7–11. When chased for 3 h, only the 110-kDa Golgi processed form was recovered from fractions 12 to 15. This shift to the monomeric form in CHO cells was determined from an upper to a lower fraction corresponds to the monomeric and dimeric forms of DDD, respectively (Jascur et al., 1991). A similar shift was observed for DDD in CHO cells, indicating that the Golgi-processed form of DDD was able to dimerize in both cell types. When cells expressing DAD were examined, the pattern of sedimentation obtained corresponded with that of DDD. From this, we can rule out the inability of DAD to form dimers as the cause of Golgi accumulation.

**DAD Is Enzymatically Active**—It has been shown that misfolded proteins might be excluded from the secretory pathway but instead are retained in the ER (Klausner and Sita, 1990). To exclude the possibility of misfolding as the reason for the observed accumulation of DAD in the Golgi, its enzyme activity was determined and compared to the native protein. Due to high background levels obtained with total cell extracts, immunoprecipitated proteins were used for the experiment. Immunoprecipitated proteins were recovered from cell extracts, a portion of which was used for the enzyme assay as described under "Experimental Procedures," and the remainder was run on an SDS-PAGE and subjected to Western blotting.

The activity of DDD in MDCK and CHO cells was 1.738 ± 0.11 and 1.756 ± 0.10 A_{285} units, respectively. This compared favorably with the activity of DAD, which was measured as 1.720 ± 0.12 and 1.687 ± 0.11 A_{285} units in MDCK and CHO cells, respectively. No enzyme activity was detected in untransfected cells. From the results of the Western blot, it was determined that the amount of protein immunoprecipitated from the transfected cells and used for the enzyme assay was approximately equal (data not shown). Thus, as the enzyme activity of the chimera was comparable to the wild-type protein, it can be concluded that DAD is properly folded.

**DISCUSSION**

The DAD Chimera Is Retarded in Its Transport to the Surface at the Trans-Golgi/TGN—All the Golgi glycosyltransferases cloned to date have been type II transmembrane proteins. Molecular dissection of these molecules by our laboratory (Wong et al., 1992; Tang et al., 1992) and others (Munro, 1991; Nilsson et al., 1991; Russo et al., 1992; Aoki et al., 1992) have delineated that sequences in and around the transmembrane domain are necessary and sufficient for Golgi retention. Furthermore, the transmembrane domain of α-2,6-ST and NT were able to confer Golgi localization to surface reporters in MDCK and COS cells. In this report, we show that the replacement of the transmembrane domain of a surface protein, DPPIV, with that from another plasma membrane protein, ApN, results in accumulation in the Golgi of the chimera. This retardation in surface transport was much more severe in MDCK cells, but only partial in CHO cells. The precise location of the transport block was de-
**Fig. 5.** Neuraminidase digestion of DAD. The cells were pulsed for 30 min and chased for various times as indicated. The immunoprecipitated DAD was divided into equal aliquots and half was treated with neuraminidase, while the other half served as a control.

**A**

Biotinylation

Chase

DDD 110 →

DAD 110 →

**B**

Surf 1/10 Total

**Fig. 6.** A, surface expression of DDD and DAD in MDCK cells. Cells were pulsed for 30 min and chased for various times as indicated. The cell surface was biotinylated with NHS biotin. Following lysis, DDD and DAD were recovered from the cell extract by immunoprecipitation and biotinylated, and 35S-labeled proteins were captured by absorption to streptavidin-agarose and resolved by SDS-PAGE. B, quantitation of the surface expression in MDCK cells. The surface to total ratio was plotted as a function of chase time for DDD (square) and DAD (diamond) in MDCK cells.
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A

Biotinylation

\[
\begin{array}{cccccccccc}
\text{Chase} & 0 & 1 & 2 & 4 & 7 & 10 & 22 & 0 & 1 & 2 & 4 & 7 & 10 & 22 \\
\hline
\text{DDD} & \text{110} & \text{100} & \text{110} & \text{100} & \text{110} & \text{100} & \text{110} & \text{100} & \text{110} & \text{100} & \text{110} & \text{100} & \text{110} & \text{100} \\
\text{DAD} & \text{110} & \text{100} & \text{110} & \text{100} & \text{110} & \text{100} & \text{110} & \text{100} & \text{110} & \text{100} & \text{110} & \text{100} & \text{110} & \text{100} \\
\end{array}
\]

Fig. 7. A, surface expression of DDD and DAD in CHO cells. The identical experiment was performed as outlined in Fig. 6, except that CHO cells were studied. B, quantitation of the surface expression in CHO cells. The surface to total ratio was plotted as a function of chase time for DDD (square) and DAD (diamond) in CHO cells.

determined by various biochemical assays to be in the trans-Golgi/TGN.

In comparison to the native protein, the DAD chimera was shown to have a similar rate of ER to Golgi transport in MDCK and CHO cells. BFA treatment resulted in redistribution of the protein from a perinuclear Golgi ring to an ER staining pattern, indicating its accumulation in the Golgi apparatus. This was confirmed with lectin binding and neuraminidase digestion experiments which further narrowed the transport block to the ST compartment in the trans-Golgi/TGN. Surface biotinylation experiments showed that DAD was directly retarded in the Golgi and not transcytosed back from the surface. In MDCK cells, the retardation in the Golgi was more severe, and Golgi to surface transport was not detected by immunofluorescence, but only by surface biotinylation after prolonged exposure (data not shown). This contrasted with findings in CHO cells, where a normal ER to Golgi transport rate of the chimera, together with a significant delay in transport to the surface, resulted in less rigorous retardation of DAD in the Golgi and significant surface expression.

The Retardation in the Golgi Is Not Due to Gross Changes in Polypeptide Folding or Oligomeric Structure—The proper folding of a protein is a prerequisite for its export from the ER to the Golgi, as misfolded and/or misassembled proteins are retained and degraded in the ER (Klausner and Sitia, 1990). The quaternary structure of the chimera was inferred indirectly by examining its peptidase activity. Since the level of enzyme activity of DAD in both cell types was comparable to that of the native protein, it can be concluded that the replacement of the transmembrane domain did not cause any gross structural alterations. In fact, the efficient ER to Golgi transport in both cell types makes the notion of severe misfolding seem unlikely. The ability of DAD to form dimers was also investigated and similarly discovered to be comparable to DDD. Together, these results prove that DAD is not substantially altered in its structure and conformation.
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**MDCK CELLS**

| Fraction | Chase time |
|----------|------------|
| 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 | 0 hr |

**DDD**

| Fraction | Chase time |
|----------|------------|
| 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 | 0 hr |

**DAD**

| Fraction | Chase time |
|----------|------------|
| 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 | 3 hr |

**CHO CELLS**

| Fraction | Chase time |
|----------|------------|
| 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 | 0 hr |

**DDD**

| Fraction | Chase time |
|----------|------------|
| 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 | 3 hr |

**DAD**

| Fraction | Chase time |
|----------|------------|
| 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 | 0 hr |

**Creation of a Golgi Retention Signal?**—We have created for the first time a Golgi retarded protein by simply replacing the transmembrane domain of a surface protein with that of another of equal length. We could interpret these results to mean that a Golgi retention signal was fortuitously created by the transmembrane domain switch. CHO cells may possess a more stringent specificity in their Golgi retention mechanism, resulting in incomplete Golgi retention for DAD and escape of the molecules to the surface. To date, no primary structure homology has been observed in the transmembrane domains of all the Golgi localized proteins cloned so far (for review, see Machamer, 1991). In fact, a construct consisting of the adjacent cytoplasmic and luminal sequences of α-2,6-ST separated by a stretch of 17 leucine residues with chicken lysozyme as the reporter, is retained in the Golgi (Munro 1991). From this, it can be deduced that the primary structure of the transmembrane domain may be unimportant for Golgi retention, but rather the structural conformation in the proper context of the adjoining sequences which is crucial.

**Possible Involvement of DPPIV Transmembrane Domain in Efficient Golgi to Surface Transport**—Another explanation would be that the transmembrane domain of DPPIV, in the
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1993

Summary of proteins defective in Golgi to surface transport

The abbreviations used are: VSV G, vesicular stomatitis virus glycoprotein; GPI, glycosylphosphatidylinositol; hGH-DAF, human growth hormone-decay accelerating factor; SIM, sucrose-isomaltase; rGH, rat growth hormone.

| Molecule                  | Alteration                      | Location of mutant protein | Location of wild-type protein | Refs.          |
|---------------------------|---------------------------------|----------------------------|--------------------------------|----------------|
| Rat growth hormone        | Transmembrane substitution      | Golgi apparatus            | DPP IV and Apn = plasma membrane rGH = secreted, G protein = plasma membrane | This report, Guan and Rose, 1984 |
| VSV G protein             | rGH fused to cyto- and transmembrane domain of G protein | Golgi apparatus            | Plasma membrane                  | Gabel and Bergmann, 1985 |
| Sucrase-isomaltase        | COOH-terminal deletion          | Golgi apparatus            | Plasma membrane                  | Sivasubramanian and Nayak, 1987 |
| Influenza virus neuraminidase | From patient with congenital SIM deficiency | Golgi apparatus            | Plasma membrane                  |                    |
| Fowl plaque virus hemagglutinin | Arg-26 to Leu in the signal anchor domain | Golgi apparatus            | Plasma membrane                  | Garten et al., 1992 |
| hGH with DAF GPI signal   | Mutated GPI signal (un- cleaved) | Golgi apparatus            | Plasma membrane                  | Moran and Caras, 1992 |

context of its adjacent flanking sequences, makes up a recognition signal allowing molecules to be packaged and transported from the Golgi to the surface. In MDCK cells, the receptors involved in surface transport may be less accommodating compared with CHO cells, causing severe intracellular retardation of DAD in the Golgi. We have also observed that several other chimeras, constructed from plasma membrane and secretory proteins, have also been found to be Golgi-localized. The fact that chimeras with vastly differing structural alterations should all be Golgi retained renders the first explanation, that a Golgi retention signal could be created so easily by simply cutting and joining, less likely. We speculate that the trans-Golg/TGN to surface step in the exocytic pathway may possess a filtering mechanism which prevents transport of incompetent proteins from exiting the Golgi apparatus.

Signal-mediated Transport Versus Transport by Default —The first explanation invoking the creation of a Golgi retention signal is in keeping with the currently accepted view of surface transport for DPPIV was twice as fast compared with DPPIV and related signals. This report.

The mechanism of vesicular budding and fusion at each junction of the exocytic pathway shares common features while having its own unique components which render specificity (Bennett and Scheller, 1993; Warren, 1993). The rab family of small GTP-binding proteins, different members of which have been localized to different parts of the exocytic pathway (Chavrier et al., 1990), have been implicated to function as molecular switches in vesicular transport, reminiscent of similar functions performed by other members of the ras superfamily. A flurry of recent reports provides evidence for the involvement of heterotrimeric G proteins in regulating vesicle formation (Leyte et al., 1992), fusion (Colombo et al., 1992), and processes which precede budding, such as binding of coat proteins to membranes (Donaldson et al., 1991) and also see Bomsel and Mostov, 1992, for a review). More than one heterotrimeric G protein may be involved in any one process. The involvement of trimeric G proteins supports the notion that vesicle budding is tightly regulated and/or may depend on some upstream signaling process. The stringency and high degree of specificity achieved by the multicomponent regulation at each step of transport would argue for the requirement of some sort of signal for a protein to be incorporated into the budding machinery, either by directly activating the budding process or indirectly interacting with components that can activate the process at each juncture. Signal-mediated transport of membrane proteins to the basolateral surface in polarized epithelial cells have been firmly established (for review, see Mostov et al., 1992). Likewise, Golgi to cell surface transport in general may also require as yet undefined signals.

Acknowledgments—We are grateful to Dr. Kai Simons (EMBL) for the MDCK (strain II) cells and Dr. D. L. Menardrick (Harvard Medical School) for monoclonal antibodies against DPPIV. We also thank Francis Leong and S. Y. Oh for excellent photographic assistance.

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