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Retention of a *cis* Golgi Protein Requires Polar Residues on One Face of a Predicted α-Helix in the Transmembrane Domain

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The first membrane-spanning domain (m1) of the model *cis* Golgi protein M (formerly called E1) from the avian coronavirus infectious bronchitis virus is required for targeting to the Golgi complex. When inserted in place of the membrane-spanning domain of a plasma membrane protein (vesicular stomatitis virus G protein), the chimeric protein ("Gm1") is retained in the Golgi complex of transfected cells. To determine the precise features of the m1 domain responsible for Golgi targeting, we produced single amino acid substitutions in m1 and analyzed their effects on localization of Gm1. Expression at the plasma membrane was used as the criterion for loss of Golgi retention. Rates of oligosaccharide processing were used as a measure of rate and efficiency of transport through the Golgi complex. We identified four uncharged polar residues that are critical for Golgi retention of Gm1 (Asn_{465}, Thr_{469}, Thr_{476}, and Gln_{480}). These residues line one face of a predicted α-helix. Interestingly, when the m1 domain of the homologous M protein from mouse hepatitis virus is inserted into the G protein reporter, the chimeric protein is not efficiently retained in the Golgi complex, but transported to the cell surface. Although it possesses three of the four residues we identified as important in the avian m1 sequence, other residues in the membrane-spanning domain from the mouse protein must prevent efficient recognition of the polar face within the lipid bilayer of the *cis* Golgi.

INTRODUCTION

The Golgi complex plays an instrumental role both in posttranslational processing of membrane-bound and secreted proteins and in sorting of membrane traffic (Farquhar, 1985; Mellman and Simons, 1992). This organelle has a unique structure in higher eukaryotic cells consisting of flattened stacks of cisternal membranes found in the central region of the cell. The stacks are polarized with respect to distribution of resident enzymes, and itinerant proteins move through the organelle in a vectorial manner.

As a first step in understanding the structural complexity of the Golgi complex, we are attempting to define the signals responsible for targeting of resident Golgi proteins. These proteins must be retained in the appropriate subcompartment of the Golgi complex in the face of substantial membrane traffic through the organelle. In the absence of specific retention signals, proteins that enter the exocytic pathway are thought to be transported to the plasma membrane by default (Rothman, 1987). We have been using the M glycoprotein from the infectious bronchitis virus (IBV),[1] an avian coronavirus, as a model protein for our studies. Although in previous reports this protein was called "E1," the name M was recently adopted for consistency in coronavirus nomenclature (Cavanagh *et al.*, 1990). The IBV M protein is targeted to *cis* Golgi membranes of animal cells when expressed from cDNA (Machamer *et al.*, 1990). Because cDNAs coding for endogenous proteins of the *cis* Golgi

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[1] Abbreviations used: DME, Dulbecco's modified Eagle's medium; ER, endoplasmic reticulum; IBV, infectious bronchitis virus; MHV, mouse hepatitis virus; TGN, *trans* Golgi network; VSV, vesicular stomatitis virus.

complex have not yet been isolated, this protein continues to be a useful model for targeting to this subcompartment. We previously found that the first of the three membrane-spanning domains of the IBV M protein was required for Golgi targeting (Machamer and Rose, 1987) and subsequently determined that this membrane-spanning domain ("m1") could retain several plasma membrane proteins when inserted in place of their own transmembrane domains (Swift and Machamer, 1991).

The targeting information for three glycosyltransferases has been analyzed in a similar way (reviewed by Shaper and Shaper, 1992). α2,6-Sialyltransferase (Munro, 1991; Colley *et al.*, 1992; Wong *et al.*, 1992), β1,4-galactosyltransferase (Nilsson *et al.*, 1991; Aoki *et al.*, 1992; Russo *et al.*, 1992; Teasdale *et al.*, 1992), and N-acetylglucosaminotransferase I (Burke *et al.*, 1992; Tang *et al.*, 1992) contain targeting information within their single transmembrane domains, although flanking sequences are also required for efficient targeting. The region flanking the lumenal side of the transmembrane domain of α2,6-sialyltransferase (the "stem" domain) may also contain targeting information (Munro, 1991; Colley *et al.*, 1992). Galactosyl- and sialyltransferase are generally considered "late" (*trans* or *trans* Golgi network [TGN]) markers, whereas N-acetylglucosaminotransferase I is considered a middle Golgi marker. No primary sequence homologies are observed when the transmembrane sequences of these and other cloned Golgi enzymes are examined (Machamer, 1991; Shaper and Shaper, 1992). Like the IBV M protein, the fact that information for retention of these transferases seems to be found in sequences buried in the lipid bilayer suggests that the membrane composition of the Golgi complex plays an essential role in appropriate retention of resident membrane proteins.

Limited mutagenesis of the IBV M protein suggested that several polar residues in m1 might be critical for Golgi retention (Swift and Machamer, 1991). Here we have further defined the sequence requirements for retention of the model Golgi protein, Gm1. This chimeric protein is composed of the ectodomain and cytoplasmic tail of the vesicular stomatitis virus (VSV) G protein (a plasma membrane protein) and the first membrane-spanning domain of IBV M. We subjected the m1 domain in Gm1 to extensive mutagenesis to test the contribution of these and other residues in the absence of other IBV M sequences. We report that residues within m1 that are critical for retention of Gm1 in the Golgi complex are uncharged polar residues that line one face of a predicted α-helix.

MATERIALS AND METHODS

Cells and Transfection

COS-7 cells were maintained in Dulbecco's modified Eagle's medium (DME) with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in 5% CO_2. Cells were plated the day before transfection in 35-mm dishes. Transfection of cells (70% confluent) with the SV40-based vector pJC119 via DEAE/dextran was as described previously (Machamer *et al.*, 1985), except 5 μg of DNA was used per plate. Expression was analyzed 40–44 h after transfection.

Mutagenesis

All general recombinant DNA techniques were as described (Sambrook *et al.*, 1989). For single amino acid substitutions, cDNA encoding Gm1 was mutagenized with degenerate oligonucleotides (30- to 35-mers) using the Kunkel method (Kunkel *et al.*, 1987) as described previously (Swift and Machamer, 1991). Degenerate oligonucleotides were designed to replace a single codon with up to eight possible different codons. After DNA sequencing, mutant inserts were subcloned into the SV40-based expression vector, pJC119. Mutant proteins were named by appending the original amino acid followed by the new amino acid in single-letter code (e.g., $Gm1QT_{480}$ is Gm1 with Gln changed to Thr at residue 480). Because a large number of mutations were produced, all were reconfirmed by double-stranded DNA sequencing after subcloning into the expression vector. The block replacement of amino acids 466–471 for M-Gm1a was produced using a 48-mer, precisely replacing codons for these amino acids with those encoding this region of the mouse hepatitis virus M. The mutations producing M-Gm1, M-Gm1b, and M-Gm1c were then introduced individually into the background of M-Gm1a.

Indirect Immunofluorescence Microscopy

COS-7 cells grown on coverslips were fixed, permeabilized, and stained for Gm1 and mutant Gm1 proteins as previously described (Swift and Machamer, 1991) using a monoclonal anti-G antibody (I1; Lefrancois and Lyles, 1982) and Texas red-conjugated goat anti-mouse IgG (Jackson Immunoresearch). For double labeling with fluorescein-conjugated *Lens culinaris* lectin (Sigma, St. Louis, MO), fixed and permeabilized cells were first incubated in 5 μg/ml lectin, followed by labeling for Gm1. Cells were visualized and photographed as described (Swift and Machamer, 1991). For the experiment shown in Figure 1, cells were treated with 5 μg/ml brefeldin A (Epicentre Technologies, Madison, WI) or 5 μg/ml nocodozole (Sigma) for 60 min before fixation.

Radiolabeling, Immunoprecipitation, Oligosaccharide Processing, and Electrophoresis

COS-7 cells expressing Gm1 or mutant Gm1 proteins were labeled 44 h post-transfection. After incubation for 15 min in methionine-free DME, cells were labeled in 0.5 ml methionine-free DME containing 100 μCi/ml of Tran^{35}S-label (>1100 Ci/mmol; ICN, Costa Mesa, CA) for 15 min. Labeling medium was removed and cells were incubated in regular growth medium with a threefold excess of the normal concentration of unlabeled L-methionine for various times before lysis. Lysis and immunoprecipitation with a polyclonal anti-VSV antibody were exactly as described previously (Swift and Machamer, 1991). Treatment of immunoprecipitated proteins with endoglycosidase H was also as described, except 0.4 mU of recombinant endoglycosidase H (ICN) was used per sample. After addition of 4× Laemmli sample buffer, proteins were electrophoresed in 10% polyacrylamide gels. Fluorograms from preflashed film were quantitated by densitometry.

RESULTS

Gm1 Is Retained in an Early Golgi Compartment

The IBV M protein is targeted to the *cis* Golgi when expressed from cDNA in animal cells (Machamer *et al.*, 1990). The chimeric protein Gm1, which has the ecto-

domain and cytoplasmic tail of the VSV G protein (a plasma membrane protein) and the first membrane-spanning domain (m1) of IBV M was targeted to the Golgi region of transfected COS-7 cells as determined by indirect immunofluorescence microscopy (Swift and Machamer, 1991). Because we found that the two N-linked oligosaccharides on Gm1 remained in an endoglycosidase H-sensitive form, it appeared that Gm1 was targeted to the same region of the Golgi complex as the original IBV M protein. However, it remained possible that the Gm1 protein was localized to a subcompartment of the endoplasmic reticulum (ER) or the intermediate compartment between the ER and the Golgi complex.

Further evidence that the Gm1 protein is targeted to the Golgi complex was obtained by treating cells with drugs that disrupt Golgi morphology. In double-label indirect immunofluorescence microscopy, the distribution of the Gm1 protein in ~75% of transfected COS-7 cells was found to overlap with the distribution of proteins that bind *Lens culinaris* lectin (Figure 1). This lectin binds to mannose residues of fucosylated N-linked oligosaccharides terminating in galactose, N-acetylglucosamine, or sialic acid (Kornfeld *et al.*, 1981) and has been used as a Golgi marker in COS-7 cells (Hsu *et al.*, 1992). The remaining 25% of COS-7 cells expressing Gm1 had ER-like reticular staining and were generally expressing much higher levels of protein. When cells were treated with brefeldin A before fixation, >95% of expressing cells showed an ER-like pattern when stained for Gm1 (Figure 1). The *Lens culinaris*–binding proteins redistributed to the ER as well. Figure 1 also shows that disruption of microtubules with nocodozole before fixation also caused a redistribution of the Gm1 protein into a dispersed, punctate pattern similar to that reported for other Golgi proteins (Kreis, 1990). These results suggested that the Gm1 protein reaches the Golgi complex in the majority of transfected COS-7 cells. This observation and our previous finding that the oligosaccharides on Gm1 are not processed by middle Golgi enzymes (Swift and Machamer, 1991), suggests that the Gm1 protein is indeed a *cis* Golgi resident protein.

Mutagenesis of the m1 Domain Reveals Amino Acids Required for Golgi Retention

To determine the amino acids in the transmembrane domain required for Golgi retention, we subjected the m1 domain to site-directed mutagenesis. We began by mutagenizing residues that are conserved in M proteins from different coronaviruses. We produced the mutations in Gm1 rather than the original M protein because we wanted to analyze the contribution of residues in m1 in the absence of other IBV M sequences. We previously showed that Gln_{480} in the membrane-spanning domain of Gm1 might be important for Golgi retention, because changing it to isoleucine resulted in loss of retention in the Golgi complex and transport to the plasma membrane (Swift and Machamer, 1991). To further test this idea, we substituted 10 other residues for Gln_{480} in Gm1 and tested the intracellular localization of the mutant proteins in transfected COS-7 cells. Indirect immunofluorescence microscopy of transfected cells showed that 9 of the substitutions at Gln_{480} resulted in transport of Gm1 to the plasma membrane (Figure 2). These substitutions (Ile, Met, Gly, Cys, Val, Ala, Thr, Tyr, and Ser) range from semi-conservative to nonconservative. Two substitutions (Asn and His) were tolerated, because these mutant Gm1 proteins remained in the Golgi region.

To test the rate at which the Gm1 proteins with nontolerated Gln substitutions moved through the Golgi complex to the cell surface, we analyzed the rate at which the oligosaccharides became resistant to endoglycosidase H in a pulse-chase labeling experiment. Gm1 proteins that were not retained were transported rapidly through the Golgi, with half-times of oligosaccharide processing of 25–30 min (Figure 3). In comparison, the wild-type G protein is processed with a half-time of 20 min. As predicted, the two substitutions that were tolerated ($Gm1QN_{480}$ and $Gm1QH_{480}$) resulted in proteins that remained endoglycosidase H-sensitive even after long times of chase. In addition, ER staining was the predominant pattern if cells expressing $Gm1QN_{480}$ or $Gm1QH_{480}$ were treated with brefeldin A for 60 min before fixation. This suggested that these two mutant proteins were retained in the same early Golgi compartment as the parent Gm1 protein.

We produced more point mutations to analyze the contribution of other residues in the m1 domain. Our results are summarized in Figure 4. Single amino acid substitutions that resulted in transport of Gm1 to the plasma membrane are shown on the right side of the m1 domain sequence, and those that were tolerated are shown on the left side. Besides Gln_{480}, three other residues were critical for retention of Gm1. These are also uncharged polar residues: Asn_{465}, Thr_{469}, and Thr_{476}. In contrast, substitutions at six other sites in m1 were tolerated, because these mutant proteins were retained in the Golgi region of transfected cells by indirect immunofluorescence microscopy. The m1 domain is predicted to form an α-helix when the primary sequence is subjected to secondary structure analysis. If m1 does form an α-helix in the membrane, the polar residues critical for retention would line up on one face. This is readily demonstrated when the m1 sequence is displayed as a helical wheel (Figure 5). Consistent with the idea that a polar face is required for retention, insertion of two Ile residues into the middle of m1 in the mutant Gm1ins also resulted in transport to the plasma membrane (Swift and Machamer, 1991). This insertion might throw the face of the helix out of register.

Pulse-chase labeling was used to determine the half-times of oligosaccharide processing for the mutant Gm1

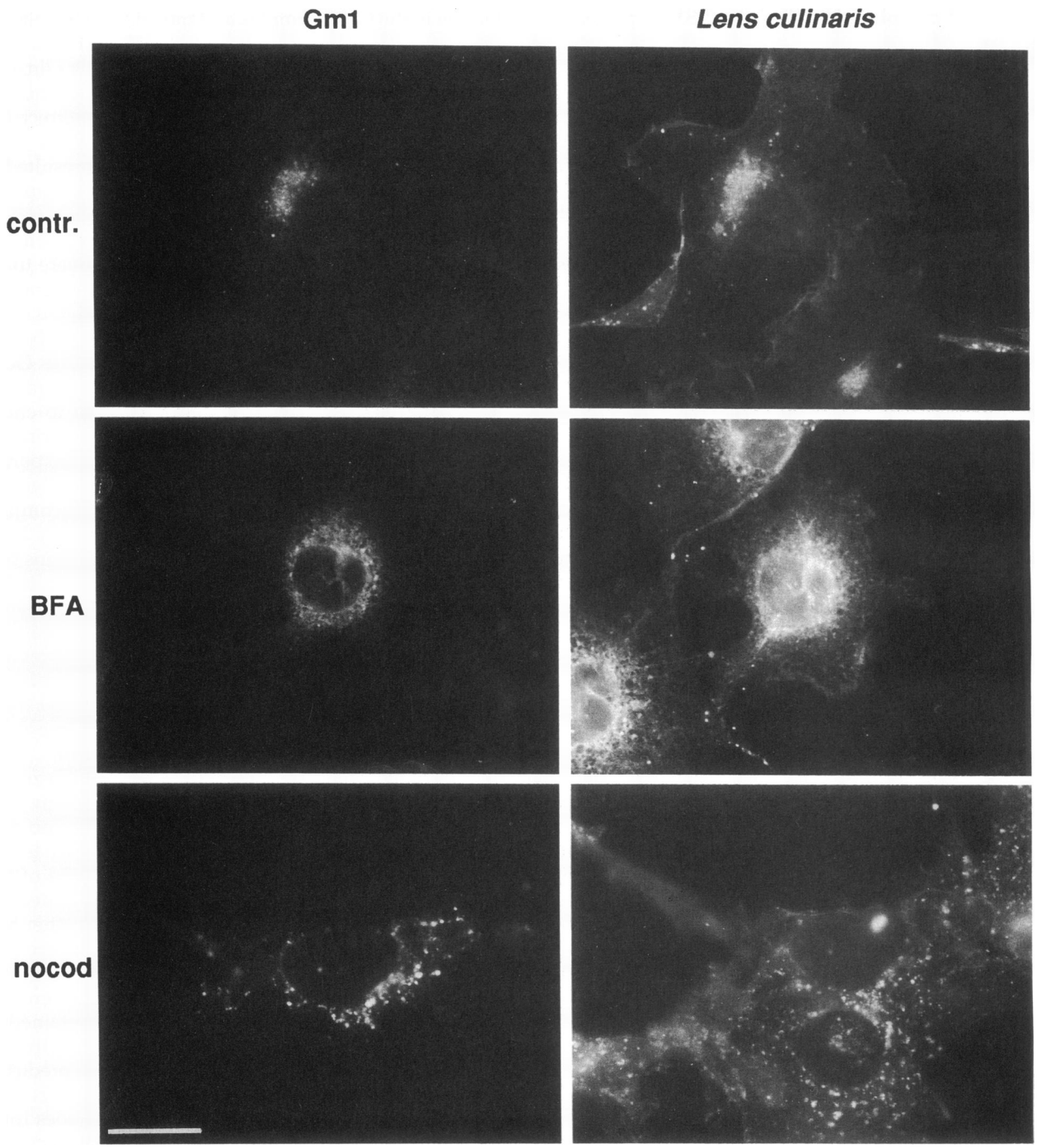

Figure 1. Gm1 behaves like a Golgi protein in transfected COS-7 cells. COS-7 cells expressing Gm1 were fixed, permeabilized, and labeled with fluorescein-conjugated *Lens culinaris* lectin as a marker for the Golgi complex, followed by anti-G antibody and Texas-red conjugated goat anti-mouse IgG. Before fixation cells were untreated (control), treated with 5 μg/ml brefeldin A for 60 min (BFA), or with 5 μg/ml nocodazole for 60 min (nocod). The same field was photographed with the appropriate barrier filters to detect Texas red (Gm1, left) or fluorescein (*Lens culinaris*, right). The distribution of Gm1 largely overlaps that of *Lens culinaris*–binding proteins under all conditions tested. Bar, 10 μm.

proteins that were transported to the cell surface. The results are summarized in Figure 6. The Gm1 proteins with substitutions at Gln_{480} are transported most rapidly (half-times of 25–30 min), and those with substitutions at Thr_{469} most slowly (half-time of 60 min). The fastest rate of oligosaccharide processing for any mutant Gm1 protein (25 min) is still slower than the parent VSV G protein (20 min). Mutant Gm1 proteins with substitu-

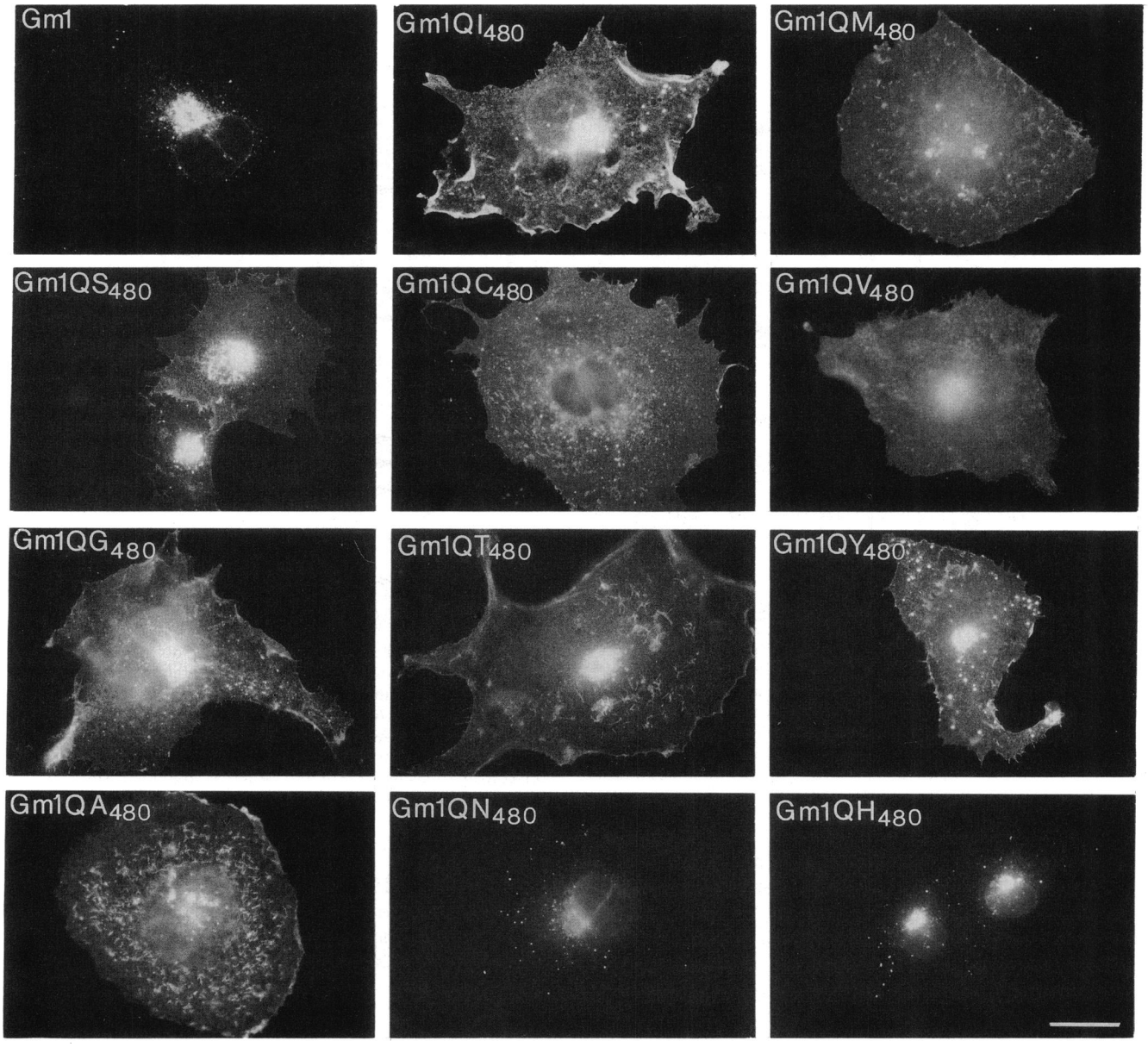

Figure 2. Localization of Gm1 proteins with substitutions at Gln_{480}. COS-7 cells expressing Gm1 or Gm1 mutant proteins were fixed, permeabilized, and stained with anti-G antibody and Texas-red conjugated secondary antibody. All substitutions with the exception of Asn ($Gm1QN_{480}$) and His ($Gm1QH_{480}$) resulted in mutant proteins that were readily detectable at the plasma membrane. Bar, 10 μm.

tions at two critical positions (e.g., NV_{465} plus QC_{480}) still had oligosaccharide processing half-times of 25 min. In preliminary experiments, $Gm1QI_{480}$ appeared to fold and oligomerize at the same rate as the VSV G protein (as measured by trimerization rates; Doms *et al.*, 1988), suggesting that the difference in the rate of transport of $Gm1QI_{480}$ was in exit from the early Golgi, not from the ER. Thus, even though the retention signal is inactivated by substitutions at Gln_{480}, it may still be functioning partially or transiently. The mutant Gm1 proteins that were retained in the Golgi complex did not acquire resistance to endoglycosidase H, consistent with the idea that they were retained in the same compartment as Gm1.

The m1 Domain from the Related Mouse Hepatitis Virus M Protein Is not Sufficient for Golgi Localization

Armstrong and Patel (1991) reported that the related M protein from the murine coronavirus mouse hepatitis virus (MHV) requires its cytoplasmic tail for retention

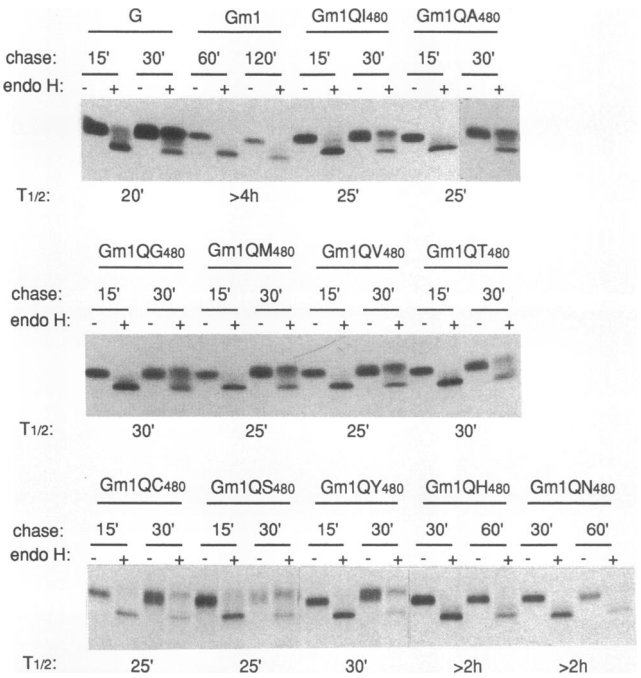

Figure 3. Transport rates of Gm1 mutant proteins with Gln substitutions. COS-7 cells were labeled for 15 min and chased for the times shown. Immunoprecipitated Gm1 proteins were split into two aliquots and left untreated (−) or incubated with endoglycosidase H (+). The half-times of processing of the N-linked oligosaccharides shown below the lanes ($T_{1/2}$) is the average from this and two other experiments and was determined by densitometry of the fluorograms. No oligosaccharide processing was observed for Gm1, $Gm1QN_{480}$, and $Gm1QH_{480}$, so the half-times are indicated as greater than the longest chase time used (time points not included in this figure).

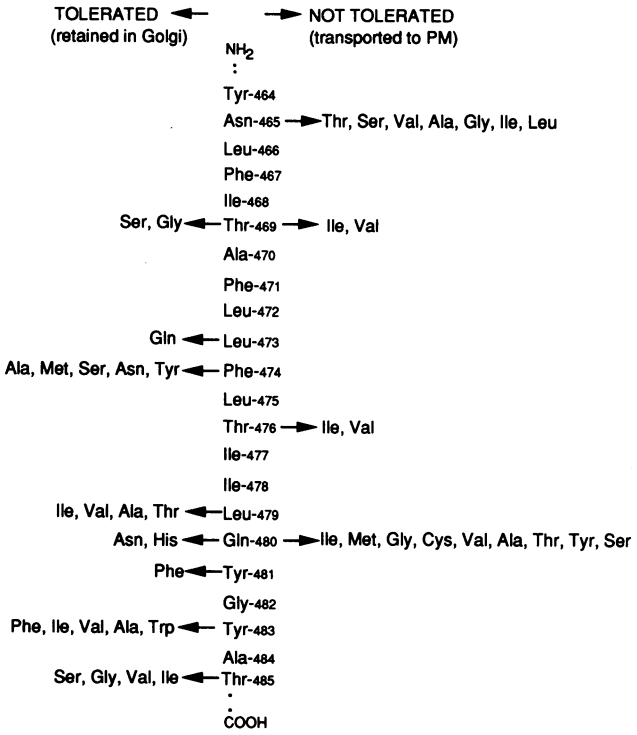

Figure 4. Summary of mutations in the m1 domain of Gm1. The effect of single amino acid substitutions on the retention of Gm1 were tested by indirect immunofluorescence microscopy and pulse-chase labeling as described for Gln_{480} substitutions in Figures 2 and 3. The sequence of m1 is shown; individual substitutions shown on the right side of the sequence were not tolerated (mutant proteins were transported to the plasma membrane), whereas individual substitutions shown on the left side were tolerated.

in the Golgi complex. In addition, a mutant MHV M protein lacking the second and third membrane-spanning domains was retained in the ER (Armstrong *et al.*, 1990), not in the Golgi complex, like a similar deletion mutant of IBV M (Machamer and Rose, 1987). The MHV results have been difficult to reconcile with our finding that the IBV M protein contains Golgi targeting information in the m1 domain. However, it appears that the MHV M protein is targeted to the *trans* Golgi and TGN when expressed from cDNA (Krijnse-Locker *et al.*, 1992). Thus, when expressed from cDNA in the absence of the other coronavirus proteins, the different localizations of the IBV and MHV proteins within the Golgi complex may explain the difference in targeting signals (Machamer, 1991).

The m1 domain of MHV M contains all three of the amino acids shown to have the strongest effect on targeting of our chimeric Gm1 protein (Asn_{465}, Thr_{476}, and Gln_{480}). We wondered why the MHV M protein, given its m1 sequence, was not retained in the *cis* Golgi. The amino acid sequences of the amino termini of IBV and MHV M proteins are shown in Figure 7A. Although the proteins share limited sequence identity, the membrane topology of the two proteins is predicted to be identical (Boursnell *et al.*, 1984). The highest regions of sequence conservation occur within the first (Figure 7A) and second membrane-spanning domains (Boursnell *et al.*, 1984). To test if the amino acid differences in the MHV

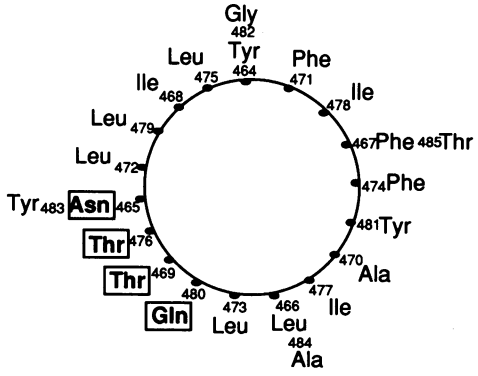

Figure 5. Residues in m1 critical for Golgi retention of Gm1 line one face of a predicted α-helix. The m1 sequence is displayed in the helical wheel format (Schiffer and Edmonson, 1967). Residues important for Golgi retention of Gm1 are boxed.

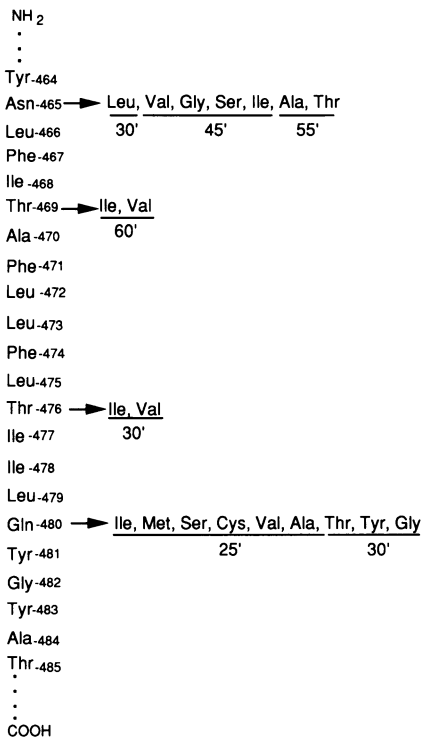

Figure 6. Transport rates for mutant Gm1 proteins that are not retained. The half-times for processing of the N-linked oligosaccharides to an endoglycosidase H–resistant form are shown. Data were acquired in pulse-chase labeling experiments like that shown in Figure 3. For reference, the wild-type VSV G protein is processed with a half-time of 20 min in COS-7 cells.

m1 domain influenced Golgi targeting, we produced a chimeric Gm1 protein that contained the MHV m1 domain instead of IBV m1. The resulting protein (M-Gm1) possessed all of the MHV m1 domain with the exception of the last two residues. When expressed in COS-7 cells, immunofluorescence staining showed that the M-Gm1 protein was not retained in the Golgi complex, but was transported to the plasma membrane (Figure 7B). In pulse-chase labeling experiments, the half-time for acquisition of endoglycosidase H-resistance was ~60 min. This result indicated that the MHV m1 domain was not sufficient for targeting of MHV M to the *trans* Golgi or TGN, consistent with results of Armstrong and Patel (1991).

To determine what amino acid differences were responsible for the lack of Golgi retention even when the three key residues were present, we produced Gm1 proteins with partial MHV m1 sequences. The most obvious difference between the MHV and IBV m1 domains is a six amino acid block near the lumenal side of the membrane (the unboxed stretch in the m1 sequences shown in Figure 7A). When this block (residues 466–471 in Gm1) was replaced with the MHV sequence, the resulting chimera was retained in the Golgi region (M-Gm1a, Figure 7B). The oligosaccharides on M-Gm1a remained endoglycosidase H sensitive, suggesting the protein was retained in an early Golgi compartment. When Tyr_{464} was changed to Trp in the presence of the 6 amino acid block replacement (M-Gm1b), the protein was still retained in the Golgi region. However, when Leu_{475} and Tyr_{481} were changed to Ile and Phe in the presence of the block replacement (M-Gm1c), the protein was transported to the cell surface, although somewhat more slowly than M-Gm1 because the half-time for oligosaccharide processing was 90 min (Figure 7B). Because changing Tyr_{481} to Phe in the original Gm1 chimera (with the IBV m1 domain) did not disrupt Golgi retention (Figure 4), we conclude that one or more of the other changes found within the 6 amino acid block in combination with Phe_{481} and/or Ile_{475} caused a loss of Golgi retention. Substitution of Trp for Tyr_{464} enhanced this loss in retention because the half-time for oligosaccharide processing was faster (60 vs. 90 min). The slow transport times for M-Gm1 and M-Gm1c may indicate that the Golgi retention signal is functioning transiently. The residues in the MHV sequence responsible for the loss of Golgi retention are not present on the polar face of m1, nor are they localized to any other face of the putative α-helix. Thus, subtle differences in the amino acid sequence of the membrane-spanning domain can influence the effectiveness of the retention signal. The inability of the MHV m1 domain to block exit from the *cis* Golgi would presumably allow signals in other regions of the full-length MHV M protein (perhaps in the cytoplasmic tail) to mediate retention in a later Golgi compartment such as the TGN.

DISCUSSION

In this paper, we have characterized the Golgi retention signal found in the first membrane-spanning domain (m1) of the IBV M glycoprotein. To analyze the residues in m1 required for retention in the absence of other IBV M sequences, we introduced single amino acid substitutions into the m1 domain of the chimeric protein Gm1. This protein consists of the ectodomain and cytoplasmic tail of the VSV G protein (a well-characterized plasma membrane protein) and the m1 domain from IBV M as the membrane-spanning domain (Swift and Machamer, 1991). The Gm1 protein was efficiently retained in the early region of the Golgi complex, most likely the *cis* Golgi. This region of the Golgi complex has been termed the *cis* Golgi network (Huttner and Tooze, 1989; Hsu *et al.*, 1991; Pelham, 1991). Transport of mutant Gm1 proteins to the plasma membrane was taken as evidence that the Golgi retention signal in m1 had been disrupted. We also determined the rate of oligosaccharide processing as a measure of the rate of intracellular transport (and thus the efficiency of release from Golgi retention). We found four residues within m1 that were critical for retention of Gm1: Asn_{465}, Thr_{469}, Thr_{476}, and Gln_{480}. Previously, we found that a single replacement (Ile) at

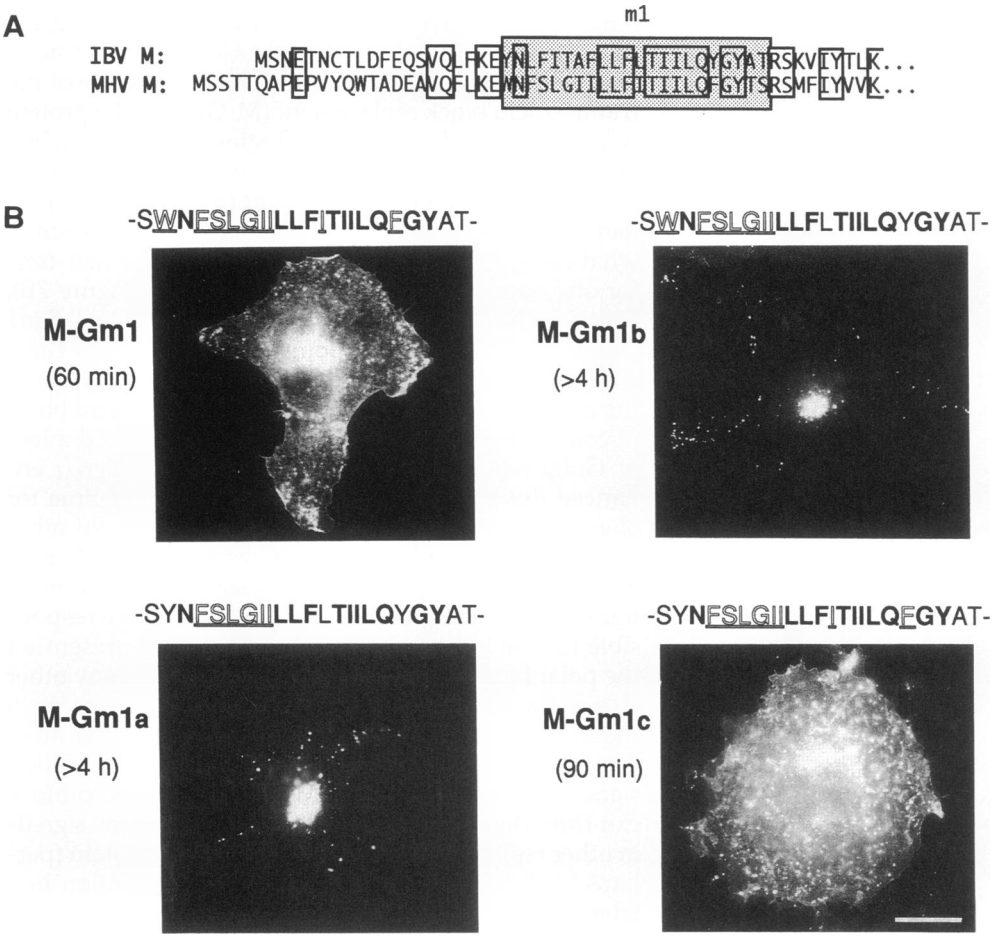

Figure 7. A Gm1 protein with the MHV m1 domain instead of the IBV m1 domain is not retained in the Golgi complex. (A) Amino acid sequence comparison of the amino-termini of IBV and MHV M proteins (in single letter code). Identities are boxed and the first membrane-spanning domain (m1) is shaded. (B) Indirect immunofluorescence of COS-7 cells expressing M-Gm1 (with the nine substitutions found in the MHV sequence) and M-Gm1 proteins with partial MHV sequence (M-Gm1a, b, and c). The sequences of the m1 domains in each of these proteins is shown above the appropriate micrograph, with conserved residues in bold and the MHV M substitutions underlined. The half-times for oligosaccharide processing are shown in parentheses under the mutant name. M-Gm1 is not retained in the Golgi complex. M-Gm1c is also transported to the plasma membrane, although more slowly than M-Gm1. Bar, 10 μm.

Asn_{22}, Thr_{33}, or Gln_{37} in the IBV M protein lacking its second and third membrane-spanning domains disrupted retention (Swift and Machamer, 1991). However, the limited mutagenesis performed and the presence of the IBV M lumenal and cytosolic domains limited our conclusions in that study. When the m1 domain is modeled as an α-helix, the uncharged polar residues that are critical for retention of Gm1 line up on one face. Our results suggest that this polar face may specify protein-protein interactions within the lipid bilayer that are important for Golgi retention.

The sequence within m1 required for retention was specific, because substitution of other polar residues at some of the critical positions inactivated the retention signal. In addition, the four polar residues did not seem to contribute equally to Gm1 retention. Substitutions at Gln_{480} resulted in the most efficient and rapid transport to the plasma membrane, followed by those at Thr_{476} and then Asn_{465}. Only two of the four substitutions made at Thr_{469} resulted in transport to the plasma membrane, and this transport was rather slow. In addition, subtle contributions from the rest of the m1 sequence must occur because substitution of the related MHV m1 sequence for the IBV m1 sequence in Gm1 (M-Gm1) resulted in a loss of retention in the Golgi. In contrast to the IBV M protein, the MHV M protein is retained in the *trans* Golgi and TGN when expressed from cDNA (Krijnse-Locker *et al.*, 1992). Although not retained in the Golgi complex, M-Gm1 contains the three most important residues for retention (Asn_{465}, Thr_{476}, and Gln_{480}). Instead of Thr at position 469, it has Gly, but this change was tolerated when tested in the original Gm1 chimera. Thus, the other differences in amino acid sequence in the MHV m1 domain (residues not on the putative polar face) must influence the recognition or function of the retention signal. Perhaps these residues influence the disposition of the membrane-spanning domain or its flanking regions. The slow transport of M-Gm1 and M-Gm1c (oligosaccharide processing half-times of 60 and 90 min, respectively) suggests that the retention signal in these proteins may function transiently. The finding that the MHV m1 domain is not sufficient for retention of the M-Gm1 protein in the *trans* Golgi or TGN is consistent with findings of Armstrong and Patel (1991).

How might a sequence buried in the lipid bilayer mediate specific targeting of a membrane protein? Two possible mechanisms are retrieval and retention (Ma-

chamer, 1991). The retrieval model invokes a constitutively recycling receptor with a ligand-binding site within its own transmembrane domain. The receptor would bind any escaped Golgi proteins and return them to the appropriate Golgi subcompartment (or to the ER for another round of vesicular transport and chance to be retained). This type of mechanism has been postulated to explain the retrieval of escaped KDEL proteins to the ER (reviewed by Pelham, 1991). The retention model implicates the formation of an oligomer or "lattice" of the retained protein mediated by its transmembrane domain and induced only in the appropriate Golgi subcompartment. This structure would be prevented from entering transport vesicles for steric reasons or by lack of mobility in the bilayer. The retrieval and retention mechanisms are not mutually exclusive, and both could operate for efficient targeting. Both models require differences in microenvironment (such as lipid composition or divalent cation concentration) between compartments, either for receptor binding and release or for inducing oligomerization.

We have not identified any potential receptor proteins that associate with Gm1. We favor the idea that the m1 domain is involved in retention rather than retrieval for several reasons. First, even with high levels of expression, we never observed "saturation" of the retention machinery with concomitant expression at the plasma membrane. Instead, high levels of expression resulted in accumulation in the ER. This has been noted by other groups studying retention of Golgi glycosyltransferases (Munro, 1991; Nilsson *et al.*, 1991). Second, we have evidence that the Gm1 protein forms large oligomers upon arrival in the Golgi complex (Weisz *et al.*, 1993). Mutant Gm1 proteins that are not retained do not form these oligomers. We do not yet know if other proteins are included in these oligomers, but it is possible that the m1 domain interacts with itself to form the Gm1 oligomers.

If oligomers or lattices of resident glycosyltransferases formed by interactions within transmembrane domains are essential for retention of these enzymes, it would seem likely that transferases found in the same Golgi subcompartment would co-oligomerize. In addition, sequences on one or both sides of the membrane could be expected to stabilize these interactions. Such ideas can be tested. An additional consideration is the observation that certain glycosyltransferases may be found in more than one compartment of the Golgi complex. Nilsson *et al.* (1993) have shown that the distribution of galactosyltransferase (a "*trans*" Golgi enzyme) and N-acetylglucosaminotransferase I (a "*medial*" Golgi enzyme) overlap substantially in HeLa cells.

In summary, we have investigated the retention signal for a model *cis* Golgi protein. The salient features are uncharged polar residues that line one face of a predicted α-helix in the transmembrane domain. Although our mutagenesis was not exhaustive, this signal remains the best characterized Golgi targeting signal to date. Thus, the Gm1 protein should continue to be a useful model for elucidating the mechanism of membrane protein retention within the Golgi complex.

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