Oligomerization of a Membrane Protein Correlates with Its Retention in the Golgi Complex

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Abstract. The first membrane-spanning domain (ml) of the M glycoprotein of avian coronavirus (formerly called El) is sufficient to retain this protein in the *cis*-Golgi. When the membrane-spanning domain of a protein which is efficiently delivered to the plasma membrane (VSV G protein) is replaced with ml, the resulting chimera (Gml) is retained in the Golgi (Swift, A. M., and C. E. Machamer. 1991. *J. Cell Biol.* 115:19–30). When assayed in sucrose gradients, we observed that Gml formed a large oligomer, and that much of this oligomer was SDS resistant and stayed near the top of the stacking gel of an SDS-polyacrylamide gel. The unusual stability of the oligomer allowed it to be detected easily. Gml mutants with single amino acid substitutions in the ml domain that were retained in the Golgi complex formed SDS-resistant oligomers, whereas mutants that were rapidly released to the plasma membrane did not. Oligomerization was not detected immediately after synthesis of Gml, but occurred gradually with a lag of ~10 min, suggesting that it is not merely aggregation of misfolded proteins. Furthermore, oligomerization did not occur under several conditions that block ER to Golgi transport. The lumenal domain was not required for oligomerization since another chimera (αmlG), where the lumenal domain of Gml was replaced by the α subunit of human chorionic gonadotropin, also formed an SDS-resistant oligomer, and was able to form hetero-oligomers with Gml as revealed by coprecipitation experiments. SDS resistance was conferred by the cytoplasmic tail of VSV G, because proteolytic digestion of the tail in microsomes containing Gml oligomers resulted in loss of SDS resistance, although the protease-treated material continued to migrate as a large oligomer on sucrose gradients. Interestingly, treatment of cells with cytochalasin D blocked formation of SDS-resistant (but not SDS-sensitive) oligomers. Our data suggest that SDS-resistant oligomers form as newly synthesized molecules of Gml arrive at the Golgi complex and may interact (directly or indirectly) with an actin-based cytoskeletal matrix. The oligomerization of Gml and other resident proteins could serve as a mechanism for their retention in the Golgi complex.

THE Golgi complex is the site of oligosaccharide processing and sorting for proteins that are destined for delivery to secretory granules, lysosomes, the plasma membrane, and the extracellular space. The Golgi-resident proteins that perform these functions must be retained in the Golgi despite the large amount of lipid and protein traffic through this organelle. Retention of these proteins is thought to involve recognition of positive signals that specify Golgi localization.

A useful model protein for studying Golgi retention signals has been the M glycoprotein (formerly called El) of the avian coronavirus infectious bronchitis virus (IBV)[1]. Coronaviruses bud into the intermediate compartment or Golgi complex of mammalian cells (Griffiths and Rottier, 1992). When cDNA encoding the M protein is expressed in animal cells, the protein is targeted to the *cis*-Golgi (Machamer et al., 1990). Structurally, the IBV M protein consists of a short glycosylated amino-terminal domain, three membrane-spanning domains, and a long carboxy-terminal cytoplasmic domain. The first membrane-spanning domain (ml) of the M glycoprotein is sufficient to retain this protein in the Golgi (Machamer and Rose, 1987). Furthermore, the ml domain can confer Golgi localization to a well-characterized plasma membrane protein (the G protein of vesicular stomatitis virus, or VSV G). VSV G is a type I membrane protein which is cotranslationally inserted into the ER, where it forms homotrimers. It is then rapidly transported through the Golgi complex (as determined by the kinetics of N-linked oligosaccharide processing) and delivered

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1. *Abbreviations used in this paper*: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; hCG, human chorionic gonadotropin; IBV, infectious bronchitis virus; VSV, vesicular stomatitis virus.

to the plasma membrane (for review see Doms et al., 1993). When the membrane-spanning domain of VSV G is replaced with the ml domain of IBV M, the resulting chimera (Gml, see Fig. 1) is retained in the Golgi complex (Swift and Machamer, 1991). Extensive mutagenesis suggests that at least four specific amino acids in the ml domain are critical for Golgi retention of Gml: asparagine 465, threonines 469 and 476, and glutamine 480 (Machamer et al., 1993). Interestingly, these residues form an uncharged polar face along one side of the alpha helix predicted for ml. The polar nature of this face of the helix suggests that protein–protein interactions along this face may mediate Golgi retention of Gml.

There are two general ways to envision the specific retention of a protein by information contained within its transmembrane domain (for review see Machamer, 1991). The first involves the recognition of this domain by a specific receptor that either blocks further movement in the exocytic pathway or retrieves escaped proteins back to the appropriate compartment. A receptor-based model also requires a mechanism for retention or recycling of the receptor itself. The second mechanism invokes changes in the tertiary or quaternary structure of the protein that are induced via the membrane-spanning domain when the protein encounters a particular microenvironment. Changes in protein structure could include oligomerization or aggregation (covalent or non-covalent) with other proteins or lipids, which would prevent movement of the protein into transport vesicles. The *cis*-Golgi may differ from the ER in lipid composition and divalent cation concentration. These changes in microenvironment could conceivably trigger a conformational shift in Golgi resident proteins upon their arrival in the appropriate compartment.

We have been investigating the mechanisms by which ml might mediate retention of Gml in the Golgi complex. We observed that Gml formed a large oligomer soon after synthesis when assayed by sucrose gradient centrifugation. Here, we show that this oligomer is unusually stable, and that its formation correlates with arrival and retention in the Golgi complex. Our findings point to a possible role for oligomerization in the retention of Gml.

Materials and Methods

Reagents

Aprotinin, colchicine, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), cytochalasin D, TPCK-trypsin, and soybean trypsin inhibitor were obtained from Sigma Chem. Co. (St. Louis, MO). Brefeldin A was from Epicentre Technologies (Madison, WI). Stock solutions of reagents were stored at −20°C.

Cells and Transfection

COS-7 and HeLa cells were maintained in DME with 10 and 5% FCS, respectively. COS-7 cells plated in 35-mm dishes (40–70% confluent) were transfected with an SV40-based expression vector using DEAE-dextran (Machamer et al., 1985). Expression was analyzed at 40–48 h posttransfection. For expression using the vaccinia-T7 system, HeLa cells (40–70% confluent) were infected with the recombinant vaccinia virus vTF7-3 encoding T7 RNA polymerase (Fuerst et al., 1986) at a multiplicity of infection of 10–20. After adsorption for 30 min at 37°C, the inoculum was replaced with 0.75 ml of serum-free medium containing 2 μg of a vector (pAR2529) encoding the appropriate gene behind the T7 promoter and 5–10 μl of the cationic lipid "TransfectACE" (GIBCO BRL, Gaithersburg, MD; Rose et al., 1991). Expression level was varied by changing the amount of DNA added per well (0.02–5 μg/dish). In coexpression experiments, cells were transfected with 1 μg of DNA encoding each construct. Expression was analyzed by metabolic labeling starting 3 h after infection.

Mutagenesis and Production of Chimeric Proteins

Gml and related mutants were generated using the Kunkel method of oligonucleotide-directed mutagenesis as described previously (Swift and Machamer, 1991; Machamer et al., 1993). Point mutants were named by appending the original amino acid (in single letter code) followed by the new amino acid. αm (Guan et al., 1988*a*) and TMR (Doms et al., 1988) were obtained from Jack Rose (Yale University). Construction of αmlG was described previously (Swift and Machamer, 1991). tGml was constructed from Gml by inserting a stop codon at position 487 using site-directed mutagenesis (Kunkel et al., 1987).

Indirect Immunofluorescence Microscopy

Indirect immunofluorescence staining of transiently transfected COS-7 cells was performed as described (Machamer and Rose, 1987; Machamer and Rose, 1988). Gml and related mutants were detected using a monoclonal anti-VSV G antibody (5 μg/ml; Lefrancois and Lyles, 1982) followed by Texas red–conjugated, affinity-purified goat anti-mouse IgG (10 μg/ml; Jackson Immuno Research Laboratories Inc., Avondale, PA).

Radiolabeling and Immunoprecipitation

Cells expressing VSV G or mutant G proteins were rinsed once with PBS, starved for 15 min in methionine-free DME, and labeled for the indicated times with 100 μCi/ml Trans^{35}S-Label (ICN Radiochemicals, Irvine, CA) or L-[^{35}S] in vitro cell labeling mix (Amersham Corp., Arlington Heights, IL) in serum-free, methionine-free DME. Cells were solubilized immediately or after the appropriate chase period in serum-free DME containing a threefold excess of unlabeled methionine. Cells were lysed in detergent solution (50 mM Tris, pH 8.0, 1% NP-40, 0.4% deoxycholate, 62.5 mM EDTA, and 0.13 TIU/ml aprotinin). Samples were immunoprecipitated using a polyclonal anti-VSV antibody (produced by immunization of rabbits with purified VSV) and fixed *Staphylococcus aureus* (Calbiochem-Behring Corp., San Diego, CA). After heating to 100°C for 3 min in Laemmli sample buffer containing 5% β-mercaptoethanol, samples were electrophoresed on 10% SDS-polyacrylamide gels as described (Laemmli, 1970). Marker proteins were ^{14}C-methylated standard molecular weight markers (Amersham Corp.). Labeled proteins were detected by fluorography (Bonner and Laskey, 1974). αm chimeras were radiolabeled and solubilized as above but immunoprecipitated using anti-human chorionic gonadotropin (hCG) antibody (Organon Teknika-Cappel, West Chester, PA) and electrophoresed on 12% SDS-polyacrylamide gels.

Cell Treatments

Brefeldin A (5 μg/ml final concentration) was diluted into serum-free medium from a 5 mg/ml stock in ethanol immediately before use. Colchicine (25 mM) was prepared in DMSO, diluted 1:10 into serum-free, methionine-free medium, and added to cells (50 μM final concentration) starting 1 h before metabolic labeling. Cytochalasin D (0.1 M in DMSO) was added to cells (100 μM final concentration) 2 h after infection and included in subsequent changes of medium. An equal volume of DMSO was added to control cells. In experiments involving cell treatment with CCCP, cells were starved in glucose-, methionine-, and cysteine-free medium for 15 min before metabolic labeling in the same medium for 5 min. CCCP (0.1 M stock in DMSO) was added at the beginning of the chase in glucose-free medium to a final concentration of 300 μM.

Cell Surface Biotinylation

HeLa cells were transfected, metabolically labeled for 15 min, and chased for 45 min as described above. Cells were rinsed three times in PBS supplemented with 1 mM $MgCl_2$ and 0.1 mM $CaCl_2$, and then biotinylated using sulfo-NHS-biotin (Pierce Chem. Co., Rockford, IL) as described (Lisanti et al., 1988). Cells were then solubilized as described above and Gml and related proteins immunoprecipitated. Samples were eluted from the *S. aureus* pellets by heating to 100°C in 50 μl 10 mM Tris, 50 mM NaCl, 1% SDS, pH 7.4, and divided into two equal aliquots. One aliquot was kept at −20°C ("total") while the other was diluted tenfold with 10 mM Tris, 50 mM NaCl, pH 7.4, and incubated with streptavidin-coupled agarose

beads (Pierce Chem. Co.) for 60 min at 4°C. The streptavidin beads were then pelleted and washed three times in RIPA buffer. Non-biotinylated proteins in the supernatant were precipitated using TCA. Samples were analyzed by SDS-PAGE followed by fluorography.

Sucrose Gradient Sedimentation

Oligomerization of the Gml protein was analyzed by velocity gradient centrifugation in sucrose performed essentially as described (Doms et al., 1987). Continuous 5–20% (wt/wt) sucrose gradients were poured over a 60% (wt/wt) sucrose cushion (0.4 ml) in SW50.1 tubes. All solutions were in MNT (100 mM NaCl, 20 mM Tris, 30 mM MES, pH 5.8) containing 0.1% Triton X-100. HeLa cells expressing either VSV G or Gml were metabolically labeled for 5 min and harvested (in MNT containing 1% Triton X-100) either immediately or after 60 min of chase. Lysates were loaded on top of the gradients and spun at 44–45,000 rpm for 15–16 h. Fractions (0.35 ml) were collected from the top using a Buchler Auto Densi-Flow IIC, immunoprecipitated with anti-VSV antibody, and electrophoresed to determine the location of the proteins in the gradient. For estimating the size of the oligomer, gradients (5–20% sucrose [wt/wt] in 100 mM NaCl, 50 mM Tris, pH 5.8, 0.1% Triton X-100) were spun at 47,000 rpm for 4 h. The markers used were thyroglobulin (19.3 $S_{20,\omega}$) and catalase (11.3 $S_{20,\omega}$).

Preparation and Proteolytic Digestion of Microsomes

Microsomes were prepared after metabolic labeling and the appropriate chase period from transfected HeLa cells plated in 6-cm dishes. Cells were rinsed in PBS and swelled in 0.8 ml swelling buffer (10 mM Tris, 15 mM NaCl, 1 mM $MgCl_2$, pH 7.4) for 5 min on ice. After scraping with a rubber policeman, 0.25 ml swelling buffer containing 40% sucrose (wt/vol) was added, and the cells were homogenized in a 1 ml Dounce homogenizer with 60 strokes of the A pestle. Lysates were centrifuged briefly (90 s 5,000 rpm) to remove nuclei and large debris. 0.3 ml swelling buffer containing 10% sucrose (wt/vol) was added, and the lysates were centrifuged for 10 min at 100,000 rpm in a Beckman TL-100 ultracentrifuge (T100.2 rotor). Pellets were resuspended gently in PBS and divided into two aliquots. TPCK-Trypsin (20 μg) was added to one aliquot, and both were incubated at 37°C for 30 min. Proteolytic digestion was stopped by adding 20 μg of soybean trypsin inhibitor to both tubes. Samples were solubilized (in detergent solution containing 0.4% SDS or in MNT as noted) and processed further as described in the text and figure legends.

Results

Oligomerization of Gml and Related Mutants Correlates with Retention in the Golgi Complex

Previous studies from this laboratory showed that the first membrane span (ml) of the M glycoprotein of IBV (previously called the E1 glycoprotein) causes the chimeric protein Gml to be retained in the Golgi. The sequence of ml is shown in Fig. 1 *A*. Using site directed mutagenesis, four key residues in the ml domain were shown to be critical for Golgi retention of Gml: asparagine (N_{465}), two threonines (T_{469} and T_{476}), and glutamine (Q_{480}). Some substitutions at Q_{480} are tolerated (e.g., $GmlQH_{480}$) whereas other changes (e.g., $GmlQL_{480}$) result in transport of the protein to the cell surface (Machamer et al., 1993). Another mutation in which two isoleucine residues were inserted near the center of the transmembrane domain (Gml_{ins}) also disrupts retention in the Golgi complex. These three chimeric proteins (Gml_{ins}, $GmlQL_{480}$, and $GmlQH_{480}$) were expressed in transiently transfected COS-7 cells and localized by indirect immunofluorescence (Fig. 1 *B*). VSV G and $GmlQL_{480}$ were readily detected at the plasma membrane, while Gml and $GmlQH_{480}$ were detected only in the Golgi region and largely colocalized with lens culinaris lectin staining, even after treatment with nocodazole or brefeldin A (Machamer et al., 1993). Gml_{ins} staining was less prominent at the plasma membrane than $GmlQL_{480}$, and a considerable amount of fluorescence was detected in the Golgi complex. The localizations of these proteins were confirmed in HeLa cells and BHK cells transfected using a vaccinia-virus-mediated expression system (not shown).

Interestingly, when we analyzed expression of these constructs in HeLa cells and COS-7 cells by metabolic radiolabeling and immunoprecipitation, we noticed the appearance of a radiolabeled SDS-resistant species that migrated at the top of the stacking gel, in addition to products migrating at the expected molecular masses. The material at the top of the stacking gel was more prevalent in samples immunoprecipitated from HeLa cells (Fig. 1 *C*) than in those from COS-7 cells (Fig. 1 *D*). This species was present only in immunoprecipitates from cells that expressed mutants with Golgi-resident populations (Gml, Gml_{ins}, and $GmlQH_{480}$) and it appeared regardless of the level of expression (over a >50-fold range). Although ~50% of the total radioactivity immunoprecipitated from Gml-expressing HeLa cells migrated at the top of the stacking gel, we have not quantitated this information routinely. One problem with quantitation is that this material could include radiolabeled components other than Gml; furthermore, some of the oligomer may not enter the gel at all and be lost during subsequent processing. Gml_{ins} was previously reported to move through the Golgi complex rapidly (with a half time of 35 min in COS-7 cells; Swift and Machamer, 1991). However, the kinetics of transport reflect only the population of protein that enters the separating gel. The SDS-resistant protein may therefore represent a stable Golgi-resident pool.

The SDS-resistant oligomerization did not depend on the type of cells used for expression, since the same results were found using transiently transfected BHK cells and stably transfected CHO cells (not shown). Because the SDS-resistant material was easiest to detect in HeLa cells, we used these cells for subsequent experiments. The material at the top of the stacking gel could be detected after transfer to nitrocellulose and probing with anti-VSV G antibodies by immunoblotting, suggesting that it contained Gml (or the related constructs; data not shown). Furthermore, this large species was efficiently precipitated by several conformation-sensitive anti-VSV G monoclonal antibodies that do not recognize grossly misfolded proteins (Doms et al., 1988). We tried to solubilize the Gml oligomer using a wide variety of lysis conditions and sample treatments. None of the modifications we tested, including changes in salt concentration, reducing agents, detergents, chaotropic agents (e.g., urea, guanidinium HCl), or temperature of solubilization or sample elution disrupted the SDS-resistant oligomer (data not shown). A very small amount of SDS-sensitive Gml could be produced when the top of the stacking gel (containing the SDS-resistant oligomer) was isolated after electrophoresis, placed in the well of a fresh gel, and re-electrophoresed (not shown). Together, these observations suggest that SDS-resistant oligomer formation is an intrinsic property of Gml and mutant Gml proteins that are retained in the Golgi complex, but not of mutants that efficiently reach the plasma membrane.

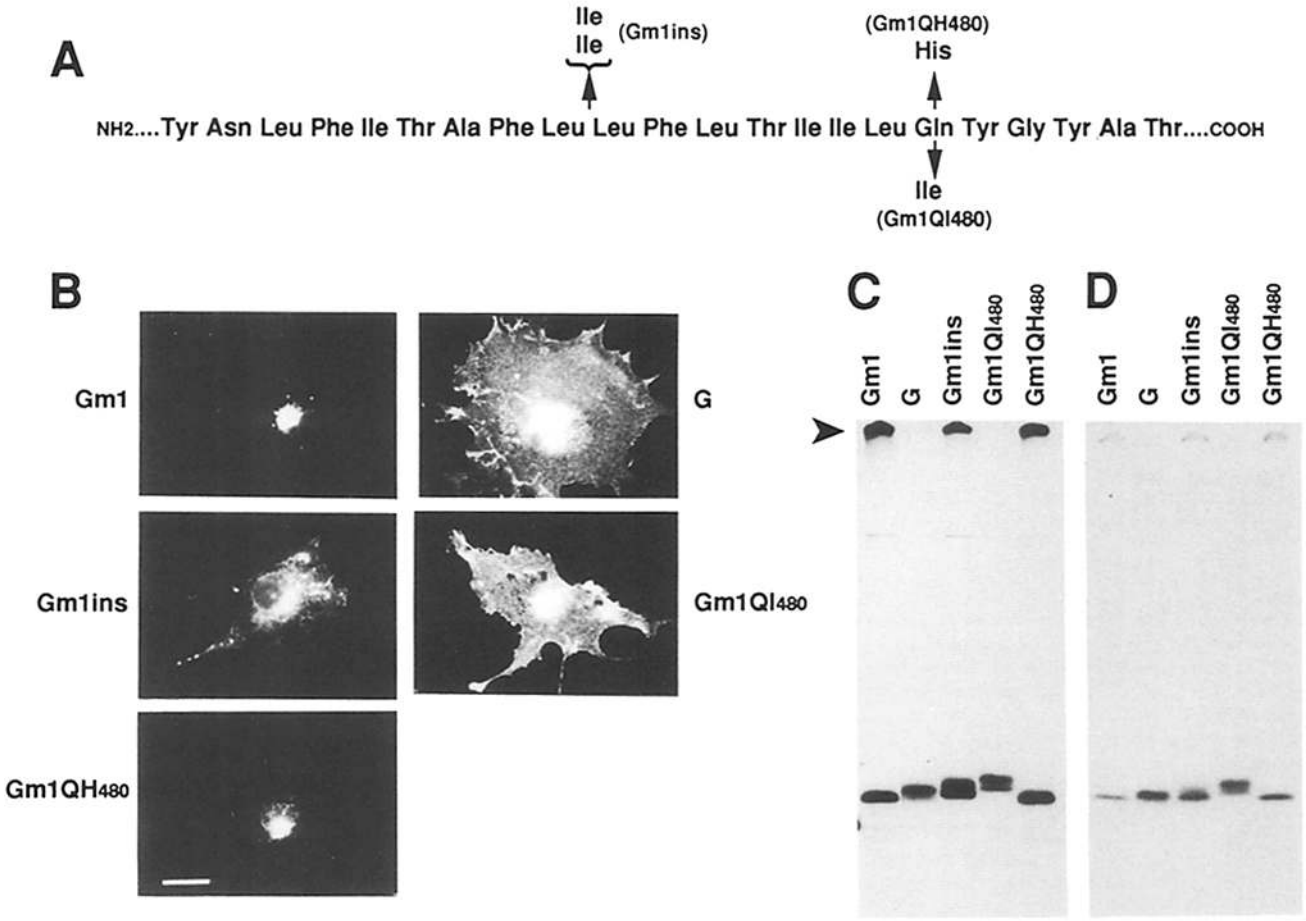

Figure 1. Formation of SDS-resistant oligomers correlates with retention in the Golgi complex. (*A*) Amino acid sequence of the m1 domain. The positions of mutations $Gm1_{ins}$, $GmlQL_{480}$, and $GmlQH_{480}$ are indicated. (*B*) Localization of mutant proteins in transiently transfected COS-7 cells. 40 h after transfection, cells were fixed and processed for indirect immunofluorescence microscopy using anti-VSV G antibody. Bar, 10 μm. (*C* and *D*) Immunoprecipitation of Gm1, VSV G, and related mutants. HeLa cells (*C*) or COS-7 cells (*D*) expressing these constructs were metabolically radiolabeled for 15 min, and then chased for 45 min, solubilized, and immunoprecipitated using an anti-VSV antibody. The immunoprecipitated proteins were electrophoresed on 10% SDS-polyacrylamide gels and analyzed by fluorography. The upper band present in samples containing proteins which are transported to the plasma membrane (VSV G, Gml_{ins}, and $GmlQL_{480}$) represents the mature (sialylated) form of the proteins. Note the SDS-resistant species at the top of the stacking gel (*arrowhead*) in lanes from mutants with Golgi-resident populations.

The SDS-Resistant Oligomer Is Not Susceptible to Cell Surface Biotinylation

If the oligomerized protein represents a distinct intracellular pool of protein(s), it should not be susceptible to cell surface biotinylation. Moreover, we predicted that an oligomer that formed nonspecifically after cell solubilization would be precipitated by streptavidin since incorporation of a few biotinylated molecules would allow precipitation of the entire oligomer. HeLa cells expressing Gm1, VSV G, or $Gm1_{ins}$ were metabolically labeled for 15 min, and then chased for 45 min before cell-surface biotinylation with sulfo-NHS-biotin. After immunoprecipitation, an aliquot of each sample was treated with streptavidin-coupled agarose, and biotinylated and non-biotinylated proteins were recovered and analyzed by SDS-PAGE (Fig. 2). Under these conditions, ~50% of the total VSV G was biotinylated and could be recovered in the streptavidin pellet. Only the mature (sialylated) form of VSV G was recovered in this fraction, suggesting that biotinylation was indeed restricted to cell surface proteins. In contrast, essentially none of the Gm1 was precipitated by streptavidin, suggesting that this protein remained in an intracellular compartment. A small amount of SDS-sensitive $Gm1_{ins}$ was recovered in the streptavidin pellet. More importantly, no SDS-resistant material was biotinylated, suggesting that the SDS-resistant $Gm1_{ins}$ constitutes an intracellular (perhaps Golgi-resident) pool that is distinct from the SDS-sensitive protein, and that only a portion of $Gm1_{ins}$ is transported to the plasma membrane where it can be biotinylated. This result strongly suggested that oligomer formation occurs before cell solubilization.

Kinetics and Cellular Site of SDS-Resistant Oligomer Formation

The time course of Gm1 oligomerization was determined. HeLa cells expressing Gm1 were metabolically labeled for 5 min, chased for the indicated times, solubilized, and immunoprecipitated with anti-VSV G antibody (Fig. 3). Oligo-

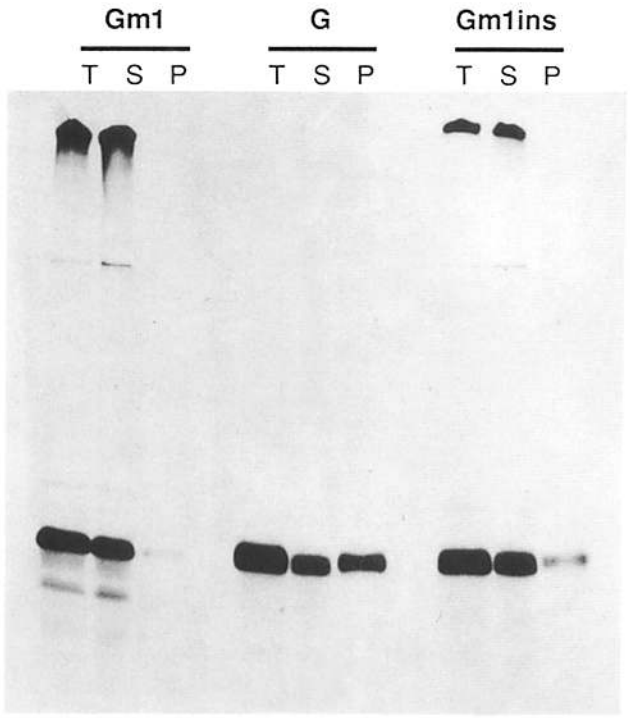

Figure 2. SDS-resistant oligomers are not subject to cell surface biotinylation. Cells were metabolically radiolabeled for 15 min, and then chased for 60 min. Cell surface-specific biotinylation was performed at 4°C using sulfo-NHS-biotin, and the cells were solubilized and immunoprecipitated with anti-VSV antibody. The bound protein was eluted from *S. aureus* pellets and divided in half. One aliquot was solubilized in sample buffer (*T*), and the remainder was incubated with streptavidin-coupled agarose beads. The supernatant ([*S*], unbound material) was collected and TCA precipitated, and the streptavidin beads (*P*) were washed and solubilized before SDS-gel electrophoresis.

merization was not detected immediately after synthesis of Gm1, but occurred gradually with a lag of ~10 min.

The kinetics of Gm1 oligomer formation were consistent with the time at which newly synthesized proteins arrive at the *cis*-Golgi. To determine if oligomerization occurred in a post-ER compartment, we asked whether the SDS-resistant oligomer would form when ER to Golgi traffic was blocked by incubation at 16°C. At this temperature, newly synthesized proteins accumulate in the intermediate compartment (Saraste and Kuismanen, 1984). HeLa cells were transfected with constructs encoding Gm1 or VSV G, metabolically radiolabeled for 5 min, and then chased at 16 or 37°C as indicated for up to 90 min (Fig. 4). No oligomer formed when cells expressing Gm1 were chased at 16°C; however, if cells chased at 16°C were subsequently transferred to 37°C, oligomer formation occurred. In separate experiments, we found that the kinetics of oligomer formation at 37°C were unaffected by the inclusion of a 16°C pretreatment step (data not shown). In addition, treatment with 0.3 mM CCCP during the chase period also inhibited SDS-resistant oligomer formation (not shown). This treatment blocks transport of newly synthesized proteins from the ER. These experiments suggested that Gm1 oligomer formation normally occurred in a post-ER compartment. However, brefeldin A pretreatment (or treatment during chase) did not block oligomer formation (not shown), although it did cause redistribution of Gm1 to an ER-like pattern (Machamer et al., 1993).

Sucrose Gradient Sedimentation Analysis of Oligomer Formation

To ask if Gm1 oligomerization was an artifact generated during SDS-PAGE, we analyzed oligomer formation using velocity gradient sedimentation in sucrose (Doms et al., 1987,

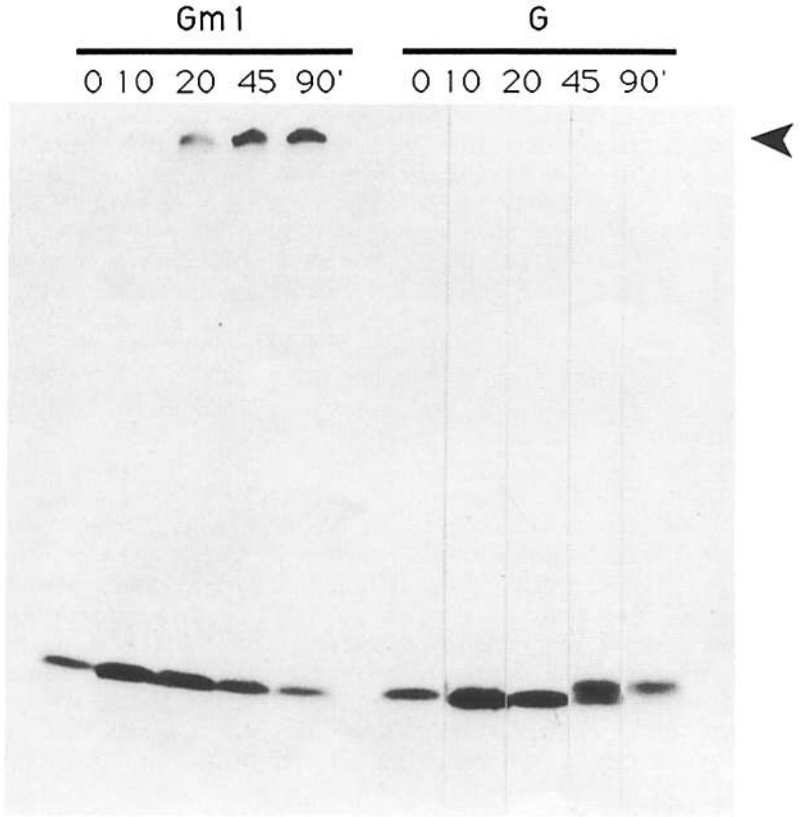

Figure 3. Time course of Gm1 oligomer formation. Transfected HeLa cells were metabolically radiolabeled for 5 min, and then chased for the indicated times. After solubilization, VSV G and Gm1 were immunoprecipitated and analyzed by SDS-PAGE as described in Materials and Methods. The arrowhead denotes the top of the stacking gel.

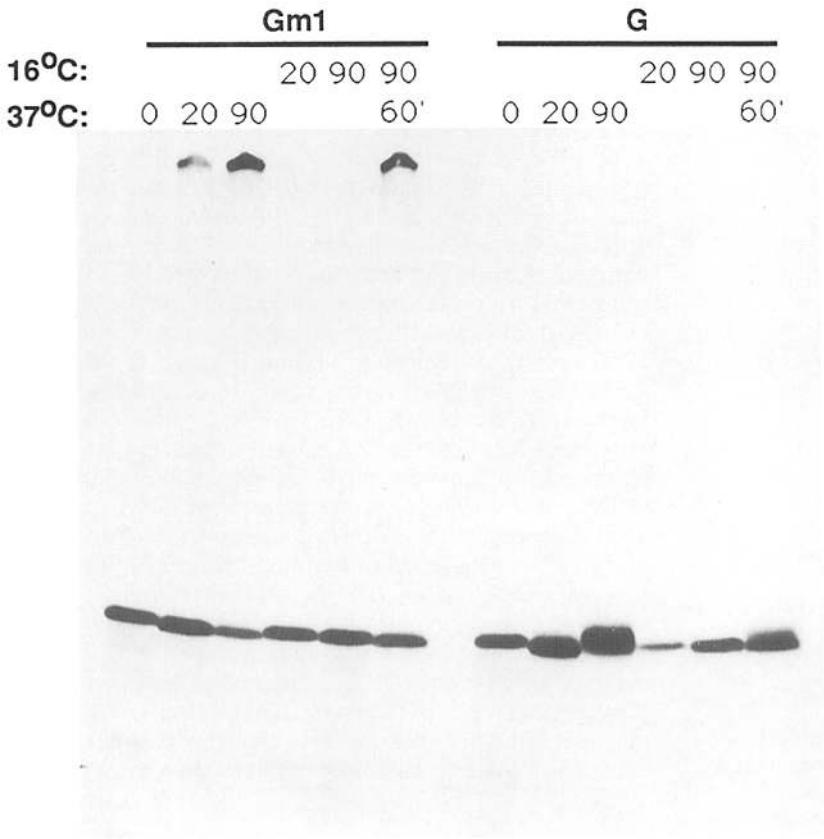

Figure 4. SDS-resistant oligomerization of Gml does not occur at 16°C. Transfected HeLa cells were metabolically radiolabeled for 5 min, and then chased at 16 or 37°C for up to 90 min. One set of dishes was incubated for 90 min at 16°C, then transferred to 37°C for 60 min. VSV G and related proteins were immunoprecipitated from solubilized cells as described in Materials and Methods.

1988). HeLa cells were transiently transfected with VSV G or Gml, and then metabolically radiolabeled for 5 min and solubilized either immediately or after 60 min of chase. Lysates were loaded onto 5–20% sucrose gradients, centrifuged, and analyzed as described in Materials and Methods. Immediately after synthesis, VSV G migrated as a $4S_{20,\omega}$ peak (Fig. 5; Doms et al., 1987). After a 60-min chase, all of the VSV G had trimerized, migrating as a discrete $8S_{20,\omega}$ peak. The behavior of Gml in these gradients was markedly different. Immediately after synthesis, Gml migrated similarly to the VSV G monomer. After a 60-min chase, however, much of the Gml was found in the pellet in an SDS-resistant form (Fig. 5; note top of lane). The time-dependent shift in the mobility of the oligomer in sucrose gradients suggests that oligomers already exist in the cells at the time of lysis and immunoprecipitation. The SDS-sensitive material in the Gml pellet fractions may represent an intermediate form of oligomer that is not yet SDS-resistant. Alternatively, this material could contain protein released from the SDS-resistant oligomer during SDS-PAGE. In contrast to our findings with Gml, misfolded mutants of VSV G that aggregate in the ER are found in the pellet (and are SDS-soluble) immediately after synthesis (Doms et al., 1988). Using different centrifugation conditions, we estimated the average size of the Gml oligomer to be approximately $22S_{20,\omega}$, or roughly 800 kD (data not shown). Therefore, if Gml (MW 66 kD) is the sole component of the oligomer, we estimate there would be ~12 molecules per oligomer.

Role of the VSV G Head and Tail Domains in SDS-Resistant Oligomer Formation or Stabilization

In contrast to the chimeric Gml protein, oligomers of the IBV M glycoprotein are not detected when assayed by sucrose gradient sedimentation (data not shown). This might suggest that oligomerization is not a retention mechanism for native M protein; alternatively, M oligomers might readily dissociate in detergent. The resistance of the Gml oligomer to solubilization suggests a structural feature of VSV G may stabilize the complex. We asked whether the lumenal domain of VSV G, which is sufficient for trimerization (Doms et al., 1988), was required for oligomer formation. To do this, we replaced the lumenal domain of VSV G or Gml with a small soluble glycoprotein, the α subunit of hCG. When expressed alone, the α subunit of hCG is secreted from cells as a monomer (Guan et al., 1988*b*). When hCGα is fused to the membrane-spanning domain and cytoplasmic tail of VSV G, the chimeric protein (αm) is membrane-bound and transported to the cell surface (Guan et al., 1988*a*). When the VSV G membrane-spanning domain of αm is replaced with the ml domain of IBV M, the resulting chimera (αmlG) is retained in the Golgi complex (Swift and Machamer, 1991). Interestingly, in metabolically labeled HeLa cells expressing αmlG, we observed the time-dependent accumulation of an SDS-resistant species at the interface between the separating and stacking gels (Fig. 6, *arrowhead*). This species was not observed in cells expressing αm. The smaller

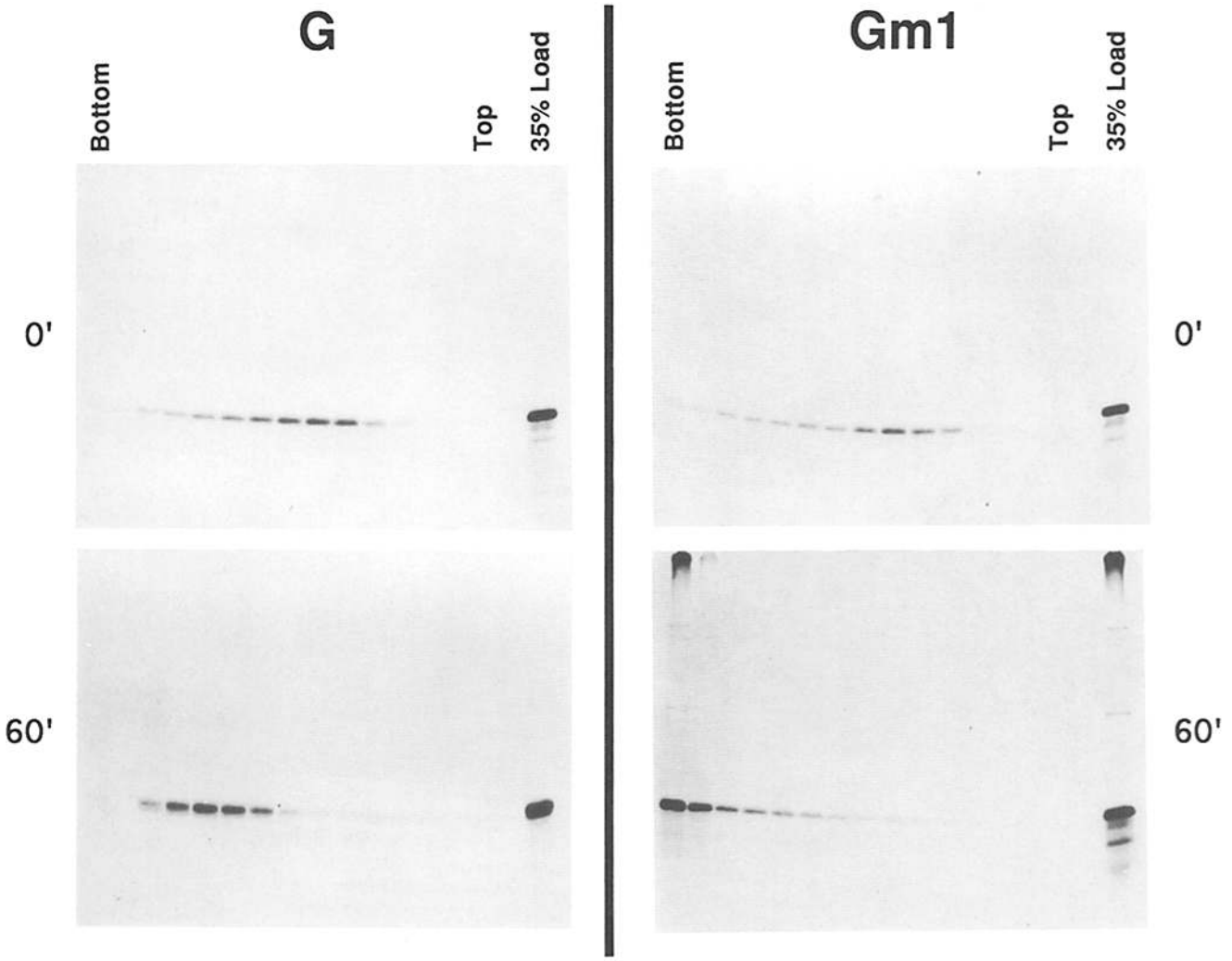

Figure 5. Sucrose gradient sedimentation of Gm1 and VSV G. HeLa cells transfected with VSV G or Gm1 were metabolically labeled for 5 min, and then solubilized immediately or after 60 min of chase. Lysates were loaded onto 5–20% sucrose gradients and centrifuged as described in Materials and Methods. Fractions were collected, immunoprecipitated using anti-VSV antibody, and analyzed by SDS-PAGE. After the 60-min chase, all of the Gm1 formed a large oligomer that pelleted under these gradient conditions, and much of it was SDS-resistant.

size of the αm1G SDS-resistant oligomer is consistent with the size difference between αm1G and Gm1 (28 vs 66 kD). On sucrose gradients, αm1G solubilized 60 min after synthesis migrated as a larger species (some of which was SDS-resistant) than newly synthesized αm1G. In contrast, the mobility of αm on sucrose gradients was unaltered during the chase (data not shown). Thus the VSV G lumenal domain was not required for SDS-resistant oligomer formation.

We used a coprecipitation assay to ask if αm1G formed hetero-oligomers with Gm1 or other related Golgi-resident proteins. HeLa cells were cotransfected to express both αm1G and either Gm1, $Gm1_{ins}$, or VSV G. After a short metabolic labeling period (5 min), cells were solubilized either immediately or after a 60-min chase. Lysates were immunoprecipitated using anti-hCG antibody. When αm1G was expressed alone, the time-dependent accumulation of SDS-resistant oligomer at the gel interface (*arrowhead*) was observed as described above (Fig. 7, lane *2*). Interestingly, when αm1G was coexpressed with Gm1 or $Gm1_{ins}$, the size of the oligomer shifted dramatically, and now migated at the top of the stacking gel (Fig. 7, lanes *4* and *8*). Furthermore, some proteins appeared to be solubilized during electrophoresis, since a small amount of material migrating with authentic Gm1 and $Gm1_{ins}$ was also detected in these lanes (Fig. 7, *arrow*). In contrast, no change in the size of the oligomer was observed when αm1G was coexpressed with VSV G (Fig. 7, lane *6*) or with $Gm1QI_{480}$ (data not shown), which are transported to the plasma membrane. Furthermore, when cells expressing Gm1 and αm (containing the VSV G membrane-spanning domain) were immunoprecipitated using anti-hCG antibody, no Gm1 was recovered (data not shown).

To test the role of the cytoplasmic tail of Gm1 in oligomer formation, we inserted a stop codon after the first residue (Arg) beyond the transmembrane domain (tGm1). The corresponding tailless VSV G (TMR; Doms et al., 1988) trimerizes with normal kinetics but is transported slowly from the ER to the plasma membrane. When tGm1 was localized by

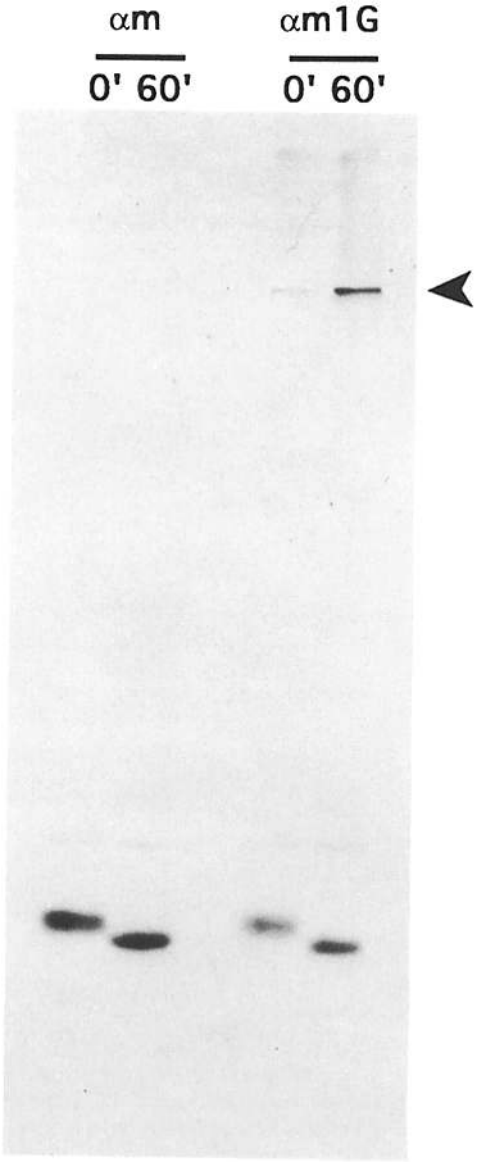

Figure 6. αm1G forms an SDS-resistant oligomer. HeLa cells transfected with αm1G or αm were metabolically labeled for 5 min, and then solubilized either immediately or after a 60-min chase and immunoprecipitated with anti-hCG antibody. Samples were analyzed by SDS-PAGE on 15% gels. Note the time-dependent accumulation of an SDS-resistant species at the interface between the separating and stacking gels (*arrowhead*). The increased mobility of αm1G and αm after 60 min of chase is due to carbohydrate trimming.

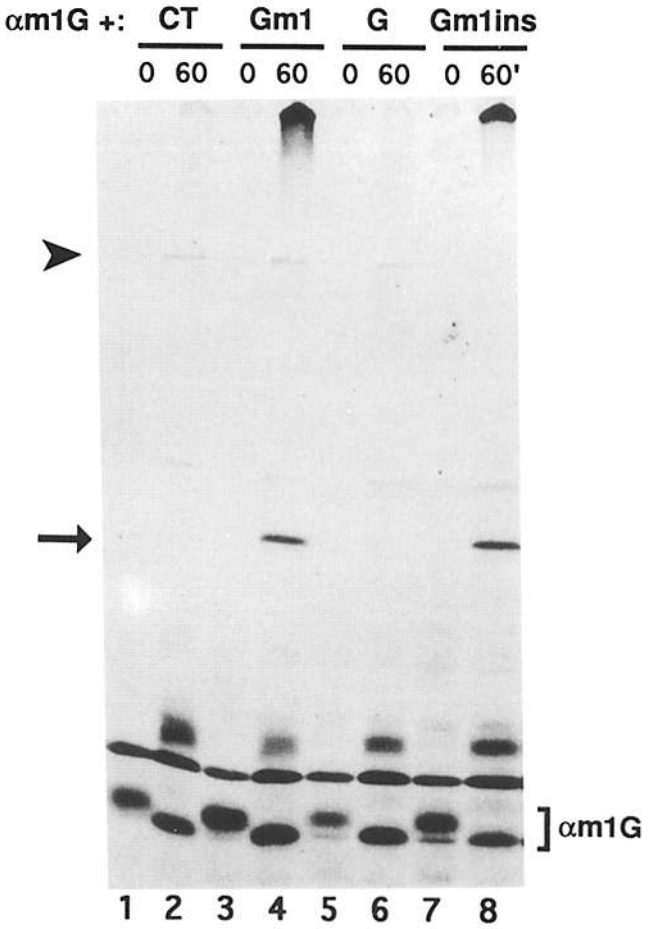

Figure 7. Gm1 and αm1G form hetero-oligomers. HeLa cells were transfected with 1 μg each of αm1G and either calf thymus DNA (*CT*), Gm1, VSV G, or $Gm1_{ins}$. After a 5-min pulse, cells were solubilized either immediately or after a 60-min chase. Aliquots of the solubilized cells were immunoprecipitated with anti-hCG antibody and electrophoresed on 12% SDS-polyacrylamide gels. The interface between the stacking and separating gels is marked with an arrowhead, and the mobility of Gm1 in the gel is denoted with an arrow. αm1G forms hetero-oligomers with Gm1 and $Gm1_{ins}$ (but not with VSV G) that could be precipitated by anti-hCG antibody.

indirect immunofluorescence in transiently transfected COS-7 cells, some Golgi staining was observed, although a significant amount of the protein was found in the ER. Unlike Gm1, tGm1 did not form an SDS-resistant oligomer when expressed in COS-7 or HeLa cells, and did not sediment as a large oligomer in sucrose gradients (although it did apparently form trimers, not shown). However, because tGm1 appears to exit the ER very slowly (like TMR), the kinetics of oligomer formation may have been too slow to detect.

Because tGm1 did not form detectable oligomers, we asked whether preformed Gm1 oligomers could be disrupted by proteolysis of the cytoplasmic tail. The 29-amino acid tail (depicted in Fig. 8 *A*) has numerous arginines and lysines that are susceptible to proteolysis by trypsin. HeLa cells expressing Gm1 or VSV G were metabolically radiolabeled for 5 min, and then chased for 0 or 60 min, and microsomes prepared as described in Materials and Methods. Microsomes were treated with TPCK-trypsin for 30 min at 37°C, and then solubilized, immunoprecipitated with anti-VSV antibody, and analyzed on SDS-polyacrylamide gels (Fig. 8 *B*). As size standards we used radiolabeled tGm1 and TMR immunoprecipitated from transfected HeLa cells. Trypsin cleaved newly synthesized VSV G protein to a species migrating slightly larger than TMR on SDS-PAGE (Fig. 8 *B*, compare lanes 7 and 10). After 60 min of chase, two proteolytic products were detected. The upper band corresponded to cleavage of the cytoplasmic tail of mature (sialylated) VSV G, as it was resistant to endoglycosidase H, whereas the lower band was sensitive to endoglycosidase H treatment (not shown). In addition, we frequently observed some residual

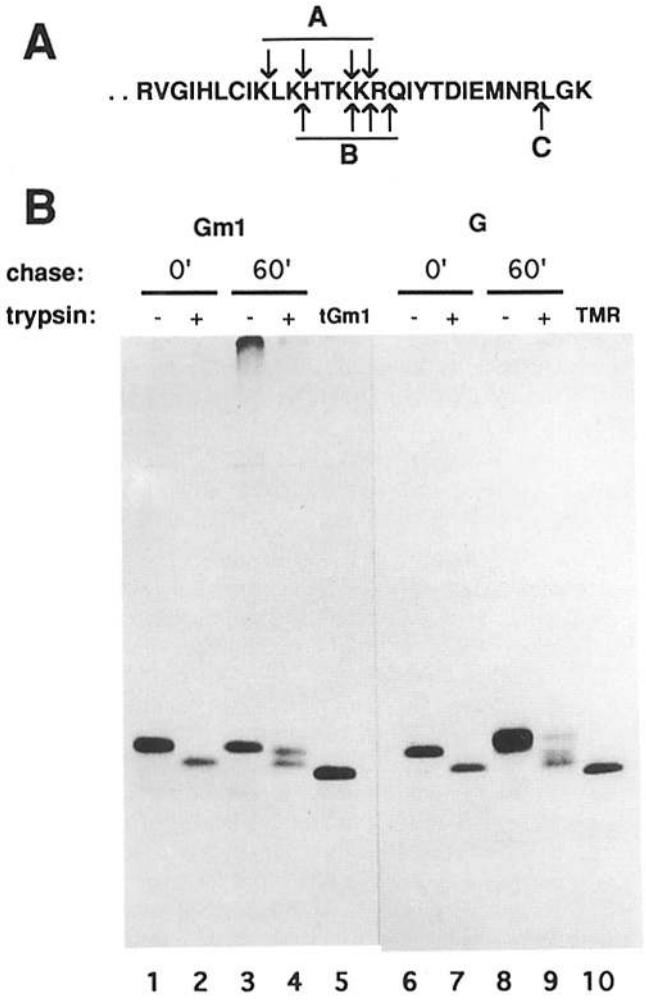

Figure 8. Trypsin treatment of preformed oligomers eliminates resistance to SDS. (*A*) Amino acid sequence (in single letter code) of the cytoplasmic tail of VSV G and Gm1. Residues susceptible to trypsin are underlined. The likely cleavage sites for VSV G (region *A*) and Gm1 (regions *B* and *C*) are indicated. (*B*) Proteolytic digestion of Gm1 and VSV G in microsomes. HeLa cells expressing Gm1 or VSV G were metabolically labeled for 5 min, and then chased for 0 or 60 min. Microsomes were prepared as described in Materials and Methods and treated with 20 μg TPCK-trypsin (lanes *2*, *4*, *7*, and *9*) or mock treated (lanes *1*, *3*, *6*, and *8*) for 30 min at 37°C. Proteolysis was stopped by the addition of trypsin inhibitor (20 μg), the microsomes were solubilized, and Gm1 (lanes *1–4*) and VSV G (lanes *6–9*) were immunoprecipitated and analyzed by SDS-PAGE. More inside-out microsomes are generated from compartments further along the secretory pathway, accounting for the loss of material recovered after trypsinization of VSV G isolated after a 60-min chase (lane *9*). tGm1 (lane *5*) and TMR (lane *10*) immunoprecipitated from HeLa cells labeled under the same conditions are included as size standards.

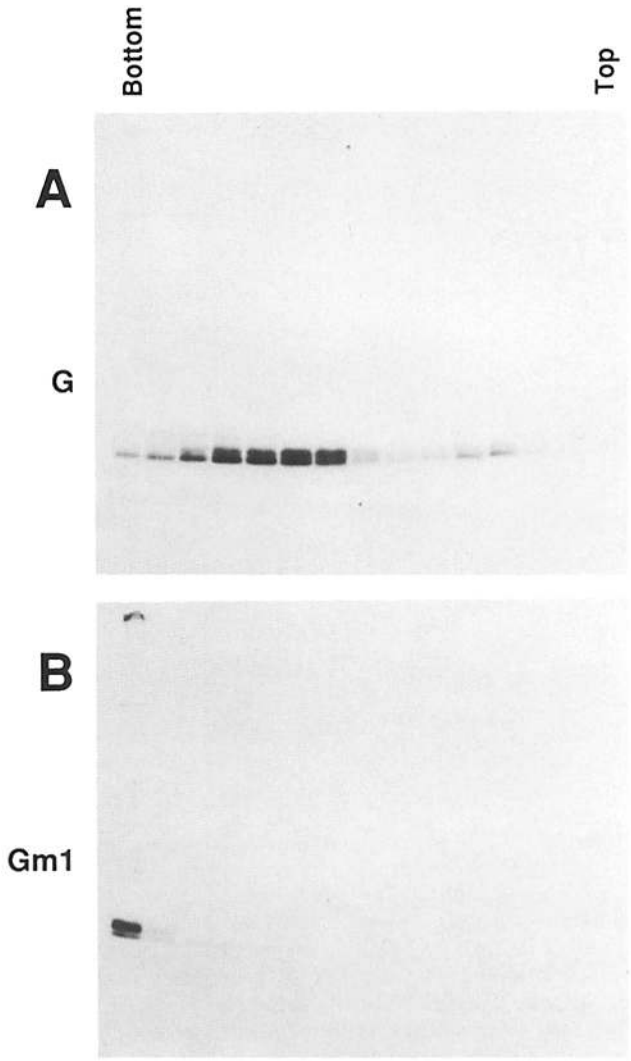

Figure 9. Trypsin-treated Gm1 is still oligomerized. Cells expressing VSV G (*A*) or Gm1 (*B*) were metabolically radiolabeled for 5 min, and then chased for 45 min. Microsomes were prepared and treated with 20 μg TPCK-trypsin for 30 min at 37°C, then solubilized in MNT and centrifuged on 5–20% sucrose gradients. Fractions were collected, immunoprecipitated using anti-VSV antibody, and analyzed by SDS-PAGE.

uncleaved mature VSV G in our experiments. Since the trypsin-cleaved form of VSV G migrated more slowly than TMR (whose tail contains a single amino acid), we deduced that trypsin cleaves the VSV G tail within region A marked on Fig. 8 *A*.

The proteolytic profile generated by trypsin cleavage of Gm1 differed from that of VSV G. Trypsin treatment of newly synthesized Gm1 yielded a single species which migrated more slowly on SDS-PAGE than the VSV G proteolytic fragment. Thus, even before oligomers are formed, the tail of Gm1 is less accessible to trypsin than that of VSV G. Interestingly, when Gm1 was digested after a 60-min chase, there was a large reduction in the amount of SDS-resistant oligomer migrating at the top of the stacking gel on SDS-PAGE (Fig. 8 *B*, compare lanes *3* and *4*). Furthermore, we saw a second, larger band in addition to the one detected immediately after the pulse label. Both bands were endoglycosidase H-sensitive, indicating that the proteins they represent had not passed through the medial Golgi (not shown). In more detailed kinetic experiments, the amount of this upper band increased with longer chase times, with a concomitant decrease in the amount of the lower band (not shown). Based on the potential trypsin cleavage sites in the

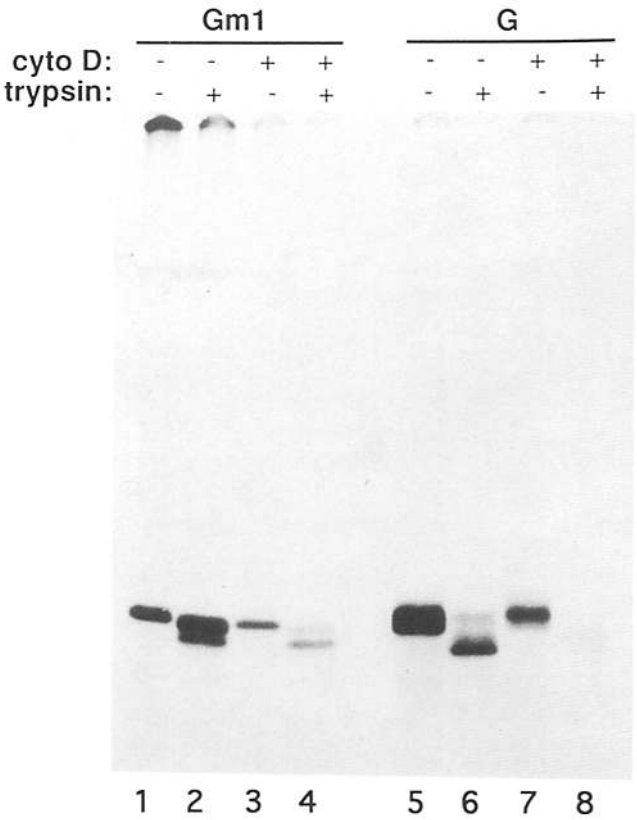

Figure 10. Trypsin treatment of Gml and VSV G recovered after treatment with cytochalasin D. HeLa cells expressing Gml or VSV G were treated with 100 μM cytochalasin D starting 1 h before radiolabeling. Because fewer microsomes were recovered from cytochalasin D-treated cells than from untreated cells, treated cells were radiolabeled with twice the usual concentration of radioactivity. Microsomes were prepared after a 15-min pulse and a 45-min chase, and aliquots were mock treated or treated with TPCK-trypsin as described in Materials and Methods. Gml and VSV G were immunoprecipitated using anti-VSV antibody and analyzed by SDS-PAGE. Cytochalasin D blocked formation of SDS-resistant oligomers and accumulation of the larger trypsin-cleaved band.

cytoplasmic tail and the relative mobility of these bands compared to the VSV G proteolytic fragment and tGml, we conclude that the upper and lower bands result from trypsin cleavage within regions C and B, respectively (Fig. 8 *A*). The upper band may represent material generated exclusively from proteolytic digestion of SDS-resistant Gml. This would suggest that the last three amino acids of the cytoplasmic tail of Gml are required for SDS resistance of the oligomer. Alternatively, trypsin may destroy another (unlabeled) component of the oligomer that is required to maintain its resistance to solubilization.

Sucrose Gradient Sedimentation of Trypsin-treated Gml and VSV G

Trypsinization of microsomes eliminated the SDS-resistant form of Gml. To ask if proteolysis dissociated the oligomers as well, we subjected trypsin-treated Gml-containing microsomes to sucrose gradient sedimentation. Microsomes prepared from HeLa cells after a 5-min pulse and a 60-min chase were mock-treated or treated with TPCK-trypsin (20 μg) for 30 min at 37°C. Samples were diluted tenfold in MNT, and aliquots were loaded on sucrose gradients and centrifuged as described in Materials and Methods. Gradient fractions were collected, immunoprecipitated, and analyzed by SDS-PAGE. The trypsin-treated samples are shown in Fig. 9. Trypsin digestion did not cause dissociation of VSV G trimers (compare Fig. 9 *A* with Fig. 5). Significantly, the larger proteolytic product of Gml migrated at the bottom of the gradient, whereas some of the smaller product pelleted and some was found throughout the gradient (Fig. 9 *B*). This suggested that removing part of the cytoplasmic tail of Gml does not disrupt the oligomer, and further supports the possibility that digestion of SDS-resistant material gives rise to the larger band.

The Cytoplasmic Tails of Oligomerized Gml May Interact with Actin

We tested the possibility of cytoskeletal involvement in SDS resistance of Gml by pretreating cells with drugs that disrupt microtubules or actin filaments. Depolymerization of microtubules with colchicine (50 μM) had no effect on the amount of SDS-resistant Gml formed (not shown). Interestingly, treatment with cytochalasin D (100 μM) starting 1 h before metabolic labeling blocked formation of SDS-resistant Gml oligomers (Fig. 10, lane *3*). The amount of immunoprecipitated radiolabel migrating at the top of the stacking gel decreased from 43% of total in untreated cells to 18% of total in cytochalasin D-treated cells (compare Fig. 10, lanes *1* and *3*). Cytochalasin D did not block the transport of VSV G since the rate of sialylation was enhanced relative to untreated cells. On sucrose gradients, Gml solubilized from cytochalasin D-treated cells migrated as an SDS-sensitive oligomer (in the pellet), and VSV G trimerization was unaffected (data not shown). This observation suggests that oligomer formation and SDS-resistance are separable characteristics, and furthermore implies that SDS-resistance may result from interaction between Gml and an actin-based matrix or cytoskeleton.

Because cytochalasin D blocked the formation of SDS-resistant oligomers of Gml, we tested whether this treatment prevented the appearance of the larger proteolytic band. HeLa cells expressing Gml or VSV G were treated with cytochalasin D (100 μM) for 1 h before a 15-min metabolic labeling. Microsomes were prepared after a 45-min chase and mock-treated or treated with 20 μg of TPCK-trypsin for 30 min at 37°C, and then solubilized and immunoprecipitated using anti-VSV antibody (Fig. 10). As mentioned above, treatment with cytochalasin D enhanced the fraction of VSV G that became sialylated during this period (compare lanes *5* and *7*). We routinely observed that trypsin degraded most of the VSV G recovered from cytochalasin D-treated cells (lane *8*), suggesting that the orientation or durability of microsomes was affected by treatment with this perturbant. Interestingly, trypsin digestion of Gml recovered from cytochalasin D-treated cells yielded predominantly the smaller proteolytic species, generated by cleavage within region B in Fig. 8 *A*. We therefore conclude that the larger Gml species does indeed represent material digested from SDS-resistant oligomers, and furthermore, that the oligomers may interact with an actin-based cytoskeleton.

Discussion

The experiments presented here support protein oligomerization as a mechanism for retaining the chimeric protein Gml in the Golgi complex. An unusual feature of the Gml oligomer (its resistance to solubilization by SDS) allowed us to easily assay oligomer formation. The specificity of oligomerization was mediated by the ml transmembrane domain, since mutant Gml proteins that were retained in the Golgi formed oligomers, while mutant proteins that were released to the plasma membrane did not. This specificity was preserved in another construct, αmlG, in which the lumenal domain of VSV G was replaced with the α subunit of hCG. Furthermore, αmlG was able to form hetero-oligomers with Gml and other Golgi-resident Gml mutants, but not with mutants that move to the plasma membrane. This suggested that the lumenal domain of VSV G was not required for oligomerization.

While the specificity of oligomerization was dictated by the sequence of the membrane-spanning region, the cytoplasmic tail of Gml was necessary for SDS-resistance and may play a role in oligomer formation. When the cytoplasmic tails of preexisting oligomers were digested with trypsin, the proteolytic products were soluble in SDS but continued to migrate as oligomers on sucrose gradients. Based on potential trypsin cleavage sites in the cytoplasmic tail of Gml and the migration of the proteolytic fragments on SDS-PAGE, it is possible that removal of the last three carboxy-terminal residues of Gml destroys its resistance to solubilization by SDS. Alternatively, trypsin digestion could remove another component of the oligomer that is required for SDS resistance. Interestingly, cell treatment with cytochalasin D blocked the formation of SDS-resistant oligomers, suggesting that the acquisition of SDS resistance may reflect association of the Gml cytoplasmic tail with an actin-based cytoskeleton. We are currently constructing a series of Gml proteins with truncated cytoplasmic tails to determine if these proteins are correctly targeted and form oligomers.

Several observations suggest that Gml oligomerization normally occurs after the protein leaves the ER. Oligomerization of Gml was not detected until ~10 min after protein synthesis, which correlates with arrival of newly synthesized proteins at the *cis*-Golgi. Oligomers were not observed when ER to Golgi traffic was blocked by incubation at 16°C or treatment with CCCP. Interestingly, SDS-resistant oligomers did form in brefeldin A-treated cells, suggesting that oligomers can form in pre-Golgi compartments under certain conditions. Brefeldin A treatment may generate the conditions required to stimulate oligomer formation in the ER. Conditions which could trigger Gml oligomerization might include its concentration in the membrane, the retrieval of glycolipids or glycoproteins typical of the Golgi complex, or changes in pH or divalent ion concentrations.

We were unable to solubilize the SDS-resistant oligomer using a wide variety of harsh conditions. It is possible that some of the Gml oligomer is covalently crosslinked to itself or to an actin-based structure via the VSV G tail. However, removal of as few as three amino acids from the cytoplasmic tail resulted in oligomers that were SDS-sensitive. This indicates that the SDS resistant phenotype (which requires the last three amino acids of Gml) can be separated from oligomerization per se, which is mediated by the membrane-spanning domain.

Transmembrane-mediated Oligomerization of Cell-Surface Proteins

The transmembrane domains of many proteins have been shown to mediate dimerization (Sternberg and Gullick, 1990; Manolios et al., 1990; for review see Bormann and Engelman, 1992). The best example is glycophorin A, whose single transmembrane domain interacts to form homodimers (Bormann et al., 1989). This dimer is resistant to solubilization by SDS at ambient temperature, although resistance is overcome by dilution or incubation at higher temperatures (Furthmayr and Marchesi, 1976). Dimerization of glycophorin A is highly sequence specific and appears to involve interactions between hydrophobic residues in the transmembrane domain (Lemmon et al., 1992). The transmembrane domains of the T-cell receptor (Cosson et al., 1991) and of class II major histocompatibility complex molecules also appear to mediate dimer formation (Cosson and Bonifacino, 1992). In the case of major histocompatibility complex molecules, α and β chains interact to form heterodimers; dimerization appears to require a pair of oppositely charged amino acids at the lumenal edge of the apposing membrane spans. A series of glycine residues on each membrane span forms a nonpolar face which allows close packing of the transmembrane domains (Cosson and Bonifacino, 1992). Similarly, the α chain of the T-cell receptor assembles with the CD3 δ chain via interaction between charged transmembrane residues (Manolios et al., 1990; Cosson et al., 1991).

A Model for the Retention of Golgi-Resident Proteins

It is not clear how the transmembrane domain of Gml molecules might interact to form a large oligomer. The large lumenal domain of Gml may sterically prevent direct interaction between many transmembrane domains to form an oligomer. Furthermore, the polar face of the ml domain itself could be expected to interact with at most one or two similar domains. The transmembrane domains of these proteins might interact to form small clusters (dimers or trimers) of Gml, mutant Gml proteins, and αmlG. These clusters could be organized into large arrays by interactions between the heads and/or tails of proteins in different clusters. These structures might contain lipids and other endogenous Golgi-resident proteins. Association into such an array could prevent Golgi-resident proteins from entering transport vesicles and thereby function as a mechanism for their retention.

The targeting signals of other Golgi resident proteins from different Golgi subcompartments also reside in their transmembrane domains (Aoki et al., 1992; Burke et al., 1992; Colley et al., 1992; Munro, 1991; Nilsson et al., 1991; Russo et al., 1992; Tang et al., 1992; Teasdale et al., 1992; Wong et al., 1992), although flanking domains also contribute to retention efficiency. No obvious homologies exist between the primary sequences of these membrane-spanning domains, even in enzymes thought to be enriched in the same subcompartment. Thus, the membrane composition of Golgi subcompartments may be responsible for specific retention of resident proteins throughout this organelle. Consistent with this, no soluble resident Golgi proteins have been detected to date.

Nilsson et al. (1993) have proposed that the cytoplasmic tails of resident proteins in sequential Golgi stacks may in-

teract (perhaps via a cytoplasmic linking protein) to maintain the stacks in close apposition. The formation of Gml oligomers may reflect association with such endogenous structures. The combination of overexpression and our metabolic radiolabeling conditions would not be expected to reveal any long-lived endogenous Golgi-resident proteins that also reside in the oligomer. However, longer radiolabeling conditions in stable cell lines expressing Gml may reveal other putative components of the oligomer. We are currently performing such experiments.

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References

Aoki, D., N. Lee, N. Yamaguchi, C. Dubois, and M. N. Fukuda. 1992. Golgi retention of a trans-Golgi membrane protein, galactosyltransferase, requires cysteine and histidine residues within the membrane-anchoring region. *Proc. Natl. Acad. Sci. USA.* 89:4319–4323.

Bonner, W. M., and R. A. Laskey. 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* 46:83–88.

Bormann, B. J., W. J. Knowles, and V. T. Marchesi. 1989. Synthetic peptides mimic the assembly of transmembrane glycoproteins. *J. Biol. Chem.* 264: 4033–4037.

Bormann, B. J., and D. M. Engelman. 1992. Intramembrane helix-helix association in oligomerization and transmembrane signaling. *Annu. Rev. Biophys. Biomol. Struct.* 21:223–242.

Burke, J., J. M. Pettitt, H. Schachter, M. Sarkar, and P. A. Gleeson. 1992. The transmembrane and flanking sequences of β1,2-*N*-Acetylglucosaminyltransferase I specify *medial*-Golgi localization. *J. Biol. Chem.* 267:24433–24440.

Colley, K. J., E. U. Lee, and J. C. Paulson. 1992. The signal anchor and stem regions of the β-galactoside α2,6-sialyltransferase may each act to localize the enzyme to the Golgi apparatus. *J. Biol. Chem.* 267:7784–7793.

Cosson, P., and J. S. Bonifacino. 1992. Role of transmembrane domain interactions in the assembly of class II MHC molecules. *Science (Wash. DC).* 258:659–662.

Cosson, P., S. P. Lankford, J. S. Bonifacino, and R. D. Klausner. 1991. Membrane protein association by potential intramembrane charge pairs. *Nature (Lond.).* 351:414–416.

Doms, R. W., D. S. Keller, A. Helenius, and W. E. Balch. 1987. Role for adenosine triphosphate in regulating the assembly and transport of vesicular stomatitis virus G protein trimers. *J. Cell Biol.* 105:1957–1969.

Doms, R. W., A. Ruusala, C. Machamer, J. Helenius, A. Helenius, and J. K. Rose. 1988. Differential effects of mutations in three domains on folding, quaternary structure, and intracellular transport of vesicular stomatitis virus G protein. *J. Cell Biol.* 107:89–99.

Doms, R. W., R. A. Lamb, J. K. Rose, and A. Helenius. 1993. Folding and assembly of viral membrane proteins. *Virology.* 193:545–562.

Fuerst, T. R., E. G. Niles, F. W. Studier, and B. Moss. 1986. Eukaryotic transient expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase. *Proc. Natl. Acad. Sci. USA.* 83:8122–8126.

Furthmayr, H., and V. T. Marchesi. 1976. Subunit structure of human erythrocyte glycophorin A. *Biochemistry.* 15:1137–1144.

Griffiths, G., and P. Rottier. 1992. Cell biology of viruses that assemble along the biosynthetic pathway. *Sem. Cell Biol.* 3:367–381.

Guan, J.-L., H. Cao, and J. K. Rose. 1988*a*. Cell-surface expression of a membrane-anchored form of the human chorionic gonadotropin α subunit. *J. Biol. Chem.* 263:5306–5313.

Guan, J.-L., A. Ruusala, H. Cao, and J. K. Rose. 1988*b*. Effects of altered cytoplasmic domains on transport of the vesicular stomatitis virus glycoprotein are transferable to other proteins. *Mol. Cell. Biol.* 8:2869–2874.

Kunkel, T. A., J. D. Roberts, and R. A. Zakour. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* 154: 367–382.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

Lefrancois, L., and D. S. Lyles. 1982. The interaction of antibody with the major surface glycoprotein of vesicular stomatitis virus. *Virology.* 121:168–174.

Lemmon, M. A., J. M. Flanagan, J. F. Hunt, B. D. Adair, B. J. Bormann, C. E. Dempsey, and D. E. Engelman. 1992. Glycophorin A dimerization is driven by specific interactions between transmembrane α-helices. *J. Biol. Chem.* 267:7683–7689.

Lisanti, M. P., M. Sargiacomo, L. Graeve, A. R. Saltiel, and E. Rodriguez-Boulan. 1988. Polarized apical distribution of glycosyl-phosphatidylinositol-anchored proteins in a renal epithelial cell line. *Proc. Natl. Acad. Sci. USA.* 85:9557–9561.

Machamer, C. E. 1991. Golgi retention signals: do membranes hold the key? *Trends Cell Biol.* 1:141–144.

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., and J. K. Rose. 1988. Influence of new glycosylation sites on expression of the vesicular stomatitis virus G protein at the plasma membrane. *J. Biol. Chem.* 263:5948–5954.

Machamer, C. E., R. Z. Florkiewicz, and J. K. Rose. 1985. A single N-linked oligosaccharide at either of the two normal sites is sufficient for transport of vesicular stomatitis virus G protein to the cell surface. *Mol. Cell. Biol.* 5:3074–3083.

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.

Machamer, C. E., M. G. Grim, A. Esquela, S. W. Chung, M. Rolls, K. Ryan, and A. M. Swift. 1993. Retention of a *cis* Golgi protein requires polar residues on one face of a predicted α-helix in the transmembrane domain. *Mol. Biol. Cell.* 4:695–704.

Manolios, N., J. S. Bonifacino, and R. D. Klausner. 1990. Transmembrane helical interactions and the assembly of the T cell receptor complex. *Science (Wash. DC).* 249:274–277.

Munro, S. 1991. Sequences within and adjacent to the transmembrane segment of α-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 β-1,4-galactosyltransferase specifies trans Golgi localization. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:3567–3575.

Nilsson, T., M. Pypaert, M. H. Hoe, P. Slusarewicz, E. G. Berger, and G. Warren. 1993. Overlapping distribution of two glycosyltransferases in the Golgi apparatus of HeLa cells. *J. Cell Biol.* 120:5–14.

Rose, J. K., L. Buonocore, and M. A. Whitt. 1991. A new cationic liposome reagent mediating nearly quantitative transfection of animal cells. *Biotechniques.* 10:520–525.

Russo, R. N., N. L. Shaper, D. J. Taatjes, and J. H. Shaper. 1992. β1,4-galactosyltransferase: a short NH_2-terminal fragment that includes the cytoplasmic and transmembrane domain is sufficient for Golgi retention. *J. Biol. Chem.* 267:9241–9247.

Saraste, J., and E. Kuismanen. 1984. Pre- and post-Golgi vacuoles operate in the transport of Semliki Forest virus membrane glycoproteins to the cell surface. *Cell.* 38:535–549.

Sternberg, M. J. E., and W. J. Gullick. 1990. A sequence in the transmembrane region of growth factor receptors with tyrosine kinase activity mediates dimerization. *Protein Eng.* 3:245–248.

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-glucosaminyltransferase I contains a Golgi retention signal. *J. Biol. Chem.* 267:10122–10126.

Teasdale, R. D., G. D'Agostaro, and P. A. Gleeson. 1992. The signal for Golgi retention of bovine β1,4-galactosyltransferase is in the transmembrane domain. *J. Biol. Chem.* 267:4084–4096.

Wong, S. H., S. H. Low, and W. Hong. 1992. The 17-residue transmembrane domain of β-galactoside α2,6-sialyltransferase is sufficient for Golgi retention. *J. Cell Biol.* 117:245–258.