Evidence for a Glycoprotein "Signal" Involved in Transport between Subcellular Organelles

TWO MEMBRANE GLYCOPROTEINS ENCODED BY MURINE LEUKEMIA VIRUS REACH THE CELL SURFACE AT DIFFERENT RATES*

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We analyzed the synthesis and intracellular transport of two plasma membrane glycoproteins encoded by murine leukemia virus, an *env* gene-encoded glycoprotein with an apparent *M*ₐ = 70,000 (gp70<sup>env</sup>) and a *gag* gene-encoded glycoprotein with an apparent *M*ₐ = 93,000 (gp93<sup>gag</sup>). These glycoproteins were specifically isolated from the surfaces of infected cells by an extracellular antibody adsorption technique in which proteins are pulse labeled with L-[<sup>35</sup>S]methionine and their arrival and turnover in plasma membranes can be kinetically studied (Krangel, M. S., Orr, H. T., and Strominger, J. L. (1979) Cell 18, 979-991). In addition, precursor and product forms of these glycoproteins were analyzed in whole cell lysates, in fractionated membranous subcellular organelles, and in released virions. Whereas gp93<sup>gag</sup> is transferred quantitatively into plasma membranes within 60 min of L-[<sup>35</sup>S]methionine incorporation, gp70<sup>env</sup> in only partially transferred at that time and is still accumulating in plasma membranes after 210 min. This difference is caused by a slow step in gp70<sup>env</sup> processing which appears to occur in the rough endoplasmic reticulum and which precedes (within several minutes) or coincides with partial proteolytic cleavage of a larger glycoprotein precursor gp90<sup>env</sup>. Once past this kinetic barrier, gp70<sup>env</sup> is rapidly processed within 15 min via the Golgi apparatus to the plasma membranes. Transfer of *env* gene-encoded glycoproteins past the kinetic barrier from the precursor pool occurs randomly rather than in the linear or cohort order in which the proteins were synthesized. The differences between gp93<sup>gag</sup> and gp70<sup>env</sup> transport kinetics indicate that intracellular transport is a selective rather than passive flow process and that structural characteristics (signals) of membrane glycoproteins determine their rates of transfer. These characteristics and their randomness with respect to time spent in the pool, support a simple model: that transport requires binding to molecule(s) which interact with different glycoproteins with distinct affinities. Binding affinities of certain glycoproteins may be modulated by covalent alterations such as partial proteolysis or glycosylation.

Plasma membrane and secretory proteins are believed to follow a similar but not necessarily identical route from their synthesis in the rough endoplasmic reticulum via the Golgi apparatus to the cell surface (1-4). Furthermore, transit between organelles involves transfer or shuttle vesicles which bud from specialized regions of the membranes and in some cases contain a clathrin coat on their cytoplasmic surfaces (5-7). At stages of this process, the proteins being transferred may be covalently altered by glycosylation (3, 8), oligosaccharide modifications (9-12), acylation with fatty acids (13, 14), or partial proteolysis (15-17). However, many plasma membrane and secretory proteins are transported intracellularly without such covalent modifications, or when these alterations are inhibited, and it is therefore believed that these modifications are not a prerequisite for protein transfer to the cell surface (18-23). However, transfer of proteins from the Golgi apparatus to lysosomes requires 6-phosphomannosyl "signal" residues on asparagine-linked oligosaccharides (24-26).

The mechanisms for selection and transfer of newly synthesized proteins between organelles are not understood. Frequently, it has been suggested that transfer to plasma membranes occurs in a linear manner as a cohort of simultaneously made proteins which passes through different organelles or processing stages during defined subsequent time intervals (4, 27), perhaps carried along by a lipid flow (1, 2). Consistent with this idea, results of previous investigations have indicated that different plasma membrane proteins are transported intracellularly with similar kinetics and that they reach the cell surface in a wave 25-50 min after synthesis of their polypeptide chains (4, 29-31). Although the processes of membrane protein transport might be expected to differ mechanistically and energetically from those of secretory proteins, it is interesting that most secretory proteins (e.g. albumin and fibronectin) are also rapidly transported to the cell surface where they are released as an apparent cohort 25-50 min after synthesis of their polypeptide chains (4, 29). However, transferrin is an exception since molecules synthesized during a brief interval are slowly secreted throughout the subsequent 25-min to 3-h period (29). Furthermore, a slow step in transferrin processing precedes the conversion of its high mannose oligosaccharides into complex structures. These modifications are known to occur in the distal or *trans* portion of the Golgi apparatus (28).

In this paper, we describe studies of the synthesis and processing of two plasma membrane glycoproteins encoded by Friend MuLV: an *env* gene-encoded glycoprotein with an

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* The abbreviations used are: MuLV, murine leukemia virus; SFFV, spleen focus-forming virus; F-MuLV and F-SFFV, the Friend strains of these viruses; *env*, the envelope glycoprotein gene of MuLV; *gag*, the core protein gene of MuLV.
apparent $M_r = 70,000$ (gp70env) and a gag gene-encoded glycoprotein with an apparent $M_r = 93,000$ (gp93env). These glycoproteins are processed into plasma membranes with widely different kinetics.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

The Overall Approach Used—Cell cultures infected with MuLV were labeled with L-$[^35]S$ methionine by a pulse-chase procedure (see "Experimental Procedures") and the virus-encoded gene products were subsequently isolated by precipitation with antisera specific for gp70env and p30env. The radioactive components were then analyzed by polyacrylamide gel electrophoresis. For the initial investigations, virus-encoded gene products were analyzed in three fractions: in whole cell lysates (Figs. 1A and 2A), on the outer surface of plasma membranes (Figs. 1B and 2B), and in released virions (see below). Careful comparison of these data and consistent data from two independent experiments reveals several important facts about the synthesis and transport of MuLV-encoded glycoproteins to the cell surface.

Analysis of Whole Cell Lysates—As shown by the whole cell lysate data in Fig. 1A, after brief labeling times (lanes 1, 2 and 7, 8) the major viral gene products are gPr90env, the precursor of gp70 and p15 E (17, 41, 42), Pr65env, the precursor of the virion internal core proteins (p15, p30, p12, and p10) (43), gPr80env, the precursor of gp93env (32, 44), and Pr180env, the precursor of reverse transcriptase (45, 46). Furthermore, in a subsequent chase with an excess of nonradioactive methionine (lanes 3–6 and 9–12), these precursors are processed to form their expected products. However, there is a striking difference in the kinetics of synthesis of gp70env compared with gp93env. Whereas gPr80env is rapidly and quantitatively processed within a 30-min chase period to form gp93env which is subsequently degraded or shed (44) from the cells, gPr90env concentration decreases throughout the several hour chase period and gp70env only slowly accumulates in the cells.

A quantitative analysis of Fig. 1A is shown in Fig. 2A. Although the radioactivity in gPr80env is quantitatively converted during the chase period into gp93env, it is apparent that gp70env contains only a portion of the radioactivity that is initially incorporated into the gPr90env precursor. This is consistent with evidence that gp70env contains only approximately two of the seven methionines that occur in gPr90env (47). The other methionines occur in the p15E protein that is formed by cleavage of gPr90env. Furthermore, as shown below, gp70env is shed from the cell surface in virion particles.

Transfer of Viral Glycoproteins to the Cell Surface—For analysis of L-$[^35]S$methionine-labeled components in the plasma membrane (Figs. 1B and 2B), the antisera were adsorbed onto the cells before lysis, the unbound serum was removed, the cells were lysed, and the antigen-antibody complexes were then isolated. Consistent with evidence previously obtained by other methods (36, 48, 49), gp93env and gp70env are the major viral gene products on the cell surface. Furthermore, this method appears to be highly specific for detection of cell surface components. For example, the gPr90env/Pr65env ratio in lanes 3–5 as determined by densitometric scanning is at least 100 times higher in the surface preparations in Fig. 1B than in the whole cell lysate in Fig. 1A. Similarly, the gp70env/gp90env ratio is at least 100 times higher in lanes 8–12 in Fig. 1B than in Fig. 1A. Presumably, therefore, the small amount of Pr65env and gp90env in Fig. 1B could have derived from a few damaged cells or from a slight dissociation-reassociation equilibrium that might occur during the isolation of the antigen-antibody complexes.

Analysis of Figs. 1B and 2B clearly indicate that gp70env and gp93env are processed to the cell surface with different kinetics. Although both first reach the cell surface between 30–60 min after addition of L-$[^35]S$methionine to cells (compare lanes 2, 3 and 8, 9) gp93env is quantitatively transferred during that time as a cohort whereas gp70env accumulates on the plasma membranes only slowly. However, within the resolution of the
sampling times used, both of these glycoproteins accumulate on the cell surface with the same kinetics that they accumulate in the whole cells (compare Fig. 1A with 1B). In other words, once gp70\\(^{\text{m}}\) and gp93\\(^{\text{m}}\) are formed intracellularly from their precursors, they can be transported to the plasma membranes without a substantial lag (i.e. within 10-15 min). Clearly, therefore, a slow step in gp70\\(^{\text{m}}\) processing to the cell surface precedes or coincides with proteolytic cleavage of the gPr90\\(^{\text{m}}\) precursor. After precursor cleavage, gp70\\(^{\text{m}}\) is processed rapidly and without hindrance.

In addition, the amounts of radioactive gp93\\(^{\text{m}}\) isolated from the cell surface are similar to the total cellular amounts (compare the band intensities in Figs. 1A and 1B and in 2A and 2B), suggesting that gp93\\(^{\text{m}}\) is transferred efficiently to the cell surface and that it does not occur intracellularly to a substantial extent. Independent evidence supporting this conclusion is described below. On the contrary, similar comparisons indicate that only approximately 16% of the total cellular quantity of radioactive gp70\\(^{\text{m}}\) is recovered in the cell surface preparations. Accordingly, either gp70\\(^{\text{m}}\) is predominantly located intracellularly or the extracellular antibody binding method only results in partial purification of the plasma membrane gp70\\(^{\text{m}}\).

In order to distinguish between these alternatives, we treated L-[35S]methionine-labeled cells with trypsin in order to degrade glycoproteins exposed to the cell exterior. As shown in Fig. 3, trypsin treatment of intact cells efficiently (\(\geq 80\%\)) degrades gPr90\\(^{\text{m}}\), Pr65\\(^{\text{m}}\), and p15E but does not affect intracellular proteins such as gPr90\\(^{\text{m}}\), Pr65\\(^{\text{m}}\), or p30\\(^{\text{m}}\). Accordingly, we infer that the majority of gp70\\(^{\text{m}}\) (80%) is located on the external surface of the plasma membranes but that it is inefficiently (\(\leq 16\%\)) isolated by the extracellular antibody adsorption technique employed in Fig. 1B. Possibly, the plasma membrane gp70\\(^{\text{m}}\) may be sterically shielded from extracellular antibodies in sites which are accessible to trypsin. Alternatively, if the antibodies had a low avidity, their complexes with gp70\\(^{\text{m}}\) might partially dissociate before they could be isolated. The results in Fig. 3 also provide independent evidence that without 60 min of adding a cold chase of nonradioactive methionine to labeled cells the radioactive gPr90\\(^{\text{m}}\) precursor has been entirely processed to form cell surface gp93\\(^{\text{m}}\), whereas the radioactive gp70\\(^{\text{m}}\) precursor has been only partially converted into cell surface gp70\\(^{\text{m}}\).

**Fig. 3.** Trypsin digestion of membranous proteins on the outer surface of intact Eveline II cells. The cells were labeled with L-\[^{35}S\]methionine for 2 h and were then chased with nonradioactive methionine for 1 h. They were then washed and were treated with extracellular trypsin in either 1X or 3X amounts as described under “Experimental Procedures.” The cells were subsequently washed, lysed, and immunoprecipitated with antibody to either p30\\(^{\text{m}}\) (lanes 1-3) or to gp70\\(^{\text{m}}\) (lanes 4-6). Lanes 1 and 4 were control cells incubated without trypsin. Lanes 2 and 5 were incubated with 1X trypsin. Lanes 3 and 6 were incubated with 3X trypsin. The extracellular trypsin efficiently degraded gp93\\(^{\text{m}}\), gp70\\(^{\text{m}}\), and p15E but did not affect the intracellular proteins Pr65\\(^{\text{m}}\), p30\\(^{\text{m}}\), or gPr90\\(^{\text{m}}\).

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Transfer of the MuLV Components into Virions—As shown in Fig. 4, we also analyzed radioactive proteins in virions released from the Eveline II cells throughout the pulse-chase experiment described above. However, the quantities of radioactivity observed in Fig. 4 are not directly comparable to those in Figs. 1 and 2 because the autoradiogram exposure times were 10 times longer and the sample volumes were four times larger. Clearly, virion cores containing L-[35S]methionine-labeled Pr65\\(^{\text{m}}\) and its products assemble from the cytoplasmic pool without a substantial lag period and are released into virions (Fig. 4, lanes 1-5). It is known that Pr65\\(^{\text{m}}\) is enriched in newly released virions which have a morphologically immature core structure and that its partial proteolysis coincides with maturation of the cores to a condensed morphology (50). On the contrary, radioactive gp70\\(^{\text{m}}\) only slowly appears in virions (Fig. 4, lanes 6-10). This is consistent with our observation that radioactive gp70\\(^{\text{m}}\) only slowly accumulates in the plasma membrane pool (Figs. 1 and 2) and with the fact that virions acquire gp70\\(^{\text{m}}\) during a process of budding from plasma membranes. A similarly slow entry of newly made gp70\\(^{\text{m}}\) into virions was previously observed by Witte et al. (51). Throughout the time period studied, we did not detect any soluble radioactive gp70\\(^{\text{m}}\)-related antigens in the culture media (data not shown). Presumably, such soluble gp70\\(^{\text{m}}\) (31, 52) derives by release from virions or from the cell surface and would therefore become labeled more slowly than gp70 in the plasma membranes. In addition, as reported earlier (53), gp93\\(^{\text{m}}\) does not occur in released virions.

*In Which Subcellular Organelle Does gPr90\\(^{\text{m}}\)* Accumu-
Membrane Glycoprotein Transport

**Fig. 4.** Incorporation of radioactive proteins into Friend MuLV particles released from Eveline cells. Cells were labeled with L-[35S]methionine as described in Fig. 1. Virus released into culture medium at the times indicated was harvested by centrifugation as described under "Experimental Procedures." The F-MuLV-encoded proteins in released virions were analyzed by immunoprecipitation with anti-p30 antiserum and were subsequently chased with nonradioactive methionine for 15 min (lanes 1 and 5) and 30-min (lanes 2 and 6), and were subsequently chased with nonradioactive methionine for either 1 h (lanes 3 and 7) or 2 h (lanes 4 and 8). Isolated membranous organelles were dissociated with lysis buffer and the radioactive proteins were precipitated using antiserum to gp70. The latter antiserum cross-reacts with gp55, a glycoprotein encoded by F-SFFV (35, 55). Each sample was adjusted to the same protein concentration before lysis and immunoprecipitation. Lanes 1--4 show the analysis of the rough endoplasmic reticulum which banded at the interface between 40 and 50% sucrose layers, whereas lanes 5--8 show the analysis of the plasma membrane containing fraction that banded at the 20--35% sucrose interface. The intermediate fraction at the 35--40% sucrose interface is not shown. It seemed to contain a diluted mixture of the adjacent fractions as shown both by electrophoretic results and by analysis of its RNA and protein contents.

**late?**—The above data are consistent with the hypothesis that there is a slow step in gPr90\(^{env}\) processing which preceeds or coincides with its partial proteolysis. Once past this barrier, however, transfer and completion of processing rapidly ensue. A key question, therefore, concerns the subcellular site for this barrier which must also be the site for accumulation of the gPr90\(^{env}\) precursor. We have approached this issue by subcellular fractionation of F4-6 erythroleukemia cells which are infected with both Friend MuLV and the Friend strain of spleen focus-forming virus. The latter cell line was used because gp70 processing kinetics appeared indistinguishable in these cells and in Eveline cells and because we had previously developed methods for fractionation and characterization of its membranous subcellular organelles.\(^3\)

The latter studies had indicated that the membrane fraction which bands in sucrose gradients between 40--50% sucrose contains ribosomes and consists of highly purified rough endoplasmic reticulum. Similarly, the fraction at the 20--35% interface is enriched in plasma membranes and in other smooth membranous organelles (38) whereas the intermediate fraction at the 35--40% sucrose interface contains relatively little material and appears to consist primarily of a mixture of membranes from the adjacent fractions. As observed by others, these preparations are enriched in their content of different membranous organelles, but they are not completely pure and each organelle is distributed among several fractions (38, 54).

Fig. 5 shows an analysis of virus-encoded glycoproteins in different membrane fractions isolated from cells that had been pulse labeled with L-[35S]methionine for 30 min and then chased with unlabeled methionine for various times. As expected, gp70\(^{env}\) is substantially enriched in the plasma membranes whereas gPr90\(^{env}\) is nearly absent from this fraction (lanes 5--8). Furthermore, the gp55 glycoprotein, which is encoded by the spleen focus-forming virus (35, 55), seems to be largely confined to the rough endoplasmic reticulum (lanes 1--4) and to be nearly absent from plasma membranes, consistent with other evidence (54, 56, 57).\(^3\)

Interestingly, L-[35S]methionine-labeled gPr90\(^{env}\) appears to occur in largest amount in the rough endoplasmic reticulum fraction and to remain in that fraction even 2 h after initiating the cold chase with nonradioactive methionine. As discussed below, the idea that the gPr90\(^{env}\) pool is confined to the rough endoplasmic reticulum is consistent with the fact that its oligosaccharides have a high mannose type structure that is susceptible to digestion with endoglycosidase H (58, 59). The gp55 glycoprotein component also contains exclusively high mannose type oligosaccharides that are susceptible to endoglycosidase H digestion and that bind strongly to concanavalin A-Sepharose columns (56).\(^3\)

Although we conclude from these studies of partially purified membranous organelles that both gPr90\(^{env}\) and gp55 are retained for up to several hours in the rough endoplasmic reticulum, we have also consistently observed substantial concentrations of these glycoproteins (approximately 30--40% of the total) in the nuclear fraction. However, this observation is difficult to interpret because our nuclear preparations are heavily contaminated with adherent rough endoplasmic reticulum and with Golgi membranes. The Golgi apparatus and portions of the rough endoplasmic reticulum are generally situated close to the nucleus in a viscous region of the cytoplasm which contains abundant microtubules (60) and is difficult to release by homogenization. Although our results suggest that gPr90\(^{env}\) and gp55 are retained in the rough endoplasmic reticulum even several hours after synthesis of their polypeptide chains, we cannot exclude the possibility that they may also be located in other subcellular organelles in the perinuclear region. Similar observations and conclusions were described previously by Lyles and McConnell (54).

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\(^3\)S. Clarke and D. Kabat, unpublished observations.
A Slow Step in Envelope Glycoprotein Processing Appears to Occur in the Rough Endoplasmic Reticulum—Our results indicate that gp70'''' is processed to the cell surface less rapidly than gp93'''' (Figs. 1B, 2B, and 3) or other plasma membrane glycoproteins which have been previously studied (27, 29–31). Furthermore, there is a slow or rate-limiting step in envelope glycoprotein processing which precedes or coincides with partial proteolysis of gpPr90''''. This conclusion derives from our observation that L-[^35S]methionine-labeled gpPr90'''' persists in the cells for a substantial time after its synthesis and is only slowly processed to form gp70'''' (Fig. 1). However, the kinetics of gp70'''' appearance on the cell surface (Fig. 1B) is not significantly or reproducibly retarded compared to the appearance of total cellular gp70'''' (Fig. 1A). Therefore, the sluggishness of gp70'''' processing to the cell surface occurs before and not after proteolytic cleavage of gpPr90''''.

Several lines of evidence suggest that the slow step in gpPr90'''' processing occurs in the rough endoplasmic reticulum. First, our subcellular fractionation studies indicate that gpPr90'''' persists in the rough endoplasmic reticulum even several hours after synthesis of its polypeptide chain (Fig. 5), in agreement with another investigation (54). Secondly, it is known that gpPr90'''' of F-MuLV contains seven asparagine-linked high mannose oligosaccharides which are susceptible to cleavage with endoglycosidase H (58, 62). However, gpPr90'''' lacks the complex-type fucosylated and sialylated oligosaccharides which occur in gp70'''' (23, 58, 63). High mannose oligosaccharides with a Glc3Man9GlcNAc2 structure are cotranslationally added onto glycoproteins in the rough endoplasmic reticulum (64, 65). These are subsequently modified in the same organelle by removal of Glc and possibly several Man residues (66, 67). However, high mannose oligosaccharides are modified in a complex-type oligosaccharide only after glycoproteins have been transferred into the distal or trans portion of the Golgi apparatus (12, 28, 66). The cis and trans Golgi membranes band in sucrose gradients at substantially lower densities than the rough endoplasmic reticulum (28, 72).

Envelope Glycoprotein Transfer Past the Kinetic Barrier Occurs Randomly Rather Than in the Linear or Cohort Order in Which Polypeptide Chains Were Synthesized—Several observations suggest that transfer of envelope glycoprotein past the slow step in gpPr90'''' processing occurs randomly rather than in the linear order in which the polypeptides were synthesized. Thus, envelope glycoprotein synthesized during a 30-min pulse period arrives slowly at the cell surface at least throughout the subsequent 30-min to 2-h period (Figs. 1, 2, and 3). Furthermore, a small amount of gpPr90'''' is still not processed after a 24-h chase period (Fig. 1A, lane 12). Consequently, it is apparent that some gp70'''' synthesized after the pulse period must have reached the cell surface before all of the radioactive gp70'''' had been transferred. Furthermore, as described previously (40), the quantity of radioactivity in gpPr90'''' declines during a cold chase period with first order kinetics and a half-life of approximately 30–45 min (Fig. 2A). Such kinetics are consistent with the idea that export from the precursor pool occurs randomly (see Mini-print, Equation 2). Finally, if exit from the precursor pool were random, it would be expected that some newly made radioactive envelope glycoprotein would pass quickly through the barrier and that its products would rapidly reach the cell surface. This expectation is consistent with the fact that radioactive gp70'''' and gp93'''' begin to reach the cell surface at the same time (i.e. 30–60 min) after addition of L-[^35S]methionine to the cells (Fig. 1B, lanes 3 and 9). The major difference is that the radioactive gp93'''' all reaches the cell surface at that time whereas the radioactive gp70'''' is transferred slowly throughout a prolonged time span.

What is the Rate-limiting Step in gp70'''' Processing?—Three events are known to be temporally closely related to the rate-limiting step in gpPr90'''' processing. These include export from the rough endoplasmic reticulum, partial proteolytic cleavage of gpPr90'''', and the oligosaccharide modifications which produce complex-type oligosaccharides. However, the oligosaccharide modifications occur in the Golgi apparatus (12, 28, 66, 67, 72) and are also not required for gp70 transport to the cell surface (68). Consequently, we believe that the rate-limiting step for gpPr90'''' processing may be its partial proteolysis or its export from the rough endoplasmic reticulum. Presumably, either of these events could be a prerequisite for the other. For example, export would be a prerequisite for cleavage if the protease was located in the Golgi apparatus. Alternatively, cleavage in the rough endoplasmic reticulum could conceivably activate the glycoprotein for export by a zymogen-like mechanism.

A Model for Membrane Glycoprotein Export from the Rough Endoplasmic Endoplasmic Reticulum—We believe that our results support three major conclusions concerning the mechanism for membrane glycoprotein export from the rough endoplasmic reticulum. 1) Selectivity: the fact that different membrane glycoproteins are exported at different rates implies that transport is a selective rather than passive flow or conveyor belt process. 2) Signal mechanism: the same evidence also indicates that structural characteristics of membrane glycoproteins influence their rates of export. Other information is also consistent with this conclusion (e.g. Refs. 31, 56, 70). We choose to call these structural characteristics signals because they are inherent in the glycoprotein, but do not mean to imply that they are necessarily identical for all glycoproteins. 3) As discussed previously, glycoproteins of a given type exit randomly rather than in order of their longevity in the precursor pool.

As shown by the mathematical analysis below (see Mini-print), these three characteristics of the transport systems are precisely what would be expected of a simple binding reaction in which export of any glycoprotein from its precursor pool required its attachment to one or more transport molecules. Furthermore, we have been unable to imagine any mechanism consistent with our observations that does not involve glycoprotein association as a controlling factor. Either the association could be long-lived to a carrier or catalytic to a transport molecule. Such transport molecules could be proteins or lipids and they would be expected to bind to different glycoproteins with different affinities. Furthermore, binding of particular glycoproteins could be facilitated by covalent modifications such as partial proteolysis or glycosylation. However, the critical binding sites on the substrate glycoproteins would probably have to include the amino acid sequence of the polypeptide chain because some proteins can be transferred efficiently to the cell surface in the absence of glycosylation (18, 22, 69). Thus, we are proposing that plasma membrane proteins contain a signal in their amino acid sequences that is required for their export from the rough endoplasmic reticulum.

It is interesting to consider the relationship between this export model and our data concerning gp93'''' and gp70'''' processing. According to this model, glycoproteins of a particular type exit randomly from their precursor pool because binding to the transport molecule(s) involves random collisions which are unrelated to the length of time a glycoprotein has remained in the pool. If the binding affinity of a glycoprotein were strong (e.g. as we propose for gpPr90''''), the pool would be small. Furthermore, such a small pool would turn over quickly and newly synthesized glycoproteins in this pool
would be rapidly exported to the Golgi apparatus without delay as an apparent cohort. On the contrary, if the binding affinity of a glycoprotein were weak (e.g. as we propose for gP90etc.), the precursor pool would be large and would turn over relatively slowly. In this case, glycoproteins synthesized during a brief interval would be diluted into the large precursor pool and their export to the Golgi apparatus would occur slowly throughout a prolonged time span.

Although our observations would be compatible with the alternative hypothesis, that binding to a matrix is required for retention in the rough endoplasmic reticulum and that transport to the Golgi apparatus automatically and passively follows release from this matrix, we consider this hypothesis inherently unlikely. For example, it cannot readily be reconciled with the fact that mutant forms of plasma membrane and secretory glycoproteins are frequently retained in the rough endoplasmic reticulum and that trans-retention in the rough endoplasmic reticulum and that transport to the Golgi apparatus is slow throughout a prolonged time span.

Numerous reports (49-52) and to a proposed mechanism for entry of newly synthesized rough endoplasmic reticulum and secretory glycoproteins are frequently retained in the rough endoplasmic reticulum and that transport to the Golgi apparatus is slow throughout a prolonged time span.
Membrane Glycoprotein Transport

S. J. Turco, P. W. Robbins, and W. R. Rothman

Membrane subcellular organelles were isolated from rabbit cells using a modification of the method of Ohashi and Singer [1980] Proc. Natl. Acad. Sci. U.S.A. 77, 7126-7130. The cells were broken in a French press, and the cell lysate was centrifuged at 10,000 x g for 10 min. The supernatant was then centrifuged at 100,000 x g for 1 h. The pellet was resuspended in 0.32 M sucrose buffer and was used for the experiment.

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