Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 253, No. 24, Issue of December 25, pp. 8667-8670, 1978
Printed in U.S.A.

A Signal Sequence for the Insertion of a Transmembrane Glycoprotein

SIMILARITIES TO THE SIGNALS OF SECRETORY PROTEINS IN PRIMARY STRUCTURE AND FUNCTION*

(Received for publication, October 3, 1978, and in revised form, October 27, 1978)

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SUMMARY

The biosynthesis of a secretory protein and a transmembrane viral glycoprotein are compared by two different experimental approaches. (*a*) NH_2-terminal sequence analysis has been performed on various forms of the transmembrane glycoprotein of vesicular stomatitis virus synthesized in cell-free systems. The sequence data presented demonstrate that the nascent precursor of the glycoprotein contains a "signal sequence" of 16 amino acids at the NH_2 terminus, whose sequence is Met-Lys-Cys-Leu-Leu-Tyr-Leu-Ala-Phe-Leu-Phe-Ile-(His-Val-Asn)-Cys. This signal sequence is proteolytically cleaved during the process of insertion into microsomal membranes prior to chain completion. The new NH_2 terminus of the inserted, cleaved, and glycosylated membrane protein is located within the lumen of the microsomal vesicles and is identical to that of the authentic glycoprotein from virions. (*b*) Nascent chain competition experiments were performed between this glycoprotein, bovine pituitary prolactin (a secretory protein), and rabbit globin (a cytosolic protein). It was found that the nascent membrane glycoprotein, but not nascent globin, competed with nascent prolactin for membrane sites involved in the early biosynthetic event of transfer across membranes. These data suggest that an initially common pathway is involved in the biogenesis of secretory proteins and at least one class of integral membrane proteins.

Secretory proteins as well as certain integral membrane proteins are synthesized on ribosomes bound to membranes. The question of how these proteins segregate to their respective final locations in the cell—*e.g.* completely extracellular, plasma membrane, or various intracellular membranes—is of central importance in cell biology. One possibility is that these distinct classes of proteins utilize different receptors and are thus synthesized on different regions of the rough endoplasmic reticulum. A second possibility is that all are biosynthesized by a common route and segregation occurs later.

Previous studies of membrane assembly using glycoprotein of vesicular stomatitis virus as a model transmembrane protein have demonstrated that insertion and subsequent glycosylation of nascent G proceeded only during translation and that the completed polypeptide was not a substrate for insertion (1, 2). Moreover, the glycoprotein synthesized in heterologous cell-free systems achieved an asymmetric orientation identical to that of pulse-labeled G found in rough microsomes of infected cells (1). This was consistent with the hypothesis (3–5) that insertion of membrane proteins into the bilayer proceeds by a mechanism, highly conserved through evolution, analogous to that described in the signal hypothesis for the transfer of secretory proteins across membranes (4, 5).

In this paper, NH_2-terminal sequence analysis of various forms of *in vitro*-synthesized G protein, as well as nascent chain competition experiments, are presented which allow us to extent the comparison of these two mechanisms. We demonstrate that this integral transmembrane glycoprotein, like most secretory proteins, is biosynthesized with a transient "signal sequence"; that this signal sequence is proteolytically removed during insertion into microsomal membranes; and that the resulting new NH_2-terminal resides exclusively within the lumen of the membrane vesicles. We also present evidence that nascent G and nascent bovine pituitary prolactin compete for membrane sites involved in chain segregation. These results are interpreted to provide further support for a conception of membrane protein biogenesis based on the signal hypothesis (5).

MATERIALS AND METHODS[1]

RESULTS

Previously, translation of VS virus[2] mRNA in the wheat germ cell-free protein-synthesizing system in the absence of a microsomal membrane supplement has yielded as major products the virus-coded proteins N, NS, M, and G. Except for the G protein, all of the proteins synthesized *in vitro* co-migrated with their authentic viral counterparts (1, 11, 12). The identical pattern of four major polypeptides was observed upon translation of VS virus mRNA in the reticulocyte lysate cell-free system (Fig. 1, *Lane 3*). Moreover, in either cell-free system, translation in the absence or in the presence of microsomal membranes during translation resulted in the synthesis of two distinct intermediates of G (1). Pre-G_0 (Fig. 1, *Lane 3, upward pointing arrow*) synthesized in the absence of microsomal membranes is a 60,000-dalton, nonglycosylated, putative precursor of the 67,000-dalton authentic glycoprotein (G_2) isolated from virions. G_1 (Fig. 1, *Lane 5, downward pointing arrow*) synthesized in the presence of membranes has been previously characterized (1) to represent a partially glycosylated form of G which is inserted into the microsomal membrane with an asymmetric, transmembrane orientation identical to that of the pulse-labeled intermediate of G observed in rough microsomes of infected cells. When membranes were added after translation, the conversion of pre-G_0 to G_1 did not take place, *i.e.* the completed pre-G_0 was not a substrate for insertion nor, hence, for glycosylation, an exclusively lumenal event.

In the case of secretory proteins, the information for translation-coupled segregation (7, 9, 13–15) usually resides in an

* This study was supported by National Institutes of Health Grants CA-12413 (to G.B.) and AI-08814 (to H.L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Portions of this paper (including "Materials and Methods, References, and Figs. 2, 3, and 6) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full-size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 78C-335, cite author(s), and include a check or money order for $1.00 per set of photocopies.

[2] The abbreviation used is: VS virus (VSV in miniprint), vesicular stomatitis virus; G, glycoprotein.

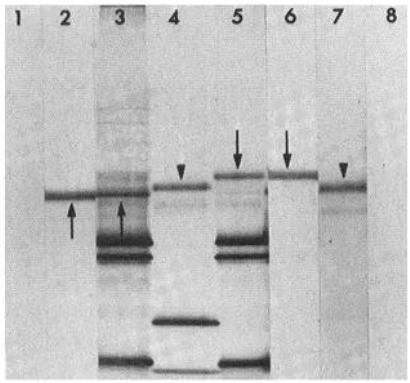

FIG. 1. Synthesis, insertion, and core glycosylation of VS virus G. VS virus mRNA was translated in the staphylococcal nuclease-treated rabbit reticulocyte lysate (6, 8) in the absence or presence of EDTA-stripped (4), nuclease-digested (6) dog pancreas microsomal membranes. This was followed by various post-translational assays as specified for each lane, and by preparation of aliquots for polyacrylamide slab gel electrophoresis in sodium dodecyl sulfate (4). Lanes were derived from two different slab gels and aligned according to protein standards on each slab gel. Incubation mixtures for translation (50 μl) contained 12 μCi of [^{35}S]Met, energy-generating system and ions as previously specified (6), 0.05 A_{260} unit of VS virus mRNA, and either no microsomal membranes (*Lanes 1* to *3*) or EDTA-stripped, nuclease-digested microsomes at a final concentration of 4 A_{260}/ml (*Lanes 4* to *8*). Incubation was for 90 min at 25°C. The following post-translational assays were performed on aliquots of translation products. *Lane 1,* a 25-μl aliquot was adjusted to 10 mM $CaCl_2$ and incubated at 0°C for 1 h with a final concentration of 250 μg/ml each of trypsin and of chymotrypsin. Digestion was terminated by the addition of 200 units of Trasylol. The entire mixture was subjected to immunoprecipitation as previously described (15) with the following modifications. Trichloroacetic acid precipitation and resolubilization in 1% sodium dodecyl sulfate was omitted. Instead, the sample was adjusted to the ionic conditions of Buffer A, namely, 1% Triton X-100, 10 mM EDTA, 50 mM Tris-HCl, pH 8.3, 150 mM NaCl; rabbit antiserum was raised against authentic G which was purified from virions by polyacrylamide gel electrophoresis in sodium dodecyl sulfate; instead of formalin-fixed *Staphylococcus aureus,* protein A-Sepharose was used as an affinity adsorbent for isolation of antigen·antibody complexes. Immunoprecipitates were dissociated from Sepharose as previously described (15). *Lane 2,* as *Lane 1,* except that the post-translational digestion with trypsin and chymotrypsin was omitted. *Lane 3,* 10-μl aliquot was treated as in *Lane 2* except that immunoprecipitation was omitted. *Lane 4,* 10-μl aliquot was treated as in *Lane 1,* except that immunoprecipitation was omitted. *Lane 5,* 10-μl aliquot was treated as in *Lane 3. Lane 6,* aliquot was treated as in *Lane 2. Lane 7,* aliquot was treated as in *Lane 1. Lane 8,* as in *Lane 1,* except that Triton X-100 was added to a final concentration of 0.5% before digestion with trypsin and chymotrypsin and subsequent immunoprecipitation. *Upward pointing arrows* indicate pre-G_0; *downward pointing arrows* indicate G_1; *downward pointing arrowheads* indicate G'_1.

NH_2-terminal signal sequence which is proteolytically removed before chain completion (4). In the case of the G protein, initial studies demonstrated a requirement for the NH_2-terminal region of the nascent chain in the process of insertion (1, 16), but were unable to resolve the question of whether proteolytic cleavage of such information also took place. Indeed, it was suggested (17, 18) that the signal sequence of integral membrane proteins might not be removed.

To define the nature and fate of the NH_2-terminal information required for insertion of this membrane protein, we undertook partial NH_2-terminal sequence determination of the various forms of the G protein.

Pre-G_0 labeled with one tritiated amino acid and either [^{35}S]Met or [^{35}S]Cys was identified and purified from total translation products by immunoprecipitation (Fig. 1, *Lane 2*) with a monospecific antiserum prepared against G_2, and by subsequent polyacrylamide gel electrophoresis in sodium dodecyl sulfate and autoradiography. Samples were prepared for sequencing as described under "Materials and Methods" and subjected to from 17 to 35 cycles of Edman degradation. The NH_2-terminal sequence was established (Fig. 2) to be:

1 5 10
Met-Lys-Cys-Leu-Leu-Tyr-Leu-Ala-Phe-Leu-Phe-Ile-His-Val-
15 20 25 30
Asn-Cys-Lys-Phe-X-Ile-Val-Phe-Pro-X-X-X-Lys-X-X-X-Lys-X-.

Due to the low specific activity of radioactive amino acids available and, hence, low recovery of radioactivity in pre-G_0, the assignments of His-Val-Asn in positions 13, 14, and 15 must be considered tentative.

Similarly, G_1 synthesized in the presence of EDTA-stripped and nuclease-digested dog pancreas microsomal membranes and radiolabeled with either [^{3}H]Lys, -Phe, or -Pro and [^{35}S]Met or -Cys, was purified from total translation products by immunoprecipitation (Fig. 1, *Lane 6*) and polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Double label counting of thiazolinones at each cycle of Edman degradation yielded a sequence (Fig. 3*a*) of:

1 5 10 15
Lys-Phe-X-X-X-Phe-Pro-X-X-X-Lys-X-X-X-Lys-X-.

Alignment with the sequence of pre-G_0 (Fig. 4) indicated that conversion of nascent pre-G_0 to G_1 was accompanied by proteolytic removal of a 16-amino-acid-long signal sequence from the NH_2 terminus of pre-G_0, as well as insertion and glycosylation of the growing chain.

In order to determine whether the cleavage of nascent pre-G_0 by dog pancreas microsomes was "correct," *i.e.* whether the authentic NH_2 terminus of G_2 had been generated, partial sequence analysis was performed on G_2 isolated from virions by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. The identification of Lys in position 1 and Phe in position 2 (data not shown), consistent with earlier peptide mapping studies,[3] suggested not only fidelity of cleavage but also, more importantly, that further processing of the NH_2 terminus does not take place during maturation of G and its transport from the rough endoplasmic reticulum to the plasma membrane.

Previously, correct asymmetric orientation of G_1 synthesized *in vitro* and inserted during translation into microsomal membranes was demonstrated by studies in which microsomal vesicles were digested with proteases. These indicated that the same COOH-terminal tryptic peptides were removed from G_1 synthesized and inserted *in vitro* as from isolated rough microsomes of infected cells. Removal of these tryptic peptides from the exposed region of the COOH terminus resulted in a partially digested form of G_1, designated G'_1, (Fig. 1, *Lane 4, arrowhead*), which is identified here by immunoprecipitation with rabbit anti-G_2 serum (Fig. 1, *Lane 7, arrowhead*). When detergent (0.5% Triton X-100) was added to solubilize the membranes, total digestion of G_1 resulted (Fig. 1, *Lane 8*). When pre-G_0 was treated with proteolytic enzymes in a similar fashion (Fig. 1, *Lane 1*), no G-related polypeptides (by immunoprecipitation) were found resistant to proteolysis. While indicating fidelity of insertion *in vitro*, the previous studies on G'_1 (1) provided no information on the location of the NH_2 terminus. In order to resolve this question, and to further characterize the orientation of G across the bilayer, automated Edman degradation of G'_1 was performed (Fig. 3*b*). The NH_2-terminal sequence was found to be identical to that of G_1 (Figs. 3*a* and 4). This strongly suggests that the NH_2-terminal sequence of G lies within the lumen of the microsomal vesicles, and moreover that G spans the bilayer only once, since a multiple transmembrane orientation would most likely have resulted in sequence heterogeneity of the NH_2 terminus after proteolytic digestion.

[3] H. F. Lodish, unpublished observations.

From the data presented here and previously (1, 16), it seems clear that the information which initiates the process of translation-coupled insertion of the G protein resides in the NH_2-terminal signal sequence, and moreover, that proteolytic removal of this information takes place before chain completion. It seemed possible however that separate sets of membranous receptors were involved in segregation and insertion, the latter set allowing only partial transfer of proteins across membranes and, hence, resulting in a transmembrane deposition. To investigate this possibility, competition experiments were designed involving nascent chains of G, or bovine pituitary prolactin (a nonglycosylated secretory protein), or rabbit globin (a cytosolic protein lacking a signal sequence). In these experiments, the behavior of nascent prolactin in the presence of varying amounts of nascent G protein is evaluated. Except where indicated, the amount of membranes was kept constant and subsaturating with respect to segregation activity. Competition was monitored by assaying for processing of prolactin which is an accurate and readily quantifiable indicator of the extent of segregation (7).

It can be seen that the presence of low (Fig. 5, *Lane 2*) or high (Fig. 5, *Lane 3*) concentrations of VS virus mRNA resulted in decreased levels of processed prolactin relative to preprolactin, when compared to translation of prolactin mRNA in the absence of added VS virus mRNA (Fig. 5, *Lane 1*). Similar experiments with increasing amounts of globin mRNA had no such effect at either low (Fig. 5, *Lane 4*) or high (Fig. 5, *Lane 5*) globin mRNA concentration. That competition between nascent pre-G_0 and nascent preprolactin

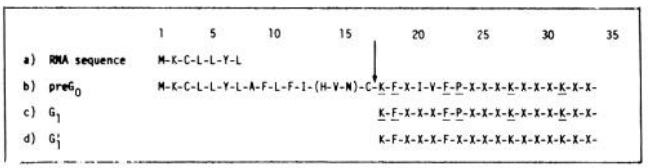

FIG. 4. Summary of sequence data on VS virus G. *a*, from partial RNA sequence (Ref. 21); *b*, VS virus pre-G_0 (see Fig. 2); *c*, VS virus G_1 (see Fig. 3*a*); *d*, VS virus G'_1 (see Fig. 3*b*). *Arrow* indicates cleavage site for signal peptidase. *Underlined residues* indicate basis for alignment of pre-G_0 with G_1. *M* = Met, *K* = Lys, *C* = Cys, *L* = Leu, *Y* = Tyr, *A* = Ala, *F* = Phe, *I* = Ile, *H* = His, *V* = Val, *N* = Asn, *P* = Pro, *X* = unknown.

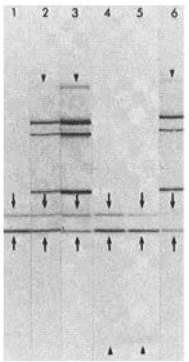

FIG. 5. Competition for membranous receptors by nascent VS virus G and nascent prolactin. 0.5 A_{260} unit/ml of prolactin mRNA was translated in the reticulocyte lysate system (*6*) containing 5 A_{260} units/ml (*Lanes 1* to *5*) or 10 A_{260} units/ml (*Lane 6*) of EDTA-stripped nuclease-digested dog pancreas microsomal membranes in the presence of 0.1 (*Lane 2*) or 0.4 (*Lanes 3* and *6*) A_{260} unit/ml of VS virus mRNA; or 0.1 (*Lane 4*) or 0.4 (*Lane 5*) A_{260} unit/ml of globin mRNA. *Downward pointing arrows* refer to preprolactin and *upward pointing arrows* refer to processed prolactin. *Downward pointing arrowheads* refer to G_1, *upward pointing arrowheads* refer to globin. *Lanes 1* to *5* were derived from one slab gel while *Lane 6* was derived from a different slab gel and realigned according to the migration of processed prolactin.

was most likely for membrane sites and not for soluble factors was supported by the demonstration (Fig. 5, *Lane 6*) that increasing membrane concentrations abolished the competition (Fig. 5, *Lane 3*). In order to quantitate the extent of inhibition of segregation of nascent prolactin by nascent pre-G_0, the radioactivity in preprolactin and in processed prolactin was determined separately and segregation was expressed as a percentage of total radioactivity in completed prolactin chains (processed prolactin/preprolactin + processed prolactin). It can be seen in Fig. 6 that increasing the amount of VS virus mRNA changed the ratio of processed prolactin to preprolactin from approximately 3:1 to 1:1 (*i.e.* reduced the percentage of processed prolactin chains from 77% to 52%). In contrast, globin mRNA had no effect on the percentage of prolactin segregation (Fig. 6). In either case, increasing the concentration of the competing mRNA resulted in an increase in both total protein synthesis (data not shown) and in specific products coded for by the competing species (*arrowheads*, Fig. 5). For example, at 16 μg/ml of globin mRNA, approximately 182,000 cpm of globin were synthesized while at the same mRNA concentration 122,000 cpm of VS virus products were made of which G comprised less than 5%. Thus, even a vast excess of nascent globin did not affect the percentage processing of prolactin.

DISCUSSION

The demonstration (1) that cell-free systems are capable of membrane assembly and that asymmetric integration into the bilayer proceeds only during translation has permitted detailed studies into this fundamental and ubiquitous intracellular process. Kinetic studies (16) have indicated a requirement for the NH_2-terminal region in the insertion process. The structural studies presented in this communication demonstrate conclusively that nascent G protein of VS virus contains a hydrophobic sequence of 16 amino acids at the NH_2 terminus which is proteolytically removed during insertion prior to chain completion.

A comparison of the VS virus signal with sequence data on signals of secretory proteins reveals several common features including a typical region of leucine-rich hydrophobic amino acids in positions 4 through 12 and the presence at either end of the signal, *e.g.* at residues 2, 3, 13, 15, and 16, of hydrophilic or polar amino acids. Similarly, the presence of a tyrosine in position 6 is consistent with the presence of at least one free hydroxyl-containing side group in most signal sequences to date. Apart from these general features, few specific homologies with other signal sequences can be discerned, which is not surprising in view of the fact that secretory signals which have been demonstrated to recognize similar receptors (19) often have little apparent homology with each other (20). For this reason, certain distinctive features of the VS virus pre-G_0 signal may not be a significant indication of functional distinction. For example, the presence of Cys in positions 3 and 16 while unusual, is not unique, in that preconalbumin has been demonstrated to contain Cys in positions 6 and 17 of its 19-residue-long signal peptide (22) and pre-MOPC 41 a and b light chains both have a cysteine, as does pre-G_0 at the penultimate site before cleavage (23).

In general, then, the primary structural studies suggest similarities between the structural prerequisites for the early events in protein secretion and those of membrane assembly, two processes which pose conceptually related biosynthetic problems. In both processes, correct topological biogenesis involves exclusively the nascent form of the polypeptide and, in both processes, the information for transfer usually resides in a cleavable hydrophobic NH_2-terminal sequence. However, that a signal sequence need be neither cleavable nor highly hydrophobic (20) in order to function in the transfer of pro-

teins across membranes in cell-free systems (19) has been demonstrated. It seems possible that hydrophobicity plays a role in the recognition by signal peptidase, a membrane-bound enzyme, and that recognition by receptors for transfer across the membrane is mediated by as yet poorly understood conformational features common to all signal sequences.

From our data, it appears that nascent G and nascent prolactin recognize at least one common membranous component. This component seems unlikely to be involved in processes of protein synthesis other than transfer across (into) membranes, since nascent globin, translated by the same pool of (initially) free ribosomes as G and prolactin, did not interfere with segregation of prolactin. Rather, it seems likely that competition was for a receptor on the cytoplasmic face of the membrane which recognizes at least in part, nascent, signal-containing chains and participates in their transfer across (into) the membrane. Although we cannot rule out competition for other components, *e.g.* signal peptidase on the lumenal aspect of the membranes, in view of previous studies which show that nascent ovalbumin (glycosylated but not processed by signal peptidase) and nascent prolactin (processed by signal peptidase but not glycoslyated) compete for common receptors, these explanations seem unlikely. Moreover, no new intermediates of G were seen (*e.g.* unprocessed but segregated and glycosylated precursors).

While detailed mechanistic studies await characterization of the membranous apparatus involved in segregation, the competition experiments presented here afford a preliminary statement on the mechanistic similarity of insertion and of segregation. These results support a biosynthetic model involving a common set of early events in membrane assembly and protein secretion, wherein similar signal sequences recognize a common set of receptors to initiate the process of ribosome attachment, tunnel formation, and initiation of chain transfer across the membrane. Subsequent to the onset of chain transfer, the events diverge; secretory chains such as prolactin are entirely transferred within the vesicle lumen while the transfer of transmembrane proteins such as G are arrested prior to completion to transfer, resulting in a polypeptide spanning the bilayer. In both cases, co-translational modification, *e.g.* cleavage of signals and glycosylation of suitable acceptor sites, proceeds during translation and, probably, prior to cessation of transfer. In such a model, the information which arrests transfer—termed a "stop transfer" sequence (24)—would be expected to reside elsewhere in nascent G other than in the NH_2-terminal sequence, which is cleaved. Such a stop transfer sequence might function by abrogating or altering the ribosome membrane junction or self-associated tunnel components, and results in retention of the COOH-terminal polypeptide region as a cytoplasmic domain. Earlier studies have firmly documented vast evolutionary conservation of the role of signal sequences in the transfer of chains across membranes (9). By corollary, it seems likely that the stop transfer role is a conserved function of a set of defined polypeptide sequences, adjacent to, or itself constituting, the region of the inserted polypeptide which actually spans the bilayer.

One implication of this model of early biosynthetic events is that information other than that involved in the initial biosynthetic disposition (*i.e.* on free *versus* bound ribosomes, and hence, cytosolic *versus* extracellular consignment of polypeptides) is required to consummate the later stages of subcellular sorting.

Acknowledgment—We thank H. Desruisseaux for help with the Beckman Sequencer.

SUPPLEMENT

to

A Signal Sequence for the Insertion of a Transmembrane Glycoprotein: Similarities to the Signals of Secretory Proteins in Primary Structure and Function.

by

Vishwanath R. Lingappa, Flora M. Katz, Harvey F. Lodish, and Günter Blobel

MATERIALS AND METHODS

Most of the procedures used in the present study have been detailed previously, including: the preparation of mRNA from rabbit reticulocytes (4), bovine steer pituitary glands (7), and BHK cells infected with VSV Indiana (1); the preparation of EDTA-stripped, nuclease-digested dog pancreas microsomal membranes (6,14); assays such as polyacrylamide slab gel electrophoresis in sodium dodecyl sulfate and subsequent autoradiography (4); and proteolysis of membranes with trypsin and chymotrypsin (4). The translation of mRNAs in a staphylococcal nuclease-treated rabbit reticulocyte lysate was as before (6,8) except that rabbit liver tRNA was added to a final concentration of 100 ug/ml. Other post-translational procedures such as immunoprecipitation are detailed in the figure legends. Sample preparation for sequence analysis: Following immunoprecipitation of G from total translation products of VSV mRNA as described in figure 1, and subsequent polyacrylamide slab gel electrophoresis, gels were dried without fixing or staining and exposed to autoradiography. G protein forms were excised from gels and electroeluted in the presence of 1 mg ovalbumin as a carrier as previously described (9). To the eluted samples in a volume of 3 ml were added serially 0.2 ml of 5 mg/ml sperm whale apomyoglobin, 0.1 ml of 10 mg/ml ovalbumin, 0.4 ml of 4.0 M KCl and 3.5 ml of 50% trichloroacetic acid. After vortexing, incubation on ice for 15 minutes, centrifugation at 600 xg for 10 minutes, and aspiration of supernatants, the resulting pellet was resuspended in 1 ml of water then precipitated with 9 volumes of acidified acetone at -20°C for 15 minutes. Resuspension, acetone precipitation, and centrifugation were repeated and the resulting pellet dissolved in 50% heptafluorobutyric acid prior to loading into the Beckman 890C sequencer running on a DMAA program. After each cycle of Edman degradation, thiazolinone derivatives were collected in minivials, air-dried and counted by standard double-label procedures in 3 ml of toluene liquifluor. Repetitive yields were calculated (10) normalized to the first cycle.

Tritiated amino acids and [^{35}S]cyatine were purchased from New England Nuclear, Boston, MA 02118, at the highest available specific activities. [^{35}S]Methionine was purchased from Amersham Corp., Arlington Heights, IL 60005. Trypsin and chymotrypsin were from Boehringer, Mannheim, Germany; Protein A Sepharose was from Pharmacia Fine Chemicals, Piscataway, NJ 08854. Trasylol was from FBA Pharmaceuticals, New York, NY; rabbit liver tRNA was from Grand Island Biological Co., Grand Island, NY 14072.

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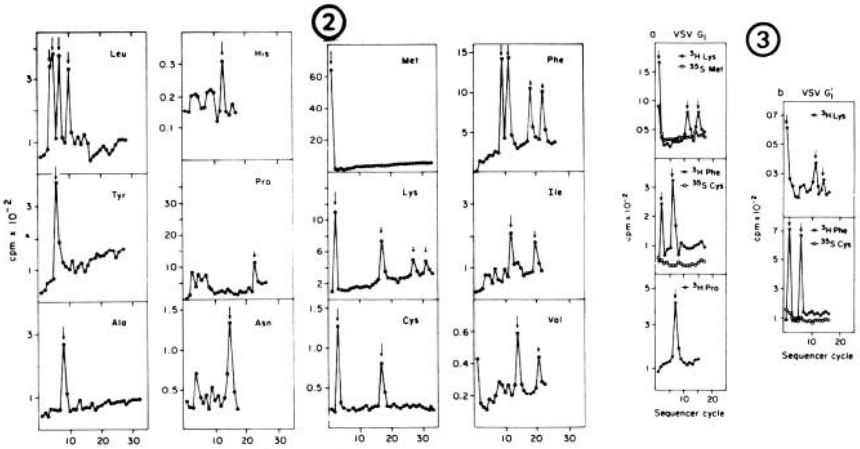

Fig. 2 Amino terminal sequence analysis of VSV preG$_0$. 1 ml translation reactions containing 250 uCi of a single tritiated amino acid, 250 uCi of [^{35}S] Met or Cys and 1.0 A_{260} u/ml of VSV mRNA were carried out as previously described (6). Sample preparation was described in methods. The preG$_0$ was taken through 20-35 cycles of Edman degradation and residues analysed as described in methods. Sequence assignments in the signal region of preG$_0$ are indicated by long arrows, those in the authentic region of G$_1$ by short arrows. Input radioactivity was: [^{35}S] Met, 120,000 cpm; [^{3}H]] Lys, [^{3}H] Pro, and [^{3}H] Phe, 40,000 cpm; [^{3}H] Leu, [^{3}H] Ile, [^{3}H] Ala, [^{3}H] Tyr, 10-15,000 cpm; [^{3}H] Val, [^{3}H] Asn, [^{35}S] Cys, and [^{3}H] His, 5,000 cpm. Repetitive yields of 96% were routinely obtained.

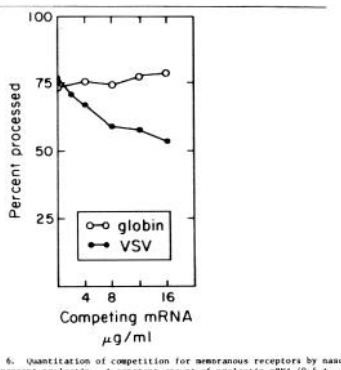

Fig. 3. Partial amino terminal sequence analysis of: a) VSV G$_1$ and b) VSV G$_1'$. 0.5 ml translation of reactions containing 100 uCi of [^{3}H] Pro, [^{3}H] Phe, or [^{3}H] Lys and 200 uCi of either [^{35}S] Met or [^{35}S] Cys, and containing 2 A_{260} u/ml of EDTA-stripped nuclease-digested dog pancreas microsomal membranes were performed as in Fig. 2. Following translation aliquots were subjected to post-translational incubations and immunoprecipitations as described in Fig. 1, lane 6 (for G$_1$) and lane 7 (for G$_1'$). Sample preparation for sequencing was described in methods. 17 cycles of automated Edman degradation were performed. Input radioactivity for G$_1$ was [^{3}H] Lys, 13,000 cpm, [^{3}H] Pro, 12,000 cpm, [^{3}H] Phe, 10,000 cpm, [^{35}S] Met, 60,000 cpm, [^{35}S] Cys, 15,000 cpm. Input radioactivity for G$_1'$ was [^{3}H] Phe, 15,000 cpm and [^{3}H] Lys, 4,000 cpm. Arrows indicate assigned positions.

Fig. 6. Quantitation of competition for membranous receptors by nascent G of VSV and nascent prolactin. A constant amount of prolactin mRNA (0.5 A_{260}u/ml) was translated in the presence of 5 A_{260}u/ml of microsomal membranes in the absence or presence, of increasing amounts of an additional mRNA (closed circles, VSV mRNA; open circles, globin mRNA). Translation products were analysed (see for example Fig. 5) and the radioactivity of gel slices containing preprolactin and prolactin was determined as previously (6). The extent of processing is expressed on the ordinate as a percentage of processed prolactin over that of total prolactin (which is preprolactin +processed prolactin) and is plotted as a function of competing mRNA concentration. As an example, 28,750 cpm were found in total prolactin and 21,800 cpm in processed prolactin when no competing mRNA was added.