Reconstitution of a Tandem Co- and Post-translational Processing Pathway with Rat Liver Subcellular Fractions*

Gary Rogers, Jeannine Gruenebaum, and Irving Boime‡

From the Department of Pharmacology, Washington University Medical School, St. Louis, Missouri 63110

Previously we showed that smooth microsomes from a variety of tissues effectively cleaved, quiescent, and "core" glycosylated nascent chains of secretory proteins. To further characterize the role of smooth membranes in the biosynthesis of secretory polypeptides, rat liver smooth microsomes were separated into rough endoplasmic reticulum and Golgi fractions. Membranes of the smooth endoplasmic reticulum cleaved the signal peptide of pre-placental lactogen, attached the high mannose core to the α-subunit of choric gonadotropin, and according to the processed polypeptides. None of these processing steps were performed by Golgi membranes. However, processing of asparagine-linked oligosaccharides and the coincident addition of terminal sugars was performed by Golgi but not by smooth endoplasmic reticulum membranes. The properties of this post-translational reaction are very similar to those described for the reactions in vivo. These observations demonstrate that the enzymes for co-translational (pre-protein processing) and post-translational (oligosaccharide maturation) processing events are localized in the endoplasmic reticulum and Golgi apparatus, respectively. This functional differentiation of Golgi and endoplasmic reticulum membranes is an important feature of the secretory process in eukaryotic cells. Restriction of the recognition and transport of nascent secretory proteins to the endoplasmic reticulum establishes the polarity necessary for the ordered sequence of post-translational steps involved in the synthesis and maturation of secretory proteins.

Eukaryotic secretory proteins travel through a number of subcellular compartments prior to their exit into the extracellular space (38). During this transit through the cell, secretory proteins frequently undergo one or more structural modifications by specific enzymes localized within the various organelles. For example, while the synthesis and translocation of the nascent chain is taking place, the NH₂-terminal 15–30 amino acids, termed the signal or prepeptide, are proteolytically removed by an activity present in the endoplasmic reticulum membrane. In the case of secretory glycoproteins, a preassembled oligosaccharide unit (30, 31) is transferred from a lipid carrier to appropriate asparagine residues in the growing polypeptide chains (22, 40, 49). This primary, or "core," glycosylation also occurs prior to completion of the polypeptide chains (2, 42). Upon release of the completed polypeptide from the ribosomes, the oligosaccharide is processed primarily by enzymes localized within the Golgi complex (40, 43).

The initial stages of protein secretion, i.e. the binding and translocation of nascent chains by the ER membrane, proteolytic removal of the pre-peptide, and core glycosylation, have been generally regarded to occur only in RER and not in SER. However, we have recently shown that smooth microsomes prepared from rat liver effectively cleave, glycosylate, and translocate nascent chains of hPL and hCGα (4). Smooth microsomes isolated from Krebs’ II ascites tumor cells and bovine adrenal cortex also processed and translocated nascent chains of hPL and hCGα very efficiently. Both of these tissues contain significantly less RER than is found in rat liver, providing strong evidence that the processing observed with smooth microsomes is not due to contamination by stripped RER that may have formed during membrane preparation. The data from the adrenal cortex are particularly important in this regard since the tissue is virtually devoid of RER (33, 39).

Smooth microsomal preparations typically contain, in addition to membranes derived from the SER, fragments of the Golgi apparatus, plasma membranes, and secretory granules. The heterogeneity of smooth microsomes raises the question of whether pre-protein processing observed with this fraction is limited to the ER or are other smooth vesicles capable of processing pre-proteins. To examine this question we have subfractionated smooth microsomes into SER and Golgi fractions that are virtually free of cross-contamination. Membranes derived from the SER, when added co-translationally to ascites tumor lysates, effectively cleaved nascent chains of both hPL and hCGα and, in the case of hCGα, transferred two high mannose oligosaccharide units to the nascent chain. Partially purified Golgi membranes did not cleave or core glycosylate either protein. Furthermore, we describe a cell-free assay for the Golgi-dependent modification of the high mannose oligosaccharide added to hCGα in the ER. The in vitro processing of oligosaccharide appears to follow the same pathway described for the in vivo maturation of complex type oligosaccharide (25).

**EXPERIMENTAL PROCEDURES**

Materials

1. The abbreviations used are: ER, endoplasmic reticulum; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; hPL, human placental lactogen; hCGα, the α subunit of human chorionic gonadotropin; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; DTT, dithiothreitol; TCA, trichloroacetic acid; SDS, sodium dodecyl sulfate; βME, β-mercaptoethanol; Pip, 1,4-piperazinediethanesulfonic acid.

2. The "Experimental Procedures" are presented in miniprint as...
RESULTS

To examine the respective roles of SER and Golgi membranes in protein secretion, a rat liver homogenate was fractionated according to the scheme shown in Fig. 1. This fractionation involves an initial isolation of crude Golgi and SER membranes. The SER preparation (fraction \( f_6 \)) contains very little galactosyltransferase activity, indicating less than 10% contamination with Golgi membranes. The overall recovery of SER is comparable to that reported by Kruppa and Sabatini (26). An estimate of ER contamination in the Golgi preparation (fraction \( f_3 \)) is provided by glucose-6-phosphatase activity, which is about 40% of that found in the SER preparation studied.

The exact level of ER contamination is difficult to assess since the tissue source was not known.

The preparation of Golgi and SER membranes involved the following steps:

1. **Preparation of Golgi Membranes**
   - Isolated rat liver homogenates were fractionated by differential centrifugation according to the scheme shown in Fig. 1.
   - The Golgi membranes were isolated from the fractions by differential centrifugation, followed by isopycnic centrifugation in a sucrose gradient.
   - The Golgi membranes were collected and assayed for their specific activities of galactosyltransferase and glucose-6-phosphatase.

2. **Preparation of SER Membranes**
   - Isolated rat liver homogenates were fractionated by differential centrifugation according to the scheme shown in Fig. 1.
   - The SER membranes were isolated from the fractions by differential centrifugation in a sucrose gradient.
   - The SER membranes were collected and assayed for their specific activities of galactosyltransferase and glucose-6-phosphatase.

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Co- and Post-translational Processing

**RAT LIVER HOMOGENATE**

**POST-NUCLEAR SUPERNATE** (PNS)

**POST-MITOCHONDRIAL SUPERNATE** (PMS)

**ISOPYCNIC CENTRIFUGATION**

![Diagram](http://www.jbc.org/)

**TABLE I**

**Distribution of protein and subcellular marker enzymes in rat liver smooth membrane fractions**

| Fraction | Glucose-6-phosphatase | Galactosyltransferase | Protein yield |
|----------|-----------------------|-----------------------|---------------|
|          | Purity | Yield | Purity | Yield | % | % |
| PNS      | 1      | 100   | 1      | 100   | 100 | 100 |
| PMS      | 0.74   | 32    | 0.97   | 41.7  | 43.1 |
| f₁       | 2.9    | 1.0   | 25.7   | 8.9   | 0.35 |
| f₂       | 3.7    | 6.3   | 9.3    | 13.1  | 1.7 |
| f₂ (GA)  | 1.7    | 5.6   | 55.5   | 8.9   | 0.16 |
| f₃       | 3.6    | 0.4   | 28.2   | 2.9   | 0.1 |
| f₄       | 4.0    | 5.3   | 5.0    | 6.5   | 1.3 |
| f₅       | 2.3    | 0.6   | 7.3    | 1.9   | 0.26 |

**TABLE II**

**Comparative yields of Golgi and SER membranes prepared by either double (Method A) or single (Method B) homogenization**

| Fraction | Protein | Glucose-6-phosphatase | Galactosyltransferase |
|----------|---------|-----------------------|-----------------------|
| PNS      | 0.118   | 0.011                 | 0.382                 |
| PMS      | 0.967   | 0.223                 | 0.287                 |
| f₁       | 0.178   | 0.013                 | 1.12                  |
| f₂       | 0.478   | 0.130                 | 0.162                 |

**FIG. 1.** Flow diagram for the preparation of smooth membrane fractions from rat liver. See under "Experimental Procedures" for details of preparation and Table I for characterization. Two membrane fractions were collected from the first equilibrium gradient: f₁, crude preparation of Golgi membranes (from the 0.5–1.0 M sucrose interface); f₂, crude SER preparation (from the 1.0–1.35 M sucrose interface). Both fractions were further purified by a second, velocity gradient centrifugation. Four fractions were collected: f₃, the final Golgi-rich fraction (from the 0.5–1.0 M sucrose interface); f₄, containing membranes originating from both Golgi and SER (1.0–1.1 M sucrose interface); f₅, SER-containing fractions (the 1.1 M sucrose interface); f₆, crude SER preparation (from the 1.0–1.35 M sucrose interface); f₇, crude preparation of Golgi membranes (from the 0.5–1.0 M sucrose interface); f₈, 2, 3, and SER membranes prepared by either double (Method A) or single (Method B) homogenization.) Experimental details were as described under "Experimental Procedures." Data are expressed as units of enzyme activity per g of liver. For glucose-6-phosphatase, 1 unit corresponds to 1 pmol of phosphate released per min at 37 °C; for galactosyltransferase, 1 unit corresponds to 1 pmol of galactose transferred to N-acetylgalactosamine per min at 31 °C; protein is in milligrams. GA, Golgi membranes; SER, smooth endoplasmic reticulum.
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FIG. 2. SDS-polyacrylamide gel electrophoresis of total [35S]methionine-labeled proteins synthesized in the presence of term placental RNA. Equivalent amounts of smooth membranes (final concentration 1 mg/ml of protein) were added to 150 μl of reconstituted ascites tumor cell-free lysates. Each reaction mixture contained approximately 2.5 μg of the human placental ribonuclease inhibitor. Mr, total ascites tumor microsomes; GA, Golgi membranes; SER, smooth ER membranes. Lane 2 indicates a reaction mixture containing no membranes. Other abbreviations are as for Fig. 1. The marker hPL (Mr = 22,000) is shown. Equivalent amounts of radioactivity (about 150,000 cpm) were added to each lane except for the minus RNA lane, which contained 50,000 cpm.

FIG. 3. Autoradiograph of hCGα immunoprecipitated protein synthesized in response to first trimester placental RNA. Conditions for the assay were the same as described in the legend to Fig. 2, except that the translation products were immunoprecipitated with hCGα subunit specific antisera. Mobilities of the cleaved and glycosylated subunit ((CHO)-hCGα) and the precursor (pre-hCGα) are shown in the left hand margin. Other abbreviations are as described in Fig. 2. Equal amounts of radioactivity (25,000 cpm) were applied to each lane.

Fractions also cleaved and glycosylated pre-hCGα (Fig. 3). The amounts of membranes added were in the linear range of cotranslational activity for each of the fractions. The highly enriched Golgi membranes of fraction f6 did not process either pre-protein, even at concentrations 2-fold higher than f5 (data not shown). The small amount of pre-protein processing observed with the crude Golgi fraction (fraction f5) is presumably due to the presence of contaminating SER fragments. There was no apparent inhibitor in the Golgi fraction since addition of f5 (1 mg/ml) to a reaction mixture containing f5 did not affect the co-translational processing of hCGα (data not shown).

It is interesting that membranes of fraction f6 also do not process nascent secretory proteins. These membranes, however, are of unknown origin, and the reason for this absence of pre-protein processing remains unclear.

Processing of nascent secretory proteins is coupled to their sequestration within the lumen of microsomal vesicles (4, 8). While the vectorial transport of the nascent chain is apparently necessary for the processing events, it has recently been shown (20) that cleavage of the prepeptide is not required for sequestration. To determine if Golgi membranes will translocate nascent secretory proteins, the pre-hCGα that accumulated in the presence of these membranes was tested for susceptibility to trypsin. Protection of the completed protein from proteolysis is indicative of sequestration within the membrane vesicles. As shown in Fig. 4, Golgi membranes (lane 7) do not protect pre-hCGα from proteolysis. However, those membranes that process hCGα also protect the processed form from trypsin digestion (Fig. 4, lanes 5, 6, 8, and 9). Thus, translocation of nascent secretory proteins does not occur in Golgi membranes. The data do not exclude the possibility that the activities responsible for proteolytic removal of the pre-peptide or attachment of the mannose-rich unit of nascent secretory glycoproteins are present in Golgi membranes.

In Vitro Processing of the Asparagine-linked Mannose-rich Oligosaccharide Precursor by Golgi Membranes—The biosynthesis of these glycoproteins is initiated by the transfer of a mannose-rich oligosaccharide precursor to the nascent polypeptide. The maturation of the oligosaccharide occurs by the stepwise removal of monosaccharides to yield a core structure common to all complex type oligosaccharides (22, 40, 49). The terminal sugars N-acetylglucosamine, galactose, and sialic acid are subsequently added by specific glycosyltransferases located within the Golgi apparatus (43, 44). Our goal in the following experiments was to determine 1) if the Golgi membranes were capable of processing the oligosaccharide precursor and adding terminal sugars to the core structure, and 2) if the compartmentation of co- and post-translational modifications of the nascent secretory proteins could be demonstrated in SER and Golgi membranes, respectively. In the course of answering these questions we have developed
a cell-free system capable of forming complex type oligosaccharides from the high mannose nascent oligosaccharide.

Direct structural analysis of in vitro-synthesized glycoprotein substrates is complicated by the relatively low amounts of material available for analysis. We have used the enzyme Endo-β-N-acetylglucosaminidase (Endo H) as an analytical probe for following the processing of asparagine-linked oligosaccharides. While Endo H will cleave the mannose-rich oligosaccharide precursor of newly synthesized glycoproteins, it will not attack oligosaccharides containing terminal sugars (50-52). Oligosaccharides which are intermediates in processing exhibit intermediate sensitivities (40, 50, 52) to Endo H.

First trimester RNA was translated in the presence of SER (fraction f2) from rat liver, and the membrane vesicles, containing the cleaved and glycosylated form of hCGα, were isolated by centrifugation. The pellets were resuspended in the presence of Golgi or SER membranes, Triton X-100, and nucleotide sugars. The products were immunoprecipitated and digested with Endo H. Endo H sensitivity is reflected by an increased electrophoretic mobility due to the loss of carbohydrate (2, 3).

After incubation in the presence of membranes derived from the Golgi complex, hCGα is converted to an Endo H-resistant form (Fig. 5, lane 2). A processing intermediate, running between the Endo H-resistant and the sensitive form of hCGα, was also observed. This intermediate presumably represents hCGα with only one of its two oligosaccharide chains rendered resistant to Endo H. Virtually all of the hCGα oligosaccharides were supplemented with Triton and deoxycholate to final concentrations of 0.8% Triton X-100, 2 mM UDP-GlcNAc, 2 mM UDP-Gal, and 100 mM PIPES-KOH (pH 6.5). These reactions were incubated for 3 h at 37 °C in the absence (lanes 4, 5) or presence of 100 μg of either Golgi (GA; lanes 1, 2) or SER (lanes 5, 6) membranes. The reactions were supplemented with Triton and deoxycholate to final concentrations of 1% for each and then centrifuged to remove ribosomes. Immunoprecipitation of the supernatant was carried out as described (14). The immunoprecipitates were dissolved in acetate buffer (pH 5.9) and incubated overnight in the presence or absence of Endo H. The products were precipitated with trichloroacetic acid and analyzed by SDS-polyacrylamide gel electrophoresis. Approximately 10,000 cpm of [35S]methionine was loaded in each lane.

**Fig. 5.** Post-translational processing of asparagine-linked oligosaccharides in vitro. Co-translational reaction mixtures containing first trimester placental RNA and 150 μg of rat liver SER were centrifuged at 135,000 × g for 15 min to pellet the membrane vesicles. The pellets were resuspended in post-translational assay media containing 0.8% Triton X-100, 2 mM UDP-GlcNAc, 2 mM UDP-Gal, and 100 mM PIPES-KOH (pH 6.5). These reactions were incubated for 3 h at 37 °C in the absence (lanes 4, 5) or presence of 100 μg of either Golgi (GA; lanes 1, 2) or SER (lanes 5, 6) membranes. The reactions were supplemented with Triton and deoxycholate to final concentrations of 1% for each and then centrifuged to remove ribosomes. Immunoprecipitation of the supernatant was carried out as described (14). The immunoprecipitates were dissolved in acetate buffer (pH 5.9) and incubated overnight in the presence or absence of Endo H. The products were precipitated with trichloroacetic acid and analyzed by SDS-polyacrylamide gel electrophoresis. Approximately 10,000 cpm of [35S]methionine was loaded in each lane.

**Fig. 6.** Nucleotide sugar requirements for processing of hCGα oligosaccharides in vitro. Post-translational assay conditions were the same as outlined in Fig. 5, except for the omission of UDP-Gal or UDP-GlcNAc where indicated. Other abbreviations are as indicated in Fig. 5. Equal amounts of radioactivity (10,000 cpm) were applied to each lane, except lane 3 (20,000 cpm).

**Fig. 7.** In vitro oligosaccharide processing in the presence and absence of 0.8% (v/v) Triton X-100. Post-translational incubations were performed in the presence of 100 μg of Golgi membranes. All other abbreviations are as previously described (Fig. 5). Each lane contains approximately 5000 cpm of [35S]methionine.
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providing strong evidence that the in vitro processing and addition of terminal sugars occurs in accordance with the proposed in vivo pathway (see Ref. 25). The processing observed in the presence of both UDP-GlcNAc and UDP-Gal was more efficient than the processing observed when only UDP-GlcNAc alone was present. No Endo H-resistant hCGa was observed in the presence of UDP-Gal alone (lane 6).

The in vitro processing of oligosaccharide required the presence of Triton X-100 (Fig. 7, compare lanes 2 and 4). It has been previously shown (3) that the asparagine-linked oligosaccharide precursor remains essentially intact while contained within the ER vesicle (the glucose residues are presumably removed in the ER; see under "Discussion"). Further processing of the mannose-rich oligosaccharide required exposure to another cellular component. Thus, the absolute requirement for detergent in our assay probably reflects the compartmentation of the co-translational (addition of the oligosaccharide precursor) and post-translational (processing and terminal sugar addition) events in the ER and Golgi complex, respectively.

DISCUSSION

Co-translational Processing of Nascent Secretory Proteins—At least four activities are known to be associated with the early stages of secretion. These are: 1) binding of nascent chains to sites in the ER membrane; 2) movement of the growing polypeptide across the ER membrane; 3) proteolytic removal of the NH2-terminal signal or pre-peptide; and 4) core glycosylation of nascent chains of secretory glycoprotein.

In a previous report we showed that three of these activities were present in smooth microsomes of ascites tumor cells, rat liver, and adrenal cortex. Nascent pre-peptides of hPL and hCGa were cleaved at the appropriate peptide bond, sequenced, and in the case of hCGa, glycosylated by these fractions. The fourth activity, binding of nascent chains to the membrane, is implied by the presence of the other three. These results strongly suggested that SER and RER cannot be distinguished on the basis of these activities.

However, in the absence of further subcellular fractionation we could not state unequivocally that the pre-protein processing observed in smooth microsomes was limited to the ER. In this study we have subfractionated smooth microsomes from rat liver into a Golgi-rich fraction and a smooth ER-containing fraction. The Golgi membranes are characterized by a high specific activity for galactosyltransferase and a low activity of glucose-6-phosphatase, and are not active in pre-protein processing or sequestration. The SER-containing fraction, on the other hand, exhibits much higher levels of glucose-6-phosphatase activity, very little galactosyltransferase activity, and is very efficient in the proteolytic processing, core glycosylation, and sequestration of nascent chains of hPL and hCGa. These data provide strong evidence that the pre-protein processing previously observed in smooth microsomes can be attributed solely to the presence of SER.

The inability of membranes derived from the Golgi complex to translocate and sequester nascent pre-structures of plasmatic peptide hormones illustrates an important feature of the subcellular organization of eukaryotic cells and of the secretory process. The organellar components involved in the recognition and transport of nascent secretory proteins are restricted to the endoplasmic reticulum. This ensures that entry of these proteins into the secretory pathway can occur only in the ER and thus establishes the polarity necessary for the ordered sequence of co-translational and post-translational events involved in the production of secretory proteins.

Post-translational Processing: Golgi-dependent Processing of Asparagine-linked Oligosaccharides—A great deal of new information has accumulated over the past few years regarding the biosynthesis of asparagine-linked, complex type oligosaccharides. The major processing intermediates have been identified, and a scheme for the sequence of events in oligosaccharide processing has been proposed (Fig. 8).

A high molecular weight oligosaccharide with the composition Glc3Man9GlcNAc2 is assembled in the ER while attached to a dolichol lipid carrier (29, 40, 54). The oligosaccharide is a branched structure with the three glucose residues arranged in a linear sequence at the nonreducing end of one branch (30, 31).

Within minutes of transfer of this intermediate to the nascent chains, processing of the asparagine-linked oligosaccharide (see Fig. 8) begins with the removal of the glucose residues (22, 40, 49) by glucosidases located within the lumen of the ER (18, 45). This intermediate is then converted to a Man9GlcNAc2 form (Fig. 8, structure III), which involves the sequential removal of four mannose residues by an α,2-specific mannosidase localized in the Golgi complex (49). Further mannose removal is dependent upon the GlcNAc-transferase I catalyzed addition of a GlcNAc residue as shown in Fig. 8 (structure IV) (19, 47). Subsequent to this step, additional trimming occurs (49) until the intermediate GlcNAc2-Man9GlcNAc2Asn is converted to a complex oligosaccharide by the addition of galactose and sialic acid residues by specific glycosyltransferases located within the Golgi apparatus (43).

We (2) have previously demonstrated that, in membrane-supplemented ascites tumor cell-free extracts, first trimester placental mRNA directs the synthesis of a glycosylated form of hCGa. Based on the ability to specifically bind concanavalin A and the sensitivity of the glycoprotein to Endo-β-N-acetylglucosaminidases H and Cn, and to α-mannosidase, it was concluded that the protein contained the mannose-rich oligosaccharide precursor shown in Fig. 8 (structure III). The glycoprotein was not sensitive to glucosidase digestion. We also showed that the oligosaccharide could undergo post-translational processing by an α-mannosidase activity present in the ascites S-100 (3). Mannose removal required the presence of 0.04% Triton X-100, suggesting that the α-mannosidase activity is localized in some subcellular component other than the ER. The oligosaccharide processing was not associated with the addition of terminal sugars.3

The data presented here clearly show that the glycosylated forms of hCGa are resistant to Endo H after incubation in the presence of Golgi membranes and nucleotide sugars. Endo H resistance was not conferred by ER membranes under identical conditions. These results are significant in at least two respects. First, the synthesis of secretory glycoproteins can be reconstituted in vitro in a manner that appears to parallel in vivo synthesis. Secondly, the data provide confirmatory evidence regarding the subcellular origin of the fractions isolated and used to examine both the co-translational and post-translational events of glycoprotein biosynthesis.

While the exact composition and structure of the various oligosaccharide intermediates of the in vitro processing were not determined, it seems likely that the same intermediates that have been observed in vivo were generated during in vitro processing. This conclusion is based on the following lines of evidence.

1) In vitro oligosaccharide processing and terminal sugar addition was demonstrable only when membranes derived from the Golgi complex were present. Oligosaccharide processing in vivo also occurs in the Golgi complex (23, 35).

2) UDP-GlcNAc is specifically required for conversion of the oligosaccharide intermediate to an Endo H-resistant form.

3 M. Biehlinska and I. Boime, unpublished observations.
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Fig. 8. Proposed sequence of events for the processing of asparagine-linked, complex oligosaccharides. Structure I is transferred en bloc from a dolichol lipid carrier to appropriate asparagine residues on the nascent polypeptide chain. Oligosaccharide transfer and removal of glucose residues (conversion of structure I to structure II) occurs in the ER. The remaining steps are carried out by enzymes located in the Golgi apparatus. The symbols are: G, glucose; M, mannose; Gal, galactose; GlcNAc, N-acetylglucosamine; NA, sialic acid; Asn, asparagine residue in the polypeptide. (Adapted from Kornfeld et al. (25).)

Endo H resistance was enhanced when both UDP-GlcNAc and UDP-galactose were present.

Fries and Rothman (17) have recently described the in vitro processing and terminal sugar addition of the asparagine-linked oligosaccharides attached to the membrane glycoprotein (G protein) of vesicular stomatitis virus. They found that cell-free processing required energy in the form of ATP. This requirement was attributed to a need for energy in the transport of G protein to the Golgi complex, rather than in the actual processing reactions, since the enzymes involved in oligosaccharide processing are not energy dependent (49). Transport of glycoprotein in vivo has previously been shown to be energy dependent (38).

The in vitro processing described here does not require ATP. However, there was an absolute requirement for detergent. Thus, while it was not possible to demonstrate transport with our assay, the compartmentation of co-translational and post-translational processing events that occur in distinct membrane preparations is evident.

The experiments described here have used glycoproteins as substrates for oligosaccharide processing and terminal sugar addition. Other in vitro studies on oligosaccharide maturation have used oligosaccharide or glycopeptides. Thus, while the presence of a protein backbone can affect the maturation of an otherwise identical oligosaccharide precursor (41, 55), the extent of these effects is not known. The in vitro assay for oligosaccharide processing described here provides an unique system well suited to the study of these problems.

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