Role of the Nuclear Envelope in Synthesis, Processing, and Transport of Membrane Glycoproteins*

(Received for publication, August 13, 1984)

Lynn Puddington‡, Mark O. Lively, and Douglas S. Lyles

From the Department of Microbiology and Immunology and the Department of Biochemistry, Wake Forest University Medical Center, Winston-Salem, North Carolina 27103

The outer nuclear membrane is morphologically similar to rough endoplasmic reticulum. The presence of ribosomes bound to its cytoplasmic surface suggests that it could be a site of synthesis of membrane glycoproteins. We have examined the biogenesis of the vesicular stomatitis virus G protein in the nuclear envelope as a model for the biogenesis of membrane glycoproteins. G protein was present in nuclear membranes of infected Friend erythroleukemia cells immediately following synthesis and was transported out of nuclear membranes to cytoplasmic membranes with a time course similar to transport from rough endoplasmic reticulum (t½ = 5–7 min). Temperature-dependence in viral membrane proteins which block transport of G protein from endoplasmic reticulum also blocked transport of G protein from the nuclear envelope. Friend erythroleukemia cells and NIH 3T3 cells differed in the fraction of newly synthesized G protein found in nuclear membranes, apparently reflecting the relative amount of nuclear membrane compared to endoplasmic reticulum available for glycoprotein synthesis.

Nuclear membranes from erythroleukemia cells appeared to have the enzymatic activities necessary for cleavage of the signal sequence and core glycosylation of newly synthesized G protein. Signal peptidase activity was detected by the ability of detergent-solubilized membranes of isolated nuclei to correctly remove the signal sequence of human preplacental lactogen. RNA isolated from the nuclear envelope was highly enriched for G protein mRNA, suggesting that G protein was synthesized on the outer nuclear membrane rather than redistributing to nuclear membranes from endoplasmic reticulum before or during cell fractionation. These results suggest a mechanism for incorporation of membrane glycoproteins into the nuclear envelope and suggest that in some cell types the nuclear envelope is a major source of newly synthesized membrane glycoproteins.

Morphological observations characterizing the structure of the nuclear envelope have led investigators to postulate that the outer membrane could be a site of membrane-bound synthesis of glycoproteins (reviewed in Franke et al., 1981). The presence of ribosomes on the cytoplasmic surface of the outer nuclear membrane and its continuity with the endoplasmic reticulum (ER) (Watson, 1955) suggest structural similarity between the two organelles and have led to the hypothesis that the nuclear envelope is functionally a specialized form of ER. The enzymatic activities of nuclear membranes and ER are similar (Franke et al., 1981). However, there are some cases of functional differentiation between nuclear membranes and ER as well as some activities that appear to be equivalent in the two membrane systems. An example of the former is the cytochrome P-450-dependent electron transport system, which appears to differ between nuclear membranes and ER with respect to isozyme pattern and drug inducibility (Fahl et al., 1978; Matsuura et al., 1981). In contrast, the glucose-6-phosphatase activity of the two membranes appears to be due to equivalent enzymes (Arion et al., 1983). These results suggest that each activity must be examined independently to determine whether the two organelles provide similar functions.

Recent studies have shown that viral and end host glycoproteins normally synthesized on ER and transported to plasma membranes are also present in the nuclear envelope (Albert and Davies, 1973; Bergmann et al., 1981; Lyles and McConnell, 1981; Rose and Bergmann, 1982). However, neither a mechanism for incorporation into the nuclear envelope nor the biochemical characteristics of these proteins in the nuclear envelope have been defined. The unexpected result of our previous experiments was that significant proportions (30–60%) of murine leukemia virus glycoproteins are present in a nuclear fraction obtained from Friend erythroleukemia cells (Lyles and McConnell, 1981). An explanation for this distribution could be that some glycoproteins, such as leukemia virus envelope proteins, are preferentially localized in the nuclear envelope of erythroleukemia cells. Alternatively, a more generalized transient association with the nuclear envelope could be common to newly synthesized membrane glycoproteins in many cell types. These hypotheses were addressed in this study through characterization of the vesicular stomatitis virus (VSV) glycoprotein, G, in the nuclear envelope of infected cells.

Biosynthesis of the VSV G protein has been well-characterized and serves as a model for many viral and cellular integral membrane proteins destined to be incorporated into the plasma membrane. G protein is synthesized on polyribo-

* This work was supported in part by National Institutes of Health Grants AI15892 and GM 32861 and by pilot grants from the Oncology Research Center, Bowman Gray School of Medicine, and from the North Carolina United Way. 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.

‡ Predoctoral trainee supported by National Institutes of Health Training Grant T32 CA09422. To whom correspondence should be addressed: Molecular Biology and Virology Laboratory, The Salk Institute, P. O. Box 8800, San Diego, CA 92138.

1 The abbreviations used are: ER, endoplasmic reticulum; VSV, vesicular stomatitis virus; endo H, endo-β-N-acetylglicosaminidase H.
Membrane Glycoprotein Biogenesis in Nuclear Membranes

soomes bound to the ER (Morrison and Lodish, 1975; Knipe et al., 1977b) and is inserted into the rough ER during synthesis (Katz et al., 1977). During translation, two high mannosylated oligosaccharides are added to the nascent chain (Robbins et al., 1977; Tabas et al., 1978). G protein remains anchored in the membrane via a hydrophobic region at the carboxyl terminus of the protein (Rose et al., 1980), and a hydrophobic signal sequence at the amino terminus is cleaved by a peptidase present in ER membranes (Lingappa et al., 1978). Soon after synthesis in the rough ER, G protein is transported to the Golgi complex (Bergmann et al., 1981) where its oligosaccharides are modified (Tabas and Kornfeld, 1979; Bretz et al., 1980; Griffiths et al., 1982; Roth and Berger, 1982). It is then transported to the plasma membrane where it is incorporated into budding virions (Knipe et al., 1977b).

We have examined the possible role of the nuclear envelope in the early stages of glycoprotein maturation. The nuclear envelope appears to contain the necessary functions for the biogenesis of G protein, including G protein mRNA, signal peptidase, and ability to glycosylate G with mannose-rich oligosaccharides. We have determined the distribution and lifetime of G protein in the nuclear envelope of cells infected with wild-type and temperature-sensitive mutants of VSV. The results obtained are consistent with the hypothesis that the nuclear envelope and rough ER are functionally equivalent in glycoprotein synthesis and processing and that in some cell types the nuclear membrane is a major source of newly synthesized membrane glycoprotein.

EXPERIMENTAL PROCEDURES AND RESULTS2

Time Course of G Protein Transport from the Nuclear Envelope of Infected Erythroleukemia Cells—The subcellular distribution and rate of transport of the VSV G protein were determined in pulse-chase experiments followed by subcellular fractionation. VSV-infected Friend erythroleukemia cells were labeled with [35S]methionine for 5 min and then incubated in medium containing unlabeled methionine for 0, 5, or 15 min. Nuclei and total cytoplasmic membranes were isolated from cells at each time point. The protein composition of each fraction was determined by electrophoresis and fluorography. Fig. 2 shows that approximately half of the G protein was present in nuclear membranes initially and was rapidly chased into cytoplasmic membranes. In addition to G protein, the nonglycosylated viral membrane protein, M, was present in the nuclear fraction. However, the higher molecular weight form of G protein, visible after modification by Golgi enzymes, and nuclocapsid proteins (N, NS, and L) were virtually absent from the nuclear fraction, thus providing further evidence that cytoplasmic contamination was negligible. Quantitation of G protein by densitometry demonstrated that 40 ± 5% of newly synthesized (t = 0) G protein was present in the nuclear membrane. The same distribution of labeled G was obtained with a 1-min incubation (data not shown) as with a 5-min incubation of cells in [35S]methionine. As discussed below, less than 10–15% of G protein observed in the nuclear fraction could have been contributed by contaminating ER. The half-time of G protein in the nuclear envelope was calculated to be 5.1 ± 2.5 min. As the quantity of labeled G protein decreased in the nuclear fraction during the chase, it increased in cytoplasmic membranes, demonstrating quantitative transport from nuclear to cytoplasmic membranes. Therefore, selective degradation of the nuclear G protein could not account for the half-time observed.

FIG. 2. Distribution and time course of transport of G protein in the nuclear envelope of erythroleukemia cells. VSV-infected Friend erythroleukemia cells were labeled with [35S]methionine for 5 min at 4 h postinfection and then incubated in medium containing unlabeled methionine for the indicated times (minutes). Nuclei and cytoplasmic membranes were obtained from cells at each time point and analyzed on a 10% sodium dodecyl sulfate-polyacrylamide gel. A fluorograph of the dried gel is shown. Proteins were extracted from unfractionated whole cells (one-third of total at each time point) (WC), purified nuclei (N), and cytoplasmic membranes (C). The VSV nucleocapsid proteins N and NS co-migrate in this electrophoresis system.

DISCUSSION

The presence of specific glycoproteins in the nuclear envelope has been demonstrated by several laboratories. Albert and Davies (1973) showed by 51Cr cytotoxicity inhibition and complement fixation tests that H-2 antigens are present in nuclear membranes obtained from mouse liver or thymus. Viral glycoproteins have been visualized in the nuclear envelope by indirect immunofluorescence microscopy of fixed, permeabilized cells, in which labeling of the nuclear envelope is indicated by ring-like staining around the nucleus. Wild-type VSV G protein was detected in the nuclear envelope of...
cells microinjected with cDNA encoding G protein, but other perinuclear structures, apparently Golgi membranes, were also heavily labeled (Rose and Bergmann, 1982). Labeling of the nuclear envelope by antibody against viral glycoproteins was much clearer in cells expressing mutant glycoproteins of VSV or vaccinia virus, blocked in transport from rough ER to the cell surface (Bergmann et al., 1981; Rose and Bergmann, 1983; Shida and Matsumoto, 1983). A mutant G protein was also detected in the nuclear envelope by ferritin immunoelectron microscopy of cells infected with the ts045 mutant of VSV, which is similar to the ts513 mutant used in this study (Bergmann et al., 1981; Bergmann and Singer, 1983). In the present study, we demonstrated the presence of wild-type G protein in the nuclear envelope using independent techniques, including immunofluorescence microscopy of isolated nuclei without fixation or permeabilization (Fig. 1) and gel electrophoresis of proteins of isolated nuclei (Fig. 2).

The present study differs from previous morphological studies because it shows that the nuclear envelope is functionally involved in glycoprotein biogenesis. Labeled G protein in the nuclear envelope was shown to be a precursor to the mature glycoprotein, rather than a mutant glycoprotein defective in post-translational modifications and/or intracellular transport. The biochemical resemblance of VSV G protein in the nuclear envelope to that in rough ER suggests a similarity of the two organelles in glycoprotein biogenesis. Several lines of evidence support this hypothesis, including presence of G in the nuclear envelope immediately after synthesis, transport of G to membranes in the cytoplasm, glycosylation of G in the nuclear envelope, and presence of signal peptidase activity and G mRNA in the nuclear envelope.

G protein was present in nuclear membranes immediately after synthesis and was transported to cytoplasmic membranes with a time course similar to transport of G protein from ER. While it is difficult to rule out the possibility that synthesis of G protein occurred on cytoplasmic rough ER followed by immediate redistribution to the nuclear envelope, this seems unlikely since nuclei purified from infected erythroleukemia cells pulse-labeled with [3S]methionine for 1 min also contained approximately half the total labeled G protein. In addition, RNA isolated from nuclei is highly enriched in mRNA for G protein, suggesting that mRNA for this protein is actively translated on the nuclear membrane in infected cells. The G protein of a temperature-sensitive mutant of VSV that is not transported from ER at the nonpermissive temperature was also not transported from nuclear membranes, in agreement with morphological observations (Bergmann et al., 1981, 1983). Mutations in other viral envelope proteins, such as the hemagglutinin of vaccinia virus, result in accumulation of the glycoproteins in both organelles (Shida and Matsumoto, 1983).

Activities required for proteolysis of signal peptides and glycosylation of G protein were demonstrated in the nuclear envelope. Detergent-solubilized extracts of nuclei contained a proteolytic activity that correctly processed preplacental lactogen to its mature form in a post-translational assay. Signal peptidases studied to date have shown to act on a variety of substrates from many species. Preplacental lactogen has been shown to be a substrate for solubilized signal peptidases from hen oviduct (Lively and Walsh, 1983) and dog pancreas (Strauss et al., 1979) and purified leader peptidase (Wolfle et al., 1982) from Escherichia coli. Therefore, the protease activity observed in Friend erythroleukemia cell nuclei appears to be a true signal peptidase.

Since glycosylation of G is a cotranslational process (Rothman and Lodish, 1977), the synthesis of G on the nuclear envelope requires that the appropriate glycosyltransferase activity also be present. Like G protein in ER, oligosaccharides on G protein in the nuclear envelope are sensitive to digestion by endo H. In addition, the endo H-sensitive G protein in the nuclear envelope was a precursor to the endo H-resistant G protein in the cytoplasm, since quantitative transport and conversion were observed. Our experiments also showed that transport from the nuclear envelope preceded acquisition of endo H resistance because nuclear G protein was always susceptible to endo H digestion. The half-time of G protein in the nuclear envelope was shorter than the half-time of conversion to the endo H-resistant, higher molecular weight form in cytoplasmic membranes (see Figs. 2 and 3).

The distribution of newly synthesized G protein between nuclear and cytoplasmic membranes was dependent on the cell type examined. Forty percent of newly synthesized G was present in nuclei from erythroleukemia cells while only 15% was present in nuclei from NIH 3T3 cells. This difference probably reflects the relative amount of ER compared to nuclear membrane in these cells. Electron micrographs of thin sections of these cells supported this hypothesis. Friend erythroleukemia cells contained very little cytoplasmic rough ER and a large nucleus, while NIH 3T3 cells contained relatively more cytoplasmic rough ER. This difference in cell types probably accounts for the unexpectedly high percentage of murine leukemia virus glycoproteins in nuclear membranes of Friend erythroleukemia cells (Lyles and McConnell, 1981). Determinations made from pulse-chase experiments of the capacity of the nuclear envelope to synthesize glycoproteins are most likely underestimates in both cell types, because portions of the outer nuclear membrane are frequently removed during isolation of nuclei (Maggio et al., 1963; Kartenbeck et al., 1973; Wishart and Fry, 1980). Consequently, a fraction of labeled glycoprotein synthesized on the nuclear envelope is found in the cytoplasmic membrane fraction. Nonetheless, these observations suggest that the relative amount of rough ER is an important factor determining distribution of glycoprotein biosynthetic capacity within cells.

The fact that Friend erythroleukemia cells contain such a large proportion of newly synthesized G protein in the nuclear membrane has proven advantageous for ruling out the contributions of other organelles. In these experiments, the most troublesome contaminant of nuclei would be rough ER. This is the most difficult contaminant to rule out by analysis of marker enzymes, since the enzyme profiles of rough ER and nuclear membranes are so similar. NADPH-cytochrome c reductase is typically used as a marker for ER, but this enzyme is also present in the nuclear envelope (Franke, 1974). However, if NADPH-cytochrome c reductase activity is taken to be an upper estimate of contamination of nuclei by ER, a limit can be placed on the extent to which contaminating rough ER could account for viral glycoproteins in the nuclear fraction of pulse-chase experiments. Nuclei purified from Friend erythroleukemia cells contain 6–10% of the total cellular NADPH-cytochrome c reductase activity (Lyles and McConnell, 1981). Since approximately 60% of total newly synthesized (labeled) glycoprotein was in cytoplasmic membranes with a distribution similar to NADPH-cytochrome c reductase, 4–6% of total labeled glycoprotein could represent cytoplasmic contamination in the nuclear fraction. Thus, 10–15% of the glycoprotein in the nuclear fraction obtained from

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3 M. O. Lively, unpublished results.

4 L. Puddington and D. S. Lyles, unpublished results.
erythroleukemia cells in radiolabeling experiments would be due to ER contamination (4–6% divided by the 40% of total labeled glycoprotein in the nuclear fraction). As indicated above, this is probably an upper limit, since some of the NADPH-cytochrome c reductase in the nuclear fraction is due to the presence of the enzyme in the nuclear membrane. The absence of significant cytoplasmic contamination in the nuclear fraction was confirmed by electron microscopy. Microsomes derived from rough ER are readily identifiable with the electron microscope. Contamination of nuclei by other organelles is less than 5% (Lyles and McConnell, 1981), and most contaminants present appear to be debris from dead cells or lysed nuclei and not recognizable ER. Contamination of nuclei with smooth cytoplasmic membranes is also negligible, since the fully glycosylated form of G protein was never detected in the nuclear fraction (see Fig. 2). Furthermore, our basic conclusion is substantiated by a technique which incorporates more stringent morphological criteria in the analysis, namely flow cytometry. The cytofluorograph was gated to measure the fluorescence intensity of those particles with light-scattering properties similar to nuclei, therefore excluding fluorescent-labeled whole cells and cytoplasmic vesicles from the analysis. The half-time of G protein in the nuclear envelope determined by this technique was indistinguishable from the half-time determined in pulse-chase experiments.

The results described here suggest a general mechanism by which membrane glycoproteins are incorporated into the outer nuclear membrane. Our recent immunoelectron microscopic results have shown that viral glycoproteins, including G protein, incorporated biosynthetically into the outer nuclear membrane, are also present on the luminal surface of the inner nuclear membrane.5 Viral glycoproteins in the nuclear envelope may have roles other than as transient intermediates in glycoprotein synthesis, particularly if they are also incorporated into the inner nuclear membrane. An example might be the gp55 glycoprotein encoded by spleen focus-forming virus, the highly oncogenic component of the Friend murine leukemia virus complex. Little, if any, gp55 is expressed on the cell surface. Transport of gp55 from rough ER (Kabat et al., 1980; Ruscetti et al., 1981) and nuclear membranes (Lyles and McConnell, 1981) appears to be blocked. It is possible that onclusion by gp55 involves interactions specific for the nuclear envelope, such as transmembrane communication with nucleoplasmic elements. Likewise, it is possible that cell surface glycoproteins are normally incorporated into the nuclear envelope by mechanisms similar to those described here for viral glycoproteins. Additional functions for these glycoproteins, such as hormone receptors, could also be expressed in the nuclear envelope.

Acknowledgments—We are grateful to Dr. David A. Bass and Pamela Szeda for helpful advice during flow cytometry experiments.

REFERENCES
5 L. Puddington, D. S. Lyles, and J. C. Lewis, manuscript in preparation.

5644 Membrane Glycoprotein Biogenesis in Nuclear Membranes

Membrane Glycoprotein Biogenesis in Nuclear Membranes

Supplemental Materials

To Role of the Nuclear Envelope in Synthesis, Processing and Transport of Membrane Glycoproteins

by

Lynne Puddington, Mark D. Lively, and Douglas S. Ylles

EXPERIMENTAL PROCEDURES

Cell lines and viruses - Clone 704-1C Friend erythroblastosis cells or clone 704-1M Friend (FIV) infected cells were obtained from Dr. E. Dinter. Friend virus-infected clone 704 cells were obtained from Dr. H. F. Schlesinger, National Cancer Institute. Cell lines were maintained in MEM containing 10% fetal bovine serum. Cells of the virus were infected with 10% fetal calf serum.

Plaque assays of SBV, D50 and New Jersey strains, were prepared by infection of baby hamster kidney (BHK) cells with cloned virus at a multiplicity of 0.001. For 18 h, the culture supernatants were used as virus stocks for experiments. In mutants, cells were propagated in the presence of 10 μg/ml G418 (Geneticin), as described in the text. Pulsed experiments were performed at 33°C for 30 min to label viral antigen until harvest.

RESULTS

Nuclear localization of the VSV G protein - The purified nuclear fraction of infected cells consisted of Friend erythroblastosis cell nuclei which are not specifically free of cytoplasmic contamination, as previously characterized by electron microscopy and differential centration (see Figure 1 and Figure 2 in Lyles and McComb, 1981). Isolated nuclei have outer and inner nuclear membranes with ribosomes present on the cytoplasmic surface of the outer nuclear membrane. There are gaps in the outer nuclear membrane which presumably are derived from blebs in discontinuities between the nuclear envelope (Weinberg et al., 1979). Treatment of nuclei with the nuclear envelope in isolation from the inner nuclear membrane is an important step in the purification of nuclei. The isolated nuclei were analyzed by exposure on the surfaces of cells are also present in the inner nuclear envelope of the outer nuclear membrane. There are gaps in the outer nuclear membrane which presumably are derived from blebs in discontinuities between the nuclear envelope (Weinberg et al., 1979). Treatment of nuclei with the nuclear envelope in isolation from the inner nuclear membrane is an important step in the purification of nuclei. The isolated nuclei were analyzed by exposure on the surfaces of cells are also present in the inner nuclear envelope.

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Nuclear localization and transport of G protein in NIH 3T3 cells -- in Friend erythroblasts cells, a large fraction (approximately half of newly synthesized protein) of the total cellular G protein is localized in the nuclear envelope of infected cells. In NIH 3T3 cells, the subcellular distribution and rate of transport of G protein were determined by pulse-labelling and analysis of purified nuclei by flow cytometry. Following exposure of NIH 3T3 cells to cycloheximide, the mean fluorescence of nuclei calculated from data similar to that in Figure 2 is shown (average of 2 experiments).

Figure 3. Analysis by flow cytometry of G protein in the nuclear envelope following inhibition of protein synthesis with cycloheximide. VSV-Indiana-infected Friend erythroblasts cells were treated with cycloheximide for 0 (A), 5 (B), 15 (C), or 30 (D) min. Immunofluorescent labeling and analysis of purified nuclei by flow cytometry were performed as described in the Experimental Procedures. Each histogram was gated on light scatter properties to exclude free G protein. The results are expressed as arbitrary units. Background fluorescence was determined by analysis of nuclei obtained from VSV-Neo adenovirus-infected cells (E). The population of nuclei was homogeneous as indicated by light scattering properties (forward versus side scatter). The flow cytometry analysis was gated on this population. (F) Mean fluorescence of nuclei calculated from data similar to that in A-D is shown (average of 2 experiments).

Figure 4. Transport of G protein from the nuclear envelope of cells infected with VSV. Friend erythroblasts cells infected with the indicated multiples of VSV were pulse-labeled at the non-permissive temperature with [3H]cycloheximide for 5 min, then chased in medium containing unlabeled methionine for the indicated time (min). Purified nuclei were isolated and analyzed electromicrographically on a Hitachi 720 transmission electron microscope. Background fluorescence was determined by analysis of nuclei obtained from VSV-Neo adenovirus-infected cells. The population of nuclei was homogeneous as indicated by light scattering properties (forward versus side scatter). Mean fluorescence of nuclei calculated from data similar to that in A-D is shown (average of 2 experiments).

Figure 5. Electron micrograph of nuclei isolated from NIH 3T3 cells. G protein present in nuclei isolated from VSV-infected Friend erythroblasts cells was treated with endo H to determine if any of the G protein had acquired resistance to cleavage as a result of processing. VSV-infected cells were labeled with [3H]cycloheximide for 30 min at 4 hr postinfection, then nuclei and total cytoplasmic membranes were isolated as before. Each subcellular fraction was treated with endo H as described in Experimental Procedures, then labelled proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose filters, and subjected to immunoblotting with 2G antibodies. Endo H treatment of G protein in the nuclear fraction resulted in a reduction of its apparent molecular weight indicative that these molecules contained oligosaccharides sensitive to endo H (compare lane 3 with lane 2). This was in contrast to G protein present in the cytoplasmic membrane fraction in which only newly synthesized G protein was susceptible to endo H cleavage. After labeling with [3H]cycloheximide for 20 min, G protein in cytoplasmic membranes is resolved into two bands with slightly different electrophoretic mobilities (Figure 5, lane 3). The slower migrating band is attributable to the complex form found on mature G protein [Eisenman et al., 1977]. Since they were not cleaved by endo H (Figure 5, lane 4), the faster migrating G
Membrane Glycoprotein Biogenesis in Nuclear Membranes

Figure 7. Treatment of nuclear and cytoplasmic membrane fractions with endo H. VSV-infected Friend erythroleukemia cells were labeled with [35S]methionine for 30 min at 4°C postinfection. Nuclear and cytoplasmic membranes were isolated immediately before analysis by SDS-polyacrylamide gel electrophoresis. A fluorograph of the dried gel is shown. Proteins extracted from purified nuclei (N) and cytoplasmic membranes (C) were incubated in the presence (+) or absence (-) of endo H. The arrow indicates the electrophoretic mobility of G protein after removal of oligosaccharides by endo H.

Protein molecules were degraded by endo H to molecules of lower apparent molecular weight (Figure 7, lane 4) as would be expected for early synthetic forms of G protein in cytoplasmic membranes.

Signal peptide release by erythroleukemia cell nuclei. G protein is initially synthesized with an extra-ribosomal signal peptide sequence that is removed by a signal peptidase during nuclear export of the precursor form. VSV-infected Friend erythroleukemia cells were labeled with [35S]methionine for 30 min at 4°C postinfection. Nuclear and cytoplasmic membranes were isolated immediately before analysis by SDS-polyacrylamide gel electrophoresis. A fluorograph of the dried gel is shown. Proteins extracted from purified nuclei (N) and cytoplasmic membranes (C) were incubated in the presence (+) or absence (-) of endo H. The arrow indicates the electrophoretic mobility of G protein after removal of oligosaccharides by endo H.

Figure 8. Signal peptide activity in solubilized erythroleukemia cell membrane fractions. Nuclei and total cytoplasmic membrane fractions from infected erythroleukemia cells were isolated and solubilized with 2 M guanidine hydrochloride and assayed for the ability to cleave [35S]methionine-labeled preproHPL as described in Experimental Procedures. The reaction products were analyzed by SDS-polyacrylamide gel electrophoresis and detected by autoradiography. The autoradiograph shows the substrate cleaved in the absence of membrane extract (-), in the presence of nuclear extract (+) of cytoplasmic extract (C), and of solubilized non-ribosomal signal peptide (+).

Figure 9. Presence of G protein mRNA in the nuclear envelope of erythroleukemia cells. VSV-infected Friend erythroleukemia cells were labeled with [35S]rribonucleotide from 2 to 5 h postinfection. Nuclear and cytoplasmic membranes were isolated with detergent. RNA was isolated from each fraction and was analyzed by electrophoresis in 1.4% agarose gel after denaturation with glyoxal. A fluorograph of the dried gel is shown. VSV mRNA species were identified by their sizes relative to standard markers. G mRNA from nuclei (15,000 cpm); C-RNA from cytoplasm (78,000 cpm).