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Intracellular appearance of a glycoprotein in VSV-infected BHK cells lacking the membrane-anchoring oligopeptide of the viral G-protein

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Infection of BHK 21 cells by vesicular stomatitis virus (VSV) results in the intracellular synthesis of the five viral proteins which are easily detectable in polyacrylamide gels after short labeling periods with [^{35}S]methionine. In addition, a 6th prominent radioactive protein band appears intracellularly in VSV-infected BHK cells. This additional polypeptide is also coded by the viral genome, because it is immunoprecipitated by antibodies against viral particles and more specifically by antibodies against purified G-protein. We propose to call this derivative of the G-protein G_{si}-protein (short intracellular G-protein). It is associated with intracellular membranes and has an apparent mol. wt. of 58 000. Both G- and G_{si}-protein have the same kinetics of appearance in the cell. The ratio of G-:G_{si}-protein in BHK 21 cells is ~85:15. The mol. wt. difference of ~6000 daltons between G- and G_{si}-protein is not due to variations in the degree of glycosylation because trypsin digestions of both [^{3}H]mannose-labeled glycoproteins gave rise to identical glycopeptide patterns. Incubation of microsomes with trypsin demonstrates that G_{si}-protein is protected in its full length by intracellular membranes. G_{si}-protein is lacking an extended carboxy-terminal region of the viral G-protein sequence because it is not modified by palmitic acid and is not immunprecipitated by specific antibodies against a C-terminal peptide of the G-protein. Limited proteolysis by endoproteinase arg C indicates that the structure of G_{si}-protein is very similar to the shedded form of the G-protein which has been previously described in the literature. The kinetic analysis demonstrates that intracellular synthesized G_{si}-protein is a precursor of the extracellular G_s-protein. Virus integrated G-protein and soluble G_{si}-protein appear extracellular with the same kinetics.

Key words: kinetics of G_{si}-protein formation/membrane association/trypsin protection/tryptic glycopeptides/pulse-chase analysis of glycoproteins

Introduction

Vesicular stomatitis virus (VSV) is a RNA-containing virus which consists of a helical ribonucleocapsid core and a lipoprotein membrane (Nakai and Howatson, 1968). The virion envelope contains host cell-derived lipids and glycolipids into which the viral glycoprotein (G-protein) is inserted as an integral membrane protein (Klenk and Choppin, 1971; McSharry and Wagner, 1971). Just internal to the envelope is the matrix or membrane (M) protein. The viral core is composed of the 42S single-stranded genomic RNA of negative polarity which is covered by the nucleoprotein (N). The virus-dependent polymerase is believed to consist of a complex of the large (L) protein and the non-structural (NS) protein which are associated with the ribonucleoprotein complex (Wagner, 1975). Studies using VSV-infected eucaryotic tissue cultures have shown that the nascent viral glycoprotein is cotranslationally inserted into the membrane of the rough endoplasmic reticulum (Rothman and Lodish, 1977; Kruppa, 1979) and then transported to the plasma membrane (Hunt *et al.*, 1978; Lodish and Rothman, 1979).

VSV-infected cells release not only viral particles into the culture medium but also virus-specific polypeptides as soluble antigens. These 'shedded' polypeptides comprise ~3% of total VSV proteins in an infected culture (Brown and Cartwright, 1966; Kang and Prevec, 1970; Little and Huang, 1977). A glycoprotein termed G_s-protein (s for shedding) is one of the major soluble antigens of VSV found in the extracellular medium. Several authors have reported that G_s-protein may arise from the viral G-protein by proteolytic cleavage at the cell surface and then be shedded into the culture medium (Little and Huang, 1978; Chatis and Morrison, 1982; Irving and Gosh, 1982). The shedding of soluble viral glycoproteins in murine leukemia virus-infected cells (Strand and August, 1976) and in mammary tumor-bearing mice (Ritzi *et al.* 1976) or tumor cell lines (Racevskis and Sarkar, 1978) has also been described. The mechanism of production and the biological function of soluble glycoproteins are not well understood. Little and Huang (1978) surmised that the G_s-protein might play a role during viral pathogenesis.

We were able to demonstrate that after VSV infection of BHK cells, a short form of the viral glycoprotein is formed intracellularly, which lacks a region of the amino acid sequence at the carboxy terminus. *In vitro* elongation experiments of membrane-bound polysomes show that this G_{si}-form (si for short intracellular) of the viral glycoprotein is detectable in the rough endoplasmic reticulum immediately after translation. The intracellular and extracellular fate of the G_{si}-protein is followed in a pulse/chase experiment. A preliminary communication of the work has been presented (Kruppa, 1981).

Results

An additional viral protein is intracellularly formed in VSV-infected BHK cells

The five viral proteins (Figure 1, lane 1) are detectable in BHK 21 cells 3 h post VSV infection (Figure 1, lane 4). Their rate of biosynthesis increases during the following 3 h (Figure 1, lanes 5–7). In the microsomal fraction of VSV-infected cells an additional [^{35}S]methionine-labeled protein band becomes detectable concomitantly with the G-protein (Figure 1, lane 4). This polypeptide migrates slightly faster in polyacrylamide gels than does the viral glycoprotein. The intensity of this additional protein band increases with time like the other virus-coded proteins (Figure 1, lanes 4–7). This short form of the viral glycoprotein was also detectable in poly-

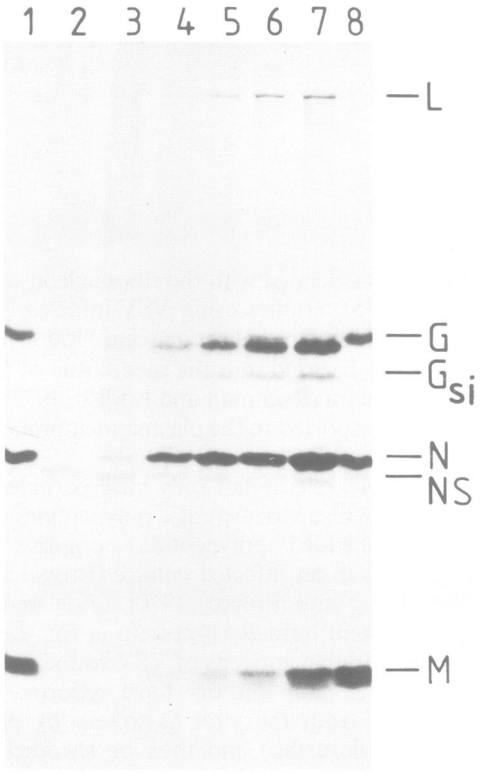

Fig. 1. Kinetics of the production of G_{si}-protein in VSV-infected BHK 21 cells. Cells were labeled with [^{35}S]methionine every hour after infection (**lanes 2–7**), membrane fractions were prepared and equal aliquots were analysed on a 10% polyacrylamide gel as described. [^{35}S]Methionine-labeled proteins of purified VSV particles were used as markers (**lanes 1** and **8**). The fluorogram was exposed for 2 days.

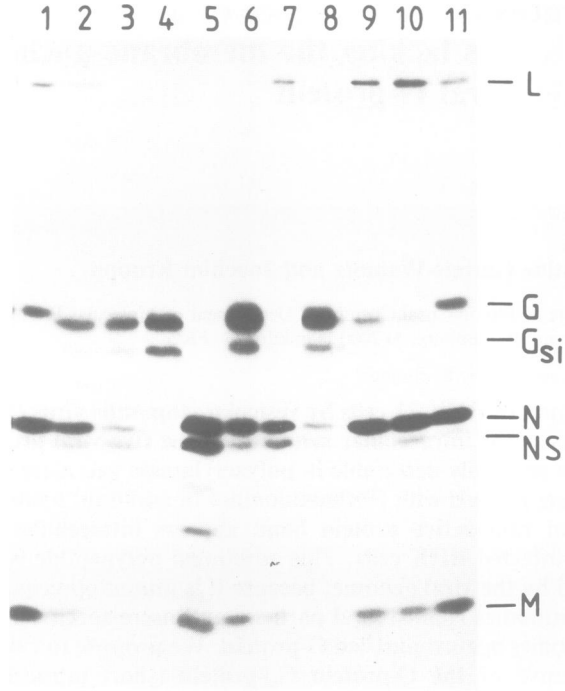

Fig. 2. Intracellular appearance of a shorter form (G_{si}-protein) of the viral glycoprotein in VSV-infected BHK 21 cells. VSV-infected BHK cells were labeled with [^{35}S]methionine for 20 min, cell fractions were prepared and analysed on a 10% polycrylamide gel as described. **Lanes 1** and **11**: purified VSV particles as markers; **lanes 2** and **3**: total cell extract before and after centrifugation at 40 000 r.p.m. in Beckman Ti50 rotor for 2 h, respectively; **lane 4**: total cell extract immunoprecipitated by antibodies against purified G-protein; **lane 5**: total cell extract immunoprecipitated by antibodies against viral core proteins; **lane 6**: total cell extract immunoprecipitated by antibodies against viral particles; **lane 7**: cytoplasmic extract after sedimentation of cellular membranes; **lane 8**: dissolved membrane fraction after removal of virus remnants (**lane 9**) by centrifugation; **lane 10**: viral particles, recovered from total cell extracts by centrifugation. The fluorogram was exposed for 2 days.

acrylamide gels when total cells were directly resuspended in hot sample buffer or precipitated by trichloroacetic acid (Kruppa and Garreis-Wabnitz, 1982). The appearance of this polypeptide raises the question of its biosynthesis: does a specific host protein become induced or is an additional viral protein translated in BHK cells after VSV infection? Antisera against viral particles, core particles and purified G-protein were raised in rabbits in order to answer this question. When detergent-treated cell extracts were incubated with these three antisera the protein was immunoprecipitated with the antibody against virus particles (Figure 2, lane 6) and more specifically with the antibody against purified G-protein (Figure 2, lane 4). Antibodies against core particles did not immunoprecipitate the additional protein band (Figure 2, lane 5). These experiments clearly indicate that the 6th protein band is of viral origin and has similar antigenic determinants to the authentic viral G-protein; we therefore propose to call this polypeptide G_{si}-protein (short intracellular glycoprotein).

Figure 2 also shows that total cytoplasmic cell extracts prepared by detergent treatment of VSV-infected BHK cells contain virions which can be removed by sedimentation in the ultracentrifuge (Figure 2, lane 10). G_{si} is completely removed from cell homogenates by centrifugation (Figure 2, lane 7) and it is quantitatively recovered in the resulting membrane fraction (Figure 2, lane 8) from which it can be released in a soluble form by detergent treatment. These results indicate that G_{si}-protein is associated with membranes. Virus particles which contaminate total cell extracts and membrane fractions can be recovered by ultracentrifugation after dissolving the membranes (Figure 2, lanes 9 and 10). Note that the pelleted virions do not contain G_{si}-protein.

G_{si}-protein is extensively glycosylated

The apparent mol. wt. of G_{si}-protein is 58 000 (Figure 6) thus it is ~6000–7000 daltons smaller than G-protein which becomes integrated into the budding virus particles. This difference in mol. wt. corresponds to the weight of both oligosaccharide chains (Reading *et al.*, 1978) of the G-protein which are linked to two asparagine residues (Robertson *et al.*, 1976). Several forms of the G-protein differing in their extent of glycosylation had been detected previously (Morrison and Lodish, 1975; Katz *et al.*, 1977; Knipe *et al.*, 1977). They have been classified according to their electrophoretic mobility in SDS-polyacrylamide gels in G_0 (unglycosylated form, Morrison and Lodish, 1975), G_1 (underglycosylated form, Katz *et al.*, 1977), and G_2 (fully glycosylated form, Knipe *et al.*, 1977). The unglycosylated G_0 form was observed after *in vitro* translation of viral mRNAs in a wheat germ system in the absence of microsomal membranes (Katz *et al.*, 1977). This form has a lower apparent mol. wt. than the virion G_2-protein and the G_1-protein (Morrison and Lodish, 1975).

To test whether the G_{si}-protein is another underglycosylated form of the G-protein, we determined its extent of glycosylation by labeling VSV-infected BHK cells with [^{3}H]monosaccharides (Table I). The experiments demonstra-

Table I. Intracellular ratios of G-:G_{si}-protein

Precursor	Incubation time	Incubation	G	G_{si}	Treatment	Sample origin
[^{35}S]Met	20 min	*In vivo*	80	20	–	Figure 6, lane 2
[^{35}S]Met	90 min	*In vitro*	85	15	Ø Trypsin	Figure 6, lane 3
[^{35}S]Met	90 min	*In vitro*	85	15	⊕ Trypsin	Figure 6, lane 4
[^{35}S]Met	20 min	*In vivo*	81	19	Antibody VSV	Figure 2, lane 6
[^{35}S]Met	20 min	*In vivo*	86	14		Figure 2, lane 8
[^{35}S]Met	20 min	*In vivo*	70	30	Antibody G	Figure 2, lane 4
[^{35}S]Cys	20 min	*In vivo*	84	16	–	Not shown
[^{3}H]Man	90 min	*In vivo*	83	17	–	Not shown
[^{3}H]GlcNH_2	90 min	*In vivo*	90	10	–	Not shown
[^{3}H]Gal	90 min	*In vivo*	90	10	–	Not shown

Extracts of VSV-infected BHK cells were metabolically labeled as indicated. Cell fractions were prepared, treated as described and analysed on 10% polyacrylamide gels. The fluorograms were quantitatively evaluated using an Ortec densitometer (Kruppa, 1983).

ted that G_{si}-protein, as well as G-protein, incorporated glucosamine, mannose and galactose. Moreover, tryptic digestions of purified [^{3}H]mannose-labeled G- and G_{si}-protein revealed identical glycopeptide pattern after elution of the DEAE-Sephadex A25 columns (Figure 3) indicating that both are also terminally modified by sialic acid (Robertson *et al.*, 1976). These experiments exclude the possibility that the lower mol. wt. of G_{si}-protein is due to a defect in glycosylation.

G_{si}-protein is completely protected by intracellular membranes

Since G_{si}-protein contained both carbohydrate chains of the normal G-protein it was reasonable to assume that the reduced mol. wt. was due to the absence of a portion of the amino acid sequence of the G-protein. Low mol. wt. forms of the glycoprotein could theoretically be formed *in vivo* by several mechanisms: either by a proteolytic fragmentation of the finished glycoprotein at the amino and/or carboxy terminus or during translation by premature termination. A third possibility would be the existence of a short mRNA with deletion of a portion of the coding sequence of G-protein. The following experiments were performed in order to elucidate the mechanism of G_{si}-protein formation.

Since G_{si}-protein is membrane associated (Figure 2) a trypsin protection experiment was performed to elucidate the topological disposition of the small glycoprotein. Membrane-integrated G-protein is trimmed by exogenous trypsin (Figure 4B and C, and Katz *et al.*, 1977). In contrast, trypsin does not cleave G_{si}-protein in the membrane fraction, whereas it becomes rapidly degraded by trypsin as soon as the integrity of the lipid bilayer is disrupted by detergent treatment (Figure 4D). This shows that G_{si}-protein is completely protected by the lipid bilayer of intracellular membranes and indicates that the molecule has no protruding carboxy-terminal region at the cytoplasmic side of intracellular vesicles. The ratio of G:G_{si} does not change after dissolving microsomes by detergent in the absence of trypsin (Figure 4E and Table I).

G_{si}-protein is lacking part of the carboxy-terminal sequence of the G-protein

Further evidence for the absence of an extended region at the COOH terminus in G_{si}-protein was obtained by two additional experiments. Extracts of VSV-infected BHK cells which had been labeled with [^{3}H]palmitic acid showed only

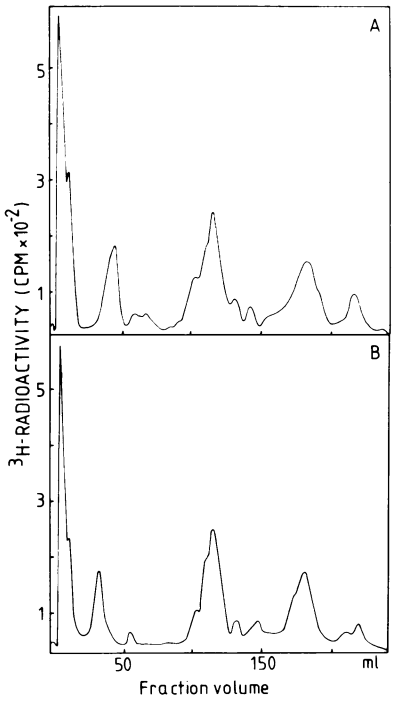

Fig. 3. Tryptic glycopeptide patterns of [^{3}H]mannose-labeled glycoproteins. VSV-infected BHK cells were labeled with [^{3}H]mannose for 20 min and G- and G_{si}-protein were electroeluted after separation by polyacrylamide gel electrophoresis. The purified proteins were cleaved by trypsin and the glycopeptides were separated on DEAE-Sephadex A25 columns (1 x 30 cm) as previously described (Robertson *et al.*, 1976). G-protein **(A)** and G_{si} protein **(B)**.

one radioactive band after polyacrylamide gel electrophoresis (Figure 5A). This band migrated in the position of G-protein as was demonstrated by co-electrophoresis with ^{35}S-labeled VSV proteins. Palmitic acid is transferred onto serine residues which are close to the luminal surface of the endoplasmic reticulum (Schmidt and Schlesinger, 1979). Since G_{si}-protein does not become acylated one has to assume that not only the highly charged COOH-terminal domain but also the hydrophobic transmembrane domain of the G-protein is lacking.

Immunoprecipitations of membrane extracts containing G- and G_{si}-protein (Figure 5B, lane 3) showed that antibodies

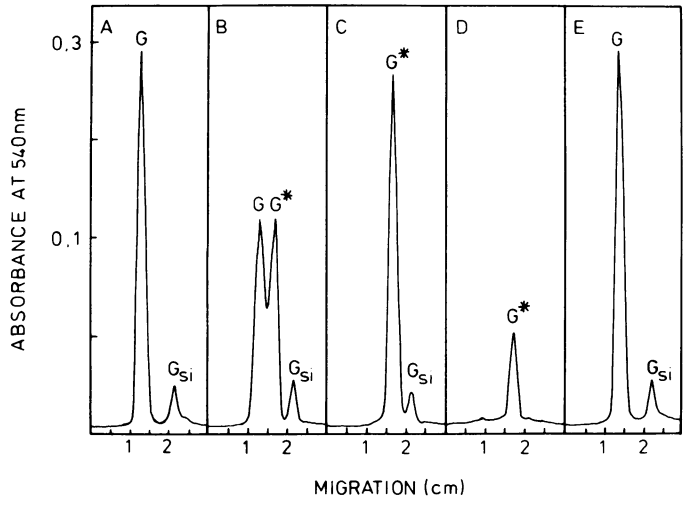

Fig. 4. Complete protection of membrane associated G_{si}-protein against exogenous trypsin degradation. [^{35}S]Methionine-labeled vesicles from VSV-infected BHK cells were incubated with trypsin for 10 min **(B)** and 30 min **(C)** as described. DOC (1% final concentration) was added to an aliquot in order to dissolve the membranes and incubation was continued for 10 min **(D)**. Intact control membrane vesicles **(A)** and detergent-treated membrane extracts **(E)** (1% DOC, final concentation) were both incubated for 40 min without trypsin addition. G_{si}: intracellular glycoprotein, G*: membrane-protected part of the viral glycoprotein.

raised against a synthetic peptide of the C terminus precipitated only G-protein (Figure 5B, lane 2) whereas monoclonal antibodies against the N-terminal region of the G-protein precipitated G-protein as well as G_{si}-protein (Figure 5B, lane 1). Taken together the experiments described up to this point indicate that ~60 amino acids of the COOH-terminal sequence of the G-protein are lacking in the G_{si}-protein.

G_{si}-protein is formed in the endoplasmic reticulum

To localize the intracellular site of G_{si}-formation, an elongation experiment was carried out. Polysomes of VSV-infected BHK cells bound to microsomal membranes were elongated in a reticulocyte lysate system. Both glycoprotein species were formed (Figure 6, lane 3). The *in vitro* produced glycoprotein form migrated in polyacrylamide gels at the same position as the *in vivo* synthesized protein species (Figure 6, lane 2, 3, and 6). The elongated glycoproteins are co-translationally translocated into the lumen of the endoplasmic reticulum suggesting that the amino-terminal leader sequence of G_{si} must be functional. The trypsin protection experiment demonstrates that the G_{si}-protein formed *in vitro* is also protected in its entire length against exogenous trypsin degradation (Figure 6, lanes 4 and 5). Identical results were obtained with a reconstitution experiment using an extracted VSV mRNA preparation, microsomes of dog pancreas, and a reticulocyte lysate system (not shown).

The shedded and the intracellular form of the glycoproteins are structurally related

The apparent mol. wt. of G_{si}-protein is similar to the previously described shedded form of the glycoprotein (Figure 6, lanes 6 and 7) from VSV which was call G_s-protein (Little and Huang, 1978; Chatis and Morrison, 1982; Irving and Gosh, 1982). Possible structural relationships were corraborated further by limited proteolytic digestions of [^{35}S]methionine-labeled G-, G_{si}-, and G_s-proteins which had

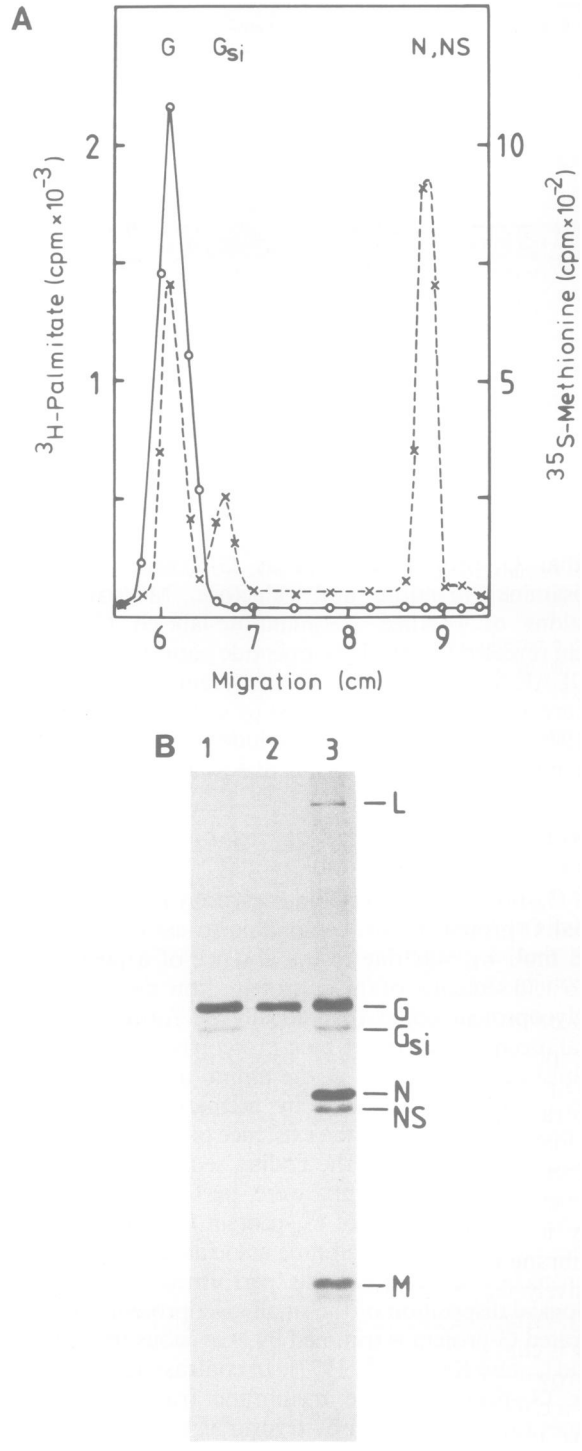

Fig. 5. A carboxy-terminal oligopeptide of the G-protein is missing in G_{si}-protein. **Panel A**: VSV-infected cells were separately labeled with [^{3}H]palmitic acid and [^{35}S]methionine. Membrane extracts were prepared and co-electrophoresed on a 10% polyacrylamide gel. Radioactivity in 1 mm gel slices was determined as described previously (Kruppa, 1983). **Panel B**: [^{35}S]methionine-labeled membrane extracts **(lane 3)** were immunoprecipitated with antibodies against the C-terminal **(lane 2)** and N-terminal **(lane 1)** regions of the viral G-protein. The fluorogram was exposed for 3 days.

been purified by gel electrophoresis (Figure 7, lanes 4–6) and incubated with endoproteinase arg C. All three glycoproteins show a similar oligopeptide pattern (Figure 7, lanes 1–3) after separation on 20% polyacrylamide gels. Only G-protein

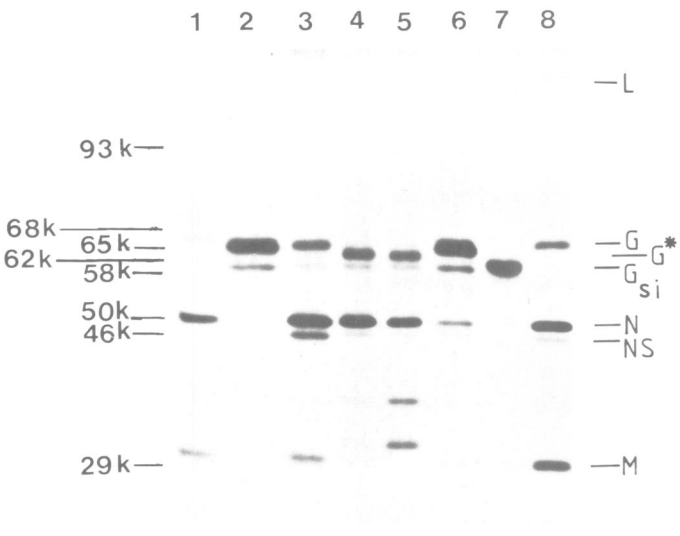

Fig. 6. *In vitro* elongation of membrane-bound polysomes. Nascent polypeptide chains of membrane-bound polysomes from VSV-infected BHK cells were elongated *in vitro* by incubating a crude membrane fraction in a reticulocyte lysate with [^{35}S]methionine. The vesicles were recovered by centrifugation and a part was treated with trypsin and subsequently analysed on a 10% polyacrylamide gel. VSV proteins were used as markers (**lanes 1** and **8**). **Lane 2**: membrane fraction of VSV-infected BHK cells after removal of VSV particles by centrifugation (90 min label). Membrane fraction after elongation before trypsin treatment (**lane 3**) and after trypsin treatment (**lanes 4** and **5**). Membrane fraction of VSV-infected BHK cells after centrifugation (**lane 6**; 20 min label). G_s-protein shedded into the growth medium (**lane 7**). Fluorogram after 24 h of exposure.

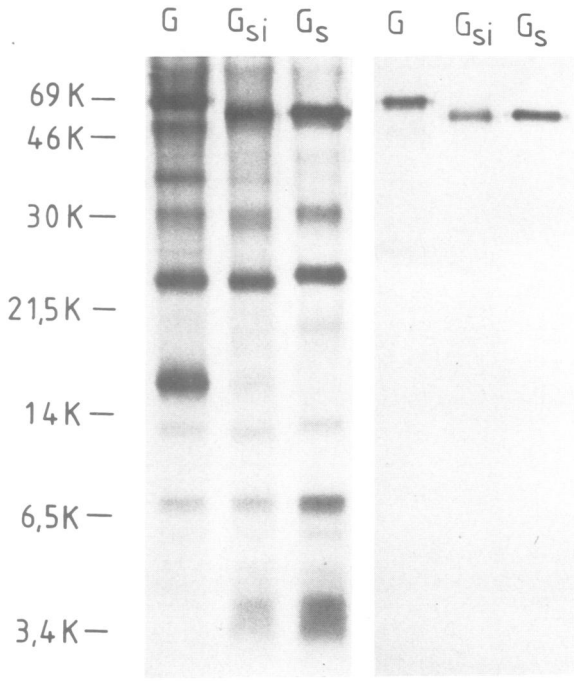

Fig. 7. Structural comparison of G-, G_s-, and G_{si}-protein by limited proteolysis. [^{35}S]Methionine-labeled G-, G_{si}- and G_s-protein were purified by polyacrylamide gel electrophoresis from a membrane fraction and from the growth medium, respectively, incubated with endoproteinase arg C and the oligopeptides were analysed on 20% polyacrylamide gels as described. A ^{14}C-labeled protein mixture was used as mol. wt. markers. The fluorogram was exposed for 14 days.

has a prominent fragment in the mol. wt. range of 14 000. Both short forms have instead a broad radioactive band at 4000 dalton which is missing in the limited digest of G-protein.

G_{si}-protein is secreted into the growth medium

An explanation for the close structural relationship between G-, G_{si}-, and G_s-protein became evident when the time course of intracellular viral protein synthesis and extracellular appearance of viral proteins were analysed in a pulse/chase experiment (Figure 8). G- and G_{si}-protein incorporate [^{35}S]methionine very rapidly giving rise to radioactive bands easily identifiable on polyacrylamide gels (Figure 8A). In membrane extracts the intensity of both glycoproteins progressively decreases with time (compare in Figure 8A, lanes 1–7). After a chase time of 1 h, ~20% of the total radioactivity is chased out of the cells and appears in the medium. In the growth medium G- and G_{si}-protein become faintly visible after a chase time of 20 min (Figure 8B, lane 3). Both protein bands increase in intensity during the following period (Figure 8B, lanes 4–7). Since the amount of G_{si}-protein is decreasing intracellularly with time, because it is transported and released into the growth medium, one has to conclude that there exists a precursor product relationship between the intracellular G_{si}-protein and the extracellular G_s-protein. The intracellular G_{si}-protein does not associate with viral particles (Figure 8C) but instead is released in a soluble form into the growth medium (Figure 8D). Both glycoproteins are transported through the intracellular compartments with the same speed, since they appear extracellularly at the same time. Since the intracellular G_{si}-protein is lacking the transmembrane domain it seems to be treated by the cell like a secretory protein.

Discussion

We have shown here that G_{si}-protein is a truncated form of the viral G-protein and can therefore be distinguished from the intracellular G_0- and G_1-forms which are unglycosylated and underglycosylated forms of the G-protein, respectively (Knipe *et al.*, 1977; Katz *et al.*, 1977). Moreover, G_{si}-protein is formed intracellularly immediately after translation on membrane-bound polysomes and translocated co-translationally into the lumen of the endoplasmic reticulum (Figure 6). Since G_{si}-protein has both carbohydrate chains the reduction in mol. wt. suggests that it lacks part of the protein sequence of the G-protein.

The carboxy-terminal region of the G-protein contains a hydrophobic sequence (Rose and Gallione, 1981) which is essential for membrane integration. The total membrane-spanning segment and the C-terminal segment of the G-protein protruding from the vesicles at the cytoplasmic side add up to 49 amino acid residues (Rose and Gallione, 1981). G_{si}-protein seems to be lacking at least this portion of its amino acid sequence. G_{si}-protein was not acylated by palmitate (Figure 5A), a modification which is assumed to occur at the luminal surface of the microsomes (Schmidt and Schlesinger, 1979). Taken together, these results suggest that G_{si}-protein is missing the membrane anchoring sequence. Since it has no contact to the lipid bilayer of the endoplasmic reticulum it cannot become inserted into the viral envelope (Figure 8).

The reduction in mol. wt. by 6000–7000 dalton is smaller than previously published for the shedded form of the glycoprotein, which was 10 000–12 000 dalton less (Kang and Prevec, 1970; Little and Huang, 1978; Capone *et al.*,

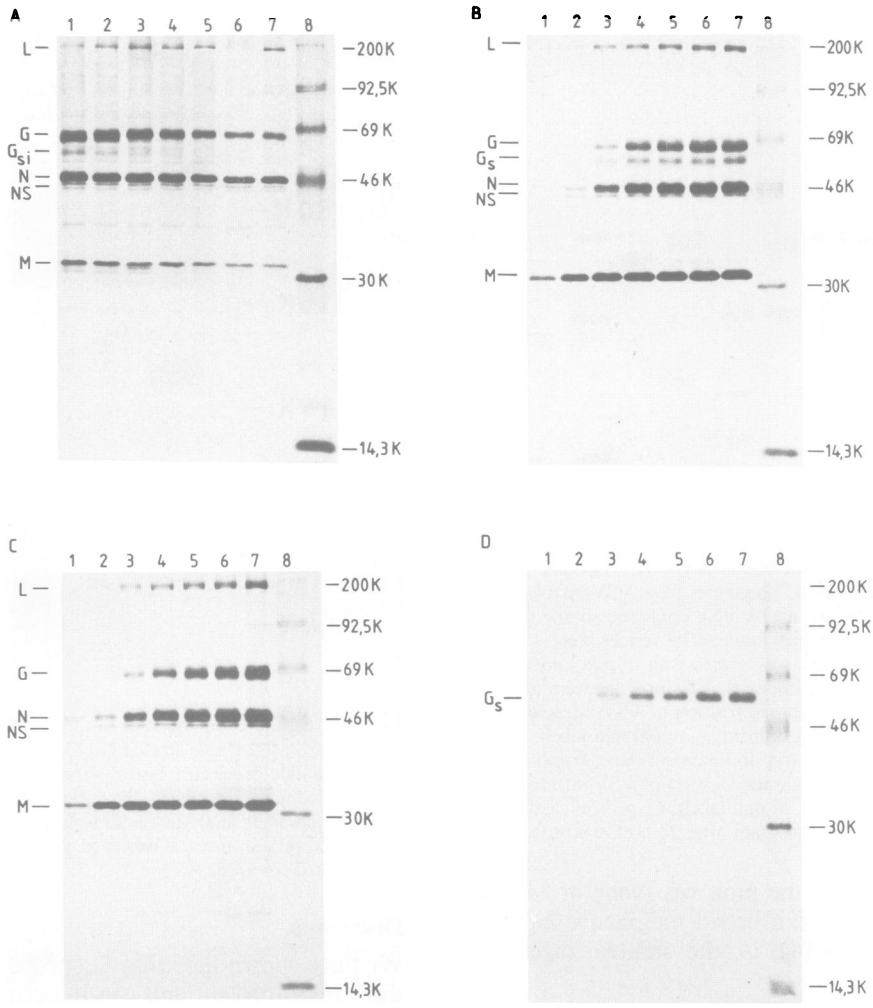

Fig. 8. Pulse chase analysis of VSV proteins. BHK cells were labeled 3.5 h after VSV infection with [^{35}S]methionine for 10 min and then chased with an excess of non-radioactive methionine for up to 60 min. Samples were taken at the beginning of the chase (**lane 1**) and then every 10 min. Commercially available ^{14}C-labeled proteins were used as mol. wt. markers (**lane 8**). The time course of protein labeling was followed in membrane extracts (**A**) and in the growth medium (**B**). Viral particles were recovered from the growth medium by centrifugation (**C**) and the particle-free medium was acid precipitated and analysed (**D**). Identical aliquots (15% of each sample) were loaded in **panels B–D** whereas, due to technical reasons, only 2% of each sample was loaded in **panel A**. The gels were fluorographed for 4 days.

1982). A glycoprotein form lacking 79 C-terminal amino acids was produced by transfection of L cells with a cloned cDNA of the G-protein from which a 3′-terminal nucleotide sequence was deleted (Rose and Bergmann, 1982). The group of Rose has obtained in addition two stable transformed mouse cell lines that express either the normal G-protein or a truncated form of the G-protein (Florkiewicz *et al.*, 1983). The artificially produced 'anchorless' G-proteins were in all cases very slowly secreted into the medium (Rose and Bergmann, 1982, 1983; Florkiewicz *et al.*, 1983) whereas the naturally-occuring G_{si}-protein showed the same secretion kinetics as the viral integrated G-protein (Figure 8).

Intracellular G_{si}-protein as well as the extracellular G_s-protein (Little and Huang, 1978) contain the same carbohydrate chains as the virus integrated glycoprotein (Figure 3). Although the intracellular G_{si}-protein and the extracellular G_s-protein have a similar mol. wt. and show a similar oligopeptide pattern upon limited digestions of [^{35}S]methionine-labeled material it has still to be demonstrated that both forms have an identical amino acid sequence at their C terminus. It is very unlikely that G_{si}-protein is a proteolytic degradation product of viral G-protein because prolonged incubation of an intracellular membrane fraction in the presence of detergent did not change the G-:G_{si}-protein ratio (Figure 4 and Table I). The intracellular ratio of G-:G_{si}-protein seems to be nearly constant irrespective of the metabolite used for labeling (Table I). Approximately a similar ratio is also found in all other tissue culture cells examined so far in which VSV is expressed (Kruppa, 1981). To understand the mechanism of G_{si}-protein formation two possibilities remain to be investigated: either G_{si}-protein is synthesized by premature chain termination during translation, or it is translated from a class of mRNA molecules which are lacking the 3′ end of the oligonucleotide sequence coding for G-protein.

Materials and methods

Chemicals

Triton X-100 and Nonidet P-40 (NP-40) were from Sigma (Munich, FRG); SDS, acrylamide, N,N′-methylenebisacrylamide, Coomassie brilliant blue and bovine serum albumin were obtained from Serva (Heidelberg, FRG); all other reagents were of analytical grade and purchased from Merck (Darmstadt, FRG).

Buffers

Buffer A: Earle's balanced salt solution (Earle, 1943); buffer B: phosphate-buffered saline (PBS), pH 7.4 (Dulbecco and Vogt, 1954); buffer C: 15 mM KCl, 1.5 mM $MgCl_2$, 2 mM dithiothreitol (DTT), and 10 mM Tris-HCl, pH 7.4; buffer D: 1.2 M KCl, 50 mM $MgAc_2$, 20 mM DTT, and 200 mM Hepes, pH 7.5; buffer E: 25 mM KCl, 5 mM $MgAc_2$, 50 mM Tris-HCl, pH 7.4; buffer F: 100 mM NaCl, 2 mM EDTA, and 10 mM Tris-HCl, pH 7.4; electrophoresis sample buffer: 80 mM Tris-HCl, pH 6.8, 2% SDS (w/v), 10% glycerol (v/v), and 0.001% bromophenol blue (w/v).

Growth and purification of VSV

BHK 21 cells were grown as monolayers in Joklik modified minimum essential medium (Flow Laboratories, Bonn, FRG) supplemented with 5% (v/v) calf serum (Seromed, Berlin, FRG). VSV (Indiana serotype) was grown on BHK 21 cells which were infected with a multiplicity of 0.1 p.f.u./cell. Virus was harvested from the cell supernatant ~18 h after infection, purified and assayed as described previously (Kruppa, 1979). Gradient fractions containing virions were layered over a 50% (v/v) glycerol cushion and sedimented at 40 000 r.p.m. for 1 h in a Beckman Ti 50 rotor. Purified viral particles were resuspended in 20 mM Tris-HCl, pH 7.4 and stored at −20°C after addition of 1 volume of glycerol.

Metabolic labeling of VSV-infected BHK cells

Monolayers of BHK 21 cells (2 x 10^7 cells/flask) were infected with VSV (30 p.f.u./cell) in 2 ml serum-free medium for 30 min at room temperature, 15 ml of medium containing 5% (v/v) calf serum was added and incubation was continued for 3.5 h if not stated otherwise. Monolayers were then washed once with pre-warmed methionine-free medium. Cells were labeled for 10 min in 2 ml methionine-free medium supplemented with 100 μCi [^{35}S]methionine (1330 Ci/mmol, Amersham-Buchler, Braunschweig, FRG) followed by a chase with non-radioactive methionine (0.05 mM) for 10 min unless stated otherwise.

For labeling with [^{3}H]monosaccharides, BHK 21 cells were washed with pre-warmed buffer A lacking glucose and serum. Cells were labeled 3.5 h post-infection with 15 μCi/ml [^{3}H]monosaccharides ([^{3}H]mannose, sp. act. 13.2 Ci/mmol; [^{3}H]glucosamine, sp. act. 39.6 Ci/mmol; and [^{3}H]galactose, sp. act. 15.4 Ci/mmol) for 45 min at 37°C in the same buffer supplemented with 5% dialysed fetal calf serum (Boehringer, Mannheim, FRG). Monolayers were washed with serum-free medium and labeled 3.5 h post-infection with 500 μCi [^{3}H]palmitic acid (sp. act. 15.2 Ci/mmol) for 2 h.

Preparation of cell fractions

Labeling of monolayers was terminated by removal of the medium and addition of ice-cold buffer B. Cells were scraped off with a rubber policeman and washed twice with ice-cold buffer B. The cells were homogenized in a Dounce homogenizer after swelling in hypotonic buffer C for 10 min. Nuclei were removed by centrifugation in a minifuge (Heraeus, Osterode, FRG) at 2000 r.p.m. and 4°C for 5 min and the membranes were subsequently sedimented at 20 000 r.p.m. and 4°C for 15 min in the Beckman JA 21 rotor. Membrane fragments adhering to the nuclei were extracted with a 1% Triton X-100 solution in buffer C. Cellular membrane fractions were contaminated by virus cores and/or virus particles which could be removed by sedimentation at 40 000 r.p.m. and 4°C in the Beckman Ti 50 rotor for 90 min after dissolving the membrane with detergent (1% Triton X-100) in buffer C. A total cytoplasmic extract was prepared by resuspending cells in buffer C, adding 1% Triton X-100 (final concentration) and removing cell nuclei by centrifugation as described above.

In vitro elongation of membrane-bound polysomes

A membrane fraction of VSV-infected BHK 21 cells was prepared by Dounce homogenization, 0.1 vol of buffer D was added and the nuclei were removed by centrifugation. Membranes were pelleted from the post-nuclear supernatant in an Eppendorf centrifuge for 15 min at 4°C and subsequently resuspended in 150 μl of nuclease-treated reticulocyte lysate (Pelham and Jackson, 1976) containing [^{35}S]methionine and incubated at 30°C for 90 min. Membranes were re-sedimented in the Eppendorf centrifuge as described above and resuspended in 100 μl buffer E. Protein concentration was determined (Hartree, 1972) and the membrane vesicles were incubated for 30 min at 37°C with trypsin using a membrane protein: trypsin ratio of 5:1 and 1:1 (w/w). Protease treatment was terminated by addition of 1 vol of 20% TCA. Precipitated proteins were pelleted, washed once with 5% TCA and twice with acetone. Protein pellets were finally dissolved by boiling for 5 min in 50 μl of electrophoresis sample buffer. [^{35}S]Methionine incorporation was determined by acid precipitation in an aliquot (Mans and Novelli, 1961).

Immunoprecipitation of viral proteins

Antibodies against purified G-protein, viral cores and viral particles were raised in rabbits. Rabbit antibodies raised against a synthetic oligopeptide corresponding to the last 15 amino acids of the C-terminal sequence of the G-protein as well as a monoclonal antibody recognizing the N-terminal region of the G-protein were gifts of Dr. T. Kreis (EMBL, Heidelberg). Immunoprecipitation of cell extracts were carried out using the *Staphylococcus aureus* procedure described by Kessler (1975). In order to reduce non-specific cellular contamination in the immunoprecipitates, the washed staphylococci were resuspended as a 10% suspension in unlabeled cell extract and in addition cell extracts were treated with serum of non-immunized rabbits before immunoprecipitation. After the 20 min incubation with staphylococcal adsorbent, the suspensions were centrifuged at 10 000 r.p.m. for 1 min. The adsorbent antibody pellets were then washed twice with 0.5% NP-40 buffer F and once with 2.5 M KCl in 0.5% NP-40 containing buffer F. The antigen-immunoglobulin complexes were dissociated from the adsorbent by boiling for 5 min in 100 μl electrophoresis sample buffer.

Proteolytic cleavage of glycoproteins

Radioactively labeled G-, G_s- and G_{si}-protein were localized in gels by autoradiography, excised from the gel and electroeluted (Green *et al.*, 1982) using Pasteur pipets as elution chambers. Proteins were recovered by acetone precipitation and digested with trypsin (TPCK treated, Worthington, Freehold, NJ) or endoproteinase arg C (Boehringer, Mannheim, FRG) an enzyme from mouse submaxillaris glands which specifically cleaves after arginine residues (Levy *et al.*, 1970). [^{3}H]Mannose labeled glycoproteins were dissolved in 50 mM triethylammonium bicarbonate, pH 8.6 and digested by trypsin at 37°C for 6 h. Glycopeptides were separated (Robertson *et al.*, 1976) on DEAE-Sephadex A25 columns (1 x 30 cm) (Pharmacia, Uppsala, Sweden). Incubation with endoproteinase arg C was carried out in 1% $NaHCO_3$ and 0.2 mg/ml glycine at 37°C for 3 h using a protein to protease ratio of 2:1. These digests were analysed on 20% polyacrylamide gels as described below.

SDS-polyacrylamide gel electrophoresis

The protein composition of cell extracts was analysed on 10% polyacrylamide gels using the discontinuous buffer system of Laemmli (1970). Electrophoretic separation was carried out under reducing conditions at 15 mA (constant current) for 11 h. Limited proteolytic digests were analysed on 20% SDS-polyacrylamide gels for which the Tris-HCl buffer concentration and the concentration of the electrode buffer were doubled (Thomas and Kornberg, 1975). All other constituents and concentrations used were the same as in the 10% SDS-polyacrylamide gels. Low mol. wt. proteins (Pharmacia, Uppsala, Sweden) or ^{14}C-methylated protein markers (Amersham-Buchler, Braunschweig, FRG) were used as standards. Gels were stained with 0.1% (w/v) Coomassie brilliant blue dissolved in 40% (v/v) methanol, 10% (v/v) acetic acid and 50% (v/v) water. After destaining in the same solvent mixture the gels were impregnanted with 2,5-diphenyloxazole, vacuum dried and fluorographed at −70°C using Kodak X-Omat AR X-ray film (Bonner and Laskey, 1974). Autoradiograms and fluorograms were quantitatively evaluated by an Ortec densitometer (Kruppa, 1983).

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