Restricted Expression of Viral Glycoprotein in Vesicular Stomatitis Virus-infected Drosophila melanogaster Cells

By FRANÇOISE WYERS, DANIELLE BLONDEL, ANNE-MARIE PETITJEAN AND SYBILLE DEZELEE

Laboratoire de Génétique des Virus, CNRS, 91198 Gif sur Yvette Cédex, France

(Accepted 7 October 1988)

SUMMARY

Vesicular stomatitis virus (VSV) establishes a non-cytopathic persistent infection in Drosophila melanogaster cells. The synthesis of the viral glycoprotein G was specifically inhibited during a post-transcriptional step, whereas the synthesis and turnover of its mRNA were not modified compared with the other viral mRNAs. Another viral glycoprotein, migrating slightly faster than G protein on an SDS-polyacrylamide gel, was detected in infected Drosophila cells. This protein showed most of the characteristics of the intracellular Gs protein found in infected vertebrate cells. The amounts of G protein integrated into mature virions and of soluble Gs protein secreted into the culture medium were reduced greatly during VSV infection in Drosophila cells.

The external glycoprotein G of vesicular stomatitis virus (VSV) is inserted into the endoplasmic reticulum during its synthesis on membrane-bound polysomes, and is transported as an integral membrane protein to the cell surface where virus budding is induced. A truncated glycoprotein, Gs, lacking the membrane-anchoring peptide of G protein, is found in VSV-infected vertebrate cells in addition to the five viral proteins (N, NS and L associated with the negative-stranded genomic RNA, and the membrane proteins M and G). This Gs protein is formed during or shortly after the translation process and is secreted into the growth medium (Garreis-Wabnitz & Kruppa, 1984; Graeve et al., 1986).

The fate of the G protein is particularly altered in Drosophila melanogaster cells where VSV establishes a non-cytopathic persistent infection (Wyers et al., 1980). Its rate of synthesis remains very low throughout the viral cycle, whereas that of the other viral proteins increases transiently during the first 8 h of infection. The released virions contain very little G protein and are found to be underglycosylated and, particularly, unsialylated (Laurent & Lafay, 1983). In the present study, we investigated the step in the synthesis of the G protein that is affected in Drosophila cells, and also whether G is preferentially converted into a soluble glycoprotein that is secreted into the culture medium.

It was first necessary to determine whether the restriction of G protein synthesis was due to a reduced rate of transcription of its mRNA. The accumulation of G protein mRNA and of nucleocapsid N protein mRNA, used as a reference, was compared throughout the multiplication of VSV Indiana in D. melanogaster cells (line 770M3) and in CER hamster cells. Hybridization experiments on dot blots were performed as previously described (Blondel et al., 1988). At various times after infection, total cellular nucleic acids were extracted and dot blots were prepared with serial dilutions of each sample. Positive polarity viral sequences, corresponding to the 3' end of the G mRNA (847 bases long) and the 5' end of the N mRNA (599 bases long), were subcloned into an M13 vector from VSV cDNAs supplied by Dr J. K. Rose (Rose & Gallione, 1981). The 32P-labelled ssDNA probes synthesized as copies of these two clones specifically detected the G or N mRNAs by hybridization on dot blots. After autoradiography (Fig. 1a and b) dots were individually counted for radioactivity. The results obtained after quantification were expressed as amount of probe hybridized per μg of total

0000-8055 © 1989 SGM
Fig. 1. Accumulation of VSV mRNAs of G and N proteins throughout infection in *Drosophila* and CER cells measured by dot blot hybridization. Hybridization experiments and quantification were performed as previously described (Blondel *et al.*, 1988). Cells were infected under one-step growth conditions. Cellular nucleic acids were extracted at the indicated times and serial dilutions of each sample were dotted onto the membrane. Dot blots were hybridized with $5 \times 10^6$ c.p.m./ml of $^{32}$P-labelled ssDNA probes (sp. act. $> 1 \times 10^8$ c.p.m./ug) synthesized from VSV sequences cloned in an M13 vector. (a, b) Examples of dot autoradiographs: detection of G mRNA in *Drosophila* (a) and CER (b) cells by hybridization with $^{32}$P-labelled ssDNA probe complementary to the 3' end of the messenger. (c) Accumulation of mRNA of N protein in *Drosophila* (●) and CER (○) cells obtained after dot blot hybridization with $^{32}$P-labelled ssDNA probe complementary to the 5' end of the messenger. The results are expressed as c.p.m. of probe hybridized with 1 ug cellular nucleic acids. (d) Accumulation of mRNA of G protein in *Drosophila* (▲) and CER (△) cells obtained after dot blot hybridization with $^{32}$P-labelled ssDNA probe complementary to the 3' end of the messenger and expressed as in (c).

cellular nucleic acids, i.e. DNA and mainly cellular RNAs. This permitted a better comparison of VSV growth in the cytoplasm of both cell types, whose size and morphology are different. *Drosophila* cells are smaller with a reduced cytoplasmic volume, and contain 66% less total nucleic acids than vertebrate cells. The variations in N and G mRNA levels throughout the viral cycle are shown in Fig. 1 (c and d). During the lytic VSV infection in CER cells each mRNA accumulated rapidly, and the decrease observed after 7 h of infection corresponded to cell decay. In *Drosophila* cells, N and G mRNAs followed the same accumulation pattern as all transcription products (Blondel *et al.*, 1988). The amounts increased during the first few hours
then decreased to a steady state maintained throughout the persistent infection. For both mRNAs, the maximal amounts were detected 5 h after infection. They represented 20 to 25% of those found in CER cells per μg of nucleic acids. At this time each Drosophila cell contained 12-fold fewer VSV transcripts than a CER cell. Since it has previously been shown that only VSV full-length mRNAs accumulate in Drosophila cells (Blondel et al., 1988), these results indicate that the transcription rate and turnover of N and G mRNAs are not markedly different. G protein synthesis is thus affected during a post-transcriptional step in which messenger maturation and also the translation process on membrane-bound polysomes could be involved.

The rate of synthesis of VSV proteins was compared 5 h after infection, when the amounts of viral mRNA were maximal in infected cells (Fig. 1). Intracellular proteins were pulse-labelled and analysed by SDS–PAGE. Viral proteins were virtually the only labelled proteins in vertebrate cells (Fig. 2a; CER hamster cells, lane 2; chicken embryo cells, lane 3). In Drosophila cells (Fig. 2a, lanes 4 and 5) N, NS and M proteins were clearly visible though cellular protein synthesis was not inhibited after VSV infection. The G protein was also detected in spite of its low rate of synthesis. However, a large difference was found in the translational efficiency of the
Fig. 3. G protein incorporated into virions and Gs protein released from VSV-infected cells. Cells were labelled 2 h after infection with [14C]leucine (6 pCi/ml) for 4 h. Growth medium was harvested; virions were pelleted by centrifugation for 2 h at 250000 g in an SW41 Spinco rotor, then soluble proteins contained in the supernatant were precipitated with TCA. Equal amounts of acid-precipitable radioactivity were analysed by 10% SDS-PAGE on a slab gel. Viruses released from chicken embryo cells (lane 1), and Drosophila cells untreated (lane 2) or actinomycin D-treated (lane 3). Soluble proteins in growth medium. Drosophila cells, uninfected (lane 4), infected (lane 5), or actinomycin-treated and infected (lane 6); chicken embryo cells, uninfected (lane 7) and infected (lane 8). Comparison of G and Gs protein migration in each cell type. Drosophila cells; soluble proteins in growth medium of uninfected (lane 9) and infected (lane 10) cells and released virions (lane 11). Infected chicken embryo cells; soluble proteins in growth medium (lane 12) and released virions (lane 13).

viral mRNA in the two cell types. The labelled proteins analysed on the gel were extracted from 9 × 10⁶ Drosophila cells and 6.4 × 10⁴ CER cells. These quantities can be expressed as amounts of nucleic acid for comparison with the results shown in Fig. 1. The Drosophila cell samples contained 45-fold more nucleic acid than the CER cell samples; they contained about 10-fold more VSV mRNAs owing to the difference found in amounts of viral messenger in the two hosts 5 h after infection. The translation rate of all the viral mRNAs was thus extremely reduced in Drosophila cells. In vertebrate cells the rapid inhibition of cellular protein synthesis after VSV infection is attributed to competition for translation machinery by large amounts of viral mRNA (Lodish & Porter, 1980). No inhibition of cellular protein synthesis occurred in infected Drosophila cells in spite of residual VSV mRNA synthesis (Fig. 1). The competition for ribosomes between virus and insect host mRNAs seemed to lead to a preferential reduction in VSV protein synthesis. This inhibition appeared to be more drastic for G protein on membrane-bound polysomes. It could be suppressed, at least in part, when cellular synthesis was partially inhibited by pretreatment with actinomycin D at low concentration (0.1 μg/ml) added 30 min before infection (Fig. 2b, lanes 1, 2 and 3). Under these conditions Drosophila cells accumulated twice as much viral mRNA (data not shown) and the protein synthesis rate was enhanced four- to five-fold for N, NS and M, and up to 14-fold for the G protein (Wyers et al., 1980).

Another viral protein migrating slightly faster than G could be detected in infected Drosophila cells (Fig. 2a, lane 4). It was synthesized in low amounts and was more clearly visible after actinomycin treatment (Fig. 2b, lane 3). Several experimental results indicated that this protein
Short communication

Table 1. Comparison of rates of synthesis of G and Gs proteins

<table>
<thead>
<tr>
<th>Host cells</th>
<th>(G + Gs)/N*</th>
<th>Gs/(G + Gs)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drosophila</td>
<td>0.21</td>
<td>0.58</td>
</tr>
<tr>
<td>Drosophila + actinomycin</td>
<td>0.98</td>
<td>0.54</td>
</tr>
<tr>
<td>Chicken embryo</td>
<td>1</td>
<td>0.43</td>
</tr>
</tbody>
</table>

* Rations obtained after scanning of gel autoradiographs as shown in Fig. 2 and calculation of molar ratio of each polypeptide; mean values of four experiments.

Table 2. Comparison of amounts of G protein incorporated into virion membrane and of soluble Gs protein secreted by cells

<table>
<thead>
<tr>
<th>Host cells</th>
<th>Gv/N*</th>
<th>Gs/(Gv + Gs)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drosophila</td>
<td>0.14</td>
<td>0.38</td>
</tr>
<tr>
<td>Drosophila + actinomycin</td>
<td>0.17</td>
<td>0.32</td>
</tr>
<tr>
<td>Chicken embryo</td>
<td>0.48</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* Gel autoradiographs (as in Fig. 3) were scanned and molar ratios of each polypeptide were determined to compare the amounts of proteins incorporated into virions (Gv, N) or secreted (Gs) by the same cell sample; mean values of three experiments.

corresponded to the intracellular Gs protein found in vertebrate cells (Garreis-Wabnitz & Kruppa, 1984; Graeve et al., 1986), and it also has been designated Gs in Drosophila cells. Like G protein, it was preferentially immunoprecipitated from 14C-labelled intracellular proteins by an antiserum prepared against native VSV particles (Fig. 2c, lanes 1 and 3). N, NS and M were also precipitated to a lesser extent, perhaps because the virus used to prepare this antiserum was partially denatured. The Gs protein was shown to be glycosylated after labelling with sugars. It was not associated with membranes following cell fractionation and was found to appear simultaneously with G protein during pulse–chase experiments (data not shown).

The relative rates of synthesis of each polypeptide were quantified after scanning of autoradiographs as shown in Fig. 2, taking into account their differing Mr values, i.e. the Mr of N was 45000, and that of G was 62000 in Drosophila cells and 64000 in vertebrate cells because of the underglycosylation in insect cells. As shown in Table 1, the total translation rate of viral glycoproteins (G + Gs protein synthesis) in Drosophila cells, where host protein synthesis occurred normally, represented about 20% of N protein synthesis. However both rates of synthesis were equivalent in the presence of actinomycin D, as in vertebrate cells. Gs was generated in slightly higher amounts in Drosophila cells, even in the presence of the inhibitor. These ratios were nearly constant irrespective of time after infection (data not shown) and would seem to be dependent on the host cell, contrary to the results obtained in different vertebrate cells (Graeve et al., 1986).

In order to measure the incorporation of the glycoprotein into virions (Gv protein) and the amounts of soluble Gs protein released into culture medium, cells were labelled from 2 to 6 h after infection with 14Cleucine. Mature virions were collected by high speed centrifugation, and soluble proteins were acid-precipitated from the culture medium. Proteins were analysed by SDS–PAGE. Gs protein was released from Drosophila cells either untreated or treated with actinomycin D (Fig. 3, lanes 5 and 6). Scanning of autoradiographs showed a shift of Mr, value between G and Gs of 4000 in Drosophila cells (Fig. 3, lanes 10 and 11) as in CER cells (Fig. 3, lanes 12 and 13). Calculation of glycoprotein ratios revealed quantitative differences (Table 2). As already described (Wyers et al., 1980), little G protein was incorporated into virions produced by Drosophila cells, approx. 25% of the amount found in vertebrate cells. The ratios between virion-incorporated Gv and extracellular Gs confirmed that in CER cells the pool of intracellular Gs protein was depleted faster than that of G protein (Strous & Lodish, 1980; Graeve et al., 1986). The latter must certainly have been inserted into the membrane before virus budding. Nevertheless, in Drosophila cells Gs secretion was still inhibited to a greater extent.
Short communication

than G incorporation into virions. Identical results were obtained in the presence of actinomycin D, even though the rates of synthesis of G and Gs were no longer restricted (Table 1). The inhibition of the extracellular transport of viral glycoproteins does not seem to be a consequence of VSV multiplication control but rather a property of insect cells (Gillies & Stollar, 1980). This inhibition could be due to the modifications of glycosylation which are known to be involved in G protein migration to the cell surface (Kotwal et al., 1986).

The establishment of persistent infection in VSV-infected Drosophila cells is clearly characterized by modifications in viral membrane proteins. As shown in the present study, the translation rate of the external membrane glycoprotein, as well as its insertion in the virion membrane or its secretion as soluble antigens, were reduced. As previously reported, the phosphorylation of the inner membrane protein M is quantitatively and qualitatively changed in insect cells (Blondel et al., 1983). Modifications specifically affecting the viral envelope proteins are also a characteristic common to RNA viruses in persistently infected vertebrate cells, e.g. Sendai virus in cell culture (Roux et al., 1984), in vivo with lymphocytic choriomeningitis virus (Oldstone & Buchmeier, 1982), and with measles virus during subacute sclerosing panencephalitis (Baczko et al., 1986). Persistent infections require adaptation of the virus for the mutual survival of the parasite and its host. Selective modifications of membrane proteins are implicated in the alteration of viral cytopathogenesis and in host cell survival. On the other hand we have shown that the continual presence of large amounts of VSV nucleocapsids in persistently infected Drosophila cells ensures the maintenance of viral information during cell division without requiring high rates of viral synthesis or production of large numbers of infectious particles (Blondel et al., 1988).

This study was supported by grants from CNRS LP 02431 and MRES 83 V 0815.

REFERENCES


(Received 18 September 1987)