Vesicular Stomatitis Virus in Drosophila melanogaster Cells: Regulation of Viral Transcription and Replication

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Vesicular stomatitis virus RNA synthesis was investigated during the establishment of persistent infection in Drosophila melanogaster cells. The transcription rate declined as early as 5 h after infection and was strongly inhibited after 7 h, leading to a decrease in viral mRNA levels and in viral protein synthesis rates. Full-length plus-strand antigenomes and minus-strand genomes were detected after a 3-h lag time and accumulated until 15 h after infection. Short encapsidated plus-strand molecules were also generated corresponding to the 5' end of viral defective antigenomes. Assembly and release of virions were not restricted, but their infectivity was extremely reduced. In persistently infected cells, an equilibrium was reached where the level of intracellular genomes maintained was constant and maximal even after the rate of all viral syntheses had decreased. These results are discussed with regard to the establishment of persistent infection.

Vesicular stomatitis virus (VSV) contains a negative-strand RNA genome in a ribonucleoprotein (RNP) particle which is a template for both transcription into the leader RNA and five mRNAs and for replication into the encapsidated full-length antigenome, itself a template for genome amplification. This virus presents an unusually wide host range spectrum and provides an excellent system for studying cell-virus relationships. It can grow in both vertebrate and invertebrate cells, although the development of viral infection and the fate of infected cells are strikingly different in both systems. The wild-type VSV is strongly pathogenic in most vertebrate cells. Infection results in a rapid inhibition of cell synthesis and in the production of high yields of infectious virions until cell death. In contrast, in insect cells such as Drosophila melanogaster cells, VSV multiplies without any apparent cytopathic effect. No cellular synthesis inhibition is observed, even transiently, although all viral functions are expressed. After an acute phase, viral production declines, the cells survive, and a persistent infection is established (32). Exceptions occur with some sublines of Aedes albopictus cells in which a strong cytopathic effect can be observed (12); we have never obtained a response to infection different from that shown by the parental population with cloned Drosophila cells (10). Occasionally, vertebrate cells can also be persistently infected. In particular, this is the case with some cell lines nonpermissive for VSV, e.g., with human B lymphoblastoid cells stably transformed by the Epstein-Barr virus (22) or with rabbit cornea cells; in the latter case, the restriction can be suppressed by coinfection with a helper virus (14). Persistence can also be artificially obtained with temperature-sensitive mutants and also under particular infection conditions with wild-type VSV in the presence of large numbers of defective interfering particles or after interferon treatment (26). In such cases, few cells survive and the viral population evolves rapidly to an altered phenotype of RNA synthesis with reduced cytopathogenicity (11).

The isolation of host range mutants has provided strong evidence that several cellular factors, such as cytoskeleton-associated proteins (15, 21), play an active role in VSV multiplication (23). In vitro studies are in progress to elucidate the nature and role of these cellular factors. But so far, their levels of interaction are not yet totally understood. Several genes which interfere with the multiplication of two rhabdoviruses, sigma (6) and pyr (4), have been identified in D. melanogaster. Taking advantage of the considerable genetic information accumulated for this organism, five of these genes have been localized and one has been cloned and its product is being investigated (D. Contamine, personal communication). In parallel, the different steps of VSV infection in Drosophila cell lines have been studied to understand the molecular basis of virus growth restriction.

Our previous results have shown that the synthesis rate of viral proteins increases transiently during the first 7 h of infection and then declines to a constant, low level which is maintained during virus persistence. The production of infectious particles follows the same kinetics, with a 3- to 4-h delay (32). In addition, membrane proteins are subjected to particular modifications. G protein, whose glycosylation is altered, is synthesized in small amounts all along the cycle (32), and M protein is hyperphosphorylated (3).

In the present study, we analyzed the viral RNA synthesis. Changes in transcription and replication were observed which are implicated in the establishment and the maintenance of persistent infection in Drosophila cells.

MATERIALS AND METHODS

Cells and virus. The D. melanogaster cell line 77 OM3 (10) and the CER hamster cell line (9) were infected at 25 and 37°C, respectively, with VSV Indiana BT78 lot 1555 (32) at a multiplicity of infection of 8 PFU per cell.

Intracellular RNA purification. Total nucleic acids were extracted from infected cells as previously described (9), quantified by optical density at 260 nm, and kept in 70% ethanol at −20°C. When RNA pulse-labeling was required, culture medium was replaced by serum-free medium to which [3H]uridine was added, at 500 μCi/ml, for 1 h just before RNA purification.

Intracellular viral RNPs were purified according to the method of Kolakovsky (16) except that cytoplasmic extracts were centrifuged through a preformed CsCl gradient as described elsewhere (9). RNPs extracted from virions and

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centrifuged in parallel were used as migration markers. Of the original cellular nucleic acids, 8% were usually purified from gradient fractions containing intracellular RNPs.

Isolation of extracellular virions. The supernatants of infected cell cultures were harvested and clarified by low-speed centrifugation for 5 min at 8,000 × g. Virions were centrifuged through a 20% sucrose pad in phosphate-buff ered saline solution for 1 h at 100,000 × g in a Beckman R40 rotor at 4°C. RNA was extracted from pellets as described above.

Electrophoresis, Northern (RNA) blots, and dot blotsof RNAs. Purified cellular RNAs were denatured in 1 M glyoxal–50% dimethyl sulfoxide–10 mM phosphate buffer (pH 7) for 1 h at 50°C and separated by electrophoresis in a 1.5% agarose gel containing 6 M urea and 25 mM sodium citrate (pH 3.5) at 90 V for 17 h at 4°C; the gel was washed twice in 10 mM phosphate buffer (pH 6.5) for 30 min. Nucleic acids were electrotransferred onto Hybond-N membrane (Amersham Corp.) in 20 mM phosphate buffer (pH 6.5) for 3 h at 40 V in a Hoefer Transphor (TE42) apparatus (Hoefer Scientific Instruments) and fixed by UV irradiation for 4 min. Northern blots were then baked for 1 h at 80°C to reverse any glyoxal reaction.

Dot blots were performed as described by Thomas (29). Purified RNAs were denatured in 1 M glyoxal–10 mM phosphate buffer (pH 6.5) for 1 h at 50°C. Samples were diluted at least 10-fold in the 10 mM phosphate buffer (pH 6.5), and applied on a gene screen membrane (New England Nuclear Research Products) by filtration without suction in a 96-hole minifold apparatus (Schleicher & Schuell, Inc.). RNAs were fixed and glyoxal was eliminated by baking for 4 h at 80°C.

Cloning of VSV sequences into bacteriophage M13. cDNAs of the N mRNA and of the 5′ half of L mRNA, inserted in plasmids PBR322 and PRL1, respectively, were supplied by Rose and Gallione (24) and Schubert et al. (25). We subcloned the 3′ end of the N gene (599 base pairs) and the 5′ end of the L gene (621 base pairs) in PstI site of the replicative form of the M13 vector (M13mp701) as described by Messing et al. (20). Single-strand clones containing plus or minus strands were selected and their polarity was determined by dot hybridization with 32P-labeled VSV genomic RNA as described below.

Labeling of probes and hybridization. VSV genomic RNA, purified as previously described (9), was uniformly labeled in vitro by T4 polynucleotide kinase with [γ-32P]ATP as described by Vlak et al. (30). Specific activity of 5 × 108 to 10 × 109 cpm/μg of RNA. Filter hybridization was performed as described by Thomas (29) with 20 ng of 32P-labeled VSV genomic RNA probe per ml during 24 h for the Northern blots and 100 ng of radioactive probe per ml during 48 h for the dot blots. After autoradiography and cutting, individual dots were counted in liquid scintillation fluid.

32P-labeled single-strand DNA probes were prepared from VSV sequences inserted in M13 vector as described by Barker et al. (2) with some modifications. The insert was copied by the Klenow fragment of DNA polymerase I in the presence of [α-32P]dATP (1,500 Ci/mmol). After a chase, the product was cut at the PvuII site in M13 DNA, located about 200 base pairs after the insert. Partially double-strand DNA was denatured in TBE buffer (90 mM Tris borate [pH 8.5], 2 mM EDTA) and 90% formamide for 10 min at 100°C, cooled and electrophoresed on a 3% polyacrylamide gel in TBE buffer and 6 M urea for 3 h at 250 V. The 32P-labeled single-strand DNA band was excised and electroeluted in TBE buffer for 2 h at 200 V with a Bio-trap apparatus (Schleicher & Schuell, Inc.). The specific activity of this probe was more than 108 cpm/μg. Prehybridization for 20 h and hybridization for 48 h were carried out at 52°C in 30 mM phosphate buffer (pH 7)-0.6 M NaCl–5 mM EDTA–1% sodium dodecyl sulfate–0.06% polyvinylpyrrolidone–0.06% Ficoll (Pharmacia Fine Chemicals)–250 μg of denatured DNA per ml. During the hybridization of the Northern blots and the dot blots, 32P-labeled DNA probe was added at a final concentration of 2 × 106 and 5 × 105 cpm/ml, respectively. The blots were washed as previously described (29).

RESULTS

Accumulation of VSV mRNAs in Drosophila cells. Viral mRNA accumulation was quantified by dot blot hybridizations to determine whether the decrease in viral protein synthesis rate observed 7 h after infection (32) was due to an inhibition of translation or to a defect in the synthesis or the stability of messengers. For comparison, an identical experiment was performed with hamster CER cells in which VSV caused an acute, cytocidal infection. At various times after infection, total intracellular nucleic acids were extracted and denatured by glyoxal, and then serial dilutions of each sample were spotted onto membranes. Viral mRNAs immobilized on the filters were specifically detected by hybridization with the 32P-labeled VSV genomic RNA probe as described in Materials and Methods. The probe also revealed the plus-strand antigenome, but since it is a minority species, this RNA was not included in quantification.

Dot blot autoradiograms are shown in Fig. 1A. Nucleic acids of noninfected cells yielded barely detectable dots which allowed us to evaluate the background to be subtracted from hybridization in infected cells. Probe amounts hybridized per dot (expressed in counts per min) versus immobilized nucleic acid amounts (in micrograms) were plotted for each sample. The relation was linear at low concentrations in viral materials, i.e., the mRNA concentration was proportional to the initial slope (Fig. 1B). The variations of messenger level during VSV growth are shown in Fig. 1C. We have chosen to express the results per microgram of cellular nucleic acids to obtain a better comparison between cell types and to compensate for variations caused by the differences in cell size and morphology. Drosophila cells are small, with a reduced cytoplasm volume, and contain about one-third as much nucleic acid as vertebrate cells do. In CER cells, viral mRNAs accumulated rapidly, reaching a maximum level as early as 4 h after infection and remained nearly constant until cellular lysis occurred 6 or 7 h after infection. In Drosophila cells, accumulation was detected as soon as the first few hours after infection but was nevertheless slower; at 6 h, it reached a maximum which was a quarter of the level shown in vertebrate cells. It then rapidly decreased to a plateau which extended beyond 20 h after infection, corresponding to a steady state between mRNA synthesis and degradation. The shape of this curve was similar to that of the curve showing kinetics of viral protein synthesis (32), with a peak at the same time. (These kinetics are also shown elsewhere [see Fig. 6 which summarizes the results obtained on VSV synthesis in Drosophila cells].) The decrease in protein synthesis rate can thus be attributed to the decline in the level of encoding mRNAs.

The nature and relative ratios of each mRNA were analyzed by Northern blot hybridization. Total intracellular glyoxylated RNAs were separated by electrophoresis through a denaturing acid-urea agarose gel, transferred onto
VOL. 62, cells and for each infected generated after infection. Serial dilutions and Methods.

Nucleic acids of infected Nucleic acids. probe hybridized with 32P-labeled VSV genomic RNA as described in Materials and Methods. Migration of the mRNAs corresponding to the five viral proteins (L, G, N, M, and NS) of viral 42 S replicative RNA and of rRNAs 18S and 28S (in CER cells and rRNAs in Drosophila cells) are indicated. In insect cells, the heavy rRNA is separated on a denaturing gel in two fragments whose migrations are very close to that of the light RNA.

membrane, and probed with 32P-labeled VSV genomic RNA. The blot autoradiogram (shown in Fig. 2) completes and confirms the results described above. In spite of a weak unspecific hybridization for ribosomal RNAs, the five messengers were the major viral RNA species detected in Drosophila cells, together with a low amount of 42 S antigeneomes. The relative ratios of these messengers, calculated after autoradiogram scanning, were very close to those found in CER cells (data not shown), indicating that attenuation of the viral sequential transcription was not affected in insect cells. Each mRNA presented the same pattern of accumulation, with a similar increase and decrease. The inhibition of viral protein synthesis therefore appears to be a direct consequence of the decrease in the amounts of mRNAs except for membrane protein G. As previously reported, this protein was always poorly translated throughout the viral cycle in Drosophila cells (32), although its messenger was present in high amounts, strongly suggesting that the synthesis was affected at a later step.

Kinetics of VSV mRNA synthesis in Drosophila cells. The general reduction in the amounts of viral mRNAs observed in persistently infected Drosophila cells could be the result of either an increase in their degradation or a decrease in their rate of synthesis. To investigate the latter possibility, the infected cells were pulse-labeled with [3H]uridine at various times after infection, and the viral plus-strand RNAs were specifically isolated by dot blot hybridization; nucleic acids were extracted just after the 1-h pulse and hybridized with an excess of unlabeled VSV genomic RNA immobilized

FIG. 1. Accumulation of VSV mRNAs throughout infection in Drosophila cells and CER cells measured by dot blot hybridization. Nucleic acids of infected cells were extracted at indicated times after infection. Serial dilutions were spotted onto membranes and probed with 32P-labeled VSV genomic RNA as described in Materials and Methods. (A) Dot autoradiograms with 3- and 12-h exposures for CER and Drosophila cells, respectively. (B) Curves were generated for each infected cell sample after individual dot counting by plotting counts per minute of bound probe versus the amount of dotted nucleic acids. (C) Accumulation of VSV mRNAs in Drosophila cells (●) and CER cells (□) expressed in counts per minute of probe hybridized with 1 µg of cellular nucleic acids.

FIG. 2. Northern blot analysis of VSV mRNAs. Cellular nucleic acids were purified at various times after infection. Glyoxalated samples, 10 and 2 µg for Drosophila and CER cells, respectively, were fractionated on an acid-urea agarose gel, transferred onto the membrane, and hybridized with 32P-labeled VSV genomic RNA as described in Materials and Methods. Migration of the mRNAs corresponding to the five viral proteins (L, G, N, M, and NS) of viral 42 S replicative RNA and of rRNAs 18S and 28S (in CER cells and rRNAs in Drosophila cells) are indicated. In insect cells, the heavy rRNA is separated on a denaturing gel in two fragments whose migrations are very close to that of the light RNA.
onto a filter. \(^3\)H-labeled RNAs of uninfected cells were used as a control. After dot counting, it was found that VSV plus-strand RNAs never represented more than 3% of the RNA synthesized in Drosophila cells, since no inhibition of host synthesis occurs (32), whereas in CER cells, radioactivity was mostly incorporated in viral messengers even soon after infection. Data were analyzed as shown in Fig. 1, and the modifications of the transcription rate throughout the viral cycle are shown in Fig. 3. In CER cells, mRNA synthesis was maximal 4 h after infection and did not significantly decline until cell monolayers decayed after 7 h. In Drosophila cells, the synthesis rate also increased during the first 4 h and then was rapidly inhibited (by approximately 50% of the peak value after 6 h and by 25 to 33% after 11 h), resulting in a steady state maintained in persistently infected cells. Since the transcripts have a limited half-life (8), the inhibition of their synthesis may be considered to be largely responsible for the decrease in mRNA amounts found in Drosophila cells 7 h after infection (Fig. 1). The maximal transcription rate reached at 4 h was fourfold lower in Drosophila cells than in CER cells. This difference was in agreement with the quantitative results shown in Fig. 1. It was certainly due in part to the lower temperature (25°C) used to grow the insect cells. However it might also be due to changes in the intracellular pools of uridine whose transport would not be affected to the same degree in the two cell types after VSV infection (27).

Accumulation of the VSV genomes and antigenomes in Drosophila cells. The two steps of VSV replication, synthesis of full-length plus-strand antigensomes and of minus-strand genomes, are absolutely dependent on continuous viral protein synthesis. We studied the characteristics of virus replication in Drosophila cells where viral protein synthesis rate was rapidly inhibited after infection.

The viral replicative RNAs encapsidated as RNPs were first separated from mRNAs on CsCl gradients after cell lysis with a nonionic detergent, since the direct quantification of the antigenomes by dot blot hybridization was impossible in the presence of VSV transcripts. Genomes or antigenomes were then specifically revealed with \(^{32}\)P-labeled single-strand DNA probes of plus or minus polarity. These probes were synthesized on DNA sequences corresponding to a part of the viral \(N\) and \(L\) genes (599 bases at the 3' end of the \(N\) gene, 621 bases at the 5' end of the \(L\) gene) which were cloned into an M13 vector as described in Materials and Methods. A qualitative analysis was performed on Northern blots prepared with nucleic acids extracted from purified RNPs. Viral minus-strand RNAs were probed with \(^{32}\)P-labeled plus-strand \(N\) and \(L\) DNAs. Full-length 42 S genomes were detected only 6 h after infection and they accumulated later (Fig. 4A). No short discrete-sized molecules were revealed, although the probes hybridized with the sequences corresponding to the two ends of the genome, indicating that no truncated particles were generated during the establishment of persistent infection. The \(^{32}\)P-labeled minus-strand \(N\) and \(L\) DNA probes displayed the full-length antigenomes which appeared to follow the same accumulation pattern as the genomes (Fig. 4B).

Each replicative RNA was quantified twice by separate hybridizations with the single-strand DNA probes complementary to either the 3' or the 5' end on dot blots prepared with the RNP-purified RNA. Examples of dot autoradiograms are shown in Fig. 5A and C. In addition, genomes packaged into mature virions were also considered. Virions were isolated by high-speed centrifugation from the supernatant of each cell culture used to purify cellular RNPs. Extracellular genomes were extracted and analyzed in parallel (Fig. 5B). To compare the results and to determine the total amounts of synthesized genomes, RNAs extracted

FIG. 3. Kinetics of VSV mRNA synthesis in Drosophila cells and CER cells. At various times after infection, cells were pulse-labeled for 1 h with 500 \(\mu\)Ci of \(^{3}\)H-uridine per ml before nucleic acid extraction was done. Increasing amounts of \(^{3}\)H-RNA samples were separately hybridized with an excess of unlabeled VSV genomic RNA (3 \(\mu\)g) dotted on filters. Identical amounts of \(^{3}\)H-labeled uninfected cell RNAs were used as controls. After counting, data were analyzed as described in the legend to Fig. 1B and C. The results were expressed in radioactivity incorporated into viral plus-strand RNAs present per microgram of cellular nucleic acids extracted from Drosophila cells (○) and CER cells (□).

FIG. 4. Northern blot analysis of VSV genome and antigenome in Drosophila cells. Viral RNPs were extracted at the indicated times after infection and 5 \(\mu\)g of RNP-purified RNAs was separated on a gel. Blots were prepared and hybridized with \(^{32}\)P-labeled single-strand DNAs as described in Materials and Methods. (A) VSV genome detected by hybridization with \(^{32}\)P-labeled plus-strand \(N\) and plus-strand \(L\) DNA probes. The 42 S genome purified from virions was used as a marker of migration (lane G). (B) VSV antigenome detected by hybridization with \(^{32}\)P-labeled minus-strand \(N\) and minus-strand \(L\) DNA probes.
from intracellular RNPs and from extracellular virions were both expressed as the original amount of cell extract that each sample represented. Quantification was performed as described in the legend to Fig. 1. Standard curves were determined for each probe from dots of known quantities of genomic RNA extracted from purified virions. They were used to extrapolate the amounts of genomes accumulated in cells (Fig. 5D, curves I) or released in the supernatants (Fig. 5D, curves E) and to assess the total synthesized quantities (Fig. 5D, curves T). In the absence of a viral plus-strand RNA which could have been used as reference, data concerning antigenomes were expressed in amounts of hybridized probe (Fig. 5E).

In CER cells, the two replicative RNAs accumulated rapidly without an important lag time. In Drosophila cells, both genomes and antigenomes were detected after a 3-h delay when de novo viral protein synthesis developed (32), and then they accumulated until 15 h after infection, whereas transcription rate declined (Fig. 3). (The time-dependent development of each viral synthesis can also be compared in Fig. 6.) These results indicated that the synthesis on genome templates switched from transcription to replication. However, the replication rate always remained low compared with the transcription rate, since viral RNAs found in Drosophila cells were mainly mRNAs throughout the cycle (Fig. 2). Later during infection, the intracellular pool of genomes and antigenomes remained constant and maximal. Several hundred genome copies were then found per cell.
TABLE 1. Quantification of genomes produced by VSV-infected Drosophila cells and CER cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Hours postinfection</th>
<th>Total genomes/μg of total cellular nucleic acids&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. released genomes/μg of cellular nucleic acids&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% Released genomes&lt;sup&gt;c&lt;/sup&gt;</th>
<th>PFU/μg of cellular nucleic acids&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Released genomes/PFU</th>
<th>No. of intracellular genome equivalents/cell&lt;sup&gt;e&lt;/sup&gt;</th>
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<tr>
<td>Drosophila melanogaster</td>
<td>6</td>
<td>1.3 x 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>5.6 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>43</td>
<td>2.4 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2,300</td>
<td>450</td>
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<tr>
<td></td>
<td>9</td>
<td>2.2 x 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>45</td>
<td>5.6 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
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<td>750</td>
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<tr>
<td></td>
<td>14</td>
<td>3.8 x 10&lt;sup&gt;8&lt;/sup&gt;</td>
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<td>950</td>
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<td>CER</td>
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<td>10</td>
<td>14,100</td>
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<sup>a</sup> Sum of released and intracellular genomes produced per microgram of total cellular nucleic acids calculated as the number of genome equivalents based upon dot hybridization data presented in Fig. 5.

<sup>b</sup> Calculated as described in footnote <sup>a</sup>.

<sup>c</sup> Calculated as percentage of total produced genomes.

<sup>d</sup> PFUs were titrated on CER cell monolayers.

<sup>e</sup> Nucleic acids (1 μg) were extracted from 5 x 10<sup>4</sup> CER cells or 1.5 x 10<sup>5</sup> Drosophila cells.
tant were only 25 to 35% less in Drosophila cells than in CER cells. These results revealed a drastic decrease in infectivity of virions produced in these insect cells, since only 0.05% were infectious compared with 10% in the vertebrate host. It has already been shown in other cell lines nonpermissive for VSV that produced virions are heterogeneous, unstable, and weakly infectious (18, 22, 31) and that the protein content of their envelopes, together with the maturation of G protein, were altered as described previously in Drosophila cells (3, 32). Whether the decrease in virion infectivity is related to the control of VSV multiplication in Drosophila cells is not yet known.

**DISCUSSION**

The establishment of persistent infection in VSV-infected Drosophila cells was characterized by the noninhibition of cellular synthesis and the control of viral synthesis. Most results indicate that in vertebrate cells the VSV plus-strand leader RNA, the first product of viral transcription, is directly involved in the inhibition of host synthesis (9, 13). The leader RNA is always found in low amounts in the cytoplasm of Drosophila cells (never more than 70 molecules per cell versus 2,000 molecules per CER cell). It follows the same pattern of accumulation as do the other transcripts, and its synthesis is also reduced soon after infection. In addition, this RNA does not migrate into the cell nucleus (9). These results probably explain the absence of host synthesis shutoff in these insect cells.

Control of viral synthesis is also essential for survival of the host. We studied virus-specific RNA synthesis in Drosophila cells. The changes observed during the first 20 h of viral multiplication are summarized in Fig. 6. The viral transcription rate rapidly declined as early as 5 h after infection and was inhibited by about sixfold 10 h after infection. This inhibition resulted in a decreasing amount of functional mRNAs, since the half-life of the transcripts is limited (8). The protein synthesis subsequently also slowed down. Thus, after an initial burst of synthesis, an equilibrium was reached after about 15 h when the production of viral messengers and proteins was minimal. This can be considered to be the state of persistent infection. The genomes and antigenomes were detected only after a 3-h lag time and from then, gradually accumulated, as long as viral protein synthesis was not greatly inhibited. The replication rate then declined following the decrease in all the other viral synthesis. This decline was not unexpected, since it is dependent on continual protein synthesis and especially on a pool of newly synthesized free N protein available in cytoplasm (1). A sixfold decrease in the synthesis of this protein certainly determined replication restriction in Drosophila cells, even under conditions in which viral polymerase activity could be still effective. Later, the number of plus- and minus-strand 42S molecules remained constant and did not decrease as did the transcription products. This finding is probably due to the fact that these RNAs were associated with proteins in a nuclease-resistant structure (27) and were more stable than mRNAs. However, the results concerning the stability and the half-life of VSV RNPs in vertebrate cells are contradictory (8, 27). The continual presence of several hundred genomes per cell throughout the divisions of persistently infected Drosophila cells was certainly sufficient to ensure the transmission of viral information, whereas virus synthesis was regulated to a level compatible with host survival.

In Drosophila cells, the first step of replication was certainly promoted over transcription by de novo protein synthesis as occurs in vertebrate cells, especially by N protein synthesis necessary for nascent RNA encapsidation (1). However this step may be affected, since high ratios of defective antigenomes were synthesized. Such short encapsidated RNAs which contain the sequence of the leader RNA and a variable part of the N gene have been already detected in Drosophila cells. They have not been isolated as free RNPs but they were found to be associated with the full-length genome in complex structures whose nature remains to be determined (9). In these structures, the synthesis on the genome templates could be stopped, leading to a global inhibition of plus-strand RNA synthesis, messengers, and antigenomes. Nevertheless incomplete minus-strand RNAs were not detected, indicating that the regulation of viral RNA-polymerases during the two replication steps might be different. This possibility has already been suggested in the increase in the ratio of minus- to plus-strand 42S RNA molecules as the infection proceeded (27).

The two membrane proteins M and G have been found to play a part in viral RNA synthesis (19). M protein acts especially as a direct transcription inhibitor in vivo (5). The synthesis and maturation of VSV membrane proteins are modified in Drosophila cells (3, 32), and it would be interesting to determine the relationship between the hyperphosphorylation of M proteins and the shutoff of the transcription.

An altered pattern of viral RNA synthesis has already been shown in other noncytopathic infections with avirulent strains of Newcastle disease virus (17) or in VSV-infected B lymphoblastoid cells. In the latter case, the results are close to ours, with a large reduction of transcription (7), in particular, in that of the leader RNA, without restriction of replication (22); possible abortive synthesis was not investigated. Truncated viral RNAs have been detected in mosquito cells infected with Semliki Forest virus (28). They are present before, during, and after the establishment of persistence but are never released from the cells. Although their polarity has not been determined, these RNAs could correspond to incomplete replicative intermediates. Moreover in persistently infected vertebrate cells, VSV populations rapidly evolve to an RNA− phenotype characterized by synthesis of far fewer mRNAs and an equal amount of genomes (11). These results and our observations suggest that this RNA phenotype would be implicated not only in the maintenance but also in the establishment of the persistent infection.

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**LITERATURE CITED**


