Translational control of viral and host protein synthesis during the course of herpes simplex virus type 1 infection: evidence that initiation of translation is the limiting step

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Herpes simplex virus type 1 (HSV-1) infection induces the selective shut-off of host protein synthesis, other than ribosomal proteins, and the successive synthesis of viral proteins. Because viral mRNAs persist in the cytoplasm after viral protein synthesis has been inhibited, we hypothesized that viral gene expression may be under translational control. Expression of genes encoding immediate early ICP27, early DBP and late US11 proteins, together with glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was monitored over the course of infection at the level of mRNA and protein synthesis. After an efficient synthesis beginning with the appearance of successive viral mRNAs in the cytoplasm, synthesis of viral proteins was shut off similarly to the synthesis of GAPDH. This shut-off was not achieved by mRNA degradation but by progressive shifts of viral mRNAs from large polyribosomes to smaller ones, then to 40S ribosomal subunits. Transient expression of the UL41 gene alone, directing synthesis of virion-associated host shut-off (VHS) protein, induced efficient mRNA degradation, but did not impair recruitment of the remaining GAPDH and β-actin mRNAs into polyribosomes. These results indicate that HSV-1 induces a selective repression of initiation of mRNA translation which is probably the main cause of the shut-off of viral protein synthesis, and which contributes to the repression of host protein synthesis. VHS protein is not directly involved in this repression, at least in the absence of other viral proteins.

Introduction

During the course of lytic infection by herpes simplex virus type 1 (HSV-1), host protein synthesis is inhibited, while three groups of viral genes, immediate early (IE), early and late, are successively expressed. HSV-1-induced inhibition of host protein synthesis is a multistep process which involves several mechanisms. The primary shut-off of host protein synthesis does not require de novo protein synthesis and is, in part, the consequence of a non-specific degradation of all cellular and viral mRNAs, induced by the virion-associated host shut-off (VHS) protein, leading to a disruption of polyribosomes. VHS protein, encoded by the viral UL41 true late gene, is a viral structural component released into infected cells by the virion (Sydskis & Roizman, 1967; Nishioka & Silverstein, 1977; Fenwick & Clark, 1982; Read & Frenkel, 1983; Schek & Bachenheimer, 1985; Strom & Frenkel, 1987; Fenwick & Owen, 1988; Oroskar & Read, 1989; Jones et al., 1995). However, the shut-off of host protein synthesis that follows infection appears to be selective and under translational control. Synthesis of β-actin becomes much less efficient, while that of ribosomal proteins continues almost unaffected, despite a similar degradation of their mRNAs (Simonin et al., 1997). This is made possible by more efficient translation of ribosomal protein mRNAs immediately after infection (Greco et al., 1997).

A secondary shut-off of protein synthesis occurs later in the course of infection, and requires viral gene expression. ICP27 IE protein and UL13 early protein contribute to this process (Nishioka & Silverstein, 1977; Fenwick & Clark, 1982; Read & Frenkel, 1983; Sacks et al., 1985; McMahan & Schaffer, 1990; Hardwicke & Sandri-Goldin, 1994; Overton et al., 1994). ICP27 inhibits gene expression at the transcriptional and post-transcriptional levels. This includes the selection of alternative termination sites of transcription, inhibition of RNA splicing and destabilization of mRNAs. This delayed inhibition of protein synthesis appears to result from selective virus-induced
translational control. During the course of infection, initiation of translation of the remaining β-actin mRNAs is selectively and progressively inhibited, while that of the remaining ribosomal protein mRNAs becomes more efficient (Greco et al., 1992; Simonin et al., 1997).

The successive expression of viral genes results primarily from transcriptional control (Batterson & Roizman, 1983; Campbell et al., 1984; Godowski & Knipe, 1986; Weinheimer & McKnight, 1987; Homa et al., 1991; Guzowski & Wagner, 1993; Roizman & Sears, 1993). Transcription of all IE genes is specifically induced by VP16, another structural protein delivered into cells by invading virions. With the exception of ICP47, all other IE proteins have been shown to be involved in the regulation of transcription of the three groups of viral genes. Transition from the IE to early phase of viral gene expression is accompanied by the shut-off of IE protein synthesis and induction of early protein synthesis. Depression of IE protein synthesis is, at least in part, controlled at the post-transcriptional level, and again involves both VHS and ICP27 viral proteins (Sacks et al., 1985; Oroskar & Read, 1989; Rice & Knipe, 1990; McLauchlan et al., 1992; Sandri-Goldin & Mendoza, 1992; Hardwicke & Sandri-Goldin, 1994).

Because IE mRNAs persist in the cytoplasm late in infection when IE protein synthesis is almost completely inhibited, the hypothesis of translational control of viral gene expression has been suggested by various groups (Honess & Roizman, 1974; Silverstein & Engelhardt, 1979; Scheik & Benchenheimer, 1985; Weinheimer & McKnight, 1987; Elshiekh et al., 1991). Although this hypothesis remains unproved, it is supported by other data. For example, inhibition of IE protein synthesis still occurs in enucleated cells (Honess & Roizman, 1973; Fenwick, 1977). Also, the rate of synthesis of some early and late proteins is not strictly correlated to the amount of their mRNAs during the course of infection (Sandri-Goldin et al., 1983; Johnson & Spear, 1984; Arsenakis et al., 1988; Yager et al., 1990; Elshiekh et al., 1991).

Consistent with the hypothesis of translational control of viral and host protein synthesis is the observation that infection by HSV-1 leads to specific alterations in the translational apparatus (Fenwick & Walker, 1979; Kennedy et al., 1981; Garcin et al., 1990; Massé et al., 1990a, b; Simonin et al., 1995a; Greco et al., 1997). The ribosomal protein S6 undergoes an irreversible phosphorylation during the adsorption step of virus infection (Kennedy et al., 1981; Massé et al., 1990a, b). An increase in S6 phosphorylation is always correlated with the preferential recruitment and translation of ribosomal protein mRNAs (Jefferies et al., 1994). Other ribosomal proteins, Sa, S2, S3a and L30, are unusually phosphorylated after infection, and two other phosphorylated proteins are associated with the ribosomal fraction (Johnson et al., 1986; Massé et al., 1990a, b; Roller & Roizman, 1992). One of these proteins has been identified as the product of the viral US11 true late gene (Diaz et al., 1993). US11 RNA-binding protein appears to be involved in post-transcriptional regulation of gene expression (Diaz et al., 1996). In addition, some cellular translation factors are modified in HSV-1-infected cells (Chou et al., 1995; Kawaguchi et al., 1997). These data and our recent results demonstrating that HSV-1 infection induces a selective translational control of host protein synthesis (Greco et al., 1997; Simonin et al., 1997) reinforce the hypothesis that viral protein synthesis is regulated at the translational level.

In order to verify this hypothesis we measured the rate of synthesis and the amount of cytoplasmic mRNA of ICP27 IE protein, double-stranded DNA-binding early protein (DBP) and US11 true late protein at different times after infection. No direct correlation was found between the rates of synthesis of these proteins and the amounts of their mRNAs. Analysis of the distribution of viral and cellular mRNAs among polyribosomes showed that all mRNA molecules, initially associated with large polyribosomes, progressively shifted to smaller polyribosomes, and then to free inactive particles during the course of infection. These results indicate that translation of the mRNAs studied was progressively inhibited at the initiation step, before binding of the 60S ribosomal subunit.

Methods

- **Cell lines and virus strain.** HeLa and Vero cells were grown as monolayers in Eagle’s minimum essential medium (MEM) supplemented with 10% heat-inactivated newborn calf serum (NBCS). The HSV-1 macroplaque strain (MP), obtained from B. Jacquemont (Lyon, France), was a gift of B. Roizman (Chicago, IL, USA) and was used throughout this study. Viruses were grown in Vero cells.

- **Infection of cells and 35S-labelling.** HeLa cells were infected just before confluence with a m.o.i. of 30 p.f.u. per cell in medium 199 (ICN Flow Laboratories) supplemented with 1% heat-inactivated NBCS. After adsorption for 1 h at 33 °C, the inoculum was replaced with medium 199 containing 1% unactivated NBCS, and cells were incubated at 37 °C for different times as indicated in the figure legends. Times post-infection (p.i.) were calculated from the time of addition of the inoculum. For 35S-labelling in vivo, the medium was removed 1 h before harvesting and replaced with methionine- and cysteine-free MEM (ICN Flow Laboratories), supplemented with 1% inactivated and dialysed NBCS, and incubated for 30 min at 37 °C. The medium was removed and replaced with the same medium supplemented with a mixture of L-[35S]methionine and L-[35S]cysteine at final concentrations of 2.75 MBq/ml (75 µCi/ml) and 0.6 MBq/ml (16 µCi/ml), respectively (NEN). In all experiments control mock-infected and infected cells were submitted to exactly the same experimental conditions.

- **Estimation of viral and host protein synthesis throughout infection.** Cells were washed three times with ice-cold PBS after labelling (130 mM NaCl, 4 mM Na2HPO4, 2H2O, 1.5 mM KH2PO4), scraped into PBS, and collected by centrifugation at 500 × g. Cells were then lysed in ice-cold buffer (0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, 150 mM NaCl, 10 mM Tris–HCl pH 7.4, 1 mM EDTA, 0.2 mM PMSF). Insoluble material was removed by centrifugation at 12,000 × g. Immunoprecipitations of proteins were performed as previously described (Simonin et al., 1995b). Aliquots of lysates prepared from 106 cells were incubated for 18 h at 4 °C with one of the following: rabbit polyclonal anti-ICP27 antibody, rabbit polyclonal anti-US11 antibody (Diaz et al., 1993), mouse monoclonal anti-DBP antibody or mouse
monoclonal antibody directed against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Interchim), each diluted 50-fold in lysis buffer. Anti-ICP27 and anti-DBP antibodies (antibody 42 and antibody Z1F11 directed against the DBP of 65 kDa, respectively) were kindly provided by H. Marsden (MRC, Glasgow, UK) (Schenck et al., 1988; Sinclair et al., 1994). Antigen–antibody complexes were collected with protein A coupled to Sepharose CL-4B beads (Pharmacia) for 30 min at 4 °C. Immuno precipitated proteins were then analysed by SDS–PAGE (Laemmli, 1970). Dried gels were submitted to autoradiography. The corresponding immunoprecipitated proteins were quantified by scanning densitometry of gels with a Storm 840 PhosphorImager (Molecular Dynamics).

Measurement of the amounts of viral and host mRNAs in the cytoplasm. Cytoplasmic RNAs from 5 × 10⁶ control mock-infected and infected HeLa cells or from transfected HeLa cells were purified after lysis of the cells with NP40 at a final concentration of 0.1% followed by proteinase K digestion and phenol–chloroform extraction. RNA was analysed on Northern blots as described (Greco et al., 1997). Hybridization was performed at 65 °C with different ³²P-labelled DNA probes by random priming. For detection of ICP27, ICP22 and DBP mRNAs, ³²P-labelled probes were prepared from the 746 bp Sall–EcoNI fragment of pSG28, the 469 bp Xhol–Poul fragment of pSG25 and the 1308 bp MdiI fragment of pSG124 (Goldin et al., 1981), respectively. UL34 and US11 mRNAs were detected with ³²P-labelled probes prepared from the 472 bp EcoRI–BamHI fragment of pG51 and the 230 bp Xhol fragment of HSV–Us11, respectively (Diaz et al., 1996). The probes used to detect the cytoplasmic GAPDH and β-actin mRNAs were prepared by labelling the 318 bp SacI–BamHI fragment of pTRI-hGAPDH (Ambion) and the 1100 bp PstI fragment of plasmid pAL41-β (Hanahan, 1983), respectively. Hybridized mRNAs were revealed by autoradiography and quantified by scanning densitometry of the blots with a Storm 840 PhosphorImager.

Distribution of mRNAs among polyribosomal fractions. Polyribosomes were prepared from about 8 × 10⁶ mock-infected and infected HeLa cells or from about 3 × 10⁶ transfected HeLa cells. Fractionation of polyribosomes on sucrose gradients was carried out from post-mitochondrial supernatants as previously described (Greco et al., 1997). In experiments with mock-infected and infected cells, twenty fractions of identical volume were collected from the top of the gradients, whereas in experiments with transfected cells, ten fractions of identical volume were collected. Each fraction was treated with 100 µg/ml proteinase K and 1% SDS for 15 min, before phenol–chloroform extraction and ethanol precipitation of the RNA. RNA from each fraction was resuspended in 10 mM Tris–HCl pH 7.4, 1 mM EDTA, 1 U RNase inhibitor (Pharmacia). The quantitative distribution of the various mRNAs among the different fractions was then analysed on Northern blots as described above.

Construction and transfection of a plasmid expressing the UL41 gene. pUL41 was prepared from pCMVβ (Clontech) by replacing the 3474 bp NotI fragment carrying the β-galactosidase gene with the 2168 bp EcoRI fragment of pSG-124 (Goldin et al., 1981), containing the entire ORF and polyadenylation signal of the UL41 gene. HeLa cells were transfected by the calcium phosphate precipitation procedure with 5 µg pUL41 to which 10 µg pUC18 was added (Sambrook et al., 1989). Control HeLa cells were transfected with 15 µg pUC18 only. After 20 h, cells were harvested and lysed to estimate the amount of GAPDH and β-actin mRNAs present in the cytoplasm and the distribution of mRNA among polyribosomal fractions as described above. Under our experimental conditions, the yield of transfection reached almost 100% (Greco et al., 1994).

Results

Synthesis of viral proteins during the course of productive infection

In order to investigate whether expression of viral genes was under translational control, it was first necessary to determine the precise pattern of synthesis of viral proteins during the course of HSV-1 infection. The synthesis of proteins representing the three groups of viral gene products was investigated: ICP27, the product of an IE gene; DBP, which is the product of the UL42 early gene; and US11, the product of a true late gene. As a control for HSV-1-induced shut-off of host protein synthesis, the synthesis of GAPDH was followed in HeLa cells that were either mock-infected or infected for the times indicated in the legend of Fig. 1. Proteins were ³⁵S-labelled during the last 30 min before harvesting of the cells. The level of protein synthesis was estimated by measurement.

![Image of Northern blots](image-url)

Fig. 1. Synthesis of ICP27, DBP, US11 protein and GAPDH during the course of HSV-1 infection. HeLa cells were either mock-infected (M) or infected for 1 to 15 h. Proteins were labelled with a mixture of [³⁵S]methionine and [³⁵S]cysteine during the last 30 min before harvesting of the cells. After immunoprecipitation with the relevant antibodies, proteins were separated by SDS–PAGE and analysed by autoradiography. Positions of proteins of interest are indicated by arrows. Times (h) after infection are indicated above each lane. In the panel DBP, lane C is a control taken at 5 h p.i. in the absence of anti-DBP antibody. This control indicates that the protein which appeared 2 h p.i. is not DBP, but the result of non-specific adsorption to the protein A–Sepharose beads. US11 protein appears as a doublet of 24 and 26 kDa (Diaz et al., 1993). No labelled protein was detected after a second round of immunoprecipitation.
Viral and GAPDH mRNAs during the course of HSV-1 infection

To determine whether the levels of protein synthesis measured above reflect the levels of mRNA in the cytoplasm, we estimated the amount of these mRNAs, together with amounts of ICP22 and UL34 mRNA, at different times after infection. Total cytoplasmic RNA was purified from either mock-infected cells or cells that had been infected for 3, 6, 9, 12 or 15 h, and RNA was analysed by Northern blotting using specific 32P-labelled DNA probes (Fig. 2).

ICP27 and ICP22 IE mRNAs exhibited similar kinetics of appearance in the cytoplasm. Present in the cytoplasm as early as 3 h p.i., they reached their maximal level at 6 h for ICP27 and between 6 and 9 h for ICP22. Levels then declined for the remainder of the experiment, but remained detectable after 15 h p.i. The early protein mRNAs DBP and UL34 also exhibited similar kinetics of appearance in the cytoplasm, starting at 6 h p.i. They reached their maximal level at 9 h, then declined slowly, and remained present in significant amounts after 15 h p.i. The use of a common probe to detect both ICP47 and US11 protein mRNAs (2 and 1.3 kb, respectively) allowed us to detect simultaneously a typical IE mRNA and a true late mRNA (Fig. 2, top right panel). The kinetics of appearance and accumulation of these mRNAs, as well as the kinetics of disappearance of ICP47 mRNA, were in complete agreement with those of other mRNAs analysed in this experiment, and are consistent with the reported kinetics of accumulation of other HSV-1 mRNAs (Weinheimer & McKnight, 1987; Goodrich et al., 1989; Oroskar & Read, 1989; Elshiekh et al., 1991; Johnson et al., 1991). As expected, the amount of GAPDH mRNA decreased progressively throughout infection, similarly to amounts of β-actin and ribosomal protein mRNAs (Greco et al., 1997; Simonin et al., 1997). However, GAPDH mRNA was still detectable after 15 h p.i. (Fig. 2, bottom right panel). Finally, the sizes of viral and GAPDH mRNAs apparently remained unchanged throughout the course of infection.

This qualitative analysis alone does not allow us to conclude definitively whether a correlation exists throughout infection between the rates of synthesis of these proteins and the amounts of the mRNA by which they are encoded.

Comparison of the levels of the newly synthesized proteins and of their mRNAs during the course of infection

Quantification of the 35S-labelled proteins and 32P-labelled probes depicted in Figs 1 and 2 was performed by scanning
Control of host and HSV-1 mRNA translation

Fig. 3. Comparison of the levels of newly synthesized proteins with those of their mRNAs during the course of infection. Results are expressed as pixels x mm². The amount of 35S incorporated into the specified proteins during the last 30 min before cell harvest is indicated to the left (histograms). The amount of bound 32P-labelled probe, proportional to the amount of the corresponding mRNA at the time of harvest, is indicated to the right (*). For each lane, the background was calculated and subtracted from the amount of radioactivity measured for each protein or mRNA. M, mock-infected.

Distribution of viral and cellular mRNAs among polyribosomes during the course of HSV-1 infection

To investigate at which step of translation viral and host protein synthesis were controlled, we traced the distribution of viral and GAPDH mRNAs among ribosomes and polyribosomes throughout infection. To the aim, postmitochondrial supernatants of HeLa cells, either mock-infected or infected for 3, 6, 9 and 15 h, were separated into twenty fractions after sucrose-gradient centrifugation. As expected, ribosomes were shifted from polyribosomes to 80S ribosomes as early as 3 h p.i. (Fig. 4A). This altered distribution of ribosomes did not change significantly up to 15 h p.i. (not shown). The distribution of mRNAs of interest among the different fractions was assessed by Northern blot with specific 32P-labelled DNA probes, and quantification of mRNA was as described above. Fig. 4(B) shows the results of a typical experiment; identical results were obtained from at least two independent experiments for each mRNA. The distributions of viral and GAPDH mRNAs among the different fractions of the sucrose gradients were not identical. This distribution varied not only with the time of infection, but also with the size of the mRNA. Table 1 summarizes the sizes of these mRNAs.

After 3 h of infection, most of the ICP27 and ICP22 IE mRNAs (65 and 83%, respectively) were associated with 80S ribosomes and polyribosomes (fractions 8 to 20) (Fig. 4B). More than 30% of the ICP27 and ICP22 mRNAs were within large polyribosomes containing five or more ribosomes per mRNA molecule (fractions 15 to 20). From the early (6 h) to the late stage (9 and 15 h) of infection, these IE mRNAs were progressively shifted to smaller polyribosomes, and again to particles sedimenting in the 40–60S region of the gradient. After 9 h of infection, less than 10% of the remaining mRNAs were within large polyribosomes. The DBP and UL34 early mRNAs behaved similarly to the IE mRNAs, although changes in their distribution occurred 3 h later (Fig. 4B). After 6 h of infection, 53% of UL42 and 70% of UL34 mRNAs were associated with 80S ribosomes and polyribosomes. From 9 to 15 h p.i., these two mRNAs were progressively shifted from large polyribosomes to smaller polyribosomes, and again to particles sedimenting in the 40–60S region of the gradient.
Fig. 4. For legend see facing page.
Table 1. Sizes of HSV-1 viral and host mRNAs

<table>
<thead>
<tr>
<th>Gene</th>
<th>mRNA</th>
<th>ORF</th>
<th>5' UTR</th>
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<tr>
<td>ICP27</td>
<td>1683</td>
<td>1536</td>
<td>138</td>
</tr>
<tr>
<td>ICP22</td>
<td>1815</td>
<td>1260</td>
<td>518</td>
</tr>
<tr>
<td>DBP</td>
<td>~1715</td>
<td>1404</td>
<td>~195</td>
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<tr>
<td>UL34</td>
<td>~1900</td>
<td>825</td>
<td>~600</td>
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<tr>
<td>ICP47</td>
<td>1967</td>
<td>264</td>
<td>530</td>
</tr>
<tr>
<td>US11</td>
<td>1320</td>
<td>483</td>
<td>214</td>
</tr>
<tr>
<td>GAPDH</td>
<td>1284</td>
<td>1005</td>
<td>75</td>
</tr>
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The ICP47/US11 probe revealed both the 2 kb ICP47 IE mRNA and the 1-3 kb true late US11 mRNA (Fig. 4B). ICP47 mRNA behaved similarly to ICP22 and ICP27 mRNAs. Association of US11 mRNA with 80S ribosomes and polyribosomes decreased from 70% after 6 h of infection to 55% after 9 h. Although US11 mRNA cannot accommodate a large number of ribosomes because of its short ORF (483 nucleotides), most US11 mRNA was associated with six to eight ribosomes at 6 and 9 h p.i. (fractions 16 to 20). At the end of the experiment, US11 mRNA was shifted to smaller polyribosomes; only 15% of the remaining US11 mRNA was still associated with five or more ribosomes, while 42% was bound to one to four ribosomes, the other US11 mRNA being no longer translated (fractions 4 to 7).

The behaviour of GAPDH mRNA was followed as a marker of host mRNA translation throughout infection (Fig. 4B). In mock-infected cells, about 70% of GAPDH mRNA was associated with 80S ribosomes and polyribosomes, 30% of it being associated with six or more ribosomes (fractions 17 to 20). Distribution of GAPDH mRNA through the gradient changed progressively during the course of infection as shown by a decrease in the amount of the remaining GAPDH mRNA found in fractions containing large polyribosomes. After 9 h of infection, just 9% of GAPDH mRNA remained associated with these large polyribosomes, while about 25% was within smaller polyribosomes. More importantly, more than half of the remaining mRNA was found among particles sedimenting in the 40S region of the gradient (fractions 4 and 5).

The partitioning of viral and GAPDH mRNAs through the gradients was compared for the two infection time-points that exhibited the largest differences (Fig. 4C). In each case analysed, viral mRNAs were initially associated with a large number of ribosomes. They were then shifted from large polyribosomes to smaller polyribosomes and also to particles sedimenting in the 40–60S region of the gradient. This observed shift in the distribution of the different mRNAs occurred simultaneously with a progressive decrease in synthesis of the proteins they encode. These results, observed for IE as well as for early and late viral proteins, demonstrate that protein synthesis was under translational control. In addition, they indicate that initiation of translation of the viral mRNAs becomes the limiting step for viral protein synthesis (Mathews et al., 1996). This was also true for GAPDH synthesis, the translation of its mRNA being similarly inhibited at the initiation step, as previously reported for β-actin (Greco et al., 1997).

Behaviour of GAPDH and β-actin mRNA following UL41 transient expression

VHS protein, the product of the true late UL41 gene, is thought to be responsible for the primary shut-off of host protein synthesis by non-selective degradation of mRNA. To determine whether this degradation also led to a shift of mRNA from polyribosomes to 40S ribosomal subunits, we investigated the behaviour of GAPDH and β-actin mRNA after transfection of the UL41 gene, allowing the transient synthesis of the VHS protein. Twenty hours after transfection, post-mitochondrial supernatants of control and of UL41-transfected HeLa cells were separated into ten fractions after sucrose-gradient centrifugation. Distribution of GAPDH and β-actin mRNAs among the different fractions was then assessed by Northern blot. Quantification of these mRNAs was as described above.

In the presence of UL41 gene expression, the amounts of cytoplasmic GAPDH and β-actin mRNAs were reduced to about 25% of the control (Fig. 5A). As expected, ribosomes were shifted from polyribosomes to 80S ribosomes, giving a sedimentation profile very similar to that observed after...
HSV-1 infection (Fig. 5B). These results indicate that VHS protein was indeed synthesized and possessed mRNA-degrading activity. However, the distribution of GAPDH and β-actin mRNAs among the different fractions of the sucrose gradients was very similar for UL41-transfected cells and for control cells (Fig. 5B). These results indicate that VHS protein was responsible for the degradation of mRNAs, but not for the HSV-1-induced accumulation of mRNAs out of the polyribosomes. Therefore, VHS protein does not affect the efficiency of host mRNA translation.

**Discussion**

These results demonstrate that HSV-1 viral protein synthesis begins as soon as viral mRNAs appear in the cytoplasm. They also show that the synthesis of viral proteins is progressively turned off during the course of infection, as is that of most host proteins. Host proteins such as GAPDH and β-actin (Greco et al., 1997; Simonin et al., 1997) exhibit a virus-induced shut-off of their synthesis beginning immediately after infection. This virus-induced shut-off of protein synthesis appears to be the consequence of translational controls affecting both host and viral proteins. The efficiency of mRNA translation is first reduced by a decrease in the number of ribosomes translating the same mRNA molecule, which occurs at the early stage of infection for host mRNAs and a few hours after their appearance in the cytoplasm for viral mRNAs. At the late stage of infection, the remaining host and viral mRNAs are generally no longer translated. They appear to be sequestered in complexes probably containing 40S ribosomal subunits, sedimenting in the 40–60S region of the sucrose gradients because of the size of the mRNAs and of their 5′ UTRs. The 5′ UTRs of the viral mRNAs examined here range from 138 (ICP27) to about 600 nucleotides (UL34), while that of GAPDH is only 75 nucleotides long (Ercolani et al., 1988) (Table 1). Therefore, whereas the 5′ UTRs of viral mRNAs can probably accommodate more than one 40S ribosomal subunit, it is unlikely that the 5′ UTR of GAPDH mRNA can bind more than a single 40S ribosomal subunit because of its small size. This probably explains why viral mRNAs were found mainly in fractions 5 to 7 at the late stage of infection, while most GAPDH mRNAs were found in fractions 4 and 5 (see Fig. 4).

Under these conditions, the shut-off of viral and host protein synthesis appears to result from a common mechanism leading to an inhibition of mRNA translation at the initiation step. This repression of mRNA translation is not caused by VHS protein, however. Indeed, in the absence of other viral proteins VHS protein does not impair efficient mRNA recruitment by ribosomes, as shown by transient expression of the UL41 gene encoding VHS protein. VHS protein contributes to the shut-off of host protein synthesis only by decreasing the amount of mRNAs available for translation. Similarly, degradation of viral mRNAs does not appear to be the major cause of inhibition of viral protein synthesis. Indeed, during the course of infection only the amount of IE mRNAs declined significantly but, in all cases, viral protein synthesis was turned off well before the decrease in the amount of viral mRNAs was apparent. This was particularly clear for US11 mRNA, the amount of which was still increasing between 9 and 15 h p.i., while US11 protein synthesis was drastically reduced during this period.
The shut-off of host protein synthesis is selective, as previously demonstrated (Greco et al., 1997). mRNAs harbouring a polypyrimidine tract at the 5′ terminus (5′-terminal oligopyrimidine; 5′ TOP), such as those encoding ribosomal proteins, are translated better after HSV-1 infection than before (Meyuhas et al., 1996; Amaldi & Pierandrei-Amaldi, 1997; Greco et al., 1997). Even though these 5′ TOP mRNAs undergo a similar pattern of degradation to that of other host mRNAs, synthesis of ribosomal proteins is not inhibited, and new ribosomes are still efficiently assembled during the course of HSV-1 infection (Greco et al., 1997; Simonin et al., 1997). These data suggest that two groups of mRNAs are affected differently by HSV-1 infection: initiation and re-initiation of the 5′ TOP mRNAs remains efficient and is even stimulated after HSV-1 infection, whereas re-initiation of other viral and host mRNAs becomes inefficient. However, the first initiation of translation of all viral mRNAs appears to be carried out efficiently. On the basis of the observation that new ribosomes are still synthesized and assembled after HSV-1 infection, it is tempting to speculate that initiation of translation of viral mRNAs, followed by their efficient translation, takes place on these new ribosomes that may be free of virus-induced modifications, such as phosphorylation of S4, S2, S3a and L30 ribosomal proteins (Massé et al., 1990b). In addition, these new ribosomes contain under-phosphorylated S6 ribosomal protein when they are first made, probably making them ineffective for 5′ TOP mRNA translation (Greco et al., 1997).

If viral mRNAs are translated by newly assembled ribosomes, there may be a specific way for these mRNAs and ribosomes to interact properly in an infected cell. Moreover, after a few rounds of translation, re-initiation of translation would appear to become progressively less likely. The mechanism involved in this inhibition of translation initiation is probably identical for viral mRNAs and for host mRNAs devoid of 5′ TOP. Therefore, selective initiation of mRNA translation would be expected to be restricted to 5′ TOP mRNAs through a step that distinguishes them from other mRNAs. Because initiation of translation appears to be blocked after the binding of 40S ribosomal subunits but before association of the 60S ribosomal subunit, it is likely that the small ribosomal subunit remains associated with the 5′ UTR except when the 5′ UTR contains a 5′ TOP region. To our knowledge, general initiation factors have not been clearly implicated in the shut-off of protein synthesis resulting from HSV-1 infection (Mathews, 1996). The best documented modification of the translational apparatus is the unusual phosphorylation of a few proteins of both ribosomal subunits after infection, making these proteins potential candidates to inhibit the initiation of translation of viral and host mRNAs, except for those harbouring a 5′ TOP tract.

We are grateful to H. Marsden (MRC, Glasgow, UK) for the generous gift of anti-ICP27 and anti-DBP antibodies. This work was supported by the Centre National de la Recherche Scientifique and Université Claude Bernard Lyon 1. A.-M. Laurent was supported by a fellowship from the Ministère de la Recherche et de l’Enseignement Supérieur and by the Association de Recherche sur le Cancer.

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Received 20 May 1998; Accepted 16 July 1998