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Differential Stability of Host mRNAs in Friend Erythroleukemia Cells Infected with Herpes Simplex Virus Type 1

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The consequences of herpes simplex virus type 1 infection on cellular macromolecules were investigated in Friend erythroleukemia cells. The patterns of protein synthesis, examined by polyacrylamide gel electrophoresis, demonstrated that by 4 h postinfection the synthesis of many host proteins, with the exception of histones, was inhibited. Examination of the steady-state level of histone H3 mRNA by molecular hybridization of total RNA to a cloned mouse histone H3 complementary DNA probe demonstrated that the ratio of histone H3 mRNA to total RNA remained unchanged for the first 4 h postinfection. In contrast, the steady-state levels of globin and actin mRNAs decreased progressively at early intervals postinfection. Studies on RNA synthesis in isolated nuclei demonstrated that the transcription of the histone H3 gene was inhibited to approximately the same extent as that of actin gene. We concluded that the stabilization of preexisting histone H3 mRNA was responsible for the persistence of H3 mRNA and histone protein synthesis in herpes simplex virus type 1-infected Friend erythroleukemia cells. The possible mechanisms influencing the differential stability of host mRNAs during the course of productive infection with herpes simplex virus type 1 are discussed.

Productive infection with herpes simplex virus type 1 (HSV-1) results in alterations in the synthesis and metabolism of cellular macromolecules. Early studies demonstrated that HSV-1 mediates the inhibition of cellular DNA (2, 36) and protein synthesis (17, 44, 45). A decrease in host RNA production also occurs; however, the degree of its inhibition is not as severe as that observed with DNA and protein synthesis (2, 3, 10, 15). The host mRNA synthesized after infection is "capped" and adenylated, but those transcripts subsequently transported to the cytoplasm seem to have lost their capacity to direct much protein synthesis (19, 46).

The steady-state levels of preexisting host mRNAs decreases in HSV-1-infected cells. Studies by Pizer and Beard (33) demonstrated that the steady-state amounts of polyomavirus mRNA in HSV-1-infected, polyomavirus-transformed BHK cells were reduced significantly. Similar results were obtained for adenovirus mRNA in adenovirus-transformed cells after infection with HSV (42, 44). Nishioka and Silverstein (30) examined the fate of preexisting globin mRNA sequences in induced Friend erythroleukemia (FL) cells infected with HSV-1 and demonstrated that these sequences were degraded early after infection. This has been confirmed by Inglis (18), who reported that HSV-1 mediated the degradation of actin and glyceraldehyde-3-phosphate dehydrogenase mRNA sequences in BHK cells. It is now known that influenza virus and vaccinia virus also mediate the degradation of host mRNAs (18, 34). These studies investigated the fate of specific classes of preexisting cellular mRNAs during the course of HSV-1 infection. Nakai et al. (27) examined the effects of HSV-1 infection on the steady-state levels of bulk mRNA sequences in polyomavirus-transformed BHK cells by using cDNA to polyadenylated cytoplasmic RNA from uninfected cells. They reported that after infection there was a reduction in the preexisting levels of cytoplasmic mRNA sequences and that increased amounts of the nonadenylated RNA fraction hybridized to the cDNA after infection. These results suggest that deadenylation of host mRNAs may be occurring.

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The experiments presented in this communication examine the fate of specific host mRNAs in FL cells productively infected with HSV-1. Initial studies by liquid hybridization analyses demonstrated that globin mRNA sequences were actively degraded in induced FL cells infected with HSV-1 (30). Using RNA blot hybridization we reexamined the fate of host mRNAs and found that, unlike globin and actin mRNA, the steady-state level of histone mRNA remained unchanged. The possible mechanisms responsible for the differential stability of host mRNAs are discussed.

MATERIALS AND METHODS

Cells and virus. FL cells (clone 745) were obtained from the Human Genetic Mutant Cell Repository (Camden, N.J.) and grown in Dulbecco modified Eagle medium supplemented with 15% fetal calf serum (Flow Laboratories). Erythroid differentiation was induced by the addition of dimethyl sulfoxide (1.8%). Vero cells were maintained in Dulbecco modified Eagle medium supplemented with 10% newborn calf serum (Flow Laboratories). The F strain of HSV-1 was propagated and titered on Vero cell monolayers.

Infection of FL cells with HSV-1. Twelve hours before infection, cells were diluted with fresh medium to 5×10^5 cells per ml. The cells were concentrated by centrifugation to 10^7 cells per ml in phosphate-buffered saline supplemented with 1% glucose, 1% heat-inactivated fetal calf serum, 0.1 mM $CaCl_2$, and 0.5 mM $MgSO_4$ and infected with HSV-1 at a multiplicity of infection (MOI) of 10 by gently shaking for 1 h at 37°C. Unadsorbed virus particles were removed by centrifugation, and the infected cells were suspended in Dulbecco modified Eagle medium to 5×10^5 cells per ml and incubated at 37°C. The elapsed time after infection was calculated from the removal of unadsorbed virus particles.

Pattern of protein synthesis. At intervals, 10^7 cells were suspended in 2 ml of methionine-free Eagle minimum essential medium supplemented with 2% dialyzed fetal calf serum. The cells were labeled with 100 μCi of [^{35}S]methionine (600 Ci/mmol; Amersham Corp.) for 1 h at 37°C. Whole cell lysates were prepared by washing the cells in phosphate-

buffered saline followed by suspension in 50 mM Tris (pH 7.0)–2% sodium dodecyl sulfate–5% β-mercaptoethanol–5% sucrose–0.005% bromophenol blue. To prepare cytoplasm, cellular membrane was lysed with 0.5% Nonidet P-40, and nuclei were removed by centrifugation. Samples were boiled for 2 min and electrophoresed on sodium dodecyl sulfate-polyacrylamide gels with the buffer system of Laemmli (21). The gels were then processed for fluorography (4), dried under vacuum, and exposed to Kodak XAR-5 films.

RNA preparation. RNA was prepared by hot phenol extraction as described by Scherrer (37), with a few modifications. Briefly, cell pellets were suspended in 0.01 M sodium acetate (pH 5.2)–0.05 M NaCl–0.001 M $MgCl_2$. Sodium dodecyl sulfate was added to a final concentration of 1%, and the samples were heated to 55°C for 3 min with an equal volume of phenol saturated with the above solution. The samples were quickly cooled before centrifugation at 5,000 rpm for 5 min in a Sorvall SS34 rotor. The phenol layer was removed, and the aqueous phase and interphase were extracted twice as described above. The final extraction was carried out at room temperature with the addition of NaCl to a final concentration of 0.3 M. The aqueous phase was pooled, and the RNA was precipitated with ethanol. The RNA pellets were dried under vacuum, dissolved in water, and stored at −70°C until needed.

Preparation of probes. The hybridization probes were a mouse α-globin gene (29), a chicken β-actin cDNA (7), and mouse histone H3 cDNA (a gift from W. Marzluff). These DNA sequences were labeled with [α-^{32}P]dCTP (800 Ci/mmol; Amersham) with a nick translation kit (Bethesda Research Laboratories, Inc.).

Rate of nucleic acid synthesis. At intervals, 2×10^6 cells were removed and exposed to 500 μCi of [^{3}H]uridine (39 Ci/mmol; Schwarz Bioresearch) or [^{3}H]thymidine (20 Ci/mmol; New England Nuclear Corp.) per ml for 15 min at 37°C. The incorporation of the radioactive precursors was monitored by measuring trichloroacetic acid-precipitable radioactivity in a liquid scintillation counter. The rate of RNA and DNA synthesis was expressed as the percentage of incorporation in infected cells compared with that in mock-infected cells.

Analysis of RNA by filter hybridization. RNA samples (20 μg) were prepared for electrophoresis by denaturation in a solution containing 50% formamide, 6% formaldehyde, and 1× MOPS buffer (20 mM morpholinepropanesulfonic acid, 5 mM sodium acetate, 1 mM EDTA, pH 7.0). The samples were heated at 55°C for 5 min and electrophoresed on agarose gels containing 6% formaldehyde and 1× MOPS buffer. The gels were incubated in 50 mM NaOH for 30 min, neutralized with 100 mM Tris-hydrochloride (pH 7.5), and equilibrated with 1× SSC (0.15 M NaCl, 0.015 M sodium citrate). The RNA was transferred to a membrane filter (Gene Screen; New England Nuclear Corp.) by the method of Southern (39). The filter was hybridized to ^{32}P-labeled probes (2×10^6 cpm/ml) for 24 to 48 h at 42°C in a solution containing 50% formamide, 1× Denhardt solution (9), 5× SSC, 1% sodium dodecyl sulfate, and denatured salmon sperm DNA (500 μg/ml). The filter was washed as suggested by the supplier (New England Nuclear Corp.), dried, and exposed to Kodak XAR-5 film.

RNA synthesis in isolated nuclei. FL cells were infected with HSV-1 at an MOI of 10 for 3 h. The cells were harvested and suspended in 10 mM Tris (pH 7.5)–10 mM KCl–10 mM $MgCl_2$ at a concentration of 5×10^7 cells per ml. After 10 min on ice, the cells were disrupted by homogenization (12 strokes in a tightly fitted Dounce homogenizer), and nuclei were purified by centrifugation through a 2 M sucrose cushion (26). Nuclei were then resuspended in 25% glycerol, 5 mM magnesium acetate, 5 mM β-mercaptoethanol, and 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (pH 7.5) at a concentration of 3×10^8 nuclei per ml and used immediately or stored in liquid nitrogen. The activity did not decrease for at least 1 month. Reactions were carried out by the method of Manley et al. (23) with 50 μCi of [^{32}P]UTP (3,000 Ci/mmol; Amersham) per 200-μl reaction mixture at 30°C for 45 min. The nuclei incorporated [^{32}P]UTP into trichloroacetic acid-precipitable material in a linear fashion for this period of incubation. RNA was extracted from the nuclei by the hot phenol procedure (37). Five microgram of pBR322 carrying actin, histone, or HSV thymidine kinase gene was immobilized onto a nitrocellulose filter and hybridized to ^{32}P-labeled RNA (8×10^6 cpm) extracted from the nuclei. Hybridization conditions were identical to that described above, except that *Escherichia coli* ribosomal RNA was used as driver at a concentration of 100 μg/ml.

RESULTS

Pattern of protein synthesis. We previously studied the pattern of protein synthesis in HSV-1-infected FL cells and showed that the synthesis of some host proteins was severely inhibited (30–32). For example, globin, a major protein synthesized in induced FL cells, represents one of the proteins extremely sensitive to HSV-1 infection. Concomitantly, there was expression of HSV-1 genes, most of which coded for high-molecular-weight proteins at 4 h postinfection.

To gain insight into the mechanism responsible for the selective expression of HSV-1 genes, it would be advantageous to identify host proteins that are refractory to HSV-1 infection. Whole cell lysates were prepared from HSV-1-infected cells and electrophoresed on a 16% polyacrylamide gel slab (Fig. 1A). The synthesis of many small proteins, including histones, progressed at apparently the same rate as that observed in mock-infected cells. This was not a result of poor infection, because the same sample showed a typical pattern of HSV-1 infection when electrophoresed longer to resolve high-molecular-weight proteins (Fig. 1B). Since histones comigrate with globin, noninduced FL cells were used in the rest of this study unless otherwise indicated.

Steady-state level of host mRNAs. We then examined the steady-state level of histone H3 mRNA in FL cells by molecular hybridization of total cellular RNA to a cloned mouse histone H3 cDNA probe. As expected from the continuous synthesis of histones, the ratio of histone H3 mRNA to total RNA remained unchanged for the first 4 h of infection (Fig. 2). Since this result contradicted the observation that actin mRNA degraded in HSV-1-infected cells (18), we quantitated β-actin sequence in the same RNA samples, using a cloned chicken β-actin cDNA probe (Fig. 2). Actin molecules have been well conserved during evolution (14), and the chicken probe recognized mouse actin mRNA under the conditions employed in this study. As previously reported (18), the ratio of β-actin mRNA to total RNA markedly decreased after infection with HSV-1.

Another mRNA known to degrade is globin mRNA. Using liquid hybridization techniques, we had previously estimated the level of these to be 49 and 15% of control at 2 and 4 h postinfection, respectively (30). To compare these two techniques, the level of globin mRNA was monitored by hybridizing a cloned mouse α-globin gene (31) to total RNA extracted from induced FL cells (Fig. 2). Although the filter

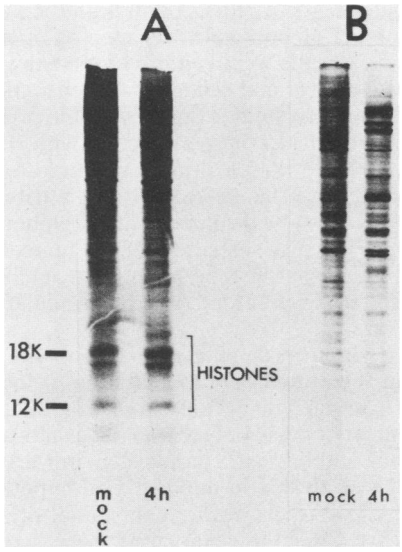

FIG. 1. Histone protein synthesis in noninduced FL cells infected with HSV-1. Noninduced FL cells were infected with HSV-1 (MOI of 10) and incubated with [^{35}S]methionine for 1 h. Whole cell lysates were prepared as described in the text. Proteins were separated by electrophoresis through a 16% sodium dodecyl sulfate-polyacrylamide gel with the buffer system of Laemmli (21). (A) Histone proteins were identified by their comigration with histone calf thymus markers. Mock refers to uninfected cells. (B) Samples shown here were the same as those used in A. The purpose of this figure is to demonstrate the HSV-1-specified proteins. Small proteins, including histones, were no longer in the gel.

hybridization technique is semiquantitative, the results were in good agreement with the previous estimates.

DNA synthesis in FL cells infected with HSV-1. Since histone synthesis and the stability of histone mRNA are tightly coupled with DNA synthesis (for review, see references 20 and 24), it is important to study DNA synthesis in FL cells infected with HSV-1. The rate of thymidine incorporation decreased to 50% by 2 h postinfection and remained at about 50% until 3 h postinfection. Thereafter there was a continuous increase in the rate of DNA synthesis; by 5 h postinfection, the rate exceeded the original level (Fig. 3A). The increase observed after 3 h postinfection most likely reflected the replication of the HSV-1 genome. This was supported by a dot-blot hybridization experiment that detected the increase in the copy number of HSV-1 thymidine kinase gene at 4 h postinfection (Fig. 3B). This pattern of DNA synthesis in HSV-1-infected FL cells was very similar to that obtained with HEp2 cells, a more permissive host (36).

The analysis of DNA synthesis indicated that the continuous presence of histone H3 mRNA was not a direct reflection of active HSV-1 DNA replication. Rather, it could be concluded that histone H3 mRNA level remained unchanged despite a decrease in DNA synthesis.

RNA synthesis in FL cells infected with HSV-1. The absence of a significant decrease in the steady-state level of histone H3 mRNA could be a result of either the stabilization of preexisting histone H3 mRNA or an increased rate of transcription that compensates for the degradation. To differentiate between these possibilities we examined RNA synthesis in isolated nuclei. Nuclei were purified from FL cells infected for 3 h with HSV-1 and labeled with [^{32}P]UTP for 45 min, and the isolated [^{32}P]RNA was hybridized to histone H3 cDNA immobilized onto a membrane filter.

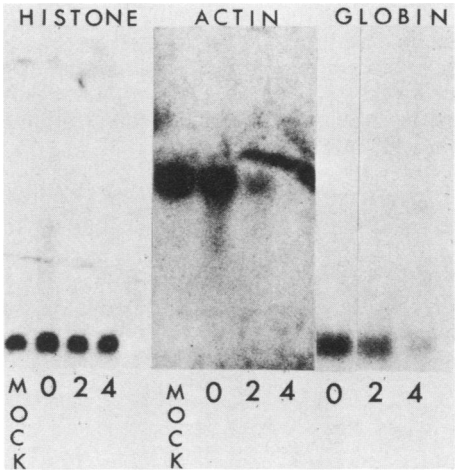

FIG. 2. RNA gel blot hybridization analyses. Total RNA was prepared from FL cells infected with HSV-1 (MOI of 10) at the indicated intervals (hours) postinfection. After denaturation, 20-μg samples in each lane were separated in a 1.5% formaldehyde–agarose gel. After transfer to nitrocellulose filter paper, the RNA was hybridized to ^{32}P-labeled, nick-translated plasmid DNA containing sequences of mouse histone H3 gene, actin cDNA, or globin.

Cloned chicken actin cDNA and HSV-1 thymidine kinase genes were used as controls (Fig. 4A). As expected, the rate of actin RNA synthesis declined, whereas that of HSV-1 thymidine kinase RNA increased. Although this is a semiquantitative procedure, it was obvious that histone H3 RNA synthesis was inhibited to approximately the same extent as that of actin RNA. Measurements of radioactivity retained on the filters confirmed that the synthesis of both actin and histone RNA was about 60% of the control level (data not shown). This is in good agreement with in vivo RNA

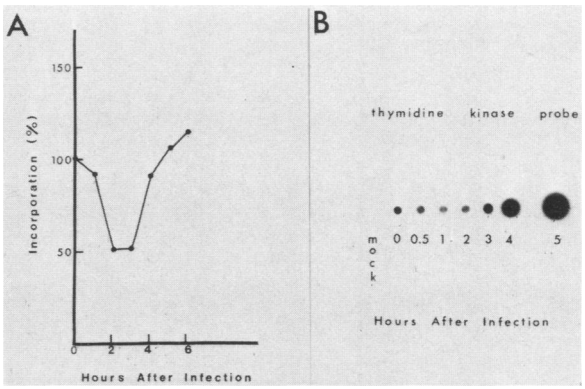

FIG. 3. DNA synthesis in HSV-1-infected FL cells. (A) Incorporation of radioactive thymidine into noninduced FL cells infected with HSV-1. Noninduced FL cells were infected with HSV-1 (MOI of 10) and incubated with [^{3}H]thymidine for 15 min at intervals postinfection. The data are the average of duplicate samples and are expressed as the percent incorporation in infected cells compared with that in mock-infected cells. (B) DNA dot-blot hybridization analysis of HSV-1 thymidine kinase (TK) DNA sequences present in noninduced FL cells infected with HSV-1. DNA was prepared from noninduced FL cells infected with HSV-1 (MOI of 10) at intervals postinfection. After denaturation, 5 g of each sample was dotted onto nitrocellulose filter paper and hybridized to ^{32}P-labeled plasmid DNA containing sequences of the HSV-1 TK gene (specific activity, 7×10^6 cpm/μg of DNA).

synthesis assayed by the incorporation of [^{3}H]uridine into trichloroacetic acid-precipitable material by FL cells (Fig. 4B). From these results, we concluded that the major factor responsible for the persistence of histone H3 mRNA was not an elevated RNA synthesis, but the stabilization of preexisting histone H3 mRNA.

DISCUSSION

To understand the mechanism responsible for the transition from host to viral protein synthesis, attention has been paid to the fate of specific host mRNAs after HSV-1 infection. Studies by Pizer and Beard (33) demonstrated that when polyomavirus-transformed BHK cells were infected with HSV-1 there was a reduction in both the synthesis and steady-state level of polyomavirus-specific mRNA. Subsequently, similar results were reported for adenovirus mRNA in adenovirus-transformed cells (41, 43). Nishioka et al. (28, 30–32) using the FL system, examined globin production and the fate of globin mRNA during infection with HSV-1. Early after infection there was a dramatic decrease in de novo synthesis of globin. This reduction was attributed to two independent events: (i) displacement of globin transcripts from polysomes and (ii) degradation of preexisting globin mRNA. We found that histone H3 mRNA, unlike actin and globin mRNA, was refractory to HSV-1 mediated degradation.

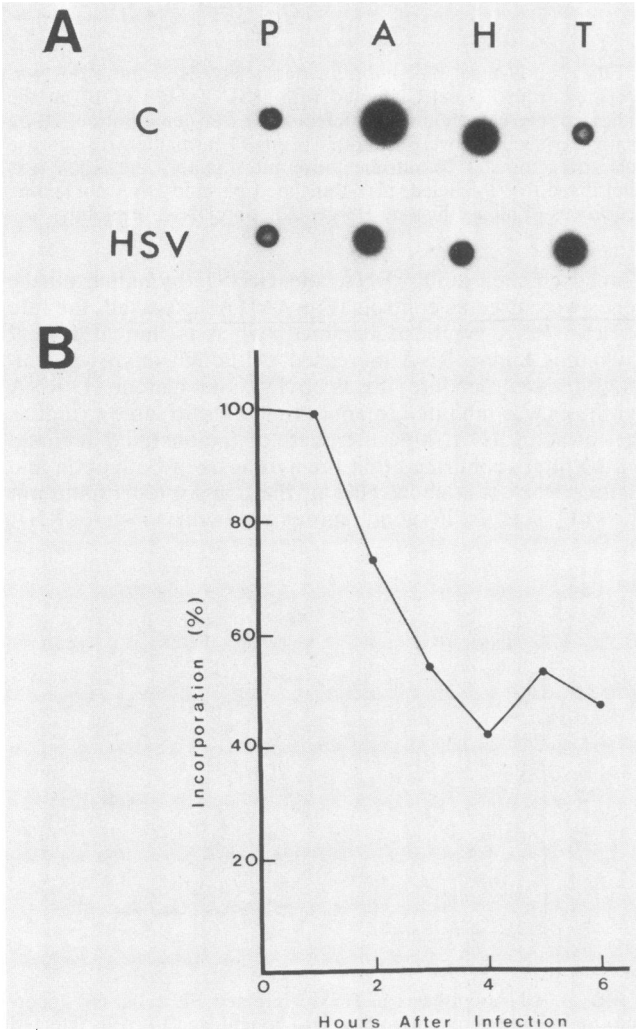

FIG. 4. RNA synthesis in HSV-1-infected FL cells. (A) Detection of specific RNAs by dot-blot hybridization. Recombinant plasmids were immobilized onto a nitrocellulose filter and hybridized to ^{32}P-labeled RNA synthesized in isolated nuclei. Abbreviations: C, mock-infected nuclei; HSV, HSV-1-infected nuclei; P, pBR322; A, actin; H, histone; T, HSV-1 thymidine kinase. (B) Incorporation of radioactive uridine into noninduced FL cells infected with HSV-1. Noninduced FL cells were infected with HSV-1 (MOI of 10) and incubated with [^{3}H]uridine for 20 min at intervals postinfection. The data are the average of duplicate samples and are expressed as the percent incorporation in infected cells compared with that in mock-infected cells.

Most eucaryotic translatable mRNAs contain polyadenylated tracts at the 3′ end, and the addition of these tracts in the nucleus is an enzymatic post-transcriptional event (22). The involvement of these polyadenylated tracts in enhancing the functional stability of cytoplasmic mRNAs has been proposed (47). With this in mind, it was hypothesized that the polyadenylated tracts might be the initial site of nuclease attack and that, after the removal of these sequences, the mRNA molecules quickly degrade. To test this hypothesis histone H3 mRNAs were quantitated during infection because histone mRNAs lack polyadenylated tracts at the 3′ end (1, 13) and may therefore escape the mechanism HSV-1 adopts to degrade host mRNAs. The results demonstrated that histone H3 mRNAs, unlike α-globin and β-actin mRNAs, persisted after infection. Additional support for this hypothesis comes from the work of Nakai et al. (27), who studied the effects of HSV-1 infection on the steady-state levels of mRNA sequences in polyomavirus-transformed BHK cells by hybridization analysis to cDNA of polyadenylated cytoplasmic RNA from uninfected cells. It was reported that after infection there was a dramatic reduction in the cytoplasmic levels of mRNA sequences and that increased amounts of the nonadenylated cytoplasmic RNA of infected cells hybridized to the cDNA. This suggested that HSV-1 infection might induce the deadenylation of host cytoplasmic mRNAs.

There are, however, other possibilities that explain the observed stabilization of histone H3 mRNAs. In most eucaryotic cells, histone synthesis and DNA replication are restricted to the S-phase of the cell cycle, and tightly coupled and histone mRNAs rapidly degrade at the end of S-phase or in S-phase cells treated with inhibitors of DNA synthesis (5, 11, 12, 35, 40). In this connection, it is important to point out that when FL cells were infected with HSV-1, the synthesis of histone persisted at times postinfection when the synthesis of DNA was inhibited. It is known that the rapid degradation of histone mRNAs after the cessation of DNA synthesis was prevented by inhibitors of protein synthesis (6, 8, 12, 38, 42), indicating that de novo protein synthesis was a requirement for the breakdown of histone mRNAs. When FL cells were infected with HSV-1 the synthesis of many host proteins was inhibited, and it is conceivable that among those proteins inhibited, one may be the protein responsible for the rapid degradation of histone mRNAs after the cessation of DNA synthesis. A practical question evolves from the observations that both histone proteins and mRNAs persist in HSV-1 infected cells. What functional role(s), if any, do histones have in infected cells? No answer to this question is available at present. It is known that histones are important not only for the organization of DNA in chromatin, but also for its replication, segregation, and expression in the cell (for review, see reference 20). In light of these facts, it is tempting to

speculate that histones may bind to the HSV-1 genome and contribute to the regulation of HSV-1 gene expression.

In conclusion, two hypotheses have been proposed to explain the mechanism(s) for the demonstrated stabilization of histone mRNAs in HSV-1-infected cells: (i) lack of polyadenylated tracts at the 3′ end and (ii) inhibition of a host protein responsible for activating a cellular RNase specific for histone mRNAs. The results reported in this communication are compatible with these two hypotheses and have built the foundation for future analysis. Finally, it should be pointed out that FL cells are a poor host for HSV-1 (30), and a different picture may emerge when RNA metabolism is examined in more permissive hosts.

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