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Requirement of Protein Synthesis for the Degradation of Host mRNA in Friend Erythroleukemia Cells Infected with Herpes Simplex Virus Type 1

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We describe experiments which demonstrate that shortly after infection of Friend erythroleukemia cells with herpes simplex virus (HSV), polyribosomes dissociate and cellular mRNA degrades. Analysis of infected cell extracts on sucrose density gradients demonstrates that the majority of the polyribosomes have dissociated to monoribosomes at 2 h postinfection. Physical measurements of infected-cell RNAs support this conclusion and demonstrate that the polyadenylated RNAs decrease in size. The degradation of mRNA is apparently a stochastic process as judged by the failure to detect a shift in the $C_rt_{1/2}$ when polyadenylated RNA extracted from infected cells at different times is hybridized to globin complementary DNA. In experiments designed to determine whether dissociation of polyribosomes is sufficient to cause degradation of globin mRNA, the amount of globin mRNA in uninfected cells did not change when cells were treated with NaF or pactamycin at concentrations sufficient to dissociate all polyribosomes. In cells infected with UV-irradiated virus polyribosomes dissociate but globin mRNA does not degrade, suggesting that it is possible to separate dissociation from degradation.

During the course of productive infection, many animal viruses inhibit the synthesis of host-specific proteins. We are using Friend erythroleukemia cells (FL cells) as the host to elucidate the viral mechanisms responsible for this effect in cells infected with herpes simplex virus (HSV). These cells synthesize large amounts of hemoglobin and globin mRNA when grown in the presence of dimethyl sulfoxide (Me_2SO) (10); each of these products is easily identified, globin by its migration in sodium dodecyl sulfate (SDS)-polyacrylamide gels and globin mRNA by hybridization to a complementary DNA (cDNA) copy (30). In a previous study we followed the fate of globin and globin mRNA during the course of productive infection of FL cells by HSV and demonstrated that globin synthesis was rapidly inhibited and that the amount of globin mRNA decreased as the infection proceeded (21).

Host mRNA is not degraded in cells infected with vaccinia virus (29), picornaviruses (5, 9, 36), or vesicular stomatitis virus (VSV) (22). These studies, and our own (21), would suggest that the decrease in abundance of globin mRNA after infection with HSV is a phenomenon unique to HSV. In this communication we demonstrate the following: (i) the shutoff of globin synthesis in vivo is due to dissociation of polysomes, rendering globin mRNA unable to associate with polysomes; (ii) host mRNA is physically degraded after infection; (iii) the effect of HSV on the dissociation of polysomes can be separated from that of the degradation of mRNA; and (iv) expression of the viral genome is required to degrade cellular mRNA.

MATERIALS AND METHODS

Cell and virus. The FL cell line, clone 19 (23), was maintained in Dulbecco-modified Eagle medium supplemented with 15% horse serum (Flow Laboratories). The growth properties of FL cells under our incubation conditions were described previously (Y. Nishioka and S. Silverstein, in press). Hemoglobin synthesis was induced by exposing the cells to 2% Me_2SO for 4.5 days. HSV type 1, strain F, was obtained from B. Roizman. The procedure for infection was as previously described (21).

Analysis of ribosomal profiles in sucrose gradients. The procedures for analysis of ribosomal profiles are based on those described by Engelhardt and Sarnoski (8). Cells were washed with phosphate-buffered saline and resuspended in RSB-K (10 mM Tris [pH 7.2], 10 mM $MgCl_2$, and 10 mM KCl) at 5×10^7 cells per ml. After swelling at 4°C for 5 min, the cells were disrupted with a tightly fitting Dounce homogenizer, and nuclei were removed by centrifugation at 2,500 rpm for 4 min at 4°C. One-tenth milliliter

of the supernatant was layered onto precooled sucrose gradients (15 to 50% in RSB-K), and the gradients were centrifuged at 35,000 rpm for 2.25 h at 4°C in a Beckman SW41 rotor. The elution profiles were obtained utilizing a density gradient fractionator equipped with a UV-flow analyzer (ISCO, Lincoln, Neb.).

Hybridization. Globin mRNA was purified from reticulocytes of DBA mice treated with phenylhydrazine. Briefly, phenol-extracted RNA was fractionated in 15 to 30% sucrose gradients (10 mM Tris, pH 7.0), and polyadenylic acid [poly(A)]-containing RNA in the 9S region was isolated by two cycles of oligodeoxythymidylic acid chromatography. The purity of this material was checked by assaying its biological activity in a wheat germ protein synthesis system, and it was then used as a template to synthesize cDNA with the reverse transcriptase from avian myeloblastosis virus, as described by Kacian and Myers (16). This cDNA back-hybridized to globin mRNA with a $C_rt_{1/2}$ of 10^{-3} mol/s. To determine the amounts of globin mRNA sequences on polyribosomes, total polyribosomal RNA was extracted from infected cells and hybridized to the cDNA according to the procedure described by Ramirez et al. (28).

Isolation of poly(A) RNA. Cytoplasm from HSV-infected cells was prepared by Dounce homogenization of cells that were swollen in RSB-K. Nuclei were removed by centrifugation at 2,000 rpm in a PR-5000 centrifuge. After addition of SDS to 0.5%, cytoplasmic RNA was extracted with phenol and then with chloroform containing 2% isoamyl alcohol. The RNA was precipitated with ethanol after the addition of NaCl to 0.3 M. This material was resuspended in 10 mM Tris-1 mM EDTA (pH 7.4), and SDS and NaCl were added to 0.2% and 0.4 M, respectively. The poly(A)-containing RNA was bound to oligodeoxythymidylic acid-cellulose (Collaborative Research, T-3), exhaustively washed with the same buffer, and then eluted with 10 mM Tris-1 mM EDTA-0.2% SDS, pH 7.4. The eluate was brought to 0.4 M in NaCl and reselected on oligodeoxythymidylic acid-cellulose. This second eluate was precipitated with ethanol, centrifuged at 10,000 rpm in the HB-4 rotor of a Sorvall centrifuge, and resuspended in water prior to hybridization.

Distribution of poly(A) sequences in sucrose gradients. Cytoplasmic RNA was extracted with phenol and stored under ethanol at −20°C prior to use. About 300 μg of the cytoplasmic RNA was layered onto precooled 15 to 30% sucrose gradients in 10 mM Tris (pH 7.0) and 0.1 N NaCl. After centrifugation at 35,000 rpm for 24 h at 4°C in the SW41 rotor, the gradients were fractionated through an ISCO UV analyzer to monitor the changes in absorbance at 254 nm. From each fraction a 0.3-ml sample was removed and hybridized to 3,000 cpm of [^{3}H]polyuridylic acid, a gift from C. Milcarek [400 cpm is equivalent to 1 ng of poly(A)], in 0.5 ml of 0.3 M NaCl, 0.1% SDS, and 30 mM Tris (pH 7.4). After 10 min of incubation at 45°C, the reaction mixtures were quickly cooled to 0°C and diluted with 2.5 ml of 10 mM Tris buffer (pH 7.4) containing 0.3 M NaCl, and nonhybridized [^{3}H]polyuridylic acid was digested with pancreatic RNase (20 μg/ml) for 10 min at 0°C. Trichloroacetic acid-precipitable material was collected onto nitrocellulose filters, and the radioactivity was determined in a Packard Tri-Carb scintillation counter.

Analysis of protein synthesis by SDS-acrylamide gel electrophoresis. At intervals, 5×10^6 cells were suspended in 0.5 ml of Dulbecco-modified Eagle medium containing 0.1× the normal concentration of leucine, valine, and threonine, 2% dialyzed horse serum, and 0.5 μCi each of [^{14}C]leucine, valine, and threonine. The cells were incubated at 37°C for 30 min and washed with phosphate-buffered saline, and the cytoplasm was prepared by treating the cells with 0.5% Triton X-100 in 10 mM HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.8) containing 5 mM NaCl, 3 mM $MgCl_2$, and 10% sucrose, as described by Bastos and Aviv (2). After removing the nuclei by centrifugation, the cytoplasm was denatured by boiling for 2 min in a solution containing 50 mM Tris (pH 7.0), 2% SDS, 5% β-mercaptoethanol, 5% sucrose, and 0.005% bromophenol blue and subjected to electrophoresis through 10 to 16% gradient SDS-acrylamide gels, using the buffer system of Laemmli (17). The gels were then processed for fluorography (4), dried under vacuum, and exposed to Cronex 2DC X-ray films.

Chemicals. The following chemicals were used in this investigation: cycloheximide (Calbiochem, San Diego, Calif.), emetine (Sigma, St. Louis, Mo.), sodium fluoride (Fisher, Fair Lawn, N.J.), and pactamycin (Upjohn, Kalamazoo, Mich.).

RESULTS

Quantitation of globin mRNA on polysomes. At early times after productive infection by HSV there is a rapid inhibition of host-directed protein synthesis accompanied by dissociation of a major fraction of the polyribosomes into monoribosomes (34, 35). The changeover from cell-specific to virus-specific polypeptide synthesis in FL cells infected with HSV is extremely rapid (21). Therefore, it was of interest to examine the fate of host mRNA on the polyribosomes of infected cells that appeared to be synthesizing virus-specified polypeptides. Figure 1 shows the polyribosome profile of infected cells at 2 h postinfection compared with a mock-infected control culture. The polyribosomes have dissociated into monoribosomes at a time when the rate of protein synthesis in infected cells is about 40% of the level observed in mock-infected cells (21).

The amount of globin mRNA that persisted on polyribosomes after infection was determined by extracting polyribosomal RNA at 2 and 4 h postinfection and hybridizing it to cDNA. The kinetics of hybridization are shown in Fig. 2 and are summarized in Table 1. Globin mRNA sequences rapidly disappeared from the polyribosomes. By 2 h postinfection, only 5% of the sequences that were present prior to infection persisted on the polyribosomes. By 4 h postin-

fection, globin mRNA could not be detected on the polyribosomes (Table 1, experiment 4). These data are compatible with our previous observations, which demonstrated that the shutoff of globin synthesis is extremely efficient shortly after HSV infection (21). Previously, we demonstrated that at 2 and 4 h postinfection, 50 and 15% of the total globin mRNA sequences remained. The rate of decrease in the amount of hybridizable sequences on polyribosomes is 10-

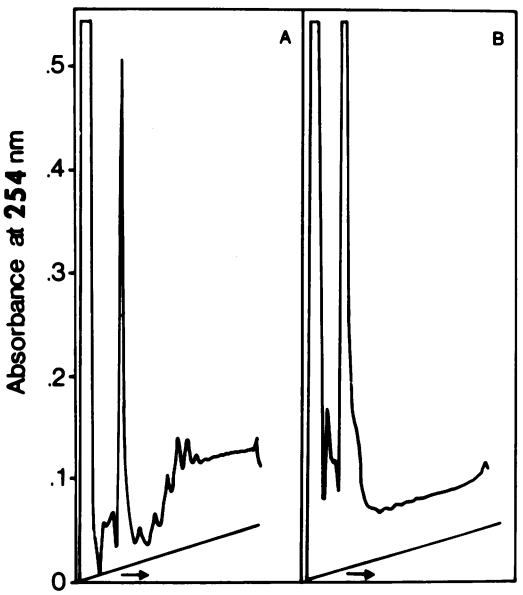

FIG. 1. *Dissociation of polysomes by HSV. Cytoplasm prepared from 5 × 10⁶ cells was layered onto 15 to 50% (wt/vol) sucrose gradients in RSB-K and centrifuged for 2.25 h at 35,000 rpm in a Beckman SW41 rotor. The absorbance profiles were obtained with an ISCO density gradient fractionator. (A) Mock-infected control; (B) 2 h after infection with HSV at an MOI of 10.*

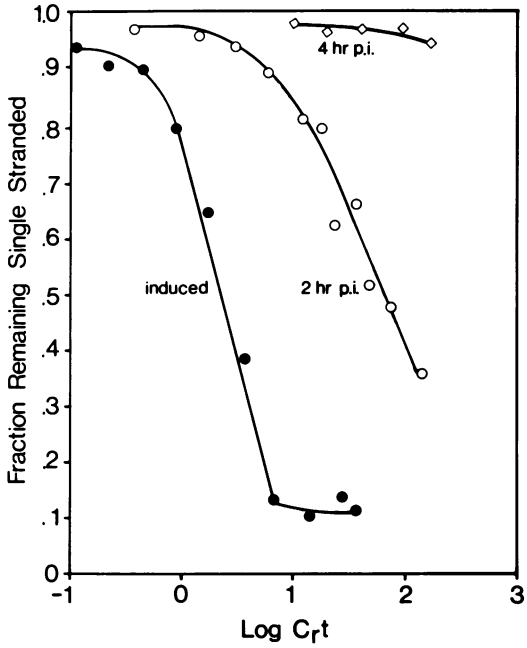

FIG. 2. *Hybridization kinetics of polyribosomal RNA with globin cDNA. At intervals postinfection, polyribosomal RNA was extracted with phenol and hybridized to globin cDNA. The results are plotted as a function of the percentage of cDNA remaining single versus C,t (concentration of RNA in moles of nucleotide per liter per time in seconds). Symbols: (●) zero time; (○) 2 h postinfection (p.i.); (◇) 4 h postinfection.*

TABLE 1. *Fate of globin mRNA in infected and uninfected cells*

Expt	$C_rt_{1/2}$				% Remaining		
	Control	2 h	3 h	4 h	2 h	3 h	4 h
1. Pactamycin[a]	8.2×10^0			8.2×10^0			100
2. NaF[b]	4.6×10^0			4.6×10^0			100
3. HSV cytoplasmic RNA	3.5×10^0	9.5×10^0		3.0×10^1	36		12
4. HSV polyribosomal RNA	3.0×10^0	6×10^1		ND[c]	5		1
5. HSV poly(A)$^+$ RNA	1.5×10^{-1}	1.5×10^{-1}		1.5×10^{-1}	100		100
6. HSV + NaF[d]	3.4×10^0		4.2×10^0			83	
7. HSV MOI = 10	1.0×10^1	3.5×10^1			29		
8. HSV MOI = 100	1.0×10^1	3.5×10^1			29		
9. HSV irradiated[e]	3.6×10^0			5.0×10^0			80

[a] Uninfected cells were treated with 10^{-5} M pactamycin for 4 h to dissociate polyribosomes; cytoplasmic RNA was extracted and hybridized to globin cDNA.

[b] Uninfected cells were treated with 5 mM NaF for 4 h, and cytoplasmic RNA was hybridized to globin cDNA.

[c] ND, Not determined; see Fig. 2 for hybridization curve.

[d] Cells were infected at an MOI of 10 in the presence of 7.5 mm NaF; in this experiment the $C_rt_{1/2}$ of infected-cell RNA in the absence of NaF was 1.3×10^1.

[e] Cytoplasmic RNA from cells infected with UV-irradiated virus was hybridized to globin cDNA; in the control, cytoplasmic RNA from cells infected for 4 h with unirradiated virus was hybridized to globin cDNA.

fold faster than that of total globin mRNA.

Effects of pactamycin and sodium fluoride on polyribosomes and globin mRNA. Because infection of FL cells with HSV results in dissociation of polyribosomes and the degradation of globin mRNA, we sought to determine if there was any relationship between these events. To examine the possibility that release from polyribosomes was sufficient to allow mRNA degradation to occur, polyribosomes of uninfected Me_2SO-treated FL cells were dissociated with either 5 mM NaF or 10 μM pactamycin; each of these agents inhibits initiation of protein synthesis (7, 19). RNA was extracted from cells treated with these drugs for 4 h and hybridized to globin cDNA. The kinetics of hybridization demonstrate that dissociation of polyribosomes is not sufficient to account for the rapid degradation of globin mRNA seen in HSV-infected cells because there was no change in the $C_rt_{1/2}$ of globin mRNA in cells exposed to either NaF or pactamycin for 4 h (Table 1, experiments 1 and 2).

mRNA degradation is stochastic. We designed two experiments to determine whether globin mRNA was uniquely degraded or if it was representative of the total mRNA population. In the first, total polyadenylated RNA was isolated from infected cells at 2 and 4 h postinfection, and the kinetics of hybridization of this class of RNA to globin cDNA was compared with the kinetics of hybridization of total polyadenylated RNA from mock-infected cells. The rationale for the interpretation of this experiment is as follows; if degradation is a stochastic process, then globin mRNA should be representative of the total adenylated mRNA population. Under these conditions the $C_tt_{1/2}$ should not change because, although globin mRNA is degraded, its abundance relative to other polyadenylated mRNA's will not change. Alternatively, if globin mRNA is selectively degraded, then the $C_tt_{1/2}$ will shift to reflect this selective degradation process. The data from one such experiment are presented in Table 1 (experiment 5). The lack of an apparent shift in $C_rt_{1/2}$ suggests that degradation is stochastic and that globin mRNA is merely a representative subset of the total population of adenylated mRNA's. In a second experiment, we measured the distribution of poly(A) sequences at 4 h postinfection. In this experiment cytoplasmic RNA was centrifuged through a sucrose gradient. The gradients were fractionated, and poly(A) sequences were assayed by hybridization to [^{3}H]polyuridylic acid. The results from one such experiment (Fig. 3) reveal that uninfected FL cell poly(A)$^+$-containing mRNA is heterogeneous in size, with

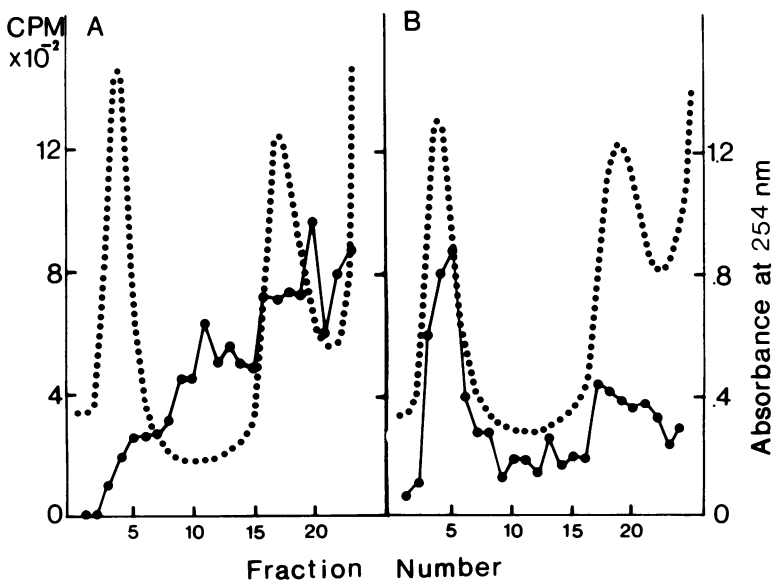

FIG. 3. *Size of polyadenylated RNA after infection. About 300 μg of cytoplasmic RNA was layered onto 15 to 30% (wt/vol) sucrose gradients in 10 mM Tris (pH 7.0) containing 0.1 N NaCl and 1 mM EDTA. After centrifugation at 35,000 rpm for 24 h in an SW41 rotor, the gradients were fractionated. From each fraction a 0.3-ml sample was removed and hybridized to 3,000 cpm of [^{3}H]poly-(U) [400 cpm is equivalent to 1 ng of poly(A)]. Symbols: (●) Absorbance at 254 nm; (●——●) cpm. Sedimentation is from left to right; the optical density markers are 4S and 18S RNA, respectively. (A) RNA from uninfected cells; (B) RNA from cells infected with HSV for 4 h.*

a distinct peak at 9S corresponding to globin mRNA (30). As predicted, infection with HSV results in a dramatic change in the distribution of poly(A) sequences (Fig. 3). The 9S peak disappears, and a major peak of poly(A)-containing RNA appears in the 4S region of the gradient. These results provide direct physical evidence for the degradation of cellular mRNA's in FL cells infected with HSV.

Degradation is MOI independent. To determine whether degradation of cellular mRNA is mediated by a virion component or if expression of genetic information is required, the effect of multiplicity of infection (MOI) on the rate of degradation of globin mRNA was examined. Cells were infected with either 10 or 100 PFU/cell, and 2 h later the cells were harvested and cytoplasmic RNA was isolated by phenol extraction. The relative amount of globin mRNA was measured by hybridization to cDNA. The results (experiments 7 and 8, Table 1) demonstrate that at 2 h postinfection there is no difference in the rate of hybridization of globin RNA to cDNA in cells infected with either 10 or 100 PFU of HSV per cell. Analysis of the kinetics of hybridization reveal that the same amount of globin sequences were degraded at either MOI (Table 1). Because there is no increase in the rate of degradation over a 10-fold increase in MOI, we postulate that degradation results from expression of the virus genome and not from a virion-associated component.

Degradation requires functional gene products. The requirement for expression of an early virus function that results in degradation of cellular mRNA was examined by two independent approaches. In the first, cells were infected with HSV whose titer was reduced 4 logs by exposure to UV irradiation. In cells infected at an MOI of 100 (original titer), polyribosomes dissociate, and the rate of amino acid incorporation decreases, as would be expected from the polyribosome profiles. When an equivalent amount of radioactivity from cells infected with UV-irradiated and unirradiated virus is subjected to electrophoresis through SDS-polyacrylamide gels, we note that the pattern of proteins synthesized remains unchanged at 2 h postinfection (Fig. 4). These results suggest that there is very little, if any, virus-specified protein synthesis. Globin continues to be synthesized, and no virus-specified polypeptides are detected in autoradiographs of cells pulse-labeled with ^{14}C-amino acids for 30 min at 2 h postinfection. When RNA extracted from cells infected with UV-irradiated virus for 4 h is hybridized to globin cDNA, no significant decrease in the quantity of globin mRNA sequences is detected. By comparison, in cells infected with unirradiated virus, only about 15% of the globin sequences remain (Table 1, experiments 3 and 9). These results are consistent with a model that requires expression of an early virus function to degrade cellular mRNA. In addition, they imply that dissociation of polyribosomes can occur in the absence of expression of an early function.

An alternate approach to show that an early protein mediates degradation is outlined in Fig. 5. In this experiment cells were infected in the presence of 100 μg of cycloheximide per ml, and at various times after infection the cycloheximide was removed by washing the cells with prewarmed medium. Under these conditions, virus-specified protein synthesis is synchronized, and only α polypeptides are synthesized immediately after removal of the drug (14). The results of inhibiting protein synthesis, reversing the inhibition, and subsequently blocking protein synthesis again are summarized in Fig. 5 and Table 2. Briefly, inhibition of virus-directed protein synthesis with cycloheximide prevents degradation of cellular mRNA for as long as 6 h postinfection. Within 2 h after reversal, the amount of globin mRNA that remains is the same as that in cells infected for 2 h with HSV in the absence of cycloheximide (cf. H and E). If at 2 h after reversal cycloheximide is restored to the infected cell culture, then the rate of degradation decreases when compared with a reversed culture (cf. F and G). These data demonstrate that synthesis of an early protein, probably of the α class, is required to degrade cellular mRNA when FL cells are infected with HSV. They also suggest that the protein responsible for degradation is likely to be somewhat labile, because in the absence of de novo synthesis the rate of degradation of globin mRNA decreases. Degradation of FL cell mRNA is prevented when either emetine, which primarily inhibits polypeptide elongation (24), or NaF is used to inhibit protein synthesis (data not shown).

Separation of dissociation from degradation. The preceding experiments demonstrate that the synthesis of early virus-specified polypeptides is required to degrade mRNA in FL cells. In FL cells infected with UV-irradiated virus, polyribosomes dissociate (Fig. 4), but globin mRNA is not degraded (Table 2, experiment 9). These data suggest that the virion supplies a product that is responsible for dissociation. To examine this possibility, [^{3}H]thymidine-labeled virus was isolated on dextran gradients (32) and heated to 56°C for 10 min; under these conditions the titer decreased from 3.8×10^{10} to 8×10^6 PFU/ml.

Heated and untreated [^{3}H]thymidine-labeled virus was allowed to adsorb to cells in suspension for 1 h. At intervals, a sample of the cells was

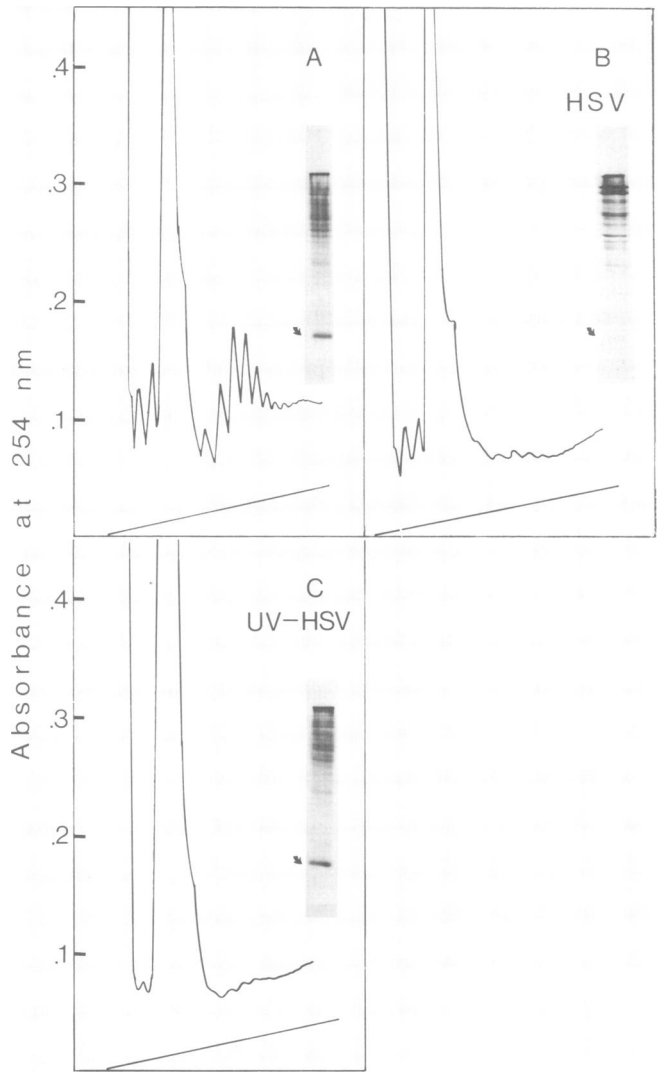

FIG. 4. *Effects of UV-inactivated HSV on polysome profile and pattern of protein synthesis. Induced FL cells were infected with HSV at an MOI of 10 or UV-inactivated HSV at the original MOI of 100. At 2 h postinfection a portion of each culture was processed to monitor polysomal profiles in sucrose gradients. The rest of the cells were pulse-labeled with ^{14}C-amino acids for 30 min, and the cytoplasmic proteins were separated by electrophoresis through 10 to 16% gradient SDS-acrylamide gel, processed for fluorography, dried under vacuum, and exposed to Cronex 2DC X-ray film. The arrows indicate the position of globin. (A) Control; (B) HSV; (C) UV-inactivated HSV.*

removed and washed twice with phosphate-buffered saline, and the amount of cell-associated virus was monitored by measuring trichloroacetic acid-insoluble counts that pelleted with the washed cells. The adsorption kinetics and amount of label that pelleted with washed cells were the same for heated and unheated virus. When FL cells are infected with heated HSV, the polyribosomes do not dissociate (Fig. 6). These data suggest that dissociation of polyribosomes requires a heat-labile, virion-associated function.

DISCUSSION

In a previous publication we demonstrated that when FL cells exposed to 2% Me_2SO were infected with HSV the synthesis of globin was rapidly shut off and the amount of globin mRNA decreased by 50% by 2 h postinfection (21). In this paper we demonstrate that globin mRNA is

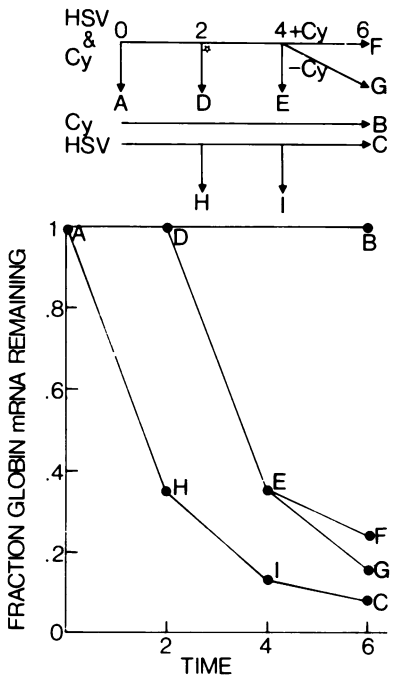

FIG. 5. *Protein synthesis is required for degradation of mRNA. Me_2SO-induced FL cells were infected in the presence or absence of cycloheximide, and the amount of globin mRNA was quantitated by hybridization with cDNA at intervals postinfection. In the first part of this experiment cells were infected with HSV (MOI = 10) in the presence of 100 μg of cycloheximide per ml [(A) uninfected cell; (D) infected in the presence of cycloheximide] and sampled at 2 h postinfection; the star refers to the removal of cycloheximide. (E) Cycloheximide was removed at 2 h postinfection, and the infected cells were cultured in complete media until the total elapsed time was 4 h; (F) cells were treated as described in (E), and at 4 h the drug was restored and the cells were allowed to incubate in the presence of drug for an additional 2 h; (G) the cells were treated as in (E) and allowed to incubate for an additional 2 h. In the second part of this experiment (B), infected cells were incubated in the presence of 50 μg of cycloheximide per ml for 6 h. In the third part of this experiment cells were infected and samples were removed for hybridization at (H) 2, (I) 4, and (C) 6 h postinfection. The plot schematically represents the amount of globin mRNA remaining in each instance as a function of the amount of globin mRNA present in (A).*

representative of the total mRNA population, early virus-specified protein synthesis is required for degradation, and dissociation of cellular polyribosomes appears to be mediated by a virion-associated component. By analyzing the change in sedimentation rate of polyadenylated mRNA after virus infection, we concluded that the loss of hybridizable sequences results from physical degradation of the mRNA, and that most of the poly(A)-containing mRNA's are degraded after infection. Pizer and Beard (26) also quantitated the amount of polyoma virus-specific mRNA's present in transformed cells infected with HSV and concluded that their abundance decreased. The results reported here extend our previous findings (21) and support theirs. Therefore, it appears plausible to postulate that degradation of polyadenylated cellular mRNA's is a feature common to cells infected with HSV. Our suggestion that degradation is apparently a stochastic process is supported by data which demonstrate that the relative abundance of adenylated globin mRNA remains con-

TABLE 2. *Degradation of globin mRNA requires the synthesis of α polypeptides*

Condition	Relative amt of globin mRNA at h:			
	0	2	4	6
HSV	1.00	0.35	0.13	0.08
HSV + cycloheximide	1.00	1.00		1.00
HSV + cycloheximide reversed[a]	1.00	1.00	0.35	0.18
HSV + cycloheximide reversed[b]	1.00	1.00	0.35	0.24

[a] Cells were infected in the presence of 100 μg of cycloheximide per ml; at 2 h the cycloheximide was removed, and 2 h later cytoplasmic RNA was extracted and assayed for globin sequences by hybridization to cDNA.

[b] Cells infected in the presence of 100 μg of cycloheximide per ml were reversed at 2 h postinfection, and after an additional 2 h of incubation in drug-free medium 100 g of cycloheximide per ml was added back to the infected culture. At 6 h postinfection cytoplasmic RNA was extracted, and globin sequences were assayed by hybridization to cDNA.

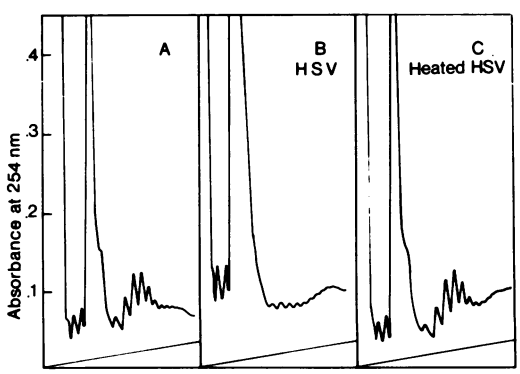

FIG. 6. *Polysome profiles of FL cells infected with heat-inactivated HSV. FL cells were infected with HSV (MOI = 10) or heated HSF (original MOI = 100) for 2 h, and the optical profiles of cytoplasmic extracts were displayed in 15 to 50% (wt/vol) sucrose gradients. (A) Mock-infected control; (B) HSV; (C) heated HSV.*

stant at 2 and 4 h postinfection. These data do not exclude the possibility that different cellular mRNA's are degraded at different rates.

The rapid decline in cell-specific protein synthesis in general and globin in particular can be explained by the disappearance of cellular mRNA's from the polyribosomes in HSV-infected cells. The data in Fig. 2 demonstrate that globin mRNA is quantitatively released from polyribosomes by 4 h postinfection. From this experiment we infer that cellular mRNA's are released from polyribosomes prior to their degradation in the cytoplasmic space.

Previous studies from a number of laboratories demonstrated that some animal viruses possess virion-associated inhibitors which effectively shut off host protein synthesis. For example, adenovirus (18), vaccinia virus (11, 20, 31), and VSV (15, 37) all can shut off cellular protein synthesis without expressing early virus functions. Alternatively, the picornaviruses appear to require the synthesis of a virus-specified function to arrest the host (1, 6, 25, 36). The possibility that a picornavirus structural component contributes to shutoff has not been completely dismissed (12, 27, 33). Infection with HSV results in the rapid shutoff of host protein synthesis and is accompanied by dissociation of the polyribosomes (13, 34, 35). The dissociated polyribosomes are subsequently recruited to form polyribosomes that are predominantly synthesizing virus-specified polypeptides (S. Silverstein and D. L. Engelhardt, submitted for publication). Earlier studies (3, 34) reported that protein synthesis is required for herpesviruses to inhibit host functions. In this study we demonstrate that UV-irradiated virus is effective at dissociating polyribosomes in the absence of any apparent virus-specified polypeptide synthesis.

We have not ruled out the possibility that only a small portion of the genome is expressed and that a protein required in catalytic amounts mediates polyribosome dissociation. However, the observation that heated virus effectively adsorbs to cells without resulting in dissociation of polyribosome suggests that dissociation is mediated by a heat-labile, virion-associated activity. The mechanism responsible for polyribosome dissociation remains open to further investigation.

In independent experiments we demonstrated that HSV-specified polypeptide synthesis was required to degrade cellular mRNA. In the first of these experiments we demonstrated that in cells doubly infected with HSV and VSV, no HSV-specified polypeptides could be detected and globin mRNA was not degraded (unpublished observation). In this paper, virus-specified protein synthesis was blocked by the addition of cycloheximide. Under these conditions degradation of mRNA does not occur. If the block is released, globin mRNA is subsequently degraded; when the block is restored, the rate of degradation decreases when compared with cells whose cycloheximide block is reversed.

In summary, we have described two events that occur early after HSV infection of FL cells. First, the dissociation of cellular polyribosomes appears to be mediated by a heat-labile, virion-associated function. Additional experiments to support this conclusion are based on the effect of superinfecting FL cells already infected with VSV. Infection of FL cells with VSV does not drastically alter the amount of ribosomes present as polyribosomes (22). When these VSV-infected cells are superinfected with HSV, (i) no HSV-specified polypeptide synthesis occurs, and (ii) globin mRNA is not degraded; however, (iii) polyribosomes are dissociated. These experiments strengthen our hypothesis that dissociation is mediated by a virion-associated function, because we are unable to detect the synthesis of any HSV-specified polypeptides and cannot detect any degradation of globin mRNA. The second early event, degradation of cellular mRNA, requires expression of the virus genome. These data are substantiated by experiments using irradiated virus and the cycloheximide experiments wherein we demonstrated that degradation of mRNA requires synthesis of a virus-specified protein.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Baltimore, D., R. M. Franklin, and J. Callender.** 1963. Mengovirus-induced inhibition of host ribonucleic acid and protein synthesis. Biochim. Biophys. Acta **76**:425–430.
2. **Bastos, R. N., and H. Aviv.** 1977. Globin RNA precursor molecules; biosynthesis and processing in erythroid cells. Cell **11**:641–650.
3. **Ben-Porat, T., T. Rakusanova, and A. S. Kaplan.** 1971. Early functions of the genome of herpes virus. II. Inhibition of the formation of cell-specific polysomes. Virology **46**:890–899.
4. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
5. **Colby, D. S., V. Finnerty, and J. Lucas-Lenard.** 1974. Fate of mRNA of L-cells infected with mengovirus. J. Virol. **13**:858–869.
6. **Collins, F. D., and W. K. Roberts.** 1972. Mechanisms of mengovirus-induced cell injury in L cells: use of inhibitors of protein synthesis to dissociate virus-specific events. J. Virol. **10**:969–978.
7. **Colombo, B., L. Felicetti, and C. Baglioni.** 1966. Inhibition of protein synthesis in reticulocytes by antibiotics. I. Effects of polysomes. Biochim. Biophys. Acta **119**:109–119.

8. **Engelhardt, D. L., and J. Sarnoski.** 1975. Variations in the cell-free translating apparatus of cultured animal cells as a function of time during cell growth. J. Cell. Physiol. **86**:15–30.
9. **Fernandez-Munoz, R., and J. E. Darnell.** 1976. Structural difference between the 5′ termini of viral and cellular mRNA in poliovirus infected cells: possible basis for the inhibition of host protein synthesis. J. Virol. **18**:719–726.
10. **Friend, C., W. Scher, J. G. Holland, and T. Sato.** 1971. Hemoglobin synthesis in murine virus-induced leukemic cells *in vitro*: stimulation of erythroid differentiation by dimethyl sulfoxide. Proc. Natl. Acad. Sci. U.S.A. **68**:378–382.
11. **Hanafusa, H.** 1960. Killing of L-cells by heat- and UV-inactivated vaccinia virus. Biken J. **3**:191–199.
12. **Holland, J.** 1964. Inhibition of host cell macromolecular synthesis by high multiplicities of poliovirus under conditions preventing virus synthesis. J. Mol. Biol. **8**:574–581.
13. **Honess, R. W., and B. Roizman.** 1973. Proteins specified by herpes simplex virus. XI. Identification and relative molar rates of synthesis of structural and nonstructural herpes virus polypeptides in the infected cell. J. Virol. **12**:1347–1365.
14. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpes virus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **14**:8–19.
15. **Huang, A., and R. R. Wagner.** 1965. Inhibition of cellular RNA synthesis by nonreplicating vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. **54**:1579–1584.
16. **Kacian, D., and J. C. Myers.** 1976. Synthesis of extensive, possibly complete, DNA copies of poliovirus RNA in high yields and high specific activities. Proc. Natl. Acad. Sci. U.S.A. **73**:2191–2195.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–684.
18. **Levine, A., and H. Ginsberg.** 1967. Mechanism by which fiber antigen inhibits multiplication of type 5 adenovirus. J. Virol. **1**:747–757.
19. **Marks, P. A., E. R. Burka, F. M. Conconi, W. Perk, and R. A. Rifkind.** 1965. Polyribosome dissociation and formation in intact reticulocytes with conservation of messenger ribonucleic acid. Proc. Natl. Acad. Sci. U.S.A. **53**:1437–1443.
20. **Moss, B.** 1968. Inhibition of HeLa cell protein synthesis by the vaccinia virion. J. Virol. **2**:1028–1036.
21. **Nishioka, Y., and S. Silverstein.** 1977. Degradation of cellular mRNA during infection by herpes simplex virus. Proc. Natl. Acad. Sci. U.S.A. **74**:2370–2374.
22. **Nishioka, Y., and S. Silverstein.** 1978. Alterations in the protein synthetic apparatus of Friend erythroleukemia cells infected with vesicular stomatitis virus or herpes simplex virus. J. Virol. **25**:422–426.
23. **Ohta, Y., M. Tanaka, M. Terada, O. J. Miller, A. Bank, P. A. Marks, and R. Rifkind.** 1976. Erythroid cell differentiation; murine erythroleukemia cell variant with unique patterns of induction by polar compounds. Proc. Natl. Acad. Sci. U.S.A. **73**:1232–1236.
24. **Oleinick, N. L.** 1977. Initiation and elongation of protein synthesis in growing cells; differential inhibition by cycloheximide and emetine. Arch. Biochem. Biophys. **182**:171–180.
25. **Penman, S., and D. Summers.** 1965. Effects on host cell metabolism following synchronous infection with poliovirus. Virology **27**:614–620.
26. **Pizer, L. I., and P. Beard.** 1976. The effect of herpes virus infection on mRNA in polyoma virus transformed cells. Virology **75**:477–480.
27. **Racevskis, J., S. S. Kerwar, and G. Koch.** 1976. Inhibition of protein synthesis in reticulocytes lysates by poliovirus. J. Gen. Virol. **31**:135–138.
28. **Ramirez, F., R. Gambino, G. M. Maniatis, R. A. Rifkind, P. A. Marks, and A. Bank.** 1975. Changes in globin messenger RNA content during erythroid cell differentiation. J. Biol. Chem. **250**:6054–6058.
29. **Rosemond-Hornbeak, H., and B. Moss.** 1975. Inhibition of host protein synthesis by vaccinia virus: fate of cell mRNA and synthesis of small poly(A)-rich polyribonucleotides in the presence of actinomycin D. J. Virol. **16**:34–42.
30. **Ross, J., Y. Ikawa, and P. Leder.** 1972. Globin messenger-RNA induction during erythroid differentiation of cultured leukemia cells. Proc. Natl. Acad. Sci. U.S.A. **69**:3620–3623.
31. **Shatkin, A. J.** 1963. Actinomycin D and vaccinia virus infection in HeLa cells. Nature (London) **199**:357–358.
32. **Spears, P. G., and B. Roizman.** 1972. Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpesvirion. J. Virol. **9**:143–159.
33. **Steiner-Pryor, A., and P. Cooper.** 1973. Temperature sensitive poliovirus mutants defective in repression of host protein synthesis are also defective in structural protein. J. Gen. Virol. **21**:215–225.
34. **Sydiskis, R. J., and B. Roizman.** 1966. Polysomes and protein synthesis in cells infected with a DNA virus. Science **153**:76–78.
35. **Sydiskis, R. J., and B. Roizman.** 1967. The disaggregation of host polyribosomes in productive and abortive infection with herpes simplex virus. Virology **32**:678–686.
36. **Willems, M., and S. Penman.** 1966. The mechanism of host cell protein synthesis inhibition by poliovirus. Virology **30**:355–367.
37. **Yaoi, Y., H. Mitsui, and M. Amano.** 1970. Effect of UV-inactivated vesicular stomatitis virus on nucleic acid synthesis in chick embryo cells. J. Gen. Virol. **8**:165–172.