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Herpes Simplex Virus Types 1 and 2 Induce Shutoff of Host Protein Synthesis by Different Mechanisms in Friend Erythroleukemia Cells

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Herpes simplex virus type 1 (HSV-1) and HSV-2 disrupt host protein synthesis after viral infection. We have treated both viral types with agents which prevent transcription of the viral genome and used these treated viruses to infect induced Friend erythroleukemia cells. By measuring the changes in globin synthesis after infection, we have determined whether expression of the viral genome precedes the shutoff of host protein synthesis or whether the inhibitor molecule enters the cells as part of the virion. HSV-2-induced shutoff of host protein synthesis was insensitive to the effects of shortwave (254-nm) UV light and actinomycin D. Both of the treatments inhibited HSV-1-induced host protein shutoff. Likewise, treatment of HSV-1 with the cross-linking agent 4,5',8-trimethylpsoralen and longwave (360-nm) UV light prevented HSV-1 from inhibiting cellular protein synthesis. Treatment of HSV-2 with 4,5',8-trimethylpsoralen did not affect the ability of the virus to interfere with host protein synthesis, except at the highest doses of longwave UV light. It was determined that the highest longwave UV dosage damaged the HSV-2 virion as well as cross-linking the viral DNA. The results suggest that HSV-2 uses a virion-associated component to inhibit host protein synthesis and that HSV-1 requires the expression of the viral genome to cause cellular protein synthesis shutoff.

A number of eucaryotic viruses have been shown to disrupt host protein synthesis after infection. In some, such as adenovirus (15), reovirus (B. Fields, personal communication), and vesicular stomatitis virus (2, 38), a virion-associated component causes shutoff of cellular protein synthesis. Others, such as the picornaviruses (8, 21), require the synthesis of virus-specified proteins to inhibit host protein synthesis, or, as with vaccinia virus, synthesize a viral RNA which induces the arrest of cellular protein synthesis (1). The effects of viral infection on cellular macromolecular synthesis induced by Herpes simplex viruses type 1 (HSV-1) or HSV-2 have been extensively documented (10, 18, 22–24, 34). Shortly after infection with either HSV-1 or HSV-2, cellular polysomes disaggregate (10, 19, 34, 35), and host protein synthesis begins an irreversible decline (10, 18, 23, 34). In addition, HSV-1 causes the dissociation of cellular mRNA from the polysomes (19), degrades host RNA (17, 19, 32), inhibits the synthesis of cellular DNA (24) and RNA (32), and reduces glycosylation of cellular proteins (30).

A consistent observation has been that HSV-2 strains shut down cellular protein synthesis much more rapidly and completely than HSV-1 strains (22, 23). Several early studies provided an adequate explanation for this difference. It was observed that viral protein synthesis was a prerequisite for inhibition of host protein synthesis with HSV-1 (34), but not with HSV-2, where a virion component was implicated in the shutoff process (10). More recent investigations have raised doubts concerning this simple picture of HSV-induced shutoff. Nishioka and Silverstein reported two distinct shutoff functions in HSV-1 (19). One, a virion-associated component, causes extensive disaggregation of host polyribosomes, but surprisingly little loss of globin synthesis in induced Friend erythroleukemia cells (FL cells). A second function, requiring expression of the viral genome, causes the degradation of host mRNAs and concomitant shutoff of host protein synthesis. Fenwick and Clark, using the *ts*B7 temperature-sensitive mutant of HSV-1 HFEM and Vero cells, have also reported on two distinct stages of viral-induced shutoff of protein synthesis, which they label "early" and "delayed" shutoff (9). Delayed shutoff requires expression of viral genes and corresponds well to that HSV-1 shutoff system described in

earlier studies. Early shutoff is apparently caused by a virion component which is temperature sensitive, being active at 34°C but not at 39°C. However, this temperature-sensitive function is unrelated to the conditional lethal mutation in *ts*B7. A revertant of *ts*B7 and an unrelated wild-type HSV-1 strain both show a heat-labile early shutoff function, suggesting that this phenotype may be characteristic of HSV-1. It is unclear at present whether the virion shutoff components described by Nishioka and Silverstein and by Fenwick and Clark are the same or similar.

At present it is difficult to draw a coherent picture of host protein shutoff as induced by HSV-1 and HSV-2 or to determine how they may differ. In large measure this difficulty arises because most investigations have focused on one virus or the other, and in part because differing host cell lines have been used. In an effort to clarify these problems, we have directly compared the effects of HSV-1 and HSV-2 infection on protein synthesis in FL cells. The FL cell system, pioneered as a model system for studying HSV-induced changes in cellular macromolecular synthesis by Nishioka and Silverstein (17–19), allows quantitation of the change in globin synthesis after HSV infection. When induced by 2% dimethyl sulfoxide, FL cells undergo erythropoetic differentiation and produce large amounts of globin and globin mRNA (20, 26). Globin is readily identified on sodium dodecyl sulfate-acrylamide gels, migrating to a region of the gel where there are no detectable viral proteins. Using this system, Nishioka and Silverstein demonstrated that HSV-1 inhibited globin synthesis completely in induced FL cells by 3 h postinfection (17, 18). We have treated HSV-1 and HSV-2 with agents which interfere with transcription, such as shortwave UV light (254 nm), actinomycin D, and 4,5′,8-trimethylpsoralen (me_3psoralen), and infected induced FL cells to determine what effects these agents have on the ability of the viruses to arrest globin synthesis. Our results show that HSV-1 requires the transcription of viral genes to effect the shutoff of host protein synthesis. HSV-2 did not require transcription, suggesting that its inhibitor of protein synthesis is introduced as part of the virion.

MATERIALS AND METHODS

Cells and viruses. Suspension cultures of FL cells were maintained in Dulbecco modified minimal essential medium (DME) containing 15% horse serum plus 50 U of penicillin per ml and 50 μg of streptomycin per ml. Monolayer cultures of rabbit skin cells and Vero cells were grown in Eagle minimum essential medium supplemented with 5% fetal calf serum and antibiotics. The KOS strain of HSV-1 was propagated and titered on rabbit skin cells, and the 186 strain of HSV-2 was grown and titered on Vero cells. Viral stocks were titered by infecting confluent monolayer cell cultures with serial dilutions of the viral stock. After a 1-h adsorption period with occasional shaking, the infected cells were overlaid with a 2% methylcellulose solution of minimum essential medium with 2% fetal calf serum, antibiotics, and 1 mM arginine.

Induction of globin synthesis in FL cells and infection with HSV. Induction of globin synthesis in FL cells with dimethyl sulfoxide (2%, vol/vol) and subsequent infection with HSV were performed as described previously (17) with some minor procedural changes. Cells were routinely infected on day 6 after induction. At 24 h before infection, cells were diluted in fresh growth medium to 4.0×10^5 cells per ml. On the day of infection, the induced cells were pelleted in a tabletop centrifuge and suspended at 1.8×10^7 cells per ml in phosphate-buffered saline (PBS) supplemented with 1% glucose, 1% γ-globulin-free horse serum (GIBCO Laboratories), 0.1 mM $CaCl_2$, and 0.5 $MgCl_2$ (infection medium). Samples of 3×10^6 cells were infected at a multiplicity of 10 PFU per cell and incubated for 30 min at 37°C with shaking. After viral adsorption, 1.0 ml of warm PBS was added, and the cells were pelleted. Infected cells were suspended at 3×10^6 cells per ml in warm DME supplemented with 2% fetal calf serum and 1 mM arginine and incubated at 37°C for various lengths of time. For labeling of proteins, cells were removed from the dish with a Pasteur pipette, and the dish was washed with an additional 1.0 ml of warm PBS to remove cells sticking to the dish. The cells were centrifuged, and the pellet was suspended in 1.0 ml of DME minus leucine, lysine, phenylalanine, proline, and tyrosine and supplemented with 2% dialyzed horse serum. High-specific-activity ^{3}H-amino acid mix (Amersham Corp.; >100 Ci/ml) containing leucine, lysine, phenylalanine, proline, and tyrosine was added to a concentration of 40 μCi/ml, and the cells were incubated for 30 min at 37°C. After labeling, the cells were transferred to a 1.5-ml microfuge tube, and the dish was washed once with 0.5 ml of cold PBS to collect any residual cells. At this time, 20 μl of the cell suspension was removed to determine the concentration of cells. To facilitate the rapid handling of the samples, all subsequent spins were performed in an Eppendorf microfuge. The labeled cells were centrifuged for 15 s, the supernatant was removed, and the cells were washed and spun two additional times in 0.5 ml of cold PBS. After the final wash, cells were resuspended in 25 μl of cold PBS containing 10% sucrose and 0.5% Nonidet P-40 and placed on ice for 5 min. The nuclei were removed by a 2-min spin in the microfuge, and 25 μl of electrophoresis buffer (0.125 M Tris-hydrochloride [pH 6.8], 20% glycerol, 6% sodium dodecyl sulfate, 10% 2-mercaptoethanol, and 0.016% bromophenol blue) was added to the cytoplasmic extract. Samples were boiled for 2 min before electrophoresis.

Cryogenic storage of induced FL cells. A 500-ml sample of exponentially growing FL cells (3×10^5 cells per ml) was induced by the addition of 2% dimethyl sulfoxide and grown in roller bottles at 37°C. On day 5 after induction, the cells were harvested by centrifugation, suspended at 1.3×10^7 cells per ml in DME supplemented with 20% fetal calf serum and 10% dimethyl sulfoxide, dispensed into 2.5-ml working samples, and immediately frozen at −70°C. At 24 h

before infection, the frozen cells were quick thawed in a 37°C water bath and suspended at 6.5×10^5 cells per ml in warm DME plus 15% horse serum. Viable induced FL cells may be stored in this manner for several months, and the cells are still capable of supporting the growth of HSV-1 and HSV-2.

Preparation of host DNA-free HSV particles. To quantitate the binding of me$_3$psoralen to HSV DNA, it was first necessary to remove any contaminating host DNA from the viral preparations since purified DNA has been shown to bind me$_3$psoralen five times faster than encapsidated viral DNA (27). Infected cells were sedimented in a tabletop centrifuge, suspended in PBS, and recentrifuged. The cell pellet was brought up in infection medium and Dounce homogenized 10 to 15 times with a tight-fitting pestle, and the nuclei were removed by centrifugation at $3,000 \times g$ for 10 min at 4°C. The supernatant was layered over a 30% sucrose cushion and centrifuged at 20,000 rpm for 4 h at 4°C in a Beckman SW27 rotor. The viral pellet was suspended in 1.0 ml of isotonic buffer (35 mM Tris-hydrochloride [pH 7.5], 146 mM NaCl, and 11 mM glucose) with 2% fetal calf serum and 1 mM arginine. Crude DNase (Worthington Diagnostics) was added to a concentration of 100 μg/ml, and the viral suspension was incubated at 37°C for 1 h. This technique gave viable virus (titer, $>10^{12}$ PFU/ml) which was free of any detectable cellular DNA as determined by CsCl banding of the viral DNA as described below.

Treatment of HSV with UV light, me$_3$psoralen, or actinomycin D. For UV light or me$_3$psoralen treatments, viral stocks of greater than 10^9 PFU/ml were diluted 10-fold in PBS plus 1% glucose before irradiation. A sterile piece of metal was used as a stirring bar during irradiation to promote mixing of the viral suspension. For the UV experiments, a shortwave UV lamp (254 nm, General Electric Co. no. G4t4-1) with an incident light intensity of 50 ergs per mm^2 per s was used. A Blak-Ray UV meter model J255 was used to measure the UV dose. Before infection of the FL cells, a small sample of the irradiated virus was removed for titering. For the me$_3$psoralen experiments, me$_3$psoralen (Paul B. Elder Co., Bryan, Ohio) at 600 to 700 μg/ml in ethanol was added to the diluted viral stock to a final concentration of 6 to 7 μg/ml. After the me$_3$psoralen was allowed to equilibrate for 5 min at room temperature, the viral suspension was chilled to 0°C and exposed to various doses of 360-nm light at an incident intensity of 1.5 kJ/m^2 per min. To determine the number of bound me$_3$psoralen photoproducts, host DNA-free viral stocks were diluted and treated with [^{3}H]me$_3$psoralen (1.16×10^6 cpm/μg; New England Nuclear Corp.) and UV light as described above. After treatment, the viral solution was brought to 10 mM EDTA and heated to 65°C for 5 min to remove any residual DNase activity. Sodium dodecyl sulfate was added to a concentration of 1%, pronase (Sigma) was added to 100 μg/ml, and the solution was incubated for 30 min at 37°C. After two phenol extractions and three chloroform extractions, the viral DNA was precipitated with 2 volumes of ethanol and suspended in 57% (wt/wt) CsCl. The DNA was centrifuged to equilibrium in a Beckman VTi65 rotor at 40,000 rpm for 12 h at 20°C. Fractions were collected, and the absorbancy at 260 nm was determined for each fraction on a Gilford 2400 spectrophotometer. A sample of each fraction was removed to determine the [^{3}H]me$_3$psoralen incorporated into the HSV DNA. For the actinomycin D experiments, actinomycin D (Sigma) in 50% ethanol was added to all media and buffers at a concentration of 1 μg/ml and was present throughout the experiment.

Electrophoresis. Protein samples were separated on linear 10 to 20% gradient sodium dodecyl sulfate-polyacrylamide gels by the method of Van Blerkom (36). Typically, extracts from 10^6 cells were loaded per well. Gels were stained overnight in 25% isopropyl alcohol–10% acetic acid–0.1% Coomassie brilliant blue (Eastman Kodak Co.). Gels were destained by successive washes in 25% isopropyl alcohol–10% acetic acid, followed by 10% isopropyl alcohol–10% acetic acid and finally a 10% acetic acid wash. The amount of protein in the globin band was quantitated with an Ortec model 4310 densitometer. Gels were then prepared for fluorography with En^{3}Hance (New England Nuclear), dried, and exposed to Cronex 4X film.

RESULTS

Infection of induced FL cells with HSV-1 and HSV-2. HSV-2 has previously been shown to inhibit host protein synthesis more rapidly than HSV-1 (22, 23). To determine that this was also the case for infected induced FL cells, FL cells were infected on day 6 after induction with HSV-1 KOS or HSV-2 186 at a multiplicity of infection of 10 PFU/cell. The cells were harvested at 2, 4, and 6 h postinfection. HSV-2 completely inhibited globin synthesis (>95%) by 2 h postinfection, whereas HSV-1 required 6 h to achieve approximately 95% inhibition of globin synthesis (Fig. 1). Our observations with respect to the time course of host protein shutoff for HSV-1 and HSV-2 are therefore consistent with those reported previously. However, Nishioka and Silverstein obtained complete globin shutoff with HSV-1 by 3 to 4 h postinfection (18, 19) compared with 6 h for HSV-1 shutoff in our hands. This difference may be due to strain differences since they employed the F strain of HSV-1 and we have used the KOS strain of HSV-1. We have also tested the HF strain of HSV-1 and found that in induced FL cells its shutoff kinetics are no different than HSV-1 KOS.

To more accurately assess the rapidity of HSV-2 host protein shutoff, the experiment was repeated with shorter time intervals between samples. Even when the ^{3}H-labeling period immediately followed the 30-min viral adsorption period, a greater than 80% loss of globin synthesis was observed in cells infected with HSV-2 (Fig. 2). HSV-1, on the other hand, shows no detectable inhibition of globin synthesis at this time. The difference in the amount of time required by the two viral types to effectively inhibit globin synthesis in FL cells suggested that the inhibitory effects of HSV-1 and HSV-2 might occur via different mechanisms. HSV-2 appeared to use a virion-associated component

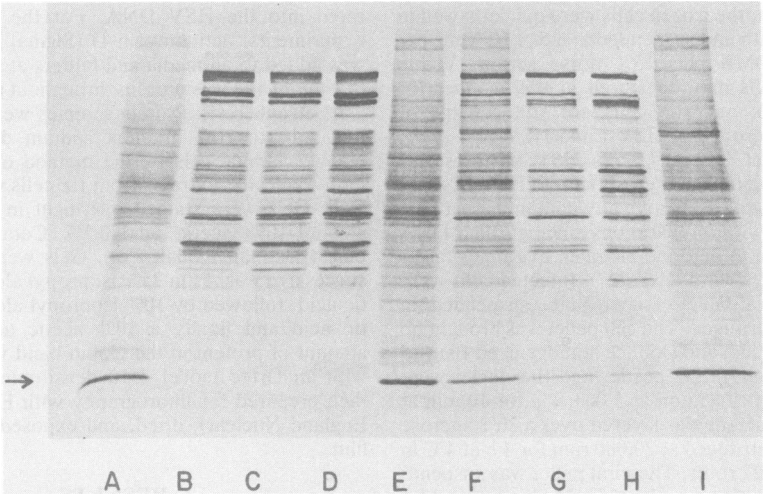

FIG. 1. Shutoff of globin synthesis by HSV-1 and HSV-2. After infection by either HSV-1 or HSV-2 at a multiplicity of 10 PFU/cell, FL cells were harvested at various times and labeled for 30 min with ^{3}H-amino acids, and the extracts were analyzed by electrophoresis. Samples of 10^6 cells were loaded per well. The arrow denotes the location of the globin band. Lanes: (A) mock infected, 2 h postinfection; (B) HSV-2 infected, 2 h postinfection; (C) HSV-2 infected, 4 h postinfection; (D) HSV-2 infected, 6 h postinfection; (E) mock infected, 6 h postinfection; (F) HSV-1 infected, 2 h postinfection; (G) HSV-1 infected, 4 h postinfection; (H) HSV-1 infected, 6 h postinfection (I) mock infected, 6 h postinfection.

to induce host protein shutoff since the inhibition of globin synthesis was initiated almost immediately after infection. HSV-1 might also employ a virion component to effect protein shutoff, however, it must be less efficient than the inhibitor produced by HSV-2. Alternately, the relatively slow rate of HSV-1-induced host shutoff may be because viral protein synthesis is required after infection to produce the inhibitor molecule.

Treatment of virus with 254-nm UV light. To distinguish between a virion component and a viral protein produced after infection, HSV-1 and HSV-2 were treated with agents which inhibit transcription and then tested to determine whether cellular protein shutoff still occurred. Both HSV-1 and HSV-2 were treated with shortwave UV light (254 nm) and titered to quantitate survival. HSV-1 and HSV-2 exhibit similar degrees of sensitivity to UV light; survival of both viruses was reduced by 10^{-4} at a dose of 16,000 ergs per mm^2. Induced FL cells were then infected with the irradiated viruses. Irradiation with 254-nm light had little effect on the inhibition of FL cell globin synthesis by HSV-2, except at the highest UV dosage (16,000 ergs per mm^2, Fig. 3a and Table 1). The slight return of globin synthesis at this highest dosage may reflect damage to the shutoff component itself induced by the UV light treatment, as observed by Fenwick and Walker (10). In contrast, HSV-1 lost virtually all of its shutoff capacity at a UV dose of 4,000 ergs per mm^2 (Fig. 3b and Table 1). Similar experiments with UV-treated HSV-1 HF demonstrated that protein shutoff induced by this strain of HSV-1 was equally sensitive to UV irradiation (data not shown). From our results it is estimated that the shutoff function of HSV-1 is about 500-fold more sensitive to 254 nm light than is the corresponding HSV-2 function and suggests that viral protein synthesis is required for HSV-1-induced shutoff of host protein synthesis.

Effect of actinomycin D on HSV-induced shutoff of globin synthesis. To confirm the result that transcription appears to be required for shutoff by HSV-1, but not for HSV-2, induced FL cells were infected in the presence of 1 μg of actinomycin D per ml. The presence of actinomycin D has no effect on HSV-2 shutoff capability, even though there is no detectable production of viral proteins (Fig. 4). This result again suggests that HSV-2 may utilize a virion component to inhibit globin synthesis. Interpretation of the results of HSV-1 infection in the presence of actinomycin D is complicated by the fact that there is an actinomycin D-sensitive translation component (11). This causes a gradual decline in cellular protein synthesis when actinomycin D is added to the culture medium. Consequently, at times late in infection (6 h), the drug alone inhibited protein synthesis in the FL cells to about the same extent as was observed in HSV-1 infected cells. In spite of this, it appears that the globin

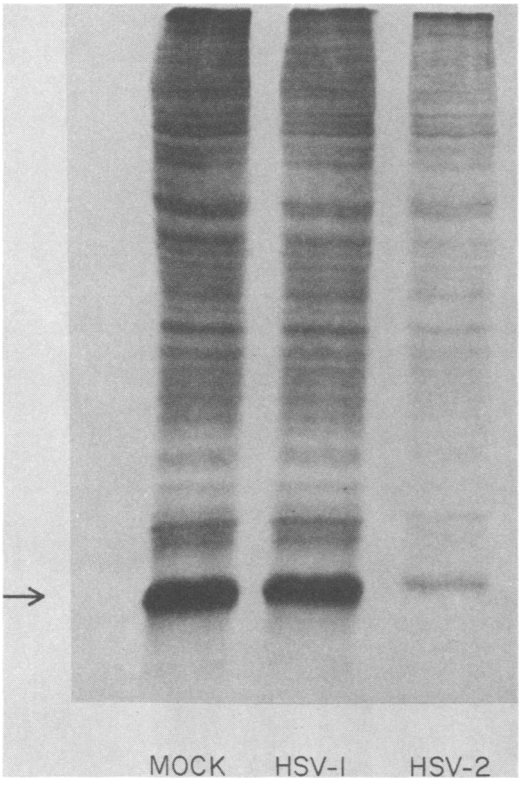

FIG. 2. Rapidity of HSV-2-induced shutoff. Induced FL cells were infected with HSV-1 or HSV-2 at a multiplicity of 10. After a 30-min viral adsorption period, the cells were labeled for 30 min with ^{3}H-amino acids, and the cell extracts were prepared for electrophoresis. The globin band is indicated by the arrow.

shutoff observed in HSV-1-infected FL cells at 2 h postinfection was inhibited by the addition of actinomycin D (Table 2). At 6 h postinfection, HSV-1 reduced globin synthesis to a greater degree than had the addition of actinomycin D to the mock-infected cells, and it was possible to discern a return of globin synthesis in cells infected with HSV-1 in the presence of the drug, although the effect was not as dramatic as with the 2-h time point.

Effect of me$_3$psoralen and 360-nm light treatment on HSV-induced shutoff. Since the results with HSV-1 shutoff with actinomycin D as an inhibitor of viral protein synthesis were complicated by the effects of the drug on cellular protein synthesis, we took an alternate approach to inhibit viral protein synthesis. Me$_3$psoralen intercalates into double-stranded DNA, and, when exposed to longwave UV light (360 nm), forms monoadducts and interstrand cross-links (5, 7). It has been shown that these interstrand cross-links inhibit DNA replication in *Escherichia coli* (6) and inactivate certain viruses including HSV-2 (12). Cross-linking of cellular DNA with me$_3$psoralen also interferes with the synthesis of RNA without affecting the ability of preexisting mRNAs to be translated (13). Under conditions described above, the yield of photoproducts introduced into HSV-1 and HSV-2 DNA was approximately 59 and 43 me$_3$psoralen molecules bound per kJ per m^2, respectively (data not shown). The two types of viruses showed equal sensitivities to the killing effects of the drug and light treatment; viability decreased 10^{-2} at a UV dose of 5×10^{-1} kJ/m^2 and more than 10^{-7} at a dose of 7.5 kJ/m^2.

When me$_3$psoralen-treated viruses were used to infect induced FL cells, HSV-1-infected cells displayed the normal rate of globin synthesis, but the HSV-2-infected cells showed less than 3% of normal globin synthesis (Fig. 5). These results are again consistent with the conclusion that HSV-1 requires the synthesis of virus-specific proteins after infection to cause the shutoff of host protein synthesis. The return of globin synthesis in HSV-2-infected cells at increasingly higher doses of longwave UV light was somewhat surprising in light of our previous results. However, since the return of globin synthesis was only partial with respect to uninfected cells, we felt that this was the result of the photobinding treatment interfering with the virion-associated shutoff component of HSV-2. To test this possibility, HSV-2 was initially treated with 254-nm light to inactivate the viral genome and then treated with either 360-nm light alone or the combination of me$_3$psoralen and 360-nm light. By first treating the viruses with 254-nm light to damage the viral DNA, any additional damages introduced into the DNA by the subsequent treatments will be negligible, and the return of globin synthesis in cells infected with these treated viruses will be the result of damage to the virion. When HSV-2 was treated in this manner, there was a significant return of globin synthesis only in cells infected with viruses which received the combined treatment of 254-nm light, me$_3$psoralen, and 360-nm light (Fig. 6). Thus, it appears that at very high doses, the me$_3$psoralen treatment impairs the ability of the HSV-2 virion to inhibit FL cell globin synthesis.

DISCUSSION

We find striking differences between the host shutoff functions of HSV-1 KOS and HSV-2 186. In FL cells, HSV-2 inhibits globin synthesis greater than 80% by 30 min after viral adsorption. This inhibition is extremely resistant to prior UV irradiation of virions (16,000 ergs per mm^2). In contrast, the HSV-1 shutoff function is not detectable until 2 h post infection and is

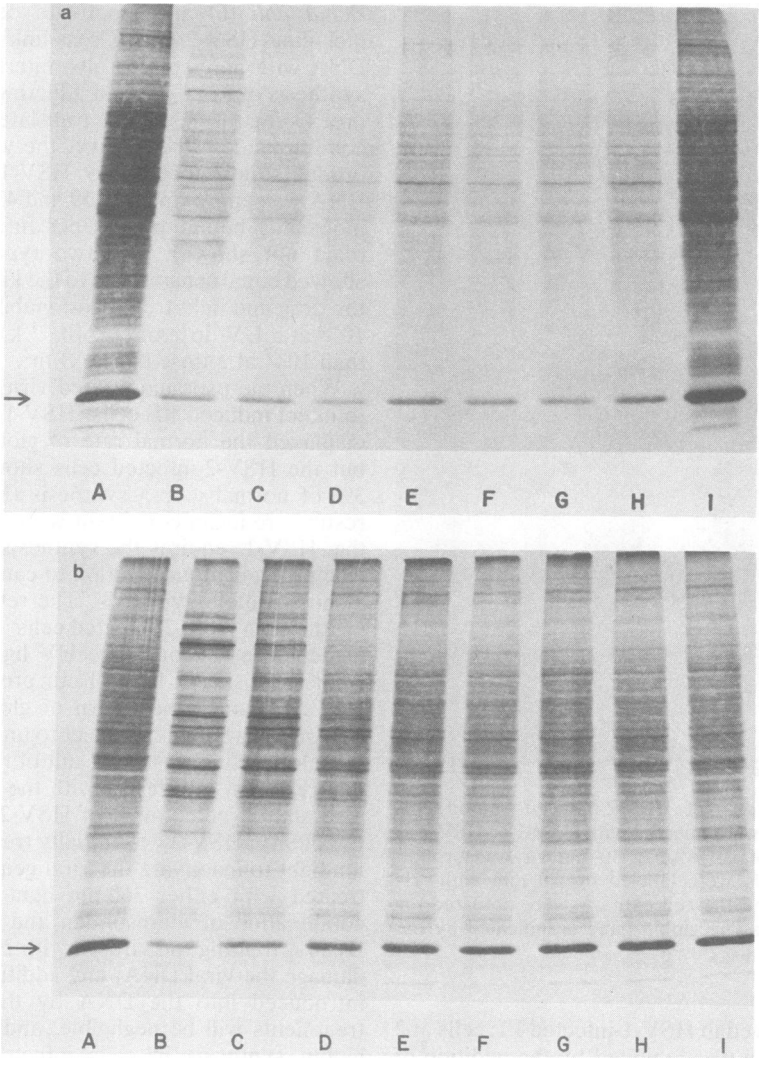

FIG. 3. (a) Effect of UV light (254 nm) on HSV-2-induced shutoff of globin synthesis. UV-treated viruses were used to infect induced FL cells at a multiplicity of 4. One hour after infection the cells were harvested and labeled, and the cell extracts were electrophoresed. Lanes (A) mock infected; (B) infected, no UV treatment; (C) infected, 500 ergs per mm^2; (D) infected, 1,000 ergs per mm^2; (E) infected, 2,000 ergs per mm^2; (F) infected, 4,000 ergs per mm^2; (G) infected, 8,000 ergs per mm^2; (H) infected, 16,000 ergs per mm^2; (I) mock infected. (b) Effect of UV light (254 nm) on HSV-1-induced shutoff of globin synthesis. UV-treated viruses were used to infect induced FL cells at a multiplicity of 10. Six hours after infection the cells were harvested and labeled, and the cell extracts were electrophoresed. Lanes correspond to treatments in a.

completely inactivated by a relatively small UV dose (4,000 ergs per mm^2). We estimate that the HSV-1 shutoff function is 500-fold more sensitive to UV irradiation than the corresponding HSV-2 shutoff function. Experiments where actinomycin D or me_3psoralen treatment was employed to block viral transcription produced similar results; HSV-2 shutoff was insensitive to such treatments, but HSV-1 shutoff was inhibited. The magnitude of difference in sensitivity of the shutoff functions suggest that the primary methods by which the two viral types inhibit host protein synthesis are different. Our results strongly suggest that HSV-1 requires transcription of viral genes to arrest cellular protein synthesis, whereas HSV-2 utilizes a virion-associated component.

Our results with HSV-2 induced shutoff are in agreement with those reported previously by Fenwick and Walker (10). Likewise, our conclu-

TABLE 1. Relative amounts of globin synthesis[a]

254-nm light dose (ergs/mm²)	HSV-2-infected FL cells		HSV-1-infected FL cells	
	Relative amt of globin synthesis (B/A)[b]	% Globin synthesis	Relative amt of globin synthesis (B/A)	% Globin synthesis
0 (mock infected)	2.05	100.0	2.08	100.0
0	0.21	10.5	0.57	27.5
500	0.24	12.0	0.70	33.5
1,000	0.14	7.0	1.19	57.0
2,000	0.23	11.5	1.57	75.5
4,000	0.14	7.0	2.09	100.4
8,000	0.22	11.0	2.34	112.1
16,000	0.37	18.0	2.21	106.0

[a] Determined by densitometric analysis of globin bands from cells infected with UV-inactivated HSV-1 and HSV-2. Data were determined from the polyacrylamide gels and fluorographs in Fig. 3a and b.

[b] B/A represents a measurement of the amount of globin synthesized during the 30-min labeling period as compared with the total amount of globin in the cells. The total amount of globin (A) was determined by densitometric analysis of the globin band in Coomassie brilliant blue-stained polyacrylamide gels. The amount of globin synthesized during the 30-min labeling period (B) was determined by densitometric analysis of the fluorograms of the same gels. The globin peaks from the tracings were cut out and weighed to calculate B/A.

sions on HSV-1 shutoff agree with early reports on herpesvirus-induced effects on cellular protein synthesis. With HSV-1 (34) and pseudorabies virus (3), it was shown that viral protein synthesis precedes the breakdown of host polyribosomes, and that the inhibition of host protein synthesis coincides with the disaggregation of polysomes containing cellular mRNA (34). In contrast to these early reports, Nishioka and Silverstein (19) found host polysome disaggregation in HSV-1 F-infected cells to be independent of viral gene expression, indicating that a virion-

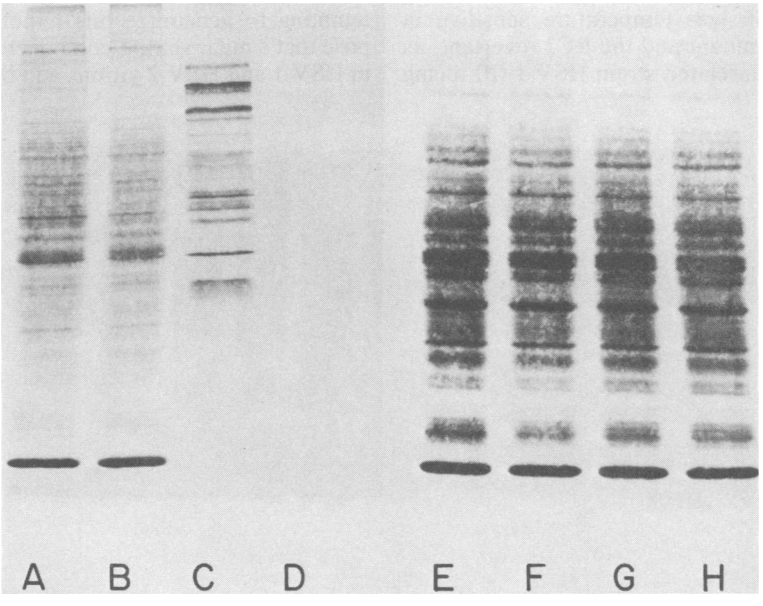

FIG. 4. Effect of actinomycin D on HSV-2-induced shutoff of globin synthesis. Induced FL cells were infected with HSV-2 at a multiplicity of 10 PFU per cell in the presence of 1 μg of actinomycin D per ml. Cells were harvested at 1 h postinfection and labeled, and cell extracts were electrophoresed. Lanes A through D show a fluorogram of the gel tracks: (A) mock infected; (B) mock infected plus actinomycin D; (C) HSV-2 infected, 1 h postinfection; (D) HSV-2 infected, 1 h postinfection, plus actinomycin D. Lanes E through H show a Coomassie brilliant blue stain of the gel tracks: (E) mock infected; (F) mock infected plus actinomycin D; (G) HSV-2 infected, 1 h postinfection; (H) HSV-2 infected, 1 h postinfection, plus actinomycin D.

TABLE 2. Effect of actinomycin D on HSV-1-induced shutoff of globin synthesis

h post-infection	Sample	Relative amt of globin synthesis (B/A)[a]	% Globin synthesis
2	Mock	1.931	100.0
	Mock + actinomycin D	1.954	101.2
	HSV-1 KOS	1.517	78.6
	HSV-1 + actinomycin D	1.838	95.2
6	Mock	2.187	100.0
	Mock + actinomycin D	0.917	41.9
	HSV-1 KOS	0.845	38.6
	HSV-1 + actinomycin D	0.901	41.2

[a] See footnote *b* of Table 1 for explanation of B/A.

associated component was responsible for this process. Curiously, the disaggregation of host polysomes did not appear to arrest the synthesis of host proteins. Complete inhibition of host protein synthesis required expression of the viral genome and coincided with the degradation of cellular mRNA. Fenwick and Clark (9), using a temperature-sensitive mutant of HSV-1 HFEM, *ts*B7 (14), and a heat-resistant revertant, RC2, were able to distinguish two distinct stages of host protein shutoff; a virion-associated early shutoff function and a delayed shutoff function that required viral gene expression. The early shutoff function was temperature sensitive in both the *ts*B7 mutant and the RC2 revertant, as well as in the unrelated strain HSV-1 (F), being active at 34°C but not at 39°C. HSV-2 G did not display a heat-labile early shutoff component. Unlike the virion-associated polysome disaggregation function described by Nishioka and Silverstein, which appeared to have little effect on cellular protein synthesis at 37°C, this early shutoff function is very effective at 34°C in suppressing host protein synthesis.

By comparison, infection of induced FL cells at 37°C with HSV-1 KOS treated with 254-nm UV irradiation, Me_3psoralen, or actinomycin D totally prevented viral inhibition of globin synthesis in our hands, suggesting that only the expression dependent delayed shutoff function was active. It would seem that either the KOS strain of HSV-1 does not possess an early virion shutoff component, or the function is inactivated at 37°C. It is to be noted that we obtained identical results with UV-inactivated HSV-1 HF, the progenitor strain to HFEM (33) and therefore to the *ts*B7 mutant employed by Fenwick and Clark. It seems unlikely that the derivative mutant *ts*B7 has regained a function missing in HSV-1 HF. A more plausible explanation posits that the HSV-1 virion component detected by Fenwick and Clark in *ts*B7 is present but labile at 37°C in strains KOS and HF. By this hypothesis, the polysome disaggregation function described by Nishioka and Silverstein could simply be an attenuated form of early shutoff, inasmuch as these workers also used 37°C. It is tempting to generalize this hypothesis to propose that similar shutoff components are present in HSV-1 and HSV-2 virions and that they differ

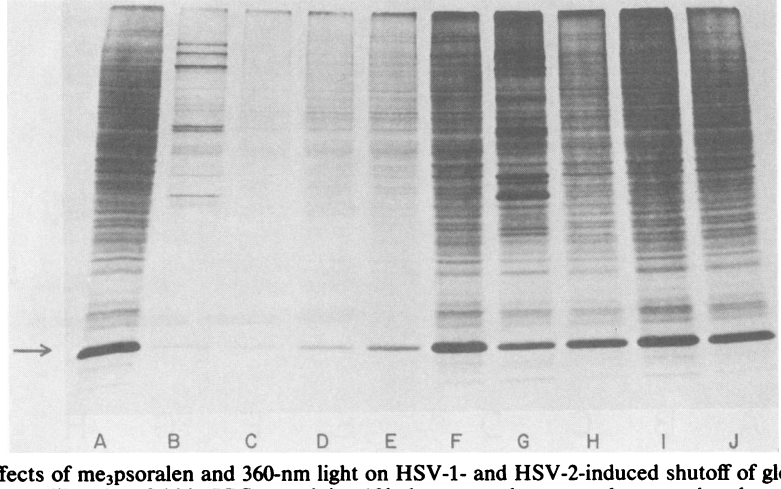

FIG. 5. Effects of me_3psoralen and 360-nm light on HSV-1- and HSV-2-induced shutoff of globin synthesis. Viral stocks were diluted 10-fold in PBS containing 1% glucose, and me_3psoralen was photobound as described in the text. Induced FL cells were infected with the treated viruses at a multiplicity of 10. HSV-2- and HSV-1-infected cells were harvested at 1 and 4 h postinfection, respectively. Lanes: (A) mock infected; (B) HSV-2 infected, no treatment; (C) HSV-2 infected, 7.5 kJ/m^2; (D) HSV-2 infected, 15 kJ/m^2; (E) HSV-2 infected, 30 kJ/m^2; (F) mock infected; (G) HSV-1 infected, no treatment; (H) HSV-1 infected, 7.5 kJ/m^2; (I) HSV-1 infected, 15 kJ/m^2; (J) HSV-1 infected, 30 kJ/m^2.

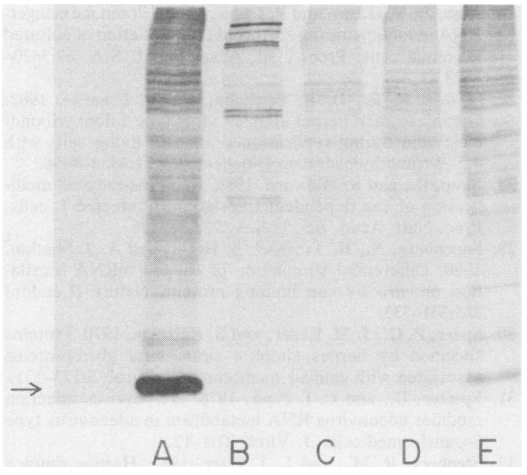

FIG. 6. Effect of me_3psoralen and 360-nm light treatment on HSV-2-induced shutoff. HSV-2 viral stocks were diluted 10-fold in PBS containing 1% glucose and exposed to shortwave UV light (254 nm). The treated viruses were subsequently exposed to either 360-nm light alone or to the combination of me_3psoralen and 360-nm light. Treated viruses were then used to infect induced FL cells at 10 PFU per cell. Lanes: (A) mock infected; (B) HSV-2 infected, no treatment; (C) HSV-2 infected. 254 nm light alone, 16,000 ergs/mm^2; (D) HSV-2 infected, 254 nm light, 16,000 ergs/mm^2 and 360 nm light, 30 kJ/m^2; (E) HSV-2 infected, 254 nm light, 16,000 ergs/mm^2, me_3psoralen (6 μg/ml), and 360 nm light 30 kJ/m^2.

only in their degrees of thermal lability. Such a unitary explanation would be consonant with the close similarities of the two viral types. It will be of interest to determine whether the early (or rapid) shutoff functions of HSV-1 and HSV-2 map to the same chromosomal coordinates (0.52 through 0.59) originally identified for the host shutoff function (16).

It appears that the inhibition of host protein synthesis by HSV-1 at 37°C may be a secondary consequence of the viral effects on cellular RNA metabolism and synthesis. Wagner and Roizman (37) have shown that HSV-1 disrupts the synthesis and processing of cellular rRNA. HSV-1 has also been shown to inhibit the synthesis and accumulation of adenovirus-specific polyadenylated RNAs in adenovirus-transformed cell lines (31, 32). Nishioka and Silverstein (17, 19) provided evidence that HSV-1 produces an immediate early viral function which degrades cellular mRNA, and Stenberg and Pizer (32) also identified an HSV-1 function which degrades adenovirus-specific transcripts in HSV-1-infected, adenovirus-transformed cells. These HSV-1 functions acting in concert will ultimately cause the cessation of the synthesis of cellular proteins. HVS-2, on the other hand, appears to exert its primary effect directly on the translational apparatus of the cell, even though it also inhibits the accumulation of cellular RNAs in much the same way as HSV-1 (Stenberg and Pizer, personal communication). We can surmise the probable target of the HSV-2 shutoff function by noting that HSV-2 can inhibit globin synthesis by greater than 80% in 30 min (Fig. 2). Only interference with the translational machinery of the cell would produce such a rapid change in the synthesis of cellular proteins. Whether this effect is mediated by ionic changes in the cell due to viral entry as suggested by Carrasco (4) or by the modification of translational factors, as seen in picornavirus infections (25, 29) and reovirus infections (28), is unclear. We are currently testing this latter possibility as a potential mechanism for HSV-2-induced shutoff by means of cell-free translation systems.

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