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Effect of Cytosine Arabinoside on Viral-Specific Protein Synthesis in Cells Infected with Herpes Simplex Virus

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The relationship between viral DNA and protein synthesis during herpes simplex virus type 1 (HSV-1) replication in HeLa cells was examined. Treatment of infected cells with cytosine arabinoside (ara-C), which inhibited the synthesis of HSV-1 DNA beyond the level of detection, markedly affected the types and amounts of viral proteins made in the infected cell. Although early HSV-1 proteins were synthesized normally, there was a rapid decline in total viral protein synthesis beginning 3 to 4 h after infection. This is the time that viral DNA synthesis would normally have been initiated. ara-C also prevented the normal shift from early to late viral protein synthesis. Finally, it was shown that the effect of ara-C on late protein synthesis was dependent upon the time after infection that the drug was added. These results suggest that inhibition of progeny viral DNA synthesis by ara-C prevents the "turning on" of late HSV-1 protein synthesis but allows early translation to be "switched off."

Expression of genetic information during productive infection by DNA-containing viruses is generally divided into at least two phases which are related to the time of viral DNA synthesis. Thus, viral proteins appearing during the early phase (before the onset of viral DNA synthesis) are replaced by a second group of proteins synthesized after the initiation of viral DNA synthesis. Compounds which inhibit the synthesis of functional viral DNA have a marked effect on this event. For example, inhibition of DNA synthesis in poxvirus-infected cells prevents both the normal "shut off" of early protein synthesis and the subsequent appearance of viral proteins normally seen only at the late phase of infection (see review, 16).

Because of the close relationship between viral DNA and protein synthesis in other DNA virus systems, it seemed of importance to determine whether such a relationship also exists for herpes simplex virus type 1 (HSV-1). Therefore, the effect of cytosine arabinoside (ara-C), a potent inhibitor of DNA synthesis, on the synthesis of HSV-1 proteins in HeLa cells was investigated. We show here that ara-C effectively prevents the transition from early to late viral protein synthesis but allows the repression of early translation which normally occurs.

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MATERIALS AND METHODS

Cells and virus. The line of HeLa cells used was obtained from P. Byatt (Clinical Laboratories, UCLA Hospital, Los Angeles, Calif.). As will be shown, these cells are very sensitive to protein synthesis inhibition by our HSV-1 strain. This sensitivity appears to be unique to these cells, since two other lines of HeLa cells available to us did not share this property. In all experiments, cells were grown in monolayer cultures in Eagle minimal essential medium containing 10% fetal calf serum.

The virus used was a prototype HSV-1 strain (MacIntyre) which has been described in detail elsewhere (4). Virus stocks were prepared by infecting monolayers of HeLa cells at low multiplicity (ca. 0.1 PFU/cell) and incubating these cultures at 34 C until cytopathic effects were complete. At this time, the cells and supernatant were sonicated and clarified by low-speed centrifugation. Samples were then frozen and stored at −70 C. A typical stock prepared in this manner possessed a titer of 3×10^7 PFU/ml.

In experiments, cell monolayers were incubated at 37 C with 10 to 50 PFU of HSV-1 per cell, the time at which virus was added being designated as the time of infection. After a 1-h incubation period, unabsorbed virus was decanted, prewarmed Eagle minimal essential medium with suitable additions was added again, and incubation of the cultures was continued.

Labeling and harvesting of infected cells. Monolayers of HSV-1-infected or mock-infected HeLa cells were maintained in Eagle minimal essential medium containing 5% fetal calf serum and one-fourth the usual concentration of amino acids, unless otherwise

specified. At the indicated times they were pulse-labeled with radioactive nucleic acid and protein precursors. The number of cells in the samples taken at various time periods depended on the amount needed to allow sufficient incorporation of radioisotope but was between 3×10^5 and 6×10^6. After the labeling period, cells were harvested and quickly cooled to 4 C with Hanks balanced salt solution (1×). Samples to be assayed for acid-precipitable radioactivity were immediately collected by centrifugation (500 × *g*, 10 min), resuspended in 0.01 M Tris, pH 8, and frozen at −20 C. These samples were later precipitated with 5% trichloroacetic acid, collected on membrane filters (0.45-μm pore size; Millipore Corp., Bedford, Mass.), and counted in a liquid scintillation spectrophotometer. Harvested cells with labeled proteins to be analyzed by polyacrylamide gel electrophoresis were washed three times at 4 C with 1× Hanks balanced salt solution, collected by centrifugation (500 × *g*, 10 min), resuspended in a minimal volume of 0.01 M Tris, pH 8, and stored at −20 C.

Polyacrylamide gel electrophoresis. The patterns of radioactive proteins found in pulse-labeled cells were analyzed by disc gel electrophoresis essentially as described by Spear and Roizman (22). In short, suspensions of labeled cells were dissolved by boiling for 2 min in buffer containing 2% sodium dodecyl sulfate (SDS), 0.05 M Tris, pH 7, 5% 2-mercaptoethanol, and 0.005% bromophenol blue and immediately layered onto discontinuous gels. The stacker gel (2 by 0.6 cm) contained 3% acrylamide, 0.075% bis-acrylamide, 0.125 M Tris, pH 7, 0.1% SDS, 0.05% (vol/vol) *N,N,N',N''*-tetramethylethylenediamine, and 0.07% (wt/vol) ammonium persulfate, whereas the separation gel (11 by 0.6 cm) consisted of 8.0% acrylamide, 0.2% bis-acrylamide, 0.375 M Tris, pH 8.9, 0.1% SDS, 0.05% *N,N,N',N''*-tetramethylethylenediamine, and 0.03% ammonium persulfate. Electrophoresis was performed at a constant voltage of 3.5 V/cm in an electrode buffer containing 0.025 M Tris, 0.192 M glycine, and 0.1% SDS at pH 8.3. When the bromophenol blue marker had migrated approximately 12 cm, the gels were fractionated using a Maizel Autogeldivider (Savant Instruments, Inc., Hicksville, N.Y.) and counted after addition of Bray solution.

Analysis of the viral DNA. Because of its greater density, HSV-1 DNA is separable from cell DNA in a CsCl equilibrium gradient (8, 9, 18). To prepare this DNA, infected cells were pulse-labeled with [^{14}C]thymidine, washed three times with 1× Hanks balanced salt solution at 4 C, centrifuged at 500 × *g* for 10 min, and resuspended in 0.15 M NaCl, 0.015 M sodium citrate (1× SSC). The samples were then incubated at 42 C for 20 min in 3 ml of 1× SSC containing 5% SDS and dialyzed at room temperature for 24 h against 1× SSC in 0.1% SDS. After dialysis, the labeled samples were incubated at 37 C for 45 min with 100 μg of predigested Pronase per ml, precipitated with 2 volumes of ethanol, and stored at −20 C. A portion of the precipitated DNA was collected by centrifugation (10,000 × *g*, 20 min) and dissolved in 2 ml of 0.1× SSC. CsCl was then added until the density of the solution was 1.70 g/ml. After centrifugation (114,000 × *g*, 48 h, 25 C, SW50.1 rotor), the labeled preparation was collected from the bottom of the tube, and the individual fractions were assayed either for density or radioactivity.

RESULTS

Transition from host to viral protein synthesis after HSV-1 infection. To characterize pertinent aspects concerning the synthesis of viral-specific proteins during HSV-1 replication, it was necessary to establish the time at which cellular protein synthesis was inhibited. This was done by two independent methods.

First, the degree to which host cell translation contributes to total protein synthesis at various times after infection was established. Others have shown that HSV-1 inhibits cellular protein synthesis in the presence of actinomycin (23). Since viral transcription is severely limited under these conditions, the rate at which total protein synthesis is reduced in such a system might be expected to indicate the viral effect on cellular translation. For this experiment, cells were infected in the presence of actinomycin and subsequently pulse-labeled with radioactive amino acids. Nearly all amino acid incorporation was eliminated by 3 h after HSV-1 infection (Fig. 1). This rapid repression of amino acid incorporation was due primarily to the virus, since there was only a slow inhibition of host protein synthesis by actinomycin acting alone (Fig. 1). From these results, it appears that host cell translation is effectively terminated 3 h after infection and that amino acid incorporation observed after this time in a normal infection should be primarily viral directed.

In the second method, the transition from host to viral protein synthesis was studied without the use of actinomycin. Here, the relative amounts of different amino acids incorporated into infected cells at various times was followed. The rationale used here was based on the fact that HSV-1 proteins generally contain less lysine and more arginine than do cellular proteins, and, as would be expected, the ratio of lysine-arginine incorporated into proteins decreases with time after infection (20). At the time that the ratio stops decreasing, the transition from host to viral protein synthesis should be complete. When cells were pulse-labeled separately with radioactive lysine or arginine, the ratio of lysine-arginine incorporation decreased rapidly after infection but changed very little after 2 to 3 h (Fig. 2). This finding reinforces the previous conclusion that the transition from host to viral protein systhesis is essentially complete by 3 h.

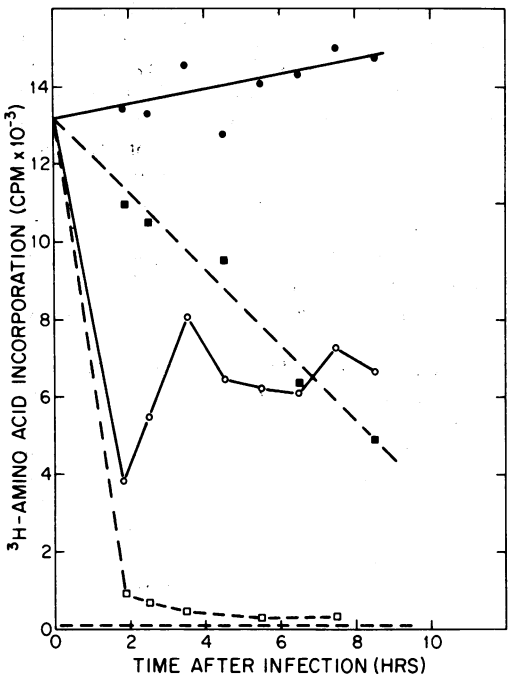

FIG. 1. *Effect of actinomycin and HSV-1 infection on amino acid incorporation in HeLa cells. Cells were pulse-labeled after HSV-1 infection or mock infection for 1-h periods with 2 μCi of ^{3}H-labeled reconstituted protein hydrolysate (Schwarz mixture) per ml and assayed for total acid-precipitable radioactivity. Actinomycin (2 μg/ml) was added to specified cultures at the time of infection. Symbols: ●, uninfected cells; ■, uninfected cells + actinomycin; ○, infected cells; □, infected cells + actinomycin; -----, uninfected cells incubated at 4 C (to determine absolute background).*

To establish the validity of this latter method, it must be shown that viral infection does not alter the specific activity of amino acids in the intracellular pool. As pointed out by Kaplan et al. (13), such a viral-induced modification could be caused either by a change in the permeability of the cells or by an alteration in the rate of turnover of cellular proteins after infection. To investigate the effect of HSV-1 infection upon the permeability of cells, the incorporation rates of labeled lysine and arginine into acid-precipitable material were determined in both normal and infected cells. The incorporation rates of both amino acids become linear immediately after their addition to uninfected cells (Fig. 3A). The same result was obtained in infected cells (Fig. 3B). Most importantly, there was no lag in lysine incorporation in infected cells, although, as expected, the rate of lysine incorporation is considerably less in these cells than in the uninfected cells. These results indicate that permeability of HeLa cells is not altered after infection.

The turnover rates of lysine and arginine were studied in cells prelabeled with [^{14}C]lysine and [^{3}H]arginine. Here, the release of total acid-soluble radioactivity after HSV-1 infection was compared with that after mock infection. The turnover rates for lysine and arginine were not altered after infection (Table 1). In agreement with the results of Saxton and Stevens (20), calculations made from these data indicate that the rates are about 2 and 3% per hour for [^{14}H]lysine and [^{3}H]arginine, respectively.

From these results it appears that the specific activities of lysine and arginine in the intracellular pool of amino acids are not altered by HSV-1 infection. The unlikely possibility that the altered ratio of lysine-arginine incorporation after infection was the result of selective repression of lysine-rich host proteins was also considered. Since this ratio did not change with time in infected cells maintained in the presence of actinomycin (unpublished data), such an explanation can be ruled out. From all these considerations then, the use of lysine-arginine ratios to determine the rate of transition from cellular to viral protein synthesis after infection is considered to be valid.

In conclusion, the experiments presented here show that host cell protein synthesis is effectively inhibited by 3 h after HSV-1 infection

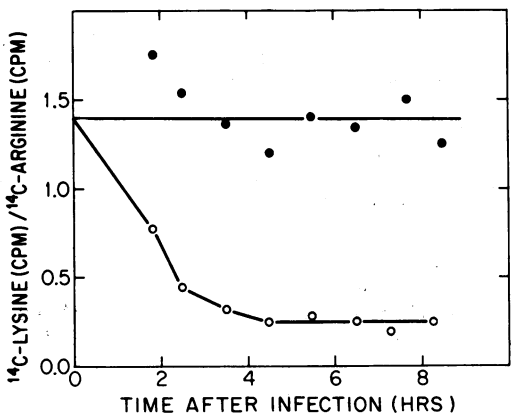

FIG. 2. *Incorporation of lysine and arginine after HSV-1 infection of HeLa cells. Infected or mock-infected cells were pulse-labeled with 0.1 μCi of either [^{14}C]lysine or [^{14}C]arginine per ml (both 312 mCi/mmol) for 1-h periods and assayed for acid-precipitable radioactivity. The ratios of lysine-arginine incorporated were calculated from these results. Symbols: ●, uninfected cells; ○, infected cells.*

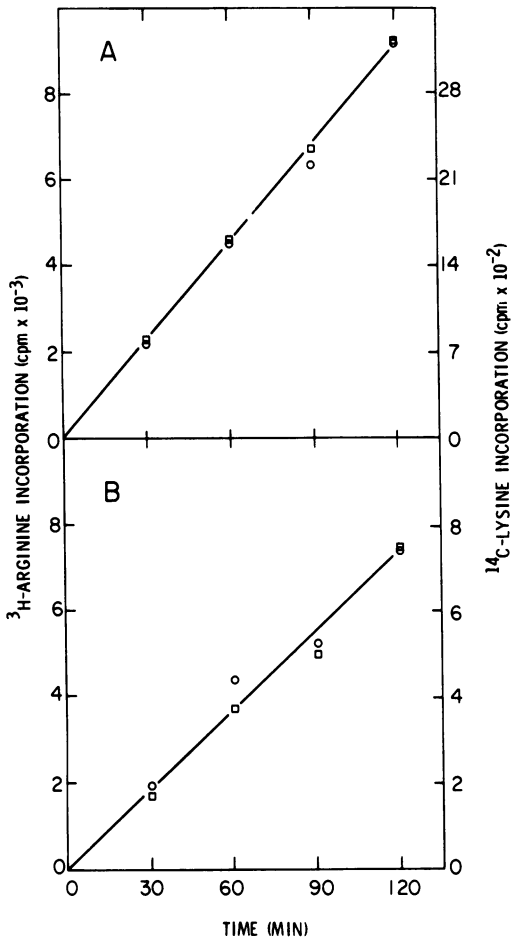

FIG. 3. *Uptake of arginine and lysine by uninfected (A) and HSV-1-infected (B) cells. Four hours after HSV-1 infection, infected or mock-infected cells were labeled with 1 μCi of [^{3}H]arginine (12 Ci/mmol) and 0.02 μCi of [^{14}C]lysine per ml (312 mCi/mmol). At 30-min intervals, samples were assayed for total acid-precipitable radioactivity. Symbols: □, [^{3}H]arginine incorporation; ○, [^{14}C]lysine incorporation.*

and suggest that viral-directed translation can be accurately studied from that time.

Time of initiation of viral DNA synthesis and characteristics of early and late HSV-1 proteins. We next determined the time at which viral DNA synthesis began and compared HSV-1 proteins made before the onset of viral DNA synthesis (early) with those made after viral DNA synthesis had been underway for some time (late). To establish the time that viral DNA synthesis was initiated in this system, cells were pulse-labeled at various times after infection for periods of 1 h with [^{14}C]thymidine, and the density of the radioactive DNA was analyzed by isopycnic gradient centrifugation. A small amount of HSV-1 DNA (1.72 to 1.73 g/ml) was first detectable during the 3- to 4-h pulse period and its rate of synthesis increased during subsequent labeling times (Fig. 4). Although this time of initiation of HSV-1 DNA synthesis is comparable to that previously found in other laboratories, it is not identical. For example, Russell et al. (19) could not detect viral DNA synthesis before 4 h after infection, whereas some HSV-1 DNA was detected as early as 1 h by Roizman (see review, 17).

Since viral DNA synthesis was noted between 3 and 4 h after infection, the ideal time to examine the synthesis of early viral proteins should be before 3 h. However, as shown in the previous section, some host cell translation is still occurring at that time. Therefore, to avoid the possibility of confusing viral with cellular proteins, proteins made between 3 and 4 h postinfection were chosen as representative products of early HSV-1 translation. This compromise is not ideal but seems justified since no new viral proteins are seen in cells analyzed between 3 to 4 h that were not also observed during the 2- to 3-h labeling period (unpublished data). This latter finding is in agreement with the results presented by Honess and Roizman (10). Viral proteins made during the 10- to 11-h labeling period were chosen as representa-

TABLE 1. *Turnover of labeled proteins in uninfected and HSV-1-infected cells*

Time after labeling[a] (h)	Acid-soluble radioactivity (uninfected cells/infected cells)	
	[^{3}H]arginine (counts/min)	[^{14}C]lysine (counts/min)
1	18,630/20,260	955/1,129
2	31,490/26,920	1,496/1,285
3	28,210/29,900	1,437/1,505
4	28,270/27,340	1,391/1,444
5	30,860/32,230	1,579/1,619
6	31,890/33,060	1,655/1,806

[a] Cells were labeled for 1 h with 4 μCi of [^{3}H]arginine and 0.1 μCi of [^{14}C]lysine per ml in Eagle minimal essential medium containing 2% fetal calf serum and 10% of the normal concentration of amino acids. After washing, the cells were infected with HSV-1, rewashed, and incubated with complete Eagle minimal essential medium containing 10% fetal calf serum. Samples were precipitated with cold trichloroacetic acid at hourly intervals, and the total acid-soluble and -precipitable radioactivity was determined. After labeling, there were about 60,000 counts of [^{3}H]arginine and 6,000 counts of [^{14}C]lysine per min in each sample as total acid-precipitable radioactivity.

tive of late proteins, since HSV-1 protein synthesis is still being carried out at a rapid rate but viral DNA synthesis has been underway for more than 6 h at this time.

Viral proteins made at an early time after HSV-1 infection were compared with those synthesized at a late time. Here, proteins pulse-labeled with [^{3}H]arginine from 3 to 4 h were co-electrophoresed in an SDS-polyacrylamide gel with [^{14}C]arginine-labeled proteins synthesized 10 to 11 h after infection. There are distinct differences between proteins made at the two times (Fig. 5). Thus, some early proteins are made in very limited quantities at late times after infection, whereas several late proteins are synthesized in very small amounts, if at all, during the early pulse period. Molar ratios of viral proteins made at the different times were not determined, since it is clear that many viral proteins were not resolved by this technique. However, to facilitate their identification, the major protein peaks in Fig. 5 have been numbered 1 to 23. In some gel patterns, all numbered peaks were resolved; in others, certain proteins only appeared as shoulders on neighboring peaks.

The majority of the numbered peaks can be further classified as belonging to one of two main groups, early or late. A summary of the peaks classified in this manner is shown in Table 2, where it can be seen that several proteins belong to each group. Finally, we would hasten to add that this classification is not meant to imply that if the same peak is found at both early and late times it is necessarily the same protein(s). For example, although peak 17 is quite prominent at early times after infection and is still found in the late gel pattern, it could be composed of entirely different proteins at each of the time periods.

Effect of ara-C on HSV-1 protein synthesis. ara-C has been shown to rapidly inhibit DNA synthesis when added to growing cells (3, 14, 21). In the system described here (Table 3), 50 μg of ara-C per ml inhibited more than 95% of the [^{14}C]thymidine incorporated into either uninfected or HSV-1-infected cells. For these experiments, the drug was added 1 h after infection and the pulse of labeled thymidine was from 4 to 7 h, a time when viral DNA synthesis is normally proceeding at a rapid rate (cf. Fig. 4). At a concentration of 100 μg/ml, ara-C had no additional effect. Furthermore, when the small amount of radioactivity incorporated in infected cells in the presence of ara-C was analyzed by centrifugation in a CsCl gradient, no peak of radioactivity was found at a density corresponding to that of viral DNA. Therefore, if any viral DNA is synthesized under these conditions, it is beyond the level

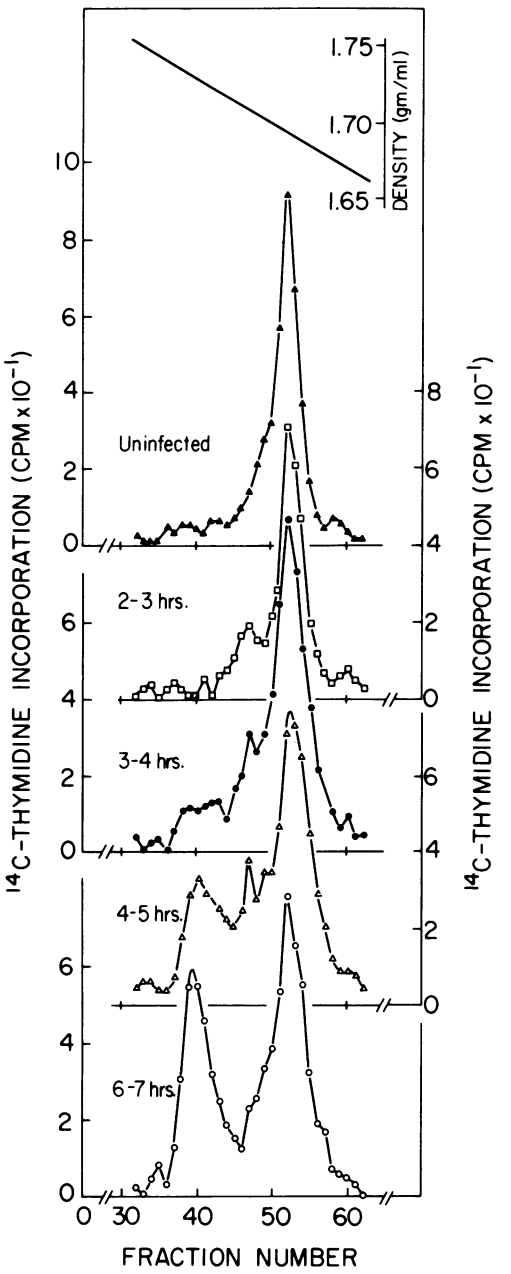

FIG. 4. *Time of initiation of viral DNA synthesis in HSV-1-infected cells. Cells were pulse-labeled with 0.13 μCi of [^{14}C]thymidine per ml (57 mCi/mmol) for 1-h periods at the times after HSV-1 infection noted on each panel. Labeled samples were prepared for analysis by isopycnic gradient centrifugation as described in Materials and Methods.*

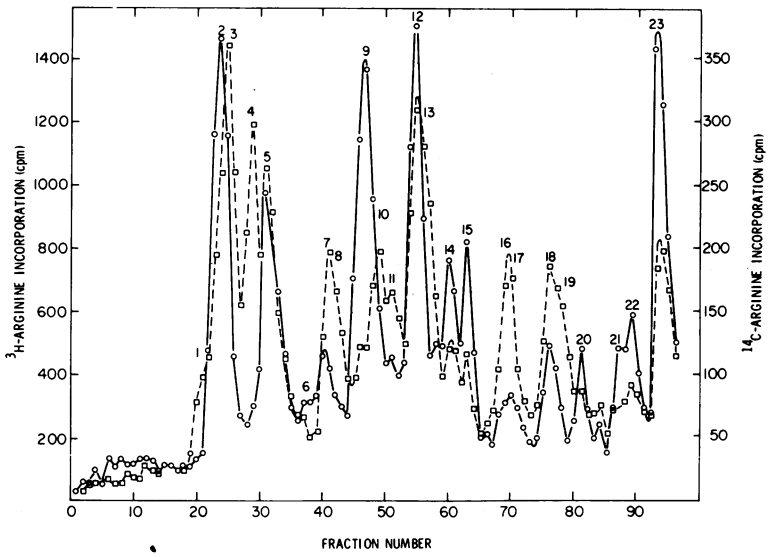

FIG. 5. *Electrophoretic patterns of HSV-1 proteins pulse-labeled at an early and a late time after infection. Cells in Eagle minimal essential medium containing 2% fetal calf serum and 10% of the normal concentration of amino acids were labeled either with 10 μCi of [^{3}H]arginine per ml from 3 to 4 h (□) or with 1 μCi of [^{14}C]arginine per ml from 10 to 11 h (○) after HSV-1 infection. The labeled samples were then analyzed by co-electrophoresis in an 8% SDS-polyacrylamide gel.*

TABLE 2. *Classification of HSV-1 proteins according to their time of synthesis*

Time of synthesis[a]	Viral protein peaks[b]
Early	1, 3, 4, 8, 10, 11, 13, 16, 17, 19
Late	2, 6, 9, 14, 15, 20, 21, 22

[a] Protein peaks from the gel pattern shown in Fig. 5 were classified according to the following criteria: early, no protein peak or a relatively small peak at this position in the 10- to 11-h pulse period; late, no protein peak or a relatively small peak at this position in the 3- to 4-h pulse time.

[b] Peaks are numbered as in Fig. 5.

TABLE 3. *Effect of ara-C on DNA synthesis in uninfected and HSV-1-infected cells*

Sample[a]	[^{14}C]thymidine incorporation (acid-precipitable counts/min)
Uninfected cells	3.65×10^4
Uninfected cells + ara-C	1.50×10^2
Infected cells	5.21×10^4
Infected cells + ara-C	1.57×10^2

[a] Cells were either infected with HSV-1 or mock infected, and 1 h later specified cultures were treated with 50 μg of ara-C per ml. Between 4 to 7 h after infection, cell cultures were pulse-labeled with 0.5 μCi of [^{14}C]thymidine per ml. Fractions of each sample were then assayed for acid-precipitable radioactivity.

which can be detected by this method.

The effect of ara-C on total HSV-1 protein synthesis was assessed by measuring the incorporation of ^{3}H-labeled amino acids into acid-precipitable material during 1-h pulses at different times after infection. In this experiment, ara-C was again added at 1 h after infection. The drug had little effect on early viral protein synthesis (Fig. 6). However, at the time when viral DNA synthesis would normally be initiated (3 to 4 h after infection), HSV-1 protein synthesis began to be depressed relative to that found in untreated, infected cells. By 10 h after infection, amino acid incorporation had dropped to less than 20% of the rate found in untreated cells. It should be noted here that the lack of effect on early HSV-1 translation is not due to the inability of ara-C to penetrate the cell, since labeled thymidine incorporation is repressed normally within 1 h after the addition of the drug to either infected or uninfected cells (unpublished data). In addition, the effect of ara-C on total late HSV-1 translation is not due to a decreased permeability of the cells to labeled amino acids, since, as will be shown below, ara-C has no effect on the rate of incorporation of these amino acids if it is added at late times after infection, when HSV-1 DNA

synthesis has been underway for several hours. From these results, we conclude that ara-C has little effect upon early HSV-1 protein synthesis but causes a rapid drop of amino acid incorporation into late HSV-1 proteins which begins at the time viral DNA synthesis is normally initiated.

To determine which HSV-1 proteins were being made at late times after infection in the presence of ara-C, viral proteins labeled with [^{3}H] arginine from 10 to 11 h after infection in cells treated with ara-C were co-electrophoresed with proteins from untreated cells labeled with [^{14}C]arginine during the same time period. The proteins labeled in the presence of ara-C are very different from those normally made between 10 and 11 h after infection (Fig. 7A). When these proteins were co-electrophoresed with early viral proteins synthesized in the absence of ara-C (Fig. 7B), the patterns were quite similar. Thus, it appears that inhibition of viral DNA replication depresses the synthesis of total late proteins and prevents the change from early to late HSV-1 translation which normally occurs.

To further investigate the relationship between late protein synthesis and the synthesis of viral DNA, the effect of ara-C added at various times after infection was studied. Addition of the drug at the time of infection or 2 h after infection had an equivalent effect on HSV-1 translation (Fig. 8). However, when the drug was not added until 4 h after infection, a time when viral DNA synthesis had been initiated, it had significantly less effect on late viral protein synthesis. Addition of the drug at 6 h after infection, when viral DNA synthesis had been going on for some time, caused only a small inhibitory effect on the production of HSV-1 proteins. Finally, addition of the drug at 8 h had no effect on viral translation. From these results, it seems that the quantity of HSV-1 proteins made at late times after infection is directly related to the amount of progeny viral DNA present in the infected cell.

The unlikely possibility that ara-C directly inhibits translation was considered. From the data already presented in Fig. 6, it is evident that ara-C does not prevent early HSV-1 translation, since normal early viral proteins synthesis occurs in the presence of the drug. Furthermore, ara-C also had little effect on late HSV-1 protein synthesis when the drug was added at 8 h after infection, a time when much progeny viral DNA was already present in the cells (Fig. 8). Thus, ara-C apparently does not directly interfere with HSV-1 translation. It should be pointed out that ara-C had no effect on the rate of cellular protein synthesis in these experiments (unpublished data).

To summarize, these results suggest that progeny viral DNA is required for the transition from early to late protein synthesis in HSV-1-infected cells but is not required for the repression of early HSV-1 translation.

DISCUSSION

In this paper, the possible relationship between viral DNA and protein synthesis during replication of HSV-1 was studied. This was done by analyzing the effect of ara-C on the amounts and types of viral proteins made in cells at different times after infection. When DNA synthesis was inhibited by ara-C, early HSV-1 proteins were synthesized normally. However, beginning at 3 to 4 h after infection (the time when viral DNA synthesis would have been initiated), a progressive and rapid decline in total viral protein synthesis occurred. When the SDS-polyacrylamide gel patterns of viral proteins were analyzed, it was found that ara-C

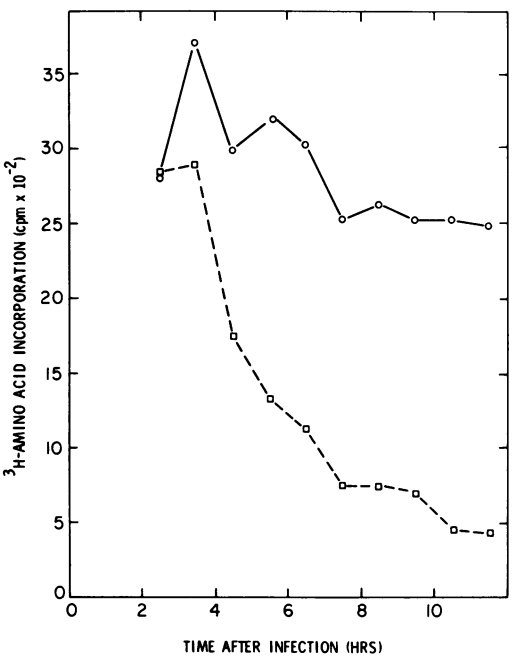

FIG. 6. *Effect of ara-C on amino acid incorporation in HSV-1-infected HeLa cells. Ara-C (50 μg/ml) was added to the specified cultures 1 h after HSV-1 infection, and all cultures were incubated with 2 μCi of ^{3}H-labeled protein hydrolysate per ml for 1-h periods. Total acid-precipitable radioactivity in each sample was then determined. Symbols:* ○, *infected cells;* □, *infected cells + ara-C.*

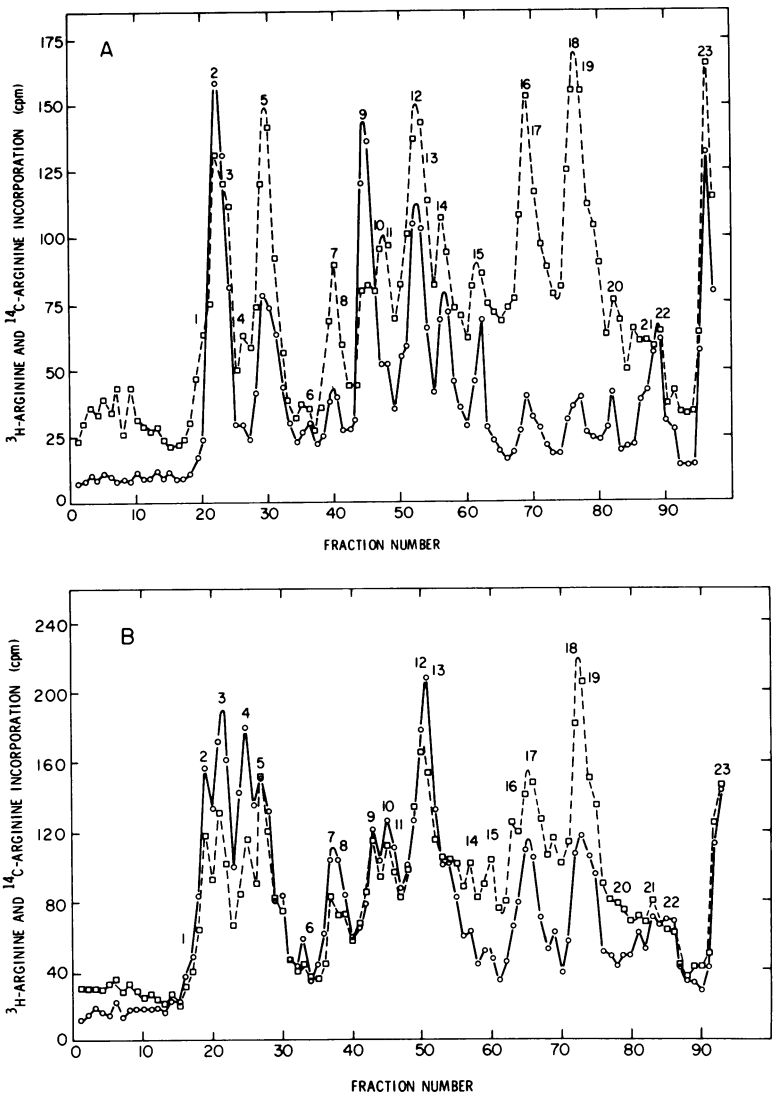

FIG. 7. *Electrophoretic pattern of proteins pulse-labeled at a late time after HSV-1 infection. Cells were incubated in Eagle minimal essential medium containing 2% fetal calf serum and 10% of the normal concentration of amino acids either in the presence or in the absence of 50 μg of ara-C per ml added at 1 h after HSV-1 infection. At the times specified below, these cells were pulse-labeled either with 10 μCi of [^{3}H]arginine or 1 μCi of [^{14}C]arginine per ml. After labeling, the proteins were analyzed by electrophoresis in 8% SDS-polyacrylamide gels. (A) Proteins pulse-labeled with [^{14}C]arginine from 10 to 11 h after infection in the absence of ara-C (○) co-electrophoresed with proteins pulse-labeled with [^{3}H]arginine in the presence of ara-C during the same time period (□). (B) Proteins pulse-labeled with [^{14}C]arginine in the absence of ara-C from 3 to 4 h after infection (○) co-electrophoresed with proteins pulse-labeled with [^{3}H]arginine in the presence of ara-C from 10 to 11 h (□).*

prevented the normal transition from early to late protein synthesis. These data suggest that inhibition of progeny viral DNA production by ara-C prevents the "turn on" of late HSV-1 protein synthesis but allows the "switch off" of early viral translation.

Studies similar to this have been conducted in other laboratories. The most extensive work concerning regulation of early viral protein synthesis in the presence of inhibitors of DNA synthesis has been carried out with poxvirus systems. In initial reports (see review, 16) it was indicated that the synthesis of early poxvirus enzymes continued beyond the normal switch

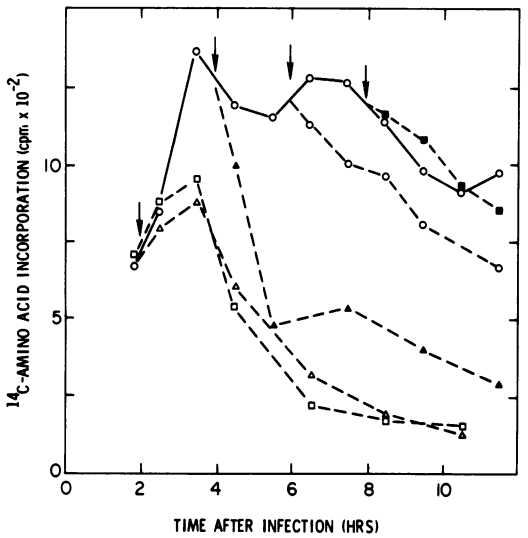

FIG. 8. *Effect of ara-C on amino acid incorporation when added to cells at different times after HSV-1 infection. Cells were incubated with 2 μCi of ^{3}H-labeled protein hydrolysate per ml for 1-h periods after HSV-1 infection. Samples were then assayed for total acid-precipitable radioactivity. Specified cultures were incubated in the presence of ara-C (50 μg/ml) added either at the beginning of infection or at the times indicated by the arrows. Symbols:* O——O, *infected cells;* □, *ara-C added at 0 h;* △, *ara-C added at 2 h;* ▲, *ara-C added at 4 h;* O- - -O, *ara-C added at 6 h;* ■, *ara-C added at 8 h.*

off time under these conditions. However, in more recent experiments in which proteins synthesized in the presence of inhibitors of DNA replication were analyzed in polyacrylamide gels, it was found that early protein synthesis was significantly reduced at times after viral DNA synthesis would normally have begun (5). An effect consistent with the early poxvirus studies was shown in pseudorabies virus-infected cells (12). There it was found that when 5-bromodeoxyuridine was employed to replace thymidine in newly synthesized DNA (presumably causing the synthesis of nonfunctional viral DNA), an early viral-induced enzyme (thymidine kinase) was made for an extended period of time. To our knowledge analogous studies have not previously been carried out with HSV.

The experiments reported here indicate that repression of early HSV-1 protein synthesis does not require viral DNA synthesis. It is important to note that this conclusion is based on a result in which total viral protein synthesis was measured. Thus, we have not ruled out the possibility that a few minor early proteins continue to be made in the absence of viral DNA synthesis.

Regulation of late protein synthesis in the presence of inhibitors of DNA synthesis has also been studied by others. For example, it was found that poxvirus-infected cells do not make late viral proteins under these conditions (see review, 16). In apparent contrast, an early paper by Ben-Porat et al. (1) concerning pseudorabies virus suggested that late viral protein synthesis is not significantly altered in cells treated with ara-C. After our own work had been completed, papers by Honess and Roizman (11) and Bone and Courtney (2) concerning HSV-1 appeared. They also indicated that, qualitatively at least, late viral protein synthesis is not inhibited in the presence of ara-C.

If one adheres to current concepts, the obvious explanation for the differences between these results and our own is that the previous authors did not completely inhibit DNA synthesis, and in their systems a small amount of DNA replication was sufficient to allow a significant quantity of late protein synthesis. Of course, it is also possible that as yet unknown differences in regulatory patterns of different virus strains and cell types could account for these contradictory results.

If the synthesis of viral DNA is not a requirement for the repression of early HSV-1 protein synthesis, some early viral product may switch off the synthesis of early proteins by either directly repressing the translation of early HSV-1 mRNA's or inhibiting the transcription of parental viral DNA, thus indirectly stopping translation. One early HSV-1 protein that could repress early translation by inhibiting transcription is the DNA polymerase. Perhaps this enzyme binds to the parental DNA molecule in such a way as to prevent further transcription before it begins to replicate viral DNA. A regulatory mechanism of this nature is probably operative during the replication of the RNA bacteriophage Qβ (15). Here it has been suggested that the viral replicase prepares the Qβ genome for replication by first inhibiting its translation.

The finding that the transition from early to late HSV-1 protein synthesis does not occur when DNA replication is inhibited indicates either that progeny viral DNA alone is transcribed into mRNA molecules used for late translation or that a product derived from the transcription of progeny DNA is required to allow the translation of pre-existent viral RNA molecules. Each explanation has support from the work of others. From the results of Wagner et al. (24), it would seem that the first explanation is the most likely. This group reported that hydroxyurea, which inhibits the synthesis

of DNA, prevents the transcription of about 60% of the viral DNA sequences normally made in the infected cell. The latter explanation is supported by the finding from Roizman's group (6, 7) that almost all HSV-1 RNA sequences are present in infected cells at early times after infection. To understand how the transition from early to late HSV-1 protein synthesis is accomplished, the differences in the results of these two laboratories should be resolved. If it is found that large quantities of viral RNA specifying proteins made only at late times after infection are present in the infected cell at the time of initiation of viral DNA synthesis, a post-transcriptional mechanism for turning off late protein synthesis during the early period of infection is indicated. If, on the other hand, few mRNA's for specifically late viral protein species are present at early times, regulation at the transcriptional level is implicated for switching from early to late HSV-1 protein synthesis.

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