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Virion Component of Herpes Simplex Virus Type 1 KOS Interferes with Early Shutoff of Host Protein Synthesis Induced by Herpes Simplex Virus Type 2 186

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Herpes simplex virus (HSV) strains HSV type 1 (HSV-1) KOS and HSV-2 186 are representative of delayed and early shutoff strains, respectively, with regard to their ability to inhibit protein synthesis in Friend erythroleukemia cells. When these cells were simultaneously infected with HSV-1 KOS and HSV-2 186, HSV-1 KOS interfered with the rapid suppression of globin synthesis induced by HSV-2 186. The observed interference was competitive and not due to exclusion of HSV-2 by HSV-1 at the level of adsorption. Furthermore, UV-irradiated HSV-1 KOS was also effective at interfering with the early shutoff function of HSV-2 186, indicating that a virion component is responsible for the observed interference.

The inhibition of cellular macromolecular synthesis by herpes simplex virus (HSV) was initially described over 20 years ago (25, 26) and the subject has been recently reviewed in detail by Fenwick (4). The current understanding of HSV-induced shutoff of cellular functions indicates that two separate viral functions are involved in the shutoff process: a virion-associated component which acts immediately after infection and which does not require viral protein synthesis; and an expression-dependent function which is active only after transcription of the viral genome. Studies with temperature-sensitive mutants of HSV type 1 (HSV-1) (6, 23) have demonstrated that the virion-associated, or early shutoff, function is dispensable for normal viral growth. One action of the virion-associated function is to disperse host polyribosomes (20, 29, 30), and other effects on host macromolecular synthesis, such as the rapid inhibition of cellular DNA (5, 7) and protein synthesis (6, 7, 10, 17), are apparent shortly after infection. Whether the multiple effects on the cell are the result of the action of a single component or several different virion components is not known. Mutants in the expression-dependent or delayed shutoff function have not been reported and its importance in the replication of HSV has therefore not been determined. The delayed shutoff function also demonstrates multiple sites of action within the cell: protein synthesis is inhibited (6, 12, 19, 20), RNA synthesis and processing are impaired (21, 28, 32), and cellular RNA species are degraded (11, 18, 20, 28). Again, it is unclear how many different HSV functions are contributing to the overall effect on the infected cell.

The gene(s) encoding early shutoff of host protein synthesis has been localized to an 11-kilobase (kb) region on the HSV genome between map units 0.52 and 0.59 (15). We had originally intended to define the limits of the early shutoff function more accurately by constructing intertypic recombinants from strains HSV-1 KOS and HSV-2 186, strains which we had shown previously to represent delayed and early shutoff strains, respectively (10). The recombinant viruses were made by inserting DNA fragments from the early shutoff region of HSV-2 186 into the thymidine kinase (TK) gene of HSV-1 KOS according to the method of Mocarski et al. (14). We assumed that the early shutoff function of the HSV-2 186 strain would be dominant in these recombinant viruses. However, none of the intertypic recombinants demonstrated early shutoff characteristics with regard to the inhibition of cellular protein synthesis (data not shown). Since the intertypic recombinant viruses were constructed in a manner which resulted in a merodiploid HSV genome, the absence of early shutoff characteristics could be the result of interference of inactive shutoff components produced from HSV-1 genes with active early shutoff components produced from HSV-2 genes. To test this possibility, we coinfected Friend erythroleukemia (FL) cells with intact HSV-1 KOS and HSV-2 186 viruses and determined the effects of mixed infections on the production of globin. Our results indicate that a virion component from the delayed shutoff strain HSV-1 KOS can interfere with the early shutoff of host protein synthesis induced by the HSV-2 186 strain.

The following techniques were used in this study. Infection of FL cells, propagation of the HSV-1 KOS and HSV-2 186 viral strains, induction of the FL cells, labeling of cellular and viral proteins with ^{3}H-amino acids or [^{35}S]methionine, and electrophoresis of cytoplasmic extracts have been described previously (10). ^{3}H-labeled HSV-2 particles were prepared by infecting Vero cells with HSV-2 186 at an input multiplicity of infection (MOI) of 10 PFU per cell and adding medium (Dulbecco modified Eagle medium supplemented with 4% dialyzed fetal bovine serum, antibiotics, and 1 mM arginine) containing 10 μCi of [^{3}H]thymidine per ml at 3.5 h postinfection. The infected cells were incubated overnight at 37°C, scraped from the plates, suspended in cold phosphate-buffered saline (PBS) plus 10% sucrose, and sonicated. Intact virions were purified on Percoll gradients according to the manufacturer's specifications (Pharmacia Fine Chemicals, Inc.). ^{3}H-labeled virions were adsorbed to 1.5×10^{6} dimethyl sulfoxide-induced FL cells in a final reaction volume of 200 μl at 2°C for 1 h. The cells were then washed twice with ice-cold infection medium and suspended in 200 μl of PBS. Sodium

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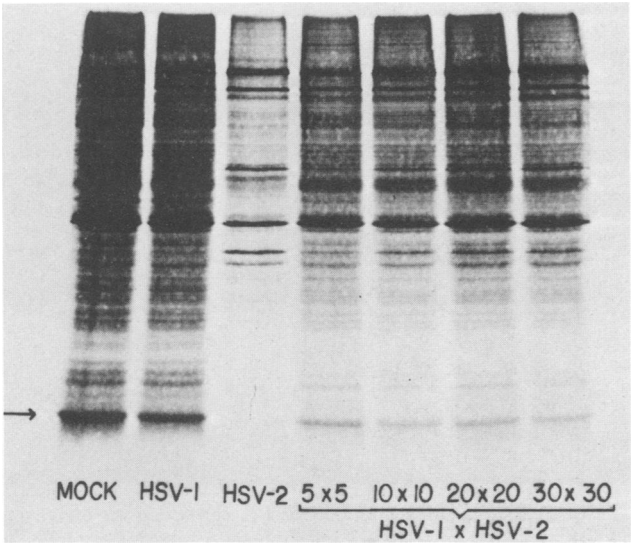

FIG. 1. Mixed infection of FL cells with HSV-1 KOS and HSV-2 186. Stocks of HSV-1 KOS and HSV-2 186 were mixed at equal-input PFUs per cell and used to infect induced FL cells. The numbers below the gel tracks represent the input PFU per cell of HSV-1 and HSV-2, respectively. Control lanes for HSV-1 and HSV-2 were infected at an input MOI of 10 PFU per cell. Cells were harvested at 1.5 h postinfection and labeled with [^{35}S]methionine. The arrow denotes the location of the globin band.

dodecyl sulfate was added to 1% and the cells were heated for 2 min at 90°C. The entire sample was transferred to a scintillation vial, 15 ml of scintillation cocktail was added, and the samples were counted.

Southern blot analysis of nuclear DNA from mixed infections of Vero cells was performed by isolating nuclei from infected cells according to the method of Roberts et al. (24). Briefly, cells were scraped from plates at 1.5 h postinfection and washed twice with ice-cold PBS. The cells were suspended at 1.5×10^7 per ml of PBS plus 10% sucrose and 0.5% each Triton X-100 and Nonidet P-40, placed on ice for 5 min, brought to 0.2% citric acid, and disrupted with a Dounce homogenizer. After centrifugation of the disrupted cells, the nuclear pellet was washed in lysis buffer plus 0.2% citric acid. The nuclei were suspended in 1.0 ml of 661 buffer (6 mM Tris, pH 7.4, 6 mM NaCl, 1 mM EDTA), sodium dodecyl sulfate was added to 1%, and pronase was added to 100 μg/ml. After overnight incubation at room temperature, the DNA was phenol extracted, chloroform extracted, and precipitated with ethanol before restriction endonuclease cleavage. DNA was electrophoresed in 1.0% agarose and transferred to nitrocellulose according to the method of Southern (27).

Coinfection of induced FL cells by HSV-1 KOS and HSV-2 186 at equal-input MOIs results in an intermediate level of globin shutoff at 1 h postinfection (Fig. 1). This suggests that the delayed shutoff strain HSV-1 KOS can interfere with the early shutoff of host protein synthesis normally induced by HSV-2 186. Since different viral types are used in these infections, one way that the HSV-1 strain might interfere with HSV-2-induced shutoff of protein synthesis is by preventing adsorption of HSV-2 at the cellular membrane or by interfering with the penetration of HSV-1 into the cell cytoplasm. Stocks of HSV-2 labeled with [^{3}H]thymidine were prepared and purified on Percoll gradients. To determine if the adsorption of HSV-2 was linear with increasing amounts of virus added, FL cells were infected with the radioactive HSV-2 at 2°C, a technique which allows viral adsorption but prevents penetration of the adsorbed viruses. Adsorption of HSV-2 particles to FL cells is linear to at least 60 PFU per cell (Fig. 2A). When HSV-1 was simultaneously added in increasing amounts relative to the HSV-2 input MOI, there was no decrease in the amount of HSV-2 adsorbed to the cells (Fig. 2B). Therefore, HSV-1 does not interfere with HSV-2 at the level of adsorption in FL cells. These results support other studies which have indicated separate receptors for HSV-1 and HSV-2 in a number of cell lines (1, 31).

An alternate method of detecting viral interference is to determine if the HSV-2 DNA reaches the cell nucleus. Vero cells were infected with HSV-1 or HSV-2, or with HSV-1 and HSV-2 simultaneously, and the nuclear DNA was extracted at 1.5 h postinfection before the onset of viral DNA replication). Southern blots of the *Bam*HI-restricted DNAs

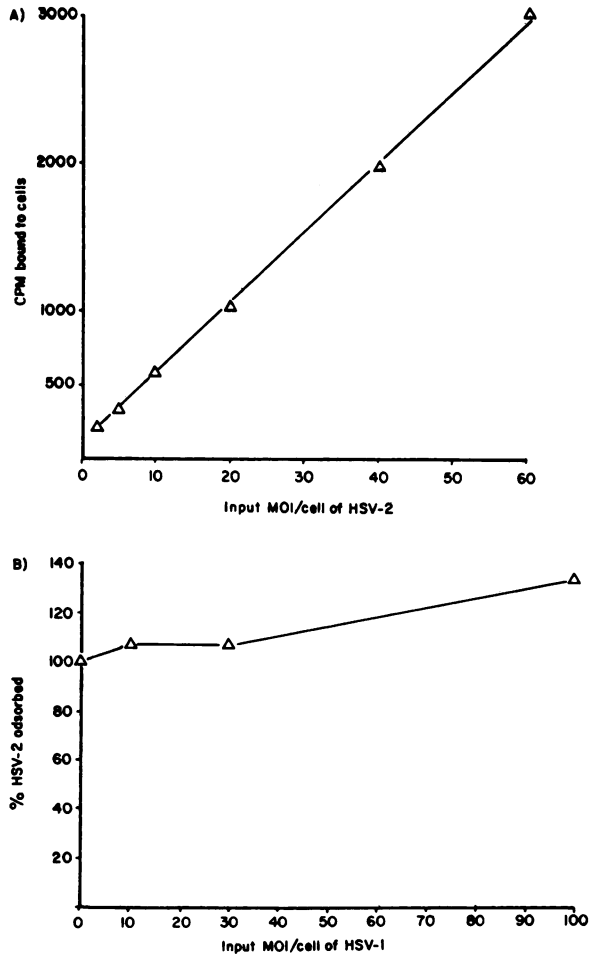

FIG. 2. Adsorption of radiolabeled HSV-2 186 to FL cells. (A) [^{3}H]thymidine-labeled virions were purified and adsorbed to FL cells at 2°C for 60 min. The cells were harvested and the number of counts bound was determined. The points represent the average of duplicate samples. (B) Adsorption of radiolabeled HSV-2 186 to FL cells in mixed infections with unlabeled HSV-1. ^{3}H-labeled HSV-2 186 (input MOI, 10 PFU per cell) was mixed with increasing amounts of HSV-1 KOS and adsorbed to cells for 60 min at 2°C. The cells were harvested and the number of counts was determined. The points represent the average of two experiments.

were prepared and probed with ^{32}P-labeled pDG504, a plasmid containing a *Bgl*II/*Hin*dIII fragment encoding the HSV-2 TK gene (9). This probe will hybridize to three HSV-2 186 *Bam*HI fragments and cross-hybridize to one predominant HSV-1 KOS *Bam*HI fragment. In Vero cells, coinfection with HSV-1 and HSV-2 did not prevent the detection of HSV-2 DNA in the nucleus of the cells (Fig. 3). Another group has also shown that HSV-2 DNA is capable of reaching the cell nucleus in mixed infections of HSV-1 and HSV-2 (S. Amundsen and D. S. Parris, personal communication). These experiments rule out the possibility that the observed effect of HSV-1 on HSV-2 inhibition of host protein synthesis is a result of viral exclusion. This conclusion supports the data of Fenwick and Walker (7), who demonstrated that coinfection of Vero cells with HSV-1 and HSV-2 results in viral proteins of both types being produced.

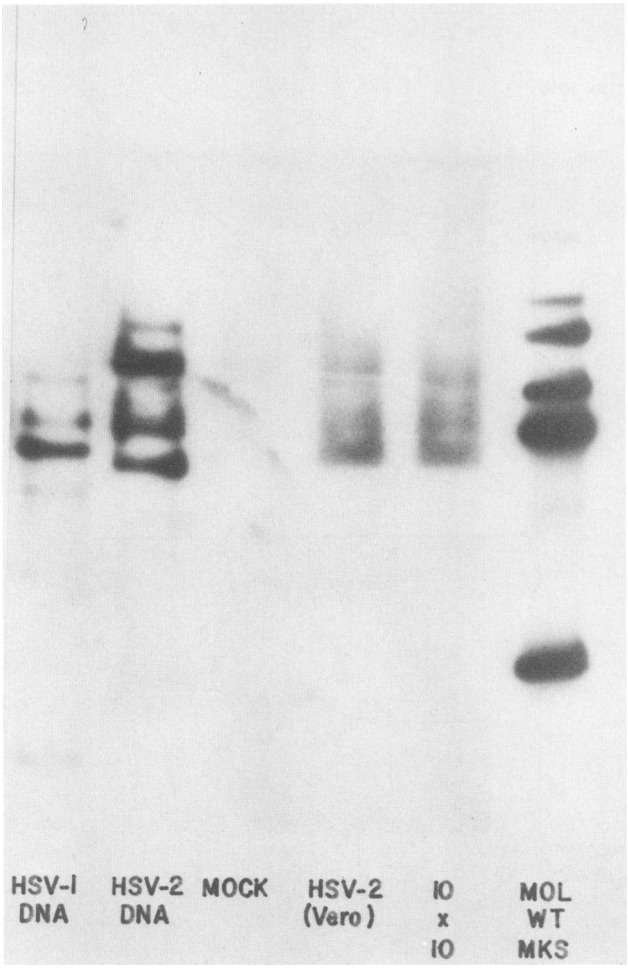

FIG. 3. Southern blot analysis of nuclear DNA preparations after simultaneous infection of Vero cells with HSV-1 KOS and HSV-2 186. Vero cells were infected with HSV-2 alone (10 PFU per cell) or simultaneously with HSV-2 186 or HSV-1 KOS (10 PFU per cell each) and incubated at 37°C for 1.5 h, and nuclear DNA was prepared. After restriction with *Bam*HI, DNAs were blotted and hybridized to ^{32}P-labeled, nick-translated plasmid pDG504 (containing the HSV-2 TK gene). As a control, purified HSV-1 KOS and HSV-2 186 DNAs were also restricted with *Bam*HI and blotted. When probed with pDG504, HSV-2 186 gives bands of 3.2, 4.2, and 7.0 kb in size and HSV-1 KOS gives a predominant band at 3.5 kb. Molecular weight markers were 22.7, 12.2, 5.8, 4.2, and 2.1 kb in size.

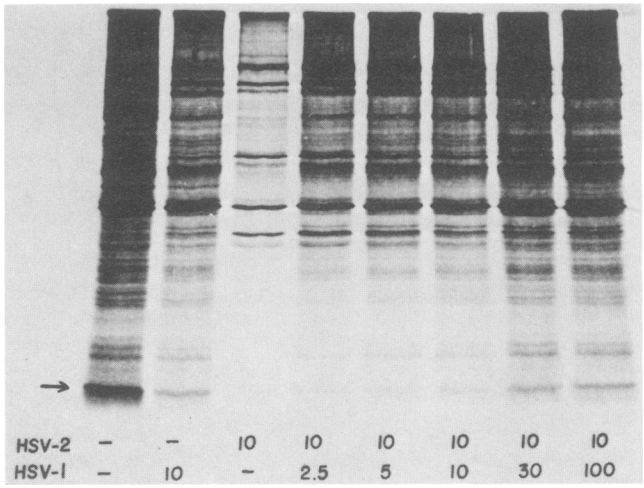

FIG. 4. Simultaneous infection of FL cells with an increasing ratio of HSV-1 KOS to HSV-2 186. Induced FL cells were infected with mixtures of HSV-1 KOS and HSV-2 186 at the indicated PFUs per cell. Cell extracts were prepared at 1.5 h postinfection and electrophoresed. The arrow denotes the position of the globin band.

To assess whether the interference of HSV-2 186-induced early shutoff of cellular protein synthesis by HSV-1 KOS was a competitive process, induced FL cells were simultaneously infected with a constant MOI of HSV-2 and an increasing MOI of HSV-1. The cells were harvested and labeled at 1.5 h postinfection. If the inhibition of HSV-2 186-induced early shutoff by HSV-1 KOS was due to competition for a target site within the cell, then a "gradient" of shutoff of globin synthesis should be observed. Increasing the amount of HSV-1 relative to HSV-2 in simultaneous infections caused a decrease in the ability of HSV-2 to inhibit globin synthesis (Fig. 4). This experiment indicated that the degree of early shutoff observed in cells coinfected with HSV-1 KOS and HSV-2 186 depends on the relative amounts of HSV-1 and HSV-2. To determine if interference with HSV-2 still occurred when HSV-1 infection was delayed until after HSV-2 adsorption, FL cells were mixed with 10 PFU of HSV-2 per cell and incubated for 30 min at 37°C, and 10 PFU of HSV-1 was added. The incubation was then continued for an additional 30 min to allow for adsorption of HSV-1. At this point, the cells were pelleted and suspended in fresh growth medium. At 2, 4, and 6 h (relative to the addition of HSV-2), the cells were labeled to determine the amount of globin synthesis. When HSV-2 infection preceded HSV-1 superinfection, a degree of shutoff characteristic of HSV-2 alone was observed (Fig. 5), in contrast to the results seen in simultaneous infections. Therefore, the interference of HSV-1 with HSV-2-induced early shutoff is ineffective if the HSV-2 component is allowed to reach its target site in the cell prior to HSV-1 infection.

Since HSV-2 186 uses a virion component to inhibit cellular protein synthesis, it follows that the component of HSV-1 KOS which interferes with the HSV-2 shutoff function would also be of virion origin, as de novo synthesis of the inhibitor would require too great a delay to be effective. To determine if HSV-1 KOS uses a virion component to inhibit the early shutoff function of globin synthesis induced by HSV-2 186, HSV-1 was irradiated with shortwave UV light prior to coinfection with HSV-2. UV irradiation of

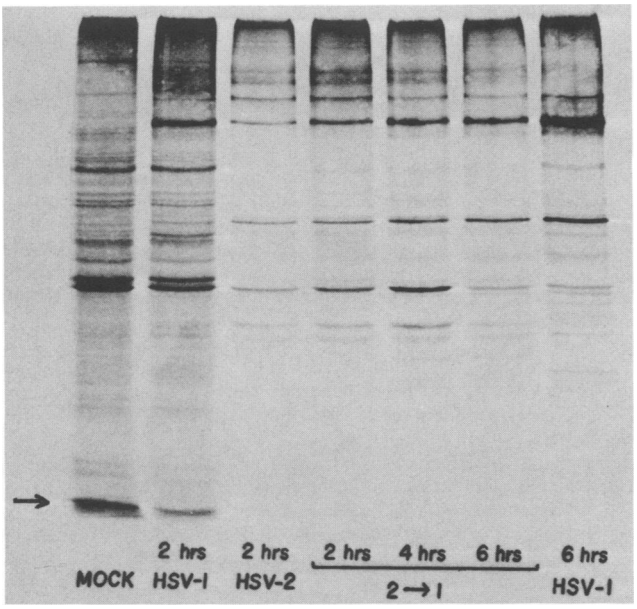

FIG. 5. Shutoff of globin synthesis in FL cells when HSV-2 186 infection precedes HSV-1 KOS superinfection. Induced FL cells were infected with HSV-2 at 10 PFU per cell for 30 min at 37°C. A total of 10 PFU of HSV-1 KOS per cell was added to the appropriate cultures, and viral adsorption was continued for an additional 30 min. At the indicated times (relative to the addition of HSV-2 186), cultures were labeled with [^{35}S]methionine and processed for electrophoresis. The arrow denotes the position of the globin band.

HSV-1 KOS impairs its ability to shut off globin synthesis and to produce detectable viral proteins (Fig. 6; cf. tracks at 6 h postinfection). However, the ability of HSV-1 to interfere with HSV-2-induced early shutoff is not impaired, since complete shutoff of globin synthesis is not observed in the tracks where HSV-2 was mixed with UV-inactivated HSV-1 before infection (cf. tracks at 2 h postinfection). This result is consistent with the hypothesis that a virion component of HSV-1 KOS is capable of interfering with the early shutoff function of HSV-2 186. In addition, it should be noted that HSV-2 proteins are produced in significant amounts in cells infected with HSV-2 and UV-irradiated HSV-1. This result provides compelling, though indirect, evidence that HSV-2 DNA is present in the nucleus of the cells after mixed infections.

Interference between strains of HSV-1 and HSV-2 is a well-established phenomenon (8, 13, 16, 22, 33; Amundsen and Parris, unpublished data), but these studies have inspected the replication of the viruses following mixed infection and have not investigated interference at other levels of viral infection. We demonstrate that the rapid shutoff of host protein synthesis induced by HSV-2 186 in FL cells can be inhibited by simultaneous coinfection with the delayed shutoff strain HSV-1 KOS. The observed inhibition of HSV-2-induced early shutoff by HSV-1 is postulated to occur by competition for target sites within the cell by both HSV-1 KOS and HSV-2 186 virion-associated components. The hypothesis that delayed and early shutoff strains of HSV contain similar shutoff components would predict that the inactive virion component of HSV-1 KOS is preventing the action of the early shutoff component of HSV-2 186. The data presented here are in agreement with such a prediction. Apparently, nonfunctional virion components of HSV-1 KOS can compete for the same substrates as functional components and prevent the action of the latter. The results demonstrating HSV-2 protein production in spite of interference by UV-irradiated HSV-1 (Fig. 6) are of particular interest since they indicate that the rapid shutoff function of HSV-2 186 is dispensable for efficient synthesis of viral proteins. Shortly after these data were obtained, Read and Frenkel (23) published their work with temperature-sensitive mutants of HSV-1 KOS. The isolate of wild-type HSV-1 KOS used in these studies showed a rapid shutoff phenotype in Vero cells. Their studies showed that mutants which had lost the virion-associated shutoff function at both 34 and 39°C were capable of growth, though at a somewhat reduced rate. In addition, those mutants lacking the early shutoff function also produced abnormally high amounts of immediate early proteins, suggesting that the early shutoff component may play a part in the efficient regulation of the expression of viral genes. Our results concur with their stated conclusion that the virion-associated shutoff function is dispensable for viral replication. In retrospect, this result is not surprising. For instance, delayed shutoff strains of HSV, such as the HSV-1 KOS isolate used in the studies presented here, grow quite well in the absence of an early shutoff function. Also, the intertypic recombinants used by Morse et al. (15) expressed HSV-2 genes normally in recombinant strains which lacked the ability to rapidly inhibit cellular protein synthesis. Had the early shutoff function been absolutely required for the expression of these HSV-2 functions, these recombinants would not have been viable. One would suspect, however, that the function would confer certain advantages on the virus. A rapid suppression of host cells may be useful in natural infections by increasing the invasiveness or virulence of the virus. Such an advantage was suggested in the experiments of Aurelian and Roizman (2) where efficient suppression of host functions was required to avert an abortive infection.

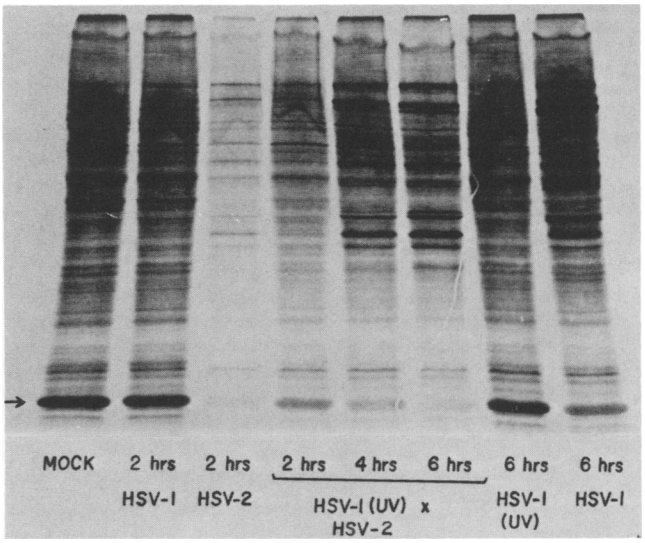

FIG. 6. Mixed infection of FL cells with HSV-2 186 and UV-irradiated HSV-1 KOS. Stocks of HSV-1 KOS were irradiated with UV light to a dose of 21,000 ergs/mm^2 as described previously (7). A total of 100 PFU (original titer) of UV-iradiated HSV-1 KOS per cell were mixed with 10 PFU of HSV-2 186 per cell, and the cells were infected. At the indicated times, cells were labeled with ^{3}H-amino acids and cell extracts were prepared for electrophoresis. The arrow denotes the location of the globin band.

Even though HSV-induced shutoff of host protein synthesis was reported 2 decades ago, many questions still remain. What is the site of action of the early shutoff component in the infected cell? Interference with the cellular translation apparatus would appear to be the most likely choice, but effects on cellular mRNA species cannot be ruled out. Also, how can an inactive shutoff component block the action of an active shutoff component? Is the virion-associated component of HSV which induces shutoff of host protein synthesis a protein or a small RNA molecule, as has been demonstrated with vaccinia virus (3)? What is the basis for the selective translation of viral over cellular mRNA species? And finally, what is the mechanism by which the selective inhibition of cellular messages occurs? Clearly, more time and effort are required to resolve this well-documented phenomenon.

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