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Interaction of Herpes Simplex Virus 1 α Regulatory Protein ICP0 with Elongation Factor 1δ: ICP0 Affects Translational Machinery

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The herpes simplex virus 1 (HSV-1)-infected cell protein 0 (ICP0) is a promiscuous transactivator, and by necessity, its functions must be mediated through cellular gene products. In an attempt to identify cellular factors interacting with ICP0, we used the carboxyl-terminal domain of ICP0 as "bait" in the yeast (*Saccharomyces cerevisiae*) two-hybrid system. Our results were as follows: (i) All 43 cDNAs in positive yeast colonies were found to encode the same translation factor, elongation factor delta-1 (EF-1δ). (ii) Purified chimeric protein consisting of glutathione *S*-transferase (GST) fused to EF-1δ specifically formed complexes with ICP0 contained in HSV-1-infected cell lysate. (iii) Fractionation of infected HEp-2 cells and immunofluorescence studies revealed that ICP0 was localized both in the nucleus and in the cytoplasm. In primary human foreskin fibroblasts, ICP0 was localized predominantly in the cytoplasm throughout HSV-1 infection even early in infection. (iv) Addition of the chimeric protein GST–carboxyl-terminal domain of ICP0 to the rabbit reticulocyte lysate in vitro translation system resulted in a dose-dependent decrease in protein synthesis. In contrast, GST alone or GST fused to the amino-terminal domain of ICP0 had no effect on the in vitro translation system. (v) The predominant forms of EF-1δ on electrophoresis in denaturing gels have apparent M_rs of 38,000 and 40,000. The higher-M_r form is a minor species in mock-infected cells, whereas in human fibroblasts and Vero cells infected with HSV-1, this isoform becomes dominant. These results indicate that ICP0 is present and may have a significant role in the cytoplasm of infected cells, possibly by altering the efficiency of translation of viral mRNAs.

Herpes simplex virus 1 (HSV-1) encodes at least 84 different proteins, several of which are regulatory proteins that affect or alter gene expression and by this definition would qualify as regulatory proteins (31). A short list would include the α-*trans*-inducing factor (α-TIF) or virion protein 16, infected cell protein 0 (ICP0), ICP4, ICP22, and ICP27 and the protein kinase encoded by the open reading frame U_L13. With the possible exception of U_L13, most of the viral regulatory proteins appear to perform several functions, some related and some quite diverse. For example, α-TIF is both an essential virion structural protein as well as a transactivator of α gene transcription (32). ICP4 functions as a transactivator and a repressor and to block apoptosis (14, 32). Both ICP22 and ICP27 have been linked to several aspects of gene expression and processing of RNA, respectively (32). ICP0 is known primarily as a monolithic transactivator of both HSV and non-HSV promoters in transient transfection systems (5, 7, 11, 22, 24, 26–28), and while the term "promiscuous transactivator" adequately describes this function, little is known of the detailed mechanisms by which it earns its appellation.

The observation that ICP0 enhances the expression of genes introduced into cells by transfection or infection suggests that its effects are mediated by a basic cellular machinery which is involved in universal gene expression. Recently, it was reported that ICP0 associates with a cellular protein with an M_r of 135,000 which is a novel member of the ubiquitin-specific proteases (9, 17, 18). It was also reported that ICP0 colocalizes with and modifies ND10, which is a nuclear multiprotein domain of unknown function (1, 4, 8, 15–17). Although the biological significance of these interactions remains to be elucidated, these observations led us to a hypothesis that ICP0 is not just a transactivator but a multifunctional protein that interacts with a variety of cellular proteins and that the functions of ICP0 result from the sum of these interactions. This hypothesis predicts that many additional targets of ICP0 interaction different from those reported to date remain, and further understanding of ICP0 function requires their identification.

In this report, we follow up studies with the yeast (*Saccharomyces cerevisiae*) two-hybrid system showing that ICP0 interacts with the translation elongation factor 1 delta (EF-1δ). Our studies indicate that (i) ICP0 localized in cytoplasm throughout HSV-1 infection, in some instances even at early stages of infection; (ii) a domain of ICP0 able to interact with EF-1δ affects translational efficiency; and (iii) HSV-1 infection causes preferential accumulation of a modified form of EF-1δ.

MATERIALS AND METHODS

Cells and viruses. HEp-2 and Vero cells were obtained from the American Type Culture Collection, whereas human foreskin fibroblast (HFF) and human lung fibroblast (HLF) cell strains were obtained from Aviron (Mountain View, Calif.). All cell lines were grown in Dulbecco's modified Eagle medium supplemented with 5% newborn calf serum (Vero cells), 5% fetal calf serum (HEp-2 cells), and 10% fetal calf serum (HFF and HLF cells). HSV-1(F), a limited-passage isolate, is the prototype strain used in this laboratory (3).

Yeast two-hybrid screen. A yeast two-hybrid screening system was used to isolate cDNAs encoding proteins able to interact with ICP0. Fragments encoding α0 codons 543 to 775 were amplified by PCR and inserted into pGBT9 (Clontech, Palo Alto, Calif.) in frame with the DNA-binding domain of GAL4. The resulting "bait" plasmid (pRB4990) and an Epstein-Barr virus-transformed human peripheral blood lymphocyte cDNA library (a gift of Aviron Inc.), which was fused to the GAL4 transcriptional activation domain in pACT, were sequentially cotransformed into yeast strain HF7c (Clontech). Double transformants were selected on SD medium (Clontech) lacking tryptophan, leucine, and histidine but containing 5 mM 3-amino-1,2,4-triazole. Positive colonies were restreaked on the

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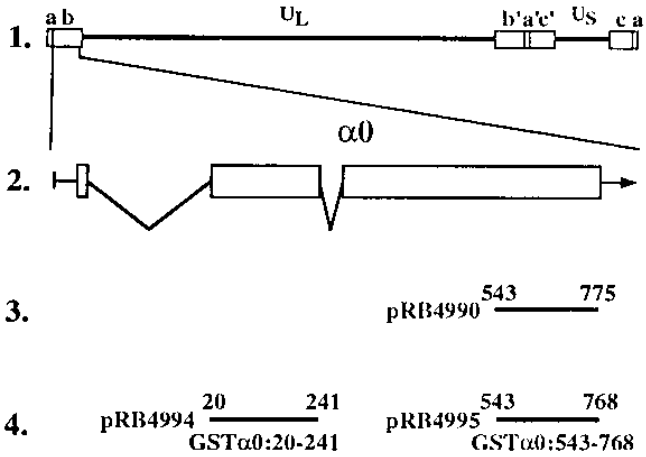

FIG. 1. Schematic diagram of the sequence arrangement of the HSV-1 genome and of the location of the α0 gene. Line 1 shows a linear representation of the HSV-1 genome. The unique sequences are represented as the unique long (U_L) and unique short (U_S) regions. The terminal repeats flanking the unique sequences are shown as open rectangles with their designation letters given above. Line 2 represents an expanded section of the domain encoding the α0 gene. The transcript and coding regions are shown for only one copy of the α0 gene. A second, identical copy is located in the internal inverted repeats flanking U_L. Line 3 shows the region used in the yeast two-hybrid screen. Line 4 shows the domains of the α0 gene used for generation of GST-ICP0 fusion proteins.

same medium and then tested for β-galactosidase activity by filter assay (as recommended by Clontech). Blue colonies were grown in SD medium (Clontech) lacking leucine, and library plasmids were isolated and electroporated into *Escherichia coli* HB101. Bacterial transformants were selected on M9 minimal medium containing ampicillin (50 μg/ml), proline (40 μg/ml), and 1 mM thiamine, 0.4% glucose and an amino acid mixture lacking leucine, and the library plasmids were repurified (as recommended by Clontech). The purified plasmids were cotransformed with pGBT9, pRB4990, pLAM5′ encoding a human lamin C (Clontech), or pVA3 encoding a murine p53 (Clontech) into HF7c cells, and transformants were assayed for β-galactosidase activity to eliminate false positives.

Production and purification of GST fusion proteins. Fragments encoding EF-1δ codons 1 to 280 were amplified by PCR and inserted into pGEX4T-3 in frame with glutathione *S*-transferase (GST). The resulting plasmid was designated pRB4993. Fragments encoding the ICP0 codons 20 to 241 generated by PCR amplification were inserted into pGEX4T-1, and the resulting plasmid was designated pRB4994 (Fig. 1). pRB4990 was cut with *Eco*RI and *Sal*I, and the 0.7-kbp fragment encoding ICP0 codons 543 to 768 was cloned into pGEX4T-1, and the resulting plasmid was designated pRB4995 (Fig. 1).

E. coli BL21 cells transformed with the plasmids encoding GST fusion proteins were induced with 0.1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) for 2 to 3 h after the optical density at 600 nm reached a value ranging from 0.6 to 0.8. The harvested cells were lysed by sonication in phosphate-buffered saline (PBS), and then Triton X-100 was added to a final concentration of 1%. After cell debris were clarified by centrifugation, GST fusion proteins were adsorbed to glutathione-agarose beads (Sigma, St. Louis, Mo.), washed with PBS, and eluted with 10 mM reduced glutathione (Sigma) in 50 mM Tris-HCl (pH 8.0). The eluted proteins were quantified with a Bio-Rad protein assay kit (Bio-Rad, Hercules, Calif.). Bovine serum albumin (BSA) was used as a protein standard.

Affinity precipitation with GST–EF-1δ fusion protein. HEp-2 cells grown in 150-cm^2 flask cultures were infected with 10 PFU of HSV-1(F) per cell. At 18 h after infection, the cells were scraped into PBS, washed with PBS, and resuspended in 500 μl of HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer (50 mM HEPES [pH 7.4], 250 mM NaCl, 10 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride [PMSF], 0.1 mM TLCK [*N*α-*p*-tosyl-L-lysine chloromethylketone], 0.1 mM TPCK [tolylsulfonyl phenylalanyl chloromethyl ketone]). Cells were lysed by addition of Triton X-100 to 1%, and cell debris was removed by centrifugation at top speed in a microcentrifuge. The supernatant fluid (200 μl) was mixed with purified GST or GST–EF-1δ immobilized on glutathione-agarose beads. After overnight incubation at 4°C with continuous mixing, the beads were collected by brief centrifugation, washed four times with HEPES buffer containing 1% Triton X-100, and resuspended in 100 μl of 2× disruption buffer (125 mM Tris-HCl [pH 6.8], 10% β-mercaptoethanol, 4% sodium dodecyl sulfate [SDS], 20% glycerol). The bound protein complexes were subjected to electrophoresis on an 8% polyacrylamide gel containing SDS, transferred to a nitrocellulose sheet, and reacted with a mouse monoclonal antibody to ICP0 (H1083).

Production of the EF-1δ rabbit antiserum. Two rabbits were inoculated by standard protocol at Jasman Laboratories (Napa, Calif.). Specifically, the protocol included five subcutaneous injections of 2.5 mg of the purified GST–EF-1δ fusion protein at 14-day intervals. The serum used in the studies reported here was collected 4 weeks after the first immunization.

Cell fractionation. HEp-2 cells grown in 150-cm^2 flask cultures were infected with 10 PFU of HSV-1(F) per cell. At 18 h after infection, the cells were scraped into PBS, washed with PBS, and resuspended in buffer A (10 mM HEPES [pH 7.4], 1.5 mM $MgCl_2$, 10 mM NaCl, 1 mM PMSF, 0.1 mM TLCK, 0.1 mM TPCK). The cells were lysed by addition of Nonidet P-40 to 0.1% or by using five strokes of a tight Dounce homogenizer, and nuclei were pelleted by centrifugation at top speed in a microcentrifuge. The supernatant fluid (cytoplasmic fraction) was transferred into a new tube. The pellet was washed with buffer A, resuspended in buffer C (10 mM HEPES [pH 7.4], 1.5 mM $MgCl_2$, 420 mM NaCl, 1 mM PMSF, 0.1 mM TLCK, 0.1 mM TPCK), and sonicated briefly. The remaining debris was removed by centrifugation as before, and supernatant fluid (nuclear fraction) was collected. To prepare a whole-cell extract, the infected cells were washed with PBS and resuspended in 500 μl of PBS* (1% deoxycholate, 1% Nonidet P-40, 1 mM PMSF, 0.1 mM TLCK, 0.1 mM TPCK in PBS), and cell debris was clarified by centrifugation. The fractions were then solubilized by addition of 2× disruption buffer and subjected to electrophoresis on an 8% polyacrylamide gel containing SDS.

Immunoblotting. In the immunoblotting procedure, proteins electrophoretically separated in denaturing gels were electrically transferred to nitrocellulose membranes. The membranes were blocked with 5% skim milk in PBS for 1 h or overnight, rinsed once, washed twice for 5 min each time in T-PBS (PBS containing 0.05% Tween 20), and reacted for 2 h with primary antibodies in T-PBS containing 1% BSA. The final dilutions were 1:500 for the mouse monoclonal antibody to ICP0 (H1083), 1:500 for a mouse monoclonal antibody to ICP4 (H640), and 1:1,000 for a rabbit polyclonal antiserum to EF-1δ. The blots were then washed in T-PBS as before, reacted for 1 h with a 1:1,000 dilution of a goat anti-mouse immunoglobulin G or 1:2,000 dilution of anti-rabbit immunoglobulin G conjugated to peroxidase (Sigma) in T-PBS containing 3% skim milk. The blots were washed in T-PBS and then further washed in Tris-buffered saline (50 mM Tris-HCl [pH 7.5], 150 mM NaCl) as before and developed in visualization buffer (100 mM Tris-HCl [pH 7.5], 0.8 mg of 3′ 3-diaminobenzidine per ml, 0.4 mg of $CoCl_2$ per ml, 0.009% H_2O_2).

Immunofluorescence. Approximately 5×10^4 cells were seeded onto glass slides (Cell-Line, Newfield, N.J.), infected with 10 PFU of HSV-1(F) per cell, incubated at 37°C, and fixed in ice-cold methanol for 20 min. The cells were blocked for 30 min in PBS containing 20% normal human serum and 1% BSA at room temperature, rinsed once with PBS, and reacted for 2 h with a 1:500 dilution of the mouse monoclonal antibody to ICP0 (H1083) in PBS containing 10% human serum and 0.2% BSA. The cells were then rinsed three times in PBS, reacted for 1 h with a 1:400 dilution of a goat anti-mouse immunoglobulin G conjugated to Texas red (Molecular Probes, Eugene, Oreg.) in PBS containing 10% human serum and 0.2% BSA, rinsed again three times with PBS, and mounted in PBS containing 90% glycerol and 1 mg of *p*-phenylenediamine per ml. The slides were examined in a Zeiss confocal fluorescence microscope. Digitized images of the fluorescent antibody-stained cells were acquired with software provided by the Zeiss confocal microscope and printed by a Codonics CP210 digital printer.

In vitro translation. A total of 8.8 μl of purified GST or indicated GST fusion proteins in the elution buffer for GST proteins was mixed with 12.5 μl of rabbit reticulocyte lysate (Promega, Madison, Wis.) and incubated for 40 min on ice. A total of 0.5 μg of luciferase RNA (Promega) was then translated with a kit obtained from Promega and the rabbit reticulocyte lysates containing the GST proteins. After incubation for 90 min at 30°C, 5 μl of reaction mixtures was subjected to electrophoresis on a 13% polyacrylamide gel containing SDS. The [^{35}S]methionine-labeled translated products were detected by autoradiography, and the relative radioactivities of the products were analyzed with a Betagen β Analyzer, Beta-Scope 603.

RESULTS

ICP0 interacts with translation factor, EF-1δ, in a yeast two-hybrid system. The results of the two-hybrid studies described in detail in Materials and Methods were as follows: Of 3.4×10^5 colonies, 52 colonies were histidine positive, and of these, 46 colonies expressed β-galactosidase activity. Retransformation of the 46 plasmids from the positive colonies yielded 43 positive clones which tested positive for interaction with ICP0 and negative for interaction with human lamin, murine p53, or the GAL4 DNA-binding domain alone. Of the 43 plasmids, the sequences at the 5′ and 3′ ends of the insert of three plasmids were determined, and all of them were found to match completely to the published sequence of the human EF-1δ subunit of EF-1—a protein complex that participates in

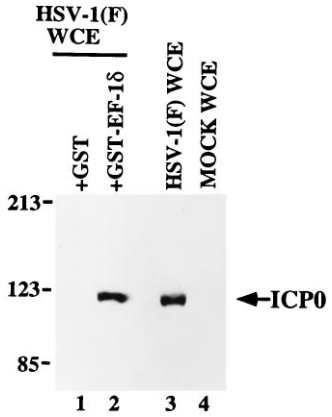

FIG. 2. Photographic image of an immunoblot of infected cell proteins bound to GST or chimeric GST fusion proteins, electrophoretically separated in a denaturing gel and reacted with a mouse monoclonal antibody to ICP0 (H1083). Lysates of infected HEp-2 cells were reacted with GST or GST–EF-1δ chimeric protein immobilized on glutathione-agarose beads. The beads were pelleted, rinsed extensively, subjected to electrophoresis on an SDS–8% polyacrylamide gel, transferred to nitrocellulose, and reacted with the ICP0 antibody. Lanes: 1 and 2, whole-cell extracts (WCE) from HSV-1-infected HEp-2 cells bound to GST and GST–EF-1δ, respectively; 3 and 4, whole-cell extracts from HSV-1-infected and mock-infected HEp-2 cells, respectively. ICP0-specific bands are indicated on the right, and molecular weights (in thousands) are shown on the left.

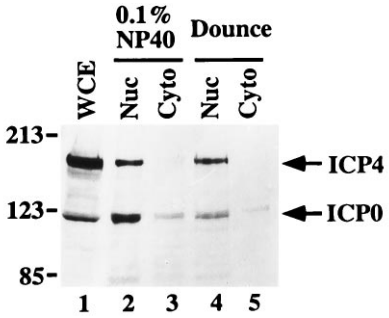

FIG. 3. Photographic image of an immunoblot of electrophoretically separated cell fractions of HEp-2 cells infected with HSV-1. Infected HEp-2 cells were harvested and lysed by addition of 1% Nonidet P-40 and 1% deoxycholate (lane 1) or 0.1% Nonidet P-40 (lanes 2 and 3), or by Dounce homogenizer (lanes 4 and 5). Whole-cell extracts (WCE; lane 1), nuclear (Nuc; lanes 2 and 4), and cytoplasmic (Cyto; lanes 3 and 5) fractions were prepared as described in Materials and Methods, subjected to electrophoresis on an SDS–8% polyacrylamide gel, transferred to nitrocellulose, and reacted with the mouse monoclonal antibodies to ICP0 (H1083) and ICP4 (H640). ICP0- and ICP4-specific bands are indicated on the right, and molecular weights (in thousands) are shown on the left.

the elongation step during translation of mRNA (19, 30, 33). The three plasmids encoded the entire open reading frame of EF-1δ, except one last codon encoding isoleucine. The remaining 40 plasmids as well as the three plasmids showed the same endonuclease restriction patterns as the published EF-1δ sequence. These results suggested that the interaction may be significant and worth pursuing.

EF-1δ specifically forms complexes with ICP0 in HSV-1-infected cell lysate. To verify and extend the binding data obtained in yeast, a GST–EF-1δ fusion protein was expressed in *E. coli*. The GST-EF-1δ or GST bound to glutathione-agarose beads was reacted with an extract from HSV-1-infected HEp-2 cells. After extensive rinsing, the beads were solubilized, subjected to electrophoresis in a denaturing gel, transferred to a nitrocellulose sheet, and reacted with the monoclonal antibody (H1083) to ICP0. As shown in Fig. 2, the GST–EF-1δ fusion protein was able to pull down ICP0, whereas GST alone did not. The electrophoretic mobility of the ICP0 bound to GST–EF-1δ fusion protein was similar to that found in extracts of whole infected cells. The results indicate that EF-1δ can interact with native, full-length ICP0.

ICP0 clearly localizes in the cytoplasm of HSV-1-infected cells. ICP0 is thought to be primarily a nuclear protein, and all functions associated with this protein are ascribed to its nuclear localization (6, 10, 12, 13). However, the results described above would suggest that ICP0 also functions in the cytoplasm, because EF-1δ is a cytoplasmic protein. The objectives of the studies described below indicate that indeed ICP0 also localizes in the cytoplasm of HSV-1-infected cells. These conclusions are based on two series of experiments.

In the first, HEp-2 cells were infected with HSV-1(F) and harvested at 18 h postinfection. The nuclei and cytoplasms were separated, as described in Materials and Methods, by two methods: by the use of nonionic detergents and by Dounce homogenization. Each fraction was subjected to electrophoresis in a denaturing polyacrylamide gel and then reacted with the monoclonal antibodies to ICP0 (H1083) and ICP4 (H640) as described in Materials and Methods. As shown in Fig. 3, ICP0 was detected both in nuclei and in cytoplasms of infected cells fractionated by either technique. In contrast, ICP4, known to be sequestered in the nuclei of infected cells (12, 13), was not detectable in the cytoplasmic fraction.

In the second series of experiments, slide cultures of HEp-2 and HFF cells were infected with HSV-1(F), fixed at times after infection indicated in the legend to Fig. 4, and reacted with the anti-ICP0 monoclonal antibody (H1083). The results were as follows.

(i) In HSV-1(F)-infected HEp-2 cells, punctate patterns of ICP0 staining were detected in the nuclei of infected cells at 3 h after infection (Fig. 4a). At later times (8 h in Fig. 4b, 18 h in Fig. 4c), both diffuse and punctate ICP0 staining were also evident in cytoplasms as well as in nuclei.

(ii) In HSV-1(F)-infected HFF cells, ICP0 was evident as both diffuse and punctate staining predominantly in cytoplasms throughout infection, including at 3 h (Fig. 4d and e) and 8 h (Fig. 4f) after infection, although punctate staining was also detected in the nuclei of only a few cells (Fig. 4e).

We conclude that ICP0 has a significant presence in the cytoplasm of infected cells, and therefore the protein could have a cytoplasmic function related to its role as a promiscuous transactivator.

Carboxyl-terminal domain of ICP0 affects translational efficiency. The EF-1 complex is known to be involved in the elongation of polypeptide chains during protein biosynthesis (19, 30). Our demonstration of the interaction between ICP0 and EF-1δ therefore raised the question of whether ICP0 affects the functions of EF-1. To elucidate this possibility, luciferase RNA was translated with rabbit reticulocyte lysate in the presence of increasing amounts of GST alone or of the carboxyl- or amino-terminal domain of ICP0 fused to GST (Fig. 5A and B). [^{35}S]methionine-labeled translated products were separated on an SDS-polyacrylamide gel and analyzed as follows. The gel was subjected to autoradiography, and the autoradiographic image is shown in Fig. 5B. In addition, the amount of radioactivity in each band was quantified with a Betagen β counter as described in Materials and Methods. The results were as follows.

Although the full-length chimeric GST-carboxyl-terminal domain of ICP0 polypeptide is seven amino acids smaller than the chimeric bait protein used in the two-hybrid system (Fig.

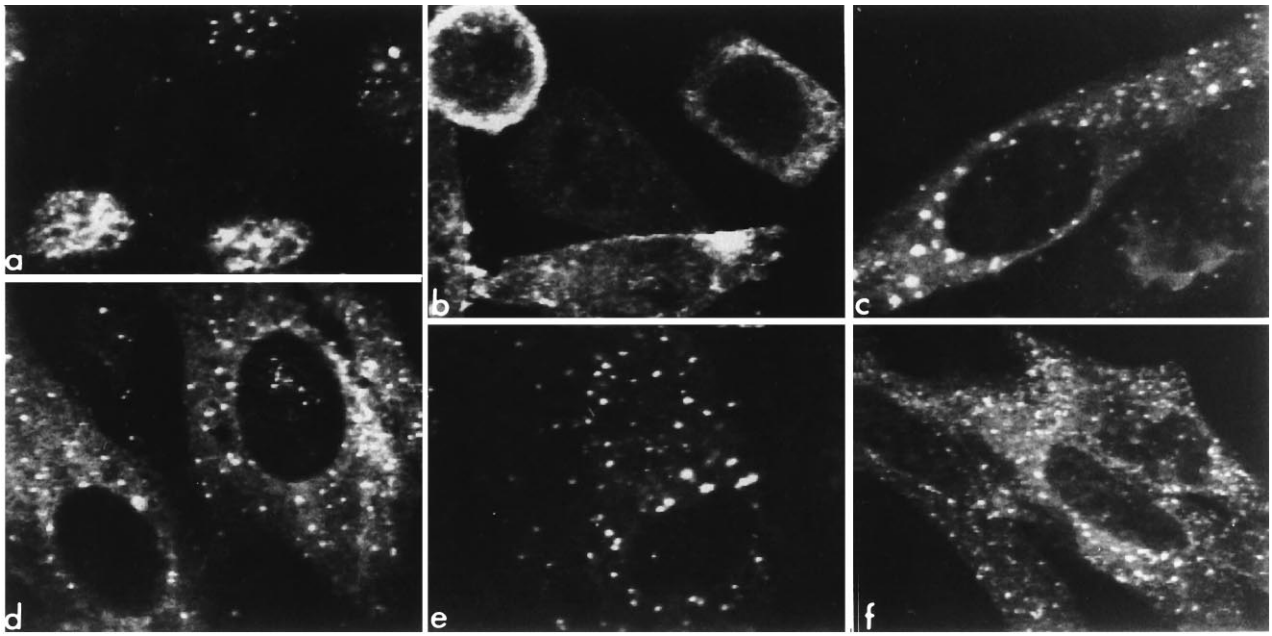

FIG. 4. Digital, unprocessed images of HEp-2 (a, b, and c) and HFF (d, e, and f) cells infected with HSV-1(F); maintained at 37°C for 3 h (a, d, and e), 8 h (b and f), or 18 h (c); and fixed and reacted with the ICP0 antibody (H1083) and anti-mouse Texas red-conjugated secondary antibody as described in Materials and Methods.

1), the GST–carboxyl-terminal domain was shown in preliminary experiments to interact with ICP0 in yeast (data not shown). The results shown in Fig. 5B and C indicate that the addition of the chimeric protein GST–carboxyl-terminal domain of ICP0 to rabbit reticulocyte lysate resulted in a dose-dependent decrease in protein synthesis. In contrast, GST alone or GST fused to the amino-terminal domain of ICP0 had no effect on the in vitro translation system. These results indicate that the domain of ICP0 shown to interact with EF-1δ in the yeast two-hybrid system is also able to affect protein synthesis in lysates of rabbit reticulocytes.

HSV-1 infection causes modification of EF-1δ. EF-1δ is known to be phosphorylated by cdc2 kinase, and the phosphorylation results in a decrease in the electrophoretic mobility of the protein on electrophoresis in denaturing polyacrylamide gels (20, 23). The objectives of the experiments described in this section were to determine whether EF-1δ is modified in the course of productive HSV-1 infection. To monitor changes in EF-1δ, Vero, HLF, HFF, or HEp-2 cells were harvested 3, 8, and 20 h after mock infection or infection with 10 PFU of HSV-1(F) per cell, solubilized, electrophoretically separated in denaturing gels, electrically transferred to nitrocellulose sheets, and reacted with the rabbit polyclonal antibody to EF-1δ. The results (Fig. 6) were as follows.

(i) EF-1δ forms three bands on electrophoresis in denaturing polyacrylamide gels. In mock-infected Vero, HLF, and HFF cells, the middle band is the dominant one. The amount of protein in the more-slowly-migrating band was smaller than that present in the middle band.

(ii) At 3 h after infection in Vero, HLF, and HFF cells, the ratios of proteins in the upper to middle bands were similar to those of mock-infected cells. At later times after infection, the relative amounts of protein in the upper bands dramatically increased as infection progressed.

(iii) In HEp-2 cells, the ratio of proteins in the upper to middle bands remained relatively constant and was not affected by the infection. We conclude that EF-1δ is modified by HSV-1 infection and that the changes are cell type dependent.

DISCUSSION

As noted in the introduction, ICP0 is a promiscuous transactivator predicted to interact with cellular factors involved in gene expression. It is a characteristic of most HSV-1 regulatory proteins examined to date that they perform several functions, and we expected that ICP0 would not be an exception. The striking feature of the two-hybrid analyses reported in this study is the number of positive colonies containing the EF-1δ cDNA. These studies indicated a potential interaction between the carboxyl terminus of the third exon of ICP0 with EF-1δ and prompted us to take a closer look at the distribution of ICP0 in infected cells and the interaction of ICP0 with EF-1δ.

EF-1δ is a subunit of EF-1—a complex of factors which mediate the elongation of polypeptide chains during translation of mRNA. EF-1α transports aminoacyl tRNA for binding to ribosomes concurrent with hydrolysis of GTP, whereas EF-1δ is a component of the EF-1–βγδ complex responsible for the GDP-GTP exchange on EF-1α (19, 21, 30, 34). EF-1δ therefore plays a key role in protein synthesis. The key observations which suggest biological significance of the interaction between ICP0 and EF-1δ are as follows.

(i) ICP0 accumulates in both nuclei and cytoplasm at various stages of infection. If ICP0 interacts with EF-1δ in infected cells, it would be necessary for ICP0 to localize in the cytoplasm. Until these studies were initiated, our impression was that ICP0 is a nuclear protein, an impression fueled in large part by a vast literature illustrating the promiscuous transactivation function of the protein in transient expression systems. In these systems, ICP0 is in nuclei. In this report, we show the existence of native ICP0 in cytoplasmic fractions both by fractionation of infected cells and by immunofluorescence. The observation that in primary human cells ICP0 localizes in the cytoplasm virtually throughout productive infection adds considerable weight to the hypothesis that ICP0 performs a cytoplasmic function.

Parenthetically, the shift of ICP0 from nuclei to cytoplasm varies, depending on the cell line. In continuous cell lines,

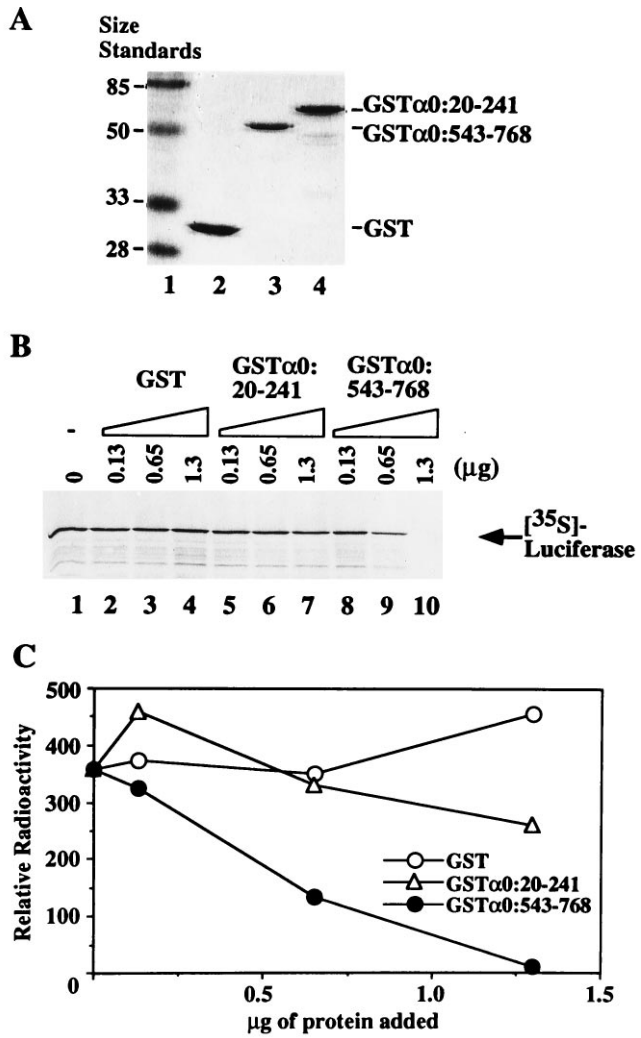

FIG. 5. Carboxyl-terminal domain of ICP0 specifically inhibits translational activity in rabbit reticulocyte lysates. (A) Photographic image of a Coomassie blue-stained SDS-polyacrylamide gel of electrophoretically separated GST proteins. Lanes: 1, molecular weight markers; 2, GST; 3, GST–carboxyl-terminal domain of ICP0 from amino acid 543 to amino acid 768; 4, GST–amino-terminal domain of ICP0 from amino acid 20 to amino acid 241. (B) Autoradiographic images of electrophoretically separated [^{35}S]methionine-labeled luciferases translated in vitro. Rabbit reticulocyte lysate was incubated with the indicated concentration of each GST protein, and then luciferase RNA was translated with the lysate. The translated products were subjected to electrophoresis on an SDS–13% polyacrylamide gel and subjected to autoradiography. (C) Quantification of the [^{35}S]methionine-labeled luciferases translated in vitro. The relative radioactivities of the translated products were quantitated with the Betagen β Analyzer, Beta-Scope 603.

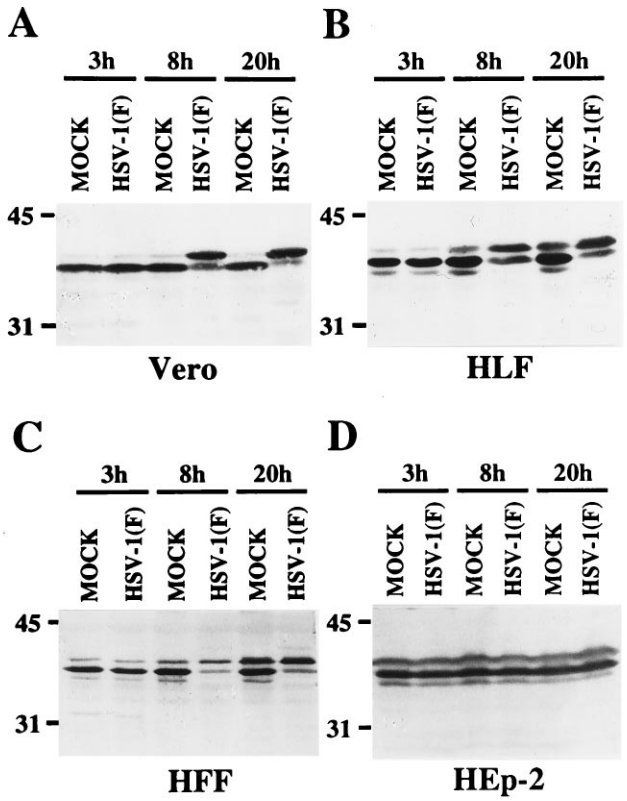

FIG. 6. Photographic image of immunoblots of electrophoretically separated cell lysates from mock- or HSV-1-infected Vero (A), HLF (B), HFF (C), and HEp-2 (D) cells. The HSV-1-infected cells were harvested at the indicated times, subjected to electrophoresis on SDS–12% polyacrylamide gels, transferred to nitrocellulose, and reacted with a rabbit antiserum raised against GST–EF-1δ. Molecular weights (in thousands) are shown on the left of each panel.

ICP0 accumulated in the cytoplasm later than in primary human fibroblasts. Recently, it has been reported that in Vero cells infected with mutants overproducing ICP27, ICP0 accumulated at 5 h after infection in both nuclei and cytoplasm, whereas in cells infected with the wild-type virus, ICP0 was detected by immunofluorescence in nuclei only (38). It is conceivable that in these studies, overexpression of ICP27 resulted in accelerated accumulation of ICP0 in the cytoplasm of Vero cells.

(ii) GST-EF-1δ chimeric protein pulls down ICP0. This experiment is in effect a reciprocal of the yeast two-hybrid system and reinforces the evidence of physical interaction between these two proteins. However, it does not address the function or requirements for this interaction.

(iii) The domain of the ICP0 interacting with EF-1δ in the context of a GST-ICP0 chimeric protein interferes with protein synthesis in vitro. We do not know the function of ICP0 in the translation of viral mRNAs. Irrespective of its function, but particularly if the interaction of native ICP0 benefits translation, it could be expected that the interaction of a truncated portion of the ICP0 capable of binding EF-1δ may have a dominant negative effect. We show that the domain of ICP0 which acted as bait in the two-hybrid system but not GST alone or another domain of ICP0 inhibits in vitro translation of a test mRNA.

(iv) EF-1δ is modified during infection in a cell line-dependent manner. In *Xenopus* oocytes, EF-1δ is known to be phosphorylated by cdc2 kinase, which controls entry into mitosis by phosphorylating various substrates (20, 23, 25). EF-1δ is detected as a singlet with an M_r of 36,000 during oogenesis, while EF-1δ is resolved into a doublet with M_rs of 36,000 and 38,000 by cdc2 kinase-induced phosphorylation during mitotic division (20). Although there is no direct evidence for the physiological role of phosphorylation, this posttranslational processing is correlated with changes in the pattern of protein synthesis (29, 37). It is also known that rabbit EF-1 activity is enhanced by phosphorylation in vivo with phorbol ester or in vitro phosphorylation with protein kinase C, in which the predominant phosphorylation is on the β and δ subunits (35, 36). Furthermore, translation initiation factor, eIF-2B, which ap-

pears to have the same function as the EF-1–βγδ complex in the initiation step of translation, is also regulated by phosphorylation. In this instance, the modification causes an increase in GDP-GTP exchange activity of the protein (2). Thus, it is conceivable that phosphorylation of EF-1δ could change activity of the protein and result in alteration of translational efficiency. In mammalian cells, EF-1δ is detected primarily as doublet with M_rs of 38,000 and 40,000. Significantly, as HSV-1 infection progressed, dramatic changes in the ratio of proteins in the dominant upper and middle bands of EF-1δ were observed. These results suggest that HSV-1 infection causes posttranslational modification of EF-1δ, and the HSV-1-induced changes could affect the activity of protein synthesis.

The salient new contribution presented in this report is that ICP0 may have a significant role in the cytoplasm of infected cells—a role overshadowed by studies of nuclear localization of the protein. The function of ICP0 in translation of viral proteins remains to be determined. Our data point to the hypothesis that ICP0 interacts with EF-1δ and thus accelerates or induces posttranslational modifications that affect the activity of translational machinery. This effect may be superfluous in some cells, but extremely important for viral gene expression in others.

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