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Eukaryotic Elongation Factor 1δ Is Hyperphosphorylated by the Protein Kinase Encoded by the U_L13 Gene of Herpes Simplex Virus 1

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The translation elongation factor 1δ (EF-1δ) consists of two forms, a hypophosphorylated form (apparent M_r, 38,000) and a hyperphosphorylated form (apparent M_r, 40,000). Earlier Y. Kawaguchi, R. Bruni, and B. Roizman (J. Virol. 71:1019–1024, 1997) reported that whereas mock-infected cells accumulate the hypophosphorylated form, the hyperphosphorylated form of EF-1δ accumulates in cells infected with herpes simplex virus 1. We now report that the accumulation of the hyperphosphorylated EF-1δ is due to phosphorylation by U_L13 protein kinase based on the following observations. (i) The relative amounts of hypo- and hyperphosphorylated EF-1δ in Vero cells infected with mutant virus lacking the U_L13 gene could not be differentiated from those of mock-infected cells. In contrast, the hyperphosphorylated EF-1δ was the predominant form in Vero cells infected with wild-type viruses, a recombinant virus in which the deleted U_L13 sequences were restored, or with a virus lacking the U_S3 gene, which also encodes a protein kinase. (ii) The absence of the hyperphosphorylated EF-1δ in cells infected with the U_L13 deletion mutant was not due to failure of posttranslational modification of infected-cell protein 22 (ICP22)/$U_S1.5$ or of interaction with ICP0, inasmuch as preferential accumulation of hyperphosphorylated EF-1δ was observed in cells infected with viruses from which the genes encoding ICP22/$U_S1.5$ or ICP0 had been deleted. (iii) Both forms of EF-1δ were labeled by $^{32}P_i$ in vivo, but the prevalence of the hyperphosphorylated EF-1δ was dependent on the presence of the U_L13 protein. (iv) EF-1δ immunoprecipitated from uninfected Vero cells was phosphorylated by U_L13 precipitated by the anti-U_L13 antibody from lysates of wild-type virus-infected cells, but not by complexes formed by the interaction of the U_L13 antibody with lysates of cells infected with a mutant lacking the U_L13 gene. This is the first evidence that a viral protein kinase targets a cellular protein. Together with evidence that ICP0 also interacts with EF-1δ reported in the paper cited above, these data indicate that herpes simplex virus 1 has evolved a complex strategy for optimization of infected-cell protein synthesis.

Viruses are totally dependent on the synthetic machinery of host cells for their replication, and as a consequence, viral gene products interact with and modify to activate, suppress, or redirect the functions of cellular proteins. Of the strategies for attaining this objective, phosphorylation of proteins by protein kinases could be expected to be among the most potent, since this is a major strategy employed by eukaryotic cells to regulate cellular functions (5). Curiously, only the large DNA viruses encode kinases (21, 34), and except for cellular oncogenes transduced into retroviruses (e.g., v-*src* and v-*erb*) (23), there is a dearth of information on employment, modification, and redirection of cellular protein kinases by viruses to modify cellular substrates. Even among the DNA viruses which encode their own kinases, most of the available data are on specific viral substrates. In this report, we show that the translation elongation factor 1δ (EF-1δ) is phosphorylated during infection by the product of the U_L13 gene of herpes simplex virus 1 (HSV-1). Although this protein has long been thought to be a protein kinase, only recently has the U_L13 gene product been purified and shown to be a kinase (3). In this report, we present the first evidence that a viral kinase phosphorylates a cellular protein. Relevant to this report are the following observations.

(i) HSV-1 encodes at least 84 different proteins expressed in three major coordinately regulated, sequentially ordered groups designated α, β, and γ (10, 11, 33, 34). The expression of α genes, the first set of genes to be expressed, is enhanced by α-*trans*-inducing factor (34). The expression of β genes requires functional α proteins, and both α and β proteins and viral DNA synthesis mediated by β proteins are required for optimal expression of γ genes that encode largely virion structural proteins (34). Six α proteins termed infected-cell protein 0 (ICP0), ICP4, ICP22, ICP27, ICP47, and $U_S1.5$ have been identified, and for the most part, they function as regulatory proteins that affect or alter viral gene expression or alter the function of cellular proteins (1, 34).

(ii) ICP0, one of the α proteins, has been shown to be a positive, promiscuous transactivator of genes introduced into cells by infection or transfection (Fig. 1) (8). Although the mechanism by which ICP0 acts in viral replication is unclear, it is likely that ICP0 is a multifunctional protein which interacts with a variety of cellular proteins and that the function of ICP0 in virus replication results from the sum of these interactions. For instance, it has been reported that ICP0 interacts with at least three different cellular proteins, the translation factor EF-1δ (13); a cell cycle regulator, cyclin D3 (14); and a ubiquitin-specific protease, HAUSP (7). Deletions of the regions of ICP0 which interact with these cellular proteins or are involved in the ability of promiscuous transactivation impair viral lytic growth (8). Therefore, ICP0 may modulate many key cellular

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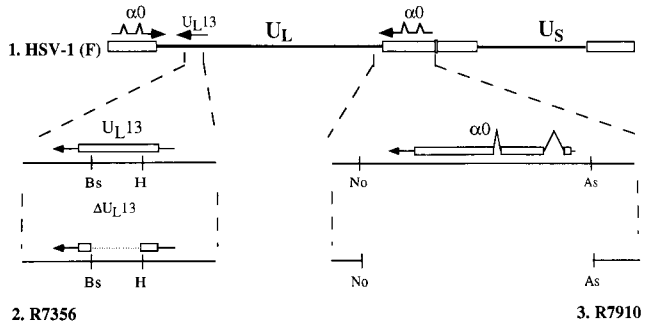

FIG. 1. Schematic diagram of the sequence arrangement of the HSV-1 genome showing the location of the U_L13 and α0 genes. Line 1 is a linear representation of the HSV-1(F) genome. The thin lines represent the unique long (U_L) and unique short (U_S) sequences. The terminal repeats flanking the unique sequences are shown as open rectangles. Line 2 is an expanded section of the coding domain of the U_L13 gene. The transcript is represented by a line capped by an arrowhead, and the coding domain of U_L13 is represented by an open rectangle. R7356 contains a deletion spanning *Hin*dIII to *Bst*EII restriction endonuclease sites within the U_L13 coding domain. Line 3 is an expanded section of the coding domain of 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 terminal inverted repeat flanking U_L. R7910 contains deletions of the entire coding regions of α0 genes spanning *Ase*I to *Not*I sites. Bs, *Bst*EII; H, *Hin*dIII; No, *Not*I; As, *Ase*I.

functions, including translation, cell cycle regulation, the protein degradation pathway, and, possibly, transcription of host cells to confer a growth advantage to the virus.

(iii) An earlier report from this laboratory showed that ICP0 interacts with EF-1δ and that the domain of ICP0 which interacts with EF-1δ affects translational efficiency in vitro (13). These observations suggest that ICP0 may regulate viral gene expression at the translational level. In the course of the studies, we also found that HSV-1 infection modifies EF-1δ (13).

EF-1δ plays an important role in regulation of protein synthesis. EF-1δ is a subunit of EF-1, a complex of proteins which mediate the elongation of polypeptide chains during translation of mRNA (18, 20, 32, 36). EF-1α transports aminoacyl tRNA for binding to ribosomes concurrent with hydrolysis of GTP, whereas EF-1δ is a component of the EF-1βγδ complex responsible for GDP-GTP exchange on EF-1α (18, 20, 32, 36). EF-1δ is phosphorylated by several cellular kinases, including casein kinase II (26), cdc2 kinase (22), and protein kinase C (38). The studies on the phosphorylation of EF-1δ suggest that the hyperphosphorylation of the protein alters translational efficiency (19, 22, 31, 37–39).

(iv) HSV encodes two major protein kinases expressed by the U_L13 and U_S3 genes, whose amino acid sequences contain motifs common to known protein kinases (2, 17, 34, 35). Whereas the amino acid sequence that encodes U_L13 protein kinase is conserved in members of all subfamilies of the family *Herpesviridae*, that of U_S3 is conserved only in the *Alphaherpesvirinae* subfamily (2, 17, 34, 35). The known substrate of the U_S3 protein kinase is ICP22 and an essential viral membrane protein encoded by U_L34 (29, 30). Recently it has been reported that U_S3 is required for protection from apoptosis induced by HSV-1 infection (16).

The U_L13 protein kinase mediates the phosphorylation of several viral proteins, such as ICP22 (29), ICP0 (25), and viral glycoprotein E (24). Unlike U_S3, the U_L13 is packaged into the virion (34). Studies with U_L13 mutants showed that the viral protein kinase affects the accumulation of ICP0 and a subset of γ proteins, suggesting that the activity of U_L13 plays a role in viral gene expression (28). We report here that U_L13 hyperphosphorylates EF-1δ.

TABLE 1. Genotype and phenotype of the viruses used in this study

Virus	Genotype[a]	Relevant phenotype
HSV-1(F)	Wild type	Wild type
HSV-1(F)	Δ305 $\Delta U_L23/\Delta U_L24$	TK^-
R7041	U_L23R/U_L24R ΔU_S3	U_S3^-
R7355	$\Delta U_L223R/\Delta U_L24R$ ΔU_L13	TK^- U_L13^-
R7356	U_L23R/U_L24R ΔU_L13	U_L13^-
R7358	$\Delta U_L23/\Delta U_L24$ U_L13R	TK^-
R325(TK^-)	$\Delta U_L23/\Delta U_L24$ Δα22	TK^- $ICP22^-$
R7910	Δα0	$ICP0^-$

[a] Δ, deletion in gene; R, repair of gene; TK, thymidine kinase. A superscript minus sign indicates the function is not expressed.

MATERIALS AND METHODS

Cells and viruses. Vero and rabbit skin cells were originally obtained from the American Type Culture Collection and J. McClaren, respectively. The cell lines were grown in Dulbecco's modified Eagle's medium supplemented with 5% newborn calf serum. An ICP0-expressing cell line, N3 (14), was grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. HSV-1(F), a limited-passage isolate, is the prototype strain used in this laboratory (6). The constructions of HSV-1 recombinant viruses HSV-1(F)Δ305, R7355, R7356, R7358, R7041, and R325 were reported previously (27–30). Construction of an ICP0 deletion mutant virus, R7910, is described below. Table 1 lists the genotypes of all of the viruses used in this study. All viruses except R7910 were propagated in Vero cells. The R7910 recombinant was grown in the ICP0-expressing cell line N3. All titrations of infectivity were done on Vero cells.

Antibody and immunoblotting. The rabbit polyclonal antibody to EF-1δ, ICP0, and U_L13 was generated as described elsewhere (13, 14, 24, 25). The sera for EF-1δ used in immunoblotting and immunoprecipitation were collected 4 and 10 weeks after the first immunization, respectively. The electrophoretically separated proteins transferred to nitrocellulose sheets were reacted with the antibody to EF-1δ or ICP0 as described previously (13).

Cosmids. Cosmids pBC1006, pBC1007, pBC1012, and pBC1013 were constructed as described elsewhere (14). Cosmid pBC1015 was constructed by cloning the product of an *Xba*I C fragment of HSV-1(F) viral DNA into the *Spe*I site of the cosmid vector pRB78 as described elsewhere (14).

Construction of α0 deletion mutant virus R7910. The recombinant virus R7910 was constructed by transfection of cosmids pBC1006, pBC1007, pBC1012, pBC1013, and pBC1015 and plasmid pRB5163 (14) into ICP0-expressing N3 cells as described elsewhere (14). Viruses isolated from individual plaques were plaque purified on N3 cells.

Metabolic labeling and immunoprecipitation. Replicate cell cultures of Vero cells in 25-cm^2 flasks were infected with 10 PFU of the appropriate virus per cell. At 6 h after infection, the cells were incubated for 1 h in Eagle's medium without phosphate. Cells were then labeled with 100 μCi of $^{32}P_i$ (New England Nuclear, Boston, Mass.) in the phosphate-free medium for 5 h. The labeled cells were scraped and washed with phosphate-buffered saline and lysed in 500 μl of lysis buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 1% Nonidet P-40, 50 mM NaF, 0.1 mM sodium orthovanadate, 10 mM β-glycerophosphate, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM TLCK [*N*α-*p*-tosyl-L-lysine chloromethyl ketone], 0.1 mM TPCK [tolylsulfonylphenylalanyl chloromethyl ketone]).

Immunoprecipitations were done as described elsewhere (14). Briefly, supernatant fluids obtained after centrifugation of cell lysates were precleared by mixing with protein A-conjugated Sepharose beads (Sigma, St. Louis, Mo.) for 30 min and were reacted with 2 μl of EF-1δ for 2 h at 4°C. Protein A-Sepharose beads were then added and allowed to react for an additional hour. Immunoprecipitates were collected by brief centrifugation, rinsed four times with the lysis buffer, and either subjected to electrophoresis on polyacrylamide gel containing 9% sodium dodecyl sulfate (SDS) or used for in vitro kinase assays as described below.

In vitro kinase assays. To assay the kinase activity of U_L13, Vero cells were harvested 24 h after infection with either HSV-1(F) or R7356, rinsed with PBS, and solubilized in the lysis buffer as described above. The infected-cell lysates were reacted with 5 μl of normal rabbit sera for 30 min and then with 30 μl of a 50% slurry of protein A-Sepharose beads twice for 30 min. The precleared lysates were then used for immunoprecipitation with U_L13 antibody as described above. The immune complexes harvested on the protein A-conjugated Sepharose beads were rinsed twice with kinase buffer (50 mM Tris-HCl [pH 8.0], 200 mM NaCl, 50 mM $MgCl_2$, 0.1% Nonidet P-40, 1 mM dithiothreitol) and subjected to the kinase assay. To obtain EF-1δ as a substrate in kinase assays,

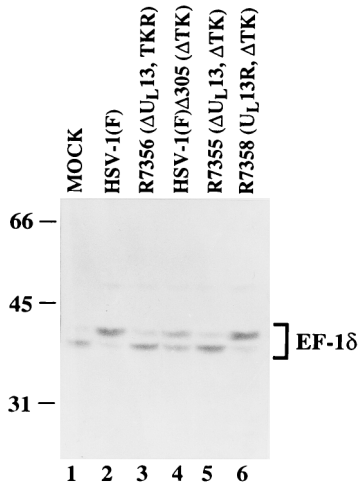

FIG. 2. Photograph of an immunoblot of electrophoretically separated lysates of Vero cells mock infected or infected with 10 PFU of the indicated virus per cell. The infected cells were harvested at 20 h after infection, solubilized, subjected to electrophoresis on an SDS–9% polyacrylamide gel, transferred to a nitrocellulose sheet, and reacted with the antibody to EF-1δ. Molecular weights (in thousands) are shown on the left. ΔTK, thymidine kinase gene deleted; TKR, thymidine kinase gene repaired.

precleared lysates of normal Vero cells with the rabbit normal sera and protein A-Sepharose were used for immunoprecipitation with EF-1δ antibody as described above. The immunoprecipitates were rinsed twice with kinase buffer and subjected to the kinase assay. The reactions were done with the mixtures of immunoprecipitates with the antibodies to EF-1δ and U_L13 at 30°C for 30 min in a total volume of 70 μl of kinase buffer containing 6.5 μM ATP and 68 μCi of [γ-^{32}P]ATP. After incubation, the beads were extensively washed with lysis buffer, and the phosphorylated proteins were resolved on a 9% polyacrylamide gel containing SDS. The gel was subjected to either Coomassie blue staining or immunoblotting with EF-1δ antibody and exposed to X-ray film.

RESULTS

The posttranslational modification of EF-1δ in HSV-1-infected cells is dependent on the presence of viral protein kinase specified by U_L13 and not on the presence of another viral protein kinase specified by U_S3. The objective of these studies was to identify the factor or factors which modify EF-1δ during HSV-1 infection. Of the known HSV-1 proteins, two protein kinases specified by U_L13 and U_S3 were the most prominent candidates for this function. To test this hypothesis, Vero cells were harvested at 20 h after mock infection or infection with 10 PFU of wild-type or mutant viruses per cell, solubilized, electrophoretically separated in denaturing gels, electrophoretically transferred to nitrocellulose sheets, and reacted with the rabbit polyclonal antibody to EF-1δ. The results (Fig. 2 and 3A) were as follows.

(i) EF-1δ harvested from mammalian cells forms two predominant bands migrating with apparent M_rs of 38,000 and 40,000 in denaturing gels (13). As reported previously (13) and shown in Fig. 2 (lanes 1, 2 and 4), in mock-infected cells, the fast-migrating band is the dominant one, whereas the relative amounts of protein in the slow-migrating band dramatically increased after infection with wild-type viruses.

(ii) In cells infected with U_L13 deletion mutant viruses (ΔU_L13), R7355 and R7356 (Fig. 2, lanes 3 and 5, respectively), the increase in the abundance of EF-1δ protein in the slow-migrating band was not observed and the fast-migrating band was still dominant, similar to what has been observed in mock-infected cells (Fig. 2, lane 1). The wild-type virus phenotype was restored in cells infected with the recombinant

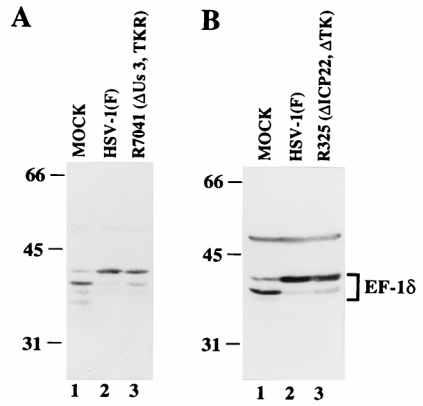

FIG. 3. Photograph of immunoblots of electrophoretically separated lysates of Vero cells mock infected or infected with 10 PFU of the indicated virus per cells. Experiments were done exactly as described in the legend to Fig. 1. ΔTK, thymidine kinase gene deleted; TKR, thymidine kinase gene repaired.

R7358, in which the U_L13 sequence was repaired (Fig. 2, lane 5). Furthermore, the amount of the modified form of EF-1δ did not increase in R7356 (ΔU_L13) virus-infected cells, even at 36 h after infection (data not shown).

(iii) The pattern of electrophoretic mobilities of EF-1δ from cells infected with the ΔU_S3 mutant virus, R7041 (Fig. 3A, lane 3), could not be differentiated from that of EF-1δ extracted from cells infected with the wild-type virus (Fig. 3A, lane 2).

These results indicate that viral protein kinase U_L13, but not U_S3, is required for the posttranslational modification of EF-1δ during HSV-1 infection.

Posttranslational modification of EF-1δ is mediated by U_L13 protein and not by ICP22. Earlier studies from this laboratory have shown that the phenotype of the ΔU_L13 mutant cannot be differentiated from that of the ΔICP22/$\Delta U_S1.5$ mutant with respect to accumulation of ICP0 and of a subset of late proteins (28). To determine whether modification of EF-1δ is mediated by ICP22/$U_S1.5$ proteins, Vero cells were harvested 20 h after mock infection or infection with 10 PFU of HSV-1(F) or R325 ($\Delta\alpha22/\Delta U_S1.5$) virus per cells. The electrophoretically separated infected-cell proteins transferred to a nitrocellulose sheet were reacted with antibody to EF-1δ. As shown in Fig. 3B, the electrophoretic pattern of EF-1δ cannot be differentiated from that observed for EF-1δ extracted from wild-type virus-infected cells. The results indicate that the posttranslational modification of EF-1δ is not mediated by ICP22.

ICP0 is not required for the posttranslational modification of EF-1δ. The observation that U_L13 is required for the modification of EF-1δ during HSV-1 infection raises the possibility that ICP0, which has shown to interact with EF-1δ, is a cofactor of the modification of EF-1δ. Inasmuch as ICP0 is posttranslationally modified by U_L13 and also binds EF-1δ (25), the hypothesis to be tested is that ICP0 binds to and brings U_L13 in apposition to EF-1δ. To address this question, we constructed the α0 null mutant R7910, as described in Materials and Methods, and examined the level of modification of EF-1δ in Vero cells mock infected or infected with HSV-1(F), R7356 (ΔU_L13), or R7910 ($\Delta\alpha0$). As shown in Fig. 4, EF-1δ extracted from HSV-1(F)-infected cells cannot be differentiated from that of cells infected with R7910 ($\Delta\alpha0$) but is readily differentiated from that of cells infected with R7356 (ΔU_L13).

U_L13 is required for phosphorylation of EF-1δ in vivo. The EF-1δ from *Xenopus* oocytes is phosphorylated by several cellular kinases. A consequence of this phosphorylation is a

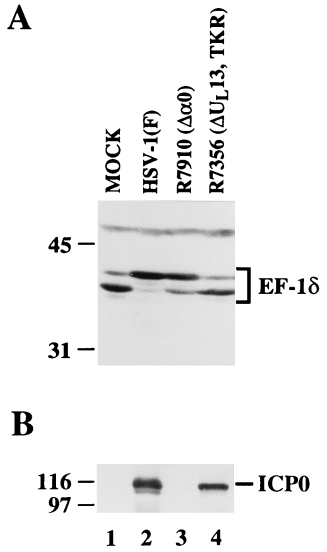

FIG. 4. Photographs of immunoblots of electrophoretically separated lysates of Vero cells mock infected or infected with 1 PFU of the indicated virus per cell. Experiments were done exactly as described in the legend to Fig. 2, except that the nitrocellulose sheet was probed with EF-1δ (A) and then with ICP0 (B). TKR, thymidine kinase gene repaired.

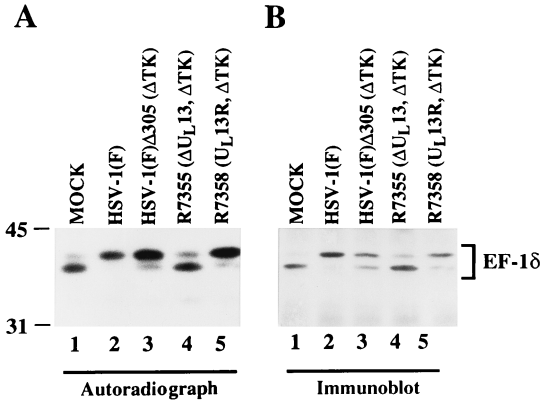

FIG. 5. Autoradiographic and photographic images of ^{32}P-radiolabeled infected-cell lysate immunoprecipitated by the antibody to EF-1δ, subjected to autoradiography, and then reacted with antibody to EF-1δ. Vero cells were mock infected or infected with the indicated virus. At 7 h after infection, the cells were labeled with $^{32}P_i$ for 5 h and then harvested, solubilized, immunoprecipitated with the antibody to EF-1δ, electrophoretically separated in an SDS–9% polyacrylamide gel, transferred to a nitrocellulose sheet, and subjected to autoradiography (A) and then reacted with the antibody to EF-1δ (B). ΔTK, thymidine kinase gene deleted; ΔU_L13, U_L13 gene deleted; U_L13R, U_L13 gene repaired.

decrease in the electrophoretic mobility of the protein on electrophoresis in denaturing gels similar to that observed in HSV-1-infected cells (22). Although the decrease in electrophoretic mobility of EF-1δ requires the presence of the protein kinase encoded by U_L13, it is not known whether the modification of EF-1δ observed in HSV-1-infected cells is associated with the phosphorylation of the protein. The objectives of the experiments described in this section were to determine whether modification of EF-1δ during the HSV-1 infection is due to phosphorylation, and if this is the case, whether U_L13 is required for the phosphorylation of EF-1δ. Vero cells were mock infected or infected with 10 PFU of HSV-1(F), HSV-1(F)Δ305 ($\Delta U_L23/\Delta U24$), R7355 (ΔU_L13), or R7358 ($U_L13R/\Delta U_L23/\Delta U_L24$) virus per cell and labeled with $^{32}P_i$ from 7 to 12 h after infection. EF-1δ immunoprecipitated from the infected cell lysates as described in Materials and Methods was then solubilized, electrophoretically separated on a denaturing gel, electrophoretically transferred to a nitrocellulose sheet, and subjected to autoradiography (Fig. 5A) and also reacted with the antibody to EF-1δ (Fig. 5B).

As previously reported for the *Xenopus* oocyte EF-1δ (22), both forms of EF-1δ with M_rs of 38,000 and 40,000 are labeled with $^{32}P_i$ in mock-infected cells (Fig. 5A, lane 1). In these cells as in cells infected with the virus lacking the U_L13 gene (R7355), the predominant form of EF-1δ was the fast-migrating form (Fig. 5A, lanes 1 and 4). In contrast, in cells infected with the parent viruses [HSV-1(F) (Fig. 5A, lane 2) or HSV-1(F)Δ305 (Fig. 5A, lane 3)], or with the mutant in which the U_L13 virus was repaired, R7258 (Fig. 5A, lane 5), the predominant form of ^{32}P-labeled EF-1δ was the slow-migrating type. Figure 5B shows the results of immunoblotting of the protein shown in Fig. 5A with antibody to EF-1δ. The results indicate that (i) phosphorylated proteins shown in Fig. 5A correspond to EF-1δ, (ii) the changes in radiolabeling profiles correspond to changes in the electrophoretic mobility of EF-1δ, and (iii) the U_L13 protein kinase is required for hyperphosphorylation of EF-1δ in HSV-1-infected cells.

The kinase activity in U_L13 immunoprecipitates phosphorylates EF-1δ in vitro. Although the data shown above strongly suggest that EF-1δ is a substrate for the U_L13 protein kinase, the experiments reported in this section were done to establish a direct link between U_L13 and phosphorylation of EF-1δ. Specifically, immunoprecipitates of EF-1δ derived from uninfected Vero cells were mixed with those of U_L13 from the cells infected with HSV-1(F) or R7356 (ΔU_L13), reacted in kinase buffer containing [γ-^{32}P]ATP, separated on a denaturing gel, electrophoretically transferred to a nitrocellulose sheet or stained with Coomassie blue (not shown), and subjected to autoradiography, and they were also reacted with the EF-1δ antibody. As reported previously, the immune complex obtained with the U_L13 antibody from HSV-1(F)-infected Vero cells contains a specific protein kinase activity attributed to the U_L13 gene (3, 24, 25). The results (Fig. 6) were as follows.

(i) Electrophoretically separated immune complex containing the U_L13 protein kinase which was allowed to react by itself in the kinase buffer did not contain labeled protein bands with an apparent M_r corresponding to that of EF-1δ (Fig. 6A, lane 1). On the other hand, in the autoradiographic images of the same immune complex reacted with immunoprecipitated EF-1δ, only the slow-migrating form of EF-1δ with an apparent M_r of 40,000 was phosphorylated (Fig. 6B, lane 2). The identity of the radiolabeled band of EF-1δ was verified in the immunoblot shown in Fig. 5C.

(ii) No proteins were labeled by the reaction of mixtures containing mixtures of immune complex formed by antibody to U_L13 with lysates of cells infected with R7356 (ΔU_L13) and the immune complex containing EF-1δ obtained from uninfected cells (Fig. 6B, lane 1). This result indicates that the kinase activity observed in Fig. 6B, lane 2, was derived from U_L13 and not from nonspecific kinase brought down by the polyclonal rabbit sera to EF-1δ or to U_L13. Figure 6C shows that EF-1δ was present in both lane 1 of Fig. 6B, in which it was not phosphorylated, and in lane 2 of Fig. 6B, in which only the slow-migrating protein was phosphorylated.

These results indicate that the immune complex with the U_L13 antibody from HSV-1(F)-infected cells possesses a kinase activity for EF-1δ, and we conclude that either U_L13 or a

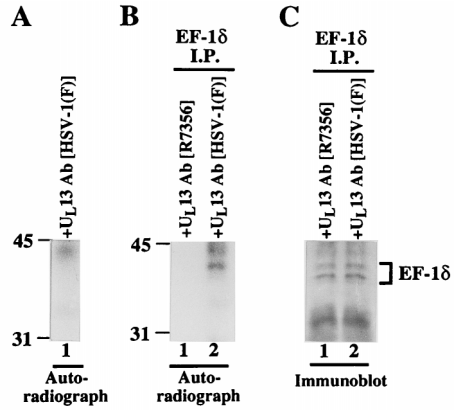

FIG. 6. Autoradiogram and photographs of a Coomassie blue-stained gel and immunoblots of proteins subjected to in vitro kinase assay. Vero cells were infected with HSV-1(F) or R7536, harvested at 24 h after infection, solubilized, precleared with normal rabbit sera and protein A-Sepharose, and then subjected to immunoprecipitation (I.P.) with U_L13 antibody (Ab). (A) The immune complex with the antibody to U_L13 from HSV-1(F)-infected cells was incubated in the kinase buffer containing [γ-^{32}P]ATP for 30 min at 30°C, separated on a denaturing gel, stained with Coomassie blue, and subjected to autoradiography. (B) The immunoprecipitated EF-1δ from normal Vero cells was incubated with the immune complex with the antibody to U_L13 from R7536 (ΔU_L13) (lane 1)- or HSV-1(F) (lane 2)-infected cells in kinase buffer containing [γ-^{32}P]ATP, separated on a denaturing gel, transferred to a nitrocellulose sheet, and subjected to autoradiography. (C) The nitrocellulose sheet corresponding to that in panel B was reacted with the antibody to EF-1δ.

complex containing U_L13 as a necessary component phosphorylates EF-1δ in vitro.

DISCUSSION

The key finding reported here is that viral protein kinase encoded by HSV-1 U_L13 phosphorylates the cellular translational regulatory protein EF-1δ, which plays a key role in protein synthesis. This is the first identification of a cellular protein target for virally encoded protein kinases of herpesviruses. The salient features of this study that support our conclusion are as follows.

(i) A hyperphosphorylated form of EF-1δ accumulates in Vero cells infected with wild-type viruses or U_L13-repaired virus, whereas in mock-infected cells or cells infected with the U_L13 deletion mutant, the predominant form of EF-1δ is a faster-migrating, hypophosphorylated form. These results indicate that U_L13 is required for the hyperphosphorylation of EF-1δ during HSV-1 infection in infected cells.

(ii) Earlier studies have shown that the phenotype in ΔU_L13 mutant-infected cells cannot be differentiated from that of $\Delta\alpha22$ mutant-infected cells in that the accumulation of ICP0 and of a subset of late proteins is reduced relative to those of wild-type virus-infected cells (28). In this report, we show that EF-1δ in $\Delta\alpha22$ mutant virus-infected cells is modified to the same extent as in wild-type-infected cells, eliminating the possibility that ICP22/U_S1.5 proteins are required for modification of EF-1δ.

(iii) The in vitro kinase assays showed that EF-1δ was phosphorylated in vitro by the mixture of independently derived immune complexes formed by lysates of cells infected with wild-type virus and the antibody to U_L13 and those of uninfected cells and antibody, whereas substitution in the mixture of the immune complex containing U_L13 protein with that formed by anti-U_L13 protein and lysates of ΔU_L13 mutant-infected cells failed to phosphorylate EF-1δ.

Taken together, these results support our conclusion that EF-1δ is phosphorylated by U_L13 protein kinase and that this form comigrates with the hyperphosphorylated form of the protein during HSV-1 infection. The relevant issues are as follows.

(i) Several lines of evidence listed below suggest that the phosphorylation of EF-1δ is involved in enhancement of the activity for protein synthesis. First, phosphorylation of EF-1δ by cellular cdc2 kinase is correlated with an increase in the activity of protein synthesis (19, 22, 31, 39). Second, EF-1δ activity is enhanced by phosphorylation of EF-1δ in vivo with phorbol esters or in vitro phosphorylation of EF-1δ with cellular protein kinase C (37, 38). Third, phosphorylation of translation initiation factor 2B (eIF-2B), which appears to have the same function as the EF-1βδγ complex in the initiation step of translation, causes an increase in GDP-GTP exchange activity of the protein (4).

(ii) Although ICP0 interacts with EF-1δ (13), it is not involved in the modification of EF-1δ. Thus, earlier this laboratory reported that ICP0 interacts physically in yeast with EF-1δ and that the sequence which binds EF-1δ fused to glutathione *S*-transferase (GST) interferes with protein synthesis, whereas GST alone had no effect (13). In this report, we show that EF-1δ is hyperphosphorylated by U_L13 protein kinase. These observations suggest that HSV-1 evolved two distinct pathways for modulating the function of EF-1δ and that EF-1δ plays an important role in efficient replication of HSV-1.

(iii) Viruses have evolved a variety of mechanisms to modulate the host cell translational apparatus to aid in their replication. In most cases reported so far, viruses have targeted proteins designed to maintain the integrity of translation initiation (15). For example, most viruses activate a double-stranded RNA-dependent kinase which phosphorylates the α subunit of eIF-2 and completely turns off protein synthesis during their replication (15). Many viruses encode proteins which are involved in blocking the phosphorylation of eIF2-α (12, 15). Viruses also change host cell translational machinery to enable preferential translation of viral mRNAs; these changes include host mRNA degradation and a change in translational specificity (15). HSV-1 has evolved both mechanisms: it encodes the $\gamma_1$34.5 protein which interacts with protein phosphatase 1α to dephosphorylate eIF2α and to preclude the shutoff of protein synthesis by double-stranded RNA-dependent protein kinase R (9, 34). HSV-1 also encodes two proteins, virion host shutoff protein (*vhs* gene) encoded by U_L41, and ICP27, encoded by α27. The *vhs*-encoded protein degrades host mRNAs, and ICP27 down-regulates expression of cellular mRNA by inhibiting splicing to cause preferential translation of viral mRNA (15, 34).

(iv) Although the physiologic role of hyperphosphorylation of EF-1δ is not known at present, it is conceivable that U_L13 protein kinase replaces a cellular function which is called upon during specific cell cycle stages or morphogenetic events to activate a higher level of protein synthesis in the infected cell. It is noteworthy that in its human host, HSV-1 causes lesions preeminently in nondividing cells. Furthermore, the infected cells used in our studies are not synchronized with respect to the cell cycle stage at which they are infected. HSV-1 may have evolved this function to compensate for a cellular function degraded during infection in order to upregulate translation to a level characteristic of dividing cells. It is noteworthy that the U_L13 gene is conserved in members of all herpesvirus subfamilies (2, 35), raising the interesting possibility that modification of EF-1δ is a conserved herpesvirus function.

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