Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 10516–10520, November 1995
Biochemistry

Association of a M_r 90,000 phosphoprotein with protein kinase PKR in cells exhibiting enhanced phosphorylation of translation initiation factor eIF-2α and premature shutoff of protein synthesis after infection with $\gamma_1$34.5$^-$ mutants of herpes simplex virus 1

(translation initiation/growth arrest and DNA damage proteins/cell stress)

JOANY CHOU*, JANE-JANE CHEN†, MARTIN GROSS‡, AND BERNARD ROIZMAN*

*Marjorie B. Kovler Viral Oncology Laboratories and ‡Department of Pathology, The University of Chicago, 910 East 58th Street, Chicago, IL 60637; and †Harvard–Massachusetts Institute of Technology Division of Health Sciences and Technology, Cambridge, MA 02139

Contributed by Bernard Roizman, July 24, 1995

ABSTRACT **The protein encoded by the $\gamma_1$34.5 gene of herpes simplex virus precludes premature shutoff of protein synthesis in human cells triggered by stress associated with onset of viral DNA synthesis. The carboxyl terminus of the protein is essential for this function. This report indicates that the shutoff of protein synthesis is not due to mRNA degradation because mRNA from wild-type or $\gamma_1$34.5$^-$ virus-infected cells directs protein synthesis. Analyses of the post-translational modifications of translation initiation factor eIF-2 showed the following: (*i*) eIF-2α was selectively phosphorylated by a kinase present in ribosome-enriched fraction of cells infected with $\gamma_1$34.5$^-$ virus. (*ii*) Endogenous eIF-2α was totally phosphorylated in cells infected with $\gamma_1$34.5$^-$ virus or a virus lacking the 3′ coding domain of the $\gamma_1$34.5 gene but was not phosphorylated in mock-infected or wild-type virus-infected cells. (*iii*) Immune precipitates of the PKR kinase that is responsible for regulation of protein synthesis of some cells by phosphorylation of eIF-2α yielded several phosphorylated polypeptides. Of particular significance were two observations. First, phosphorylation of PKR kinase was elevated in all infected cells relative to the levels in mock-infected cells. Second, the precipitates from lysates of cells infected with $\gamma_1$34.5$^-$ virus or a virus lacking the 3′ coding domain of the $\gamma_1$34.5 gene contained an additional labeled phosphoprotein of M_r 90,000 (p90). This phosphoprotein was present in only trace amounts in the immunoprecipitate from cells infected with wild-type virus or mutants lacking a portion of the 5′ domain of $\gamma_1$34.5. We conclude that in the absence of $\gamma_1$34.5 protein, PKR kinase complexes with the p90 phosphoprotein and shuts off protein synthesis by phosphorylation of the α subunit of translation initiation factor eIF-2.**

Herpes simplex virus 1 (HSV-1) encodes a gene, $\gamma_1$34.5, that functions to preclude a host response which terminates all protein synthesis after the onset of viral DNA synthesis (1). We show here that in cells infected with viruses lacking $\gamma_1$34.5 gene the eIF-2 α subunit of translation initiation factor eIF-2 is extensively phosphorylated and that a M_r 90,000 phosphoprotein associates with protein kinase PKR, which normally phosphorylates eIF-2α. Relevant to this report is the following information:

(*i*) The $\gamma_1$34.5 gene maps in the sequences flanking the long unique sequence of HSV-1 DNA, and therefore it is present in two copies per genome (2). The 263-amino acid protein encoded by HSV-1(F) (the prototype strain used) $\gamma_1$34.5 gene consists of three domains, an amino-terminal domain of ≈160 amino acids, 10 repeats of three amino acids $(Ala\text{-}Thr\text{-}Pro)_{10}$, and a 73-amino acid carboxyl-terminal domain (3). A stretch of 64 amino acids at the carboxyl terminus of the $\gamma_1$34.5 protein is homologous to a corresponding stretch of amino acids at the carboxyl terminus of a murine protein known as MyD116 and a Chinese hamster protein known as GADD34 (for growth arrest and DNA damage) (1, 4). MyD116 protein belongs to a set of proteins expressed in myelogenous leukemia cells induced to differentiate by interleukin 6 (5). GADD34 protein, structurally closely related to MyD116 protein, is also one of a subset of proteins induced after DNA damage or cell-growth arrest (6–8).

(*ii*) Infection of human cells, but particularly of human neuroblastoma cell line SK-N-SH or primary human foreskin fibroblasts, results in gradual shutdown of protein synthesis before completion of the viral replicative cycle (9). This premature cessation of protein synthesis is not seen when these cells are treated with inhibitors of viral DNA synthesis or in Vero cells (1). The capacity to preclude total premature shutoff of protein synthesis maps to the carboxyl-terminal domain of $\gamma_1$34.5 protein that is homologous to MyD116 protein (10). Indeed, the carboxyl terminus of MyD116 protein successfully substitutes for the corresponding domain of $\gamma_1$34.5 protein (B. He, J.C., D. A. Liebermann, and B.R., unpublished work). Viruses lacking the $\gamma_1$34.5 gene or unable to express the carboxyl terminus of protein are totally avirulent in the murine encephalitis model of HSV-1 infections (11–13).

(*iii*) The premature shutoff of protein synthesis raises several interesting questions—i.e., what triggers the host response, what is the mechanism by which protein synthesis is turned off, what is the mechanism by which $\gamma_1$34.5 gene precludes the host response, and ultimately, whether or not the function of GADD34 genes is related in some way to the apparent function of $\gamma_1$34.5 gene. In the studies reported here we show that the activity of a protein kinase responsible for phosphorylation of the α subunit of translation initiation factor eIF-2 and a phosphorylated M_r 90,000 protein, which we designate p90, coprecipitated with anti-PKR kinase antibody from lysates of cells infected with mutants lacking the $\gamma_1$34.5 gene.

MATERIALS AND METHODS

Cells and Viruses. The African green monkey kidney cells (VERO) and the human neuroblastoma cells (SK-N-SH) (American Type Culture Collection) were propagated in Dulbecco's modified Eagle's medium supplemented with 5% and 10% fetal bovine serum, respectively. HSV-1(F) is the prototype HSV-1 strain used in this laboratory (14). The HSV-1

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Abbreviation: HSV-1, herpes simplex virus 1.

recombinants R3616, R4002, and R3939 have been described (1, 10). R3616 mutant virus lacks 1 kb from the coding domains of both $\gamma_1$34.5 genes. R4002 mutant virus lacks 500 bp from the coding domain of thymidine kinase (U_L23) gene, and the first 28 codons from both $\gamma_1$34.5 genes. R3939 mutant virus contains a six-way stop codon inserted in the *Dra* III site of the $\gamma_1$34.5 gene and, therefore, its carboxyl-terminal domain is not expressed.

eIF-2. Purification of rabbit reticulocyte eIF-2 was as described (15).

RESULTS

Sedimentation Profiles of Polyribosomes Extracted from the Cytoplasm of VERO or of Neuroblastoma Cells Infected with HSV-1(F) (Wild Type) or R3616 ($\gamma_1$34.5$^-$) Viruses. The objective of these studies was to determine the status of polyribosomes in VERO cells and in SK-N-SH neuroblastoma cells infected with HSV-1(F) or R3616 mutant. Cytoplasmic extracts prepared from infected VERO or SK-N-SH cells were centrifuged in a 15–50% (wt/wt) sucrose-density gradient. Fractions were collected with a flow-through spectrophotometer, and A_{260} was measured. Fig. 1 shows the spectrophotometric tracings of the separated ribosomal subunits and polyribosomes from infected cells. As shown in Fig. 1*A*, the sedimentation profiles of polyribosomes extracted from VERO cells infected with HSV-1(F) and R3616 viruses were similar, consistent with the observation that in VERO cells wild-type and mutant viruses cannot be differentiated with respect to levels of intracellular protein synthesis. In contrast, the profile of polyribosomes from neuroblastoma cells infected with R3616 mutant consists largely of 80S monosomes, 40S ribosomal subunit, and 60S ribosomal subunit, whereas the sedimentation profile of the cytoplasm of cells infected with wild-type virus exhibited polyribosomes and substantially fewer monosomes and ribosomal subunits.

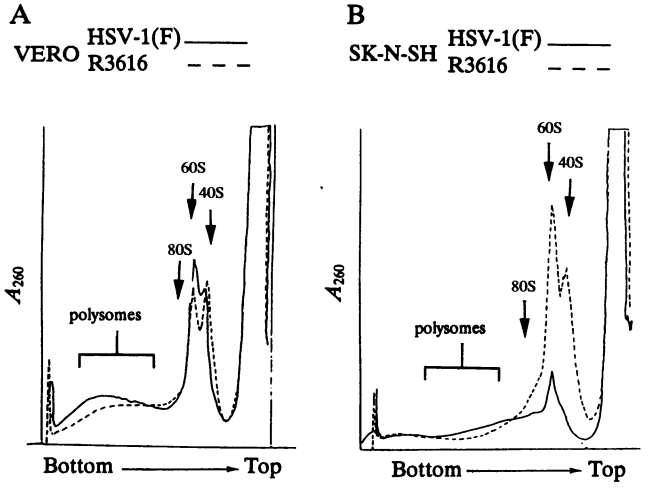

FIG. 1. UV absorbance profiles of cytoplasmic fraction from VERO or human SK-N-SH neuroblastoma cells infected with wild-type or mutant viruses. Replicate VERO or human SK-N-SH neuroblastoma cell cultures were infected with 5 plaque-forming units of wild-type HSV-1(F) or R3616 ($\gamma_1$34.5$^-$) mutant virus. At 13 hr after infection, cells were harvested, rinsed with phosphate-buffered saline, and lysed with 0.5% of Triton X-100. After a brief centrifugation to remove nuclei, cytoplasm was loaded onto a 15–50% (wt/wt) sucrose gradient and centrifuged at 40,000 rpm for 90 min at 4°C in a Beckman SW41 rotor. After centrifugation, gradients were harvested through a flow-through spectrophotometer, and A_{260} values were recorded. (*A*) VERO cells. (*B*) SK-N-SH cells. Bottom and top of the gradient are indicated. The 40S and 60S ribosomal subunits and the 80S monosomes are indicated.

The sedimentation profiles of polyribosomes from SK-N-SH cells infected with R3616 mutant virus are consistent with a gross decrease in the number of ribosomes engaged in the translation of proteins late in infection. The most likely explanations for the observed phenomenon are (*i*) a decrease in competent mRNA available for translation or (*ii*) a decrease in the rate of initiation of protein synthesis.

Viral Messages Are Not Degraded in Human SK-N-SH Neuroblastoma Cells Infected with $\gamma_1$34.5$^-$ Virus. One hypothesis to explain the shutoff of protein synthesis in neuroblastoma cells infected with $\gamma_1$34.5$^-$ virus is that viral mRNA is degraded in the absence of the $\gamma_1$34.5$^-$ protein. To test this hypothesis, RNA transcripts, either total RNA or purified poly(A)$^+$ mRNA from SK-N-SH human neuroblastoma cells infected for 13 hr with wild-type HSV-1(F) or R3616 mutant virus, were translated in the rabbit reticulocyte lysate cell-free system. Both total and poly(A)$^+$ RNAs from cells infected with R3616 ($\gamma_1$34.5$^-$) mutant virus yielded all the same major viral proteins as those expressed by RNA from wild-type infected cells (Fig. 2). These results do not support the hypothesis that mRNA is selectively degraded in $\gamma_1$34.5$^-$-infected cells; these results do indicate that the transcripts present in $\gamma_1$34.5$^-$-infected cells are functional templates for protein synthesis.

It is noteworthy that some proteins (e.g., ICP25, encoding the α trans-inducing factor, or VP16 and members of the ICP35 family of proteins) were translated *in vitro* more efficiently than others (e.g., ICP5 and ICP11). One possible explanation for this phenomenon is that larger polypeptides tend to be prematurely terminated in *in vitro* systems.

An eIF-2α Kinase Activity Is Associated with the Ribosomal Fraction in Cells Infected with $\gamma_1$34.5$^-$ Virus. The purpose of several series of experiments described below was to determine

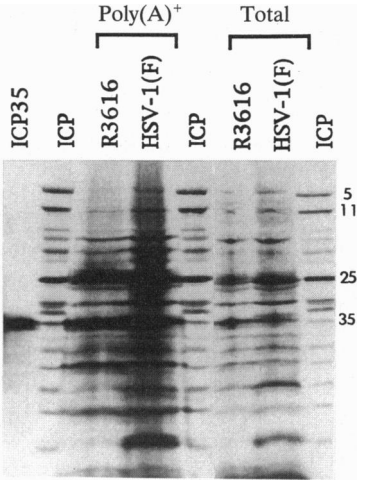

FIG. 2. Autoradiographic images of proteins translated *in vitro* from mRNA extracted from SK-N-SH cells infected with wild-type and deletion mutants. Replicate cultures were infected with HSV-1(F) or with R3616 mutant. At 13 hr after infection cells were harvested, and RNA was prepared from these cells with the RNA STAT-60 kit from Tel-Test (Friendswood, TX) according to procedures specified by the manufacturer. Poly(A)$^+$ RNAs were then selected from total RNA with a PolyATract mRNA isolation systems kit from Promega. Five micrograms of the total RNA and poly(A)$^+$-selected RNAs were then translated *in vitro* with a rabbit reticulocyte lysate system (Promega) in the presence of [^{35}S]methionine and RNase inhibitor. Translated products were then solubilized, electrophoretically separated on a denaturing 10% polyacrylamide gel crosslinked with *N'*,*N'*-diallyltartardiamide, transferred electrically onto a nitrocellulose membrane, and subjected to autoradiography. The lanes marked ICP represent viral proteins specified by the wild-type HSV-1(F) virus in infected VERO cells. The lane marked ICP35 contained the product of transcription and translation of the HSV-1 protease substrate encoded by plasmid pRB4103 (16) by the SP6 polymerase transcription and translation kit provided by Promega.

whether the premature shutoff of protein synthesis in human neuroblastoma cells infected with R3616 mutant virus is due to modification of the α subunit of translation initiation factor eIF-2. The experiments used HeLa cells because eIF-2 appears more stable in lysates of this cell line. HeLa cells, like most human cell lines studied to date, are also affected by premature shutoff of protein synthesis in cells infected with $\gamma_1 34.5^-$ viruses.

This series of experiments was designed to determine whether the lysates of cells infected with R3616 mutant virus contained any eIF-2α kinase activity. Purified translation initiation factor eIF-2 from rabbit reticulocytes (0.2 μg per reaction mixture) was mixed in the presence of [γ-^{32}P]ATP with S10 (excluding mitochondria) or S100 (postribosomal) fractions prepared from replicate cultures of HeLa cells that were either mock-infected or infected with HSV-1(F) or R3616 viruses. Fig. 3*A* shows that eIF-2α subunit was extensively phosphorylated in the S10 extracts of R3616-infected cells, minimally phosphorylated in wild-type- or mock-infected cells and not phosphorylated at all in the absence of cell extracts. These results indicate that the S10 fraction of the $\gamma_1 34.5^-$ virus-infected cells contained an eIF-2α kinase activity that was much reduced or absent in similar extracts of mock-infected or wild-type-infected cells. This activity was absent from all S100 fractions tested, suggesting that the kinase activity is associated with ribosomes. In the absence of exogenous eIF-2, minimal phosphorylation of endogenous eIF-2 was seen in all extracts due to steady-state phosphorylation and dephosphorylation processes in the cell. Fig. 3*B* shows that the level of eIF-2α was similar in all samples to which it had been added.

Phosphorylation of eIF-2α and of a M_r 90,000 Protein in Cells Infected with Mutants Lacking the $\gamma_1 34.5$ Gene or Containing a 3′ Truncation of the $\gamma_1 34.5$ Coding Sequence. Earlier studies have shown that the carboxyl-terminal domain of $\gamma_1 34.5$ protein is necessary to preclude the premature shutoff of protein synthesis in the human neuroblastoma cell line, whereas amino-terminal domains of the protein were dispensable (10). To test the hypothesis that the PKR kinase is the eIF-2α kinase activity described above and that this activity is elevated in cells infected with viruses containing truncations of the carboxyl terminus of $\gamma_1 34.5$ protein but not those carrying amino-terminal truncations of the protein, two series of experiments were done.

In the first series, extracts containing endogenous eIF-2 from mock-infected or infected HeLa cells were prepared in the presence of NaF to inhibit phosphatase activity and analyzed on slab isoelectric focusing gels to determine the extent of eIF-2α phosphorylation. As shown in Fig. 4*B*, eIF-2α remained largely or totally unphosphorylated in extracts of mock-infected cells (Fig. 4*B*, lane 1) or in extracts of cells infected with wild-type or R4002 mutant virus from which the first 28 codons of both $\gamma_1 34.5$ gene copies had been deleted (Fig. 4*B*, lanes 2 and 4). As expected from earlier studies, these extracts were derived from cells fully competent to synthesize protein, as evident from [^{35}S]methionine incorporation patterns in lanes 1, 2, and 4 of Fig. 4*C*. In contrast, eIF-2α was fully phosphorylated in extracts of HeLa cells infected with R3616 mutant carrying a 1000-bp deletion in $\gamma_1 34.5$ gene or with R3939 mutant lacking codons for the carboxyl terminus of $\gamma_1 34.5$ protein (Fig. 4*B*, lanes 3 and 5). The concordant finding was that protein synthesis reflected by incorporation of [^{35}S]methionine was largely absent in these cells (Fig. 4*C*, lanes 3 and 5).

The objective of the second series of experiments was to determine whether phosphorylation of eIF-2α correlates with activation of PKR kinase. In these experiments, PKR kinase was precipitated with a specific rabbit polyclonal antibody from S10 extracts prepared from HeLa cells that were either mock-infected or infected with HSV-1(F), R3616, R4002, or R3939 virus and allowed to react with [γ-^{32}P]ATP. Fig. 4*A*, lane 1, shows that protein complexes precipitated by the PKR

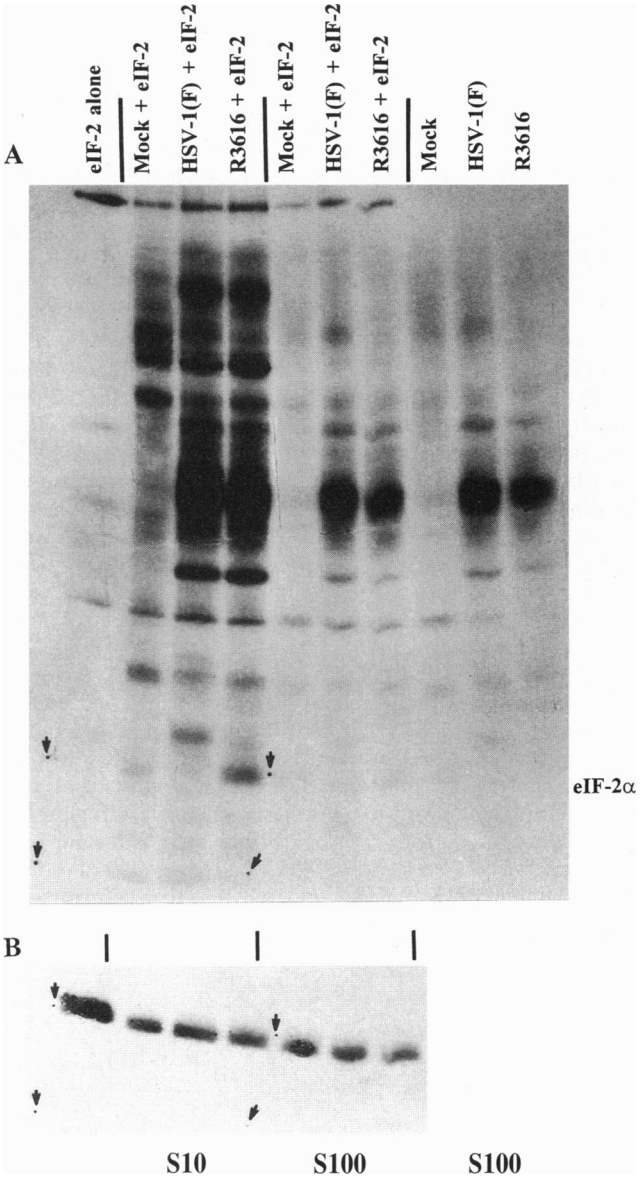

FIG. 3. eIF-2α kinase activity in infected cell extracts. Replicate cultures of HeLa cells were either mock-infected or infected with HSV-1(F) or R3616 viruses. At 7 hr after infection cells were harvested, and S10 fractions, which include all cytoplasmic materials, were prepared according to published methods (17). S100 fractions were supernatant fluids prepared from S10 fractions after centrifugation at 29,000 rpm for 3 hr at 4°C in a Beckman SW41 rotor. This fraction contained most of the soluble proteins, but it was free of ribosomes. eIF-2 purified from rabbit reticulocyte lysate was used to detect eIF-2α kinase activity in those extracts. (*A*) eIF-2 (0.2 μg) was treated with the S10 or S100 fractions in the presence of [γ-^{32}P]ATP (100 μCi per sample, specific activity 6000 Ci/mmol; 1 Ci = 37 GBq DuPont/NEN) at 30°C for 20 min. Reaction mixtures were then solubilized, electrophoretically separated on a denaturing 12% polyacrylamide gel crosslinked with *N′N′*-diallyltartardiamide, transferred onto nitrocellulose sheets, and set for autoradiography as described (1). Lanes with exogenous eIF-2 added are indicated at top; position of eIF-2α is shown at right. (*B*) The nitrocellulose sheet containing separated proteins was stained with monoclonal antibody specific to eIF-2α (18). After reaction with an appropriate secondary antibody, the blot was developed with color reagents provided by Promega to identify eIF-2α polypeptide. Arrows indicate the dots used to orient and align the phosphorylated eIF-2α polypeptide in *A* with the antibody-stained eIF-2α polypeptide in *B*.

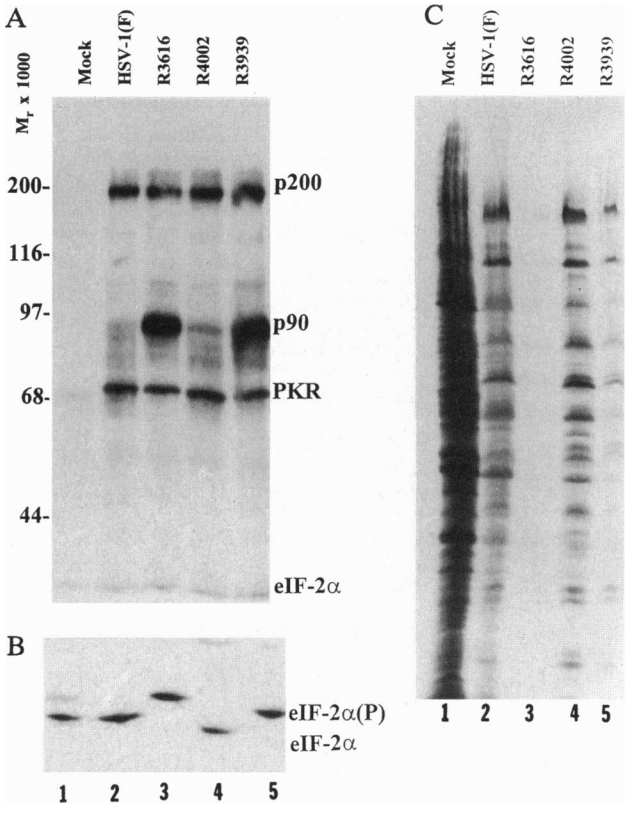

FIG. 4. Autoradiographic images of proteins separated by electrophoresis or by isoelectric focusing. (*A*) Autoradiographic images of electrophoretically separated immune precipitate of PKR kinase. S10 fractions, prepared as described in the Fig. 2 legend from replicate cultures of HeLa cells either mock-infected or infected for 7 hr with wild-type HSV-1(F) virus, R3616 mutant carrying a 1000-bp deletion in the $\gamma_1$34.5 genes, R4002 mutant carrying a deletion in the first 28 codons of the coding sequence of the $\gamma_1$34.5 genes, or R3936 mutant carrying a deletion in the 3′ domain of the $\gamma_1$34.5 gene, were allowed to react with [γ-^{32}P]ATP (100 μCi per sample) for 20 min at 30°C. Fractions were then mixed with antibody to PKR kinase from Santa Cruz Biotechnology. Precipitated proteins were collected, washed, solubilized, electrophoretically separated in a denaturing gel as described above, transferred onto a nitrocellulose sheet, and exposed to x-ray autoradiography. Positions of molecular weight standards (New England Biolabs) are shown at left. p200 and p90 refer to proteins with an apparent M_r of 200,000 and 90,000, respectively. Bands containing PKR kinase (M_r of 68,000) and eIF-2α are also identified. (*B*) Immunoblot of eIF-2α separated by slab isoelectric focusing. Sample preparation and all reagents used in this procedure have been described (19). HeLa cells grown in 6-well Costar dishes were mock-infected or infected with the same viruses described for Fig. 3 for 7 hr, and extracts were prepared with NaF to inhibit endogenous phosphatase activity. The lysate of $\approx 2.5 \times 10^5$ cells was loaded per lane, and gels were run overnight at room temperature in a pH gradient of 4.5–6.5 and then electrically transferred to an Immobilon-P membrane. eIF-2α was visualized by probing the membrane with monoclonal antibody specific for eIF-2α and developed with chemiluminescence reagents as specified by the manufacturer (Amersham). eIF-2α(*P*) and eIF-2α, representing the phosphorylated and unphosphorylated species of eIF-2α, respectively, are identified at right. Lanes 1–5 represent infection with the same viruses as shown in *A*. (*C*) Autoradiographic image of electrophoretically separated proteins from lysates of HeLa cells infected with the viruses described in *A* and identified at the top. Cells were mock-infected or infected with 5–10 plaque-forming units per cell. At 12 hr after infection cells were labeled with [^{35}S]methionine (20 μCi per sample, specific activity >1000 Ci/mmol; Amersham) for 1 hr. Cells were then harvested, electrophoretically separated in denaturing gels as described in *A*, transferred onto a nitrocellulose sheet, and subjected to autoradiography.

kinase antibody from the mock cell extract were mostly unlabeled, whereas three or four phosphorylated polypeptides were precipitated from extracts of infected cells. These included PKR kinase, a protein with an apparent M_r of 200,000 (p200), and the α subunit of eIF-2 (Fig. 4*A*, lanes 2–5), suggesting that PKR kinase was activated in cells infected with all viruses, both mutant and wild type, whereas eIF-2α was extensively phosphorylated only in cells infected with R3616 or R3939 mutant viruses. However, in addition to these phosphoproteins, cells infected with R3616 or R3939 mutant viruses (Fig. 4*A*, lanes 3 and 5) contained an additional phosphorylated protein with an apparent M_r of 90,000 (p90). This protein was not phosphorylated in cells infected with the wild-type or the mutant viruses lacking the amino-terminal 28 codons of the $\gamma_1$34.5 gene.

DISCUSSION

The salient features of our results are as follows:

(*i*) The disappearance of polyribosomes observed in SK-N-SH cells infected with the mutants from which the $\gamma_1$34.5 gene had been deleted indicated either degradation of the mRNA or a failure in translation initiation. We have shown that the mRNA extracted from SK-N-SH cells can direct synthesis of viral proteins in the rabbit reticulocyte cell-free system, indicating that the protein synthesis block is at the level of translation initiation.

(*ii*) Lysates of HeLa cells infected with $\gamma_1$34.5$^-$ virus contained a ribosome-associated kinase that phosphorylated eIF-2α in exogenously added eIF-2. This activity was not present in mock-infected or wild-type-infected cells. eIF-2 binds Met-tRNA and GTP in a ternary complex, which then associates with ribosomal subunits in a preinitiation complex (20, 21). After initiation, eIF-2 is recycled off the 80S complex with the help of translation factor eIF-2B. One of the events that regulates translation is the phosphorylation of eIF-2 complex α subunit. Phosphorylation of eIF-2α correlates with the shutoff of protein synthesis in heme-regulated hemopoietic cells in response to growth inhibition, or as a consequence of cellular stresses caused by virus infections, heat shock, heavy metals, and deprivation of serum, amino acids, or glucose (22, 23). These effects have been linked to the phosphorylation of Ser-51 of eIF-2α either by PKR kinase, a M_r 68,000 protein also known as dsl, a double-stranded RNA-activated eIF-2α kinase that autophosphorylates itself, or as HRI, a heme-regulated eIF-2α kinase (20, 21). A primary role of phosphorylation of Ser-51 in regulation of protein synthesis is supported by the observation that the Ser-51 → Ala substitution precludes phosphorylation and maintains protein synthesis (23).

(*iii*) The phosphorylation of eIF-2α was associated with a kinase present in a fraction enriched for ribosomes of cells infected with R3616 virus and in which there was no protein synthesis. Immunoprecipitation of PKR kinase from the same fraction after reaction with [γ-^{32}P]ATP yielded several phosphorylated polypeptides. The immunoprecipitates of all infected cells contained PKR kinase and a M_r 200,000 (p200) protein. The immunoprecipitates of cells infected with total or carboxyl-terminal deletion mutants of the $\gamma_1$34.5 gene contained, in addition, a heavily labeled M_r 90,000 (p90) protein. Three issues are of relevance here.

(*i*) Significantly more PKR enzyme was phosphorylated in cells infected with either wild-type or $\gamma_1$34.5 mutants than in mock-infected cells. These results indicate that elevation of PKR kinase activity may be a general characteristic of HSV-infected cells, possibly induced by double-stranded RNA made during infection.

(*ii*) Complete phosphorylation of eIF-2α in lysates of cells infected with mutants that lack the sequences capable of encoding all or the carboxyl terminus of $\gamma_1$34.5 protein was particularly striking and does not appear to correlate with the

activated PKR kinase levels. Numerous reports have shown that phosphorylation of as much as 30–60% of total cellular eIF-2α could account for the shutoff of 90–95% of total protein synthesis (22). Complete phosphorylation of eIF-2α suggests that the mechanism for phosphorylation of eIF-2α differ from those mechanisms described so far.

(*iii*) The function and nature of p90 protein is unknown, but the perfect correlation between p90 phosphorylation and the premature shutoff of protein synthesis exclusively in cells lacking either the entire $\gamma_1$34.5 gene or the domain encoding the carboxyl terminus of the protein is particularly striking. The presence of phosphorylated p90 also correlates with the presence of excess activity capable of phosphorylating eIF-2α in exogenously added eIF-2. In cells lacking a functional $\gamma_1$34.5 protein the p90 is apparently a component of a complex containing PKR kinase. Possibly p90 regulates PKR activity in the phosphorylation of eIF-2α and $\gamma_1$34.5 protein blocks this interaction. Further experiments to investigate the nature of p90 and its interaction with other proteins will shed light on the mechanism by which $\gamma_1$34.5 protein enables protein synthesis during HSV infection.

Note Added in Proof. In recent studies done after submission of the manuscript we discovered that two antibodies to the PKR kinase react with the p90 protein. This observation suggests that the p90 protein is at least immunologically related to the p68 human protein PKR kinase. Similar relationship between PKR kinase and p90 protein was observed in a human system (24).

We thank Dale Mobley for technical assistance. These studies were aided by grants from the National Cancer Institute (CA47451), the National Institute of Allergy and Infectious Diseases (AI124009), and by an unrestricted grant from the Bristol–Myers Squibb Program in Infectious Diseases to B.R., National Institutes of Health Grant HL30121 to M.G., and National Institutes of Health Grant DK-16272 to J.-J.C.

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