The γ₁34.5 Protein of Herpes Simplex Virus 1 Has the Structural and Functional Attributes of a Protein Phosphatase 1 Regulatory Subunit and Is Present in a High Molecular Weight Complex with the Enzyme in Infected Cells*

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The carboxyl-terminal domain of the γ₁34.5 protein of the herpes simplex virus 1 binds to protein phosphatase 1α (PP1) and is required to prevent the shut-off of protein synthesis resulting from phosphorylation of the α subunit of eIF-2 by the double-stranded RNA-activated protein kinase. The corresponding domain of the conserved GADD34 protein homologous to γ₁34.5 functionally substitutes for γ₁34.5. This report shows that γ₁34.5 and PP1 form a complex in the infected cells, that fractions containing this complex specifically dephosphorylate eIF-2α, and that both γ₁34.5 and GADD34 proteins contain the amino acid sequence motif common to subunits of PP1 that is required for binding to the PP1 catalytic subunit. An oligopeptide containing this motif competes with γ₁34.5 for binding to PP1. Substitution of Val₁⁸³ and Phe₁⁹₅ in the PP1-binding motif abolished activity. These results suggest that the carboxyl-terminal domain of γ₁34.5 protein has the structural and functional attributes of a subunit of PP1 specific for eIF-2α, that it has evolved to preclude shut-off of protein synthesis, and that GADD34 may have a similar function.

The γ₁34.5 gene of herpes simplex virus 1 (HSV-1) encodes two functions. The first enables the virus to replicate in vivo and particularly to multiply and spread in the central nervous system of experimental animal systems (1, 2). This function appears to map throughout the coding domain of the gene (3, 4). The second blocks the shut-off of protein synthesis resulting from phosphorylation of the α subunit of the translation initiation factor eIF-2 by the double-stranded RNA-activated protein kinase (PKR). This function maps in the 3′-terminal domain of the 263-codon gene (5, 6). Earlier studies have shown that in HSV-1-infected cells PKR is activated but that in cells infected with wild-type virus or virus carrying in-frame deletions of the 3′-terminal coding domain of the gene eIF-2α was not phosphorylated (7). In subsequent studies (8) we have shown that the γ₁34.5 protein interacts with the protein phosphatase 1α (PP1). Indeed, infected cells contain a phosphatase activity that specifically dephosphorylates eIF-2α at a rate 3000-fold greater than that measured in uninfected cells. This phosphatase activity is inhibited by inhibitors of PP1. The hypothesis that emerged from these studies is that transcription of complementary sequences of the HSV-1 DNA results in the formation of double-stranded RNA, that PKR is activated in cells infected with both wild-type and mutant viruses, and that a domain of the γ₁34.5 protein binds PP1 and redirects its activity to dephosphorylate eIF-2α. This report centers on one aspect of this hypothesis: we show that in infected cells γ₁34.5 protein is a component of a multi-protein, cytoplasmic complex containing PP1 and that the interacting domain of the γ₁34.5 protein is near the carboxyl terminus of the protein and has an amino acid motif shared with accessory proteins or subunits interacting with the catalytic subunit of PP1.

Relevant to this report are the following: (i) The HSV-1 γ₁34.5 gene encodes a protein consisting of a 159-amino acid amino-terminal domain, the amino acids Ala-Thr-Pro repeated 5–10 times depending on virus strain, and a 74-amino acid carboxy-terminal domain (9–11). The gene maps in the inverted repeat sequence ab and b′a′ flanking the unique long (UL) sequence, between the terminal a sequence and the gene encoding the regulatory infected cell protein number 0. Earlier studies described a series of mutants in which in-frame deletions truncated the amino-terminal domain or prematurely terminated the translation of the carboxy-terminal domains. The key observation was that protein synthesis was shut off after the onset of synthesis of viral DNA in cells infected with mutants either lacking the entire γ₁34.5 coding domain or that are unable to express the carboxy-terminal domain (6). (ii) The γ₁34.5 gene is conserved in very few herpesviruses, suggesting that other herpesviruses have evolved different methods for blocking the consequences of double-stranded RNA accumulation in infected cells. Homologs of the γ₁34.5 carboxy-terminal domain have been found, however, in the African swine fever virus (12), a DNA virus belonging to the unrelated, Iridovirus family, and in the corresponding domain of a highly conserved mammalian protein known as GADD34 (growth arrest and DNA damage protein 34) (13–16). The GADD proteins are induced in cells subjected to growth arrest as a consequence of serum deprivation or damage to their DNA or in the course of differentiation (14–16). It has been reported that overexpression of GADD34 results in apoptosis (17). It is of interest, however, that the carboxyl-terminal domain of the mouse GADD40 protein substituted for the HSV-1 γ₁34.5 carboxy-terminal domain in blocking the shut-off of protein synthesis (18). This observation suggests that the shared sequences are sufficient to block the shut-off of protein synthesis and that the
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Corresponding GADD34 protein domain may perform upon induction a similar function. The homologous domains of known GADD34 proteins and of the HSV-1 and HSV-2 and African swine fever virus homologs are shown in Fig. 1. (iii) The catalytic subunit of PP1 is a highly conserved protein with a M₉ of 38,000 (19). PP1 exists in holoenzyme complexes with noncatalytic or regulatory components that modulate catalytic activity or restrict the subcellular localization of the catalytic subunit. In essence, PP1 is regulated by different cellular proteins. Many of such regulatory proteins have been described (20–25), and these complexes have diverse functions within the cell (19, 26). While this work was in progress, it has been reported that these subunits share an amino acid sequence required for binding to the catalytic subunit of PP1 (27, 28).

MATERIALS AND METHODS

Cells and Viruses—The HeLa and SK-N-SH cell lines were obtained from American Type Culture Collection and propagated in Dulbecco’s modified Eagle’s medium supplemented with 5% (HeLa) or 10% (SK-N-SH cells) fetal bovine serum, respectively. The rabbit skin cell line originally obtained from J. McClaren was propagated in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum. HSV-1(F) is the prototype HSV-1 strain used in this laboratory (29).

Plasmids—In plasmid pRB4892 the coding domain of glutathione S-transferase (GST) is fused to the entire coding domain of PP1 except for the initiator methionine codon (8). In plasmid pRB4893 the coding domain of GST is fused to codons 146–263 of the g134.5 gene (9). To construct plasmid pRB4895, an oligonucleotide linker, AATCCCGAG-CAGTGTACGGTTCTCGCTCACGAGGTAGTACGTCACG, and its complement, TCAGCGTGACGTACTCAGCTGACGTAGGACAAACG- TACGCTGTCCGG, were cloned into the EcoRI and SacI sites of pGEX4T-1. In this plasmid, codons 190–203 of the γ134.5 gene were fused in frame to the coding sequences of GST. To construct plasmid pRB4894, an oligonucleotide linker, AATCCCGAG-GTACGGTTCTCGCTCACGAGGTAGTACGTCACG, and its complement, TCAGCGTGACGTACTCAGCTGACGTAGGACAAACG-TACGCTGTCCGG, were inserted into the EcoRI and Sall sites of pGEX4T-1. In this plasmid, codons 190–203 of the γ134.5 gene were fused in frame to the coding sequences of GST. To construct plasmid pRB4896, an oligonucleotide linker, AATCCCGAG-GTACGGTTCTCGCTCACGAG-GTACGGTTCTCGCTCACGAGGTAGTACGTCACG, and its complement, TCAGCGTGACGTACTCAGCTGACGTAGGACAAACG-TACGCTGTCCGG, were inserted into the EcoRI and Sall sites of pGEX4T-1. In this plasmid, codons 190–203 of the γ134.5 gene were fused in frame to the coding sequences of GST.

To construct plasmid pRB4897, a BstEII-DraIII fragment encoding codons 28–205 of γ134.5 was amplified by polymerase chain reaction from plasmid pRB143 with primers CCACCCCGGCGACGCTCTCTGT and its complement, TCGACTGACC, were inserted into the DraIII and BstEII sites of pRB4896. To construct plasmid pRB4898, a BstEII-DraIII fragment encoding codons 28–205 of γ134.5 amplified by polymerase chain reaction from plasmid pRB4897 was ligated into the DraIII and BstEII sites of pRB4896.

Expression of GST fusion proteins was induced by the addition of isopropyl β-D-thiogalactoside to cultures of Escherichia coli BL21 cells transformed with plasmid pRB4892, pRB4893, pRB4894, or pRB4895, followed by affinity purification of the fusion proteins from bacterial lysates on agarose beads conjugated with glutathione. Purified PP1 was obtained by cleavage of GST-PP1 fusion protein with thrombin (Sigma).

Construction of the Recombinant Virus R8321—Recombinant virus R8321 was constructed by cotransfection of the intact viral DNA of R3659 (30) with the plasmid pRB4897 on rabbit skin cells. In the parent virus R3659 (31), a 1-kilobase fragment from the coding sequences of γ134.5 was replaced with the chimeric a27tk gene. The progeny of the recombinant was selected and plaque-purified on 143 TK mutant cells in medium containing mixture 199V supplement with 100 μg of bromodeoxyuridine/ml and 2% fetal calf serum. Preparation of viral stocks and titrations of infectivity were done with Vero cells.

Cell Lines—HeLa cells either mock-infected or infected with viruses were harvested at 18 h after infection, rinsed with phosphate-buffered saline, lysed in lysis buffer containing 10 mM Hapes (pH 7.6), 250 mM NaCl, 10 mM MopsCl, 1% Triton X-100, 0.5 mM phenylmethylsulfonyl fluoride, and 2 mM benzamidine, stored on wet ice for 30 min, and subjected to low speed centrifugation to remove nuclei. The supernatant fluids (S10 fractions) were saved for analysis.

Immunoblotting—Samples were solubilized in disruption buffer containing 50 mM Tris-HCl (pH 7.6), 5% 2-mercaptoethanol, 2% SDS, and 2.75% sucrose, sonicated and boiled, subjected to electrophoresis in

FIG. 1. Amino acid sequence alignment of the carboxyl-terminal domains of hamster (15), mouse (14), and human (17) GADD34 proteins and of the γ134.5 homologs of HSV-1 (11) and HSV-2 (4) and of the NL protein of African swine fever virus (12).

FIG. 2. A, schematic representation of the genome structure and sequence arrangements of HSV-1(F) and of the γ134.5 deletion mutants. The top line represents the two covalently linked components of HSV-1 DNA, L and S, each consisting of unique sequences (Uₗ and Uₛ, respectively) flanked by inverted repeats (42, 43). The reiterated sequences flanking Uₛ, designated b and b’ , are each 9 kilobase pairs in size, whereas the repeats flanking Uₗ designated a’ c and ca, are 6.3 kilobase pairs in size (43). The location of the γ134.5 gene is shown in an expanded portion of the inverted repeat sequences b and b’ . The shaded bar and the arrow indicate the coding region and the direction of transcription of γ134.5, respectively. Because the b sequence is repeated in an inverted orientation, the HSV-1 genome contains two copies of the γ134.5 gene. The thick lines indicate the γ134.5 deletion mutants. The numbers above the lines indicate the position of amino acid residues. The gaps between numbers indicate the deletions in the γ134.5 gene. B, schematic diagram of GST-γ134.5 chimera proteins. Vertical shaded bars indicate the domains of the γ134.5 protein, and numbers indicate the terminal γ134.5 amino acids of the portion present in the chimeric protein. The designation of the plasmids encoding the chimeric proteins are shown to the left of the schematic diagrams.
**RESULTS**

Localization of Protein Phosphatase 1-binding Site(s) in γ34.5 Protein—Earlier studies have shown that the carboxy-terminal domain of the γ34.5 protein (Fig. 1) is required to prevent the shut-off of protein synthesis, that it interacts with PP1, and that it is highly conserved among several viral and GADD34 proteins. The objective of this series of experiments was to determine the PP1-binding sites in the γ34.5 protein.

In the first series of experiments, purified GST-PP1 protein bound to beads was incubated with cell extracts prepared from HeLa cells infected with 20 pfu of wild-type virus or mutants carrying γ34.5 genes from which various domains of the gene had been deleted (Fig. 2A). The proteins bound to GST-PP1 were solubilized, electrophoretically separated in denaturing gels, transferred to nitrocellulose, and reacted with antibody to the Ala-Thr-Pro repeat of the γ34.5 protein (9). The results in
that PP1 bound strongly to the main (deletions from amino acids 1–146 at the amino-terminal domain) and protein (amino acids 205–263) but with a lower affinity.

In the second set of experiments, purified PP1 was reacted with beads carrying GST or GST fused to the sequence of amino acids 146–263, 190–203, or 205–263 of the γ34.5 protein (Fig. 2B). The proteins bound to GST fusion proteins were solubilized, subjected to electrophoresis on a denaturing gel, and reacted with anti-PP1 antibody. The results in Fig. 2B indicate that a PP1-binding site is located between amino acids 146 and 205.

In the third series of experiments, a competition assay was done to test whether a synthetic test peptide containing amino acids 185–211 could block the interaction between γ34.5 mutants with the nested deletions from amino acids 1–146 at the amino-terminal domain (lanes 1–3 and 5), and the γ34.5 protein lacking the amino acids 205 to 263 (lane 4). These results indicate that a PP1-binding site is located between amino acids 146 and 205.

The purpose of this series of experiments was to determine whether mutations in the PP1-binding site abolish the ability of the γ34.5 protein to prevent the shut-off of protein synthesis in HSV-1-infected cells. To address this question, we constructed the recombinant

**PP1-binding Site Is Required to Prevent Shut-off of Protein Synthesis in HSV-1-infected Cells**—In this series of experiments replicate cultures of SK-N-SH cells were exposed to 10 pfu/cell of wild-type and mutant viruses and incubated at 37 °C. At 14 h after infection, the cultures were labeled with [35S]methionine for 1 h. As shown in Fig. 5, protein synthesis continued in cells infected with wild-type or with the mutants with deletions in the amino-terminal domain, whereas protein synthesis was shut off in cells infected with either R3616, from which 1 kilobase pair of coding sequences of the γ34.5 had been deleted, or R8301, from which 244 base pairs of the carboxyl-terminal domain had been deleted. It is noteworthy that the γ34.5 protein encoded by R8301 bound PP1 but was unable to prevent the shut-off of protein synthesis. We conclude from this experiment that both the PP1-binding site described above and the functions encoded by the carboxyl-terminal domain of the γ34.5 protein are required to prevent the shut-off of protein synthesis.

**The γ34.5 Protein Carboxyl-terminal to the**
virus R8321 in which the conserved amino acids Val193 and Phe195 in the PP1-binding motif were mutated to Glu and Leu, respectively, and created the novel restriction site EcoRII. The inserted mutations were verified by sequencing of the plasmid and the presence of the restriction site in the viral DNA (data not shown).

Replicate cultures of human neuroblastoma SK-N-SH cells were either mock-infected or infected with HSV-1(F), R3616, or R8321 at 10 pfu/cell. At 14 h after infection, the cells were labeled for 1 h with [35S]methionine and then harvested, solubilized, subjected to electrophoresis in denaturing polyacrylamide gel, and transferred to a nitrocellulose sheet. The autoradiogram shown in Fig. 6A indicates premature shut-off of protein synthesis in cells infected with R8321 mutant but not in mock-infected cells or those infected with HSV-1(F).

To determine whether R8321 expressed the mutant γ34.5 in SK-N-SH cells, the nitrocellulose sheet described above was reacted with the anti-γ34.5 antibody. As shown in Fig. 6B, the lysates of cells infected with HSV-1(F) or R8321 expressed full-length γ34.5 protein. The decrease in the amount of the γ34.5 protein made in cells infected with the R8321 mutant was expected because γ34.5, as its name indicates, is made mostly after the onset of DNA synthesis, and its accumulation would be affected by the shut-off of protein synthesis. The antibody did not react with lysates of mock-infected cells or cells infected with the R3616. On the basis of these experiments, we conclude that Val193 and Phe195 in the PP1-binding motif of γ34.5 are essential for the function of γ34.5.

Cytoplasmic PP1 and γ34.5 Protein Cofractionation in a Complex with an Apparent Molecular Weight of 340,000—The purpose of this experiment was to determine whether PP1 forms a complex with γ34.5 protein in cells infected with wild-type virus. Replicate 150-cm² flask cultures of HeLa cells were exposed to 20 pfu of HSV-1(F)/cell. At 15 h after infection, the cells were harvested and lysed, and the S10 fraction was chromatographed on a Superdex 200 column. The fractions were then assayed for their ability to dephosphorylate eIF-2α(32P) and eIF-2(β,γ,δ-P) (8, 32). The smooth tracing represents the absorbance at 280 nm. The Superdex 200 column was calibrated by chromatographing 200 μg of molecular size marker proteins individually using conditions that were identical to the chromatography of HeLa cell S10 fraction on this column, except the absorbance was monitored at 10-fold greater sensitivity. The molecular weight size markers and their M₉ values were: horse spleen apoferritin (465,000); aldolase (150,000); bovine serum albumin (69,000); ovalbumin (45,000); and rabbit reticulocyte thioredoxin (11, 600).

Fig. 7. Gel filtration analysis of cytoplasmic extracts from HSV-1(F)-infected cells on a Superdex 200 column. HeLa cells were exposed to 20 pfu of HSV-1(F)/cell and harvested 15 h after incubation at 37 °C. The S10 fraction (0.5 ml) prepared as described under “Materials and Methods” was chromatographed on a Superdex 200 column (1.0 × 30 cm), and the fractions were assayed for their ability to dephosphorylate eIF-2α(32P) and eIF-2(β,γ,δ-P) (8, 32). The smooth tracing represents the absorbance at 280 nm. The Superdex 200 column was calibrated by chromatographing 200 μg of molecular size marker proteins individually using conditions that were identical to the chromatography of HeLa cell S10 fraction on this column, except the absorbance was monitored at 10-fold greater sensitivity. The molecular weight size markers and their M₉ values were: horse spleen apoferritin (465,000); aldolase (150,000); bovine serum albumin (69,000); ovalbumin (45,000); and rabbit reticulocyte thioredoxin (11, 600).
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DISCUSSION

The salient features of this report are as follows. First, in an earlier article we reported that the γ134.5 protein binds to PP1 and that the cells infected with wild-type virus but not the cells infected with the γ134.5 mutant contain a highly potent, phosphatase activity specific for the α subunit of the translation initiation factor eIF-2. In this report we link the two observations by showing that the cytoplasm of wild-type virus-infected cells contained a high molecular weight complex that contained both γ134.5 protein and PP1 and that this fraction specifically dephosphorylated eIF-2α.P. The approximate molecular weight of the complex (340,000) is significantly higher than the combined molecular weight of single copies of γ134.5 protein and PP1. The results suggest that either one or both interacting proteins are present in several copies or that the complex has additional as yet unknown polypeptides.

Although we have not specifically addressed the issue, the results of our studies do not support the hypothesis that the amount of PP1 increased after infection. In several experiments we have not observed significant change in the level of protein phosphatase 1, as measured by immunoblotting with anti-PP1 antibody in HeLa cells infected with HSV-1 when compared with HeLa cells that were mock-infected or infected with the γ134.5 deletion mutant (data not shown). Rather, the results of this and preceding studies are consistent with the hypothesis that the γ134.5 protein binds to PP1 and that the activity is enhanced and redirected to dephosphorylate eIF-2α.

Second, we have identified the domain of the γ134.5 protein that binds to PP1. Specifically, the sequence of amino acids 190–203 of the γ134.5 protein fused to GST strongly bound and pulled down PP1. Moreover, a peptide containing amino acids 185–211 of the γ134.5 protein competed with the protein for binding to PP1 and substitution of Val203 with Glu and Leu, respectively, abolished the activity of the γ134.5 protein. The significance of these observations stems from the fact that this sequence is highly conserved among homologs of GADD44 and is also present in the NL protein of African swine fever virus (Fig. 1). More important, this sequence, exemplified in greater detail in Table I, contains a motif ((Arg/Lys)-(Val/ Ile)-Xaa-Phe) present in all of the subunit, accessory, or regulator proteins that bind directly to the catalytic subunit of PP1 (27, 28). These proteins interact with the catalytic subunit of PP1 (PP1c) in a mutually exclusive manner. Among the interacting proteins studied to date are the G-subunit (Gα), which targets the PP1 to glycogen particles in muscle (35), DARPP-32 (dopamine and cAMP-regulated phosphoprotein) (36), inhibitor 1 (37), splicing factor poly (38), p53BP2 (39), and NIPP-1 (40).

| Table I | Sequence alignment: PP1 binding domains |
|---------|---------------------------------------|
| PP1-binding protein | Sequence | Reference |
| Proteins known to bind PP1 | | |
| Gα subunit | ggr RV s F adn | 22 |
| Gβ subunit | vkk RV s F adn | 24 |
| Gγ-subunit | akk RV v F ads | 25 |
| p35BP2 | hqm RV k F npl | 37 |
| NIPP-1 | kns RV t F sed | 38 |
| Splicing factor PSF | rgl RV f at h | 36 |
| Inhibitor 1 | spr KI q F tvp | 35 |
| DARPP32 | dkz KI q F avp | 34 |
| M1 subunit | kzt KV k F dgg | 21 |
| HSV-1 γ134.5 | tpa RV f F sph | This work |
| GADD44 mouse | kar KV h F seek | 8 |
| GADD44 human | kar KV f F seek | Unpublished data |

Proteins predicted to bind PP1

HSV-2 γ134.5 | prg KV c F spr |
GADD44 hamaster | rar KV h F sen |

To characterize this complex further, we chromatographed crude HSV-1(F)-infected HeLa cell lysate or the purified complex obtained from gel filtration on Superdex 200 (Figs. 7 and 8) on a Mono Q column. To our surprise, none of the fractions from either chromatographic procedures yielded any phosphatase activity specific for eIF-2α. Assays of the aliquots of fractions 2–40 from the Mono Q chromatography indicated that the γ134.5 protein eluted in fractions 28–34 with peaks at 29 and 32–33 (data not shown). Because the KCl in these fractions is approximately 0.53–0.68 M, our inability to recover activated eIF-2α (P) phosphatase was probably the result of the dissociation of the complex by the high KCl concentrations required to elute the γ134.5 protein.
Peptides containing this motif have been shown to bind PP1c and to disrupt or attenuate the effects of binding of interacting subunits (27, 28). In this report, we also showed that the γ34.5 peptide containing this motif disrupted the binding of the γ34.5 protein to PP1.

The current definition of PP1 regulatory or subunit proteins is that they bind to the catalytic domain of the PP1 and that they regulate or direct PP1 activity to specific substrates. Inasmuch as (i) a γ34.5 protein binds PP1, (ii) the binding site has the motif common to PP1 subunits that bind the catalytic subunit, and (iii) the function of PP1 is redirected to dephosphorylate eIF-2α(P), the carboxyl-terminal domain of the γ34.5 protein has the structural and functional attributes of a PP1 subunit.

Third, as shown in Fig. 5, the recombinant virus carrying a truncated γ34.5 protein that contains the PP1-binding motif did not block the shut-off of protein synthesis associated with activated PKR. This result indicates that the binding site is necessary to enable binding but not sufficient to redirect PP1 to dephosphorylate eIF-2α(P). We have also noted that the domain of the γ34.5 protein carboxyl-terminal to the binding motif (amino acids 205–263) also binds PP1 but weakly. Relevant to the interpretation of these data are two observations: (i) subunits of PP1 may contain catalytic subunit-binding sites in addition to the (Arg/Lys)-(Val/Ile)-Xaa-Phe binding motif (28) and (ii) as noted in the Introduction, the carboxyl terminus of the GADD43 protein can substitute for the carboxyl terminus of the γ34.5 protein in blocking the shut-off of protein synthesis and also binds PP1. GADD43 and γ34.5 proteins share significant homology in their amino acid sequences carboxyl-terminal to the (Arg/Lys)-(Val/Ile)-Xaa-Phe binding motif. We interpret these results to suggest that like other subunits of the PP1, γ34.5 protein contains sequences that interact with the PP1 catalytic domain and also, possibly, with other proteins to bring about the direct interaction of PP1 with eIF-2α. As is the case with many other subunits of the PP1 protein, this aspect of the three-body interaction (the catalytic PP1 domain, the regulatory subunit, and the substrate) remains to be elucidated.

Fourth, the question remains as to the function of GADD43. Of the various GADD proteins, GADD43 is the least well understood. Our studies suggest that at least the carboxyl-terminal domain of the protein has functions consistent with those of a PP1 subunit similar to that of the γ34.5 protein. The conclusion that GADD43 can perform this function does not exclude the possibility that this protein has other functions as well.

Lastly, it seems appropriate to stress that activation of PKR is a common obstacle facing most viruses studied to date. In turn, viruses have evolved a variety of mechanisms to block the shut-off of protein synthesis resulting from phosphorylation of the eIF-2α. The herpes simplex viruses and possibly, African Swine fever virus, have a mechanism very different from any studied to date in that they have expropriated a piece of a cellular protein to cause the dephosphorylation of eIF-2α rather than to block the activation of PKR or the phosphorylation of eIF-2α. It is also important to note that this mechanism is not universally used by all herpesviruses because the γ34.5 gene is conserved among very few herpesviruses sequenced to date. These observations are consistent with the hypothesis that GADD43 does indeed enable the dephosphorylation of eIF-2α in response to stress and that the transient expression of the gene may explain why so few viruses captured the essential domain of the GADD43 gene to nullify the effect of activated PKR.

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