

Herpes simplex virus 1716, an ICP 34.5 null mutant, is unable to replicate in CV-1 cells due to a translational block that can be overcome by coinfection with SV40

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Herpes simplex virus (HSV) mutants lacking the gene encoding infected cell protein (ICP) 34.5 exhibit an attenuated phenotype in models of pathogenesis and have been used for experimental cancer therapy. Recently it was shown that the HSV ICP 34.5 protein functions to prevent the host cell-induced double-stranded RNA-activated protein kinase (PKR)-dependent translational block that normally occurs during virus infection. We now report that an HSV ICP 34.5 mutant called HSV-1716 is unable to replicate in the simian kidney cell-derived line CV-1, due to a translational block. Moreover, we find that this block can be overcome by simian virus 40 (SV40). This has been shown directly by infecting CV-1 cells with SV40 and HSV-1716 simultaneously, and indirectly via HSV-1716

infection of COS-1 cells (CV-1 cells transformed by an origin-defective mutant of SV40 that codes for wild-type T antigen). The translational block is restored when infections are done in the presence of the phosphatase inhibitor okadaic acid. These results support, but do not directly prove, contentions that HSV ICP 34.5 interacts with the PKR pathway to restore translation in non-permissive cells, and that SV40 large T antigen has a similar functional role, but acts downstream of the site of ICP 34.5 interaction (eIF2 α) in the pathway. Study of this CV-1/COS-1 system should allow further clarification of the virus–host interactions that underlie the restricted replication of HSV-1 ICP 34.5 gene null mutants.

Introduction

Herpes simplex virus type 1 (HSV-1) infected cell protein (ICP) 34.5 gene null mutants exhibit severely attenuated replication in animal models of HSV pathogenesis, but replicate as well as wild-type HSV in many malignant cells *in vitro* and *in vivo* (Bolovan *et al.*, 1994; Chou *et al.*, 1990; Javier *et al.*, 1987; Kucharczuk *et al.*, 1997; MacLean *et al.*, 1991; McGeoch & Barnett, 1991; Mckie *et al.*, 1996; Randazzo *et al.*, 1995; Spivack *et al.*, 1995; Taha *et al.*, 1990; Valyi-Nagy *et al.*, 1994). Capitalizing on this selective lytic replication, we and others have used ICP 34.5 mutants to successfully treat brain tumours, including melanoma, intraperitoneal human mesothelioma and subcutaneous human melanoma, in various immunodeficient and immunocompetent mouse models (Chambers *et al.*, 1995; Kesari *et al.*, 1995; Kucharczuk *et al.*, 1997; Lasner *et al.*, 1996;

Markert *et al.*, 1993; Randazzo *et al.*, 1995, 1996, 1997). The ability of ICP 34.5 mutants to carry out lytic replication has been shown to correlate with host cell type and cell cycle state (Brown *et al.*, 1994; Mckie *et al.*, 1996). The fundamental reasons for the attenuated phenotype of these HSV mutants *in vivo* remain unclear.

It was noted previously that an HSV-1 ICP 34.5 gene null mutant replicates poorly in the African green monkey kidney cell-derived line CV-1 (Perng *et al.*, 1995); however, the reason for this restriction was not identified. Recently, it was determined that the ICP 34.5 gene product is directly involved in allowing the virus to overcome the double-stranded RNA-activated protein kinase (PKR)-mediated block in translation that the host cell mounts during HSV infection (He *et al.*, 1997). Simian virus 40 (SV40) large T antigen also interacts with the PKR pathway to overcome host cell-induced translational block (Rajan *et al.*, 1995). The interaction of large T antigen with the PKR pathway is believed to occur downstream of the

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site of HSV interaction, although the details of the interaction are not clearly defined at present (Swaminathan *et al.*, 1996). Since HSV and SV40 appear to overcome host attempts to block translation at different points in the PKR-mediated pathway, we reasoned that SV40 should be able to rescue the replication of an HSV-1 ICP 34.5 null mutant if in fact the block to replication of HSV-1716 in CV-1 results specifically from a PKR-mediated translational block.

We now report that restricted replication of the HSV-1 ICP 34.5 mutant HSV-1716 in CV-1 cells is due to an HSV-induced block in translation related to the absence of the ICP 34.5 protein. Moreover, we show that this block can be overcome by coinfection with SV40. This is further supported by the fact that COS-1 cells, which are CV-1 cells transformed by an origin-defective mutant of SV40 that codes for wild-type T antigen (Gluzman, 1981), are fully permissive for HSV-1716 replication. Study of this CV-1/COS-1 system will allow further clarification of the virus–host interactions that underlie the neuroattenuated phenotype of HSV-1 ICP 34.5 gene null mutants.

Methods

■ **Cells and viruses.** SV40 was a generous gift from James Alwine (University of Pennsylvania, Pa., USA). To produce HSV stocks, subconfluent monolayers of baby hamster kidney 21 clone 13 (BHK) cells were infected with HSV strain 1716 or wild-type 17⁺. Virus was concentrated from the culture and titrated by plaque assay (Spivack & Fraser, 1987). All virus stocks were stored frozen at -70°C , and thawed rapidly just prior to use.

CV-1 cells and COS-1 cells, which are CV-1 cells that are transformed by an origin-defective mutant of SV40 that codes for wild-type T antigen (Gluzman, 1981), were obtained from the ATCC (Rockville, Md., USA) and were grown in Dulbecco's modified Eagle's medium (DMEM) containing penicillin, streptomycin and 5% calf serum.

■ **Virus growth kinetics assay.** Subconfluent monolayers (5×10^5 cells) in six-well plastic tissue culture plates were infected with the appropriate virus (HSV-1 and/or SV40) in 1 ml DMEM containing penicillin and streptomycin. For the 0 h measurement the cell monolayer was scrapped off into the virus inoculum suspension immediately and frozen at -70°C . For the remaining time-points, the virus inoculum was incubated on the cell monolayer at 37°C for 1 h with gentle rocking, and then aspirated off. The infected monolayers were washed twice with medium and resuspended in 1 ml DMEM containing penicillin, streptomycin and 5% calf serum. At the appropriate times post-infection, the monolayers were harvested with a cell scraper and the suspension frozen at -70°C . Following a total of two cycles of freezing and thawing, each sample was cleared by centrifugation at 3000 g for 5 min at 4°C and the supernatant was titred in triplicate on BHK cells using a rabbit anti-HSV-1 polyclonal antibody (American Qualex)-based immunoperoxidase assay (Hung *et al.*, 1992).

■ **RT-PCR detection of viral gene transcripts.** Detailed methods for RT-PCR including primer sequences, standardization, determination of primer sensitivity and validation were described previously (Tal-Singer *et al.*, 1997). Briefly, RNA was isolated using the TRizol reagent (Gibco BRL) followed by extensive digestion with RNase-free DNase I (BMB) and ethanol precipitation. cDNA was generated using a Superscript Pre-amplification kit priming with oligo (dT) and random hexamers (Gibco BRL). HSV-17⁺ DNA used for positive controls was

purified from infected Vero cells. PCR was performed in $25\ \mu\text{l}$ vols containing 4% total cDNA (or $10\ \mu\text{g}$ DNA), $200\ \mu\text{M}$ of each deoxynucleoside triphosphate (Pharmacia), $1\ \mu\text{M}$ of each primer and $2.5\ \text{U}$ *Taq* polymerase with PCR Buffer A (Fisher). Cycling reactions were performed with a Perkin Elmer thermal cycler. After one cycle of 4 min denaturation at 94°C , a total of 35 cycles were performed as follows: (i) 1 min denaturation at 94°C ; (ii) annealing at 60°C for 1 min; (iii) extension for 2 min at 72°C . The final cycle was terminated with a 7 min extension at 72°C . Aliquots of the amplification products were fractionated on 2.5% NuSieve agarose (FMC). Gels were stained with ethidium bromide (Sigma) and imaged by fluorimetry (Molecular Dynamics). Images were assembled for viewing using Photoshop software (Adobe) on an Apple Macintosh computer.

■ **Detection of protein translation.** Subconfluent CV-1 or COS-1 cultures (5×10^5 cells) in six-well plastic tissue culture plates grown in DMEM containing penicillin, streptomycin and 5% calf serum were infected with the appropriate virus at an m.o.i. of 10 in 1 ml serum-free DMEM with gentle rocking. After 1 h the medium was aspirated off and replaced with 1 ml DMEM containing penicillin, streptomycin and 5% calf serum. At this point, appropriate cultures were treated with okadaic acid sodium salt (Sigma) at a dose of 20 ng/ml for COS-1 and 30 ng/ml for CV-1. At 14 h after infection, cultures were pulsed with $50\ \mu\text{Ci}$ [^{35}S]methionine/cysteine labelling mix (specific activity $< 1000\ \text{Ci/mmol}$, NEN) in 1 ml methionine/cysteine-free DMEM for 90 min, washed twice with ice-cold PBS, and lysed in $250\ \mu\text{l}$ lysis buffer ($8.2\ \text{mM}$ Na_2HPO_4 , $1.5\ \text{mM}$ KH_2PO_4 , $0.14\ \text{M}$ NaCl, $2.5\ \text{mM}$ KCl, 1% NP-40, 1% sodium deoxycholate, $10^{-5}\ \text{M}$ *N* α -*p*-tosyl-L-lysine chloromethyl ketone and $10^{-5}\ \text{M}$ *N* α -*p*-tosyl-L-phenylalanine). Lysates were spun at $15000\ \text{g}$ for 5 min and the supernatant was reserved. Equal volumes of cell lysate were reduced and run on 10% NuPAGE gels (Novex), transferred to nitrocellulose and imaged using a phosphorimager.

■ **Western blotting for viral proteins.** Subconfluent CV-1 or COS-1 cultures (5×10^5 cells) in six-well plastic tissue culture plates grown in DMEM containing penicillin, streptomycin and 5% calf serum were infected with the appropriate virus at an m.o.i. of 10 in 1 ml serum-free DMEM with gentle rocking. After 1 h the medium was aspirated off and replaced with 1 ml DMEM containing penicillin, streptomycin and 5% calf serum. At 20 h post-infection, the monolayers were washed twice with ice-cold PBS and lysed in $250\ \mu\text{l}$ lysis buffer ($8.2\ \text{mM}$ Na_2HPO_4 , $1.5\ \text{mM}$ KH_2PO_4 , $0.14\ \text{M}$ NaCl, $2.5\ \text{mM}$ KCl, 1% NP-40, 1% sodium deoxycholate, $10^{-5}\ \text{M}$ *N* α -*p*-tosyl-L-lysine chloromethyl ketone and $10^{-5}\ \text{M}$ *N* α -*p*-tosyl-L-phenylalanine). Lysates were spun at $15000\ \text{g}$ for 5 min and the supernatant was reserved. Equal volumes of cell lysate were reduced and run on 10% NuPAGE gels (Novex) and electroblotted onto nitrocellulose. The blots were probed first with the monospecific polyclonal rabbit antibody R-45 (a generous gift from Drs Gary Cohen and Roz Eisenberg, University of Pennsylvania, Pa., USA) and then with horseradish peroxidase-conjugated goat anti-rabbit antibody (Sigma). Following incubation with a chemiluminescent substrate, the blots were exposed to X-ray film.

■ **Statistical analysis.** Data analysis including calculations of means and standard deviations was performed using StatView statistical software (Abacus Concepts) on an Apple Macintosh computer.

Results

HSV-1716 exhibits severely attenuated replication in CV-1 cells

Our first experiment was designed to confirm the fact that the HSV-1 ICP 34.5 gene null mutant HSV-1716 would

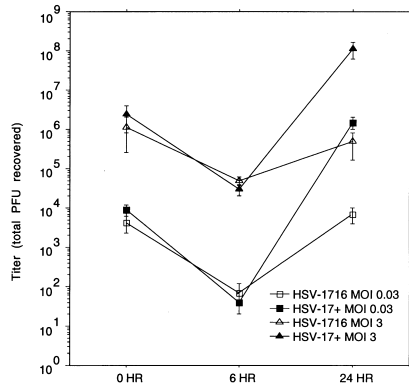


Fig. 1. Replication of the ICP 34.5 mutant HSV-1716 is restricted in CV-1 cells relative to wild-type HSV-17⁺. Subconfluent monolayers of CV-1 cells were infected with HSV-1716 or HSV-17⁺ at an m.o.i. of 0.03 or 3. At the various times shown, the infected monolayers were harvested with a cell scraper and the suspension frozen at -70 °C. Each sample was titred in triplicate on BHK cells using an immunoperoxidase-based assay. Each data-point represents the mean ± standard deviation of three independent triplicate determinations. This experiment was repeated twice with essentially identical results.

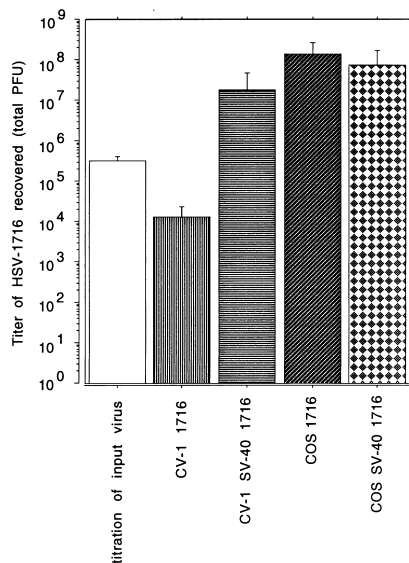


Fig. 2. Replication of the ICP 34.5 mutant HSV-1716 in CV-1 cells is rescued by coinfection with SV40 and is normal in COS-1 cells. Subconfluent monolayers of CV-1 or COS-1 cells were infected with HSV-1716 at an m.o.i. of 10 with or without coinfection of SV40 at an m.o.i. of 10. An aliquot of infecting HSV-1716 was frozen immediately for titring. At 24 h post-infection the infected monolayers were harvested with a cell scraper and the suspension frozen at -70 °C. Each sample was titred in triplicate on BHK cells using an immunoperoxidase-based assay. Each bar represents the mean ± standard deviation of three independent triplicate determinations. This experiment was repeated three times with essentially identical results.

replicate poorly in CV-1 cells (Perng *et al.*, 1996). We compared the kinetics of virus replication of HSV-1716 and the wild-type parental virus 17⁺ in CV-1 cells at an m.o.i. of 0.03 or 3. As shown in Fig. 1, the replication of HSV-1716 was severely impaired relative to wild-type virus at both low and high

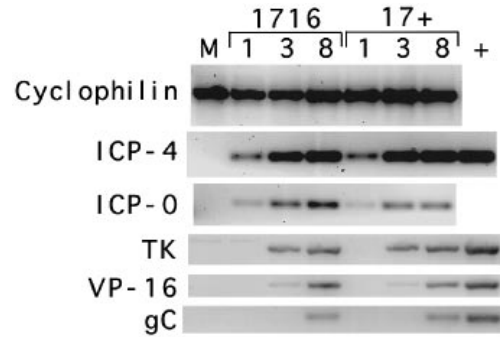


Fig. 3. Viral gene transcription of the ICP 34.5 mutant HSV-1716 in CV-1 cells is similar to that of wild-type HSV-17⁺. Subconfluent monolayers of CV-1 cells were infected with either HSV-1716 or HSV-17⁺ at an m.o.i. of 0.1 or mock-infected (M). At the times indicated (1, 3 or 8 h), infected cells were lysed in TRIzol reagent and RNA was isolated. After extensive digestion with RNase-free DNase I, cDNA was generated. HSV-17⁺ DNA used for positive controls to verify the size of expected fragments (lane +) was purified from infected Vero cells. PCR was performed and aliquots of the amplification products were fractionated on 2.5% agarose. Gels were stained with ethidium bromide and imaged by fluorimetry. The data shown in the figure are from a single representative experiment from a series of two, in which essentially identical results were obtained.

multiplicities of infection. The fact that the titre of virus was higher at 24 h than at 6 h post-infection at both low and high multiplicities of infection indicates that the block to replication of this mutant virus, while substantial, is not absolute.

SV40 rescues the replication of HSV-1716 in CV-1 cells

In order to directly address the effect of SV40 on the replication of HSV in CV-1 cells, we examined the kinetics of replication of HSV-1716 in the absence and presence of coinfecting SV40. As shown in Fig. 2, coinfection of HSV-1716 and SV40 boosted the production of infectious HSV-1716 by over 3 logs at 24 h relative to CV-1 cultures infected with HSV-1716 alone. In a second set of experiments we addressed the same issue with another approach, and compared replication of HSV-1716 in CV-1 cells and COS-1 cells (CV-1 cells that are transformed by an origin-defective mutant of SV40 that express wild-type T antigen). Approximately 4 logs more progeny HSV-1716 was produced in COS-1 cells than in CV-1 (Fig. 2), providing additional indirect data on the ability of SV40 to complement replication of HSV-1716 in CV-1. Coinfection of COS-1 cells with HSV-1716 and SV40 did not boost HSV-1716 production above the level produced by HSV-1716 infection alone.

HSV-1716 gene transcription appears unaltered in CV-1 cells relative to wild-type HSV

In order to determine the level of the block of replication of HSV-1716 in CV-1 cells, we determined the relative abundance and temporal expression of HSV gene transcripts by RT-PCR for HSV-1716 relative to wild-type HSV-17⁺ in CV-1 cells. We

cell line:	CV-1			COS-1		
virus:	mock	1716	17+	mock	1716	17+
okadaic acid:	-	+	-	+	-	+

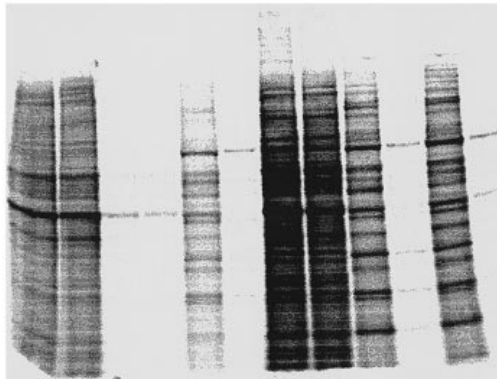


Fig. 4. Translation is blocked in CV-1 cells infected with the ICP 34.5 mutant HSV-1716, but proceeds normally in CV-1 cells infected with wild-type HSV-17⁺ and in COS-1 cells infected with HSV-1716. Subconfluent CV-1 or COS-1 monolayers were infected with HSV-1716 or HSV-17⁺ at an m.o.i. of 10 or mock-infected in serum-free medium. After 1 h the medium was replaced with medium containing 5% calf serum. At this point, appropriate cultures were treated with okadaic acid sodium salt at a dose of 20 ng/ml for COS-1, and 30 ng/ml for CV-1. At 14 h into the infection, cultures were pulsed with [³⁵S]methionine/cysteine labelling mix for 90 min, washed twice with ice-cold PBS and lysed in 250 µl of lysis buffer. Equal volumes of cell lysate were reduced and run on 10% NuPAGE gels, transferred to nitrocellulose and imaged using a phosphorimager. This representative blot is from a series of four, in which essentially identical results were obtained.

have previously shown that RT-PCR gives quantitatively valid results under the conditions utilized (Tal-Singer *et al.*, 1997). This technique is particularly convenient for evaluating the level of multiple transcripts simultaneously. As shown in Fig. 3, the level of RNA for each representative transcript from the various temporal classes of HSV genes (immediate early or α , ICP-0 and ICP-4; early or β , TK; late or γ , VP-16 and gC) was comparable for HSV-1716 and wild-type HSV-17⁺.

Translation is blocked in CV-1 cells infected with HSV-1716, but not in COS-1 cells

We performed metabolic labelling experiments at 14 h post-infection to determine the level of protein translation in HSV-infected CV-1 and COS-1 cells. As shown in Fig. 4, there was a dramatic inhibition of protein translation in CV-1 cells infected with HSV-1716, whereas translation in CV-1 cells infected with wild-type HSV-17⁺ remained intact. In contrast, this translational block was not seen in COS-1 cells infected with either HSV-1716 or HSV-17⁺.

An HSV-induced translational block occurs in both CV-1 and COS-1 infected with either HSV-17 or HSV-1716 in the presence of okadaic acid

It has been shown that okadaic acid, a potent inhibitor of serine/threonine-specific protein phosphatases 1 and 2A,

	COS-1		CV-1	
M	1	7	1	7
o	1	1	1	1
c	6	7	6	7
k				

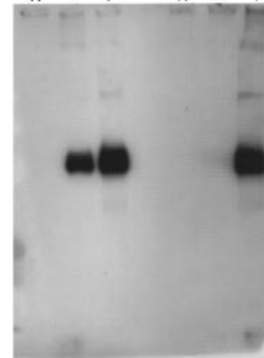


Fig. 5. The level of HSV-1 glycoprotein D is markedly decreased in lysates of CV-1 cells infected with HSV-1716. Subconfluent CV-1 or COS-1 monolayers were infected with HSV-1716 or HSV-17⁺ at an m.o.i. of 10 or mock-infected in serum-free medium. After 1 h, the monolayers were washed twice and the medium was replaced with medium containing 5% calf serum. At 20 h into the infection, cultures were washed twice with ice-cold PBS and lysed in 250 µl lysis buffer. Equal volumes of cell lysate were reduced, run on 10% NuPAGE gels and electroblotted onto nitrocellulose. The blots were probed first with rabbit anti-gD antibody and then with horseradish peroxidase-conjugated goat anti-rabbit antibody (Sigma). Following incubation with a chemiluminescent substrate, the blots were exposed to X-ray film. This representative blot is from a series of eight, in which essentially identical results were obtained.

blocks both HSV and SV40-mediated rescue of translation (He *et al.*, 1997; Rajan *et al.*, 1995). Thus, we tested the effect of okadaic acid on protein translation in HSV-infected CV-1 and COS-1 cells. As shown in Fig. 4, translation in CV-1 cells infected with wild-type HSV-17⁺ was blocked when the cells were exposed to okadaic acid. Moreover, translation in COS-1 cells infected with either HSV-17⁺ or HSV-1716 was also dramatically inhibited in the presence of okadaic acid. Okadaic acid had no effect on translation in mock-infected CV-1 or COS-1 cells.

Levels of viral proteins are markedly decreased in lysates of CV-1 cells infected with HSV-1716

Western blots were performed on cell lysates at 20 h post-infection to specifically determine whether a block of translation of viral proteins was present. The blot shown in Fig. 5 demonstrates a marked decrease of glycoprotein D (gD) in lysates of CV-1 cells infected with HSV-1716 relative to CV-1 cells infected with HSV-17⁺, or COS-1 cells infected with either HSV-1716 or HSV-17⁺. Similar marked decreases in the abundance of the viral proteins thymidine kinase and glycoprotein C were also seen (data not shown).

Discussion

Prevention of translational initiation is a commonly utilized mechanism of cellular anti-viral defence (for review see Katze, 1995). Diverse strategies have been adopted by viruses to

circumvent host cell attempts to terminate protein translation in the face of infection. The mechanisms utilized by viruses such as HSV, SV40 and adenoviruses (Ad), relevant to our present work, are known in some detail and the host cell enzyme PKR is a central mediator in these mechanisms. PKR is normally present in cells in a latent form. Upon induction by interferon following virus infection, PKR is activated via autophosphorylation. Activated PKR can phosphorylate the α subunit of the initiation factor eIF-2 (eIF2 α), which generally results in prevention of translational initiation and thus halts protein synthesis.

The HSV-1 ICP 34.5 gene encodes a protein of 263 amino acids consisting of a large amino-terminal domain, a linker region of three amino acid repeats (Ala-Thr-Pro, ATP codons) and a carboxy-terminal domain (Chou & Roizman, 1992). The carboxy-terminal domain is homologous to domains of MyD116 and growth arrest and DNA damage gene 34 (GADD 34) (Lord *et al.*, 1990; McGeoch & Barnett, 1991), cellular genes involved in cell cycle regulation and programmed cell death. ICP 34.5 forms a physical complex with the host enzyme protein phosphatase 1 α (PP1 α ; He *et al.*, 1997). He *et al.* (1997) show that the α subunit in purified eIF2 α -P phosphorylated *in vitro* is specifically dephosphorylated by specific lysate fractions of wild-type HSV-infected cells at a rate 3000 times that of mock-infected cells, whereas the eIF2 α -P phosphatase activity of ICP 34.5 gene null virus-infected cells is lower than that of mock-infected cells. Presumably when complexed to ICP 34.5, PP1 α -directed dephosphorylation of the α subunit of eIF2 α activity is enhanced, and thus the translation block is overcome despite the presence of activated PKR.

Recent data suggest that SV40 has adopted a strategy for overcoming host cell PKR-mediated attempts at halting protein translation that is related to, but different from, that described for HSV above (Swaminathan *et al.*, 1996). The details have been defined in a system in which SV40 coinfection rescues the translation defect observed in cells infected with Ad mutants lacking a small virus-encoded RNA species designated virus-associated RNA I (VAI RNA). During wild-type Ad infection VAI RNA binds to and blocks the activation of PKR, and thus prevents phosphorylation of eIF2 α (reviewed by Mathews & Shenk, 1991). Cells infected with Ad mutants lacking the VAI RNA are severely defective for translation. As in HSV infection, protein translation continues during SV40 infection despite phosphorylation of PKR, and it is known that the effect is mediated by a domain in the carboxy-terminal region of SV40 large T antigen (Rajan *et al.*, 1995). However, the rescue of translation by SV40 in cells infected with Ad mutants lacking the VAI RNA occurs despite no detectable difference in the level of phosphorylated eIF2 α between those cells coinfecting by SV40 and those which are mock-infected (Swaminathan *et al.*, 1996). Thus, it appears that SV40 large T antigen-mediated translational rescue occurs at a step downstream of eIF2 α phosphorylation.

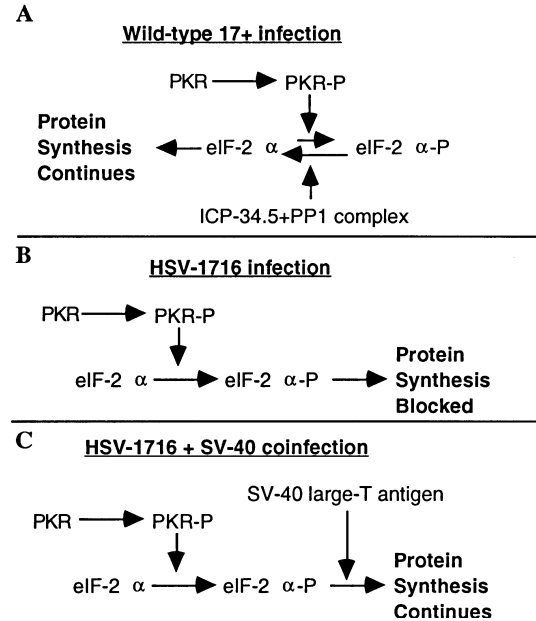


Fig. 6. Diagram of proposed regulation of protein synthesis by the PKR pathway in HSV/SV40-infected CV-1 cells. The state of PKR pathway intermediates are based on data of He *et al.* (1997), Swaminathan *et al.* (1996) and our findings. (A) Despite activation of PKR to PKR-P, eIF2 α -P is ultimately dephosphorylated by the PP1 α -ICP 34.5 complex in CV-1 cells infected with wild-type HSV-17+, and protein synthesis proceeds. (B) During infection of CV-1 cells with the ICP 34.5 null mutant HSV-1716, PKR activation leads to phosphorylation of eIF2 α -P and protein synthesis is blocked. (C) In COS-1 cells infected with HSV-1716, and in CV-1 cells coinfecting with HSV-1716 and SV40, protein synthesis continues despite phosphorylation of PKR-P and eIF2 α -P, since SV40 large T antigen unblocks the pathway downstream of eIF2 α -P.

We found that CV-1 cells were unable to replicate the mutant HSV-1716. At both low and high multiplicities of infection very little infectious virus was produced. The profile of viral gene expression documented by RT-PCR in CV-1 cells infected with HSV-1716 was similar temporally and quantitatively to that occurring during wild-type HSV-17+ infection, and thus it appears that HSV-1716 enters CV-1 cells and viral gene transcription proceeds normally. Since it had been previously reported that infection of human skin and neuronally derived cells with an HSV-1 ICP 34.5 gene null mutant led to a PKR-mediated translational block (He *et al.*, 1997), we wondered whether the same process could be occurring in CV-1. Our metabolic labelling and Western blotting experiments support the fact that the block to HSV-1716 replication is at the level of protein translation. Although the details of the interaction between SV40 large T antigen and the PKR pathway are not clearly defined at present, our coinfection data and HSV-1716 COS-1 infection experiments both agree with the previously published contention that the interaction of SV40 is downstream of eIF2 α (Swaminathan *et al.*, 1996). A diagrammatic representation of our interpretation of these data in the context of previously published work on the interactions of HSV ICP 34.5, SV40 large T antigen and the PKR pathway is shown in Fig. 6. Okadaic acid is a potent

inhibitor of serine/threonine-specific protein phosphatases 1 and 2A (Cohen *et al.*, 1990). The block to translation in COS-1 cells infected with either HSV-17⁺ or HSV-1716 that we observed in the presence of okadaic acid is compatible with a paradigm in which COS-1 cells are permissive to HSV-1716 because of a phosphatase-dependent SV40 large T antigen-mediated effect, analogous to the case of COS-1 complementation of Ad VA-negative mutants (Rajan *et al.*, 1995). However, this remains to be rigorously proven.

An intriguing area beyond the scope of this present work is the nature of factors that make most transformed cells permissive to ICP 34.5 mutant HSV. It would seem that endogenous factors present in most cell lines can obviate the need for ICP 34.5-mediated bypass of host cell translational block. CV-1 and COS-1 are readily available, well-characterized cell lines. One could envision that these easily transfected cell lines could be used to screen potential complementing cellular genes. It is our hope that further studies utilizing these two cell lines will help clarify the role of the HSV-1 ICP 34.5 gene product in virulence.

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