The Herpes Simplex Virus Type 2 R1 Protein Kinase (ICP10 PK) Functions as a Dominant Regulator of Apoptosis in Hippocampal Neurons Involving Activation of the ERK Survival Pathway and Upregulation of the Antiapoptotic Protein Bag-1

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Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) can trigger or block apoptosis in a cell type-dependent manner. We have recently shown that the protein kinase activity of the large subunit of the HSV-2 ribonucleotide reductase (R1) protein (ICP10 PK) blocks apoptosis in cultured hippocampal neurons by activating the extracellular signal-regulated kinase (ERK) survival pathway (Perkins et al., J. Virol. 76:1435-1449, 2002). The present studies were designed to better elucidate the mechanism of ICP10 PK-induced neuroprotection and determine whether HSV-1 has similar activity. The data indicate that apoptosis inhibition by ICP10 PK involves a c-Raf-1-dependent mechanism and induction of the antiapoptotic protein Bag-1 by the activated ERK survival pathway. Also associated with neuroprotection by ICP10 PK are increased activation/stability of the transcription factor CREB and stabilization of the antiapoptotic protein Bcl-2. HSV-1 and the ICP10 PK-deleted HSV-2 mutant ICP10ΔPK activate JNK, c-Jun, and ATF-2, induce the proapoptotic protein BAD, and trigger apoptosis in hippocampal neurons. c-Jun activation and apoptosis are inhibited in hippocampal cultures infected with HSV-1 in the presence of the JNK inhibitor SP600125, suggesting that JNK/c-Jun activation is required for HSV-1-induced apoptosis. Ectopically delivered ICP10 PK (but not its PK-negative mutant p139) inhibits apoptosis triggered by HSV-1 or ICP10ΔPK. Collectively, the data indicate that ICP10 PK-induced activation of the ERK survival pathway results in Bag-1 upregulation and overrides the proapoptotic JNK/c-Jun signal induced by other viral proteins.

Apoptosis is a tightly regulated, irreversible process that results in cell death in the absence of inflammation. It is necessary for the proper development of the nervous system, but when it is inappropriate in timing or extent, apoptosis in the central nervous system can trigger or account for progression of neurodegeneration in various diseases, including stroke, trauma, and virus infection (37, 89). Morphological changes associated with apoptosis include nuclear and cytoplasmic condensation, intranucleosomal DNA cleavage, and blebbing of the cell into membrane-bound apoptotic bodies. Apoptosis is primarily mediated by caspases, which are cysteine proteases with aspartate specificity that are activated by the cleavage of inactive zymogens (procaspases).

Caspase 3 is one of the key executioners of apoptosis. Its activation requires proteolytic cleavage of the inactive procaspase 3 into 17- to 20-kDa and 12-kDa fragments. Activated caspase-3 is, in turn, responsible for the proteolytic cleavage of many key proteins, such as the nuclear poly(ADP-ribose) polymerase, which is involved in DNA repair. Poly(ADP-ribose) polymerase cleavage to an 85-kDa fragment is a critical event in the commitment to undergo apoptosis (48).

Signal transduction pathways are linked to the apoptotic machinery (9, 49). Activation of the c-Jun N-terminal kinase (JNK) (also known as stress-activated protein kinase) and the p38 mitogen-activated protein kinase protein kinase cascade is associated with increased expression of proapoptotic proteins (49, 51). JNK and its target c-Jun play an important role in triggering neuronal apoptosis (35, 60). Survival stimuli cause the membrane-bound G protein Ras to adopt an active, GTP-bound state, and it, in turn, coordinates the activation of a multitude of downstream effectors. The ERK survival pathway involves c-Raf-1 kinase and activation of mitogen-activated protein kinase kinase (MEK) and mitogen-activated protein kinase (ERK). A variety of genes are ERK targets, including those required for cell cycle progression (54). The ERK survival pathway overrides the effects of apoptotic signals, apparently by upregulating antiapoptotic Bcl-2 proteins through a transcription dependent mechanism which involves activation of the calcium/cyclic AMP response element binding (CREB) protein (49, 80, 86, 87).

Bcl-2 proteins are a family of apoptosis regulators that includes members with antiapoptotic (Bcl-2) or proapoptotic (BAD) activity. Antiapoptotic Bcl-2 proteins prevent caspase activation by sequestering their proforms, inhibiting the release of cytochrome c, or preventing mitochondrial dysfunction. Susceptibility to a given apoptotic stimulus is determined by the interaction between pro- and antiapoptotic family members (70). The Bcl-2-associated athanogene (Bag-1) interacts with Bcl-2 to cooperatively interfere with the apoptotic cascade at the level of caspase activation (79). Bag-1 also has Bcl-2-independent antiapoptotic activity (79), and it enhances c-
Raf-1 kinase activity through a Ras-independent mechanism (82).

Viruses depend on cells for their replication and can differentially affect various signaling pathways. Herpes simplex virus type 1 (HSV-1) and HSV-2 can trigger or counteract apoptosis in a cell-specific manner (1, 15, 38, 47, 61, 69). The mechanism by which they trigger apoptosis, and the identity of the proapoptotic genes are still poorly understood. Antiapoptotic activity, on the other hand, has been ascribed to the HSV-1 and HSV-2 gene US3 (38, 47, 61), the HSV-1 genes γ34.5, US5, ICP27, and LAT (2, 15, 47, 69), and the HSV-2 gene ICP10 (66, 67). In nonneuronal cells, the HSV-1 US3 protein kinase (PK) phosphorylates, and thereby inactivates, the proapoptotic Bcl-2 family member BAD (61). However, the activity of the antiapoptotic HSV-1 genes in hippocampal neurons, if any, is still unclear.

The large subunit of HSV-2 ribonucleotide reductase (R1, also known as ICP10) has an intrinsic PK activity that is required for immediate-early (also known as α) gene expression and HSV-2 growth in nonneuronal cells, involving activation of the ERK survival pathway (73, 75, 76). Our laboratory first demonstrated that ICP10 PK blocks apoptosis of hippocampal neurons caused by loss of trophic support, genetic defects, or virus infection by activating MEK/ERK (66, 67). Antiapoptotic activity does not require de novo viral protein synthesis and appears to be mediated by the ICP10 PK protein in the virion tegument (66, 74).

Here we report that ERK activation by ICP10 PK in hippocampal neurons upregulates the antiapoptotic protein Bag-1 and consequently overrides a JNK/c-Jun signal(s) responsible for virus-induced apoptosis.

**MATERIALS AND METHODS**

**Viruses and plasmids.** HSV-2 (G), HSV-1 (F), HSV-2 mutants ICP10Δ2PK and ICP10ΔARR, which have deletions in the PK and RR domains of ICP10, respectively, and the revertant virus HSV-2 (R) have been described previously (75). HSV-1 mutants ICPαΔ, which is deleted in the R1 (also known as the ICP0) coding sequence, and brR3, which retains only 38% of the N-terminal domain, were obtained from Sandra K. Weller (University of Connecticut Health Center, Farmington). Their generation and properties have been described (31, 32). The expression vectors for ICP10 and its PK-negative mutant p139 (pW17 and pH215, respectively) have been described (16, 55, 66, 73, 76). The Flag-Raf1 expression vector for the c-Raf-1 dominant negative mutant protein K375M (18, 78) was obtained from Bernard Weinstein (Columbia University, New York). Expression vector pG45-mBag-1, for the murine Bag-1 protein (73), was purchased from Science Reagents Inc (Atlanta, Ga.).

**Cells, virus infection, and plasmid transfection.** Vero (African green monkey kidney) cells were grown in minimal essential medium with 10% fetal bovine serum and 100 μg of penicillin-streptomycin (Gibco-BRL, Gaithersburg, Md.) per ml and used for virus infection (73–76). Primary cultures of central nervous system neurons were established as previously described (1, 66). Cells dissociated from the hippocampi or cerebral cortex of 16- to 19-day-old rat fetuses (Sprague-Dawley) were plated at a density of approximately 750,000/2 ml on collagen-coated 35-mm dishes (Nunc, Rochester, N.Y.) or glass coverslips coated with poly-l-lysine (Sigma, St. Louis, Mo.). The studies described in this report were done with 6-day-old cultures in which ≥88% of the cells were postmitotic neurons, as determined with the 5-bromo-2′-deoxyuridine and detection kit (Roche Molecular Biochemicals, Indianapolis, Ind.) and staining with class III  β-tubulin (TUJ1) antibody (23).

Virus infection was done with 10 PFU/cell in medium containing 10% horse serum, a condition that allows growth of the mutants in nonneuronal cells (31, 32, 75, 76). After adsorption for 1 h at 38.5°C, the virus inoculum was removed, and the cultures were overlaid with growth medium (66). Transient transfection was performed as previously described (66, 67) with the FuGene 6 transfection reagent (Roche Molecular Biochemicals) according to the manufacturer’s instructions.

**Antibodies.** TUJ1 antibody was the gift of Paul Yarowsky (University of Maryland, Baltimore). The Flag monoclonal antibody M2 was purchased from Cayman Chemicals, Ann Arbor, Mich. The polyclonal antibody to HSV-1 pUL18 was described previously. It recognizes amino acids 13 to 26 on ICP10 and p139 but does not recognize HSV-1 proteins (3, 16, 55, 66, 73–76). The following polyclonal antibodies were purchased and used according to the manufacturer’s instructions: antibodies to cleaved caspase-3 (D175), recognizes the 17- to 20-kDa fragment of the active caspase (63, 66, 67) (caspase3p20) (Cell Signaling Technology, Beverly, Mass.); cleaved poly(ADP-ribose) polymerase (cPARP) (p85kDa) (Cell Signaling Technology) (66, 67) and confirmed in our laboratory (data not shown) (Promega, Madison, Wis.); phosphorylated JNK (recognizes the dually phosphorylated form of JNK1/2 (Promega), actin, Bcl-2 (ΔC21, and Bag-1 (FL-274) (Santa Cruz Biotechnology, Santa Cruz, Calif.); antibodies to c-Jun phosphorylated on Ser63 (p-Jun(Ser63)) or Ser73 (p-Jun(Ser73)) and nonphosphorylated c-Jun (part of the PhosphoPlus c-Jun(Ser63) II c-Jun(Ser73) antibody kit); antibodies to CREB phosphorylated on Ser133 and nonphosphorylated CREB (part of the PhosphoPlus CREB(Ser133) antibody kit); and antibody to ATF-2 phosphorylated on Thr646 (phosphorylated ATF-2) were purchased from Cell Signaling Technology.

**Chemicals.** The c-Raf-1 kinase inhibitor I (52) and the JNK inhibitor SP600125 (7, 36) were purchased from Calbiochem (La Jolla, Calif.). The MEK-specific inhibitor U0126 (22) was purchased from Promega.

**Hoechst staining.** Cells were fixed in 4% paraformaldehyde (pH 7.4) for 1 h at room temperature, followed by permeabilization in 0.1% Triton X-100 (in 0.1% sodium citrate) for 2 min on ice. DNA breaks were labeled by addition of terminal deoxynucleotidyl transferase and nucleotide mixture containing fluorescein isothiocyanate-isothiocyanate-conjugated dUTP, and incubation was continued for 60 min at 37°C. Coverslips were mounted in phosphate-buffered saline–glycerol, and cells were analyzed by fluorescence microscopy. After extensive washes in phosphate-buffered saline, cells were incubated for 30 min at 37°C with an anti-fluorescein isothiocyanate antibody conjugated with alkaline phosphatase.

Chromogenic reaction was carried out by adding alkaline phosphatase substrate solution containing 0.4 mg of nitroblue tetrazolium chloride per ml and 0.2 mg of 5-bromo-4-chloro-3-indolylphosphate toluidine salt (Roche Molecular Biochemicals) per ml in 0.1 M Tris-HCl (pH 9.5)–0.05 M MgCl2–0.1 M NaCl–1 mM levamisole for 10 min at room temperature. Coverslips were mounted in phosphate-buffered saline–glycerol and analyzed by light microscopy. Apoptotic cells were characterized by a dark nuclear precipitate) and nonapoptotic cells (unstained or displaying a diffuse, light, and uneven cytoplasmic staining) were counted in five randomly chosen microscopic fields (containing at least 250 cells). Results are expressed as percent apoptotic cells ± standard error of the mean.

**Caspase activation.** The CaspACE fluorescein isothiocyanate Val-Ala-Asp-fluoromethylketone (VAD-FMK) in situ marker (Promega) was used according to the manufacturer’s instructions. Cells were cultured with the fluorescein isothiocyanate-conjugated permeable irreversible caspase inhibitor VAD-FMK for 20 min to allow binding to activated caspases. Cells were subsequently fixed with 10% buffered formaldehyde (1 h, room temperature) and visualized by fluorescence microscopy.

**Immunofluorescence and immunohistochemistry.** The identity of the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick-end labeling (TUNEL)-positive cells in primary hippocampal cells was determined by double immunofluorescence, as previously described (66). Briefly, the cultures were incubated with terminal deoxynucleotidyltransferase and the nucleotide mixture (containing fluorescein isothiocyanate-conjugated dUTP) for 1 h at 37°C and stained (1 h, room temperature) with TUJ1 antibody followed by phycoerythrin-conjugated anti-mouse immunoglobulin G (30 min, room temperature). Stained cells were visualized with an epifluorescence confocal microscope fitted with an argon ion laser (Zeiss LSM 410).

The Dako LSAB 2 kit horseradish peroxidase (Dako Corporation, Carpinteria, Calif.) was used for immunoperoxidase staining. Cells were exposed overnight (4°C) to primary antibodies (caspase-3 p20, phosphorylated JNK, Flag, or c-Jun (p133kDa), and immunolabeled cells were subsequently detected with the streptavidin-biotin method according to the manufacturer’s instructions. Counterstaining was done with Mayer’s hematoxylin (Sigma) (66, 67). Five randomly
chosen microscopic fields (containing at least 250 cells) were counted, and the percentage of mock-infected positive cells was subtracted from each average. Results are expressed as percent positive cells ± standard error of the mean.

**Immunoblotting.** Immunoblotting was done as previously described (55, 66, 73–76). Briefly, cells were lysed with radioimmunoprecipitation assay (RIPA) buffer (20 mM Tris-HCl [pH 7.4], 0.15 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate) supplemented with phosphatase and protease inhibitor cocktails (Sigma) and sonicated for 30 s at 25% output power with the Sonicator ultrasonic processor (Misonix, Inc., Farmingdale, N.Y.). Total protein was determined by the bicinchoninic assay (Pierce, Rockford, Ill.), and proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The blots were incubated (1 h, 37 °C) in TN-T buffer (0.01 M Tris-HCl [pH 7.4], 0.15 M NaCl, 0.5% Tween 20) containing 1% bovine serum albumin to block nonspecific binding and exposed overnight at 4 °C to the appropriate antibodies diluted in TN-T buffer with 0.1% bovine serum albumin. After three washes with TN-T buffer, the blots were incubated with protein A-peroxidase for 1 h at room temperature. Detection was done with the ECL kit reagents (Amersham Life Science, Arlington Heights, Ill.) and exposure to high-performance chemiluminescence film (Hyperfilm ECL; Amersham). Quantitation was done by densitometric scanning with the Bio-Rad GS-700 imaging densitometer.

**Statistical analyses.** Student’s t test and analysis of variance with the Tukey-Kramer posttest were performed with GraphPad InStat version 3.01 for Windows 95/NT (GraphPad Software, San Diego, Calif.).

**RESULTS**

**HSV-2 but not HSV-1 blocks apoptosis in primary cultures of central nervous system neurons.** We have previously shown that the HSV-2 ICP10 PK blocks apoptosis in hippocampal neurons (66, 67). To determine whether this activity is shared by HSV-1 and extends to other types of neurons, primary hippocampal and cerebral cortical cultures were mock-infected or infected with 10 PFU of HSV-1, HSV-2, ICP10ΔPK, or ICP10ΔRR per cell and assayed by TUNEL [widely accepted to be specific for apoptosis (28, 30)] at 24 h postinfection.

![Image](https://via.placeholder.com/150)

**FIG. 1.** ICP10 PK blocks and HSV-1 triggers apoptosis in central nervous system neurons. (A) Hippocampal cultures were mock infected or infected with HSV-1, HSV-2, ICP10ΔPK, or ICP10ΔRR (multiplicity of infection, 10) and analyzed by TUNEL at 24 h postinfection, as described in Materials and Methods. Results represent the average of three independent experiments and are expressed as mean percent apoptotic cells ± standard error of the mean. The number of apoptotic cells in mock-infected cultures (−10%) was subtracted from each average. *, P < 0.01 versus HSV-2 or ICP10ΔRR; +, P < 0.05 versus HSV-2 by analysis of variance. (B) Cortical cultures were mock infected or infected with HSV-1, HSV-2, ICP10ΔPK, or ICP10ΔRR and analyzed by TUNEL as in A. **, P < 0.05 versus HSV-2 or ICP10ΔRR by analysis of variance). (C and D) Hoechst staining of representative nuclei from HSV-1-infected (C) and HSV-2-infected (D) cultures. (E and F) Hippocampal cultures infected with HSV-1 (E) or HSV-2 (F) were assayed by TUNEL and stained with TUJ1 antibody. Colocalization was seen only for HSV-1.
ICP10PK (61.6 ± 2.5%, respectively) than HSV-2 (5.7 ± 3.2% and 17.9 ± 2%, respectively) or ICP10RR (19.7 ± 2 and 23.5 ± 1.2%, respectively). Hoechst staining of HSV-1-infected cells evidenced nuclear fragmentation characteristic of apoptosis (Fig. 1C) that was similar to that described previously for ICP10PK-infected cultures (Fig. 1D) and was not seen in HSV-2-infected cells (Fig. 1D).

TUNEL-positive cells were neurons, because they stained with the neuron-selective TUJ1 antibody. The TUJ1 signal (phycoerythrin) was found in the cell bodies and projections, while the fluorescein isothiocyanate signal (TUNEL) was primarily nuclear (Fig. 1E). Mock- and HSV-2-infected neurons (TUJ1 positive) were mostly TUNEL negative (Fig. 1F). The data indicate that HSV-1 triggers and ICP10 PK blocks apoptosis in both hippocampal and cerebral cortex neurons that differ in their response to other apoptotic (85) and survival (43) stimuli. By contrast, the percentage of TUNEL-positive cells was higher for ICP10RR than HSV-2 only in hippocampal cultures, suggesting that the contribution of ICP10 RR to HSV-2 ant apoptotic activity (66) is neuron-type specific.

**HSV-1 and ICP10ΔPK activate caspase-3 and induce poly-(ADP-ribose) polymerase cleavage.** Primary hippocampal cultures mock-infected or infected with HSV-1, HSV-2, ICP10ΔPK, or ICP10ΔRR (multiplicity of infection, 10) were reacted with fluorescein isothiocyanate-VAD-FMK, which binds to the active sites of caspases 3, 6, and 7 (12), and examined by fluorescence microscopy. The fluorescein isothiocyanate signal was seen in 40 to 60% of the cells in cultures infected with HSV-1 (Fig. 2C) or ICP10ΔPK (Fig. 2D), compared to 10 to 20% of the cells in mock-infected cultures (data not shown) or cultures infected with HSV-2 (Fig. 2A) or ICP10ΔRR (Fig. 2B). We conclude that the higher percentage of caspase-3 cleaved poly(ADP-ribose) polymerase (p85PARP)-positive cells was similar for HSV-1 (33.8 ± 2.2%) and ICP10ΔPK (39.5 ± 2.5%) than HSV-2- (9.9 ± 0.2%), ICP10ΔRR- (7.4 ± 0.9%), or mock-infected cells (4.2 ± 1.3%) (P < 0.001 by analysis of variance) (Fig. 3E). As reported previously (17), p85PARP staining was primarily but not exclusively intranuclear for both HSV-1 (Fig. 3A) and ICP10ΔPK (Fig. 3B). HSV-2- (Fig. 3C), ICP10ΔRR- (Fig. 3D), and HSV-2(R)- (data not shown) infected cultures were essentially negative. The percentage of p85PARP-positive cells was similar for HSV-1 (33.8 ± 2.2%), ICP6a (44.8 ± 9.1%), and hrR3 (36.8 ± 2.7%) (Fig. 3E), indicating that ICP6, the HSV-1 homologue of ICP10, is not involved in apoptosis modulation.

**Ectopically delivered ICP10 PK protects from HSV-1- or ICP10ΔPK-induced apoptosis.** To examine whether ICP10 PK overrides the apoptotic signal induced by HSV-1 or ICP10ΔPK in the absence of any other HSV-2 proteins, hippocampal cultures were transfected with the expression vectors for ICP10 (pJW17) or its PK-negative mutant p139 (pJHL15) and infected with HSV-1 or ICP10ΔPK (multiplicity of infection, 10) at 48 h after transfection to allow transgene expression. They were stained with p85PARP antibody 24 h later (72 h after transfection), and the percentage of staining cells was calculated as described in Materials and Methods. Cultures similarly
transfected with pJW17 or pJHL15 were mock-infected with growth medium and studied in parallel.

Uninfected cultures were essentially negative, whether untransfected (4.2 ± 1.3%) or transfected with pJW17 (1.4 ± 0.4%) or pJHL15 (2 ± 0.4%) (P > 0.05 by analysis of variance). HSV-1 infection increased the percentage of p85PARP-positive cells in untransfected cultures (33.8 ± 2.2%) and in cultures transfected with pJHL15 (35.9 ± 3.5%) but not with pJW17 (5.1 ± 2.4%) (P < 0.001 by analysis of variance). Similar results were obtained for ICP10 PK (Fig. 4) and by TUNEL (data not shown). The data indicate that ICP10 PK protects from apoptosis induced by HSV-1 or ICP10 PK by a kinase-dependent mechanism which does not require other HSV-2 proteins. Significantly, however, the percentage of cells staining with ICP10 antibody (28 to 35% for both pJW17 and pJHL15) was approximately threefold lower than the reduction (approximately 85%) in apoptotic cells seen in pJW17-transfected cultures. Because postmitotic neurons in primary culture extend processes, form synapses with one another, are electrically active, and secrete and respond to neurotransmitters (5, 13, 44, 62, 65), the data suggest that ICP10 PK-transfected neurons synthesize and secrete neurotrophins and/or form synapses that promote the survival of surrounding neurons.

HSV-2 antiapoptotic activity is Raf dependent and involves activation of MEK/ERK. Two series of experiments were done to further investigate factors involved in the ability of ICP10 PK to block apoptosis. First, we took advantage of previous findings that the Flag-Raf1 K375M protein is a dominant negative mutant that inhibits the kinase activity of its endogenous c-Raf-1 counterpart (18, 78) in order to examine whether antiapoptotic activity is Raf dependent. Primary hippocampal cultures were transfected or not with the Flag-Raf1 K375M expression vector. Twenty-four hours later, duplicate cultures were examined for transgene expression by staining with Flag antibody or infected with HSV-2 (multiplicity of infection, 10) and examined by TUNEL at 24 h after virus infection (48 h posttransfection). Cultures transfected or not with Flag-Raf1 K375M and mock-infected with growth medium served as the control.

The percentage of TUNEL-positive (apoptotic) cells in untransfected cultures infected with HSV-2 (11.8 ± 3.2%) was similar to that seen in untreated cultures (7.1 ± 2.6%). It was significantly (P < 0.01 by analysis of variance) increased in HSV-2-infected cells that had been transfected with Flag-Raf1 K375M (49.0 ± 4.3), but not in similarly transfected mock-infected cells (14.5 ± 3.5%), although the same proportion of cells stained with Flag antibody (36 ± 2.2% and 33.7 ± 3.6%, respectively) (Fig. 5A). Ongoing studies indicate that c-Raf-1 kinase activity is inhibited by Flag-Raf1 K375M transfection and by treatment with the pharmacologic inhibitor of c-Raf-1 kinase (c-Raf-1 kinase inhibitor I), which also causes a significant increase in the percentage of TUNEL-positive cells in HSV-2- but not ICP10ΔPK-infected cultures (unpublished data).

The data suggest that Raf kinase is involved in HSV-2 antiapoptotic activity is Raf dependent and involves activation of MEK/ERK. Two series of experiments were done to further investigate factors involved in the ability of ICP10 PK to block apoptosis. First, we took advantage of previous
with Flag antibody presumably re-
percentage of neurons undergoing apoptosis than that staining
kinase activity is increased by HSV-2 infection. The higher
(ICP10 PK) antiapoptotic activity, but it is still unclear whether
expression.

In a second series of experiments, we asked whether the
antiapoptotic activity of ICP10 PK is MEK/ERK dependent. Hip-
campal cultures were mock-infected with growth me-
dium or infected with 10 PFU of HSV-2 per cell in the absence
of presence of 20 μM U0126, a MEK-specific inhibitor (22),
and examined for ERK activation (phosphorylation) at 0.5 h
postinfection and by TUNEL at 24 h postinfection, as previ-
sely described (66). Cultures infected with ICP10ΔPK or
HSV-1 (10 PFU/cell) were studied in parallel. Relative to
mock-infected cultures (Fig. 5B, lane 1), the levels of phos-
phorylated (activated) ERK (P-ERK1/2) were signi-
ificantly increased in cultures infected with HSV-2 (Fig. 5B, lane 2) but
not HSV-2 with U0126 (Fig. 5B, lane 3), HSV-1 (Fig. 5B, lane 4), or ICP10ΔPK (Fig. 5B, lane 5). U0126 treatment caused a
significant increase in the percentage of TUNEL-positive cells
in HSV-2 but not mock-infected cultures (9 ± 1% and 49 ± 3.8%, respectively) (Fig. 5C). Collectively, the data confirm
and extend previous findings (66) that activation of the ERK
survival pathway is required for HSV-2 (ICP10 PK) antiapo-
ptotic activity but not for the survival of uninfected hip-
campal neurons.

**ERK survival pathway activated by ICP10 PK induces Bag-1 expression.** The exact relationship between activated survival
pathways and antiapoptotic Bcl-2 family proteins is still poorly
understood. To determine whether activation of the ERK
pathway results in increased expression of antiapoptotic pro-
teins, we focused on Bag-1, which has neuroprotective activity
and inhibits apoptosis by itself or by cooperating with the
antiapoptotic protein Bel-2 (9, 72, 79). Bag-1 also binds c-Raf-1
and activates its kinase activity (82), thereby providing a po-
tential positive feedback loop for the ERK survival pathway.

Hippocampal cultures were mock-infected or infected with
10 PFU of HSV-1, HSV-2, ICP10ΔPK, or ICP10ΔARR per cell and
examined for Bag-1 expression by immunoblotting with
specific antibody at 0.5, 6, and 24 h postinfection. These time
points were selected because ERK is activated within 30 min
postinfection with HSV-2, but TUNEL-positive cells are seen
at 12 to 24 h postinfection. Bag-1 was not seen in mock-
infected cultures (Fig. 6A, lane 1) or at 0.5 h postinfection (Fig.
6A, lanes 2 to 5). At 6 and 24 h postinfection, Bag-1 was seen
in cultures infected with HSV-2 (Fig. 6A, lanes 6 and 10) or
ICP10ΔRR (Fig. 6A, lanes 8 and 12) but not ICP10ΔPK (Fig.
6A, lanes 7 and 11) or HSV-1 (Fig. 6A, lanes 9 and 13),
indicating that it is induced by ICP10 PK. We conclude that
Bag-1 is upregulated by the activated ERK pathway, because
its levels were not increased in cultures infected with HSV-2 in
the presence of the MEK-specific inhibitor U0126 (20 μM)
(Fig. 6B, lane 3) or the c-Raf-1 kinase-specific inhibitor c-Raf
kinase inhibitor I (50 μM) (Fig. 6B, lane 4). Actin levels were
identical in all cultures (Fig. 6).

**Ectopically expressed Bag-1 inhibits HSV-1- and ICP10ΔPK-
induced apoptosis.** To test the hypothesis that Bag-1 is
involved in the ability of ICP10 PK to inhibit apoptosis caused by
HSV-1 or ICP10ΔPK, hippocampal cultures were transfected
or not with an expression vector for Bag-1 (pJG4-5m Bag-1) and
mock-infected or infected with 10 PFU of HSV-2, HSV-1,
or ICP10ΔPK per cell 24 h later. Cultures were examined by
TUNEL at 24 h after virus infection (48 h postransfection),
and the percentage of positive cells was determined as de-
scribed in Materials and Methods.

Transfection did not modify the percentage of TUNEL-
positive cells in uninfected cultures (5.8 ± 2.4% and 7.1 ± 2.5%
for transfected and untransfected cells, respectively) or
HSV-2-infected cultures (9.2 ± 1.0% and 9.3 ± 3.3% for
transfected and untransfected cells, respectively). However,
the percentage of TUNEL-positive cells in cultures infected
with ICP10ΔPK (58.2 ± 3.8%) or HSV-1 (55.5 ± 3.6%) was
significantly reduced by Bag-1 transfection (11.8 ± 2.5% and
8.1 ± 1.8% for ICP10ΔPK and HSV-1, respectively) (P <
0.001 by analysis of variance) (Fig. 7), and similar results
were obtained by staining with p85PARP antibody (data not shown).
The data indicate that Bag-1 is involved in the antiapoptotic
activity of ICP10 PK. Furthermore, as with ICP10 PK, Bag-1
transfection had a bystander trophic effect exemplified by the
higher reduction in TUNEL-positive cells (approximately
80%) than Bag-1 staining cells (38 to 45%).

**ICP10 PK increases Bel-2 stability and inhibits BAD expres-
sion.** Having seen that ICP10 PK induces Bag-1 expression, we
wanted to know whether it also modulates Bel-2, which inter-
acts with Bag-1 (79), is associated with neuroprotection (39),
and is proteolytically inactivated by other apoptosis-inducing
viruses (33). Hippocampal cultures were mock-infected or
infected with 10 PFU of HSV-2, or HSV-1 per cell and immu-
noblotted with Bel-2 antibody at 0.5 and 24 h postinfection.

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**FIG. 4.** Ectopically delivered ICP10 PK protects from ICP10ΔPK-
or HSV-1-triggered apoptosis. Primary hippocampal cultures were
transfected with pJW17 or pH1L15, expressing ICP10 and its PK-
negative mutant p139, respectively, and mock infected or infected with
HSV-1 or ICP10ΔPK (multiplicity of infection, 10) at 48 h after trans-
fection. Twenty four hours later, cultures were stained with p85PARP
antibody. Results (average of three independent experiments) are per-
cent p85PARP-positive cells ± standard error of the mean. *, P <
0.001 versus mock-infected untransfected, mock-infected pJW17-
transfected, ICP10ΔPK-infected pJW17-transfected, and HSV-1-in-
fected pJW17-transfected cultures by analysis of variance.
Bcl-2 levels were essentially similar in mock- (Fig. 8A, lane 1) and HSV-2-infected cultures at 0.5 h postinfection (Fig. 8A, lane 2) and 24 h postinfection (Fig. 8A, lane 4). They were slightly lower in HSV-1-infected cultures at 0.5 h postinfection (Fig. 8A, lane 3) and no longer visible at 24 h postinfection (Fig. 8A, lane 5). Similar results were obtained for ICP10/H9004PK (data not shown), but actin levels were identical in all samples.

By contrast to Bcl-2, the proapoptotic protein BAD, which is associated with glutamate-induced neuronal apoptosis and neurodegenerative disorders (50, 84, 88), was not expressed in mock-infected cells (Fig. 8B, lane 1). Its expression was rapidly induced (within 30 min) by infection with HSV-1 (Fig. 8B, lane 3) or ICP10/H9004PK (Fig. 8B, lane 4) and was no longer detected at 24 h postinfection (Fig. 8B, lane 5). BAD expression was not induced by HSV-2 (Fig. 8B, lane 2) or ICP10/H9004RR (Fig. 8B, lane 5) at any time postinfection (0.5 to 24 h). We interpret the data to indicate that Bcl-2 is stabilized in HSV-2- but not HSV-1- (or ICP10/H9004PK)-infected cells and that ICP10 PK blocks BAD upregulation.

ICP10 PK activates or stabilizes CREB and blocks ATF-2 activation. Activated ERK promotes cell survival by transcription-dependent (induction of survival genes) or -independent (Bcl-2 phosphorylation [10]) mechanisms. Because ICP10 PK induces Bag-1 expression by an ERK-dependent mechanism,
we reasoned that its ability to block apoptosis is primarily transcription dependent. Two series of experiments were done to begin testing this interpretation. In the first series, we focused on CREB, which is the primary Bcl-2 transcriptional activator (70) and is involved in neurotrophin-mediated gene transcription associated with neuronal survival (24). Hippocampal cultures were mock-infected or infected with 10 PFU of HSV-2, ICP10ΔPK, or HSV-1 per cell 24 h after infection. They were assayed by TUNEL at 24 h after infection. Nontransfected but similarly infected cultures were studied in parallel. Results (average of three independent experiments) are expressed as percent apoptotic cells ± standard error of the mean. *, P < 0.001 versus transfected and infected (mock, HSV-1, HSV-2, and ICP10ΔPK) and versus nontransfected and infected (mock and HSV-2) by analysis of variance.

Phosphorylated CREB was seen in mock-infected cultures (Fig. 9A, lane 1), but its levels were significantly increased in HSV-2-infected cultures at 4 h (Fig. 9A, lane 2) and 8 h (Fig. 9A, lane 4) postinfection, and they were still somewhat increased at 24 h postinfection (Fig. 9A, lane 6). Phosphorylated CREB levels were not increased in ICP10ΔPK-infected cultures at 4 and 8 h postinfection (Fig. 9A, lanes 3 and 5), and they were significantly decreased at 24 h postinfection (Fig. 9A, lane 7). This decrease was accompanied by the appearance of a 30-kDa nonphosphorylated species (p30) (Fig. 9A, lane 7), which is consistent with a previously reported fragment generated by caspase 3-mediated cleavage (25).

In a second series of experiments to examine the effect of infection on transcription factors, we asked whether ATF-2 (also known as CRE-BP) is activated (phosphorylated on Thr\textsuperscript{69/71}) in virus-infected hippocampal cultures. We focused on ATF-2 because it is activated by the JNK apoptotic pathway in response to stress stimuli (34). Extracts of hippocampal cultures, mock-infected or infected with HSV-2 or ICP10ΔPK (multiplicity of infection, 10) for 0.5, 8, or 24 h, were immunoblotted with antibody to phosphorylated ATF-2. Consistent with previous reports (40), a major 72- to 74-kDa band and a smaller 67- to 68-kDa band were seen in mock-infected cultures (Fig. 9B, lane 1). Their levels decreased in cultures infected with HSV-2 (Fig. 9B, lanes 2, 4, and 6) and increased in those infected with ICP10ΔPK (Fig. 9B, lanes 3 and 5), in which a 60-kDa band was also evident. Phosphorylated ATF-2 was no longer detected at 24 h postinfection with either virus (Fig. 9B, lanes 6 and 7), and actin levels were similar in all samples (Fig. 9B). Collectively, the data indicate that ICP10 PK activates and stabilizes CREB while it inhibits ATF-2 activation. The role of signaling pathways in these alterations remains to be documented.

ICP10ΔPK and HSV-1 activate the JNK/c-Jun pathway in hippocampal cultures. HSV-1 was previously shown to activate JNK/c-Jun in nonneuronal cells (57, 90, 91). Having seen that HSV-1 and ICP10ΔPK upregulate BAD and activate ATF-2, both of which are targets of the apoptotic JNK/c-Jun pathway (11, 12, 34, 81), we wanted to know whether JNK/c-Jun activation is associated with apoptosis triggered by these viruses (i.e., in the absence of ICP10 PK). Two series of experiments...
were done. First, hippocampal cultures were mock infected or infected with 10 PFU of HSV-1 or HSV-2 per cell and examined for JNK activation at 24 h postinfection by immunoblotting with phosphorylated JNK antibody (recognizes phosphorylated JNK1/2/3). Antibody to nonphosphorylated JNK1/2/3 was used as a control.

JNK1 was the only isotype expressed in mock-infected cultures (Fig. 10A, lane 1). In most experiments, it was not phosphorylated (Fig. 10A, lane 1), but minimal (barely detectable) levels of phosphorylated JNK1 were observed in rare experiments (data not shown). Both HSV-2 (Fig. 10A, lane 2) and HSV-1 (Fig. 10A, lane 3) induced expression of JNK2/3. However, while the levels of phosphorylated JNK1/2 in HSV-2-infected cultures were low (Fig. 10A, lane 2), JNK1 and -2 were intensely phosphorylated (activated) in HSV-1-infected cultures (Fig. 10A, lane 3). Actin levels were virtually identical in all extracts (Fig. 10A), indicating that these results are not an artifact due to improper gel loading or other technical problems. The percentage of cells staining with phosphorylated JNK antibody was also significantly ($P < 0.05$ by analysis of variance) higher for HSV-1- than mock- or HSV-2-infected cultures as early as 0.5 h postinfection (Fig. 10B).

In a second series of experiments, hippocampal cultures were mock infected or infected (24 h, 10 PFU/cell) with HSV-1, HSV-2, ICP10△PK, or ICP10△RR and assayed for c-Jun activation by immunoblotting with antibody to c-Jun phosphorylated on Ser63 [P-Jun(Ser63)] or Ser73 [P-Jun(Ser73)], both of which are phosphorylated by phosphorylated JNK (59). Antibody to nonphosphorylated c-Jun was studied in parallel and served as a control. The levels of P-Jun(Ser63) and P-Jun(Ser73) were significantly increased in HSV-1-infected (Fig. 10C, lane 5) and ICP10△PK-infected (Fig. 10C, lane 3) relative to mock-infected (Fig. 10C, lane 1) cultures. They were minimally increased in HSV-2-infected (Fig. 10C, lane 2) or ICP10△RR-infected (Fig. 10C, lane 4) cultures. Actin levels were virtually identical in all cultures.

The levels of nonphosphorylated c-Jun were also increased in HSV-1- and ICP10△PK- but not HSV-2- or ICP10△RR-infected cultures (Fig. 10C), consistent with previous findings that c-Jun is subject to positive autoregulation (19, 59). P-Jun(Ser63) and P-Jun(Ser73) levels were similar for HSV-1, ICP6α, and hrR3 (Fig. 10D), suggesting that ICP6 is not involved in c-Jun activation.

**Activation of JNK/c-Jun pathway is required for HSV-1-induced apoptosis.** To determine the relationship between HSV-1-induced JNK activation and apoptosis, duplicates of hippocampal cultures were mock infected or infected with 10 PFU of HSV-1 per cell in the absence or presence of 10 μM SP600125, the JNK inhibitor (7, 36). They were examined for (i) c-Jun activation by immunoblotting with P-Jun(Ser63) and P-Jun(Ser73) antibodies and (ii) TUNEL reaction at 24 h postinfection The levels of P-Jun(Ser63) and P-Jun(Ser73) were significantly higher in HSV-1-infected (Fig. 11A, lane 2) than mock-infected (Fig. 11A, lane 1) cells. The levels of P-Jun in cells infected with HSV-1 in the presence of SP600125 (Fig. 11A, lane 3) were similar to those in mock-infected cells, supporting the conclusion that SP600125 is a JNK inhibitor.

We conclude that JNK activation is required for HSV-1-induced apoptosis because the percentage of TUNEL-positive cells was significantly ($P < 0.001$ by analysis of variance) decreased in cultures infected with HSV-1 in the presence of SP600125 (7.3 ± 1.4% and 43.6 ± 5.8% for SP600125-treated and untreated cultures, respectively), while it was not altered in mock-infected cultures (3.8 ± 1.5% and 6 ± 1% for SP600125-treated and untreated cultures, respectively) (Fig. 11B).

**DISCUSSION**

Cell type-specific antiapoptotic activity has been attributed to various HSV-1 genes, most of which function by a still poorly understood mechanism (2, 15, 38, 47). However, to the extent of our knowledge, ICP10 PK is the first viral gene shown to inhibit apoptosis by activating the ERK survival pathway.

![Figure 9](http://jvi.asm.org/content/130/4/1300/F9.large.jpg)
The following comments seem pertinent with respect to these findings.

We used previously established conditions to examine the mechanism responsible for the ability of ICP10 PK to block apoptosis in central nervous system neurons and compare the results to those obtained for HSV-1. These included HSV-2 mutants deleted in the PK (ICP10ΔPK) or RR (ICP10ΔRR) domains of ICP10, transfection with expression vectors for ICP10 (pJW17) or its PK-negative mutant p139 (pJHL15) (3, 55, 66–68, 73–76), and primary hippocampal cultures that contained >88% postmitotic neurons, which have definite exons and dendrites, form synapses, are electrically active, and closely resemble neurons in vivo (5). Apoptosis was determined by TUNEL, morphological alterations, caspase activation, and poly(ADP-ribose) polymerase cleavage, a crucial factor in the cell’s commitment to undergo apoptosis (48), with a good degree of correlation between the various assays. The specificity of the antibodies used in these studies was previously confirmed in our and other laboratories (3, 16, 55, 63, 66–68, 73–76). Normal rabbit serum was negative in all assays.

Apoptosis was seen in hippocampal and cortical neurons infected with HSV-1 or ICP10ΔPK but not with HSV-2, ICP10ΔRR, or HSV-2(R), supporting previous conclusions that ICP10 PK blocks apoptosis in hippocampal neurons (66, 67) and extending them to indicate that HSV-1 triggers apoptosis in these cells. Studies of ectopically delivered ICP10 PK indicated that its ability to block apoptosis is independent of other HSV-2 proteins, is kinase dependent, and overrides apoptosis triggered by HSV-1 or ICP10ΔPK. The percentage of apoptotic cells in HSV-2-infected cultures was significantly increased by transfection with the dominant negative Flag-c-Raf-1 mutant K375M, which inhibits c-Raf-1 kinase activity (18, 78), or by treatment with a pharmacologic inhibitor of c-Raf kinase, suggesting that Raf kinase is involved in antiapoptotic activity.

We conclude that ERK activation is causally related to Bag-1 induction and apoptosis inhibition because (i) ERK was activated and Bag-1 was induced by the antiapoptotic viruses HSV-2 and ICP10ΔRR but not the proapoptotic viruses ICP10ΔPK and HSV-1, (ii) ERK activation Bag-1 induction, and ICP10 PK antiapoptotic activity were abrogated by the MEK-specific pharmacologic inhibitor U0126, and (iii) apoptosis induced by ICP10ΔPK or HSV-1 was blocked by ectopic delivery of ICP10 PK or Bag-1. Bcl-2, which cooperates with Bag-1 to interfere with the apoptotic cascade (39, 72, 79, 82), is also likely to contribute to the antiapoptotic activity of ICP10 PK. Indeed, Bcl-2 was stabilized in HSV-2- and ICP10ΔRR-infected cells, while its expression was decreased in cultures infected with HSV-1 or ICP10ΔPK as early as 0.5 h postinfection, and it was no longer detected at 24 h postinfection.

![JNK and c-Jun activation](http://jvi.asm.org/content/83/16/1301/F10.large.jpg)
FIG. 11. HSV-1-mediated apoptosis depends on JNK/c-Jun activation. (A) Hippocampal cultures were mock infected (lane 1) or infected with 10 PFU of HSV-1 per cell in the absence (lane 2) or presence (lane 3) of 10 μM SP600125, the JNK inhibitor. Cell extracts obtained at 24 h postinfection were immunoblotted with antibodies specific for P-Jun(Ser73), followed by P-Jun(Ser63), c-Jun, and actin. (B) Duplicates of the cultures in A were assayed by TUNEL. The results of three independent experiments are expressed as mean TUNEL-positive cells ± standard error of the mean.

may reflect protein cleavage by caspases or other proteases (21, 33) or proteasome-dependent degradation and may be due to activation of the proapoptotic JNK/c-Jun pathway (91). We favor the interpretation that in HSV-2- and ICP10ΔRR-infected cells, Bcl-2 is stabilized by interacting with Bag-1 (79). However, the contribution of other factors, such as c-Raf-1-mediated phosphorylation (9, 21, 83), cannot be excluded.

Significantly, protection by transfection with pJW17 (ICP10) or pJG4-5m (Bag-1) was approximately two- to threefold higher than the percentage of transfected cells, suggesting that neurons rescued from apoptosis by ICP10 PK or Bag-1 produce trophic factors and/or form synapses that stimulate the survival and adaptive responses of surrounding neurons. Such a bystander effect is consistent with previous reports that (i) hippocampal neurons secrete and respond to nerve growth factor (5, 13), (ii) ICP10 PK protects hippocampal neurons from apoptosis caused by nerve growth factor withdrawal (67), and (iii) the levels of apoptosis induced by the dominant negative c-Raf-1 mutant K375M were higher than predicted by the transfection efficiency, presumably reflecting previous documented findings that apoptotic neurons release glutamate, which in turn triggers apoptosis in surrounding neurons (44, 62, 65). Ongoing studies are designed to test the validity of these interpretations and further define the role of Raf kinase in apoptosis inhibition, with emphasis on the contribution of neuronal cell receptors and survival pathways in uninfected neurons.

Also under investigation is the antiapoptotic activity of ICP10 PK in stably transfected neural cell lines similar to our previously established nonneuronal lines (73, 76). However, neural cell lines do not reflect the properties of neurons in vivo (5) and provide potentially misleading information, as suggested by recent findings that Bcl-2 overexpression is neuroprotective in such lines but not in primary cultures of postmitotic neurons (14, 46). This underscores the significance of the finding that ICP10 PK blocks apoptosis in postmitotic neurons and its promise as a therapeutic agent for neurological disorders with an apoptotic component (42).

The ERK pathway imparts survival by transcription-dependent and/or -independent mechanisms (11, 87, 92). We reason that ICP10 PK functions primarily by a transcription-dependent mechanism, because it activates MEK/ERK, which are required for Bag-1 induction and are probably also responsible for increased CREB activation in HSV-2-infected cultures. Presumably, the low levels of phosphorylated CREB in mock-infected cultures reflect CREB activation by the phosphatidylinositol 3-kinase/Akt pathway, which is required for basal maintenance of hippocampal neurons (49, 66) and is associated with neuronal cell survival (24). Indeed, CREB plays a key role in neurotrophin-dependent survival, including Bcl-2 transcriptional activation (10, 49, 71), and it is activated by both the ERK and phosphatidylinositol 3-kinase/Akt pathways (89). Because CREB has antiapoptotic activity even when it is not phosphorylated, presumably by competing with c-Jun for alkaline phosphatase-1 binding sites on target genes (25, 56), its apparent cleavage in ICP10ΔPK-infected cultures is likely to contribute to apoptosis induction. However, the exact role played by CREB in apoptosis inhibition and its relationship to Bag-1, if any, are still unclear.

Previous studies of nonneuronal cells showed that HSV-1 activates JNK/c-Jun and that this is required for virus replication (57, 90, 91). We found that HSV-1 also activates JNK in hippocampal neurons, beginning at 0.5 h postinfection. However, this is unrelated to virus replication, since JNK was activated equally well by ICP10ΔPK, which does not replicate in these cells (66). JNK activation by HSV-1 and ICP10ΔPK (but not HSV-2 or ICP10ΔRR) was accompanied by c-Jun phosphorylation at Ser^63 and Ser^73, conditions that increase its ability to activate the transcription of target genes, including c-Jun itself (6, 59). We conclude that activation of the JNK/c-Jun pathway is involved in HSV-1 (and ICP10ΔPK)-induced
apoptosis, because both Jun phosphorylation and TUNEL were inhibited with the JNK-specific inhibitor SP600125.

The transcription factor ATF-2, which dimerizes and cooperates with c-Jun to induce neuronal cell apoptosis (19, 34, 35, 56, 60, 81), and the antiapoptotic protein BAD also seem to be targets of the JNK/c-Jun pathway activated by HSV-1 and ICP10ΔPK. The role of ATF-2 in HSV-1 (and ICP10ΔPK)-induced apoptosis is still unclear, but our findings for BAD are consistent with a previous report that it is phosphorylated by the HSV-1 US3 protein kinase and is involved in HSV-1-induced apoptosis of nonneuronal cells (61). In hippocampal neurons, BAD expression was induced by HSV-1 or ICP10ΔPK (but not HSV-2 or ICP10ΔRR), suggesting that US3 does not have antiapoptotic activity in these cells. It remains to be shown that BAD expression is also induced by a JNK/c-Jun-dependent mechanism (11, 12, 49) in our system. Notwithstanding, the absence of Bag-1 and the inhibition/degradation of Bcl-2 in HSV-1- and ICP10ΔPK-infected cultures shifts the balance between the anti- and proapoptotic functions in favor of apoptosis.

The finding that only 40 to 60% of the cells in the HSV-1- or ICP10ΔPK-infected cultures undergo apoptosis is difficult to reconcile with the high multiplicity of infection used in these experiments, which ensures that all cells are infected. It may reflect the contribution of cellular proteins that differ in their function as JNK/c-Jun targets, such as the Fas ligand (60), death cytokine tumor necrosis factor alpha (41, 45), and transcription factors p53 and c-Myc, the phosphorylation of which (by JNK) induces their proapoptotic function (26, 58, 64). Notwithstanding, the data indicate that ICP10 PK activates the ERK survival pathway, which overrides the apoptotic JNK/c-Jun signal (53, 80), apparently triggered by proteins shared by the two HSV serotypes.

Collectively, the data are consistent with the model schematically represented in Fig. 12. It posits rapid activation of the apoptotic JNK/c-Jun pathway in central nervous system neurons infected with HSV-2 or HSV-1. Dotted lines represent potentially involved factors. pi, postinfection.

FIG. 12. Schematic representation of apoptosis modulation in central nervous system neurons infected with HSV-2 or HSV-1. Dotted lines represent potentially involved factors. pi, postinfection.
when extrapolating our findings to apoptotic cell death in HSV-1- or HSV-2-infected neurons of the peripheral nervous system.

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