Herpes Simplex Virus Type 1 Infection Stimulates p38/c-Jun N-terminal Mitogen-activated Protein Kinase Pathways and Activates Transcription Factor AP-1*

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Eukaryotic cells respond to environmental stress and proinflammatory cytokines by stimulating the Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and the p38 mitogen-activated protein kinase cascades. Infection of eukaryotic cells with herpes simplex virus type 1 (HSV-1) resulted in stimulation of both JNK/SAPK and p38 mitogen-activated protein kinase after 3 h of infection, and activity reached a maximum of 4-fold by 9 h post-infection. By using a series of mutant viruses, we showed that the virion transactivator protein VP16 stimulates p38/JNK, whereas no immediate-early, early, or late viral expressed gene is involved. We identified the stress-activated protein kinase kinase 1 as an upstream activator of p38/JNK, and we demonstrated that activation of AP-1 binding proceeded p38/JNK stimulation. During infection, the activated AP-1 consisted mainly of JunB and JunD with a simultaneous decrease in the cellular levels of Jun protein. We suggest that activation of the stress pathways by HSV-1 infection either represents a cascade triggered by the virus to facilitate the lytic cycle or a defense mechanism of the host cell against virus invasion.

Eukaryotic cells respond to extracellular stimuli by recruiting signal transduction pathways, many of which are mediated through activation of distinct mitogen-activated protein (MAP) kinase cascades. The best characterized of these are the extracellular signal regulated kinase (ERK), the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), and the p38 MAP kinase pathways (1, 2). Most proliferative stimuli activate the ERK pathway, primarily through the small GTP-binding protein Ras. Active Ras recruits to the plasma membrane and activates the MAP kinase kinase kinase Raf-1. Active Raf-1 phosphorylates the MAP/ERK kinases (MEKs) 1 and 2, which in turn stimulate the respective ERKs (3, 4). In a similar fashion, UV irradiation, environmental stress, and proinflammatory cytokines stimulate the JNK/SAPK and the p38 MAP kinase (p38 MAPK) cascades. These kinases have been shown to phosphorylate a number of transcription factors (5–7), including c-Jun (specifically phosphorylated by JNK/SAPK) and ATF-2 (phosphorylated by both JNK/SAPK and p38 MAPK). Although there is coordinate regulation of JNK and p38, they have distinct upstream activators as follows: p38 is activated by MAP kinase kinases (MKKs) 3 and 6, whereas MKK 4 (SKK 1) and MKK 7 (SKK 4) activate both JNK and p38 (8, 9).

Two of the major classes of control elements that contribute to transcriptional regulation of cellular genes by extracellular signals are the activator protein 1 (AP-1)-binding site, also known as the phorbol 12-O-tetradecanoate-13-acetate response element (AP-1/TRE), and the activating transcription factor (ATF)-binding site, also known as the cAMP response element (ATF/CRE) (10, 11). The AP-1/TRE is recognized by a group of proteins encoded by the c-jun and c-fos gene families. TRE-binding proteins form homodimers or heterodimers (12, 13), which are induced by mitogenic stimuli, stress- and virus-induced alterations (14, 15). Dimerization partners are affected by signal-regulated protein kinases and alterations in the composition of the AP-1 complex result in differential binding, growth, and oncogenic potential of AP-1 (12, 13, 16, 17). The ATF/CRE site is recognized by the ATF/CRE-binding (CREB) proteins and is implicated in cAMP-, calcium-, and virus-induced alterations (11). TRE- and CRE-binding proteins preferentially recognize the corresponding sequences; however, cross-family dimerization has been reported (18).

Herpes viruses are ubiquitous eukaryotic pathogens that possess a common basic structure consisting of an icosahedral nucleocapsid containing a linear, double-stranded DNA genome, surrounded by a proteinaceous tegument and a membranous envelope. Herpes simplex virus type 1 (HSV-1) is the best characterized member of the family, and during the lytic cycle, viral gene expression can be divided into three temporal stages, based upon the appearance of the gene products (for review see Ref. 19). Immediate-early (IE) genes are transcribed in the absence of de novo protein synthesis, and their products act to orchestrate the expression of the early and late genes. Transcription of the five viral IE genes is initiated by the virion tegument protein VP16 (Vmw65) through formation of a multiprotein-DNA complex on viral promoters that includes the pre-existing cellular proteins Oct-1 and host cell factor (HCF) (Ref. 20 and for review see Ref. 21).

Four of the five IE gene products (Vmw175, Vmw63, Vmw110, and Vmw68) are phosphorylated nucleoproteins with regulatory activities that prime the cell for efficient HSV-1
infection and control the expression of viral early and late genes. Proteins Vmw175 and Vmw63 are absolutely essential for lytic virus replication, whereas Vmw110 is not essential but it is important for the efficient entry into the lytic phase of infection and interacts with a number of cellular components (22–25). Vmw68 plays roles in the efficient late gene expression and phosphorylation of the cellular RNA polymerase II (26, 27).

The fifth IE gene product (Vmw12) is a cytoplasmic protein postulated to assist virus in avoiding immune detection (28). An additional HSV gene, UL39, that encodes the large subunit (R1) of the viral ribonucleotide reductase is expressed during IE and early times, with a concomitant rise in AP-1 binding stress pathways by viral protein VP16 during virus immediate-early and early phases of viral invasion. Our infection and a detailed analysis of the host cell response during virus immediate-early times, but its function at this stage of infection is not yet understood (29, 30). Expression of early gene products occurs approximately 4–5 h postinfection, and they are mostly virally encoded enzymes involved in DNA synthesis and replication. Efficient expression of late genes that encode mostly structural proteins commences approximately 6–7 h postinfection, and their full expression requires viral DNA synthesis.

Recently, much attention has focused on the potential use of HSV-1 as a gene delivery vector for the nervous system and other tissues (31). Production of effective vectors requires a full understanding of the biological mechanisms activated by viral infection and a detailed analysis of the host cell response during the immediate-early and early phases of viral invasion. Our study shows stimulation of the JNK/SAPK and p38 MAPK stress pathways by viral protein VP16 during virus immediate-early and early times, with a concomitant rise in AP-1 binding activity.

**EXPERIMENTAL PROCEDURES**

**Cells and Viruses**—Baby hamster kidney (BHK) cells were grown in BHK medium (Life Technologies, Inc.) supplemented with 10% newborn calf serum. Chinese hamster ovary and HeLa cells were grown in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum. Cells were grown as monolayers, at 37 °C, in an atmosphere of 5% CO2.

Virus d403 (22) and D30EBA (32) that fail to express Vmw110 and Vmw175, respectively, were kindly provided by Dr. R. E. Everett, Institute of Virology, University of Glasgow, Glasgow, UK. Virus IC6delta (33), which fails to express the R1 subunit of the viral ribonucleotide reductase, was a gift from Professor S. Weller, University of Connecticut Health Center, Farmington, CT. The HSV-1 temperature-sensitive mutant ts1 expresses an inactive form of Vmw175 at the non-permissive temperature of 38.5 °C (34). In the 27laZ virus, the gene encoding Vmw63 is inactivated by insertion of a lacZ cassette (35). Virus in1814 expresses an inactive form of virion protein VP16 which is defective in interaction with cellular factors Oct-1 and HCF and was a gift from Dr. C. M. Preston, Institute of Virology, University of Glasgow, Glasgow, UK (36).

**Proteins, Antibodies, and Plasmids**—Escherichia coli for expression of a GST/Jun(1–79) fusion protein and a JNK/SAPK immunoprecipitating antisera were provided by Dr. D. Gillespie, CRC Beatson Institute for Cancer Research, Glasgow, UK. E. coli for expression of a GST/ATF-2(19–96) fusion protein, purified GST/ATF-2(19–96), purified recombinant 6-his-JNK/SAPK, and antibodies for the immunoprecipitation of SKK 1 and SKK 4 (9) were obtained from Dr. S. Lawler, MRC Protein Phosphorylation Unit, Department of Biochemistry, University of Dundee, Dundee, UK. Antibodies for the detection of Vmw110 and Vmw175 in Western blotting were obtained from Dr. R. E. Everett (37, 38). Antibodies specific for R1 and Vmw63 proteins were as described (39, 40). Polyclonal antibodies against Jun, JunB, JunD, Fos, and ATF-2 were obtained from Santa Cruz Biotechnology. A nonradioactive kit for detection of p38 MAPK activity was used as directed by the manufacturer (New England Biolabs, Hitchin, UK). Plasmids pMCI, expressing full-length VP16 and pMCI del, encoding a truncated form lacking the transactivation domain, were kindly provided by Dr. C. M. Preston.

**Virus Infection and UV Irradiation of Cell Monolayers**—Subconfluent cell monolayers were infected with either wild-type HSV-1 strain 17+ or mutant HSV-1 viruses at multiplicity of infection of 10 pfu per cell, unless stated otherwise. Infected cells were grown at 37 °C, 5% CO2, before harvesting. In experiments with the tk virus, infected cells were grown at 38.5 °C (non-permissive temperature) or at 32 °C (permissive temperature) before harvesting.

For UV irradiation of BHK cells, the medium was removed and the cell monolayer was exposed to 80 J/m2 in a UV cross-linker. The medium was replaced, and the cells were returned to the incubator for 30 min before harvesting.

**Transfection of Cells**—BHK cells were grown in 30-mm dishes and transiently transfected using LipofectAMINE (Life Technologies, Inc.), as indicated by the manufacturer. Cells were harvested at 48 h posttransfection, and pull down kinase assays were performed.

**Preparation of Cell Extracts**—For immunocomplex and pull down kinase assays, harvested cells from a 60-mm plate were incubated on ice for 30 min, in 250 μl of lysis buffer J (25 mM HEPES, pH 8.0, 75 mM NaCl, 2.5 mM MgCl2, 0.1 mM EDTA, 0.5 mM DTT, 20 mM β-glycerophosphate, 0.1 mM NaVO4, 25 mM NaF, 1% Nonidet P-40). Cell debris was removed by centrifugation at 13,000 × g.

For Western blotting, cells were lysed in buffer W (20 mM HEPES, pH 7.6, 0.4 mM KCl, 5 mM EDTA, 1 mM DTT, 1 mM NaVO4, 5 mM NaF, 10% glycerol, 0.4% Triton X-100, 1 μM okadaic acid) and treated as described above.

For DNA binding assays, nuclear extracts were prepared as described by Dignam et al. (41).

**Pull Down Kinase Assays**—Induction of GST fusion protein production in E. coli was achieved by treating cultures with 150 μg/ml isopropyl-1-thio-β-D-galactopyranoside for 3 h. Harvested bacteria were resuspended in NETN buffer (50 mM Tris, pH 8.0, 10 mM NaCl, 1 mM EDTA, 0.1% Nonidet P-40) and lysed by sonication, and cell debris was removed by centrifugation at 5000 × g. Bacterial extracts were incubated at 25 °C for 30 min, with glutathione-agarose (Sigma) previously swollen and washed extensively in NETN buffer. The glutathione-agarose with bound GST fusion protein was washed 4 times with 10 volumes of NETN buffer, and the gel was resuspended in equal volume of NETN buffer to give a 50% slurry.

Kinase activity in the cell extracts was analyzed by using the glutathione-agarose with bound GST/Jun or GST/ATF-2 to pull down the associating kinases and then determining incorporation of [32P]PO4 into the fusion proteins. Briefly, 60 μl of the 50% matrix slurry were added to 250 μl of cell extract and incubated overnight, at 4 °C, with constant agitation. The matrix was washed 4 times in wash buffer comprising 20 mM HEPES, pH 7.6, 50 mM NaCl, 2.5 mM MgCl2, 0.1 mM EDTA, 0.05% Triton X-100 and resuspended in 30 μl of kinase assay buffer (20 mM HEPES, pH 7.6, 20 mM MgCl2, 20 mM β-glycerophosphate, 0.1 mM NaVO4, 2 mM DTT, 0.1 μM ω-maleic acid, 0.125 mM [γ-32P]ATP). The phosphorylation reaction was allowed to proceed for 30 min, at 25 °C, and samples were electrophoresed on SDS-polyacrylamide gel electrophoresis. [32P]PO4 incorporation was observed by autoradiography and quantified by scintillation counting.

**Immunocomplex Kinase Assays**—10 μl of a JNK/SAPK-specific anti-serum were added to 250 μl of cell extract, and the volume was adjusted to 500 μl with buffer P (20 mM Tris, pH 8.0, 40 mM sodium pyrophosphate, 50 mM NaF, 0.1 mM NaVO4, 10 mM EDTA, 1% Triton X-100, and 0.5% sodium deoxycholate). Samples were incubated overnight at 4 °C, with constant agitation. 50 μl of a 50% slurry of protein A-Sepharose (Sigma), previously swollen and extensively washed in buffer P, were used to bind the immunocomplexed JNK/SAPK. 20 μl of GST/Jun bound to glutathione-agarose were added to the matrix and resuspended in 40 μl of kinase assay buffer, at 25 °C, for 30 min. Samples were electrophoresed by SDS-polyacrylamide gel electrophoresis, and [32P]PO4 incorporation was observed by autoradiography and quantified by scintillation counting.

**Measurement of SKK 1 and SKK 4 Activity**—SKK 1 and SKK 4 activities were measured in cell extracts as described previously (9). Briefly, SKK 1 or SKK 4 were immunoprecipitated with 10 μg of the relevant antibody coupled to protein G-Sepharose 4B (Sigma), washed extensively with 0.5 mM NaCl, and incubated with 1 μl of 20 μM 6-his-SAPK/JNK and 2.5 μl of 40 mM magnesium acetate and 0.4 μM unlabeled ATP for 30 min, at 25 °C. Activated JNK/SAPK was then assayed in 40 μl of a solution containing 31.25 mM Tris, pH 7.4, 0.125 mM EDTA, 1.25 mM NaVO4, 1 mM DTT, 12.5 mM magnesium acetate, 0.125 mM [γ-32P]ATP, and 0.25 mg/ml purified GST/ATF-2 as substrate. Samples were incubated at 25 °C, for 30 min, electrophoresed, and visualized by autoradiography.

**Western Blotting**—Antibodies against Jun, JunB, JunD, Fos, and ATF-2 were used at dilution of 1:300. Antibodies for R1 and Vmw63 were used at dilutions of 1:1000 and 1:100, respectively. Mouse monoclonal antibodies for detection of Vmw110 and Vmw175 were used at dilutions of 1:5000.

**DNA Binding Assays**—The AP-1 probe, encompassing binding sites for the AP-1 complex, and the CRE probe, encompassing a binding site
Infection with Wild-type HSV-1 Stimulates JNK/SAPK—The effects of wild-type HSV-1 infection of BHK cells on p38 MAPK activity were determined by immunocomplex assays, using ATF-2 as a substrate (Fig. 2A). Immunocomplexed p38 MAPK activity was determined in samples harvested at 2, 4, 6, 8, and 10 h postinfection (Fig. 2A, lanes 2–6, respectively) and in untreated cells (lane 1); Western blots were probed with an antibody specific for the phosphorylated form of ATF-2 and demonstrated an increase in phosphorylation by 6 h, with a maximum activity detected at 10 h. ATF-2 phosphorylation by p38 MAPK was also stimulated in UV-irradiated cells (lanes 7 and 8).

Additionally, a GST/ATF-2 fusion protein was used as a substrate to detect ATF-2 phosphorylation by both p38 MAPK and JNK/SAPK pathways (Fig. 2B). Compared with untreated cells (lanes 3 and 4), a marginal increase in ATF-2 phosphorylation was detected by 3 h postinfection (lanes 5 and 6), a 3-fold increase was observed by 6 h (lanes 7 and 8), with a further increase to 5-fold by 9 h (lanes 9 and 10). A 5-fold activation was also observed in control UV-irradiated cells (lanes 1 and 2). These results indicate that both p38 MAPK and JNK/SAPK are stimulated by wild-type HSV-1 infection, and we considered the possibility that a viral protein was implicated in this phenomenon.

Virion Protein VP16 Triggers Activation of JNK/SAPK during HSV Infection—We examined whether some component of
Expression of the VP16 protein in transient transfection assays in a virus-free environment using different amounts of the pMCI plasmid (Fig. 3C, lanes 3–8) was sufficient for activating JNK/SAPK, compared with cells transfected with the empty vector (lanes 1 and 2). Induction of JNK/SAPK by UV irradiation is shown in lanes 9 and 10. Furthermore, expression of a truncated VP16 protein lacking the transactivation domain but still capable of interacting with the cellular factors retained the ability to activate JNK (Fig. 3D, lanes 3 and 4, compared with lanes 1 and 2). Thus, VP16 activates JNK/SAPK through the domain required for interaction with cellular factors.

**Viral IE, Early, or Late Genes Do Not Trigger Stimulation of JNK/SAPK**—The primary function of VP16 is to activate transcription of the IE genes. We therefore investigated the possible contribution of IE genes in stimulating JNK. BHK cells were infected with mutant viruses ICP6delta, 27lacZ, dI403, and E30DBA that fail to express functional forms of R1, Vmw63, Vmw110, and Vmw175 proteins, respectively. Pull down kinase assays in extracts of cells harvested 9 h postinfection were performed using GST/Jun as substrate. All mutant viruses stimulated JNK/SAPK to approximately the same levels as those obtained with extracts from cells infected with wild-type virus (data not shown). In addition, similar kinetics of JNK/SAPK stimulation were observed with the different mutant viruses and wild-type HSV-1 (data not shown). Overall, our results indicate that immediate-early proteins Vmw63, Vmw110, Vmw175, and R1 are not directly responsible for the induction of JNK/SAPK activity.

The role of IE gene products in JNK/SAPK activation was further studied in cells infected with the temperature-sensitive mutant tsk. The tsk virus has a mutation in the gene encoding Vmw175 such that the protein fails to transactivate early and late gene expression at the non-permissive temperature of 38.5 °C and IE gene products accumulate (34). At the permissive temperature of 32 °C the tsk virus replicates as wild type. GST/Jun pull down kinase assays were performed using cellular extracts after infection with tsk. Identical patterns of JNK/SAPK activation were observed at both the permissive and non-permissive temperatures (data not shown). Thus, an accumulation of IE gene products at 38.5 °C did not augment the induction of JNK/SAPK activity, suggesting that none of these proteins is the direct cause of this phenomenon.

Furthermore, viruses failing to produce functional Vmw175 or Vmw63 also fail to express efficiently early and late genes (34, 35, data not shown). We can therefore exclude early and late viral proteins from causing activation of JNK/SAPK.

The Upstream Activator of p38/JNK, SKK1, Is Stimulated during HSV-1 Infection—The activities of SKK 1 and SKK 4, which are upstream activators of p38/JNK, were assayed after infection with wild-type HSV-1 virus (Fig. 4). Levels of GST/ATF-2 phosphorylation were stimulated by SKK 1 immunoprecipitated from extracts prepared at 5 (Fig. 4A, lanes 1 and 2) and 8 h postinfection (lanes 3 and 4) and by exposure to UV light (lanes 7 and 8), compared with untreated cells (lanes 5 and 6). SKK 4 activity was increased in samples extracted after UV irradiation (Fig. 4B, lanes 7 and 8); however, the levels of GST/ATF-2 phosphorylation were similar in infected (lanes 1–4) compared with untreated cells (lanes 5 and 6). These results implicate SKK 1, but not SKK 4, in stimulating p38/JNK during HSV-1 infection.

**Elevated AP-1 Binding Proceeds Stimulation of the Stress Pathways during HSV-1 Infection**—Members of the Jun/Fos and ATF/CREB families of transcription factors are common targets for activation by JNK/SAPK and p38 MAPK pathways. BHK cells infected with wild-type HSV-1 virus were examined for AP-1 and CREB binding activity, by means of gel retarda-
tion assays, at various time points of postinfection (Fig. 5). Increased DNA binding by the AP-1 transcription factor to its consensus oligonucleotide was observed at 6 (lane 5) and 9 h (lane 6) postinfection and reached a maximum by 11 h postinfection (lane 7), compared with untreated cells (lane 3). No alterations in the DNA binding activity of the ATF/CREB family of transcription factors to the consensus CRE probe was observed (lanes 10–13). UV irradiation induced DNA binding of both AP-1 and CREB factors (lanes 1 and 2 and 8 and 9, respectively).

In order to identify the proteins that participate in the activated AP-1 complex, we used antisera specific for the most abundant Jun/Fos family members (Jun, JunB, JunD, and Fos) and for ATF-2, in electrophoretic mobility shift assays, in untreated cells and cells infected with HSV-1, at various times postinfection. In untreated cells (Fig. 6, lanes 1–6), we detected Jun (lane 2), JunB (lane 3), and JunD (lane 4) as major components of the AP-1 complex. Neither Fos (lane 5) nor ATF-2 (lane 6) was detected. Following induction of AP-1 binding activity at 6 h postinfection, the relative amounts of JunB and JunD were unchanged, whereas those of Jun decreased (data not shown). At 11 h postinfection (Fig. 6, lanes 7–12), the activated AP-1 complex was composed mainly of JunB (lane 9) and JunD (lane 10). Jun became a minor participant (lane 8), and Fos (lane 11) was detected in very small amounts, only after overexposure of the film, and ATF-2 was not detected at all (lane 12).

Intracellular levels of Jun/Fos family members and of ATF-2 were examined by Western blotting, at several times postinfection (Fig. 7). Levels of Jun protein decreased between 6 and 11 h postinfection (lanes 1–5), whereas JunB (lanes 6–9), JunD and ATF-2 (data not shown) levels remained constant.

Our results suggest that the AP-1 transcription factor, but not CREB, is a downstream candidate for mediating signals from the activated p38/JNK pathways to the target genes. They also show increased participation of JunB and JunD in the AP-1 complex during HSV infection, with a simultaneous decrease in both relative contribution in the complex and total cellular amounts of Jun protein.

DISCUSSION

Two independent yet parallel signaling cascades are responsible for coordinating the cellular responses to environmental stress. JNK/SAPK and p38 MAPK play a central role in these pathways, and their substrates include proteins of the Jun/Fos and ATF families, forming the AP-1 transcription factor (1–7, 18).

In our study, we showed that HSV-1 infection of cells stimulated both JNK/SAPK and p38 MAPK activity after 3 h of infection and that activity levels increased up to a maximum of 4-fold between 3 and 9 h postinfection; these increased levels were maintained throughout the remainder of the virus replicative cycle.

By using a series of mutant viruses, we identified the virion transactivator protein VP16 as the activator of the p38/JNK pathway and mapped this activity to the domain that is responsible for interaction with host cellular factors. We also showed that VP16 is both necessary and sufficient for activating the stress pathways even in the absence of any viral context. Preparations of UV-irradiated virus retained their ability to stimulate JNK/SAPK, which is consistent with a role for VP16 in activating JNK/SAPK (data not shown). No structural protein of the capsid nor any viral IE, early, or late gene product was found to be involved in triggering stimulation of p38/JNK. However, the fact that the kinetics of the JNK/SAPK activation apparently coincided with the accumulation of IE proteins between 3 and 9 hours postinfection, may suggest a secondary, complementary role for some viral IE proteins.

VP16 regulates viral IE genes expression; however, unlike most transcriptional activators, it is recruited to IE gene promoters by association with the cellular proteins Oct-1 and HCF (20, 21). VP16 is reported to mimic Luman protein in its interaction with HCF. Luman is a human transcription factor of the CREB/ATF family that requires HCF to activate transcription from CRE sites (43). HSV VP16 mimics this interaction with HCF to monitor the physiological state of the host cell (44), and this may result in the observed activation of the stress pathway as a host cell response. Alternatively, stimulation of the stress pathway may occur via interaction of VP16 with an unknown
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a simultaneous decrease both in the contribution of Jun to AP-1 and in the total cellular levels of Jun protein. Thus, Jun does not appear to be a target for stimulated p38/JNK.

Effective JNK substrates require a separate docking site and specificity-conferring residues flanking the phospho-acceptor (16). JunB has a functional JNK-docking site but lacks specificity-conferring residues, whereas JunD lacks a JNK-docking site, requiring heterodimerization with docking competent partners in order to be phosphorylated by JNK (16). Therefore, JunD may be a substrate for JNK during activation by HSV-1 infection and JunB may serve as the JNK docking partner. Significantly, progressive exclusion of Jun from the activated AP-1 complex could confer different attributes to AP-1 compared with the uninduced complex. Members of the Jun/Fos family differ in their characteristics (12, 17); Jun behaves as a positive regulator of cell growth and may cause transformation when overexpressed (12, 17, 46), whereas JunD antagonizes both of these effects and is linked to AP-1-induced apoptosis (46, 47).

The biological role of p38/JNK and subsequent AP-1 activation by HSV-1 infection is unknown. Stimulation of p38/JNK and AP-1 activation may represent a mechanism by which the virus manipulates cellular processes to promote successful virus replication. There is evidence to suggest that JNK/SAPK activation regulates the cell cycle (48, 49). In addition, a subset of cellular genes transactivated by AP-1 may ensure efficient viral gene expression and DNA replication and facilitate virus growth. Alternatively, stimulation of stress-activated signaling pathways could represent a spontaneous cellular defense mechanism to viral invasion, with the aim of aborting virus replication by programmed cell death (50, 51).

The emergence of HSV-1 as a candidate for gene delivery (31) makes further investigations regarding cellular stress response to viral infection of great importance.

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SKK 1 and SKK 4 are upstream activators of p38 and JNK. SKK 4 is known to be activated by proinflammatory cytokines, whereas stressful stimuli like UV irradiation and osmotic stress activate both SKK 1 and SKK 4 (9). Surprisingly, we identified SKK 1, but not SKK 4, as upstream activator of p38/JNK, and these findings suggest that HSV-1 infection activates stress pathways by a different mechanism than other stress stimuli.

Gel retardation assays demonstrated that induction of AP-1 binding proceeded stimulation of p38/JNK. AP-1 activation was detected at 6 h and reached a maximum by 11 h postinfection. Activation of AP-1 might occur via the ras/ERK pathway (44), but induction of ERKs during HSV-1 infection was not observed using kinase assays in myelin basic protein polyacrylamide gels (data not shown), as described by Shackelford (45). The in vivo targets of virus-stimulated p38/JNK do not include ATF-2, since CREB binding activity was not elevated and ATF-2 was not part of the activated AP-1 complex. Furthermore, induction of AP-1 binding coincided with an increased participation of JunB and JunD in the AP-1 complex and with cellular protein.

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