

Herpes Simplex Virus 1 Upregulates p35, Alters CDK-5 Localization, and Stimulates CDK-5 Kinase Activity during Acute Infection in Neurons

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The cyclin-dependent kinase 5 (CDK-5) activating protein, p35, is important for acute herpes simplex virus 1 (HSV-1) replication in mice. This report shows that HSV-1 increases p35 levels, changes the primary localization of CDK-5 from the nucleus to the cytoplasm, and enhances CDK-5 activity during lytic or acute infection. Infected neurons also stained positive for the DNA damage response (DDR) marker γ H2AX. We propose that CDK-5 is activated by the DDR to protect infected neurons from apoptosis.

Herpes simplex virus 1 (HSV-1) is a human pathogen that establishes lifelong latent infection in sensory neurons (1, 46). It is not well understood how the virus switches from lytic to latent infections in neurons and the mechanisms involved in reversing this switch under stress stimuli to initiate reactivation. It is vital that the neurons survive an infection by HSV-1 if the virus is to efficiently establish a latent infection or reactivate. Not surprisingly, HSV-1 has developed countermeasures to prevent neuronal apoptosis, in part, with the expression of the latency-associated transcripts (LATs) (2–4). While the contribution of viral gene products that possess antiapoptotic activities has been a major area of interest in HSV-1 research, the role of cellular factors involved in neuronal survival has received limited attention.

One neuronal factor that is highly active in postmitotic neurons and is required for neuronal survival during stress is cyclin-dependent kinase 5 (CDK-5) (5, 6). CDK-5 regulates many neuronal processes (reviewed in reference 7) and has been shown to play important roles in both neuronal survival and death. Inactivation of CDK-5 has been shown to trigger neuronal death (8–11). On the other hand, a survival function for CDK-5 is evident when neurons are stressed (12–16). For its activation, CDK-5 binds to p35 or a related protein, p39, which also modulate CDK-5's subcellular localization (17–20). Notably the activity of CDK-5 directly correlates with the levels of its major activator, p35, and both are expressed predominantly in neurons (21), which likely explains why CDK-5 is mainly active in neurons, although it is constitutively expressed in many cell types (17, 22).

We have previously shown that HSV-1 acute replication is impaired in the eyes and trigeminal ganglia (TG) of p35 knockout mice, reducing the establishment of latency and reactivation (23). Given this previous result, we sought to determine whether HSV-1 altered the expression or subcellular localization of the p35 and CDK-5 proteins during acute infection.

HSV-1 infection induces p35 protein levels. To examine how HSV-1 acute TG infection affects p35, wild-type HSV (KOS)-infected TG were harvested 3 days postinfection, as previously described (24, 25). TG sections were immunostained for both the HSV-1 immediate early protein, ICP0, used as a marker for productively infected neurons, and p35. As shown in Fig. 1A, HSV-1-infected neurons showed an increase in p35 staining

compared to mock-infected cells, with a distribution that was primarily cytoplasmic. To confirm this result in cell culture, the human neuronal cell line SK-N-SH was mock infected or infected with KOS. As shown in Fig. 1B, p35 was not detected in mock-infected cells, whereas it was readily detected in KOS-infected cells and reached maximal levels of expression at 24 h postinfection (H. H. Mostafa, J. M. van Loben Sels, and D. J. Davido, unpublished data).

HSV-1 infection changes the localization of CDK-5 and enhances its activity. Because p35 function is linked to its binding partner, CDK-5, we next wanted to determine if HSV-1 acute infection affects CDK-5 expression and/or subcellular localization. For these studies, TG sections from 3-day KOS-infected mice were immunostained for ICP0 and CDK-5. As shown in Fig. 2A, in contrast to the mock-infected neurons, where CDK-5 localization was predominantly nuclear with diffuse cytoplasmic staining in most cells, HSV-1-infected neurons had a CDK-5 localization that was primarily punctate and both cytoplasmic and nuclear in ~70% of cells that stained positive for ICP0. It was difficult to detect CDK-5 localization in SK-N-SH cells, as CDK-5's staining was faint (H. H. Mostafa and D. J. Davido, unpublished data); CDK-5 protein levels, however, were apparent in HSV-1-infected SK-N-SH cells and at comparable levels to those in mock-infected cells (Fig. 1B). Thus, alterations in CDK-5 localization do not appear to correlate with decreased protein levels. In order to test whether HSV-1 infection modulated CDK-5's kinase activity, SK-N-SH cells were mock infected or infected for 24 h with KOS. CDK-5 was immunoprecipitated and incubated with the CDK-5 substrate Tau (26) for 30 min at 37°C. As shown in Fig. 2B, KOS

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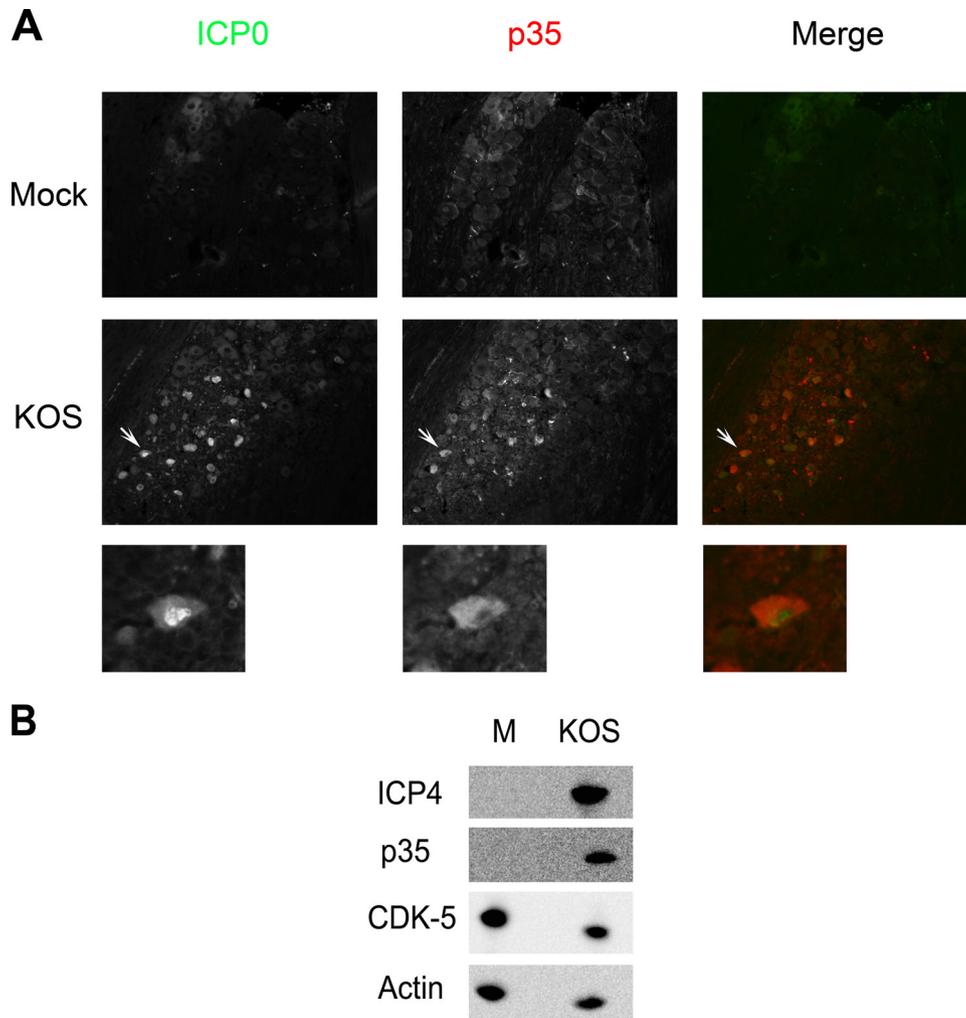


FIG 1 Alterations in p35 expression by HSV-1. (A) p35 staining in response to HSV-1 infection. CD-1 mice were infected with 2×10^5 PFU of HSV-1 (strain KOS) per eye. Three days postinfection, mice were sacrificed, and TG were collected, fixed, paraffin embedded, and processed for immunofluorescence staining of ICP0 and p35. More than 10 sections from two independent experiments were examined, and the image shown is a representative section. Magnification, $200\times$. Arrows point to the expanded view shown below each panel. (B) p35 and CDK-5 protein levels after HSV-1 infection. SK-N-SH cells were mock infected (M) or infected at a multiplicity of infection (MOI) of 2 with KOS. Twenty-four hours postinfection, cells were harvested, and protein levels from cell extracts were determined by Western blot analysis.

infection increased the amount of phosphorylated Tau by 4- to 5-fold as detected with the monoclonal antibody AT8 (27). This indicates that HSV-1 infection enhances CDK-5 kinase activity in neuronal cells.

HSV-1 infection induces DDR in TG. CDK-5 is important for neuronal survival in response to stressful conditions, including the DNA damage response (DDR) (12–16). In this context, HSV-1 infection triggers and counteracts the DDR in nonneuronal cell lines (28–32). However, the induction of the DDR in cultured neurons (28) or in the neurons of HSV-1-infected animals has not been reported. To determine whether HSV-1 infection triggers DDR in infected neurons, KOS-infected TG were isolated 3 days postinfection, sectioned, and immunostained with γ H2AX, a histone variant and one of the earliest markers of the DDR (33–35). Interestingly, neurons that stained positive for HSV-1 antigens were positive for γ H2AX, whereas mock-infected neurons had no

apparent γ H2AX staining (Fig. 3). This indicates that acute HSV-1 neuronal infection can trigger early DDR events.

Model of CDK-5 alteration and p35 induction during HSV-1 infection. p35 levels are induced secondarily to neurotrophic factors that stimulate extracellular-signal-regulated kinase 1/2 (ERK1/2), activating the transcription factor Egr1 (36–38). Interestingly, a previous report showed that HSV-1 infection activates the ERK/mitogen-activated protein kinase (MAPK) signaling pathway (39). Moreover, it has been shown that HSV-1 lytic infection induces Egr1 in rabbit corneal cells (40). Oxidative stress is capable of initiating the DDR (41) and is reported to enhance the expression of Egr1 (42), and HSV-1 has the ability to induce neuronal oxidative stress (43). With these observations and our data, we propose that oxidative stress and/or activation of DDR during acute HSV-1 infection of neurons stimulates p35 levels via Egr1 (Fig. 4).

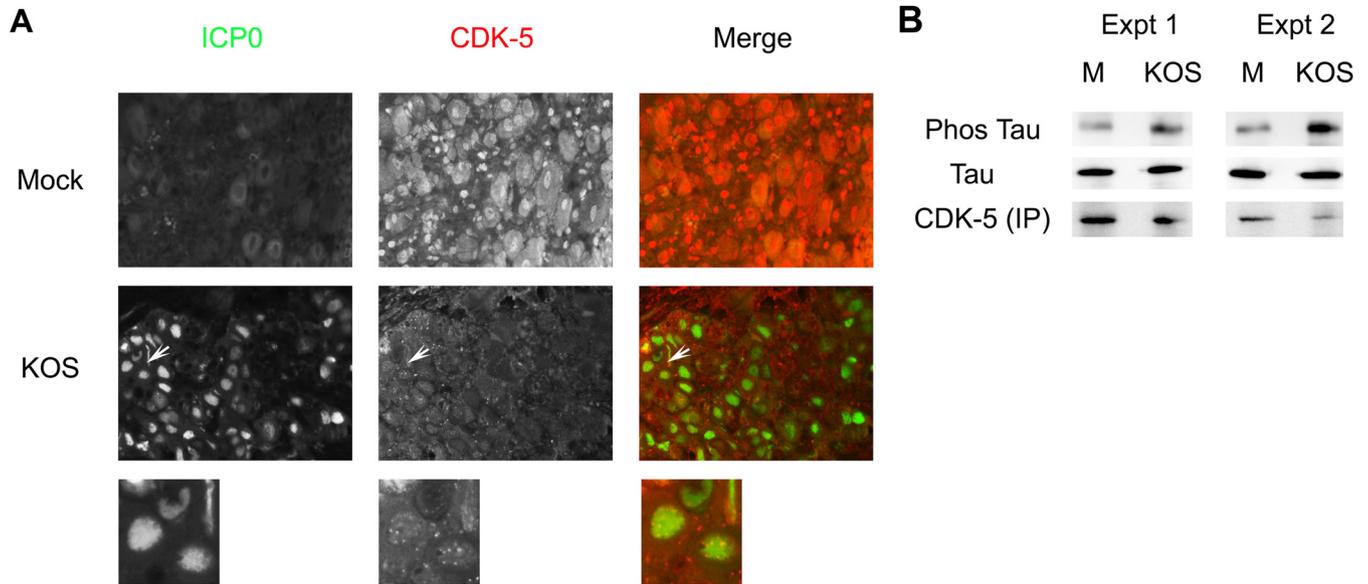


FIG 2 HSV-1 affects CDK-5 localization and kinase activity. (A) CDK-5 localization in response to HSV-1 infection. CD-1 mice were infected with 2×10^5 PFU of KOS per eye. Three days postinfection, mice were sacrificed, and TG were collected, fixed, paraffin embedded, and processed for immunofluorescence staining of ICP0 and CDK-5. More than 10 sections from two independent experiments were examined, and the image shown is a representative section. Magnification, 400 \times . Arrows point to the expanded view shown below each panel. In about 30% of the ICP0-positive neurons, CDK-5 colocalized with ICP0 in the nucleus but showed punctate staining, as evident in the expanded view. (B) *In vitro* CDK-5 kinase assay. SK-N-SH cells were mock infected or infected with KOS for 24 h. CDK-5 protein was immunoprecipitated (IP) and incubated with bacterially purified Tau protein. Phosphorylated (Phos) versus total Tau was determined by Western blot analysis and quantified by densitometry. Data from two independent experiments are shown.

It has been shown that cytoplasmic CDK-5 inhibits apoptosis by preventing neurons from entering mitosis (15, 44), and its protein levels are stabilized by p35 (45). Our data allow us to propose a model in which HSV-1 induces CDK-5 kinase activ-

ity to protect lytically infected neurons from dying (Fig. 4). In support of this possibility, we examined acutely infected TG for signs of apoptosis 3 days postinfection using terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end label-

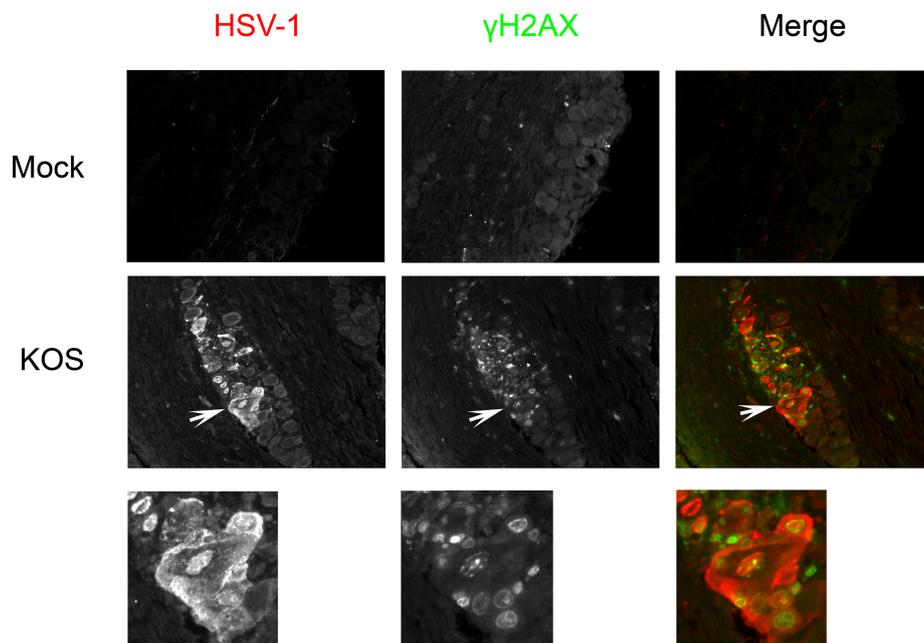


FIG 3 DNA damage response to HSV-1 infection. CD-1 mice were infected with KOS at 2×10^5 PFU per eye. Three days postinfection, mice were sacrificed, and TG were collected, fixed, paraffin embedded, and processed for immunofluorescence staining of HSV-1 and γ H2AX. More than 10 sections from two independent experiments were examined, and the image shown is a representative section. Magnification, 200 \times . Arrows point to the expanded view shown below each panel.

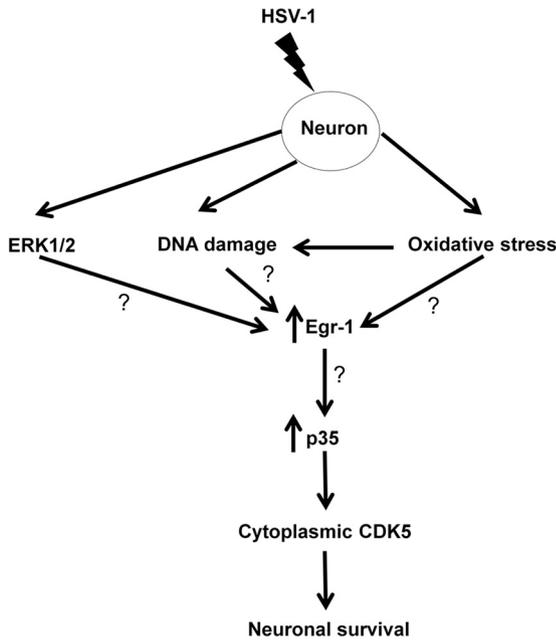


FIG 4 Proposed model of p35 upregulation in response to HSV-1 infection. HSV-1 infection of neurons stimulates the ERK pathway, activates at least one component of the DDR, and triggers the oxidative stress response. These stimuli increase Egr-1 expression, leading to the induction of p35. Elevated p35 protein levels result in the cytoplasmic localization of CDK-5 and enhance its kinase activity, which promotes neuronal survival of the infected neuron.

ing (TUNEL) staining and did not detect an increase in apoptotic neurons in KOS-infected cells (H. H. Mostafa and D. J. Davido, unpublished data), consistent with other studies (2–4). In the future, we will determine how p35 is upregulated to promote lytic replication.

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