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STAT1 binds to the herpes simplex virus type 1 latency-associated transcript promoter

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The authors hypothesized that environmental stimuli induce cytokines that act through an intracellular cascade, which includes signal transducers and activators of transcription (STATs), to change herpes simplex virus (HSV) gene expression, thereby inducing viral reactivation. The HSV type 1 (HSV-1) latency-associated transcript (LAT) gene regulates viral reactivation within neurons via an unknown mechanism. HSV-1 deletion mutants that are missing key portions of the LAT gene, particularly the 3′ region of the LAT promoter, do not reactivate normally *in vivo*. The authors hypothesized that STAT transcription factors may bind in this region to regulate viral reactivation. Electrophoretic mobility shift assay (EMSA) experiments were performed by incubating mouse trigeminal ganglion (TG) nuclear extracts with each of three overlapping sequences representing the 3′ region of the HSV-1 LAT promoter (referred to as oligos L1, L2, and L3). The ganglionic nuclear extracts bound specifically to oligos L1 and L3, but not L2. Oligos L1 and L3 contain predicted STAT binding sequences whereas L2 does not. Specific binding to oligo L3 (including the TATA box sequence) was supershifted by incubating with anti-STAT1 antibodies, but not by incubating with anti-STAT3 or anti-STAT5a antibodies. Specific L3 binding was reduced by competing with excess unlabeled STAT1 consensus sequences. These results indicate that STAT1, probably as part of a complex, is capable of binding to the LAT promoter on or near the TATA box. Further studies are required to determine if STAT1 is required for LAT expression *in vivo*. This work supports the hypothesis that interferons act through STAT1 to regulate the expression of HSV-1 LAT. *Journal of NeuroVirology* (2004) **10,** 12–20.

Keywords: herpes simplex virus; HSV; latency; reactivation; signal transducers and activators of transcription

Introduction

Herpes simplex virus (HSV) type 1 (HSV-1) and HSV type 2 (HSV-2) cause orolabial herpes, ocular herpes, and genital herpes. These diseases are the most common recurrent viral infections in humans, affecting millions of persons worldwide. At least 20% of the U.S. population, over 40,000,000 people, experience periodic vesicular HSV lesions on the lip, face, or mouth (Spruance, 1995). Approximately 400,000 persons have experienced herpes keratitis (corneal infection), an important subset of HSV-1 infections (Dawson, 1995). HSV infects epithelial surfaces andthen enters sensory nerves where it establishes viral latency. Latent HSV exists as episomes (circular double-stranded DNA) within the nuclei of sensory neurons. These latently infected sensory neuron cell bodies are located predominantly within the trigeminal ganglion (TG), the major site of HSV-1 latency, and the sacral (dorsal root) ganglia, the predominant site of HSV-2 latency.

Only one region of the HSV genome is abundantly transcribed during latency, producing several viral RNAs called latency-associated transcripts (LATs) (Stevens, 1990; reviewed by Block and Hill, 1997; Fraser *et al*, 1992). The abundant expression of LATs from the latent viral genome is unique to HSV. The

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LAT region appears to play an important role in HSV reactivation because deletion of this region prevents most reactivations, at least in animal models (Hill *et al*, 1990; Perng *et al*, 1994, 1996). Some investigators have shown that LAT expression is important for the efficient establishment of latency (Perng *et al*, 2000b; Sawtell, 1998; Sawtell *et al*, 1998), but this observation does not explain why LAT is transcribed after viral latency is fully established. Although no LAT-encoded protein has ever been positively identified (Drolet *et al*, 1998), recent efforts suggest that LAT encodes a phosphorylated protein that forms punctate structures within cell nuclei (Thomas *et al*, 1999, 2002). Other studies show that LAT may act as a repressor of viral immediate-early gene expression (Mador *et al*, 1998) and/or as an antiapoptotic gene, preventing the death of neurons latently infected with HSV (Ahmed *et al*, 2002; Henderson *et al*, 2002; Perng *et al*, 2000a, 2002). The precise function of the HSV-1 LAT remains controversial.

The LAT domain, including its promoter region, is 8.3 kb in length (Figure 1). The large primary LAT transcript is processed into stable 1.5- and 2.0-kb introns, which accumulate and persist in neuronal nuclei. Two promoters have been identified within the LAT domain, including LAP1, responsible for LAT expression during latency, and LAP2, responsible for expression during lytic infection (Chen *et al*, 1995). Reporter construct studies show that the 3′ end of the LAT promoter (LAP1) is primarily responsible for ongoing LAT expression (Ackland-Berglund *et al*, 1995; Dobson *et al*, 1995; Perng *et al*, 1996; Zwaagstra *et al*, 1991). Transcription factors previously shown to bind in this region include cAMP response element–binding protein (CREB) and the LAT promoter–binding factor (LPBF; see Figure 1). Mutation studies show that deletions within the 3′ LAT promoter region inhibit viral reactivation, whereas the reactivation is preserved when the LAT intron region is deleted (Hill *et al*, 1990; Kriesel *et al*, 1998). This accumulated data suggest that it is LAT *transcription*, rather than accumulation of the stable intron, that controls HSV-1 reactivation.

HSV can be induced to reactivate in humans and animal models by different stimuli, including ultraviolet (UV) radiation and fever. The exact sequence of events that leads from the stimulus to viral reactivation is unknown, but recent studies suggest that the cytokines interleukin (IL)-6, ciliary neurotrophic factor (CNTF), nerve growth factor, and interferon (IFN) are involved (Cantin *et al*, 1999; Halford *et al*, 1996; Hendricks *et al*, 1991, 1992; Kriesel *et al*, 1994, 1997, 1998). Each of these cytokines can alter cellular gene expression through the Janus kinase–activator of transcription (JAK-STAT) intracellular signal cascade (Chatterjee-Kishore *et al*, 2000; Rajan *et al*, 1998). We hypothesized that these reactivating stimuli may work through cytokines and JAK-STAT signaling to induce the expression of HSV genes. We have recently demonstrated the presence

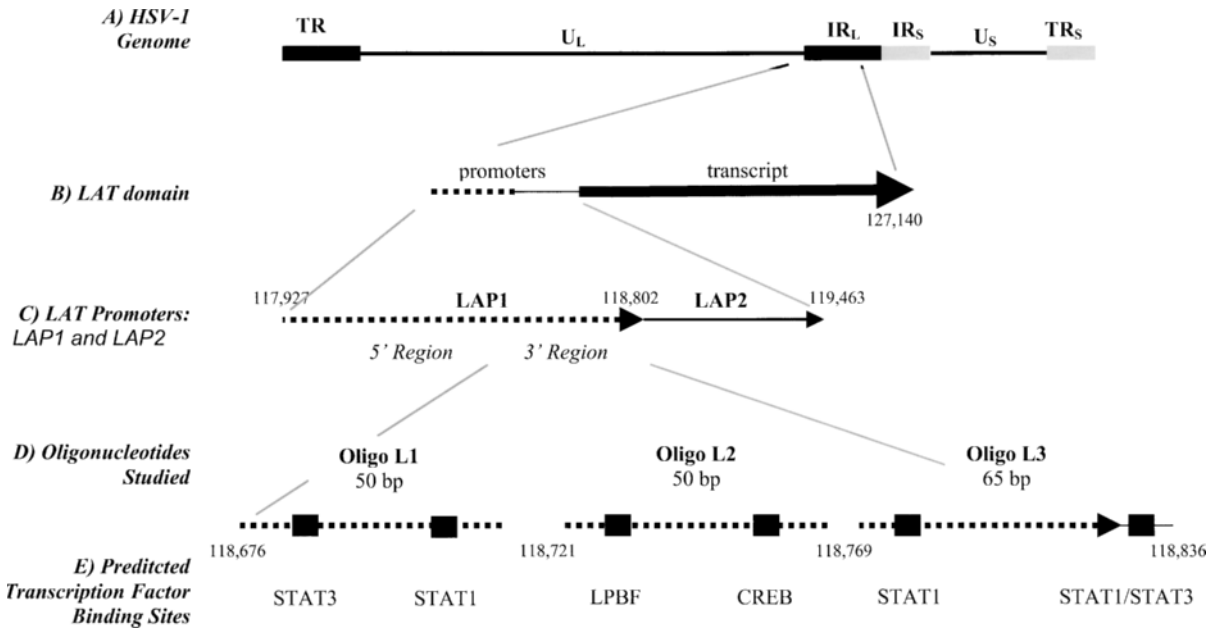

Figure 1 The linearized HSV-1 genome (**A**), the LAT domain (**B**), and the LAT promoter region (**C–E**). The regulatory terminal (TR_L, TR_s) and inverted (IR_L, IR_s) repeat regions flank the unique long (U_L) and short (U_S) regions, which contain the majority of HSV-expressed genes. The LAT domain is transcribed from the inverted repeat regions (IR_L, IR_s). Nucleotide numbers within the Genbank HSV-1 genomic sequence are shown. The LAT promoter LAP1 was arbitrarily divided into 5′ and 3′ regions (**C**). The 3′ region encodes important transcription regulatory elements. The 165-bp 3′ LAT promoter region was divided into three oligonucleotides (L1, L2, and L3) for EMSA (gel shift) analysis (**D**). Transcription factor binding sites (**E**) were either predicted from the sequence in this region (STAT1 and STAT3) or were suggested by previous studies (LPBF and CREB).

of several STATs in TG neurons, both in mice and humans (Kriesel *et al*, 2001). Using the GenBank HSV-1 database (McGeouch *et al*, 1988), we identified four possible STAT binding sites within the HSV-1 LAT promoter (Akira *et al*, 1995; Kriesel *et al*, 1998; Schindler and Darnell, 1995). Two of these four sites lie between nucleotides 118,676 and 118,836, within the 3′ LAT promoter (see Figure 1). To explore the potential role of cytokines and the JAK-STAT cascade in HSV reactivation, electrophoretic mobility shift assay (EMSA) was performed to determine whether TG nuclear extracts (containing STATs) could bind to the 3′ LAT promoter region, arbitrarily divided into the three overlapping oligonucleotides L1, L2, and L3 (Figure 1). Mouse TG nuclear extracts specifically bound to oligonucleotides L1 and L3, but not L2, corresponding to predicted STAT1 and STAT3 binding sites. Evidence of specific STAT1 binding to the L3 oligonucleotide, including a transcription start site (TATA box), is presented below.

Results

EMSA

Three radiolabeled HSV-1 LAT promoter double-stranded DNA probes, 50 to 65 bp in length (oligos L1, L2, and L3), were incubated with 1 μg of mouse TG nuclear extracts. DNA–TG nuclear extract complexes were separated by polyacrylamide gel electrophoresis (PAGE) and confirmed by adding 200× excess of cold, unlabeled probe (Figure 2). EMSA using oligo L1 (representing HSV-1 bp 118676 to 118726) revealed three specific HSV-1 DNA–TG nuclear extract complexes. Two specific HSV-DNA complexes were demonstrated in the oligo L3 region (representing HSV-1 bp 118769 to 118834). EMSA with oligo L2 (representing HSV-1 bp 118721 to 118771) revealed a faint band with the addition of TG nuclear extracts to the probe. However, this weak binding did not disappear with the addition of cold probe, indicating that this observed binding was nonspecific. This faint nonspecific binding was observed with the addition of 10×, 50×, and 200× cold probe (data not shown). No specific binding of TG nuclear extracts occurred in this region of the LAT promoter under these conditions.

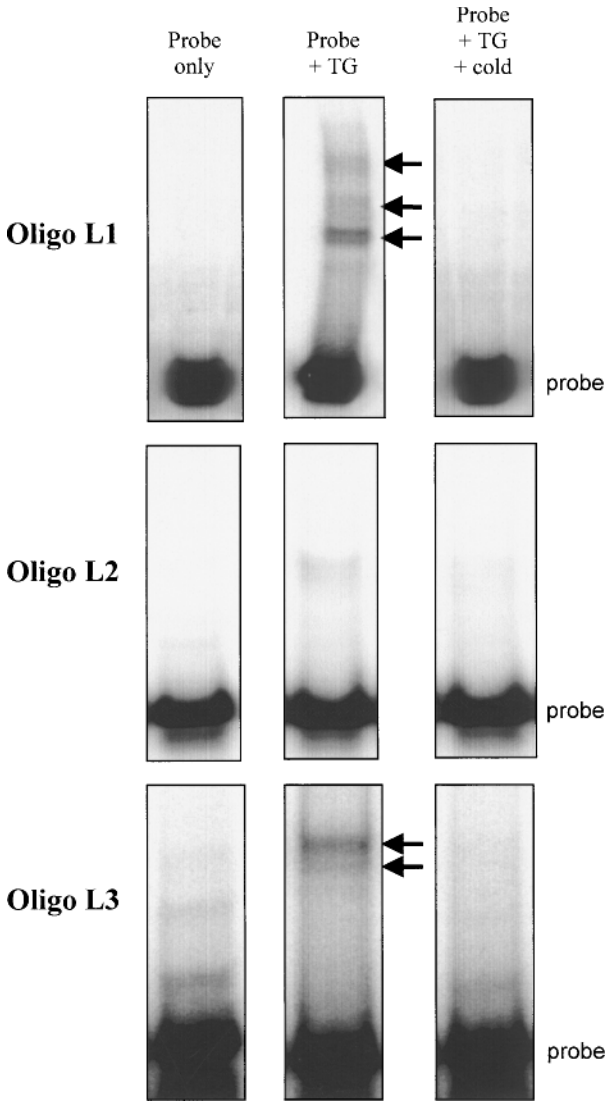

Figure 2 Mouse TG nuclear extracts bind specific to HSV-1 LAT promoter DNA. Radiolabeled double-stranded DNA probes from the 3′ region of the HSV-1 LAT promoter (Oligos L1, L2, and L3) were incubated with uninfected TG nuclear extracts with or without 200× competing cold probe. The control (probe only), test (probe + TG), and cold probe competitor control (probe + TG + cold) lanes displayed were run simultaneously through single native polyacrylamide gels for each oligo. Specific DNA–nuclear extract complexes *(arrows)* were observed with Oligos L1 and L3.

Supershifting

Oligos L1 and L3 were incubated with TG nuclear extracts and 2 or 8 μg of anti-STAT1, anti-STAT3, or anti-STAT5b antibodies. Specific HSV DNA-TG complexes were again observed with oligos L1 and L3. Addition of anti-STAT1 antibodies to the reaction mixture caused supershifting of the oligo L3–TG DNA complex (Figure 3), but not of the oligo L1–TG DNA complex. Neither anti-STAT3 nor anti-STAT5b antibodies cause supershifting of the oligo L3–TG DNA complexes (data not shown). STAT1 supershifting of the oligo L3–TG DNA complex was consistently observed in several experiments during these studies. The results suggest that STAT1 forms part of a DNA-protein complex that can bind only to the extreme 3′ end of the HSV-1 LAT promoter.

Site-directed mutagenesis

Mutation of the STAT1 and STAT1/3 consensus sites in oligo L3 was performed to determine whether this would affect the specific binding observed. To switch purines and pyrimidines in the putative active sites, adenine was changed to cytosine and thymidine was changed to guanine where possible within the

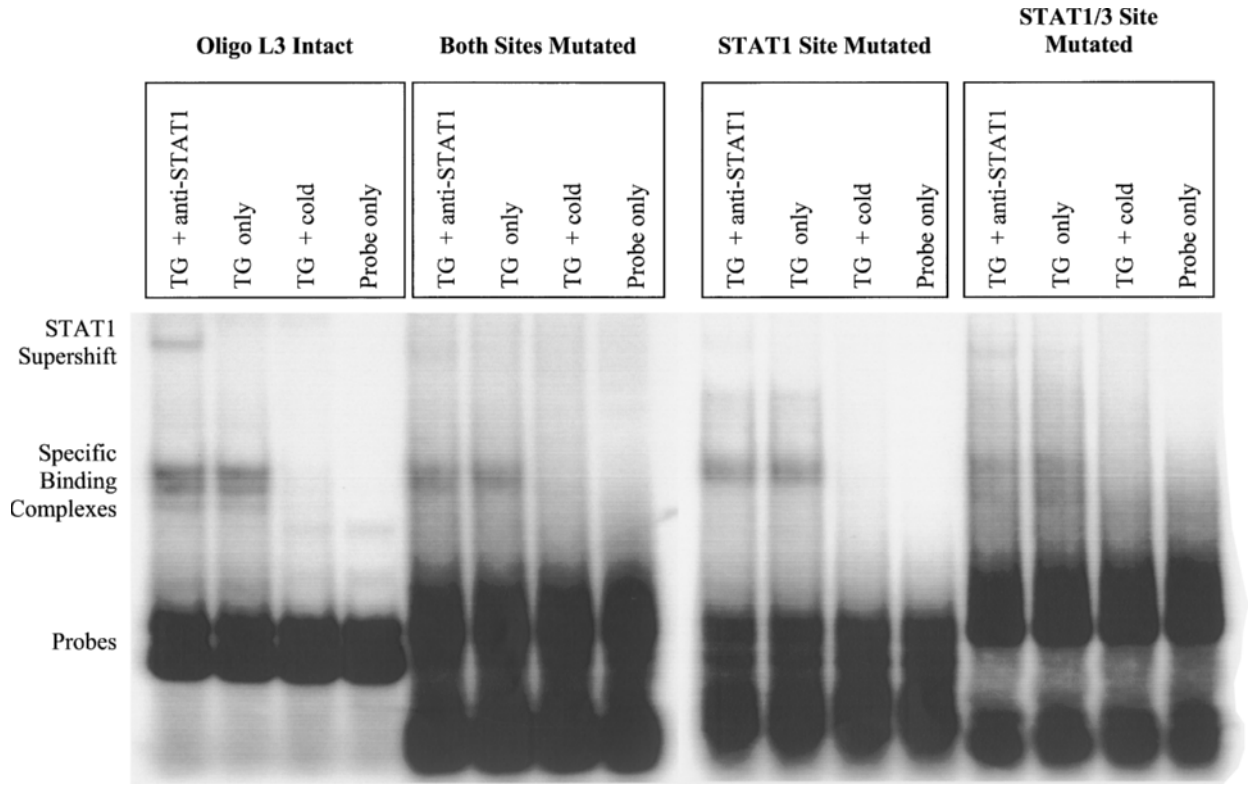

Figure 3 Mutation of the LAT promoter Oligo L3 affects STAT1 supershifting. Uninfected TG nuclear extracts were incubated with site-specific oligo L3 mutants with and without anti-STAT1 antibodies. The sequence of Oligo L3 is 5′-CAGCC**TTTATAAAA**GCGG GGGCGCG-GCCGT GCCGATCGCG GGTGGTGCG**AAAGACT*TTCCGG***GCGCG-3′ with the predicted STAT binding sites in bold. Mutation of either the predicted (upstream) STAT1 or (downstream) STAT1/3 binding sites decreased STAT1 supershifting of the oligo. Mutation of both sites virtually eliminated STAT1 supershifting. Non–STAT1-specific binding was not appreciably affected by these site-directed mutations.

predicted STAT consensus binding sites. Mutation of either these predicted binding sites decreased the intensity of the STAT1 supershifted band (Figure 3). Mutation of both sites virtually eliminated STAT1 supershifting in this system. The pattern of TG-DNA complex binding not attributable to STAT1 (the lower bands in Figure 3) was altered somewhat by mutating oligo L3 in this manner, but this binding was still detectable and was competed out by cold oligonucleotide. Because mutation of both the STAT1 and STAT1/3 sites affected supershifting, these studies suggest that STAT1 binding to oligo L3 is complex and involves both sites.

Competition studies

Competition studies using 10- and 50-fold excesses of commercially available STAT1, STAT3, or STAT5 consensus binding oligonucleotides were also performed (Figure 4). These experiments showed that the STAT1 consensus oligonucleotide, but not the STAT3 or STAT5 consensus oligonucleotides, effectively competed with TG nuclear extracts for the binding of oligo L3. These consensus STAT oligos did not significantly compete for TG nuclear extract binding of oligo L1 (data not shown). The STAT1 consensus oligonucleotide only partially inhibited the binding TG nuclear extracts to L3, but the level of inhibition (about 30%) was approximately equivalent to direct inhibition of binding by a 10-fold excess of cold L3 oligo. These results support the supershifting studies, indicating that STAT1 in TG nuclear extracts binds to the distal LAT promoter oligo L3 *in vitro*.

Effects of HSV-1 infection, LAT expression, and UV exposure

It was hypothesized (1) that the presence of the LAT itself might up-regulate STAT1 expression and binding in the TG, and (2) that corneal UV exposure would reduce local interferon expression and, therefore, down-regulate STAT1 expression and binding in the TG. Groups of mice were latently infected with parent HSV-1 (strain 17 syn+), a LAT-negative mutant (strain 17-deltaPST), or its LAT-expressing rescuant (17-deltaR1). The left TGs from these mice were harvested 0 (unexposed), 4, or 24 h after left corneal UV exposure. Densitometry performed on the L3 oligo STAT1 supershifted complexes revealed no effects of latent HSV-1 infection, LAT expression, or previous corneal irradiation (Figure 5). The experiment was performed twice and similar results were obtained each time. These experiments confirmed the anti-STAT1 supershifting of L3 oligo complexes in the

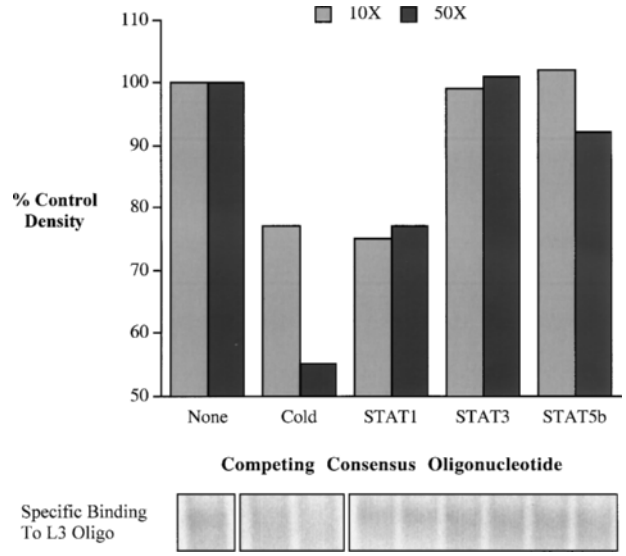

Figure 4 The STAT1 consensus binding oligonucleotide competes for binding of the LAT promoter oligo L3. Ten- and 50-fold molar excesses of commerically obtained STAT consensus binding oligonucleotides were added to TG nuclear extracts and the LAT promoter oligo L3. (A 200-fold excess was cost prohibitive.) Densitometry was performed to determine the intensities of the specific binding observed. Band intensities for the competing oligonucleotides are shown as percent of the positive, uncompeted control value (e.g., "None"). Specific binding of TG nuclear extracts to the L3 oligo was consistently decreased in the presence of STAT1 consensus binding oligonucleotide, similar to competition with the cold L3 oligo. STAT3 and STAT5b consensus binding oligonucleotides did not affect specific binding of TG nuclear extracts to the L3 oligo.

presence and absence of latent HSV-1 infection, with or without LAT production.

Discussion

These studies show that STAT1 is present in TG nuclear extracts and is capable of binding the 3′ region of the HSV-1 LAT promoter *in vitro*. The other STATs studied, STAT3 and STAT5b, did not bind in this region, despite the presence of some predicted binding sites identified in this nucleotide sequence. STAT1 binding to the 3′ region of the HSV-1 LAT promoter was supported by several lines of evidence, including (1) anti-STAT1 antibody supershifting of HSV DNA-TG nuclear extract complexes; (2) site-directed mutation of the putative STAT1 binding site; and (3) STAT1 consensus binding oligonucleotide competition experiments. These studies demonstrated STAT1 binding to TG nuclear extracts taken from live animals, not cell cultures.

The present study does not prove that STAT1 binds HSV-1 LAT *in vivo* or drives its expression, but these possibilities are raised for future investigation. We know from a recent immunohistochemical study that the STATs, including STAT1, are present primarily in TG neurons both in mice (as used for this study) and in humans (Kriesel *et al*, 2001). STAT1 is not prominent in non-neuronal cells or connective tissue within these ganglia, so these results are relevant to TG neurons, the site of HSV-1 latency.

This study appears to contradict the findings of Zwaagstra *et al* (1991), who demonstrated a binding site they designated "LPBF" in the region we studied. As shown in Figure 1, we expected the LPBF site to fall within the L2 oligo region, yet no specific binding to mouse TG nuclear extracts was demonstrated. A possible explanation for this difference in results is the use of cell culture extracts by Zwaagstra *et al* versus murine TGs in the present study.

Many potential STAT1 binding sites (TTNNNN NAA) exist within the HSV-1 genome. However, not all these sites will actually bind STAT1, as we showed with the L1 oligo, which, despite the presence of a predicted site, did not appear to bind STAT1 in our TG nuclear extract system. STAT1 may have multiple sites of activity on the HSV-1 genome, analogous to its activity as a mediator of the interferon response at multiple sites on the human genome.

Although this study demonstrates the presence of STAT1 in TG neurons and its ability to bind the distal LAT promoter on or near the transcription initiation site (TATA box), there are important limitations. First, these studies were conducted *in vitro* using quiescent TGs, not TGs from acutely infected animals. Binding of neuronal proteins to the LAT promoter might be quite different during acute infection when a number of inducible factors would be present. Demonstrating STAT1 binding to latent HSV-1 LAT *in vivo*, perhaps using DNA footprinting, before, during, and after acute HSV infection would be a logical extension of these studies. Second, even if we assume that binding of STAT1 to LAT promoter does occur *in vivo*, the effects of such binding on viral reactivation remain unknown. These effects could be investigated by making viral mutants with small deletions in the putative STAT1 binding sites or a scrambled distal LAT promoter, then infecting animals and measuring reactivation. It should be noted that the existing HSV-1 mutant 17ΔPST has a 202-bp deletion in this area, beginning at HSV-1 bp 118664 and extending through 188866, including most of the region we studied (all of oligos L1 and L2, half of L3) (Block and Hill, 1997; Bloom *et al*, 1994). 17ΔPST has a markedly impaired reactivation phenotype in animal models.

Neither latent HSV-1 infection nor a corneal UV stimulus appeared to affect STAT1 binding to the distal LAT promoter *in vitro* (Figure 5). These results suggest that the STAT1–LAT promoter complexes formed are stable and unaffected by these various conditions. It was anticipated that ocular UV radiation, a stimulus known to cause HSV-1 reactivation, might drive a change in neuronal STAT1 transcription or binding via interferon signaling. However, the data presented here do not support a direct link between ocular UV and neuronal STAT1 activity at

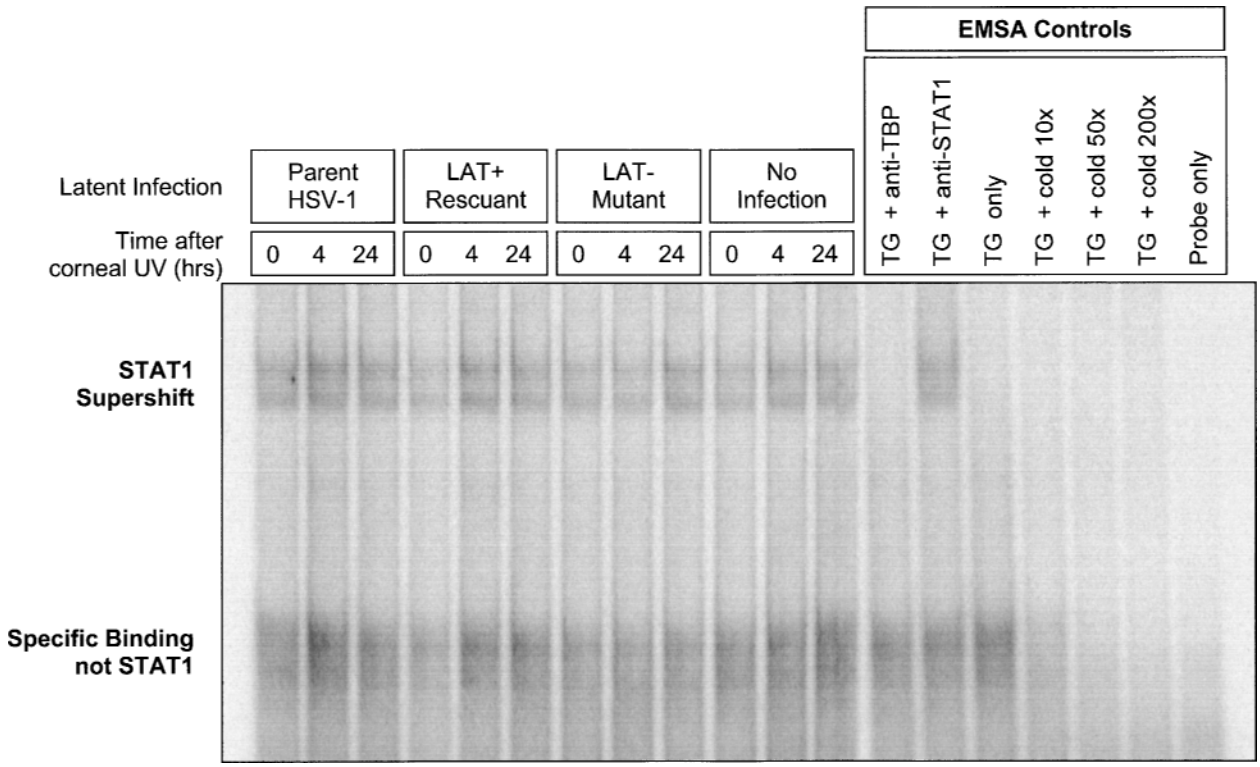

Figure 5 STAT1 supershifting of the L3 oligo in TGs latently infected with HSV-1, with and without LAT expression and reactivation stress. Groups of 3 mice each were latently infected with parent HSV-1 (strain 17-syn+), a LAT-negative mutant (strain 17-ΔPST), or its LAT-expressing rescuant (17-ΔR1). The left TGs from these mice were harvested 0 (unexposed), 4, or 24 h after left corneal UV exposure given at a dose previously determined to cause HSV-1 reactivation in this model. EMSA using oligo L3 as the probe and STAT1 supershifting were performed on nuclear extracts from the harvested TGs. Controls included probe only, cold competitor, and TG nuclear extracts with and without STAT1 antibodies, and TG nuclear extracts with anti–TATA binding protein (TBP) antibodies.

the time points we studied. Whole TGs were used in these experiments, not just the neurons responsible for corneal innervation. Therefore, it is possible that there is a limited effect of ocular UV on STAT1 expression or binding within the few TG neurons that innervate the cornea. Such a limited, specific effect would have been undetectable by the methods used here. Alternatively, ocular UV exposure may exert its immunosuppressive effects only at the corneal surface (McKenzie and Sauder, 1994), leaving STAT1 binding or expression in the TG unaffected. Finally, it is possible that the wrong time points were chosen after UV exposure and that effects on LAT binding are seen much later, perhaps 2 to 4 days after exposure, the time required for HSV-1 to appear at the corneal surface. LAT-producing neurons comprise only 1% to 5% of the sensory neurons in the TG. If LAT did have an effect on STAT1 binding to the HSV-1 genome, but only within LAT-producing neurons, this would have been overwhelmed by the 95+% of neurons that are LAT negative, and, therefore, undetectable in our system. Studies quantitating LAT expression in the neurons responsible for innervating the cornea would be helpful to sort this out. Also, LAT expression in STAT1 knockout mice or cells might help to settle this question.

STATs, intracellular effectors of the immune system, are present within latently infected neurons where they may regulate HSV LAT expression. STAT1 is the transcription factor activated by interferons, antiviral proteins made to halt the spread of viral infections. It was demonstrated here for the first time that activated STAT1 is present in the mouse TG where it is capable of binding the 3′ HSV-1 LAT promoter. These studies are a prelude to further investigation of the role of binding of STAT1 to LAT in HSV-1 neuronal latency and reactivation.

Materials and methods

TG harvests and nuclear extractions

The experiments shown in Figures 2 to 4 were performed using uninfected Balb/c mice. The ganglia were removed and immediately frozen for subsequent nuclear extractions. These animal procedures conform with United States Department of Agriculture (USDA) guidelines and have been approved by the University of Utah Animal Care and Use Committee. TGs harvested from the mice were gently homogenized and suspended using a Kontes pellet pestle (Kontes, Vineland, NJ). The cell suspensions

were washed twice with ice-cold phosphate-buffered saline containing 1 mM phenylmethylsulfonyl fluoride (PMSF), resuspended, and homogenized in 1 ml (low salt) buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 μg/ml aprotinin, 100 μM leupeptin, 1 mM PMSF, 1 mM dithiothreitol [DTT], and 0.5% Nonidet P-40). After centrifugation at 500 $\times$ *g* for 5 min, the cytoplasmic fraction (supernatant) was collected. The nuclear pellets were then washed in buffer A without Nonidet P-40. The samples were homogenized in 50 μl of (high salt) buffer B (10 mM HEPES, pH 7.9, 420 mM NaCl, 25% glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM EGTA, 10 μg/ml aprotinin, 100 μM leupeptin, 1 mM PMSF, and 1 mM DTT) and centrifuged at 13,000 $\times$ *g* for 10 min to remove debris. The supernatant was collected and labeled as nuclear fraction. The nuclear extracts were assayed for protein concentration using the BCA Protein Assay Kit (Pierce, Rockford, Illinois).

HSV-1 infections and ocular UV exposure
TG harvested from groups of three anesthetized mice each before (no infection) or 30 days after left ocular infection with HSV-1 strains 17 syn+ (LAT-expressing parent strain), 17ΔR1 (LAT+ rescuant), or 17ΔPST (LAT deletion mutant). The TGs were taken 0 (unexposed), 4, or 24 h after 250 mJ/cm^2 of ocular UV exposure *in vivo* ($n = 3$ mice = 3 TGs per group), a dose previously shown to induce HSV-1 ocular reactivation in this system (Kriesel *et al*, 1997; Laycock *et al*, 1991).

Probes and antibodies
The published HSV-1 McKrae strain LAT sequence was used (McGeouch *et al*, 1988). The following probes and their complements were synthesized and purified at the University of Utah core facility. A search for STAT1 binding sites (the GAS motif, 5′-**TTNNNNNAA**-3′), the STAT3 consensus binding sequence (5′-***CYGGRAA***-3′), and their complements (5′-**AANNNNNTT**-3′ and 5′-***TTYCCRG***-3′, R = A or G; Y = C or T) was performed (Akira *et al*, 1995; Kriesel *et al*, 1998; Schindler and Darnell, 1995). No mismatches were allowed.

- Oligo L1, 50 bp (HSV-1 oligonucleotide no. 118676): 5′-CCGCG***CCCGGAAA***TCCATTAGGCCG CAGACGAGGAA**AATAAAATT**ACATC-3′
- Oligo L2, 50 bp (HSV-1 oligonucleotide no. 118721): 5′-ACATCACCTACCCACGTGGTGCTGT GGCCTGTTTTTGCTGCGTCATCTCA-3′
- Oligo L3, 65 bp (HSV-1 oligonucleotide no. 118769): 5′-CAGCC**TTTATAAAA**GCGGGGGCGCG GCCGTGCCGATCGCGGGTGGTGCG**AAAGACT*TT CCGG***GCGCG-3′

Polyclonal rabbit anti-mouse STAT1, STAT3, and STAT5b antibodies were obtained from Santa Cruz Biotechnology, Santa Cruz, CA. Supershifting was performed by adding 2 or 8 μg of antibody into the reaction mixture.

Site-directed mutagenesis
Mutation of the (upstream) STAT1 and (downstream) STAT1/3 consensus binding sites was performed to determine whether this would affect the specific binding of the L3 oligo. To switch purines (AG) and pyrimidines (CT), A was changed to C and T to G where possible. The sequence of the parent L3 oligo is shown above. The predicted STAT1 binding site was mutated from TTTATAAAA to GGGCGCCCC. The predicted STAT1/3 binding site was mutated from TTTCCGG to GGGAATT, but this sequence is similar to a frame-shifted STAT3 consensus binding sequence, so it was mutated again to GGGCCTT. Full-length L3 oligonucleotides and their complements with mutated predicted STAT1, STAT1/3, or both binding sites were synthesized and used in the experiments shown.

EMSA
Purified double-stranded DNA oligonucleotides L1 to L3 were 5′ end-labeled using T4 Kinase (Gibco BRL, Grand Island, NY) and [^{32}P]ATP (Amersham Pharmacia Biotech, Piscataway, NJ). A standard count of labeled oligo (50,000 cpm/ml) were annealed and incubated with 0.5 to 3.0 μg of TG nuclear extract, 1.5 ng poly dI-dC (Amersham), 10% glycerol, in 4 μl of binding buffer (Tris-HCl 50 mM, KCl 250 mM, DTT 5 mM, bovine serium albumin [BSA] 1 mg/ml, and glycerol 25%), with a final volume of 20 μl, kept at 23°C for 30 min. The mixture was loaded and run through a 5% nondenaturing polyacrylamide gel. The gel was dried and imaged with a Storm system (Molecular Dynamics, Sunnyvale, CA). Competition studies were performed by adding 10- and 50-fold molar excesses of commercially available STAT1, STAT3, and STAT5b consensus binding oligos (Santa Cruz Biotechnology) into the reaction mixture. Densitometry was performed on the specifically binding bands observed using ImageQuant Version 1.2 (Molecular Dynamics).

References

Ackland-Berglund C, Davido D, Leib D (1995). The roles of cAMP-response element and TATA box expression of the HSV-1 LATs. *Virology* **210:** 141–151.

Ahmed M, Lock M, Miller CG, Fraser NW (2002). Regions of the herpes simplex virus type 1 latency-associated transcript that protect cells from apoptosis in vitro and protect neuronal cells in vivo. *J Virol* **76:** 717–729.

Akira S, Nishio Y, Tanaka T, Inoue M, Matsusaka T, Wang XJ, Wei S, Yoshida N, Kishimoto T (1995). Transcription

factors NF-IL-6 and APRF are involved in gp130-mediated signaling pathway. *Ann N Y Acad Sci* **762:** 15–28.

Block T, Hill J (1997). The latency associated transcripts (LAT) of herpes simplex virus: still no end in sight. *J NeuroVirol* **3:** 313–321.

Bloom D, Devi-Rao G, Hill J, Stevens J, Wagner E (1994). Molecular analysis of HSV-1 during epinephrine induced reactivation of latently infected rabbits in vivo. *J Virol* **68:** 1283–1292.

Cantin E, Tanamachi B, Openshaw H (1999). Role for gamma interferon in control of HSV-1 reactivation. *J Virol* **73:** 3418–3423.

Chatterjee-Kishore M, VanDenAkker F, Stark J (2000). Association of STATs with relatives and friends. *Trends Cell Biol* **10:** 106–111.

Chen X, Schmidt MC, Goins WF, Glorioso JC (1995). Two herpes simplex virus type 1 latency-active promoters differ in their contributions to latency-associated transcript expression during lytic and latent infections. *J Virol* **69:** 7899–7908.

Dawson C (1995). Management of herpes simplex eye diseases. In: *Clinical management of herpes viruses*, Vol. 1. Sacks SL, Strauss SE, Whitley RJ, Griffiths PD (eds). Amsterdam: IOS Press, pp 127–136.

Dobson A, Margolis T, Gomes W, Feldman L (1995). In vivo deletion analysis of the HSV-1 LAT promoter. *J Virol* **69:** 2264–2270.

Drolet BS, Perng GC, Slanina SM, Yukht A, Nesburn AB, Wechsler SL (1998). The region of HSV-1 LAT gene involved in spontaneous reactivation does not encode a functional protein. *Virology* **242:** 221–232.

Fraser NW, Block TM, Spivack JG (1992). The latency-associated transcripts of HSV: RNA in search of function. *Virology* **191:** 1–8.

Halford WP, Gebhardt BM, Carr DJ (1996). Mechanisms of herpes simplex virus type 1 reactivation. *J Virol* **70:** 5051–5060.

Henderson G, Peng W, Jin L, Perng G, Nesburn A, Wechsler S, Jones C (2002). Regulation of caspase 8 and caspase 9-induced apoptosis by the HSV-1 LAT. *J NeuroVirol* **8 (suppl 2):** 103–111.

Hendricks RL, Tumpey TM, Finnegan A (1992). IFN-gamma and IL-2 are protective in the skin but pathologic in the corneas of HSV-1-infected mice. *J Immunol* **149:** 3023–3028.

Hendricks RL, Weber PC, Taylor JL, Koumbis A, Tumpey TM, Glorioso JC (1991). Endogenously produced interferon alpha protects mice from herpes simplex virus type 1 corneal disease. *J Gen Virol* **72:** 1601–1610.

Hill JM, Sedarati F, Javier RT, Wagner EK, Stevens JG (1990). Herpes simplex virus latent phase transcription facilitates in vivo reactivation. *Virology* **174:** 117–125.

Kriesel J, Jones B, Hwang I, Dahms K, Spruance S (2001). Signal transducers and activators of transcription (STATs) are detectable in mouse trigeminal ganglion neurons. *J Interferon Cytokine Res* **21:** 445–450.

Kriesel J, Ricigliano J, Spruance S, Garza H, Hill J (1998). Neuronal reactivation of herpes simplex virus may involve interleukin-6. *J NeuroVirol* **3:** 441–448.

Kriesel JD, Araneo BA, Petajan JP, Spruance SL, Stromatt S (1994). Herpes labialis associated with recombinant human ciliary neurotrophic factor. *J Infect Dis* **170:** 1046.

Kriesel JD, Gebhardt BM, Hill JM, Maulden SA, Hwang IP, Clinch TE, Cao X, Spruance SL, Araneo BA (1997). Anti-Interleukin-6 antibodies inhibit herpes simplex virus reactivation. *J Infect Dis* **175:** 821–827.

Laycock KA, Lee SF, Brady RH, Pepsose JS (1991). Characterization of a murine model of recurrent HSV keratitis induced by ultraviolet B radiation. *Invest Ophthamol Vis Sci* **32:** 2741–2746.

Mador N, Goldenberg D, Cohen O, Panet A, Steiner I (1998). HSV-1 LAT's suppress viral replication and reduce immediate-early gene mRNA levels in a neuronal cell line. *J Virol* **72:** 5067–5075.

McGeouch DJ, Dalrymple MA, Davison AJ, Dolan A, Frame MC, McNab D, Perry LJ, Scott JE, Taylor P (1988). The complete DNA sequence of the long unique region in the genome of HSV-1. *J Gen Virol* **69:** 1531.

McKenzie RC, Sauder DN (1994). Ultraviolet radiation: effects on the immune system. *Ann RCPSC* **27:** 20–26.

Perng G, Jones C, Ciacci-Zanella J, Stone M, Henderson G, Yukht A, Slanina S, Hofman F, Ghiasi H, Nesburn A, Wechsler S (2000a). Virus-induced neuronal apoptosis blocked by HSV LAT. *Science* **287:** 1500–1503.

Perng G, Slanina S, Nesburn A, Wechsler S (1996). The spontaneous reactivation function of the HSV-1 LAT gene resides completely within the first 1.5 kilobases of the 8.3 kilobase primary transcript. *J Virol* **70:** 976–984.

Perng G, Slanina S, Yukht A, Ghiasi H, Nesburn A, Wechsler S (2000b). The LAT gene enhances establishment of HSV-1 latency in rabbits. *J Virol* **74:** 1885–1891.

Perng GC, Dunkel EC, Geary PA, Slanina SM, Ghiasi H, Kaiwar R, Nesburn AB, Wechsler SL (1994). The latency-associated transcript gene of herpes simplex virus type 1 (HSV-1) is required for efficient in vivo spontaneous reactivation of HSV-1 from latency. *J Virol* **68:** 8045–8055.

Perng GC, Maguen B, Jin L, Mott KR, Osorio N, Slanina SM, Yukht A, Ghiasi H, Nesburn AB, Inman M, Henderson G, Jones C, Wechsler SL (2002). A gene capable of blocking apoptosis can substitute for the herpes simplex virus type 1 latency-associated transcript gene and restore wild-type reactivation levels. *J Virol* **76:** 1224–1235.

Rajan P, Gearan T, Fink J (1998). LIF and NGF regulate STAT activation in sympathetic ganglia: convergence of cytokine- and neurotrophin-signaling pathways. *Brain Res* **802:** 198–204.

Sawtell NM (1998). The probability of in vivo reactivation of herpes simplex virus type 1 increases with the number of latently infected neurons in the ganglia. *J Virol* **72:** 6888–6892.

Sawtell NM, Poon DK, Tansky CS, Thompson RL (1998). The latent herpes simplex virus type 1 genome copy number in individual neurons is virus strain specific and correlates with reactivation. *J Virol* **72:** 5343–5350.

Schindler C, Darnell J (1995). Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. *Annu Rev Biochem* **64:** 621–651.

Spruance SL (1995). Herpes simplex labialis. In: *Clinical management of herpes viruses,* Vol. 1. Sacks SL, Strauss SE, Whitley RJ, Griffiths PD (eds). Amsterdam: IOS Press, pp 3–42.

Stevens JG (1990). Transcripts associated with herpes simplex virus latency. *Adv Exp Med Biol* **278:** 199–204.

Thomas S, Gough G, Latchman D, Coffin R (1999). HSV LAT encodes a protein which greatly enhances virus growth,

can compensate for deficiencies in IE gene expression, and is likely to function during reactivation from viral latency. *J Virol* **73:** 6618–6625.

Thomas S, Lilley C, Latchman D, Coffin R (2002). A protein encoded by the HSV-1 2 kb LAT transcript is phosphorylated, localized to the nucleus, and overcomes the repression of expression from exogenous promoters when inserted in the quiescent HSV genome. *J Virol* **76:** 4056–4067.

Zwaagstra JC, Ghiasi H, Nesburn AB, Wechsler SL (1991). Identification of a major regulatory sequence in the LAT promoter of HSV-1. *Virology* **182:** 287–297.