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Glucocorticoid receptor and specificity protein 1 (Sp1) or Sp3 transactivate HSV-1 ICP0 promoter sequences but a GC-rich binding antibiotic, Mithramycin A, impairs reactivation from latency

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ABSTRACT

Glucocorticoid receptor (GR) activation enhances Human alpha-herpes virus 1 (HSV-1) replication and explant-induced reactivation from latency. Furthermore, GR and Krüppel-like factor 15 (KLF15) cooperatively transactivate cis-regulatory modules (CRMs) that drive expression of infected cell protein 0 (ICP0), ICP4, and ICP27. KLF and specificity protein (Sp) family members bind GC-rich or C-rich sequences and belong to the same superfamily of transcription factors. Based on these observations, we hypothesized CRMs spanning the ICP0 promoter are transactivated by GR and Sp1 or Sp3. CRM-A (-800 to -635), CRM-B (-485 to -635), and CRM-D (-232 to -24), but not CRM-C, were significantly transactivated by GR, DEX, and Sp1 or Sp3 in mouse neuroblastoma cells (Neuro-2A). Mutagenesis of Sp1/Sp3 binding sites were important for transactivation of CRM-A and CRM-B. Chromatin immunoprecipitation studies revealed significantly higher levels of GR occupied ICP0 promoter sequences when Sp1 or Sp3 was over-expressed suggesting these transcriptions factors recruit GR to ICP0 CRM sequences. Mithramycin A, an antibiotic that preferentially binds GC-rich DNA and impairs Sp1/Sp3 dependent transactivation and reduced virus shedding during reactivation from latency in mice latently infected with HSV-1. These studies indicate GR and certain stress-induced cellular transcription factors preferentially bind GC rich DNA, which stimulates HSV-1 gene expression and reactivation from latency in trigeminal ganglia of latently infected mice.

1. Introduction

Following acute infection of oral, ocular, and/or nasal cavity, sensory neurons in trigeminal ganglia (TG) and/or neurons in the central nervous system are important sites for herpes simplex virus-1 (HSV-1) latency (Perng, 2010; Al-Dujaili et al., 2011). HSV-1 is the leading cause of infectious blindness worldwide and approximately 40,000 people develop severe monocular visual impairment or blindness each year. Approximately 20 % of latently infected people develop recurrent herpetic stromal keratitis, which is due to HSV-1 reactivation from latency, reviewed in (Newell et al., 1989). Furthermore, reactivation from latency is responsible for most cases of HSV-1 induced encephalitis (Sekizawa and Openshaw, 1984). Hence, the ability of HSV-1 to reactivate from latency is crucial for virus transmission and recurrent disease. Identifying cellular and viral factors that trigger reactivation from latency and lytic cycle viral replication may yield novel anti-viral strategies designed to reduce the incidence of reactivation from latency.

HSV-1 encodes five immediate early (IE) viral mRNAs expressed in the absence of de novo protein synthesis during productive infection: ICP0, ICP4, ICP22, ICP27, and ICP47 (Honess and Roizman, 1974). The viral tegument protein (VP16) interacts with two host transcription factors, Oct1 and host cellular factor 1 (HCF1), to specifically transactivate IE gene expression, reviewed in (Kristie, 2007; Kristie, 2015). ICP0 is a promiscuous activator of promoters, an E3 ubiquitin ligase, reviewed in (Boutell and Everett, 2013; Everett, 2000), and reduces histone occupancy in viral DNA (Cliffe and Knight, 2008). ICP0

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expression also stimulates reactivation from latency (Halford et al., 2001; Halford and Schaffer, 2001), in part because it impairs innate immune responses and promotes viral replication (Linderman et al., 2017).

HSV-1 reactivation from latency in humans correlates with increased stress; including UV light exposure, trauma, and heat stress (Perng, 2010; Cassidy et al., 1997; Glaser et al., 1985; Padgett et al., 1998; Rooney et al., 1992; Jones, 1998; Jones, 2003). Stress generally increases corticosteroid levels, which binds and activates the glucocorticoid receptor (GR) or mineralocorticoid receptor (MR), reviewed in (Oakley and Cidlsowski, 2013). The MR or GR corticosteroid complex enters the nucleus, binds a glucocorticoid response element (GRE), alters chromatin conformation, and activates transcription. In response to stress, GR and Krüppel like factor 15 (KLF15) regulate gene expression via a positive feed-forward loop (Mangan and Alon, 2003; Sasse et al., 2015; Sasse et al., 2013). Notably, primary human gingival fibroblasts treated with the synthetic corticosteroid dexamethasone (DEX) prior to HSV infection yield significantly higher levels of virus relative to controls only infected with HSV-1 (Erlandsson et al., 2002). Our recent studies revealed HSV-1 infection of mouse neuroblastoma cells (Neuro-2A) treated with DEX also yield higher levels of infectious virus (Ostler et al., 2019). Consistent with this study, a corticosteroid specific antagonist (CORT-108,297) significantly reduces HSV-1 replication in Neuro-2A cells but does not reduce cell viability (Harrison et al., 2019). We and others demonstrated DEX accelerates explant-induced reactivation from latency, as judged by increased virus production (Harrison et al., 2019; Du et al., 2012; Du et al., 2011). A mouse strain containing a mutant GR that does not efficiently activate transcription exhibits reduced virus shedding during TG explant-induced reactivation in females but not male mutant mice (Harrison et al., 2023). However, parental C57Bl/6 mice do not exhibit sex-dependent differences during explant-induced reactivation.

The objectives of this study were to test whether GR and specificity protein 1 (Sp1) or Sp3 cooperate with GR to stimulate cis-regulatory modules (CRMs) within the ICP0 promoter. The rational for these studies are the ICP0 promoter and ICP0 CRMs are cooperatively transactivated by GR and KLF15 (Ostler et al., 2019; Wijesekera et al., 2022). Conversely, the effect GR, DEX, and KLF4 or KLF6 had on ICP0 promoter activity was much lower (Ostler et al., 2019). The 18 KLF family members and 9 Sp family members belong to the same super family of transcription factors (Black et al., 2001; Kaczynski et al., 2003). Since the viral genome is GC-rich, it is likely the KLF and Sp1 superfamily of transcription factors have multiple effects, positively and negatively effects, on viral gene expression. These current studies provide evidence GR and Sp1 or Sp3 transactivate ICP0 promoter sequences, and Sp1 consensus binding sites were important. Finally, an antibiotic that preferentially interacts with GC-rich DNA, Mithramycin A (Campbell et al., 1994), reduced explant-induced reactivation from latency in a mouse model of latency, These studies support the premise that stress-induced cellular transcription factors that bind GC-rich sequences, including Sp1 and KLF family members, expedite reactivation from latency.

2. Results

2.1. GR, DEX, and Sp1 or Sp3 transactivate ICP0 cis-regulatory modules

The ICP0 gene is located within the long repeats (TR_L and IR_L) of the HSV-1 genome (Fig. 1A), and the ICP0 promoter (Fig. 1B) contains numerous consensus transcription factor binding sites (Figure C) (Kushnir et al., 2009). To identify ICP0 non-coding sequences that stimulate transcription, four ICP0 cis-regulatory modules (CRMs) upstream of the TATA box were examined (A, B, C, and D; Fig. 1B) (Wijesekera et al., 2022). These CRMs were cloned upstream of a minimal promoter in the luciferase reporter construct of the luciferase reporter plasmid (pGL4.24[luc2P/minP]). Transcriptional activation by GR, DEX treatment, and Sp1 or Sp3 were examined in Neuro-2A cells. Neuro-2A cells were used because they have neuronal like properties, can be differentiated into dopamine-like neurons, and approximately 50 % of Neuro-2A cells are transfected (Tremblay et al., 2010; El-Mayet et al., 2017). A mouse GR-α expression plasmid was transfected into Neuro-2A cells because endogenous GR proteins expressed in Neuro-2A cells are smaller than GR-α. GR mRNA is differentially spliced yielding distinct GR proteins, and GR-α stimulates transcription better than the other GR isotypes (El-Mayet et al., 2017; El-Mayet and Jones, 2024). Hereafter, we denote the GR-α expression construct as GR.

Relative to the CRM-A construct alone, co-transfection with GR, Sp1 and DEX treatment significantly increased CRM activity 5-fold (Fig. 2A). CRM activity of B and D fragments were also significantly increased when co-transfected with GR, Sp1 and treated with DEX by ~3.5 and ~1.5- fold respectively relative to the respective CRM constructs alone. Conversely, the C fragment luciferase construct did not significantly change CRM activity levels even when co-transfected with GR and/or Sp1, regardless of DEX treatment. Similar trends were observed when ICP0 CRM constructs were co-transfected with GR, Sp3, and DEX (Fig. 2B). Although co-transfection of the CRM-C fragment with GR, Sp3,

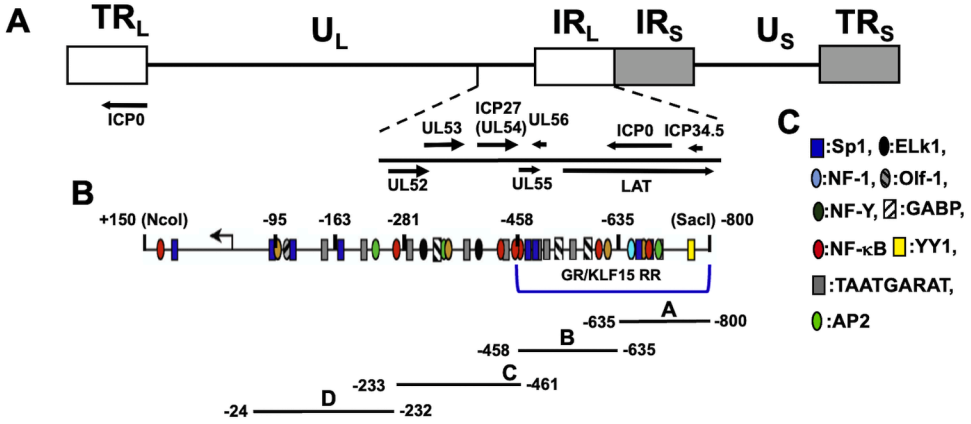

Fig. 1. Location of ICP0 gene within IR_L and TR_L repeats of the HSV−1 genome and cis-regulatory modules within the ICP0 promoter.
Panel A: Schematic of HSV-1 genome. Unique long (U_L) and unique short (U_S) segments are flanked by long internal or terminal repeats (IR_L and TR_L: white rectangles) and short internal or terminal (IR_S and TR_S: gray rectangles). ICP0 and other genes in IR_L are shown. A copy of ICP0 is also present in the TR_L. **Panel B:** Schematic of ICP0 promoter, and location of cellular transcription factor binding sites (**Panel** C) relative to the start site of ICP0 mRNA (arrow). Four ICP0 CRM fragments (A–D) were cloned upstream of the pGL4.24[luc2/minP] firefly luciferase reporter plasmid (Promega: Madison, WI) as previously described (Panel B) (Wijesekera et al., 2022).

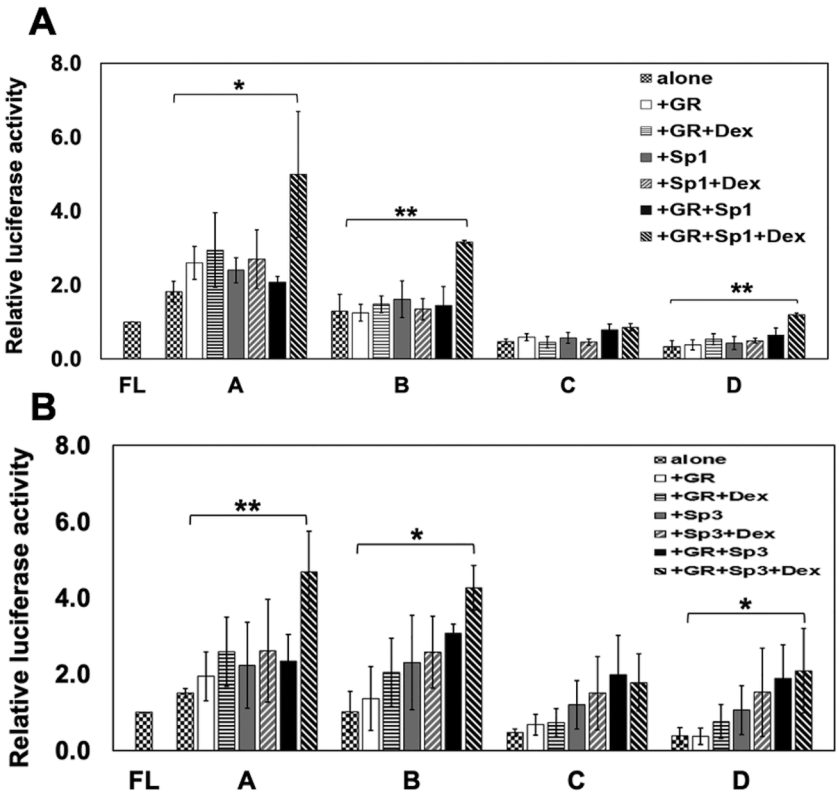

Fig. 2. Transactivation of ICP0 CRM constructs by GR, DEX, and Sp1 or Sp3.
Panel A: Neuro-2A cells were transfected with a plasmid encoding *Renilla* luciferase (0.05 μg DNA), denoted CRM fragment constructs (0.5 μg DNA), a plasmid that expresses the mouse GR-α protein (1.0 μg DNA), DEX and Sp1 (1.0 μg DNA) as described in the Materials and Methods. **Panel B**: Neuro-2A cells were transfected with a plasmid encoding *Renilla* luciferase (0.05 μg DNA), denoted CRM fragment constructs (0.5 μg DNA), a plasmid expressing the mouse GR-α protein (1.0 μg DNA), DEX and Sp3 (1.0 μg DNA) as described in the Materials and Methods. Empty vector was included in certain samples to maintain the same amount of DNA in each sample. At 24 h post-transfection, MEM was changed, and denoted cultures were treated with water-soluble DEX (10 μM). Cells were harvested 48 h post-transfection, and luciferase activity measured. Basal transcriptional activity of cells transfected with the respective CRM construct plus empty expression vector normalized to 1, and fold activation values for other samples calculated. The results are the average of three separate experiments, and error bars denote standard errors. Asterisks denote significant differences (*, $p \leq 0.05$; **, $p \leq 0.01$) between the indicated treatments as determined by Student's *t*-test.

and DEX treatment yielded higher results than just the CRM-C construct, the differences were not statistically significant relative to CRM C alone.

2.2. Localization of CRM A fragment activity

The ability of GR, DEX, and Sp1 or Sp3 to transactivate the wt-A fragment was compared to CRM-A mutants in Neuro-2A cells (Fig. 3A). Wt-A fragment encompasses −800 to −635 and contains three consensus Sp1 binding sites, two complements of a consensus Sp1 binding site (denoted cSp1: CCGCCC), and a 15-base alternating purine/pyrimidine motif (CGCGCATATATACGCTTG), which has the potential to form Z-DNA (Fig. 3A). The Sp1 and cSp1 binding sites have the potential to form a stem-loop structure. CRM fragment A1 contains the 5′ half of the wt-A CRM fragment, which contains a 15-base alternating purine/pyrimidine and one Sp1 binding site. Transactivation of the A1 construct was significantly reduced in Neuro-2A cells relative to the wt-A fragment alone regardless of whether GR and Sp1 were cotransfected regardless of DEX treatment (Fig. 3B). Results obtained were the same for A1 when co-transfected with GR and/or Sp3, regardless of DEX treatment in Neuro-2A cells (Fig. 3C). GR, KLF15, and DEX also did not transactivate the A1 CRM (Wijesekera et al., 2022).

Fragment A2 is derived from the 3′ half of the wt-A fragment, which contains an Sp1 and cSp1 binding site. In contrast to the A1 CRM construct, transcriptional activity of A2 alone was not significantly different than the wt-A fragment (Fig. 3B). Furthermore, A2 was significantly transactivated by GR+DEX or GR+Sp1+DEX by 3–4-fold when compared to the A2 construct alone. Although co-transfection with GR and Sp3 yielded ~4-fold increase regardless of DEX treatment, it was not statistically different because there was high variability from experiment to experiment (Fig. 3C).

Fragment A3 contains all Sp1 and cSp1 binding sites but lacks the 15-base alternating purine/pyrimidine motif. Fragment Z encompasses −762 to −635, contains the alternating purine/pyrimidine motif,and all Sp1/cSp1 binding sites. Fragments Z and AΔSp1 exhibited similar CRM activity, even when co-transfected with GR and/or Sp1 or Sp3 or regardless of DEX treatment. The AΔSp1 construct spans the same sequences as the wt-A fragment but contains mutations in all Sp1 and cSp1 binding sites. Like A2, CRM activity of A3, Z, or AΔSp1 did not significantly change when transfected alone compared to the wt-A fragment. Co-transfection with GR and/or Sp1 or Sp3, regardless of DEX treatment, did not significantly increase CRM activity of the respective fragments when compared to wt-A CRM fragment. Although these results indicated that mutating the Sp1 and cSp1 binding sites in the A fragment were important for GR, DEX, and Sp1 or Sp3 mediated transactivation, sequences between −800 to −762 were also important.

2.3. Transactivation of fragment B mutants by GR, DEX, and Sp1 or Sp3

The CRM-B fragment was further examined because it contains two consensus Sp1 and three C-rich Sp1 binding sites (cSp1; Fig. 4A). The Sp1 and cSp1 binding sites are crucial for GR-, KLF15-, and/or DEX-mediated transactivation (Wijesekera et al., 2022), suggesting they are important for Sp1 and Sp3 mediated transactivation. Basal transcriptional activity of B-ΔSp1, B-ΔcSp1, or B-ΔAll was not significantly

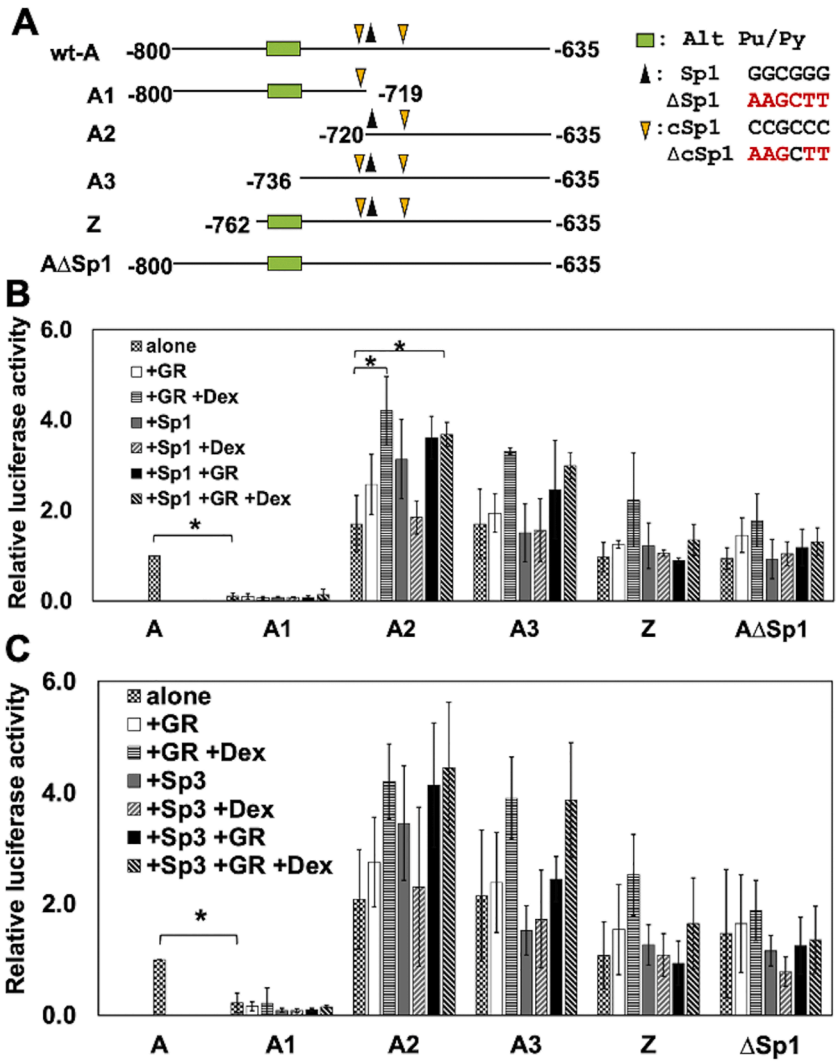

Fig. 3. Transactivation of ICP0 CRM-A mutants by GR, DEX, and Sp1 or Sp3.
Panel A: Schematic of CRM-A wt fragment and mutants that were previously described (Wijesekera et al., 2022).
Panel B: Neuro-2A cells were transfected with a plasmid encoding *Renilla* luciferase (0.05 μg DNA), denoted CRM-A fragment constructs (0.5 μg DNA), a plasmid that expresses the mouse GR-α protein (1.0 μg DNA), DEX and Sp1 (1.0 μg DNA) as described in the Materials and Methods. **Panel C**: Neuro-2A cells were transfected with a plasmid encoding *Renilla* luciferase (0.05 μg DNA), denoted CRM-A fragment constructs (0.5 μg DNA), a plasmid expressing the mouse GR-α protein (1.0 μg DNA), DEX and Sp3 (1.0 μg DNA) as described in the Materials and Methods. Empty vector was included in certain samples to maintain the same amount of DNA in each sample. At 24 h post-transfection, MEM was changed, and the denoted cultures were treated with water-soluble DEX (10 μM). Cells were harvested 48 h post-transfection, and luciferase activity measured. Basal transcriptional activity of cells transfected with the respective CRM construct plus empty expression vector normalized to 1, and fold activation values for other samples calculated. The results are the average of three separate experiments, and error bars denote standard errors. Asterisks denote significant differences (*, $p \leq 0.05$; **, $p \leq 0.01$) between the indicated treatments as determined by Student's *t*-test.

different than the wt-B fragment alone (Fig. 4B). Cotransfection of B-ΔSp1 with GR, Sp1, and DEX treatment significantly increased transcriptional activity. Conversely, transcriptional activity of B-ΔcSp1 or B-ΔAll was not significantly increased by GR, Sp1, regardless of DEX treatment. Notably, GR, Sp3, and DEX treatment significantly increased transcriptional activity of B-ΔSp1 or B-ΔcSp1. However, transcriptional activity of the B-ΔAll construct was not significantly increased when cotransfected with GR, DEX, and Sp3 (Fig. 4C). These findings suggest that the three cSp1 binding sites are important for GR, DEX, and Sp1-mediated transactivation of the B fragment, but not for GR, DEX, and Sp3. When all Sp1 and cSp1 binding sites were mutated (B-ΔAll), transcriptional activity was not significantly higher when transfected with GR, DEX, and Sp1 or Sp3.

Since CRM-C was not transactivated by GR, DEX, and/or Sp1/Sp3, mutants in CRM-C were not examined. CRM-D mutants were not examined because transactivation was only ~2-fold by GR, DEX, and/or Sp1/Sp3.

2.4. *Sp1 and Sp3 occupy ICP0 promoter sequences in Neuro-2A cells*

Chromatin immunoprecipitation (ChIP) studies were performed in Neuro-2A cells transfected with the FL ICP0 construct using Sp1- or Sp3-specific antibodies. Since previous studies demonstrated GR binds to the ICP0 promoter (Ostler et al., 2021), the focus of these studies was to test whether Sp1 or Sp3 occupy sequences within the FL ICP0 promoter. Sp1 (Fig. 5B; black columns) and Sp3 (Fig. 5E; black columns), and GR (white columns) occupied ICP0 promoter at higher levels when compared to the non-specific isotype control antibody when just an empty expression vector was co-transfected with the FL ICP0 promoter construct. Occupancy of GR and Sp1 (Fig. 5C) or Sp3 (Fig. 5F) to ICP0 promoter sequences was significantly increased when the Sp1 or Sp3 expression plasmid was co-transfected with the ICP0 promoter. Sp1

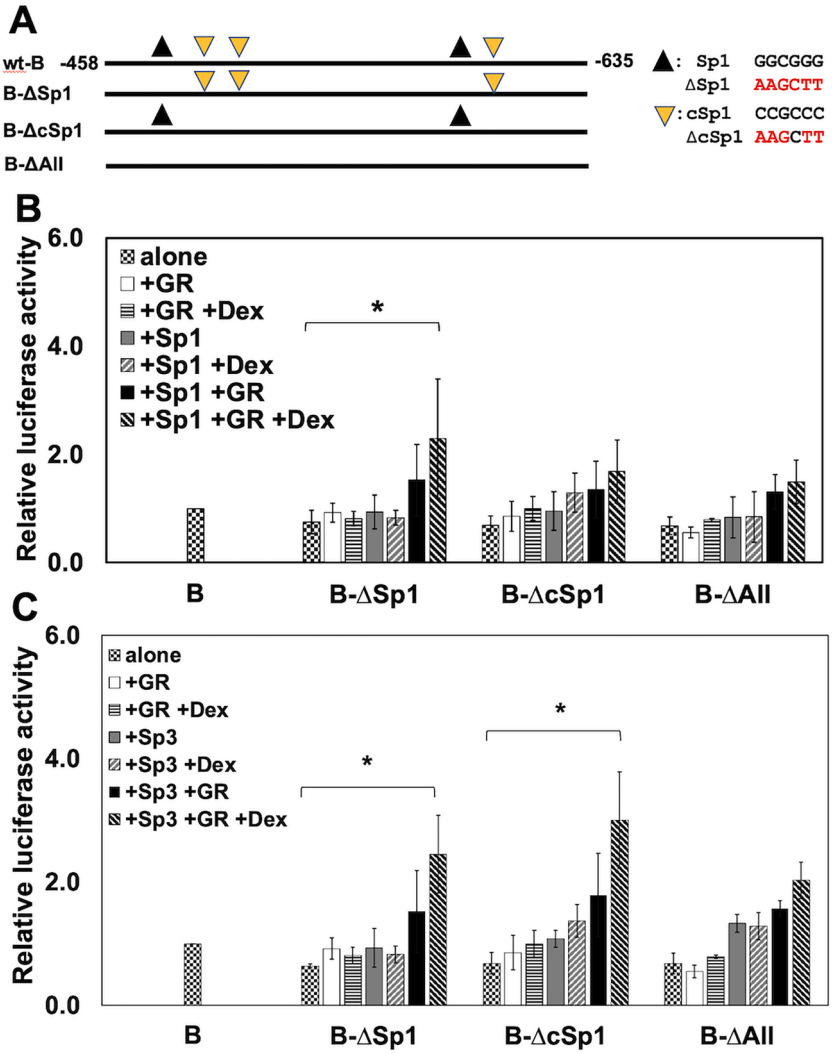

Fig. 4. Transactivation of ICP0 CRM-B mutants by GR, DEX, and Sp1 or Sp3.
Panel A: Schematic of CRM-B wt fragment and mutants that were previously described.
Panel B: Neuro-2A cells were transfected with a plasmid encoding *Renilla* luciferase (0.05 μg DNA), denoted CRM-B fragment constructs (0.5 μg DNA), a plasmid that expresses the mouse GR-α protein (1.0 μg DNA), DEX and Sp1 (1.0 μg DNA) as described in the Materials and Methods. **Panel C**: Neuro-2A cells were transfected with a plasmid encoding *Renilla* luciferase (0.05 μg DNA), denoted CRM-A fragment constructs (0.5 μg DNA), a plasmid expressing the mouse GR-α protein (1.0 μg DNA), DEX and Sp3 (1.0 μg DNA) as described in the Materials and Methods. Empty vector was included in certain samples to maintain the same amount of DNA in each sample. At 24 h post-transfection, MEM was changed, and the denoted cultures treated with water-soluble DEX (10 μM). Cells were harvested 48 h post-transfection, and luciferase activity measured. Basal transcriptional activity of cells transfected with the respective CRM construct plus empty expression vector normalized to 1, and fold activation values for other samples calculated. The results are the average of three separate experiments, and error bars denote standard errors. Asterisks denote significant differences (*, $p \leq 0.05$; **, $p \leq 0.01$) between the indicated treatments as determined by Student's *t*-test.

occupancy also increased significantly when transfected with GR and cells treated with DEX (Fig. 5D): however, GR occupancy was not significantly different compared to cells transfected with Sp1. When the ICP0 promoter was transfected with GR, Sp3 and DEX (Fig. 5G), Sp3 occupancy was not significantly different compared to cells co-transfected with just Sp3. Agarose gels of the ChIP studies (Supplementary Figure 1) are consistent with the scanning of the PCR products. In summary, this study demonstrated when Sp1 or Sp3 were over-expressed GR occupancy of ICP0 promoter sequences significantly increased even in the absence of DEX treatment.

2.5. *Mithramycin A impairs explant-induced reactivation*

Recent studies demonstrated bovine herpesvirus 1 (BoHV-1) (El-Mayet and Jones, 2024) and HSV-1 (El-mayet et al., 2024) replication in cultured cells were significantly reduced by Mithramycin A. Furthermore, Mithramycin A significantly reduced ICP0 promoter activity in Neuro-2A cells (El-mayet et al., 2024). Notably, the ability of Sp1 to stimulate the BoHV-1 immediate early transcription unit 1 (IEtu1) promoter was also significantly reduced by Mithramycin A (El-Mayet and Jones, 2024). Mithramycin A is an anti-tumor drug that binds with high affinity to GC-rich DNA sequences, which culminates in displacement of Sp1 family transcription factors and subsequently inhibiting transcription (Miller et al., 1987; Choi et al., 2014; Ray et al., 1989).

To evaluate the effect Mithramycin A has on HSV-1 reactivation from latency, TG from mice latently infected with HSV-1 were explanted in MEM + 2 % charcoal-stripped fetal bovine serum (FBS) in the presence of DEX to accelerate virus reactivation (Du et al., 2012; Harrison et al., 2023). Where indicated, 1 or 4 μM of Mithramycin A was added to TG explants. Aliquots (1 ml) of supernatant were collected at 4, 6, 8, and 10 days after explant to measure shedding of infectious HSV-1. In male control mice, virus shedding from TG cultures was readily detected day 6 after explant and continued until day 10 (Fig. 6). Conversely, viral

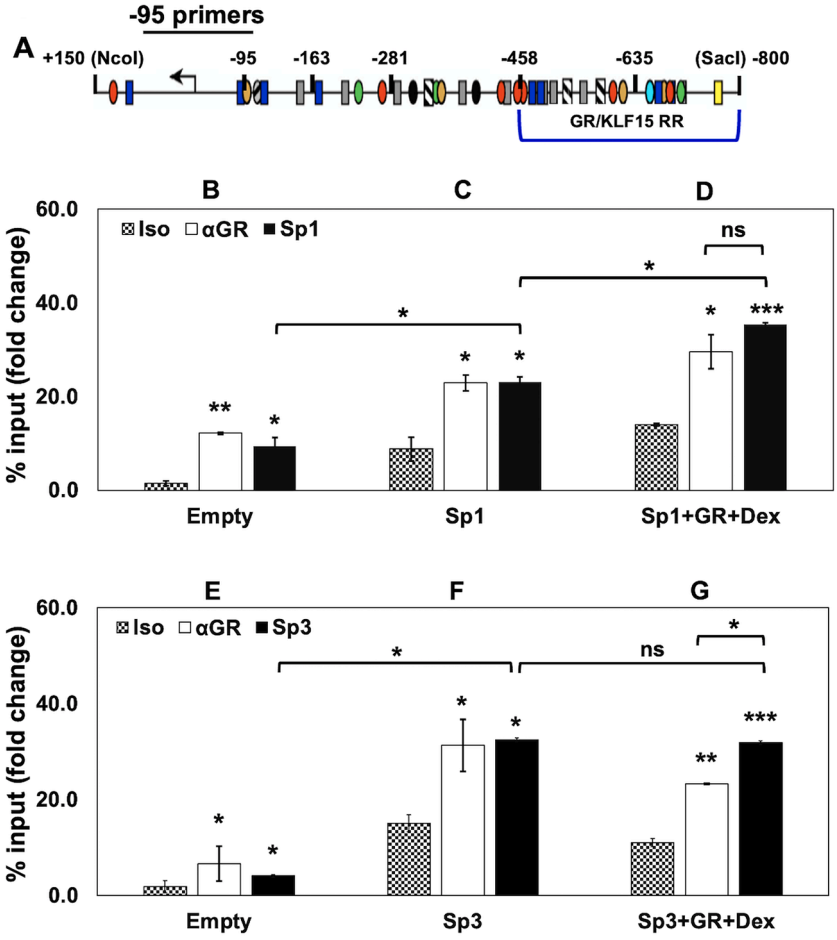

Fig. 5. ChIP studies to examine GR and Sp1 or Sp3 that occupy the ICP0 promoter.
Panel A. Schematic of ICP0 promoter that includes sequences amplified by the PCR primer used in this study.
Panels B and E: Neuro-2A cells were grown in MEM containing 2 % charcoal-stripped FBS following transfection with plasmid containing just the FL ICP0 promoter construct (1.5 μg DNA). **Panel C**: Neuro-2A cells were grown in MEM containing 2 % charcoal-stripped FBS following transfection with plasmids containing the FL ICP0 promoter construct (1.5 μg DNA) and a Sp1 expression plasmid (3.0 μg DNA).
Panel D: Neuro-2A cells were grown in MEM containing 2 % charcoal-stripped FBS followed by transfection with plasmids containing the FL ICP0 promoter construct (1.5 μg DNA), Sp1 expression plasmid (3.0 μg DNA), GR-α expression plasmid (3.0 μg DNA), and DEX.
Panel F: Neuro-2A cells were grown in MEM containing 2 % charcoal-stripped FBS following transfection with plasmids containing the FL ICP0 promoter construct (1.5 μg DNA) and a Sp3 expression plasmid (3.0 μg DNA).
Panel G: Neuro-2A cells were grown in MEM containing 2 % charcoal-stripped FBS followed by transfection with plasmids containing the FL ICP0 promoter construct (1.5 μg DNA), Sp3 expression plasmid (3.0 μg DNA), GR-α expression plasmid (3.0 μg DNA), and DEX. Designated samples were treated with DEX (10 μM) for 4 h prior to harvesting cells (48 h after transfection). Cells were cross-linked with formaldehyde and harvested as described in the materials and methods. ChIP was performed as described in the materials and methods using the isotype control antibody, GR antibody, Sp1 antibody, or Sp3 antibody. Target DNA was amplified using PCR primers that amplify the ICP0 −**95** region (forward, 5′-GCCATTGGGGGAATCGTC-3′; reverse, 5′-TGTGGTGATGCGGAGAGG-3′) that were previously described (Ostler et al., 2019). Individual bands were quantified using Image Lab software and presented as a percentage of the input sample, representing 13.3 % of the cell lysate used for each sample. Data presented are the means from two separate transfection studies that were performed on different days. Asterisks denote a significant difference between the GR-, Sp1-, or Sp3-specific antibody and respective isotype control, and as indicated between samples (*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ns, not significant). Student's *t*-test was performed to determine significant differences.

shedding was not readily detected in TG cultures of male mice treated with 1 or 4 uM Mithramycin A.

In control female mice, viral shedding from TG cultures was detected as early as 4 days after explant and increased as a function of time until day 10. In contrast, viral shedding was not readily detected in TG cultures prepared from female mice treated with 1 or 4 uM Mithramycin A up to day 10 after explant. In summary, these studies revealed Mithramycin A significantly signficantly reduced viral shedding in TG cultures from male and female mice during explant-induced reactivation from latency.

3. Discussion

With the exception of LAT, the HSV-1 genome is relatively quiescent during latency, in large part because lytic cycle viral genes exist as heterochromatin (Cliffe et al., 2013; Knipe and Cliffe, 2008). Surprisingly, low levels of viral lytic cycle gene expression were reported to be expressed in TG neurons during latency (Ma et al., 2014; Margolis et al., 2007). However, viral protein synthesis and virus shedding were not readily detected. For "successful" reactivation from latency to occur, extensive viral gene expression and virus production must occur. ICP0, ICP4, or VP16 expressed from an adenovirus vector triggers reactivation from latency in primary cultures of latently infected trigeminal ganglion cells (Halford et al., 2001). These same viral transcriptional regulators

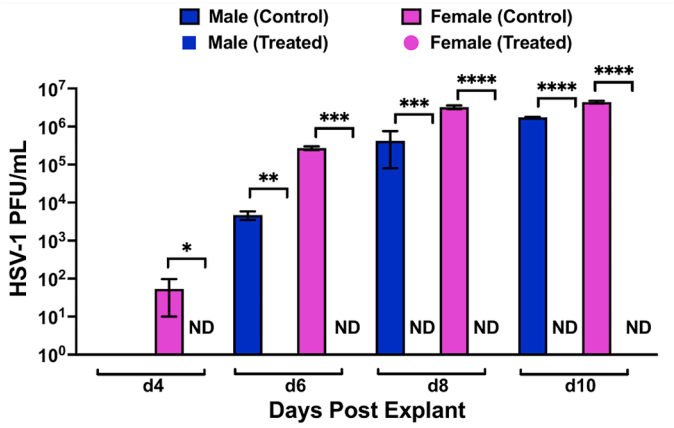

Fig. 6. Effect of Mithramycin A on DEX-induced reactivation in latently infected TG explants. TG from C57Bl/6 mice latently infected with wt HSV-1 McKrae were harvested ~30 dpi (latency). TG were minced and explanted in MEM with l-glutamine, antibiotics, 2 % charcoal-stripped FBS, and 10 μM DEX. Where indicated, 1 or 4 μM Mithramycin A reconstituted in methanol was added to TG explants. At 4, 6, 8, or 10 days post explant, aliquots (1 mL) of supernatant were removed and used to plaque for infectious virus on Vero monolayers. Data are shown as mean ±SEM for duplicate wells of triplicate experiments using the same aliquots. Asterisks denote significant difference (*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$) calculated by Students *t*-test.

For the control group, one experiment was performed with 5 males and 5 female C57Bl/6 mice latently infected with HSV-1. Plaque assays were conducted 3x in duplicate wells from the same sample. These results are consistent with previous studies (Harrison et al., 2019; Harrison et al., 2023). 1 uM Mithramycin A treated explanted TG came from 1 experiment with 5 male or female mice. For the 4 uM Mithramyicin A treated TG explants, 2 independent experiments were performed using 4 or 5 male or female mice in each experiment. Plaque assays were conducted 3x in duplicate wells from the same aliquot/sample. **ND:** no plaques were detected.

are detected during early stages of explant-induced reactivation from latency but not prior to explant (Harrison et al., 2019; Harrison et al., 2023) suggesting expression of one or more of these viral genes can trigger reactivation from latency. Based on the finding that DEX and GR activation accelerates explant-induced reactivation from latency (Harrison et al., 2019), cellular transcription factors that interact with GC-rich promoters are predicted to stimulate promoters that drive expression of VP16, ICP0, ICP4, viral transcriptional regulators subsequently activate lytic cycle viral gene expression and virus production. KLF family members, KLF4, KLF6, KLF15, promyelocytic leukemia zinc finger (PLZF) (Workman et al., 2012; Sinani et al., 2013), and Sp3 (El-mayet et al., 2024) are induced in TG neurons following treatment with the synthetic corticosteroid DEX. Furthermore, these transcription factors cooperate with GR to transactivate CRMs within ICP0, ICP4, or ICP27 promoters (Ostler et al., 2019; Wijesekera et al., 2022; OStler and Jones, 2021; Ostler et al., 2021).

GR, Sp1 or Sp3, and DEX treatment were previously demonstrated to transactivate a full-length ICP0 promoter (−800 to + 150) luciferase reporter construct (El-mayet et al., 2024). These studies revealed CRM-A and CRM-B were significantly transactivated by GR, Sp1 or Sp3, and DEX treatment: however, CRM-C was not significantly transactivated and the CRM-D construct was only transactivated ~2-fold. CRM-C and CRM-D fragments contain consensus Sp1 binding (Fig. 1B) suggesting sequences adjacent to Sp1/Sp3 binding sites are important for transactivation. Mutating the GC-rich Sp1 binding site and C-rich consensus Sp1 binding sites in the CRM-A construct significantly reduced GR, DEX, and Sp1 or Sp3 mediated transcriptional activation when compared to mutating just the GC-rich Sp1 binding site or both C-rich binding sites. Consistent with CRM-A, mutating both GC-rich and the three C-rich Sp1 binding sites significantly reduced GR, Sp1 or Sp3, and DEX mediated transactivation of the CRM-B fragment. However, mutating the three C-rich Sp1 binding sites impaired GR, Sp1, and DEX mediated transactivation whereas mutating just the GC-rich Sp1 binding sites in the CRM-B fragment did not significantly reduce GR, Sp3, and GR mediated transactivation. Notably, the bovine herpesvirus 1 (BoHV-1) immediate early transcription unit 1 (IEtu1) promoter is cooperatively transactivated by GR and Sp1, in part because these transcription factors are stably associated (El-Mayet and Jones, 2024). These observations imply additional GC-rich herpesvirus promoters can also be transactivated by GR and Sp1.

Sp1 and Sp3 are expressed in most cells and are generally believed to have the same transcriptional activation properties. However, this study and other studies revealed there are differences between Sp1 and Sp3. For example, significantly more TG neurons expressed Sp3 during early stages of DEX-induced explant-induced reactivation (El-mayet et al., 2024) whereas Sp1+ TG neurons was only a modest increase in female C57Bl/6 mice. Furthermore, Sp1 is important for early stages of hematopoietic morphogenesis in mice (Gilmour et al., 2014) whereas Sp3 (Bouwman et al., 2000) is important for post-natal survival and tooth development. Notably, Sp1 over-expression has been reported to induce (Chuang et al., 2009; Kavurma and Khachigian, 2003) apoptosis whereas other studies concluded Sp1 and Sp3 impair apoptosis (Ryu et al., 2003). Since apoptosis was reported to accelerate reactivation from latency (Du et al., 2012; Hunsperger and Wilcox, 2003) we suggest Sp1 and Sp3 expression can enhance reactivation by stimulating HSV-1 replication (El-mayet et al., 2024) or inducing apoptosis.

Surprisingly, the ICP0, ICP4, and ICP27 CRMs do not contain a consensus GRE suggesting binding of GR to these promoters depends on stable interactions with KLF15 (El-Mayet et al., 2017; Takeda et al., 2016), Sp1 (El-Mayet and Jones, 2024), and/or other transcriptional coactivators that tether GR to these CRMs. This prediction is supported by the finding that mutating Sp1 binding sites impaired GR and KLF15 mediated transactivation of the ICP4 and ICP27 CRMs and GR binding (OStler and Jones, 2021; Ostler et al., 2021). Like the results for ICP0 CRMs, an ICP4 CRM upstream of the TATA box was transactivated by GR, DEX, and Sp1 or Sp3 (Santos and Jones, unpublished data). In contrast to the three IE promoters, the VP16 CRM upstream of the VP16 TATA box contains a ½ GRE that is crucial for GR and Slug mediated transactivation (Santos et al., 2023). Furthermore, mutating the 3 Sp1 binding sites in the VP16 CRM did not significantly reduce GR and Slug-mediated transcriptional activity of the VP16 CRM. Hence, we suggest GR interactions with the ½ GRE and binding of Slug to the consensus enhancer (E)-box are important for recruiting other transcription factors necessary for increased VP16 expression.

Mithramycin A significantly reduces HSV-1 (El-mayet et al., 2024) and bovine herpesvirus 1 (BoHV-1) (El-Mayet and Jones, 2024) replication in part because Mithramycin A specifically interacts with GC-rich double stranded DNA (Campbell et al., 1994), impairs Sp1 dependent transcription (Miller et al., 1987), and reduces Sp1 steady state protein levels (Choi et al., 2014). Mithramycin A also inhibits Sp1 and Sp3 binding to the dynamin promoter, which correlates with reduced promoter activity (Yoo et al., 2002). Consistent with these observations, the simian virus 40 (SV40) early promoter contains GC-rich sequences comprised of several Sp1 binding sites and Mithramycin A inhibits this promoter (Ray et al., 1989). Based on these observations, we anticipated Mithramycin A would reduce virus shedding during explant-induced reactivation from latency. However, we did not expect Mithramycin A to reduce virus shedding to the point that infectious virus was not readily detected during explant-induced reactivation from latency. Although this finding warrants additional studies, this result demonstrated Mithramycin A has the potential to impair reactivation from latency and virus spread. Since there are no commercially available HSV-1 or HSV-2 vaccines, Mithramycin A may be an alternative antiviral drug. In summary, these studies demonstrated GR, stress-induced KLF family members, and/or Sp1/Sp3 play important roles during reactivation from latency.

3.1. Materials and methods

Cells and Virus Cultures: Mouse neuroblastoma cells (Neuro-2A; ATCC, CCL-131) and Monkey kidney cells (Vero; ATCC, CCL-81) were obtained from American Type Culture Collection ATCC (Manassas, VA USA). Cells were grown in Minimal Essential Media (MEM) supplemented with 10 % fetal bovine serum (FBS), 2 mM l-glutamine, Streptomycin (100 mg/mL), and penicillin (10 U/ml).

The HSV-1 McKrae strain was obtained from the late Dr. Steven Wechsler (University of California, Irvine, CA, USA), and stock cultures were prepared in African green monkey kidney (Vero) cells.

Transfection and dual-luciferase reporter assay: Neuro-2A cells ($\sim 8 \times 10^5$) were seeded into 60-mm dishes containing MEM (Corning; catalogue no 15–010-CV) with 10 % FBS at 24–48 h prior to transfection until 70–80 % confluence was reached. Two hours before transfection, the medium was replaced with MEM containing 2 % charcoal-stripped FBS lacking antibiotics. Cells were co-transfected using Lipofectamine 3000, according to the manufacturer's instructions, with the designated promoter firefly luciferase gene reporter construct (0.5 μg plasmid DNA) and a plasmid encoding Renilla luciferase under the control of a minimal herpesvirus thymidine kinase (TK) promoter (50 ng DNA). Where indicated, plasmids expressing GR, Sp1, or Sp3 (1.0 μg plasmid DNA) were included in the transfection mixture. To maintain equal amounts of plasmids in transfection mixtures, empty expression vector was added as needed. Transfection mixtures were incubated at 37 °C in 5 % CO_2. At 24 h after transfection, certain cultures were treated with water-soluble DEX (10 μM) (Sigma; catalogue no D2915). Forty-eight hours after transfection, cells were harvested, and protein extracts subjected to a dual-luciferase assay using a commercially available kit (Promega catalogue no E1910). Luminescence was measured using a GloMax 20/20 luminometer (Promega; catalogue no E5331).

Plasmids: The full-length ICP0 promoter (−800 to +150) was previously described , and provided by the late Dr. Priscilla Schafer (Kushnir et al., 2009). The respective CRM constructs were synthesized by Genscript and inserted into pGL4.23[luc2/minP] at SacI and XhoI unique restriction enzyme sites. A mouse GR-α expression vector was obtained from John Cidlowski, NIH. The Sp1 (plasmid #27,264) and Sp3 (plasmid #24,541) expression vectors were purchased from Addgene. All plasmids were prepared from bacterial cultures by alkaline lysis and two rounds of cesium chloride centrifugation.

Chromatin Immunoprecipitation (ChIP) Assay: Neuro-2A were cultured in 100 mm dishes until ~80 % confluency. At 2 h prior to transfection, cells were washed with PBS and antibiotic free media with 2 % stripped FBS was added. Cells were transfected with pGL4.23 [luc2P/minP] plasmid containing FL ICP0 promoter construct (1.5 μg) using Lipofectamine 3000 (Invitrogen) according to manufacturer's instructions. Where indicated, cells were co-transfected with GR-α (3 μg DNA) and Sp1 (3 μg DNA) or Sp3 (3 μg DNA). Designated cultures were treated with DEX (10 μM) for 4 h prior to harvesting cells. At 48 h post-transfection, cells were formaldehyde crosslinked and harvested for ChIP.

Formaldehyde cross-linked cells were lysed in buffer A (50 mM HEPES [pH 7.5], 140 mM NaCl, 1 mM EDTA [pH 8.0], 1 % Triton X-100, 0.1 % sodium deoxycholate, 0.1 % SDS) containing protease inhibitors. Cell lysate was sonicated to shear DNA and cleared with agarose beads and salmon sperm DNA (Millipore) to reduce non-specific binding. Input samples were collected (50 μl) from the precleared sonicated DNA-protein complexes (750 μl sample).

Cleared samples were immunoprecipitated and incubated with GR-α (Cell Signaling; catalogue no 3660S), Sp1 (Invitrogen; catalogue no PA5–29,165), Sp3 (Invitrogen; catalogue no PA5–78,176), or non-specific isotype control rabbit IgG (Abcam; catalogue no ab171870) in radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris–HCl [pH 8.0], 150 mM NaCl, 2 mM EDTA [pH 8.0], 1 % NP-40, 0.5 % sodium deoxycholate, 0.1 % SDS). Protein A-conjugated magnetic beads (Dynabeads) (Invitrogen; catalogue no 10002D) were added to samples and allowed to incubate at 4 °C. Beads were washed twice with a low-salt wash buffer (20 mM Tris–HCl [pH 8.0], 150 mM NaCl, 2 mM EDTA [pH 8.0], 1 % Triton X-100, 0.1 % SDS), twice with a high-salt wash buffer (20 mM Tris–HCl [pH 8.0], 500 mM NaCl, 2 mM EDTA, 1 % Triton X-100, 0.1 % SDS), and once with a LiCl wash buffer (10 mM Tris–HCl [pH 8.0], 1 mM EDTA [pH 8.0], 1 % NP-40, 1 % sodium deoxycholate, 250 mM LiCl). DNA-protein complexes were eluted off the beads at 30 °C, and cross-linking was reversed at 65 °C in the presence of RNase A and proteinase K. DNA was purified using phenol-chloroform-isoamyl alcohol and amplified by PCR using primers specific to ICP0 −95 construct (forward, 5′-GCCATTGGGGGAATCGTC-3′; reverse, 5′-TGTGGTGATGCGGAGAGG-3′). Following agarose gel electrophoresis, DNA bands were quantified using Image Lab software (Bio-Rad) as a percentage of the input sample.

Mouse infections and explant-induced reactivation: Male and female, 8-week old, C57BL/6 J mice were purchased from Jackson Labs and housed and handled in agreement with Oklahoma State University Institutional Animal Care and Use Committee guidelines (protocol VM-21–86). The mice were acclimated for 1 week prior to ocular infection using $\sim 2 \times 10^5$ PFU wt HSV-1 in 3 μl MEM per eye without scarification, as described previously (Santos et al., 2023). TG from ~5 animals/treatment were harvested approximately 30 days post-infection in MEM containing 2 % stripped FBS and antibiotics. Tissue was explanted into 60 mm dishes, minced into 3 or 4 pieces each, and incubated at 37 °C, 5 % CO_2, with 10 μM water-soluble DEX (Sigma; catalog no D2915). Where indicated, 1 or 4 μM Mithramycin A, reconstituted in methanol was added to the TG explants. At 4, 6, 8, and 10 days post explant, 1 mL supernatant was removed and used to perform plaque assays on Vero cell monolayers as previously described (Santos et al., 2023).

CRediT authorship contribution statement

Vanessa Claire Santos: Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation. **Nishani Wijesekera:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis. **Fouad S. El-Mayet:** Writing – review & editing, Formal analysis. **Clinton Jones:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2024.199487.

Data availability

No data was used for the research described in the article.

References

Al-Dujaili, L.J., Clerkin, P.P., Clement, C., McFerrin, H.E., Bhattacharjee, P.S., Varnell, E. D., Kauffman, H.E., Hill, J.M., 2011. Ocular herpes simplex virus: how are latency, reactivation, recurrent disease, and therapy interrelated. Future Microbiol 6, 877–907.

Black, A.R., Black, J.D., Azizkhan-Clifford, J., 2001. Sp1 and Kruppel-like transcription factor family of transcription factors in cell growth and cancer. J. Cell. Physiol. 188, 143–160.

Boutell, C., Everett, R.D., 2013. Regulation of alphaherpesvirus infections by the ICP0 family of proteins. J. Gen. Virol. 94, 465–481.

Bouwman, P., Gollner, H., Elsasser, H.-P., Eckhoff, G., Karis, A., Grosveld, F., Philipsen, S., Suske, G., 2000. Transcription factor Sp3 is essential for post-natal survival and late tooth development. The EMBO J 19, 655–661.

Campbell, V.W., Davin, D., Thomas, S., Jones, D., Roesel, J., Tran-Patterson, R., Mayfeild, C.A., Rodu, B., Miller, D.M., Hiramoto, R.A., 1994. The G-C specific DNA binding drug, mithramycin, selectively inhibits transcription of the c-myc and c-ha-ras genes in regenerating liver. Am. J. Med. Sci. 307, 167–172.

Cassidy, L., Meadows, J., Catalan, J., Barton, S., 1997. Are stress and coping style associated with frequent recurrence of genital hereps? Genitourin. Med. 73, 263–266.

Choi, E.-Y., Nam, J.-S., Jung, J.-Y., Cho, N.-P., Cho, S.-D., 2014. Modulation of specificity protein 1 by mithramycin A as a novel therapeutic strategy for cervical cancer. Scientifc Reports 4, 7162.

Chuang, J., Wu, C.H., Lai, M.D., Chang, W.C., Hung, J.J., 2009. Overexpression of Sp1 leads to p53-dependent apoptosis in cancer cells. Int. J. Cancer 125, 2066–2076.

Cliffe, A., Knight, D., 2008. Herpes Simplex Virus ICP0 Promotes both Histone Removal and Acetylation on Viral DNA during Lytic Infection. J. Virol. 82, 12030–12038.

Cliffe, A.R., Coen, D.M., Knipe, D.M., 2013. Kinetics of Facultative Heterochromatin and Polycomb Group Protein Association with the Herpes Simplex Viral Genome during Establishment of Latent Infection. mBio 4, e00590, 00512.

Du, T., Zhou, G., Roizman, B., 2011. HSV-1 gene expression from reactivated ganglia is disordered and concurrent with suppression of latency-associated transcript and miRNAs. Proc. Natl. Acad. Sci. USA 108, 18820–18824.

Du, T., Zhou, G., Roizman, B., 2012. Induction of apoptosis accelerates reactivation from latent HSV-1 in ganglionic organ cultures and replication in cell cultures. Proc. Natl. Acad. Sci. USA 109, 14616–14621.

El-Mayet, F.S., Jones, C., 2024. Specificity protein 1 (Sp1) and glucocorticoid receptor (GR) stimulate bovine alphaherpesvirus 1 (BoHV-1) replication and cooperatively transactivate the immediate early transcription unit 1 promoter. J. Virol. 98 e01436-01423.

El-mayet, F.S., Santos, V.C., Wijesekera, N., Lubbers, S., Harrison, K.S., Sadeghi, H., Jones, C., 2024. Glucocorticoid receptor and specificity protein 1 (Sp1) or Sp3, but not the antibiotic Mithramycin A, stimulates Human alphaherpesvirus 1 (HSV-1) replication. Antiviral Res. 225, 105870.

El-Mayet, F.S., Sawant, L., Thungunutla, P., Jones, C., 2017. Combinatorial effects of the glucocorticoid receptor and Krüppel-like transcription factor 15 on bovine herpesvirus 1 transcription and productive infection. J. Virol. 91, e00904–e00917, 91.

Erlandsson, A.C., Bladh, L.-C., Stierna, P., Yucel-Lindberg, T., Hammersten, O., Modeer, T., Harmenberg, J., Wikstrom, A.-C., 2002. Herpes simplex virus type 1 infection and glucocorticoid treatment regulate viral yield, glucocorticoid receptor and NF-kB levels. J. Endocrinol. 175, 165–176.

Everett, R.D., 2000. ICP0, a regulator of herpes simplex virus during lytic and latent infection. Bioessays 22, 761–770.

Gilmour, J., Assi, S.A., Jaegle, U., Kulu, D., van de Werken, H., Clarke, D., Westhead, D. R., Philipsen, S., Bonifer, C., 2014. A crucial role for the ubiquitously expressed transcription factor Sp1 at early stages of hematopoietic specification. Development 141, 2391–2401.

Glaser, R., Kiecolt-Glaser, J.K., Speicher, C.E., Holliday, J.E., 1985. Stress, loneliness, and changes in herpesvirus latency. J. Behav. Med. 8, 249–260.

Halford, W.P., Kemp, C.D., Isler, J.A., Davido, D.J., Schaffer, P.A., 2001. ICP0, ICP4, or VP16 expressed from adenovirus vectors induces reactivation of latent herpes simplex virus type 1 in primary cultures of latently infected trigeminal ganglion cells. J. Virol. 75, 6143–6153.

Halford, W.P., Schaffer, P.A., 2001. ICP0 is required for efficient reactivation of herpes simplex virus type 1 from neuronal latency. J. Virol. 75, 3240–3249.

Harrison, K., Zhu, L., Thunuguntla, P., Jones, C., 2019. Antagonizing the glucocorticoid receptor impairs explant-induced reactivation in mice latently infected with herpes simplex virus 1. J. Virol. 93, e00418–e00419.

Harrison, K.S., Wijesekera, N., Robinson, A.G.J., Santos, V.C., Oakley, R.H., Cidlowski, J. A., Jones, C., 2023. Impaired Glucocorticoid Receptor Function Attenuates Herpes Simplex Virus 1 Production During Explant-Induced Reactivation from Latency in Female Mice. J. Virol. 97, e01305–e01323.

Honess, R.W., Roizman, B., 1974. Regulation of herpes virus macromalecular synthesis: cascade regulation of three groups of viral proteins. J. Virol. 14, 8–19.

Hunsperger, E.A., Wilcox, C.L., 2003. Caspase-3-dependent reactivation of latent herpes simplex virus type 1 in sensory neuronal cultures. J. Neurovirol. 9, 390–398.

Jones, C., 1998. Alphaherpesvirus latency: its role in disease and survival of the virus in nature. Adv. Virus Res. 51, 81–133.

Jones, C., 2003. Herpes simplex virus type 1 and bovine herpesvirus 1 latency. Clin Micro Rev 16, 79–95.

Kaczynski, J., Cook, T., Urrutia, R., 2003. Sp1- and Kruppel-like transcription factors. Genome Biol. 4, 206.201-206.208.

Kavurma, M.M., Khachigian, L.M., 2003. Sp1 inhibits proliferation and induces apoptosis in vascular smooth muscle cells by repressing p21WAF1/Cip1 transcription and cyclin D1-Cdk4-p21WAF1/Cip1 complex formation. J. Biol. Chem. 278, 32537–32543.

Knipe, D.M., Cliffe, A., 2008. Chromatin control of herpes simplex virus lytic and latent infection. Nat. Rev. Micro. 6, 211–221.

Kristie, T.M. Early pre-initiation of alphaherpesvirus viral gene expression. in *Human herpesviruses: biology, therapy, and Immunoprophylaxis.*, Vol. 1 (ed. A. Arvin, G. Campadelli-Fiume, E. Mocarski, P.S. Moore, R. Whitley, and K. Yamanishi) 112–127 (Cambridge University Press, Cambridge, NY, 2007).

Kristie, T.M., 2015. Dynamic modulation of HSV chromatin drives initiation of infection and provides targets for epigenetic therapies. Virology 479-480, 555–561.

Kushnir, A.S., Davido, D.J., Schaffer, P.A., 2009. Role of nuclear factor Y in stress-induced activation of the herpes simplex virus type 1 ICP0 promoter. J. Virol. 84, 188–200.

Linderman, J.A., Kobayashi, M., Rayannavar, V., Fak, J.J., Darnell, R.B., Chao, M.V., Wilson, A.C., Mohr, I., 2017. Immune Escape via a Transient Gene Expression Program Enables Productive Replication of a Latent Pathogen. Cell Rep. 18, 1312–1323.

Ma, J., Russell, T.A., Spelman, T., Carbone, F.R., Tscharke, D.C., 2014. Lytic gene expression is frequent in HSV-1 latent infection and corrleates with the engagement of a cell-intrinsic transcriptional response. PLoS Pathog. 10, e1004237.

Mangan, S., Alon, U., 2003. Structure and function of the feed-forward loop network motif. Proc. Natl. Acad. Sci. USA 100, 11980–11985.

Margolis, T.P., Elfman, F., Leib, D., Pakpour, N., Apakupakul, K., Imai, Y., Voytek, C., 2007. Spontaneous reactivation of herpes simplex virus type 1 in latently infected murine sensory gangalia. J. Virol. 81, 11069–11074.

Miller, D., Polansky, D., Thomas, S., Ray, R., Campbell, V., Sanchez, J., Koller, C., 1987. Mithramycin selectively inhibits transcription of G-C containing DNA. Am. J. Med. Sci. 294, 388–394.

Newell, C.K., Martin, S., Sendele, D., Mercadal, C.M., Rouse, B.T., 1989. Herpes simplex virus-induced stromal keratitis: role of T-lymphocyte subsets in immunopathology. J. Virol. 63, 769–775.

Oakley, R.H., Cidlsowski, J.A., 2013. The biology of the glucocorticoid receptor: new signaling mechanisms in health and disease. J. Allergy Clin. Immunol. 132, 1033–1044.

Ostler, J., Harrison, K.S., Schroeder, K., Thunuguntla, P., Jones, C., 2019. The glucocorticoid receptor (GR) stimulates Herpes Simplex Virus 1 productive infection, in part because the infected cell protein 0 (ICP0) promoter is cooperatively transactivated by the GR and Krüppel-like transcription factor 15. J of Virol 93, e02063, 02018.

OStler, J.B., Jones, C., 2021. Stress induced transcription factors transactivate the herpes simplex virus 1 infected cell protein 27 (ICP27) transcriptional enhancer. Viruses 13, 2296.

Ostler JB, P.T., Hendrickson, B.Y., Jones, Clinton, 2021. Transactivation of Herpes Simplex Virus 1 (HSV-1) Infected Cell Protein 4 Enhancer by Glucocorticoid Receptor and Stress-Induced Transcription Factors Requires Overlapping Krüppel-Like Transcription Factor 4/Sp1 Binding Sites. J. Virol. 95, e01776, 01720.

Padgett, D.A., Sherida, J.F., Dorne, J., Berntson, G.G., Candelora, J., Glaser, R., 1998. Social stress and the reactivation of latent herpes simplex virus type 1. Proc. Natl. Acad. Sci. USA 95, 7231–7235.

Perng, G.-C.a.C.J., 2010. Towards an understanding of the Herpes Simplex Virus Type 1 latency-reactivation cycle. Interdiscip Perspect Infect Dis 2010, 1–18.

Ray, R., Snyder, R.C., Thomas, S., Koller, C.A., Miller, D.M., 1989. Mithramycin Blocks Protein Binding and Function of the SV40 Early Promoter. J. Clin. Invest. 83, 2003-2007.

Rooney, J.T., Straus, S.E., Mannix, M.L., Wohlenberg, C.R., Banks, S., Jagannath, S., Brauer, J.E., Notkins, A.L., 1992. UV light-induced reactivation of herpes simplex virus type 2 and prevention by acyclovir. J of Infectious Diseases 166, 500–506.

Ryu, H., Lee, J., Zaman, K., Kubilis, J., Ferrante, R.J., Ross, B.D., Neve, R., Ratan, R.R., 2003. Sp1 and Sp3 Are Oxidative Stress-Inducible, Antideath Transcription Factors in Cortical Neurons. The Journal of Neuroscience 23, 35907, 33606.

Santos, V.C., Ostler, J.B., Harrison, K.S., Jones, C., 2023. Slug, a stress-induced transcription factor, stimulates herpes simplex virus type 1 replication and transactivates a cis-regulatory module within the VP16 promoter. J. Virol. 97.

Sasse, S., Mailoux, C.M., Barczak, A.J., Wang, Q., Altonsy, M.O., Jain, M.K., Haldar, S.M., Gerber, A.N., 2013. The glucocorticoid receptor and KLF15 regulate gene expression dynamics and integrate signals through feed-forward circuitry. Molec Cell Biol 33, 2104–2115.

Sasse, S., Zuo, Z., Kadiyala, V., Zhang, L., Pufall, M.A., Jain, M.K., Phang, T.L., Stormo, G. D., Gerber, A.N., 2015. Response element composition governs correlations between binding site affinity and transcription in glucocorticoid receptor feed-forward loops. J. Biol. Chem. 290, 19756–19769.

Sekizawa, T., Openshaw, H., 1984. Encephalitis resulting from reactivation of latent herpes simplex virus in mice. J. Virol. 50, 263–266.

Sinani, D., Cordes, E., Workman, A., Thunuguntia, P., Jones, C., 2013. Stress induced cellular transcription factors expressed in trigeminal ganglionic neurons stimulate the herpes simplex virus type 1 (HSV-1) infected cell protein 0 (ICP0) promoter. J. Virol. 87, 1183–1192.

Takeda, K., Yahagi, N., Aita, Y., Murayama, Y., Sawada, Y., Piao, X., Toya, N., Oya, Y., Shikama, A., Takarada, A., Masuda, Y., Nishi, M., Kuobota, M., Izumida, Y., Yamamoto, T., Sekiya, M., Matsuzaka, T., Nakagawa, Y., Urayama, O., Kawakami, Y., Iizuka, Y., Gotoda, T., Itaka, K., Kataoka, K., Nagai, R., Kadowaki, T., yamada, N., Lin, Y., Jain, M.K., Shimano, H., 2016. KLF15 enables switching between lipogenesis aand gluconeogenesis during fasting. Cell Rep. 16, 2373–2386.

Tremblay, R., Sikorska, M., Sandhu, J.K., Lanthier, P., Ribecco-Lutkiewicz, M., Bani-Yaghoub, M., 2010. Differentiation of mouse Neuro-2A cells into dopamine neurons. J of Neuroscience Methods 186, 60–67.

Wijesekera, N., Hazell, N., Jones, C., 2022. Independent Cis-Regulatory Modules within the Herpes Simplex Virus 1 Infected Cell Protein 0 (ICP0) Promoter Are Transactivated by Krüppel-like Factor 15 and Glucocorticoid Receptor. Viruses 14, 1284.

Workman, A., Eudy, J., Smith, L., Frizzo da Silva, L., Sinani, D., Bricker, H., Cook, E., Doster, A., Jones, C., 2012. Cellular transcription factors induced in trigeminal ganglia during dexamethasone-induced reactivation from latency stimulate bovine herpesvirus 1 productive infection and certain viral promoters. J. Virol. 86, 2459–2473.

Yoo, J., Jeong, M.-J., Kwon, B.-M., Hur, M.-W, Park, Y.-M., Han, M.Y., 2002. Activation of Dynamin I Gene Expression by Sp1 and Sp3 Is Required for Neuronal Differentiation of N1E-115 Cells. J. Biol. Chem. 277, 11904–11909.