Simian varicella virus gene 28 and 29 promoters share a common upstream stimulatory factor-binding site and are induced by IE62 transactivation

Yang Ou and Wayne L. Gray

Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, 4301 W. Markham Street, Little Rock, AR 72205, USA

INTRODUCTION

Outbreaks of a natural, varicella-like disease occur sporadically in facilities housing non-human primates (Gray, 2004). A primate herpesvirus, simian varicella virus (SVV), is responsible for this highly contagious disease, which is characterized by fever and vesicular skin rash. SVV is related antigenically and genetically to varicella-zoster virus (VZV), which causes varicella (chickenpox) and herpes zoster (shingles) in humans (Fletcher & Gray, 1992; Gray & Oakes, 1984). The SVV and VZV genomes are similar in size and structure, share extensive DNA similarity and are collinear with respect to gene organization (Gray et al., 2001). The pathogenesis of simian varicella in monkeys, including the ability of SVV to establish latent infection in neural ganglia and to reactivate to cause secondary disease, parallels human VZV infections (Gray, 2003; Mahalingam et al., 1992). Therefore, SVV infection of non-human primates offers an experimental model for studying varicella pathogenesis and latency and for evaluating antiviral strategies (Gray, 2004).

Little is known concerning SVV and VZV gene regulation, due to the cell-associated nature of the viruses. However, like that of other alphaherpesviruses, SVV and VZV gene expression during lytic infection is considered to be regulated coordinately into immediate-early (IE), early and late phases (Cohen & Straus, 2001). The IE genes, such as open reading frames (ORFs) 4, 62 and 63, play an important regulatory role during viral infection by transactivating the expression of other viral genes. The VZV IE62 protein, encoded by ORFs 62 and 71, is the major transactivator, capable of stimulating expression of viral genes of all three kinetic classes (Inchauspé et al., 1989; Perera et al., 1992). Early genes are generally involved in viral DNA replication and include genes such as ORFs 28 and 29, which encode the viral DNA polymerase and a single-stranded DNA-binding protein, respectively. Following the onset of DNA replication, the viral late genes, which encode structural products such as capsid proteins and envelope glycoproteins, are expressed.

During lytic infection, all or most VZV and SVV genes are expressed. However, during latent infection of neural ganglia, VZV gene expression is restricted to a subset of viral genes. Several studies, involving analysis of human cadaver tissues and animal models, have demonstrated transcription
and sometimes translation of VZV ORFs 4, 21, 29, 62, 63 and 66 within latently infected ganglia, although these reports of specific gene expression during latency have varied considerably and further studies are warranted (Kennedy, 2002a, b).

The VZV 28 and 29 genes appear to be expressed differentially during acute and latent infection. Both genes are expressed during productive viral infection (Meier et al., 1993). However, during viral latency, VZV ORF 29 transcripts and protein are detected in neural ganglia, but ORF 28 is not expressed (Kennedy, 2002a; Lungu et al., 1998). A common 221 bp intergenic region controls expression of the VZV ORFs 28 and 29 during lytic infection and probably during latent infection (Meier & Straus, 1993; Yang et al., 2004). Therefore, study of the ORF 28/29 intergenic element may provide clues for elucidating factors affecting differential viral gene expression during acute and latent infection. Unfortunately, animal models to investigate the molecular basis of VZV pathogenesis and latency are limited.

The simian varicella model provides an experimental approach to investigate viral gene expression throughout acute infection and during viral latency (Gray, 2004). In this study, we analysed the features of the intergenic region that regulate expression of the SVV ORF 28 and 29 during lytic infection of Vero cells. The results demonstrate that SVV 28 and 29 gene expression is activated by a viral trans-activator (IE62) and indicate that a binding site for a cellular transcription factor, the upstream stimulatory factor (USF), is important for this induction. USF is a helix-loop-helix-leucine zipper protein that binds to a specific DNA sequence (5’-CACGTG-3’) and helps to regulate expression of a variety of viral and cellular genes (Meier et al., 1994; Rahaus et al., 2003).

**METHODS**

**Cells and virus.** Vero (African green monkey kidney) cells were grown in Eagle’s minimal essential medium (EMEM) supplemented with 5% newborn bovine serum, penicillin (5000 U ml⁻¹) and streptomycin (5000 U ml⁻¹). The Delta herpesvirus strain of SVV was originally isolated from an infected patas monkey at the Tulane National Primate Center in Covington, LA, USA (Allen et al., 1974).

**Plasmid constructs.** The entire 185 bp SVV ORF28/29 intergenic region or portions of it were amplified by PCR and cloned into the pGL3 basic vector (Promega), which contains the firefly luciferase reporter gene. The specific nucleotide primers included SacI and NcoI sites to aid in the directional cloning into pGL3. Total cellular DNA isolated from SVV-infected cells was used as template DNA for PCR. Cycling-reaction conditions were 94°C for 2 min, 55°C for 1 min, 72°C for 1 min for 30 cycles, followed by 72°C for 7 min. The resulting PCR product was digested with SacI and NcoI enzymes and cloned into the corresponding sites of pGL3. Site-specific mutations were incorporated into the intergenic region by using specific PCR primers or with a commercial kit (QuikChange II; Stratagene). The SVV 61-pCI, 63-pCI and 4-pCI expression vectors were constructed by PCR amplification of SVV ORFs 61, 63 and 4, respectively, and insertion of the amplification products into vector pCI (Promega), which includes the human cytomegalovirus (HCMV) immediate-early gene promoter/enhancer. SVV 62-pCI expressing ORF 62 was provided by Dr Ravi Mahalingam (University of Colorado Health Sciences Center, CO, USA).

**Transfection and reporter-gene assays.** Vero cell monolayers, at 90–95% confluence in 12-well plates, were transfected with plasmid DNA constructs by using Lipofectamine 2000 transfection reagent (Invitrogen). Approximately 4 µl transfection reagent was used per 1-6 µg transfected DNA. In most cases, the pGL3 reporter construct (1-4 µg) was co-transfected with 0.2 µg of the pCI vector expressing the SVV ORF 62, 61, 63 or 4 or the null-pCI vector. At 48 h post-transfection, cell extracts were prepared in lysis buffer (80 µl per well) and luciferase activity was determined by using a commercial luciferase-assay system (Promega). Luciferase activities were expressed as fold increase over basal level. To monitor transfection efficiency, 0.2 µg pCMVβ vector DNA (BD Biosciences Clontech) was included in transfection reactions and β-galactosidase activity was measured by using a commercial assay system (Promega).

**Determination of transcription start sites by rapid amplification of cDNA ends (RACE) and DNA sequence analysis.** The 5’ ends of SVV ORF 28 and 29 transcripts were determined by using a commercial RACE kit (Invitrogen Life Technologies). Briefly, total cellular RNA was isolated from SVV-infected Vero cells by using RNAzolB reagent (Tel-Test). First-strand cDNA synthesis was performed with reverse transcriptase and ORF 28 or ORF 29 gene-specific primers (GSP1), which anneal 438 or 423 bp downstream of the ORF 28 or ORF 29 ATG initiation codons, respectively. After degradation of the RNA template with RNase, the 3’ end of the cDNA was dCTP-‘tailed’ by using terminal deoxynucleotidyl transferase (TdT). The cDNAs were amplified with an SVV ORF 28 (ORF 29) nested GSP2 primer and the 5’ RACE abridged anchor primer (AAP), which contains 3’ sequence complementary to the homopolymeric tail at the 3’ end of the first cDNA strand and additional 5’ sequence that encodes an adapter region. An abridged universal amplification primer (AUAP), which contains the adapter region homologous to that of the anchor primer (AAP), and a nested ORF 28 or ORF 29 GSP3 primer were used to reamplify the 5’ RACE PCR products, which were then ligated into the pGEM-T Easy vector (Promega). Escherichia coli cells were transformed, DNA was isolated from individual clones and DNA sequencing analysis was used to reveal the transcription start sites for SVV ORF 28 and ORF 29. The 185 bp ORF 28/29 intergenic sequence was analysed for potential transcription factor-binding sites by using the TESS (Schug & Overton, 1997) and MatInspector (Wingender et al., 2000) software programs.

**Construction of SVV recombinant viruses.** SVV recombinant viruses expressing the green fluorescent protein (GFP) under the control of the SVV 29 promoter were generated by using a cosmid recombination system as described previously (Gray & Mahalingam, 2005). Briefly, a cassette was constructed that included the GFP gene expressed from the SVV ORF 29 promoter (from nt −160 to the ORF 29 ATG initiation codon) along with a simian virus 40 polyadenylation signal sequence (pr29-GFP-polyA). This cassette was bracketed by AscI restriction-endonuclease sites, permitting ligation and insertion into a unique AscI site created in cosmid A within the intergenic region of SVV ORFs 12 and 13 [nt 19148 on the SVV genome (Gray et al., 2001)]. The cassette was inserted into cosmid A in both possible orientations, either on the same DNA strand as SVV ORFs 12 and 13 or on the opposite DNA strand. The cosmid A-pr29-GFP-polyA was co-transfected into Vero cells along with cosmids B, C and D and infectious recombinant virus clones were isolated on day 10 post-transfection. PCR and DNA sequence analysis confirmed the orientation of the cassette within the SVV-pr29-GFP recombinant virus genomes.
RESULTS

SVV ORF 28 and 29 promoters are active in SVV-infected cells

A study was conducted to compare activation of the ORF 28 and 29 promoters in uninfected and SVV-infected Vero cells. The 185 bp intergenic sequence (Fig. 1a, b) was PCR-amplified and cloned into the pGL3 vector such that the luciferase reporter gene was under the control of the ORF 28 (pr28-pGL3) or the ORF 29 (pr29-pGL3) promoter. A preliminary study determined that the basal luciferase activities of pr28-pGL and pr29-pGL3 in transfected Vero cells were the same as that of the null-pGL3 vector, confirming that the 28 and 29 promoters are not active in uninfected Vero cells (data not shown). The pr28-pGL3 and pr29-pGL3 constructs were transfected into uninfected or SVV-infected Vero cells and cell lysates were processed for luciferase activity at 24 h post-transfection. Expression of the reporter gene was increased by 170- and 129-fold in SVV-infected cells transfected with pr28-pGL3 and pr-29-pGL3, respectively, compared with expression in uninfected Vero cells (Fig. 1c). The result indicates that viral infection provides specific factors or conditions for specific stimulation of the SVV 28 and 29 gene promoters.

Identification of viral transactivators of the SVV ORF 29 promoter

SVV ORFs 4, 61, 62 and 63 are predicted to encode putative transcriptional activators of viral gene expression, based upon their VZV and herpes simplex virus 1 homologues (Cohen & Straus, 2001). To identify the SVV transactivator(s) responsible for the activation of SVV ORF29 promoter, pr29-pGL3 was co-transfected into Vero cells along with pCI vectors expressing the individual SVV 4, 61, 62 or 63 genes under control of the HCMV IE promoter/enhancer (SVV ORF4-pCI, ORF61-pCI, ORF62-pCI, ORF63-pCI) or with the null-pCI expression vector. At 48 h post-transfection, cell lysates were analysed for luciferase activity. The ORF 62 gene product (IE62) induced a 135-fold activation of the gene 29 promoter, compared with the lack of activation by the null-pCI vector (Fig. 2a). Further study confirmed that IE62 stimulates the 29 promoter in a dose-responsive manner, with as little as 5–10 ng ORF62-pCI DNA inducing strong activation (Fig. 2b). In contrast, SVV ORF61-pCI and ORF4-pCI induced a modest two- to sixfold increase, whilst ORF63-pCI induced little or no transactivation of the 29 promoter. The ORF 4, 61 and 63 expression vectors did not exhibit strong transactivation, regardless of dose (data not shown).

Deletion and mutagenesis analyses of the SVV ORF 29 promoter

To identify the minimal functional sequence for the SVV 29 promoter, the 185 bp intergenic region in the pr29-pGL3 construct was shortened progressively at the 5’ end and the effect on the ability of SVV ORF62-pCI to transactivate the promoter was analysed (Fig. 3). Deletion of 24 bp (F1 construct) or 62 bp (F2 construct) had minimal effect on the ability of SVV IE62 to transactivate the gene 29 promoter. However, deletion of 88 bp, resulting in a 97 bp upstream element (F3 construct), resulted in a nearly tenfold reduction in transactivation compared with induction by the 185 bp intergenic promoter. The results indicated that a 26 bp element (present in the F2 construct, but absent from the F3 construct) is important for IE62-mediated transactivation. Analysis of this sequence identified a 16 bp palindrome (5’-TATATCACGTGATATA-3’) that includes a consensus binding site (5’-CAGTG-3’) for the USF cellular basic helix–loop–helix transactivation factor.

To explore the role of the palindrome and the USF-binding site in the IE62-mediated transactivation of the SVV gene 29 promoter, two mutants were constructed (Fig. 4a). The pr29mut 1-pGL3 construct (mutant 1) included substitution of three bases within the USF hexameric core (TCA to GTC), resulting in disruption of the putative hairpin, although there is no direct evidence that the palindrome forms a stem–loop structure in the pr29-pGL3 construct or in viral DNA. The pr29mut 2-pGL3 construct (mutant 2)
included an additional three-base substitution (TGA to GAC), which further changed the hexameric-core sequence, but repaired the hairpin structure. As shown in the Fig. 4(b), SVV ORF62-pCI transactivated the wild-type SVV 29 promoter by 138-fold, but disruption of the palindrome (mutant 1) reduced the transactivation by >15-fold. In addition, mutant 2, which includes additional changes to the hexameric sequence, but retains the hairpin, resulted in

![Graph](image)

Fig. 2. Induction of the gene 29 promoter by potential SVV transactivators. (a) Vero cells were co-transfected with pr29-pGL3 DNA (1·4 μg) and 0·2 μg null-pCI, ORF62-pCI, ORF63-pCI, ORF61-pCI or ORF4-pCI. pCMV/l DNA (0·2 μg) was also included in each transfection to monitor transfection efficiency. (b) Dose effect of ORF62-pCI transactivation of the gene 29 promoter. Vero cells were co-transfected with pr29-pGL3 DNA (1·4 μg) and with various amounts of pCI-62 (1–400 ng). Null-pCI DNA was included in transfections as needed to bring the final pCI vector concentration to 400 ng. Cell lysates were processed at 48 h post-transfection and luciferase activity was determined. Promoter activities are presented as fold induction normalized to the luciferase activities expressed from cells co-transfected with pr29-pGL3 and the null-pCI vector. Error bars represent SD from triplicate experiments.

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Fig. 3. Deletion mutagenesis of the intergenic region and the effect on IE62-mediated transactivation of the gene 29 promoter. Full-length pr29-pGL3 or constructs with 5′ deletions (F1-, F2-, F3-pGL3) (0·8 μg) were co-transfected into Vero cells with 0·1 μg ORF62-pCI (shaded bars) or the null-pCI vector (filled bars). Cell lysates were processed at 48 h post-transfection and luciferase activity was determined. Promoter activities are presented as fold induction normalized to the luciferase activities expressed from the full-length pr29-pGL3 construct co-transfected with the null-pCI vector. Arrows indicate the site of the 16 bp palindrome. Values represent the mean from duplicate experiments.

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Fig. 4. Mutagenesis of the intergenic-region palindrome and the effect on IE62-mediated transactivation of the gene 29 promoter. (a) DNA sequence of the wild-type (wt) palindrome sequence and the sequence of mutants 1 and 2, with asterisks denoting altered nucleotides. (b) Vero cells were co-transfected with wt pr29-pGL3 or one of the mutant pGL3 constructs (1·4 μg) and with 0·2 μg ORF62-pCI or with the null-pCI vector. Cell lysates were processed at 48 h post-transfection and luciferase activity was determined. Promoter activities are presented as fold induction normalized to the luciferase activities expressed from the wt pr29-pGL3 construct co-transfected with the null-pCI vector. Error bars represent SD from triplicate experiments.
a 20-fold reduction in IE62-induced transactivation. The results demonstrate that the USF hexameric-core sequence, and not simply a hairpin structure, is important for IE62-mediated transactivation of the ORF 29 promoter.

SVV IE62 induction of SVV ORF 28 promoter

The ability of IE62 to transactivate the SVV ORF 28 promoter was analysed. Vero cells were co-transfected with pr28-pGL3, which includes the 185 bp intergenic region oriented to express the luciferase gene from the ORF 28 promoter, and pCI-62 or the null-pCI vector. The results demonstrated that the IE62 transactivated the gene 28 promoter strongly compared with the non-activation by the null-pCI vector (Fig. 5a).

To identify the minimal functional sequence for the SVV 28 promoter, the 185 bp intergenic region in the pr28-pGL3 construct was shortened progressively at the 5′ end and the effect on the ability of SVV ORF62-pCI to transactivate the promoter was analysed (Fig. 5a). Deletion of 18 bp (F1 construct) had no effect and deletion of 51 bp (F2) caused less than a twofold reduction in the ability of SVV IE62 to transactivate the gene 29 promoter. However, further shortening of the 5′ upstream element caused progressive reductions in IE62-mediated transactivation. Deletion of 80 bp (F3) and 107 bp (F4) resulted in four- and 25-fold reductions, respectively. Deletion of 123 bp (F5), including the 16 bp palindrome, caused complete abrogation of IE62-mediated transactivation of the SVV 28 promoter.

The importance of the USF-binding site in IE62-mediated induction of the ORF28 promoter was confirmed by a tenfold reduction in activation of a pr28-pGL3 mutant that includes a 3 bp mutation within the USF-binding site (Fig. 5b).

The results of this study indicate that, whilst the palindrome and USF-binding site are essential, an additional 56–89 bp upstream of the palindrome is needed for optimal IE62-mediated transactivation of the ORF 28 promoter.

Fig. 5. IE62-mediated induction of the ORF 28 promoter and mutagenesis to determine the 5′ upstream sequence required for optimal transactivation. (a) Full-length pr28-pGL3 or constructs with 5′ deletions (F1-, F2-, F3-, F4-, F5-pGL3) (0–8 μg) were co-transfected into Vero cells with 0-1 μg ORF62-pCI (shaded bars) or the null-pCI vector (filled bars). Cell lysates were processed at 48 h post-transfection and luciferase activity was determined. Promoter activities are presented as fold induction normalized to the luciferase activities expressed from the full-length pr28-pGL3 construct co-transfected with the null-pCI vector. Arrows indicate the site of the 16 bp palindrome. Error bars represent SD from triplicate experiments. (b) Effect of mutagenesis of the USF-binding site on IE62-mediated transactivation of the ORF 28 promoter. Vero cells were co-transfected with 1·4 μg wt pr28-pGL3 or with a pr28-pGL3 mutant along with 0.2 μg ORF62-pCI or the null-pCI vector and cell lysates were processed as described above.
Transcriptional and DNA sequence analysis of the 28/29 intergenic region

RACE analysis was used to determine the 5' transcriptional start sites of the ORF 28 and ORF 29 mRNAs. Two ORF 29 RNA start sites located 20 or 56 (-20 and -56) bp upstream of the ATG start codon were identified (Fig. 6). A transcriptional start site for ORF 28 was detected 20 bp upstream of the ATG start codon. An unexpected ORF 28 RNA start site at 190 bp upstream of the ORF 28 ATG start codon and within the ORF 29 was also identified. Each of these transcriptional start sites was preceded (within 30 bp) by a potential TATA-box sequence. The palindrome sequence containing the USF-binding site was centred at nt 51338 on the SVV genome within the 185 bp intergenic region. Several other binding sites for cellular transcription factors were also identified, including potential binding sites for activator protein 1 (AP-1, centred at nt 51322 and also at 51411), cAMP-responsive element-binding protein (CREB, nt 51338) and octamer-binding factor 1 (Oct-1, nt 51351). The importance of these additional potential binding sites in regulation of the SVV ORF 28/29 promoter is unknown.

Construction and analysis of SVV recombinant viruses expressing GFP from the SVV 29 promoter

SVV recombinant viruses were constructed to demonstrate the ability of the 29 gene promoter, located in a novel site within the SVV genome, to drive expression of a heterologous gene during viral infection. Cassettes including the ORF 29 promoter (160 bp) controlling the GFP gene were inserted into a unique AscI site located within the intergenic region of ORFs 12 and 13 by using the SVV cosmid genetic-recombination system (Gray & Mahalingam, 2005) (Fig. 7a). Recombinant viruses with the pr29-GFP situated in both orientations (same strand as ORFs 12 and 13 or the opposite strand) were constructed and isolated. The presence and orientation of the pr29-GFP within the recombinant virus genomes were confirmed by PCR and DNA sequence analysis (data not shown). Analysis of infected cells by fluorescent microscopy indicated that the GFP gene was expressed under control of the 29 gene promoter, regardless of orientation (Fig. 7b, c).

DISCUSSION

This study demonstrates that the SVV ORF 28/29 intergenic region includes overlapping, divergent promoters, which share a common USF-binding site and are transactivated by SVV IE62. Deletion and mutagenesis analysis indicated that a 123 bp 5' upstream sequence ending with the 16 bp palindrome and including the USF-binding site is sufficient for efficient IE62-mediated transactivation of the gene 29 promoter. In contrast, optimal transactivation of the gene 28 promoter requires a 134–167 bp 5' upstream element, which includes the palindrome plus an additional 56–89 bp upstream sequence. The results provide a comparison between the SVV and VZV ORF28/29 promoter elements and indicate that these viral genes are

Fig. 6. DNA sequence of the 185 bp SVV ORF28/29 intergenic region, located on the SVV genome between nt 51269 and 51453 (GenBank accession no. AF275348) (Gray et al., 2001). The ORF 28 and 29 ATG initiation codons are indicated in bold. RNA start sites mapped by RACE with direction of transcription are designated by arrows. Putative TATA boxes upstream of each RNA start site are underlined. The 16 bp palindrome, including the consensus USF-binding site, is indicated by italics.

Fig. 7. Generation of SVV recombinant viruses expressing GFP under control of the SVV ORF 29 promoter. (a) The SVV genome and the four overlapping cosmids (32–38 kb) that are used to generate infectious SVV clones (Gray & Mahalingam, 2005). A cassette including the ORF 29 promoter controlling the GFP gene was inserted into cosmid A at the unique Ascl site (nt 19148), which is located within the SVV ORF12/13 intergenic region. Recombinant SVV clones with the cassette inserted in either of the two possible orientations (each DNA strand) were generated. Analysis of infected Vero cells by fluorescent microscopy indicated that the GFP gene was expressed whether the pr29-GFP cassette was inserted into the same DNA strand as ORFs 12 and 13 (b) or was inserted into the opposite strand (c).
controlled in a similar fashion in these closely related herpesviruses.

Previous studies demonstrated that the 221 bp VZV ORF 28/29 intergenic element consists of two independent, but overlapping, unidirectional promoters that are transactivated by the VZV IE62 (Meier & Straus, 1993; Yang et al., 2004). The VZV 28 and 29 gene promoters have independent TATA boxes and transcription start sites, arranged closely back-to-back (Yang et al., 2004). The intergenic region includes several potential binding sites for cellular transcription factors and the USF, Sp1 and TBP (TATA-box-binding protein) cellular proteins each bind to this region (Yang et al., 2004). The USF site and an Sp1-binding site, centred 60 bp upstream of the VZV ORF 28 initiation codon, are critical for VZV IE62-mediated transactivation in both directions.

DNA sequence and transcriptional analyses of the 185 bp SVV ORF 28/29 intergenic region revealed similarities with the VZV ORF28/29 regulatory region. Like VZV, the SVV ORF 29 has two transcription start sites, one located proximal (−20 bp) and one located distal (−56 bp) to the ATG initiation codon. The SVV ORF 28 transcription start site is situated 26 bp upstream of the initiation codon and is in a location relatively similar to that of the VZV ORF 28 RNA start site. The additional SVV ORF 28 transcription start site detected at −190 bp will require further analysis for confirmation, but the 5′ deletion analysis of the gene 28 promoter demonstrated that this RNA start site is not essential for optimal IE62-mediated transactivation. Like VZV, each of the ORF 28 and 29 transcriptional start sites is preceded by potential TATA boxes. The SVV ORF 28/29 intergenic element includes potential binding sites for several cellular transcriptional activators, including USF and AP1. The SVV USF site is located within a 16 bp palindrome, compared with the 12 bp VZV palindrome, but the hexameric-core sequences (CACGTG) are identical. The SVV ORF28/29 intergenic sequence does not have a consensus Sp1 site corresponding to the critical VZV Sp1 site, although a high-G+C sequence is located in an analogous position just proximal to the SVV ORF 28 TATA box (28-1) centred at nt 51317.

Expression of the SVV 28 and 29 genes is dependent on viral (IE62) and cellular (USF) transcription factors, as is the case for VZV ORF28/29 expression. The USF hexameric-core sequence, rather than a double-stranded hairpin structure per se, is critical for IE62-mediated transactivation of the SVV 28 and 29 genes. Mutagenesis of the USF hexameric-core sequence inhibited IE62-mediated transactivation of the SVV 28 and 29 genes. Similar alteration of the USF hexameric-core sequence was demonstrated to abrogate binding of USF to the VZV ORF 28/29 promoter (Meier et al., 1994). The precise mechanism by which IE62 and USF interact to stimulate the SVV and VZV 28 and 29 genes is unclear, but a direct physical interaction between VZV IE62 and USF has been confirmed (Rahaus et al., 2003; Ruyechan et al., 2003). USF binding may prepare or target the ORF 28/29 promoters and other viral promoters for IE62-mediated transactivation (Meier et al., 1994). The IE62–USF complex in association with cellular TATA-associated transcription factors, such as TFIID, may stabilize the transcription complex and promote cellular RNA polymerase binding, thus stimulating viral gene transcription.

VZV gene 29, along with VZV ORFs 4, 21, 62, 63 and 66, is reported to be expressed in ganglia derived from latently infected humans and experimentally infected animals (Kennedy, 2002b). VZV ORF 29 RNA has been detected in latent ganglia by a variety of methods, including cDNA enrichment, in situ and Northern blot hybridization analyses and real-time RT-PCR (Cohrs et al., 1996, 2000; Kennedy et al., 2000, 2001; Meier et al., 1993). In addition, VZV gene 29 protein expression was detected in latently infected human and animal ganglia (Grinfeld & Kennedy, 2004; Kennedy et al., 2001; Lungu et al., 1998). In contrast, VZV ORF 28 transcripts were not identified in two studies that attempted to detect gene 28 RNA in latently infected human ganglia (Kennedy et al., 2000; Meier et al., 1993). These studies suggest differential expression of VZV ORFs 28 and 29 during latency. However, experimental studies involving human cadaver tissues have technical restrictions and animal models of VZV latency are limited, as the virus does not reactivate from latent ganglia to cause secondary disease.

The simian varicella model offers a relevant experimental approach to investigate the molecular basis of varicella latency and reactivation (Gray, 2004; White et al., 2001). The similarities between the SVV and VZV ORF28/29 intergenic elements indicate that analysis of SVV 28 and 29 gene expression in ganglia derived from acutely and latently infected animals may provide clues to explain the possible differential expression of these genes during viral latency. Studies are in progress to detect SVV gene 28 and 29 expression in acutely and latently infected tissues, including ganglia.

The SVV ORF 29 promoter controlling a heterologous gene (GFP) was expressed in the context of viral infection when inserted into a novel site (ORF 12/13 intergenic region) within the viral genome of a recombinant SVV. Previous studies employing recombinant SVV have demonstrated expression of foreign genes under the control of the HCMV IE and Rous sarcoma virus promoter/enhancers (Gray & Byrne, 2003; Gray & Mahalingam, 2005; Mahalingam et al., 1998) and the present study is the first example of expression of a foreign gene from a natural SVV promoter. Recombinant viruses provide an opportunity to investigate varicella vaccines expressing antigens of other pathogens and also an approach to study the molecular basis of varicella pathogenesis and latency. It will be of interest to determine whether experimental infection of animals with the SVV recombinant virus expressing GFP from the ORF 29 promoter results in expression of the foreign gene in acutely and latently infected ganglia.
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REFERENCES


