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Differential effects of Sp cellular transcription factors on viral promoter activation by varicella-zoster virus (VZV) IE62 protein

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ABSTRACT

The immediate early (IE) 62 protein is the major varicella-zoster virus (VZV) regulatory factor. Analysis of the VZV genome revealed 40 predicted GC-rich boxes within 36 promoters. We examined effects of ectopic expression of Sp1-Sp4 on IE62- mediated transactivation of three viral promoters. Ectopic expression of Sp3 and Sp4 enhanced IE62 activation of ORF3 and gI promoters while Sp3 reduced IE62 activation of ORF28/29 promoter and VZV DNA replication. Sp2 reduced IE62 transactivation of gI while Sp1 had no significant influence on IE62 activation with any of these viral promoters. Electrophoretic mobility shift assays (EMSA) confirmed binding of Sp1 and Sp3 but not Sp2 and Sp4 to the gI promoter. Sp1–4 bound to IE62 and amino acids 238–258 of IE62 were important for the interaction with Sp3 and Sp4 as well as Sp1. This work shows that Sp family members have differential effects on IE62-mediated transactivation in a promoter-dependent manner.

Introduction

Varicella-zoster virus (VZV) is an alphaherpesvirus that causes two diseases, varicella or chickenpox during primary infection and herpes zoster or shingles upon reactivation from latency. It has a 125 kb linear double-stranded DNA genome that encodes at least 71 genes (Arvin and Gilden, 2013). VZV gene expression during lytic infection is thought to occur in three kinetic stages, immediate early (IE), early and late. VZV utilizes the host cell RNA polymerase II (RNA Pol II) and the general transcription machinery of the cell for viral gene transcription as do the other herpesviruses. A few VZV regulatory proteins, including IE62, IE4, ORF61, IE63 and ORF10 are responsible for efficient viral gene expression (Arvin and Gilden, 2013).

IE62, a tegument protein, is the major VZV transactivator, regulates the expression from all of the VZV promoters tested to date (Kinchington et al., 2000; Arvin and Gilden, 2013) and also activates a variety of cellular promoters (Perera, 2000). It is a 1310 amino acid protein with five distinct domains, based on comparisons with other herpesvirus IE transactivators (Cheung, 1989). Of these domains, II and IV are the most conserved at the amino acid level, while domain I, III and V show significant variability. Among the functions mapped to the various domains of IE62, domain I (aa 1–467) contains an acidic activation domain essential for the transcription activation function of IE62. Domain II (aa468–640) contains a highly conserved DNA binding and dimerization domain. These two domains have been shown to be a platform for the physical interaction of IE62 with several cellular transcription factors and VZV proteins. The VZV proteins ORF9 and IE4 bind to the acidic activation domain (Cilloniz et al., 2007; Spengler et al., 2000) while IE63 interacts with the DNA binding and dimerization domain (Lynch et al., 2002). The cellular TATA box binding protein (TBP) also interacts with the DNA binding domain while the cellular transcription factors USF and Sp1 bind to the acidic activation domain (Peng et al., 2003; Rahaus et al., 2003).

The Sp/KLF family contains at least twenty members identified thus far, with the prototypes being Sp1, Sp2, Sp3 and Sp4. Members of this family bind with varying affinities to sequences designated as 'Sp1 sites' (e.g., GC-boxes and CACCC-boxes). Sp family proteins are characterized by a highly conserved C-terminal DNA-binding domain containing three zinc fingers (Philipsen and Suske, 1999). Although conservation within their DNA binding domains means that Sp1-related proteins can interact with the same DNA sequences, the family members vary in their interaction with different sequences. For example, Sp1, Sp3, and Sp4 bind with

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higher affinity to GC-boxes than to CACCC-boxes (Hagen et al., 1992; Hagen et al., 1995; Kingsley and Winoto, 1992; Thiesen and Bach, 1990), whereas Sp2 binds preferentially to CACCC-boxes over GC-boxes (Crossley et al., 1996; Matsumoto et al., 1998; Shields and Yang, 1998). While Sp1, Sp2 and Sp3 are ubiquitously expressed in different tissues, Sp4 is expressed primarily in neurons and cells of neuronal origin.

Sp1 binds to several VZV promoters (Peng et al., 2003; Ruyechan et al., 2003). The GC rich sequence that binds Sp1 has been found in the promoters of VZV ORF61, viral glycoproteins gI and gE, the VZV major single-strand binding protein and the VZV DNA polymerase catalytic subunit (Wang et al., 2009; Berarducci et al., 2007; Peng et al., 2003; Yang et al., 2004). Recently we reported that Sp3, in addition to Sp1, binds to the downstream region of VZV oriS and to the ORF3 promoter. Mutation of their binding sites reduced ORF62, ORF63 and ORF3 expression in reporter gene assays (Khalil et al., 2012, 2013). The contributions of Sp2 and Sp4 to VZV gene expression and replication remain unknown. The binding of Sp3 to the Sp1 binding sites in some VZV promoters, raised a specific question; is the involvement of these GC-rich sequences in IE62-mediated transactivation due to the binding of Sp3 rather than or in concert with Sp1?

In the work presented here, we extend our study of the role of Sp family members in VZV replication by examining the influence of the ectopic expression of four Sp family members, Sp1, 2, 3 and 4, on IE62 regulation of viral promoters that we showed previously to contain functional Sp1 sites. We demonstrate that Sp3 increases IE62-mediated transactivation of ORF3 and gI promoters as well as the model luciferase reporter pSp1-TA-Luc, while it reduces IE62 activation of the ORF28/29 promoter. In contrast, Sp2 reduced IE62-mediated transactivation of model promoters and the gI promoter, a finding consistent with the inhibitory effects typical of Sp2. Surprisingly, the ectopic expression of Sp1 had no effect on IE62-mediated transactivation of any of the VZV promoters or model promoters. Of particular interest, the ectopic expression of Sp3, which inhibited the dual ORF28/29 promoters, also inhibited oriS dependent DNA replication in DpnI assays. This work suggests that the Sp family members have significant differential effects on IE62-mediated activation of VZV genes and, contrary to previous hypotheses, Sp3 may play a much more important role than Sp1.

Results

Predicted Sp1 binding sites within putative VZV promoters

The number of predicted Sp1 binding sites in putative VZV promoters was determined using the two Sp1 binding sites previously identified in the VZV ORF3 and gI promoters as the template for a bioinformatics search. These were 5′–CCCGCCC-3′ in the ORF3 promoter and 5′–CACGCCCC-3′ in the gI promoter (Peng et al., 2003; Khalil et al., 2013). The search was done with NCBI Blastn program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and a conservative promoter size of 200–300 bp upstream from the translational start sites of the VZV genes was chosen. Forty Sp1 sites were identified within putative promoter regions (Table 1). Of the 36 promoters identified, we chose three that we had studied previously, (ORF3, ORF28/29 and gI), to assess how over-expression of Sp family members affected VZV genes transcription.

Ectopic expression of Sp family members influences IE62-mediated transactivation differentially in a promoter dependent manner

In the first set of experiments, reporter gene assays were done in the context of pCMV-62 transfection in the presence and absence of plasmids expressing Sp1, Sp2, Sp3 and Sp4, using two reporters containing model promoters. One model reporter, pTA-Luc, is a minimal promoter including only a TATA element. The second model reporter contains the Sp1 site identified in the downstream region of VZV oriS (Khalil et al., 2008) at a distance of 25 nucleotides upstream of the same TATA element (Fig. 1A). Luciferase activities obtained from each reporter plasmid in the absence of ORF62, but with ectopic expression of Sp family members, represented basal activity levels and were normalized to 1. Reporter gene activities in the presence of ORF62 transfection and/or plasmids over-expressing Sp1, Sp2, Sp3 and Sp4 were displayed as induction (*n*-fold) of luciferase activities relative to the basal activity.

As shown in Fig. 1B and C, IE62 activated the two model reporters, as did the Sp family members Sp1, Sp3 and Sp4, but to a lesser extent. Ectopic expression of Sp2 reduced IE62 activation of the two reporters significantly while Sp1 had no statistically significant influence. In contrast, Sp3 activated the IE62-mediated transactivation of pSp1-TA-Luc reporter significantly but had no significant effect on IE62 activation of the pTA-Luc reporter. Sp4 overexpression reduced IE62 activation of the pTA-Luc reporter significantly but had no statistically significant influence on IE62-mediated transactivation of the pSp1-TA-Luc reporter.

In the next series of experiments, we tested the ORF3, gI and ORF28/29 promoters in reporter gene assays. IE62 activated all of these reporters, while Sp1, Sp3 and Sp4 activated significantly ORF3 and gI promoters, as shown in Fig. 2. Sp1 overexpression activated ORF3 and gI promoter 4- and 8-fold respectively while Sp3 overexpression activated the two promoters 7- and 11-fold respectively. Also, Sp4 ectopic expression activated ORF3 and gI

Table 1
List of the VZV promoters that contain predicted Sp1 binding site(s).

VZV gene	No. of Sp1 sites	Sp1 site sequence
ORF3	1	CCCGCCC
ORF4	1	CCGCC
ORF6	2	GGAGCCGCCC
		CCGCC
ORF10	1	CGGCGGG
ORF13	1	GGCGG
ORF18	1	GGAGGCGGG
ORF23	1	GCACCGCCCC
ORF25	1	CGGCGG
ORF28&29	1	CCCCACGCCC
ORF32	1	CCGCACCCG
ORF33	4	GGCGGCG
		CGGCGGC
		CGGCGG
		GCCGCCC
ORF35	1	CCGCCGCCG
ORF37	2	CCCGCC
		GCCGCC
ORF38&39	1	GGCGGGG
ORF40	2	CCGCCCCCG
		CCCGCAC
ORF41	1	CCACGCCC
ORF44	1	CCCGGGCGGC
ORF45	1	CCGCC
ORF46	1	CCGCC
ORF50	1	CGCCGCCG
ORF51	2	GAGGCGGC
		CCGCACCC
ORF52	1	CGGGGCGG
ORF56	1	CGGCGG
ORF61	1	GGCGG
ORF62 & ORF71	1	CGAGGCGGG
ORF63 & ORF70	1	CTCCCGCCCCGG
ORF64	1	CCGCCCCCCC
ORF65&66	1	CTCCGCCCTC
ORF67	1	CACGCCCC
ORF68	1	GGGCGGGG
ORF69	1	GGGGGGGCGG