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Cellular transcription factor YY1 mediates the varicella-zoster virus (VZV) IE62 transcriptional activation

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ABSTRACT

Several cellular transcription factors have been shown to be involved in IE62-mediated activation. The YY1 cellular transcription factor has activating and repressive effects on gene transcription. Analysis of the VZV genome revealed 19 postulated YY1 binding sites located within putative promoters of 16 VZV genes. Electrophoretic mobility shift assays (EMSA) confirmed the binding of YY1 to ORF10, ORF28/29 and gI promoters and the mutation of these binding sites inhibited YY1 binding and the promoter activation by IE62 alone or following VZV infection. Mutation of the ORF28/29 YY1 site in the VZV genome displayed insignificant influence on virus growth in melanoma cells; but it inhibited the virus replication significantly at day 5 and 6 post infection in HELF cells. This work suggests a novel role for the cellular factor YY1 in VZV replication through the mediation of IE62 activation of viral gene expression.

Introduction

Varicella-zoster virus (VZV) is a neurotropic herpesvirus and member of the family *alphaherpesvirinae*. It causes two diseases, varicella (chickenpox) during primary infection and herpes zoster (shingles) upon reactivation from latency in sensory ganglia. The VZV genome consists of a 125 kb linear double-stranded DNA molecule that encodes at least 71 genes (Cohen et al., 2007). All of the VZV genes are believed to be expressed during lytic infection in three kinetic classes, immediate early (IE), early and late. Like other herpesviruses, VZV uses the host cell RNA polymerase II (RNA Pol II) and the general transcription apparatus of the cell for viral gene transcription. A few VZV proteins, including IE62, IE4, ORF61, IE63 and ORF10 are responsible for efficient viral gene expression (Cohen et al., 2007). IE62, the primary viral transactivator, regulates the expression of genes from all three putative kinetic classes (Kinchington et al., 2000).

Several cellular transcription factors have been shown to be involved in the regulation of VZV gene expression mediated by IE62. Sp1 family members target the GC rich sequence within VZV promoters and interact with IE62 (Peng et al., 2003; Ruyechan et al., 2003). The presence of a GC rich sequence that binds Sp1 has been found in gE, gI and ORF28/29 promoters (Beraraducci et al., 2007; Peng et al., 2003; Yang et al., 2004). Sp1 and Sp3 bind to the downstream region of VZV oriS and to the ORF3 promoter; mutation of their binding sites inhibited ORF62, ORF63 and ORF3 expression in reporter gene assays (Khalil et al., 2012, 2013).

The upstream sequence factor, USF, is another cellular factor involved in IE62-mediated gene expression (Rahaus et al., 2003). The USF consensus binding sequence (5′-CACGTG-3′) is present in some VZV promoters and IE62 interacts with USF (Rahaus et al., 2003). The ORF10 and ORF28/29 promoter sequences have USF sites and mutation of these sites inhibited gene expression in reporter gene assays (Che et al., 2007; Yang et al., 2004, 2006). Yang et al., (2008) showed that the human mediator complex is also an essential component for efficient VZV gene expression. The physical interaction between the N-terminal acidic activation domain (TAD) of IE62 and the factors involved in the formation of the human mediator complex has been demonstrated (Yang et al., 2008; Yamamoto et al., 2009).

Other classes of mammalian transcription factors have been identified but their role in transcriptional regulation is not well understood. Yin Yang1 or YY1 is a cellular transcription factor

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discovered about 22 years ago (Shi et al., 1991). YY1 belongs to the GLI-Kruppel class of zinc finger proteins that are ubiquitously expressed and play an important role in biological processes such as embryogenesis, differentiation, replication and cellular proliferation (Yang et al., 1995; Gordon et al., 2006). The YY1 protein contains four C2H2 type zinc finger domains near the C-terminal half of the protein responsible for the DNA binding activity and two acidic activation domains near the N-terminal half that are capable of transcriptional activation (Hyde-DeRuyscher et al., 1995; Yant et al., 1995). YY1 protein is responsible for activating or repressing a diverse group of promoters depending on the promoter architecture, which is the origin of the name Yin Yang (Shi et al., 1991). YY1 has also been shown to interact with other cellular factors including Sp1, Histone deacetylase2, ATF6 and Notch1 (Seto et al., 1993; Yao et al., 2001; Li et al., 2000; Yeh et al., 2003).

Two YY1 protein consensus binding sites were identified recently in the VZV genome; the first one is in the downstream region of VZV oriS (5′CAAATGGCG-3′) and the other is within the ORF3 promoter (5′CCCATATAT-3′) (Khalil et al., 2008, 2012, 2013). The mutation of the YY1 site downstream of VZV oriS had no significant effect on VZV DNA replication or ORF62 and ORF63 transcription efficiency in DpnI replication and reporter gene assays respectively although it increased the VZV growth in skin xenografts slightly at day 21 post-infection (Khalil et al., 2012). In contrast, mutation of the YY1 site in the ORF3 promoter inhibited expression in reporter gene assays (Khalil et al., 2013).

In the work presented here, we investigated the role of YY1 binding sites within VZV promoters in activation of IE62-dependent gene expression in order to better understand mechanisms that IE62 uses to exploit the host cell environment for efficient viral gene expression. The binding of YY1 to sites within the putative VZV promoters of ORF10, ORF28/29 and gI was established using EMSA and supershift assays. The mutation of these YY1 sites ablated formation of the YY1 specific complex and inhibited activation of these promoters. A VZV recombinant virus with a mutation of the YY1 site in the ORF28/29 promoter was constructed and effects on virus growth were studied. This mutation was found to have no significant effect on VZV replication in melanoma cells and on the ORF29 expression. On the other hand, this mutation inhibited VZV replication in HELF significantly at day 5 and 6 post infection. This work suggests that the cellular transcription factor YY1 acts as a cellular factor involved in the IE62-mediated activation of VZV genes.

Results

Predicted YY1 binding sites within putative VZV promoters

As the first step in studying the role of YY1 in regulation of viral gene expression, the number of predicted YY1 binding sites in putative VZV promoters was determined. Since there is no degenerate consensus binding site for the YY1 protein, we used the YY1 binding sites previously identified in the VZV genome as the template in the bioinformatics search. These were 5′-CAAATGG-3′ in the downstream region of VZV oriS and 5′-CCCATATAT-3′ in the ORF3 promoter (Khalil et al., 2008; 2013). The search was done with NCBI Blastn program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). In order to estimate the number of predicted YY1 sites, we used a conservative promoter size of 200 to 300 bp upstream from the translational start sites of the VZV genes. Nineteen YY1 sites were identified within putative promoter regions (Table 1). Three of the 19 promoters that had been studied previously, including those of ORF10, ORF28/29 and gI genes were selected to study the binding of YY1 and the influence of these predicted YY1 binding sites on regulation of the expression of these genes.

Table 1
List of the VZV promoters containing predicted YY1 binding site.

VZV gene	No. of YY1 site	YY1 site sequence
ORF3	1	CCCATATA
ORF4	1	AATGGG
ORF7	1	CCCATTTTT
ORF10	1	CCCCATT
ORF11	1	CCCATATA
ORF15	1	CCCCCCCATTTT
ORF28/29	1	CCCCCCATT
ORF30	1	CCCATAT
ORF35	1	AAAAATGGG
ORF42	1	CCCATTT
ORF49	1	CCCATT
ORF61	1	CCCATTT
ORF63, 70	3	CCCCCATT, CCATTTT, AAATGG
ORF67	1	CCCCATT

YY1 binds to the predicted YY1 binding sites within ORF10, ORF28/29 and gI promoters

In the first set of experiments, we used EMSA and supershift assays to establish the binding of YY1 to its predicted binding sites within VZV ORF10, ORF28/29 and gI promoters. We performed our experiments using 40 bp duplex oligonucleotides containing wild type and mutant YY1 sites within these promoters and nuclear extracts from VZV infected MeWo cells. 30 bp duplex oligonucleotides containing the origin binding protein (OBP) box A sequence in the ORF62/ORF63 intergenic region was used as the nonspecific competitor (Khalil et al., 2008). The sequence of the duplex oligonucleotides used in the EMSA and supershift assays are listed in Table 2.

EMSA and supershift assays using the oligonucleotides containing the predicted YY1 binding site of the ORF10 promoter revealed multiple complexes including two major faster migrating complexes and several minor slowly migrating complexes (Fig. 1A). Antibody supershift assays were then performed to assess the binding of the YY1 cellular transcription factors to this site. The anti-YY1 antibody supershifted one of the minor complexes formed (Fig. 1A).

In the next series of experiments, EMSAs were performed using oligonucleotides containing the mutation in the YY1 binding site of the ORF10 promoter that AliBaba2.1 and Patch programs (http://www.gene-regulation.com/pub/programs.html; Biobase) predicted to inhibit the binding of YY1. The mutant probe has a substitution of the CC residues with AA in the YY1 binding site. As shown in Fig. 1B, this mutation inhibited the formation of the identified YY1 complex and also affected the formation of some of the major complexes suggesting that these complexes may need the presence of the YY1 binding site.

To test the specificity of the formation of the YY1 containing complex with ORF10 containing nucleotides, competition EMSA experiments were done using unlabeled oligonucleotides containing the YY1 binding site of the ORF10 promoter as the specific competitor and a 30-bp oligonucleotides containing the origin binding protein (OBP) box A sequence described above. As shown in Fig. 1C, the cold specific competitor efficiently competed away the formation of the YY1 containing complex and some major complexes, while the presence of the nonspecific competitor had no effect on the shift pattern. These experiments indicated that cellular transcription factor YY1 binds specifically to the ORF10 promoter element.