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Regulation of the varicella-zoster virus ORF3 promoter by cellular and viral factors

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ABSTRACT

The varicella zoster virus (VZV) immediate early 62 protein (IE62) activates most if not all identified promoters of VZV genes and also some minimum model promoters that contain only a TATA box element. Analysis of the DNA elements that function in IE62 activation of the VZV ORF3 promoter revealed that the 100 nucleotides before the translation start site of the ORF3 gene contains the promoter elements. This promoter lacks any functional TATA box element. Cellular transcription factors Sp1, Sp3 and YY1 bind to the promoter, and mutation of their binding sites inhibited ORF3 gene expression. VZV regulatory proteins, IE63 and ORF29, ORF61 and ORF10 proteins inhibited IE62-mediated activation of this promoter. Mutation of the Sp1/Sp3 binding site in the VZV genome did not alter VZV replication kinetics. This work suggests that Sp family proteins contribute to the activation of VZV promoters by IE62 in the absence of functional TATA box.

Introduction

Varicella-zoster virus (VZV) is a human alphaherpesvirus that belongs to the family *Herpesviridae*. It is the causative agent of two diseases, varicella (chickenpox) and herpes zoster (shingles). The VZV genome is a linear double-stranded DNA molecule of 125 kb and encodes at least 71 genes which are identified numerically according to the location of their open reading frames (ORF) relative to the 5′ end of the VZV genome (Cohen et al., 2007). Upon its entry into the infected cell nucleus, the VZV genome is transcribed by the host cell RNA polymerase II (RNA Pol II) and the general transcription apparatus of the cell. By analogy with herpes simplex virus-1 (HSV-1), the VZV ORFs are presumed to be expressed as three distinct kinetic classes during viral infection: immediate early (IE), early and late. All of the IE gene products appear to be transcriptional regulatory proteins. Expression of these genes permits transcription of the early genes that are involved in DNA replication and nucleotide metabolism and the late genes encoding structural proteins, such as the capsid proteins and glycoproteins, which are required for assembly of virions (Cohen et al., 2007).

The expression of VZV early and late genes is thought to be mediated primarily through functions of the IE62 protein acting in conjunction with viral and cellular factors. Although several other proteins encoded by VZV, including the IE4, IE63, ORF10, and ORF61 proteins have been shown to be capable of transactivating and/or transrepressing specific viral promoters, (Baudoux et al., 2000; Defechereux et al., 1997; Moriuchi et al., 1992, 1993a, 1994, 1995; Sato et al., 2003 and Spengler et al., 2000; Wang et al., 2009;), IE62 is considered to be the primary viral transactivator driving expression of genes from all three putative kinetic classes (Kinchington et al., 2000).

To date, only a few VZV promoters have been analyzed in enough detail to determine their functional elements. However, these analyses as well as predictions of the positions of putative promoters within the VZV genome indicate that they are relatively typical RNA Pol II promoters with TATA-like elements and binding sites for ubiquitous cellular transcription factors upstream of the TATA elements (Smale and Kadonaga, 2003). No promoters showing dependence on the presence of an initiator element (INR) at or just downstream of the start site of transcription are known. All of the VZV promoters studied thus far have been shown to depend on either canonical or non-canonical TATA elements for activation by IE62 (Peng et al., 2003; Ruyechan et al., 2003 and Yang et al., 2004) except for the VZV ORF10 promoter as shown by Che et al. (2007). The ORF10 promoter required the presence of a USF site and the mutation of this *cis*-element inhibited the IE62-mediated transactivation of this promoter.

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This circumstance most likely reflects the fact that only a few VZV promoters have been analyzed at a functional level rather than that VZV lacks these possible gene regulatory mechanisms.

The biology of VZV infection indicates that control of expression of the viral genes is essential to the ability of the virus to replicate productively in cell types that it targets during primary infection, which include skin and T cells, and to establish and preserve latency in neurons (Cohen et al., 2007). However, the molecular mechanisms by which VZV gene expression is regulated are not well understood. Functional analysis of additional VZV promoters representing all three putative kinetic classes of VZV genes and exhibiting differential regulation of viral gene expression is needed to provide more information about this fundamental process. Based on this consideration, the work presented here examines aspects of the regulation of the ORF3 promoter, which results in expression of ORF3 protein, a late gene product.

VZV ORF3 encodes a predicted 179 amino acid protein with no known function. ORF3 protein was dispensable for VZV replication in melanoma cells and skin organ culture in vitro as well as in human T cells in thymus/liver xenografts in SCID-hu mice infected in vivo (Zhang et al., 2007, 2010). UL55, the HSV homologue of ORF3, has been shown to be dispensable for viral replication and for establishment of latency (Nash and Spivack, 1994).

In the work presented here, we analyzed the ORF3 promoter to better understand the molecular mechanism of its activation by IE62. The results demonstrated that ORF3 promoter lacks any functional TATA box and requires the cellular transcription factors Sp1, Sp3 and YY1 for its activation. However, the ORF3 promoter was down regulated by the presence of a suppressor cis-acting element located in the nucleotide segment from 100 to 120 before the ORF3 gene translation start site. VZV ORF10, ORF29, ORF61 and IE63 proteins had inhibitory effects on IE62-mediated transactivation of the ORF3 promoter. Mutating the Sp1/Sp3 site in the ORF3 promoter in the context of the VZV genome had no effect on viral replication in MeWo cells. This work suggests that an IE62 and Sp1/Sp3 mechanism is an alternative to VZV promoter activation by IE62 through interaction with a TATA-binding protein (TBP).

Materials and methods

Cells and viruses

MeWo cells, a human melanoma cell line were grown in Eagle's minimal essential medium supplemented with 10% fetal bovine serum (Spengler et al., 2000). VZV strains MSP and pOka were propagated in MeWo cell monolayers as described by Lynch et al. (2002) and Peng et al. (2003).

Nuclear and whole cell lysate preparation and immunoblot analysis

Nuclear extracts of VZV infected MeWo cells were prepared as previously described (Lynch et al., 2002). MeWo cells were incubated in buffer A (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol) at 4 °C on ice for 15 min to lyse the cells and release the cytoplasmic fraction. After centrifugation, the crude nuclear pellet was incubated on ice in buffer C (20 mM HEPES, pH 7.9, 25% (v/v) glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol). After centrifugation the nuclear extract was dialyzed against buffer D (20 mM HEPES, pH 7.9, 20% (v/v) glycerol, 0.1 M KCl, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol).

Whole cell lysates of VZV infected and pCMV-ORF3 transfected MeWo cells were prepared in lysis buffer (50 mM Tris–HCl, pH 7.5, 0.15 M NaCl, 1 mM EDTA, 0.1% Triton X-100 and protease inhibitor cocktail (Roche, Mannheim, GE) added per the manufacturer's instructions) and analyzed for ORF3 protein by immunoblot (10% SDS-PAGE) using a rabbit polyclonal antiserum against a GST fusion full length ORF3 protein (peng et al., 2003; and Yang et al., 2006) and IE63 protein using rabbit polyclonal antibody against full length IE63 protein (Zuranski et al., 2005). Rabbit polyclonal antibody against β-tubulin was obtained from Santa Cruz Biotechnology (Santa Cruz, CA.) and mouse monoclonal antibody against α-tubulin was obtained from Sigma-Aldrich. Quantification of the relative amounts of ORF3, IE63 α-tubulin and β-tubulin was done using a BioRad GS700 Imaging Densitometer (BioRad Hercules, CA). Statistical significance was determined by one-way ANOVA analysis of variance followed by Tukey's post hoc test.

Plasmids

A set of luciferase reporter plasmids containing the ORF3 promoter flanked by firefly luciferase was constructed using the pGL2 basic vector (Promega, Madison, WI). The 336 bp intergenic region between ORF3 and ORF4 was amplified by PCR using these two primers containing a HindIII restriction site at the 5′ end and a XhoI restriction site at the 3′ end, respectively; the primer sequences were 5′-ATCAAGCTT TAATTAAACGTTCGGTACACGTCT-3′ and 5′-ATCCTCGAGAAATAAAAAATACCTT TTTCATGC-3′. The PCR product was digested and inserted into the pGL2 basic vector multiple cloning sites between the HindIII and XhoI restriction sites. The ORF3 promoter truncation that contained the 120 nucleotides from the translation start codon of ORF3 gene was cloned by amplification of the first 120 bp by PCR using the first primer as above; the second primer was: 5′-ATCCTCGAGTTTT-TAAGGCGACGTTG GGGATAT-3′. This PCR product was inserted into the basic pGL2 plasmid. The other ORF3 promoter truncations containing 87, 94 and 100 nucleotides from the translation start codon were constructed from the 120 nucleotides truncation construct using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, LaJolla, CA).

The plasmids containing mutations of the Sp1/Sp3 and YY1 sites and TATA box within the ORF3 promoter were generated from the wild type pGL2-ORF3 plasmid containing the ORF3/ORF4 intergenic region using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, LaJolla, CA). The primer sets for these mutations were: Sp1/Sp3 site: 5′-TGGTTTGAAAGCAATGTAAT CC**TT**CCCATATATCCCCAACGTCGC-3′ and 5′-GCGACGTTGGGGATA-TATGGG**AA**GGATTACATTGCTTTCAAACCA-3′; YY1 site: 5′-TGAAAG CAATGTAATCCCGCCC**G**TATATCCCCAACGTCGCCTTAA-3′ and 5′-TTAAGGCGACGTTGGGGATATA**C**GGGCGGGATTACATTGCTTTCA-3′; TATA box1: 5′-AGTACCGGAATGCCAAGCTTTA**GC**TAAACGTTCGG-TACACGTCTG-3′ and 5′-CAGACGTGTACCGAACGTTTA**GC**TAAAG CTTGGCATTCCGGTACT-3′; TATA box2: 5′- TGAAAGCAATG-TAATCCCGCCCATAT**CC**CCCCAACGTCGCCTTAA-3′ and 5′- TTAAG GCGACGTTGGGG**GG**ATATGGGCGGGATTACATTGCTTTCA-3′.The mutated nucleotides are indicated in bold. All primers were synthesized by IDT (Coralville, IA). The mutations were verified by sequencing at the Roswell Park Cancer Institute sequencing facility, Buffalo NY.

The pCMV62 plasmid expressing ORF62 under the control of the cytomegalovirus immediate-early (IE) promoter and the pCMV-ORF63 and pCMV-ORF29 constructs have been described previously (Perera et al., 1992a, 1993; Stevenson et al., 1996 and Zuranski et al., 2005). The pCMV-ORF10, pCMV-ORF61 and pCMV-ORF4 constructs were kindly provided by P. R. Kinchington (University of Pittsburgh, Pittsburgh, PA).

The pCMV-ORF3 was constructed using pcDNA empty vector (Invitrogen, Carlsbad, CA). The 540 bp of ORF3 gene was amplified by PCR using these two primers containing a HindIII restriction