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Brief Communication

Varicella-zoster virus (VZV) origin of DNA replication oriS influences origin-dependent DNA replication and flanking gene transcription

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ABSTRACT

The VZV genome has two origins of DNA replication (oriS), each of which consists of an AT-rich sequence and three origin binding protein (OBP) sites called Box A, C and B. In these experiments, the mutation in the core sequence CGC of the Box A and C not only inhibited DNA replication but also inhibited both ORF62 and ORF63 expression in reporter gene assays. In contrast the Box B mutation did not influence DNA replication or flanking gene transcription. These results suggest that efficient DNA replication enhances ORF62 and ORF63 transcription. Recombinant viruses carrying these mutations in both sites and one with a deletion of the whole oriS were constructed. Surprisingly, the recombinant virus lacking both copies of oriS retained the capacity to replicate in melanoma and HELF cells suggesting that VZV has another origin of DNA replication.

Introduction

Varicella-zoster virus (VZV), a neurotropic herpesvirus, 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 (Straus et al., 1982). The linear sequence of VZV genes is similar to that of herpes simplex virus type 1 (HSV-1) and includes the coding sequences for orthologues of the seven HSV-1 proteins required for origin-dependent DNA replication (Arvin and Gilden, 2013).

By analogy to HSV, two origins of DNA replication (oriS) within the internal repeats (IRs) and terminal repeats (TRs) bounding the U_S segment are identified within the VZV genome. These two copies of oriS are located in the intergenic region between the two immediate early genes encoding IE62 and IE63 proteins. HSV has another origin of DNA replication oriL near the center of the HSV-1 U_L region (Davison and Scott, 1985, 1986; Stow, 1982; Stow and McMonagle, 1983; Stow and Davison, 1986). HSV-1 oriL has been shown to be dispensable for replication of HSV DNA (Balliet et al., 2005; Weller et al., 1985) but has been implicated in HSV pathogenesis and reactivation from latency (Balliet and Schaffer, 2006). The equivalent region in the VZV genome is comprised of a bidirectional promoter that regulates the transcription of the VZV DNA polymerase catalytic subunit (ORF28) and DNA binding protein (ORF29) genes and does not contain any origin of DNA replication (Meier and Straus, 1993; Stow and Davison, 1986; Yang et al., 2004).

VZV oriS contains a 46-bp AT-rich palindrome and three consensus binding sites for the VZV origin-binding protein (OBP) (ORF51). All three OBP-binding sites (Boxes A, B, and C) for VZV [5′-C(G/A)TTCGCACT-3′] are upstream of the palindrome (Fig. 1). This is in contrast to the structure of HSV oriS, where the OBP-binding sites (Boxes I, II, and III) are located both upstream and downstream of the AT-rich element (Hay and Ruyechan, 2007; Stow and Davison, 1986). Using the in vitro DpnI assay, Stow et al. (1990) showed previously that the A site is absolutely required for DNA replication and that the deletion of the C site results in a decreased level of replication in VZV. In contrast, the deletion of the B site showed no effect on the extent of replication. In contrast, all three OBP-binding sites in HSV are required for efficient DNA replication (Stow and McMonagle, 1983; Balliet and Schaffer, 2006; Deb and Doelberg, 1988; Hernandez et al., 1991; Martin et al., 1991; Olsson et al., 2009; Weir and Stow, 1990).

Stow et al. (1990) also showed that the CGC motif within the 10 nucleotides forming Box A, C and B is crucial for the binding of

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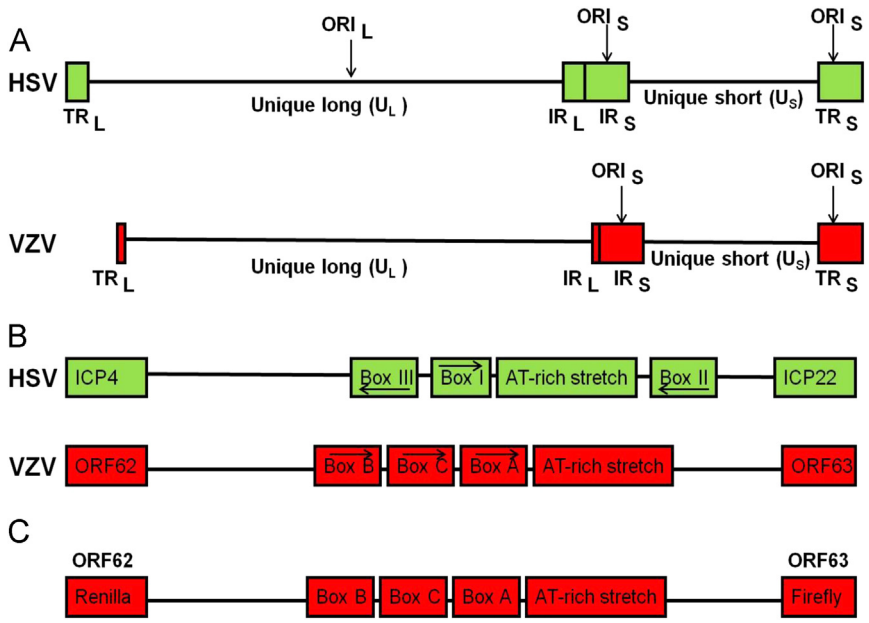

Fig. 1. The structure of the HSV-1 and VZV (A) genome and (B) oriS origins of DNA replication. (A) IRs and TRs are the internal and terminal repeat regions that bound the unique short segment of the genome. IR_L and TR_L are the internal and terminal repeat regions that bound the unique long segment of the genome. Two oriS copies are present in the IRs and TRs of the unique short segment in both HSV-1 and VZV. The HSV-1 oriL is present in the intergenic region between UL28 and UL29. (B) The positions of OBP binding site boxes and their orientations in HSV and VZV genomes. (C) A schematic diagram shows the structure of the wild type pLitmus R62/63 F plasmid used in the experiments where *Renilla* and firefly luciferase genes were placed in the position of ORF62 and ORF63 respectively.

ORF51 to these sites. Previous studies showed that either the complete deletion of the 10 nucleotides of Box A or the CGC mutations to AAA inhibits the VZV origin dependent DNA replication using the DpnI replication assay (Stow et al., 1990; Khalil et al., 2008, 2011, 2012). The deletion of the 10 nucleotides of Box C inhibited VZV origin dependent DNA replication 50–80% while the deletion of Box B has insignificant effect on VZV DNA replication (Stow et al., 1990). In this study we expand our understanding of the role of Box A, C and B by investigating the influence of the CGC motif mutation in these three origin binding protein boxes on VZV origin dependent DNA replication and the flanking gene transcription.

Our results suggest the presence of another functional origin of DNA replication within the VZV genome. This origin(s) might be analogous to the HSV oriL.

Results

Origin binding protein Boxes A, C and B influence origin-dependent DNA replication

The first set of experiments employed the pLitmus R62/63 F plasmid which contains the whole intergenic region (~1.5 kb) between ORF62 and ORF63 genes and includes the complete VZV oriS structure. The assays were performed as described previously, by transfection of plasmids containing the wild type or mutant oriS sequence followed by VZV superinfection (Khalil et al., 2008, 2011, 2012).

DpnI replication assays using the wild type pLitmus R62/63 F plasmid showed a detectable signal at the position predicted for the replicated DNA (Fig. 2A and B). In contrast, assays performed under the same conditions using the Box A mutant plasmid resulted in loss of the replicated DNA. This confirmed the results of Stow et al. (1990) who showed that Box A is required for VZV origin-dependent DNA replication. The CGC motif mutation of Box C inhibited oriS-dependent DNA replication by about 80% which was a statistically significant inhibition. On the other hand, the CGC motif substitution to AAA of Box B had no effect on the VZV DNA, as shown in Fig. 2A and B. The effect of CGC motif mutation of Box C and B on oriS-dependent DNA replication is in agreement with the effect of the deletion of both boxes shown by Stow et al. (1990).

Influence of Box A, C and B on the expression of ORF62 and ORF63 genes

To determine the involvement of the origin binding protein Boxes A, C and B in the expression of the flanking genes ORF62 and ORF63, we carried out a series of luciferase reporter gene assays examining this effect in the presence and absence of the VZV DNA polymerase inhibitor phosphonacetic acid (PAA). DpnI replication assays confirmed that the presence of 400 μg/ml of PAA completely inhibited oriS-dependent DNA replication (data not shown). The results of the luciferase reporter assays are shown in Fig. 2C and D.

The Box A mutation inhibited the expression of ORF62 and ORF63 genes by about 7 and 5-fold, respectively, compared to the wild type level, in the absence of PAA. The Box C mutation inhibited the expression of both ORF62 and ORF63 by about 2-fold in the absence of PAA. In contrast, the Box B mutation did not have a significant effect on either ORF62 or ORF63 expression in the absence of PAA. The presence of PAA inhibited the expression of ORF62 and ORF63 genes by about 7 and 5-fold respectively in the experiments using the wild type pLitmus R62/63 F plasmid (Fig. 2C and D). Mutations in any of the origin binding protein Boxes A, C or B had no statistically significant effect on the expression of ORF62 and ORF63 genes compared to the wild type level in the presence of PAA. These results suggest that the effects of the Box A and C mutations on the expression of ORF62 and ORF63 reporter genes are due to their role in oriS-dependent DNA replication, and not because they are part of the promoters of these genes.