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Mapping of human herpesvirus 6 immediate–early 2 protein transactivation domains

Andru Tomoiu, Annie Gravel, Louis Flamand *

Laboratory of Virology, Rheumatology and Immunology Research Center, Centre de Recherche du CHUL and Faculty of Medicine, Laval University, 2705 Laurier Blvd., Room T1-49, Québec, Qc, Canada G1V 4G2

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Abstract

The immediate–early 2 (IE2) protein of human herpesvirus 6 (HHV-6) is a potent transactivator of multiple cellular and viral promoters. Deletion mutants of HHV-6 variant A IE2 allowed us to map functional transactivation domains acting on complex and minimal promoter sequences. This mapping showed that both the N-terminal and C-terminal domains of IE2 are required for efficient transactivation, and that deletion of the C-terminal (1397–1466) tail of IE2 drastically reduces both transactivation and the intranuclear distribution of IE2. Moreover, we determined that the ATF/CRE binding site within the HHV-6A polymerase promoter is not required for efficient transactivation by IE2, whereas the R3 repeat region of the putative immediate–early promoter of HHV-6A is responsive to and positively regulated by IE2. These results contrast sharply to that of human cytomegalovirus (HCMV) IE2, which down-regulates its promoter. Our characterization of HHV-6 IE2 transactivating activity provides a better understanding of the complex interactions of this protein with the viral and cellular transcription machinery and highlights significant differences with the IE2 protein of HCMV.

Keywords: HHV-6; IE2; Immediate–early; Transactivation; Promoter; Polymerase; Nuclear localization sequence

Introduction

Human herpesvirus 6 (HHV-6) is a betaherpesvirus initially isolated from human immunodeficiency virus (HIV)-infected individuals and from patients suffering from lymphoproliferative disorders (Salahuddin et al., 1986). Two HHV-6 variants (A and B) have been identified. Although they share a high degree of homology for the majority of their genes (95–99%), these two variants are considered distinct infectious agents in view of their biological properties (such as gene expression and splicing pattern, *in vitro* cell tropism, reactivity to monoclonal antibodies), tissue distribution and disease association (Ablashi et al., 1991). The greatest genetic variability between the two HHV-6 variants is observed within the immediate–early 1 and 2 genes (60–70% amino acid identity), variations that may account for the observed differing biological properties. HHV-6B is the etiologic agent of the childhood disease roseola or *exanthema subitum* (Yamanishi et al., 1988). Links between HHV-6 infection and other pathologies such as meningoencephalitis (Ishiguro et al., 1990), organ transplant rejection (Carrigan et al., 1991; Yoshikawa et al., 1991) and AIDS (Knox and Carrigan, 1995; Saito et al., 1995) have also been suggested. Evidence for a co-factorial role of HHV-6A in AIDS come from several observations, including that co-infection of T cells with HIV and HHV-6 leads to the activation of HIV long terminal repeat (LTR)-directed viral gene expression and accelerates cytopathic effects (Lusso et al., 1989).

Several HHV-6 gene products have been identified as potential transcriptional activators. Among them, products of open reading frames (ORFs) DR7 (Thompson et al., 1994a, 1994b), U16 (Geng et al., 1992), U27 (Zhou et al., 1994), U90/U86 (Gravel et al., 2003), U89 (Martin et al., 1991) and U94 (Thompson et al., 1994a, 1994b) were found to transactivate the HIV LTR promoter *in vitro*. ORFs U86 and U89 are comprised within the HHV-6 immediate–early A (IE-A) locus. IE-A

* Corresponding author. Fax: +1 418 654 2765.
E-mail addresses: Andru.Tomoiu@crchul.ulaval.ca (A. Tomoiu), Annie.Gravel@crchul.ulaval.ca (A. Gravel), Louis.Flamand@crchul.ulaval.ca (L. Flamand).

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includes two genetic units termed IE1 and IE2, corresponding to ORFs U90/U89 and U90/U86, respectively (Fig. 1A). We have previously characterized the IE1 variant B (Gravel et al., 2002) and IE2 variant A (Gravel et al., 2003) proteins translated from spliced transcripts of the IE-A locus.

It has been reported that the IE2 protein is a potent transcriptional activator of heterologous promoters (Flamand et al., 1998; Gravel et al., 2003). Moreover, cotransfection experiments in T cells indicated that IE2 variant A can induce the transcription of a complex promoter such as the one present in the HIV LTR, as well as simpler promoters, whose expression is driven by a unique set of responsive elements (CRE, NF-AT, NF-κB) (Duprez et al., 1999; Gravel et al., 2003). Finally, the C-terminal domain encompassing the final 436 residues of HHV-6A IE2 was shown to bind a DNA fragment containing the transcription initiation site, TATA box and upstream sequence of the putative IE-A promoter (Papanikolaou et al., 2002).

Transcriptional activators must possess at least two functional domains: a DNA binding domain that allows attachment of the transactivator to its target sequence within a gene promoter, and an activation domain that promotes the transcription of the target genes. For HHV-6A IE2, the activation and DNA binding domains have not been defined yet, but clues pertaining to the nature of its functional domains could perhaps be deduced from studies of human cytomegalovirus (HCMV) immediate–early protein IE2. HCMV like HHV-6 is a betaherpesvirus and shares limited amino acid sequence similarities, immunological cross-reactivity and overall gene organization with HHV-6 (Lawrence et al., 1990; Neipel et al., 1991; Yasukawa et al., 1993). HCMV gene UL122, encoding for protein IE2, is a positional homologue of HHV-6 ORF U86 (Nicholas, 1994) and corresponds to the C-terminal portion of HHV-6 IE2. The similarity between HCMV IE2 and the carboxy-terminal region of HHV-6 IE2 is 45% (Nicholas, 1994). HCMV IE2 is a 86-kDa protein whose biological functions are well defined and include transactivation of heterologous promoters (Pizzorno et al., 1988), repression of its own promoter (Hermiston et al., 1990), association with the viral DNA replication compartment (Ahn et al., 1999), blocking of cell cycle progression (Wiebusch and Hagemeier, 1999) and modulation of apoptosis (Zhu et al., 1995).

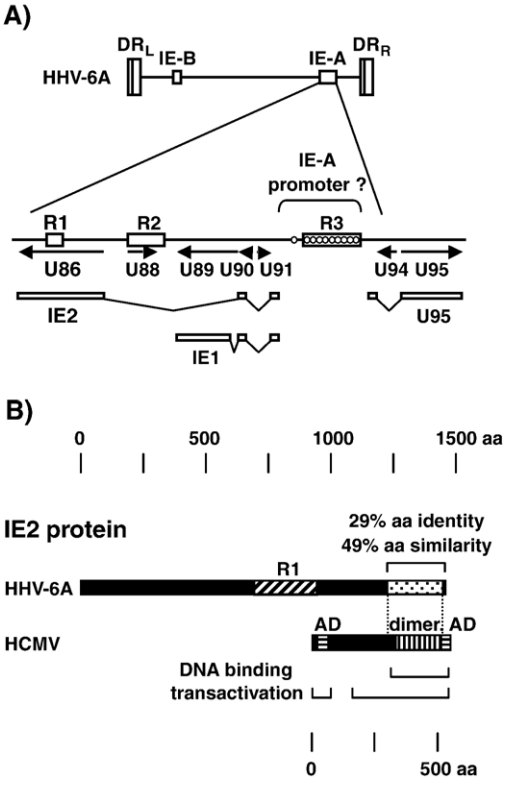

Fig. 1. (A) Schematic representation of HHV-6A immediate–early A locus. Top drawing represents HHV-6A genome including left and right direct repeats (DR_L and DR_R) and immediate–early loci (IE-A and IE-B). Lower drawing represents IE-A locus including repeat regions (R1–R3), orientation and approximate position of major ORFs (arrows) and coding exons of key transcripts (IE1, IE2 and U95). U95 exon prediction is based on HHV-6B data. White circles denote known transcription factor binding sites, including NF-κB/AP2/PEA3 (circa 28 copies in R3) and AP1 (2 overlapping putative binding sites upstream of R3 on the sense strand). (B) Schematic representation of similarities between HHV-6A and HCMV IE2 proteins. The two proteins share a significant identity in the C-terminal domain. AD=activation domain; dimer.=dimerization domain; lower brackets indicate HCMV IE2 regions required for DNA binding and transactivation.

Mapping studies have revealed that HCMV IE2 contains two distinct acidic activation domains, one at the N terminus and one at the C terminus (Fig. 1B). Both domains are required for transactivation of most target reporter genes within the context of wild-type IE2 (Pizzorno et al., 1991). The activator domains of HCMV IE2 do not seem to fall within the conserved region with HHV-6A IE2. However, minimal dimerization and DNA binding domains have been identified in the HCMV IE2 region having significant similarity with HHV-6 (Chiou et al., 1993).

In the present work, we used deletion mutants to map functional transactivation domains of HHV-6A IE2 using complex and minimal promoter sequences. This mapping allowed us to determine that both the N-terminal and C-terminal domains of IE2 are required for efficient transactivation, and that deletion of the C-terminal (1397–1466) tail of IE2 drastically reduces both transactivation and the nuclear patchy distribution of IE2. Moreover, we determined that the ATF/CRE binding site in the HHV-6A polymerase promoter is not required for efficient transactivation of the promoter by IE2, whereas the R3 repeat region of the putative immediate–early promoter of HHV-6A strongly enhances IE2 transactivation of this promoter. This and future characterization of IE2 should provide a better understanding of this complex viral protein. Furthermore, this study underscores important functional differences between HCMV and HHV-6A IE2 proteins.

Results

Mapping of HHV-6A IE2 domains required for transactivation

The main function currently known of HHV-6A IE2 is to promiscuously promote transcriptional activation (Gravel et al., 2003). IE2 being a large protein, we generated various deletion mutants in order to determine which domains are essential for transactivation. We arbitrarily divided IE2 into three major