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The Varicella-zoster virus DNA encapsidation genes: Identification and characterization of the putative terminase subunits

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Abstract

The putative DNA encapsidation genes encoded by open reading frames (ORFs) 25, 26, 30, 34, 43, 45/42 and 54 were cloned from Varicella-zoster virus (VZV) strain Ellen. Sequencing revealed that the Ellen ORFs were highly conserved at the amino acid level when compared to those of 19 previously published VZV isolates. Additionally, RT-PCR provided the first evidence that ORF45/42 was expressed as a spliced transcript in VZV-infected cells. All seven ORFs were expressed *in vitro* and full length products were identified using a C-terminal V5 epitope tag. The *in vitro* products of the putative VZV terminase subunits encoded by ORFs 30 and 45/42 proved useful in protein–protein interaction assays. Previous studies have reported the formation of a heterodimeric terminase complex involved in DNA encapsidation for both herpes simplex virus-type 1 (HSV-1) and human cytomegalovirus (HCMV). Here we report that the C-terminal portion of exon II of ORF45/42 (ORF42-C269) interacted in GST-pull down experiments with *in vitro* synthesized ORF30 and ORF45/42. The interactions were maintained in the presence of anionic detergents and in buffers of increasing ionic strength. Cells transiently transfected with epitope tagged ORF45/42 or ORF30 showed primarily cytoplasmic staining. In contrast, an antiserum directed to the N-terminal portion of ORF45 showed nearly exclusive nuclear localization of the ORF45/42 gene product in infected cells. An ORF30 specific antiserum detected an 87 kDa protein in both the cytoplasmic and nuclear fractions of VZV infected cells. The results were consistent with the localization and function of herpesviral terminase subunits. This is the first study aimed at the identification and characterization of the VZV DNA encapsidation gene products.

Keywords: Varicella-zoster virus; DNA encapsidation; Terminase; ORF30; ORF45/42

1. Introduction

The ongoing clinical need to prevent or treat infections caused by members of the family *Herpesviridae* merits continued investigation of novel antiviral agents (Biron, 2006; Bogner, 2002; Casper, 2006; Casper and Wald, 2007; De Clercq, 2001; Griffiths, 2006; Luck et al., 2006; Schang, 2006; Visalli, 2004; Visalli and van Zeijl, 2003). Proteins that play a role in the DNA encapsidation process have become promising novel targets (van Zeijl et al., 2000; Visalli et al., 2003; Visalli and van Zeijl, 2003). Studies on herpes simplex virus-type 1 (HSV-1) have contributed greatly to our understanding of the proteins involved in encapsidation. A total of seven genes have been shown to be essential in the HSV-1 DNA encapsidation process: ULs 6, 15, 17, 25, 28, 32 and 33 (Beard et al., 2002; Chang et al., 1996; Goshima et al., 2000; Koslowski et al., 1999; Lamberti and Weller, 1996; Lamberti and Weller, 1998; McNab et al., 1998; Newcomb et al., 2001a; Ogasawara et al., 2001; Patel and MacLean, 1995; Patel et al., 1996; Reynolds et al., 2000; Salmon and Baines, 1998; Sheaffer et al., 2001; Taus and Baines, 1998; Yu and Weller, 1998b). These genes are conserved throughout the herpesvirus family (Alba et al., 2001) and when any of the seven were deleted from the HSV-1 genome, empty capsids accumulated in the nucleus. There is mounting evidence that at least two of the seven encapsidation proteins form an essential terminase complex that likely functions as both an endonuclease and a DNA translocase during DNA cleavage and packaging (Bogner, 2002; Hwang and Bogner, 2002; Scheffczik et al., 2002; Scholz et al., 2003). During viral replication, progeny DNA genomes are synthesized in the nucleus as long, branched head-to-tail concatemers (Severini

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et al., 1996; Zhang et al., 1994). The capsid proteins are synthesized in the cytoplasm, transported to the nucleus, and assembled into procapsids (Brown and Homa, 2002). The tight packing of one unit length of herpesviral genomic DNA into preformed procapsids is likely mediated by multi-protein complexes that include the viral terminase subunits.

The human cytomegalovirus (HCMV) terminase subunits pUL56 and pUL89, encoded by the UL56 and UL89 genes, have been extensively studied. Both gene products formed toroidal structures, possessed DNA binding and nuclease activities, and specifically recognized genomic packaging (pac) sequences (Bogner et al., 1998; Scheffczik et al., 2002). The subunit pUL56 was shown to have ATPase activity that was enhanced by the addition of the pUL89 subunit (Hwang and Bogner, 2002; Scholz et al., 2003). These subunits interacted *in vitro* (Hwang and Bogner, 2002; Thoma et al., 2006) and could be co-immunoprecipitated from HCMV infected cell extracts (Hwang and Bogner, 2002). Additionally, pUL56 formed a complex with the HCMV portal protein, pUL104 (Dittmer et al., 2005). This latter interaction was abolished in the presence of the benzimidazole-D-ribonucleoside inhibitors BDCRB and CL4RB (Dittmer et al., 2005). It was postulated that disruption of the pUL56/pUL104 complex was responsible for the anti-HCMV activity exhibited by these compounds. Little work has been done on the remaining HCMV encapsidation gene products.

Based on evidence for their homologous counterparts in HCMV, the UL15 and UL28 genes encode the putative HSV-1 terminase subunits. The HSV-1 UL15 and UL28 gene products were shown to interact with each other (Abbotts et al., 2000; Jacobson et al., 2006; Koslowski et al., 1999). The UL15/UL28 complex is presumed to direct the cleavage and packaging of genomic DNA, but neither subunit has been formally shown to exhibit ATPase activity. Detailed studies have shown that the UL6 gene encodes a portal protein found at one vertex of each preassembled capsid and that the portal is intimately involved in "guiding" viral DNA into the pre-capsid (Newcomb et al., 2001b). The HSV-1 portal protein was shown to interact with both the UL15 and UL28 proteins (White et al., 2003) and a series of thiourea compounds were shown to prevent both the UL6 and UL15 proteins from associating with capsids (Newcomb and Brown, 2002). Additionally, replicating DNA, localized to nuclear replication compartments, is probably associated with a complex containing the UL28, UL15 and UL33 gene products (Beard et al., 2002; Wills et al., 2006; Yang and Baines, 2006). The evidence thus far suggests that multiple proteins act in concert during various steps of the encapsidation process.

Very little is known about the encapsidation process in Varicella-zoster virus (VZV). The interactions previously described between the heterodimeric terminase subunits for both HSV-1 and HCMV provided a basis for our studies seeking to define protein-protein interactions important in the VZV DNA encapsidation process. This report is the first to describe the cloning, sequencing, and *in vitro* expression of the seven ORFs encoding the putative VZV encapsidation proteins. Consistent with studies reported previously for both HSV-1 and HCMV, the VZV terminase subunit homologs, encoded by ORF30 and ORF45/42, were shown to interact and form stable complexes *in vitro* and both were shown to localize in the nucleus of infected cells.

2. Materials and methods

2.1. Cells and virus

Monolayer cultures of human foreskin fibroblast (HFF), human lung fibroblast (IMR-90), African green monkey kidney (Vero), or human melanoma (MeWo) cells were used for propagation of VZV strain Ellen (ATCC VR-1367). Vero, IMR-90, and HFF cells were grown in Dulbecco's Modified Eagles Medium (DME) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 1.5 g/l sodium bicarbonate, 100 units/ml of penicillin, 100 mg/ml streptomycin sulfate, and 50 μg/ml ciprofloxacin. MeWo cells were grown in Minimal Essential Medium (MEM) supplemented with 8% fetal calf serum, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 100 units/ml of penicillin, and 100 mg/ml streptomycin sulfate. Cells infected with VZV were incubated in either DME or MEM containing 3% serum. Infections were performed with VZV-infected cell stocks applied to monolayers of uninfected cells.

2.2. RT-PCR

IMR-90 cells were infected with VZV strain Ellen and total infected cell RNA was prepared at 48 h post-infection using the RNAqueous-4PCR kit (Ambion Inc., Austin, TX). RT-PCR was performed on RNA samples using the SuperScript One-Step RT-PCR kit with Platinum Taq (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations with the following primer pair: (ORF45 5′-CAAGTCTCGCCTGGAACAGT-3′, ORF42 5′-CAAGCTGTGACATCGCTATG-3′). The primer pairs were expected to yield a product of 386 nucleotides if ORF45 (exon I) and ORF42 (exon II) were contained within a spliced transcript. A synthetically fused ORF45/42 gene lacking an intron was used as a control template with the same primer set.

2.3. Cloning of Varicella-zoster virus strain Ellen open reading frames

Six of the seven VZV encapsidation ORFs (25, 26, 30, 34, 43, and 54) were amplified from VZV strain Ellen genomic DNA using gene specific oligonucleotide primers (IDT Inc., Coralville, IA): ORF25: forward 5′-CACCATG TACGAATCGGAAAATG-3′, reverse 5′-AGCATCCTTCAA TATTTCATG-3′; ORF26: forward 5′-CACCATGGATCGGG TAGAATCAGA-3′, reverse 5′-GACATACTTCGATAGG GTGTG-3′; ORF30: forward 5′-CACCATGGAATTGGAT ATTAATCG-3′, reverse 5′-TGAAAACGCCGGGTCCGTTG-3′; ORF34: forward 5′-CACCATGACGGCGAGATATGGGTT-3′, reverse 5′-CGGTGTGGAGGCAAACTGAGG-3′; ORF43: forward 5′-CACCATGGAAGCCCATTTGGCAAAT-3′, reverse 5′-TTTATGGGGGTTGGGAATAGAGAA-3′; ORF54: