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Original article

The varicella–zoster virus–mediated delayed host shutoff: open reading frame 17 has no major function, whereas immediate–early 63 protein represses heterologous gene expression

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Abstract

We reported that varicella–zoster virus (VZV) causes a delayed host shutoff during its replicative cycle. VZV open reading frame 17 (ORF17) is the homologue of the herpes simplex virus (HSV) UL41 gene encoding the virion host shutoff (vhs) protein which is responsible for the shutoff effect observed in HSV-infected cells. In the present study, we demonstrated that ORF17 is expressed as a late protein during the VZV replicative cycle in different infected permissive cell lines which showed a delayed shutoff of cellular RNA. A cell line with stable expression of VZV ORF17 was infected with VZV. In these cells, VZV replication and delayed host shutoff remained unchanged when compared to normal infected cells. ORF17 was not capable of repressing the expression of the β-gal reporter gene under the control of the human cytomegalovirus immediate–early gene promoter or to inhibit the expression of a CAT reporter gene under the control of the human GAPDH promoter, indicating that ORF17 has no major function in the VZV-mediated delayed host shutoff. To determine whether other viral factors are involved in the host shutoff, a series of cotransfection assays was performed. We found that the immediate–early 63 protein (IE63) was able to downregulate the expression of reporter genes under the control of the two heterologous promoters, indicating that this viral factor can be involved in the VZV-mediated delayed host shutoff. Other factors can be also implicated to modulate the repressing action of IE63 to achieve a precise balance between the viral and cellular gene expression.

Keywords: Varicella–zoster virus; ORF17; Host shutoff; Herpes simplex virus UL41; Reporter gene expression; Immediate–early 63 protein

1. Introduction

Varicella–zoster virus (VZV), a member of the human alphaherpesvirus family, is the etiologic agent of two distinct clinical syndromes: varicella (chickenpox) occurring during the primary infection and zoster (shingles) appearing after reactivation from latency. Infection of host cells by VZV begins with the entry of the virion's nucleocapsid into the cell after which the viral DNA is translocated into the nucleus. The expression of the viral genes occurs according to a precise cascade. The immediate–early (IE) genes are transcribed first, in absence of the novo protein synthesis and are implicated in viral gene regulation. Then, the early (E) gene expression occurs and provides most of the components necessary for the viral DNA replication. Finally, after the DNA synthesis has began, late (L) genes are expressed, which mainly encode virion structural proteins and the glycoproteins [1].

Infection of cells with alphaherpesviruses generally results in a shutoff of host macromolecular metabolism. During the herpes simplex virus (HSV) infection, host DNA synthesis is blocked, cellular protein synthesis is inhibited and glycosylation of cellular proteins is interrupted [2]. Among the numer-

Abbreviations: β-Gal, β-galactosidase; BHV-1, bovine herpesvirus type 1; E gene, early gene; EHV-1, equine herpesvirus type 1; EtBr, ethidium bromide; FCS, fetal calf serum; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; HSV, herpes simplex virus; IE gene, immediate–early gene; L gene, late gene; NEAA, non-essential amino acids; ORF, open reading frame; PAA, phosphonoacetic acid; PDH, pyruvate dehydrogenase; p.i., post-infection; PRV, pseudorabies virus; P/S, penicillin/streptomycin; RT-PCR, reverse transcription-polymerase chain reaction; vhs, virion host shutoff; VZV, varicella–zoster virus.

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ous strategies developed by HSV to achieve the host shutoff, there are two particularly important components, the UL41 protein designated as virion host shutoff (vhs) factor and the infected cell protein 27 (ICP27). The HSV UL41 encodes a 58-kDa polypeptide which is produced at late times in infection and packaged into the virion [3,4]. The vhs protein enters the host cell as components of infecting virions and causes the degradation of cellular mRNAs, contributing to an overall decrease in host protein synthesis. In addition, the vhs protein accelerates the turnover of viral mRNAs belonging to all kinetic classes with the aim of facilitating the expression in cascade of the viral genes [5]. It was recently shown that the vhs protein itself is an RNase which interacts with the mammalian translation initiation factor eIF4H, possibly for targeting vhs to mRNAs and regions of translation initiation [6,7]. The second HSV protein implicated in the shutoff of the host is ICP27 which is the product of the α27 ORF. ICP27 contributes to the decrease of cellular mRNA levels during the infection by blocking pre-mRNA splicing [8]. Moreover, ICP27 mediates the export of viral intronless mRNAs by shuttling between the nucleus and cytoplasm [9].

Homologue sequences of vhs factor and ICP27 have been found in other alphaherpesvirus like VZV as well as in equine herpesvirus type 1 (EHV-1) and bovine herpesvirus type 1 (BHV-1) [10–13]. VZV ORF4 is homologous to HSV ICP27 but it is functionally distinct and does not complement HSV ICP27 [11,14].

VZV ORF17 is homologous to the HSV UL41 (vhs) [15]. ORF17 presents 39% amino acid identity to HSV UL41 and the two proteins share four domains that are highly conserved [16]. Sato et al. [17] published that ORF17 protein cleaves RNA to a substantially lesser extent than HSV-1 vhs, but fails to inhibit expression from a β-galactosidase reporter plasmid. Moreover, in opposition to HSV UL41, VZV ORF17 protein is not detectable in virions [17]. Recently, we reported that VZV causes a delayed host shutoff effect. However, it seemed likely that the ORF17 protein does not contribute to this effect with a specific function [18].

In the present study, we demonstrated that ORF17 is expressed in different VZV-infected cell lines, which showed a shutoff of cellular RNA during the viral replication cycle. We also demonstrated that ORF17 is expressed as a late gene since its expression is inhibited in presence of phosphonoacetic acid (PAA), a DNA synthesis inhibitor. The presence of ORF17 in the cells prior to the infection with VZV did not alter neither the viral yield nor the delayed host shutoff. To determine whether other viral factors are responsible for the VZV-induced host shutoff effect, cotransfection assays were performed with reporter vectors and a plasmid encoding the IE gene product ORF4, homologous to HSV ICP27. Due to yet unknown functions regarding direct or indirect transregulation or induction of signaling pathways leading to host shutoff, the three other IE gene products (ORF61, ORF62 and ORF63) and the trans-inducing protein encoded by ORF10, homologue of HSV VP16 which binds the HSV vhs protein, were also analyzed.

2. Materials and methods

2.1. Cells and virus

MeWo cells (human melanoma cell line, ECACC 93082609) were grown in Earle's modified Eagle's medium (EMEM) supplemented with 10% fetal calf serum (FCS), 1% penicillin/streptomycin (P/S) and 1% non-essential amino acids (NEAA). VZV strain Ellen (ATCC VR-586) was propagated by passage of infected cells showing 70–80% cytopathic effect onto uninfected monolayers. When needed, following a 60 min adsorption step, 25 μg/ml of phosphonoacetic acid (PAA, ICN), a DNA synthesis inhibitor, were added to the culture medium.

Human B-lymphocytes cells (EBV-transformed, IARC 721) and H9 cells (human T-cell lymphoma, ATCC HTB 176) were grown in RPMI 1640 supplemented with 10% FCS and 1% P/S. When needed, these two cell lines were infected with cell-free VZV which was prepared as previously described [19].

Chinese hamster ovary cells (CHO cell line, ECACC 85050302) were grown in Dulbecco's MEM supplemented with 10% FCS and 1% P/S.

2.2. Reverse transcription and PCR analyses

Total RNA was isolated using TRIZOL reagent (Life Technologies) according to the manufacturer's instructions followed by a DNase treatment. Reverse transcription-polymerase chain reaction (RT-PCR) was performed as a two step procedure. Reverse transcription was done using 1.0 μg of the respective RNA sample and the "Omniscript RT Kit" (Qiagen, Germany) according to manufacturer's instructions.

Resulting cDNA from reverse transcription was used for PCR amplification of VZV ORF17 by using the primers identified in Table 1. The reaction mixture contained 1 μg template DNA, 50 pmol of each primer, 100 μM dNTP, 3 mM $MgCl_2$, 67 mM Tris–HCl (pH 8.8), 0.1% Tween-20 and 2.5 U BioTherm DNA polymerase (Genecraft, Germany). The thermocycler conditions were 4 min 94 °C initial denaturation followed by 30 s 94 °C, 30 s 55 °C and 1 min 72 °C. For the amplification of VZV ORF4, ORF21, ORF63, ORF68, pyru-

Table 1
PCR primers used to amplify ORF17, ORF4, ORF10, ORF61 and ORF63

Primer	Sequence (5′ → 3′)
ORF17 forward	CGCGTACAGTGGCTTTGGTTTAT
ORF17 reverse	AGGATGAGGGTGG CAGAAGGTT
ORF4 forward	CCAAGCTTAAGATGGCCTCTGCTTCAATTCCAAC
ORF4 reverse	CCCTCGAGTTAGCAGTTAAAGGTACTA
ORF10 forward	GGGAATTCATTATGGAGTGTAATTTAG
ORF10 reverse	CCCTCGAGTTAACGCGTTAAAAAC
ORF61 forward	GGGAATTCATTATGGATACCATATTA
ORF61 reverse	CTTCTAGACTAGGACTTCTTCATC
ORF63 forward	CCAAGCTTAGTATGTTTTGCACCTCACCG
ORF63 reverse	CACTCGAGCTACACGCCATGGG