JOURNAL OF VIROLOGY, Mar. 1994, p. 1987–1992
0022-538X/94/$04.00+0
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Vol. 68, No. 3

Varicella-Zoster Virus Open Reading Frame 4 Protein Is Functionally Distinct from and Does Not Complement Its Herpes Simplex Virus Type 1 Homolog, ICP27†

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Received 11 August 1993/Accepted 24 November 1993

Varicella-zoster virus (VZV) open reading frame 4 (ORF4) encodes a putative immediate-early protein which is homologous to herpes simplex virus type 1 (HSV-1) ICP27 on the basis of gene location and similarity in amino acid sequence. In transient expression assays, however, ORF4 and ICP27 exhibit different properties. ICP27 alone has little activity on target plasmids, but it acts as a transactivator or a transrepressor in the presence of other HSV-1 transactivators. In contrast, ORF4 directly transactivates plasmids containing homologous or heterologous promoters and has no apparent transrepressing activity. To further illuminate the functional similarities and differences between ORF4 and ICP27, Vero cell lines which express ORF4 under the inducible metallothionein promoter were constructed. Cell lines expressing functionally active ORF4 protein upregulated the expression of transfected VZV target plasmids but were unable to efficiently complement HSV-1 ICP27 mutants. These results indicate that, despite structural similarities, VZV ORF4 and HSV-1 ICP27 behave differently in transient expression assays and may play different roles in virus replication.

Varicella-zoster virus (VZV), the etiologic agent of chicken pox and shingles, is a member of the alphaherpesvirus family. VZV has physiochemical properties and genomic organization (3) similar to those of herpes simplex virus type 1 (HSV-1), another member of the alphaherpesvirus family. The functions of many VZV gene products remain undefined but have been predicted by analogy to those of their HSV-1 counterparts, which are better characterized.

VZV expresses four genes, open reading frame 4 (ORF4), ORF61, ORF62, and ORF63, each of which is homologous to an HSV-1 immediate-early (IE) gene on the basis of genomic location and similarity in predicted amino acid sequence. Previous studies revealed that all four VZV putative IE gene products possess transregulatory activity.

VZV ORF62 transactivates all three putative kinetic classes of VZV gene promoters in transient expression assays (10, 11, 18, 24) and autorepresses or autostimulates its own promoter, depending on the experimental conditions (5, 25). The predicted amino acid sequence of ORF62 is highly homologous to that of HSV-1 ICP4, and cell lines stably expressing VZV ORF62 complement HSV-1 ICP4 mutants (7), indicating that VZV ORF62 and HSV-1 ICP4 are functional homologs.

VZV ORF61 protein also transactivates VZV promoters in the absence of other viral transactivators (21) but represses the ORF62- or ORF4-mediated transactivation in Vero cells (21, 22). Amino acid homology between ORF61 protein and its HSV-1 counterpart, ICP0, is restricted to the amino-terminal cysteine-rich region; nevertheless, cell lines stably expressing ORF61 complement an HSV-1 ICP0 mutant (19), indicating that VZV ORF61 and HSV-1 ICP0 are functional homologs.

VZV ORF63 represses the ORF62 promoter in transient expression assays (12). At present, it is uncertain whether ORF63 is the functional equivalent of HSV-1 ICP22, with which it shares genomic location and limited amino acid sequence homology.

VZV ORF4 encodes a protein which shares considerable amino acid sequence homology with HSV-1 ICP27. Although ICP27 is essential for HSV-1 replication (27), the role of ORF4 in viral replication is not well understood. ICP27 and ORF4 manifest distinct properties in transient expression assays. ICP27 has little or no transregulatory effect on target plasmids by itself (6, 29); however, in combination with ICP4 and ICP0, ICP27 acts as a transactivator or a transrepressor, depending on the target construct. In contrast, ORF4 alone transactivates plasmids containing VZV or other viral promoters (4, 11, 22), and no transrepressing activity has been reported.

To further evaluate the regulatory capacity of the VZV ORF4 protein, we cotransfected Vero cells with a plasmid expressing the VZV ORF4 gene along with plasmids containing promoters from VZV, HSV-1, or other viruses linked to the chloramphenicol acetyltransferase (CAT) gene (Table 1). In these experiments, plasmid pGORF4 (ORF4 driven by its own promoter [10]) transactivated each of the target constructs tested, with an increase in CAT activity from 5- to 59-fold. Thus, ORF4 transactivates target constructs containing diverse promoters.

Recently, Sandri-Goldin and Mendoza (28) showed that the regulatory activity of HSV-1 ICP27 is independent of the target gene promoter sequences but depends on the presence of different mRNA processing signals. Activation by ICP27 correlated with different polyadenylation sites in the target gene, while repression by ICP27 correlated with the presence of an intron either 5′ or 3′ to the target gene. To determine whether VZV ORF4 and HSV-1 ICP27 have similar functions in transient expression assays, we cotransfected cells with plasmids expressing VZV ORF4 or HSV-1 ICP27 along with HSV-1 target plasmids. pTK-CAT SV40 A contains the HSV-1 thymidine kinase gene promoter followed by the CAT gene, the simian virus 40 (SV40) small-t-antigen intron, and the SV40 early polyadenylation region (28). pMC150 (which ex-

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† This paper is dedicated to the memory of Holly A. Smith.

TABLE 1. Transactivation of target plasmids containing promoters from VZV, HSV-1, and unrelated viruses by VZV ORF4

Promoter (virus, class, and gene)[a]	Plasmid[b] (reference)	Fold induction by pGORF4[c]
VZV		
IE		
ORF62	p62CAT (25)	15.0 ± 4.2
ORF61	p61CAT (24)	13.0 ± 7.1
ORF4	p4CAT (24)	58.5 ± 28.0
E		
ORF29	p29CAT (17)	44.0 ± 5.7
dPK	p2tkCAT (11)	4.5 ± 3.5
L gpIV	pgpIVCAT (13)	15.5 ± 7.1
HSV-1		
IE		
ICP0	pIGA-65 (9)	11.0 ± 1.4
ICP4	pPOH2 (23)	29.5 ± 2.1
E TK	pPOH3 (23)	21.5 ± 7.8
LAT	pf280 (16)	33.5 ± 4.2
HCMV IE	pCMV-CAT (14)	12.0 ± 2.8
SV40 E	pSV2CAT (22)	20.7 ± 3.5
HIV LTR	HIV-LTR-CAT (22)	20.0 ± 2.8

[a] E, early; L, late; dPK, deoxypyrimidine kinase; TK, thymidine kinase; LAT, latency-associated transcript; HCMV, human cytomegalovirus; HIV, human immunodeficiency virus; LTR, long terminal repeat.

[b] The amount of transfected target plasmid was chosen so that the basal level of CAT activity was less than 5%. The total amount of plasmid DNA was adjusted to 10 μg by adding pGEM2.

[c] Five micrograms of pGORF4 was cotransfected with target plasmids. Each transfection was done at least twice, and the fold inductions are means ± standard deviations.

presses ICP27 from its own promoter [2]) alone had little or no effect on pTK-CAT SV40 A (Fig. 1A) but repressed the ICP0- and ICP4-mediated transactivation of pTK-CAT SV40 A in accord with earlier findings (28). In contrast, pGORF4 transactivated pTK-CAT SV40 A by itself and did not repress the ICP0- and ICP4-mediated transactivation of pTK-CAT SV40 A. pTK-CAT SYN A contains the HSV-1 thymidine kinase gene promoter followed by the CAT gene and a synthetic oligonucleotide linker 3′ to the CAT-coding sequences (28). This oligonucleotide contains the highly conserved polyadenylation recognition signal AATAAA; however, a second signal, termed the G/U box, is not present. Both pMC150 and pGORF4 had little or no effect on pTK-CAT SYN A (Fig. 1B) but enhanced the ICP0- and ICP4-mediated transactivation of pTK-CAT SYN A. Thus, VZV ORF4 and HSV-1 ICP27 behave differently in transient expression assays.

To further compare the roles of VZV ORF4 and HSV-1 ICP27 in the regulation of herpesvirus gene expression, we constructed plasmids which inducibly express VZV ORF4 (Fig. 2). Plasmids expressing ORF4 were constructed by digesting recombinant VZV DNA with *Hgi*AI and *Nco*I (which cut at nucleotides 2267 and 4141, respectively [3]), producing blunt ends with T4 DNA polymerase, ligating a double-stranded *Kpn*I linker oligonucleotide (19), digesting with *Kpn*I, and inserting the DNA into the *Kpn*I site of pMTP-3H (which contains the human metallothionein promoter [19]). Each of the resulting plasmids, pMTPORF4R and pMTPORF4I, contains the VZV ORF4 gene with the consensus sequence for initiation of translation at −1 to −4 bases upstream of the ATG codon, in the right and inverted orientations, respectively, under the control of the human metallothionein promoter which is inducible by cadmium chloride ($CdCl_2$). Cotransfection of pMTPORF4R with p62CAT (containing the VZV ORF62 promoter followed by the CAT gene [25])

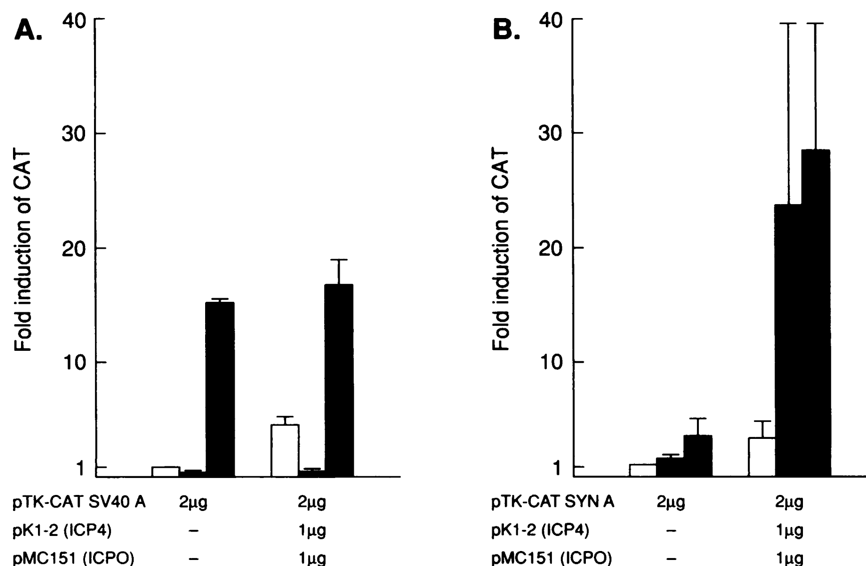

FIG. 1. Comparison of VZV ORF4 and HSV-1 ICP27 in transient expression assays. Vero cells were cotransfected with 2 μg of pTK-CAT SV40 A (CAT under the control of the HSV-1 thymidine kinase gene promoter followed by the SV40 small-t-antigen intron and SV40 early polyadenylation region) (A) or with 2 μg of pTK-CAT SYN A (CAT under the control of the HSV-1 thymidine kinase gene promoter followed by a synthetic oligonucleotide containing the conserved AATAAA sequence but not the G/U box) (B). Each plasmid was used alone and in combination with 1 μg of pMC151 (expressing HSV-1 ICP0) and 1 μg of pK1-2 (expressing HSV-1 ICP4), along with 5 μg of pGEM2 (open bars), 5 μg of pMC150 (expressing HSV-1 ICP27 [closed bars]), or 5 μg of pGORF4 (expressing VZV ORF4 [shaded bars]). Plasmids used for the experiments are indicated below the figure. Fold induction of CAT is the CAT activity relative to that obtained for plasmid pTK-CAT SV40 A or pTK-CAT SYN A alone. The values are the averages of duplicate transfections, and standard deviations are shown.

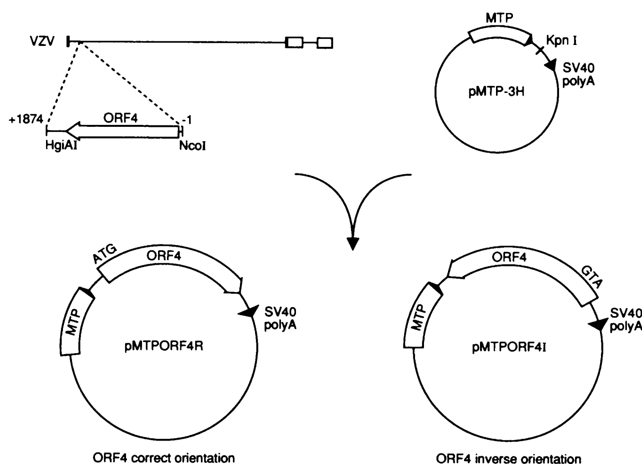

FIG. 2. Construction of VZV ORF4 expression vectors. pMTP-3H contains the human metallothionein promoter (MTP) followed by a *Kpn*I restriction endonuclease site and an SV40 polyadenylation (polyA) sequence. The 1.9-kb *Nco*I-*Hgi*AI fragment from the VZV genome contains the protein-coding sequence of ORF4 but no upstream transcriptional regulatory sequences. pMTPORF4R contains the VZV ORF4 gene inserted into the *Kpn*I site of pMTP-3H in the right orientation driven by the MTP with an SV40 polyA sequence downstream, while pMTPORF4I contains ORF4 in the inverted orientation.

resulted in upregulation of CAT expression in a dose-dependent manner in Vero cells (Fig. 3). Similar results were obtained with pGORF4 (ORF4 driven by its own promoter) (Table 1).

To generate cell lines which inducibly express VZV ORF4, Vero cells were cotransfected with pSV2neo (containing the G418 resistance gene as a selection marker [30]) and a 20-fold molar excess of pMTPORF4R or pMTPORF4I, and G418-resistant colonies were selected and subcloned (19). Cell lines stably transformed with ORF4 were maintained in medium containing dialyzed fetal bovine serum before the experiments to reduce the level of expression from the metallothionein

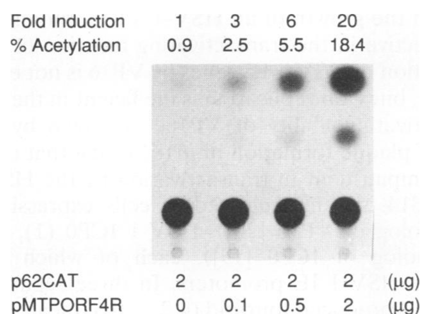

FIG. 3. Transactivation of the VZV ORF62 promoter by ORF4 expressed from transfected plasmids. Vero cells were cotransfected with 2 μg of p62CAT (CAT under the control of the VZV ORF62 promoter) alone or in combination with 0.1 to 2 μg of pMTPORF4R (ORF4 driven by the human metallothionein promoter). $CdCl_2$ (10 μM) was added immediately after transfection, cells were harvested 2 days later, and cell lysates were assayed for CAT activity. Fold induction of CAT (shown above the autoradiogram) is the CAT activity relative to that obtained for plasmid p62CAT alone. The experiments were performed three times, and a representative result is shown.

promoter. $CdCl_2$ was added to a final concentration of 5 μM (for viral infection experiments) or 10 μM (for other experiments) immediately after transfection to induce expression from the metallothionein promoter.

Twenty colonies obtained from transfection with pMTPORF4R and ten colonies from transfection with pMTPORF4I were analyzed by Northern (RNA) blotting (19). Two cell lines obtained from transfection with each plasmid expressed VZV ORF4 RNA after induction by $CdCl_2$ (Fig. 4A). The V4R-5 and V4R-13 cell lines (which express ORF4) and the V4I-8 and V4I-10 cell lines (which express antisense ORF4) were further characterized.

Southern blotting of cellular DNAs from the four cell lines indicated that each cell line contained ORF4 DNA (Fig. 4B). The V4R-5 and V4R-13 cell lines were further examined for expression of VZV ORF4 protein by Western blotting (immunoblotting). To induce expression from the metallothionein promoter, $CdCl_2$ (10 μM) was added to the medium the day before cells were harvested. Cell extracts from V4R-5 and V4R-13 cells were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane, and incubated with rabbit anti-ORF4 antibody directed against a peptide corresponding to the carboxy-terminal 14 amino acids (amino acids 439 to 452) of VZV ORF4. After being washed, the membrane was incubated with goat anti-rabbit antibody conjugated with alkaline phosphatase and was then developed with buffer containing 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium (Immuno-Blot assay kit; Bio-Rad, Richmond, Calif.). In the absence of $CdCl_2$, the cell lines contained no detectable ORF4 protein (18). However, the addition of $CdCl_2$ led to expression of ORF4 protein which was detected with anti-ORF4 antibody (Fig. 4C).

Each of the cell lines described above was examined in transient expression assays. To verify that the inducible expression of ORF4 in these cell lines upregulates the VZV ORF62 promoter, transfections were performed with p62CAT in the presence or absence of $CdCl_2$ (10 μM). Induction of ORF4 expression by $CdCl_2$ in V4R-13 cells (which express VZV ORF4 [Fig. 4C]) increased CAT activity (eightfold) (Fig. 5). Less activation (fourfold) of p62CAT was seen after transfection of V4R-5 cells (which express less ORF4 protein than V4R-13 cells [Fig. 4C]). In the absence of $CdCl_2$, little or no activation of p62CAT was observed after transfection of V4R-5 and V4R-13 cells (Fig. 5). No activation of p62CAT was observed in the presence of $CdCl_2$ in VM-3 cells (which were transformed with vector plasmid pMTP-3H and pSV2neo [19]) or in V4I-8 and V4I-10 cells (which express antisense ORF4 RNA [Fig. 4A]).

Activation of the VZV ORF62 promoter in ORF4-expressing cell lines was dependent upon the concentration of $CdCl_2$. The CAT activity of p62CAT in V4R-13 cells increased with increasing concentrations of $CdCl_2$ and reached a maximum at 10 to 40 μM $CdCl_2$ (18). Above 10 μM $CdCl_2$, the cells developed a granular appearance after 2 to 3 days, suggesting toxicity due to the added heavy metal. In contrast, the CAT activity of p62CAT in VM-3 cells was not enhanced but slightly reduced with addition of $CdCl_2$, probably because of the toxicity of the heavy metal. Since concentrations of 10 μM or more $CdCl_2$ were toxic to cells, 5 μM $CdCl_2$ was added to induce expression from the metallothionein promoter in the viral infection experiments described below.

Studies with HSV-1 ICP27 temperature-sensitive and deletion mutants demonstrated that ICP27 is indispensable for HSV-1 growth in vitro (15, 27). HSV-1 ICP27 temperature-sensitive mutants grow only at the permissive temperature,

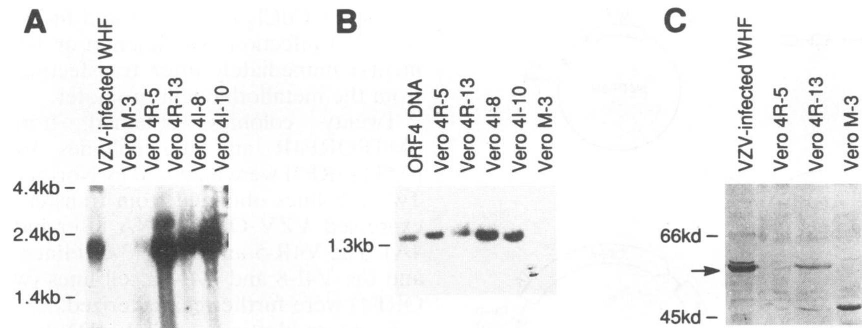

FIG. 4. (A) Northern blot analysis of ORF4 transcripts in transformed Vero cell lines. Each lane contains 15 μg of cellular RNA. The blot was hybridized with the ^{32}P-labelled 1.8-kb *Kpn*I ORF4 DNA fragment of pMTPORF4R. The leftmost lane contains RNA from VZV-infected human fibroblast (WHF) cells. Markers indicate sizes in kilobases. A 2.1-kb transcript was detected in VZV-infected WHF and ORF4-transformed cells, and a 4-kb transcript was noted in VZV-infected WHF cells (as described previously [26]). An additional 2.4-kb transcript detected in ORF4-transformed cells may be due to the additional (SV40) polyadenylation signal in the ORF4-expressing vector (Fig. 2). (B) Southern blot analysis of ORF4-specific sequences in transformed Vero cell lines. Electrophoretically separated *Kpn*I digests (10 μg) of cellular DNAs were transferred to a nylon membrane and probed with the probe used for the Northern blot. The leftmost lane contains 50 pg of the same fragment used for the probe. Marker indicates size in kilobase pairs. (C) Western blot analysis of ORF4 proteins in transformed Vero cell lines. Each lane contains lysate from 5×10^5 cells. Cellular proteins were transferred to a nitrocellulose membrane, incubated with rabbit anti-ORF4 serum, and then incubated with alkaline phosphatase-conjugated anti-rabbit serum. ORF4 protein is present as a 55-kDa protein. Markers indicate sizes in kilodaltons.

while ICP27 deletion mutants grow only in ICP27-expressing cells. To determine whether VZV ORF4 can functionally substitute for HSV-1 ICP27, we infected cell lines expressing VZV ORF4 with HSV-1 ICP27 mutants. Single-step growth experiments with HSV-1 KOS (wild type), *ts*E6 (an HSV-1 ICP27 temperature-sensitive mutant [27]), and *5dl*1.2 (an HSV-1 ICP27 deletion mutant [27]) were performed to determine the yields of virus 24 h after infection. While HSV-1 KOS (18) and *ts*E6 at 33°C (Table 2) grew to similar titers on all cell lines tested, *ts*E6 at 39.5°C (Table 2) grew to 10^6-fold-higher levels in 3-3 (ICP27-expressing [15]) cells than in control cells. *ts*E6 at 39.5°C grew to peak titers of only three- or sevenfold more in V4R-13 (VZV ORF4-expressing) cells than in VM-3 (control) cells after induction by $CdCl_2$ (Table 2). *ts*E6 at 39.5°C grew to similar titers in V4R-5 cells (which express less ORF4 protein than V4R-13 cells) and in VM-3 cells. *5dl*1.2 grew well only in 3-3 (ICP27-expressing) cells and grew to similar titers in V4R-13, V4R-5, and VM-3 cells (18).

To further determine whether VZV ORF4 can complement

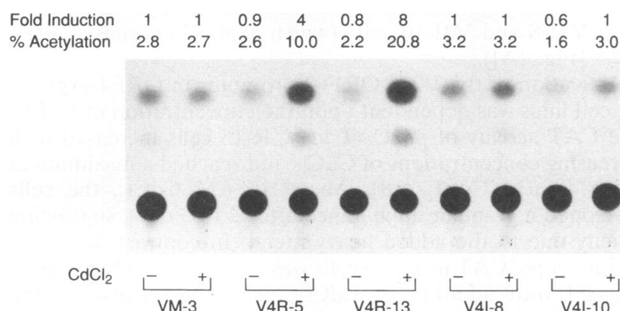

FIG. 5. Transactivation of the VZV ORF62 promoter by ORF4 expressed from stably transformed cell lines. Cell lines were transfected with p62CAT and maintained in either the absence (−) or the presence (+) of $CdCl_2$ (10 μM). Fold induction of CAT (shown above the autoradiogram) is the CAT activity relative to that obtained for VM-3 cells in either the absence or the presence of $CdCl_2$. The experiments were performed three times, and a representative result is shown.

the growth of HSV-1 ICP27 mutants, the plaque formation efficiencies of the mutants were tested in various cell lines. HSV-1 *ts*E6 (at 39.5°C) and *5dl*1.2 yielded few or no plaques in ORF4-expressing cells or in control cells, while these mutants produced numbers of plaques similar to that of wild-type virus (KOS) in 3-3 cells (18). Likewise, cell lines expressing other VZV regulatory proteins (FI-14 [ORF62-expressing] [7], V61R-21 [ORF61-expressing] [19], and V10R-18 [ORF10-expressing] [20] cells) were unable to complement the HSV-1 ICP27 mutants (18), suggesting that none of these VZV regulatory proteins was able to efficiently substitute for HSV-1 ICP27.

In the single-step growth study (Table 2), there was a suggestion that ORF4 assisted replication of *ts*E6; however, the plaque titration assays did not confirm this finding. Furthermore, ORF4 never assisted replication of another ICP27 mutant, *5dl*1.2. To help assess whether ORF4 could complement another HSV-1 transactivator, we studied the influence of ORF4 on the growth of an HSV-1 VP16 mutant, *in*1814 (a mutant defective in the transactivating function of VP16 [1]). Transactivation of HSV-1 IE genes by VP16 is not essential for viral growth, but viral replication is inefficient in the absence of the transactivating ability of VP16, as shown by the lower efficiency of plaque formation of *in*1814 than that of wild-type virus. The impairment in transactivation by the HSV-1 VP16 mutant *in*1814 is complemented in cells expressing ORF10 (VZV homolog of VP16 [20]), HSV-1 ICP0 (1), or ORF61 (VZV homolog of ICP0 [19]), each of which is able to transactivate HSV-1 IE promoters. In three separate experiments, *in*1814 produced fourfold (4.2 ± 0.6 [mean ± standard deviation]) more plaques on V4R-13 cells than on control cells when induced by $CdCl_2$, while *in*1814 produced sevenfold (7.4 ± 1.5) more plaques on 16-8 (HSV-1 VP16-expressing [31]) cells than on control cells (18). Since VZV ORF4 transactivates HSV-1 IE promoters in transient expression assays (Table 1), the increase of HSV-1 *in*1814 growth by ORF4 was probably due to the overall upregulation of HSV-1 IE genes by ORF4. Thus, V4R-13 cells produce ORF4 protein that is functionally active in the context of herpesvirus infection and slightly activates the growth of an HSV-1 mutant.

TABLE 2. Single-step growth study of an HSV-1 ICP27 temperature-sensitive mutant (*ts*E6) on Vero cell lines expressing VZV ORF4 or HSV-1 ICP27[a]

Cell line	$CdCl_2$	Result(s) (expt 1, expt 2) at: 33°C Titer (10^9 PFU/ml)	33°C Fold increase	39.5°C Titer (PFU/ml)	39.5°C Fold increase
VM-3	−	3.6	1.0	4.4×10^3	1.0
	+	3.0, 4.3	1.0	1.5×10^2, 1.2×10^2	1.0, 1.0
V4R-5	−	2.9	0.81	3.5×10^3	0.80
	+	2.7	0.90	2.4×10^2	1.6
V4R-13	−	4.0	1.1	7.1×10^3	1.6
	+	3.3, 3.9	1.1, 0.91	4.2×10^2, 8.8×10^2	2.8, 7.3
V4I-8	−	2.8	0.78	4.7×10^3	1.1
	+	2.5	0.83	$<1.5 \times 10^2$	<1.0
3-3	−	2.7	0.75	8.4×10^9	1.9×10^6
	+	ND, 2.0	ND, 0.47	ND, 7.6×10^8	ND, 6.3×10^6

[a] Monolayers of indicated cell lines were infected at a multiplicity of infection of 2 PFU per cell and incubated at 33 or 39.5°C for 24 h in either the absence (−) or the presence (+) of 5 μM $CdCl_2$. Cell-free and cell-associated virus was pooled, and titers were determined by plaque assays on Vero cells at 33°C. ND, not done.

Although VZV ORF4 and HSV-1 ICP27 have similar predicted amino acid sequences, our data indicate that VZV ORF4 is not a functional homolog of HSV-1 ICP27. Transient expression assays showed that the two proteins exert somewhat different regulatory functions. Moreover, cell lines expressing functionally active ORF4 protein were unable to efficiently complement HSV-1 ICP27 mutants.

VZV ORF4 and HSV-1 ICP27 exhibit different properties in transient expression assays. ICP27 appears to impair splicing of pre-mRNA (28). HSV-1 encodes several IE proteins (ICP0, ICP22, and ICP47) whose mRNAs are known to be spliced. Therefore, downregulation of these genes by ICP27 may be critical for the transition from the IE phase to the early phase of infection. In contrast, ORF4 has no known repression activity and only one VZV gene, ORF42/45 (presumably not an IE gene), is thought to be spliced (3). Thus, the different properties of ORF4 and ICP27 in transient expression assays may reflect evolutionary differences in VZV and HSV-1 IE genes.

VZV ORF4-expressing cells produced ORF4 protein that was functionally active in transient expression assays (as shown by upregulation of transfected p62CAT [Fig. 5]) and in the context of herpesvirus infection (as shown by enhancement of plaque formation of an HSV-1 VP16 mutant *in*1814 [18]). By using cell lines stably expressing specific genes, our laboratory showed that VZV ORF62 (7), ORF61 (19), and ORF10 (20) are functional homologs of their HSV-1 counterparts, ICP4, ICP0, and VP16, respectively. In contrast, cell lines stably expressing VZV ORF4 were unable to complement HSV-1 ICP27 mutants efficiently (only three- to sevenfold [Table 2]). Moreover, cell lines expressing other VZV regulatory proteins (ORF62, ORF61, or ORF10) were also unable to complement HSV-1 ICP27 mutants (18). Felser et al. (8) found that VZV coinfection could complement *ts*E6 (an HSV-1 ICP27 temperature-sensitive mutant) only 3-fold but that *ts*B21 (an HSV-1 ICP4 temperature-sensitive mutant) was complemented up to 83-fold. These results, taken together, indicate that neither ORF4 nor the complete repertoire of VZV proteins can efficiently substitute for HSV-1 ICP27.

In conclusion, VZV ORF4 behaves differently from HSV-1 ICP27 in transient expression assays and is unable to efficiently complement HSV-1 ICP27 mutants, indicating that ORF4 is not a functional homolog of ICP27. Thus, despite the similarities in their amino acid sequences, VZV ORF4 and HSV-1 ICP27 may have different roles in herpesvirus gene regulation.

We thank R. M. Sandri-Goldin for plasmids pTK-CAT SV40 A and pTK-CAT SYN A, S. Nagpal and J. M. Ostrove for rabbit anti-ORF4 antibody, and P. A. Schaffer for HSV-1 *ts*E6 and 5*dl*1.2 and for 3-3 cells.

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