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Varicella-Zoster Virus Open Reading Frame 10 Protein, the Herpes Simplex Virus VP16 Homolog, Transactivates Herpesvirus Immediate-Early Gene Promoters

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The varicella-zoster virus (VZV) open reading frame 10 (ORF10) protein is the homolog of the herpes simplex virus type 1 (HSV-1) protein VP16. These are two virion tegument proteins that have extensive amino acid sequence identity in their amino-terminal and middle domains. ORF10, however, lacks the acidic carboxy terminus which is critical for transactivation by VP16. Earlier studies showed that VZV ORF10 does not form a tertiary complex with the TAATGARAT regulatory element (where R is a purine) with which HSV-1 VP16 interacts, suggesting that ORF10 may not have transactivating ability. Using transient-expression assays, we show that VZV ORF10 is able to transactivate VZV immediate-early (IE) gene (ORF62) and HSV-1 IE gene (ICP4 and ICP0) promoters. Furthermore, cell lines stably expressing ORF10 complement the HSV-1 mutant *in*1814, which lacks the transactivating function of VP16, and enhance the de novo synthesis of infectious virus following transfection of HSV-1 virion DNA. These results indicate that ORF10, like its HSV-1 homolog VP16, is a transactivating protein despite the absence of sequences similar to the VP16 carboxy-terminal domain. The transactivating function of the VZV ORF10 tegument protein may be critical for efficient initiation of viral infection.

Varicella-zoster virus (VZV), the causative agent of chickenpox and shingles, is a member of the human alphaherpesvirus family, along with herpes simplex virus types 1 and 2 (HSV-1 and HSV-2). Although the poor growth characteristics of VZV in cell culture have severely impeded detailed analyses of VZV gene regulation, the functions of many regulatory VZV genes have been inferred by homology with their HSV-1 counterparts (4). Similarly, putative kinetic classes have been assigned for VZV genes by comparison with their HSV-1 gene homologs.

HSV-1 gene expression is regulated coordinately and ordered sequentially progressing from immediate-early (IE) to early to late viral genes (15). The α, or IE, genes are the first to be expressed, and their transcription does not require de novo protein synthesis. Four of the five IE gene products, ICP0, ICP4, ICP22, and ICP27, are known to play critical roles in HSV-1 gene regulation. HSV IE gene transcription is induced by a virion structural protein designated variously as VP16, Vmw65, and α-TIF. VP16 is a 490-amino-acid viral phosphoprotein synthesized during the late phase of HSV-1 replication. It is incorporated into the tegument during virion assembly, is released during infection of host cells, and stimulates transcription of HSV-1 IE genes (2, 14). Transactivation by VP16 involves recognition of IE gene-specific TAATGARAT consensus motifs (where R is a purine) present in the 5′ upstream region of each of the five HSV-1 IE genes (19). Although HSV-1 VP16 does not bind DNA directly, it interacts with cellular factors, one of which, the octamer-binding protein Oct-1, can bind directly to the TAATGARAT sequence element (10, 31). Mutational analyses of VP16 showed that it contains two separable and modular domains (12, 13, 34). One domain, at the amino terminus, interacts with Oct-1 to form an IE complex that binds the TAATGARAT element on IE promoters. The second domain, at the acidic carboxy terminus, activates transcription.

VZV open reading frame 10 (ORF10) encodes a protein of 410 amino acids that is homologous to HSV-1 VP16 (4). Like VP16, VZV ORF10 protein is incorporated into the tegument of virions (18); however, additional functions of ORF10 are unknown. While VZV ORF10 protein and HSV-1 VP16 show considerable amino acid homology, VZV ORF10 protein is 80 amino acids shorter, lacking sequence similar to that of the VP16 acidic carboxy-terminal tail. Furthermore, McKee et al. (21) showed that VZV ORF10 protein does not form an IE complex comprising the TAATGARAT sequence element and cellular proteins. Thus, VZV ORF10 protein had been thought to lack VZV gene-regulatory activity.

To determine the function of VZV ORF10 protein, we expressed ORF10 from its cognate promoter or from a heterologous promoter in transient-expression assays and established cell lines that stably expressed this gene. Our studies indicate that VZV ORF10 transactivates VZV and HSV-1 IE promoters. Furthermore, VZV ORF10-expressing cell lines enhance the de novo synthesis of infectious virus following transfection of HSV-1 virion DNA and complement an HSV-1 mutant (*in*1814) which lacks the transactivating ability of VP16.

MATERIALS AND METHODS

Cells and viruses. African green monkey kidney cells (Vero; American Tissue Culture Collection, Rockville, Md.) and human malignant melanoma (MeWo) cells, provided by Charles Grose, were propagated as described previously (23). The Vero VM-3 and MeWo MM-1 cell lines, transformed with both pSV2neo and pMTP-3H (vector), were described previously (23). The 16-8 cell line (HSV-1 VP16-expressing Vero cells), kindly provided by Steven P. Wein-

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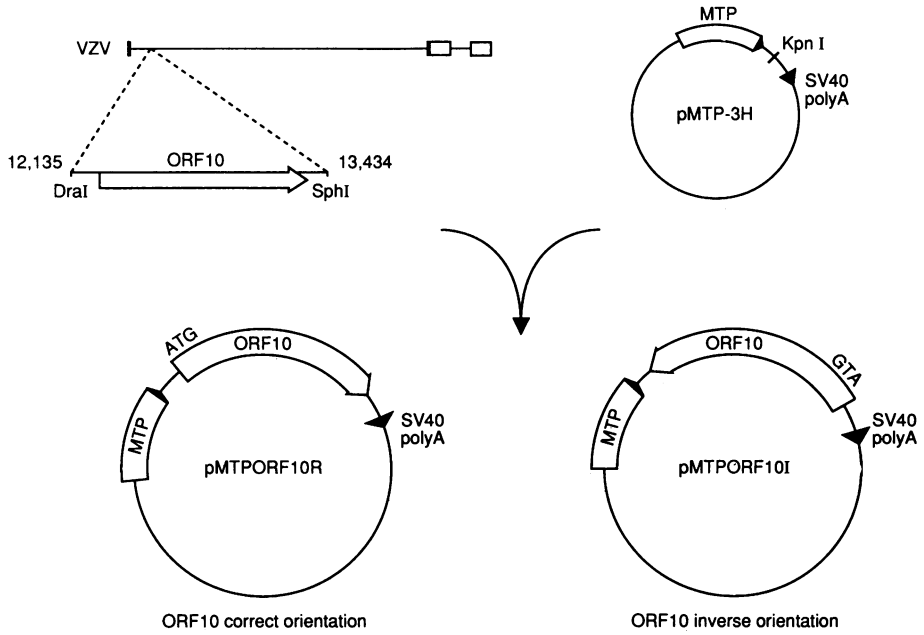


FIG. 1. Construction of VZV ORF10 expression vectors. pMTP-3H contains the human metallothionein promoter (MTP) followed by a *Kpn*I restriction site and an SV40 polyadenylation sequence. The 1.3-kb *Dra*I-*Sph*I fragment from the VZV genome contains the protein-coding sequence of ORF10 with 25 bases upstream of the AUG initiation codon. pMTPORF10R contains the VZV ORF10 gene inserted into the *Kpn*I site of pMTP-3H in the right orientation, driven by the MTP with an SV40 polyadenylation (polyA) sequence downstream, while pMTPORF10I contains ORF10 in the inverted (antisense) orientation.

heimer (35), was maintained in a 1:1 mixture of Eagle's minimal essential medium and medium 199 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, penicillin G (100 U/ml), streptomycin (100 μg/ml), and active G418 (400 μg/ml) (geneticin; Life Technology, Inc./GIBCO, Grand Island, N.Y.).

HSV-1 *in*1814, carrying a VP16 insertion mutation that abolishes the transactivation function of the protein (1), and wild-type HSV-1 strain KOS were propagated in Vero cells. HSV-1 virion DNA was isolated from nucleocapsid preparations of infected cells and purified as described previously (32).

Plasmids. Plasmid p62CAT, provided by Liyanage P. Perera (28), contains the VZV ORF62 promoter sequence (nucleotides −1440 to +68 respective to the transcription start site) upstream from the chloramphenicol acetyltransferase (CAT) coding sequence. p61CAT, also provided by L. P. Perera (27), contains the VZV ORF61 promoter sequence (nucleotides −1400 to +65 respective to the transcription start site) upstream from the CAT coding sequence. pGORF4CAT contains the VZV ORF4 promoter sequence (nucleotides −895 to +192 relative to the AUG initiation codon) upstream of the CAT coding sequence (25). pIGA-65, a gift from Saul Silverstein (9), contains the HSV-1 ICP0 gene promoter sequence (−548 to +149 relative to the transcription start site) upstream of the CAT gene. pPOH2, kindly provided by Gary S. Hayward (26), contains the HSV-1 ICP4 gene promoter sequence (nucleotides −1900 to +26 relative to the transcription start site) upstream from the CAT gene. pCH110 (Pharmacia LKB Biotechnology, Piscataway, N.J.) was used as an internal control to correct for differences in transfection efficiency among various cell lines. pCH110 contains the *lacZ* gene driven by the simian virus 40 (SV40) early promoter. pSV2neo encodes the G418 resistance gene under the control of the SV40 early promoter (30).

pGORF10 (22), which expresses ORF10 from its cognate promoter, contains the 3.3-kb *Sac*I-*Eco*RI fragment from recombinant VZV DNA (nucleotides 11433 to 14726 [4]) inserted into plasmid pGEM2 (Promega Biotech, Madison, Wis.). A plasmid expressing VZV ORF10 from a heterologous promoter was constructed by digesting pGORF10 with *Dra*I and *Sph*I (which cut at nucleotides 12135 to 13434), producing blunt ends with T4 DNA polymerase, ligating a double-stranded *Kpn*I linker oligonucleotide (5′-GCTGCA GGTACCTGCAGC-3′) to both ends, digesting with *Kpn*I, and inserting the fragment into the *Kpn*I site of pMTP-3H. The resulting plasmids, pMTPORF10R and pMTPORF10I (Fig. 1), contain a single copy of the VZV ORF10 gene with 25 bases upstream of the AUG initiation codon and 45 bases downstream from the stop codon of ORF10 in the right (R) and inverted (I) orientations, respectively, under the control of a human metallothionein promoter. pMTPVP16, which expresses HSV-1 VP16 under the control of a human metallothionein promoter, was constructed by amplifying the UL48 gene from HSV-1 strain 17+ virion DNA by polymerase chain reaction (5′-end primer, 5′-CCCCCGGAAT TCCACCCAATGGACCTCTTGGTC-3′; 3′-end primer, 5′-CCCCCGGAATTCCCTACCCACCGTACTCGTCAA-3′) and inserting the UL48 gene into the *Eco*RI site of pMTP-3H.

Transfections and CAT assays. Transfections of plasmid DNAs for transient-expression assays were carried out in 60-mm tissue culture dishes. Cells were plated the day before transfection at about 60% confluency (approximately 5×10^5 Vero cells or 1.2×10^6 MeWo cells per dish). Vero cells were transfected by the calcium phosphate procedure (11). MeWo cells were transfected as described previously

(23) except that the cells were shocked with 30% (vol/vol) dimethyl sulfoxide for 4 min. Cadmium chloride ($CdCl_2$) was added to the cell culture medium at a final concentration of 10 μM immediately after transfection to induce expression from the metallothionein promoter. Cells were harvested after incubation at 37°C for 48 to 72 h. CAT assays were performed as described previously (23).

For transfection with HSV-1 (KOS) viral DNA, Vero cells expressing VZV ORF10 were seeded at a density of 6×10^5 cells per 60-mm dish on the day before transfection. Cells were transfected with 0.5 μg of purified HSV-1 DNA together with 10 μg of sheared salmon sperm DNA by the calcium phosphate procedure.

Establishment and characterization of Vero and MeWo cell lines expressing VZV ORF10. Vero and MeWo cells were cotransfected with pSV2neo and pMTPORF10R or pMTPORF10I. G418-resistant cell colonies were selected, subcloned, and screened as described previously (23).

Southern and Northern (RNA) blot analyses of total cellular DNA and RNA, respectively, were performed as described previously (23). For Southern blots, total cellular DNA was digested with *Kpn*I, separated by agarose gel electrophoresis, and transferred to a nylon membrane. $CdCl_2$ was added to the cells to a final concentration of 10 μM to induce expression from the metallothionein promoter 1 day before cells were harvested for Northern blots. The probe used for both Southern and Northern blots was the VZV ORF10 DNA *Kpn*I fragment from pMTPORF10R that was radiolabeled by random priming with [α-^{32}P]dCTP (Amersham Corp., Arlington Heights, Ill.).

Viral complementation studies. Virus titers were determined by plaquing on various cell lines as described previously (8). Cells were maintained in medium containing dialyzed fetal bovine serum before and throughout the experiments to reduce the level of expression from the metallothionein promoter. $CdCl_2$ was added to a final concentration of 10 μM (Vero cells) or 5 μM (MeWo cells) immediately after infection with virus to induce expression from the metallothionein promoter.

Computer analysis. The predicted amino acid sequences of VZV ORF10, HSV-1 VP16, bovine herpesvirus 1 (BHV-1) B-αTIF, and equine herpesvirus type 1 (EHV-1) gene 12 protein were compared simultaneously with the Pileup and Pretty Plot Programs from the Sequence Analysis Software Package (5) (Genetics Computer Group, Inc., Madison, Wis.).

RESULTS

VZV ORF10 transactivates VZV and HSV-1 IE promoters in transient-expression assays. HSV-1 VP16, the homolog of VZV ORF10, transactivates several HSV-1 IE genes. Therefore, we determined whether VZV ORF10, like its HSV-1 homolog VP16, could transactivate expression from putative VZV IE gene promoters.

We constructed plasmids encoding sense (pMTPORF10R) and antisense (pMTPORF10I) VZV ORF10 under the control of an inducible (human metallothionein) promoter. Transient-expression studies were then performed with these ORF10 constructs and reporter plasmids containing promoter regions for VZV regulatory genes of the putative IE class: ORF62, the homolog of HSV-1 ICP4 (6, 7); ORF4; and ORF61, the homolog of HSV-1 ICP0 (23).

pMTPORF10R transactivated the VZV ORF62 promoter up to 35-fold when induced by $CdCl_2$ (Fig. 2). pMTPORF10R transactivated the VZV ORF62 promoter only fivefold in the absence of $CdCl_2$ and lost its transactivating activity after digestion with *Sty*I, which cuts twice within the protein-coding sequence of ORF10. pMTPORF10I was unable to transactivate the ORF62 promoter (Fig. 2). Similar results were obtained with MeWo cells (24). When driven by its cognate promoter, VZV ORF10 had little activity to transactivate the ORF62 promoter. pGORF10 transactivated p62CAT only up to twofold in Vero cells (24).

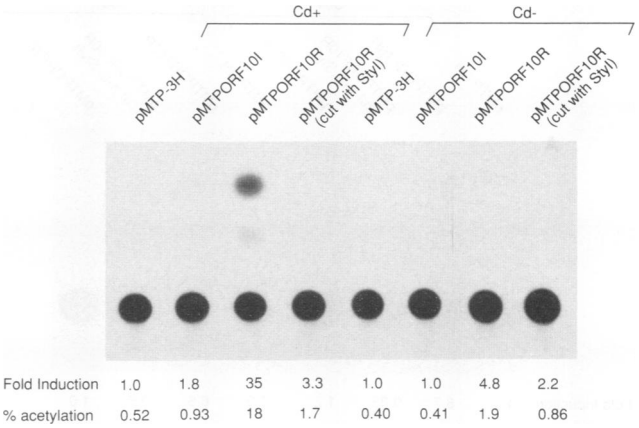


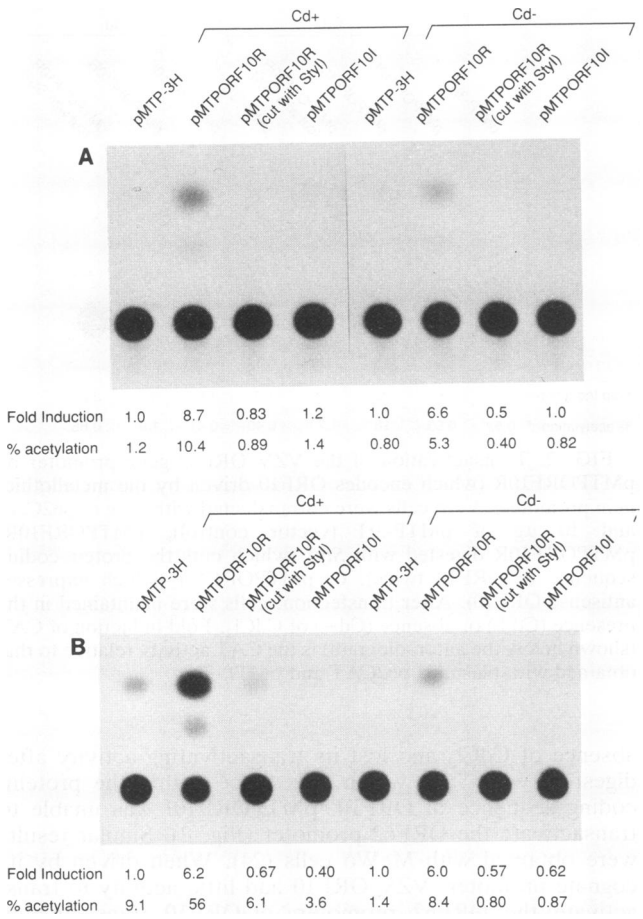
FIG. 2. Transactivation of the VZV ORF62 gene promoter by pMTPORF10R (which encodes ORF10 driven by the metallothionein promoter). Vero cells were cotransfected with 2 μg of p62CAT and 10 μg of pMTP-3H (vector control), pMTPORF10R, pMTPORF10R digested with *Sty*I (which cuts the protein-coding sequence of ORF10 twice), or pMTPORF10I (which expresses antisense ORF10). After transfection, cells were maintained in the presence (Cd+) or absence (Cd−) of $CdCl_2$. Fold induction of CAT (shown below the autoradiogram) is the CAT activity relative to that obtained with plasmids p62CAT and pMTP-3H.

Despite strong activation of the ORF62 promoter, pMTPORF10R was unable to transactivate two other VZV putative IE gene (ORF4 and ORF61) promoters (24). These two promoters could be transactivated by a plasmid expressing VZV ORF62 (25, 27).

To determine whether VZV ORF10 transactivates other alphaherpesvirus promoters, we assessed its effects on HSV-1 IE gene promoters. pMTPORF10R transactivated pPOH2 (CAT gene under the control of the HSV-1 ICP4 gene promoter; Fig. 3A) and pIGA-65 (CAT gene under the control of the HSV-1 ICP0 gene promoter; Fig. 3B). A dose-dependent response was observed with increasing amounts of transfected pMTPORF10R (24). Transactivation of these HSV-1 promoter constructs was not observed after transfection with pMTPORF10R digested with *Sty*I or with pMTPORF10I (which expresses ORF10 in the antisense orientation). Thus, like HSV-1 VP16, VZV ORF10 transactivates both VZV and HSV-1 IE genes.

To compare the transactivating ability of VZV ORF10 with that of HSV-1 VP16, Vero cells were cotransfected with p62CAT and either pMTPORF10R or pMTPVP16 (HSV-1 VP16 driven by a human metallothionein promoter). Induction of the VZV ORF62 promoter by VP16 was more than threefold higher than that by ORF10 from the same promoter (Fig. 3C).

VZV ORF10 expressed in stably transformed cell lines transactivates IE promoters of VZV and HSV-1. Having demonstrated that ORF10 has transregulatory activity in transient-expression assays, we sought to define its activity in regulating gene expression in the course of virus infection.



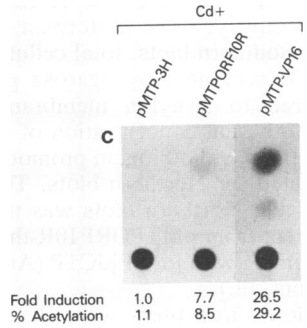


FIG. 3. Transactivation of the HSV-1 IE gene (ICP4 and ICP0) promoters by VZV ORF10 in transient-expression assays. Vero cells were transfected with (A) 2 μg of pPOH2 (CAT gene under the control of the HSV-1 ICP4 gene promoter) or (B) 1 μg of pIGA-65 (CAT gene under the control of the HSV-1 ICP0 gene promoter); 10 μg of pMTP-3H, pMTPORF10R, pMTPORF10R digested with *Sty*I, or pMTPORF10I was cotransfected in each case. After transfection, cells were maintained in the presence or absence of $CdCl_2$. Fold induction of CAT (shown below the autoradiogram) is the CAT activity relative to that obtained with plasmids pPOH2 or pIGA-65 plus pMTP-3H. (C) Comparison of transactivation of the VZV ORF62 promoter by VZV ORF10 and HSV-1 VP16. Vero cells were cotransfected with 2 μg of p62CAT and either 5 μg of pMTPORF10R or 5 μg of pMTPVP16 in the presence of $CdCl_2$.

To determine whether VZV ORF10 is able to facilitate HSV-1 infection in the absence of functional VP16, we established cell lines that stably expressed VZV ORF10 and performed complementation studies with HSV-1 *in*1814, in which the transactivation ability of wild-type VP16 is defective.

To construct cell lines which stably express VZV ORF10, Vero cells were cotransfected with pSV2neo (containing the G418 resistance gene as a selection marker) and a 20-fold molar excess of pMTPORF10R (yielding the V10R cell lines) or pMTPORF10I (yielding the V10I cell lines) and G418-resistant cells were selected. In a similar manner, MeWo cells were cotransfected with pSV2neo and pMTPORF10R (M10R cell lines). G418-resistant cell colonies were isolated and subsequently amplified. Eighteen of 30 V10R, 15 of 20 V10I, and 16 of 20 M10R cell lines analyzed by Southern blotting contained VZV ORF10 DNA. Figure 4 shows the presence of VZV ORF10 DNA in four of the cell lines (V10R-18, V10I-14, M10R-15, and M10R-21) chosen for further characterization. Northern blot analysis detected ORF10 RNA as a faint band in M10R-15 cells and as a prominent band in VZV-infected cells, but failed to detect ORF10 RNA in any of the other transformed cell lines (24). Western immunoblot analyses, which detected ORF10 protein in VZV-infected cells, failed to detect ORF10 protein in transformed cell lines shown to contain ORF10 DNA by Southern blotting (24).

The stably transformed Vero and MeWo cell lines express functionally active ORF10. To verify this, the cells were transfected with the p62CAT reporter plasmid (CAT gene under the control of the VZV ORF62 promoter). They were cotransfected with plasmid pCH110 (which carries the *Escherichia coli lacZ* gene under the control of the SV40 early promoter) as an internal control to correct for differences in transfection efficiency among cell lines.

These experiments revealed that CAT expression was upregulated up to 28-fold in V10R-18 cells transfected with p62CAT and treated with $CdCl_2$ compared with CAT expression in transfected control VM-3 cells (Table 1). Little or no activation was seen in transfected V10R-18 cells in the absence of $CdCl_2$. CAT expression was also upregulated in 16-8 cells (Vero cells stably transformed with the HSV-1 VP16 gene under the control of the Moloney murine sarcoma virus long terminal repeat [35]) transfected with p62CAT, although to a much lower level than in V10R-18 cells (3- to 5-fold versus 28-fold). There was no upregulation of CAT activity in V10I-14 cells transfected with p62CAT. CAT expression was also upregulated by up to 11- and 4-fold in M10R-15 and M10R-21 cells, respectively, after transfection with p62CAT and addition of $CdCl_2$ (Table 1). This activation was diminished in M10R-15 cells in the absence of $CdCl_2$. Similar results were obtained after transfection of ORF10-expressing cell lines with pPOH2 (CAT gene under the control of the HSV-1 ICP4 gene promoter; Table 1). CAT expression in 16-8 cells was upregulated up to fivefold after transfection with pPOH2 in the absence of $CdCl_2$. Less transactivation (up to twofold) was seen in the presence of $CdCl_2$, probably because of the toxic effect of $CdCl_2$ on these

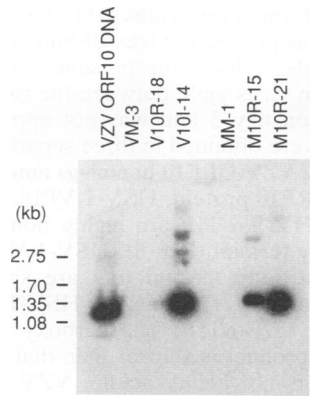


FIG. 4. Southern blot analysis of VZV ORF10 DNA in transformed Vero and MeWo cell lines. Electrophoretically separated *Kpn*I digests (10 μg) of cellular DNAs were transferred to a nylon membrane and probed with the ^{32}P-labeled 1.3-kb *Kpn*I fragment of pMTPORF10R shown in Fig. 1. The leftmost lane contains 100 pg of the same fragment, used as the probe. VZV ORF10 sequences were detected in Vero cells (V10R-18 and V10I-14) and MeWo cells (M10R-15 and M10R-21) transformed with ORF10, but not in cells containing only vector sequences (VM-3 and MM-1). Additional bands in cell line V10I-14 are due to multiple copies of VZV ORF10 DNA of various sizes, probably because of rearrangement of transfected DNA with integration into the cellular genome. Markers indicate sizes in kilobase pairs.

TABLE 1. Transactivation of VZV ORF62 and HSV-1 ICP4 promoter by VZV ORF10 expressed in stably transformed Vero and MeWo cell lines[a]

Cell line	$CdCl_2$	Relative CAT activity (fold induction[b])	
		p62CAT	pPOH2
VM-3	+	1	1
	−	1	1
V10R-18	+	28	4
	−	2	2
V10I-14	+	2	ND[c]
	−	1	ND
16-8	+	3	2
	−	5	5
MM-1	+	1	1
	−	1	1
M10R-15	+	11	4
	−	7	2
M10R-21	+	4	2
	−	4	2

[a] Cell lines were transfected with 2 μg of p62CAT (CAT gene under the control of the VZV ORF62 promoter) or pPOH2 (CAT gene under the control of the HSV-1 ICP4 promoter). Cells were cotransfected with plasmid pCH110 (which carries the *lacZ* gene under the control of the SV40 early promoter) to correct for differences in transfection efficiency. After transfection, cells were maintained in the presence or absence of $CdCl_2$.

[b] Fold induction is the CAT activity relative to that obtained with transfected control cells in the presence or absence of $CdCl_2$, as indicated.

[c] ND, not determined.

TABLE 2. Titration of HSV-1 wild-type (KOS) and VP16 mutant (*in*1814) stocks on Vero and MeWo cell lines expressing VZV ORF10[a]

Cell line	$CdCl_2$	KOS titer (PFU/ml)	Fold increase[b]	*in*1814 titer (PFU/ml)	Fold increase
VM-3	+	5.9×10^8	1.0	3.5×10^4	1.0
	−	6.4×10^8	1.0	3.6×10^4	1.0
V10R-18	+	5.3×10^8	0.9	2.8×10^5	8.0
	−	5.6×10^8	0.9	1.2×10^5	3.3
V10I-14	+	5.4×10^8	0.9	3.6×10^4	1.0
	−	6.1×10^8	1.0	3.9×10^4	1.1
16-8	−	ND[c]		5.0×10^5	14
MM-1	+	3.8×10^8	1.0	1.1×10^4	1.0
	−	4.3×10^8	1.0	1.4×10^4	1.0
M10R-15	+	6.1×10^8	1.6	8.5×10^4	7.7
	−	5.8×10^8	1.3	1.6×10^4	1.1
M10R-21	+	6.2×10^8	1.6	2.2×10^5	20
	−	5.6×10^8	1.3	3.1×10^4	2.2

[a] Stocks of the indicated viruses were generated in Vero cells; titers were determined on the indicated cell line, as described previously (8). Cells were maintained in the presence or absence of $CdCl_2$ (10 μM for Vero cells, 5 μM for MeWo cells) immediately after infection with virus.

[b] Ratio of virus titer on the indicated cell line to the titer on VM-3 or MM-1 cells in the presence or absence of $CdCl_2$, as indicated.

[c] ND, not determined.

cells. These results indicate that the ORF10 protein stably expressed in V10R-18, M10R-15, and M10R-21 cells is a functionally active transactivator for the IE promoters of VZV and HSV-1.

VZV ORF10-expressing cell lines complement an HSV-1 VP16 mutant (*in*1814) that lacks the transactivating function of VP16. Since VZV ORF10 transactivated herpesvirus IE promoters, we determined whether cell lines expressing VZV ORF10 could complement an HSV-1 VP16 mutant. HSV-1 *in*1814 contains a 12-bp insertion in the VP16 ORF, which abolishes the ability of the protein to transactivate IE genes or form IE complexes. Infection of Vero cells with HSV-1 *in*1814 results in less plaque formation than with the wild-type virus, indicating that the transactivating function of VP16 is not essential for virus growth but that viral replication is inefficient in the absence of VP16. That defect is complemented in cell lines expressing HSV-1 VP16, such as 16-8 cells (Table 2) (36). HSV-1 *in*1814 yielded three- to eightfold more plaques on Vero cells expressing VZV ORF10 (V10R-18) than on control cells (VM-3) in the absence or presence of $CdCl_2$, respectively (Table 2). In contrast, cells expressing VZV ORF10 in an antisense orientation (V10I-14) failed to influence the growth of HSV-1 *in*1814. Similarly, HSV-1 *in*1814 produced 8- to 20-fold more plaques on MeWo cells expressing VZV ORF10 than on control cells (MM-1) in the presence of $CdCl_2$. In the absence of $CdCl_2$, MeWo cells expressing ORF10 showed little or no change in the yield of HSV-1 *in*1814 compared with control cells (MM-1). No discernible differences were noted in the growth of HSV-1 wild-type KOS (Table 2) and VZV wild-type Ellen (24) on cell lines expressing VZV ORF10 compared with growth on control cell lines.

VZV ORF10-expressing cell lines enhance the infectivity of HSV-1 virion DNA. HSV-1 virion DNA, devoid of all struc-

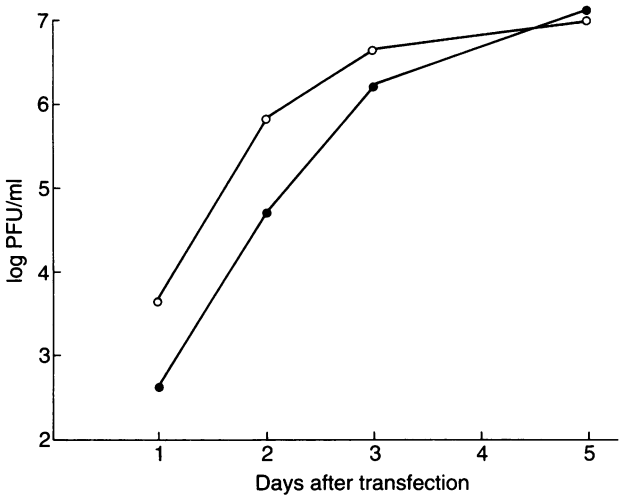


FIG. 5. Comparison of virus production after transfection of HSV-1 virion DNA in VZV ORF10-expressing Vero (V10R-18) and control (VM-3) cells. VM-3 cells (●) and V10R-18 cells (○) were transfected with 0.5 μg of HSV-1 wild-type KOS DNA. $CdCl_2$ (10 μM) was added immediately after transfection. Intracellular and extracellular virus was harvested and pooled at 24, 48, 72, and 120 h after transfection, and titers were determined by plaque assay on Vero cell monolayers. The experiment was repeated twice, and a representative experiment is shown.

tural proteins including VP16, is infectious when transfected into cells in culture; however, initiation of viral replication by HSV-1 DNA is inefficient compared with infection by virions. An initial delay in the onset and progression of the replicative cycle after transfection with HSV-1 DNA is reduced after the first round of replication, presumably because the progeny virions contain VP16.

To further confirm that VZV ORF10 can substitute for HSV-1 VP16, HSV-1 wild-type (KOS) DNA was isolated and transfected into ORF10-expressing (V10R-18) or control (VM-3) cells in the presence of $CdCl_2$. At various times posttransfection, cultures were harvested and the virus yield was determined on Vero cells. On days 1 and 2 after transfection, virus production was 10-fold higher in V10R-18 cells than in VM-3 cells in the presence of $CdCl_2$ (Fig. 5). The difference in virus yield between the two cell lines was less prominent on day 3 and was not apparent on day 5. Similar results were obtained in three separate experiments.

Comparison of VZV ORF10 homologs among alphaherpesviruses. VZV ORF10 protein, HSV-1 VP16, EHV-1 gene 12 product, and BHV-1 α-TIF are highly homologous except for their carboxy termini (Fig. 6). HSV-1 VP16 and BHV-1 B-αTIF have carboxy termini that are similar in length; however, the carboxy terminus of HSV-1 VP16 is more acidic than that of B-αTIF. The carboxy terminus of the EHV-1 gene 12 product is shorter than that of HSV-1 VP16 or B-αTIF and is not highly acidic. VZV ORF10 has the shortest carboxy terminus and is also not highly acidic. Since the transactivating activity of VP16 has been shown to reside in the acidic carboxy terminus, which is not conserved in VZV ORF10, it is unknown at present which portion of the ORF10 molecule is responsible for transactivation.

DISCUSSION

We have shown that VZV ORF10 protein acts as a transactivator for VZV and HSV-1 IE promoters in transient-expression assays. VZV ORF10 expressed by transfected plasmids or from integrated DNA in stable cell lines transactivated the VZV ORF62, HSV-1 ICP4, and HSV-1 ICP0 promoters. Cell lines stably expressing ORF10 complemented an HSV-1 mutant which lacks the transactivating function of VP16 and enhanced the de novo synthesis of infectious virus after transfection of HSV-1 virion DNA. Thus, VZV is now known to express five genes, ORF4 (16, 27), ORF10 (this study), ORF61 (25, 27), ORF62 (16, 27), and ORF63 (17), each homologous to an HSV-1 gene, that regulate gene expression in transient-expression assays.

In contrast to this study, an earlier study of VZV ORF10 failed to show transactivating activity for VZV or HSV-1 promoters (21). In that study, the HSV-1 VP16 promoter was used to drive VZV ORF10, and the failure to demonstrate transregulatory activity by VZV ORF10 may have been due

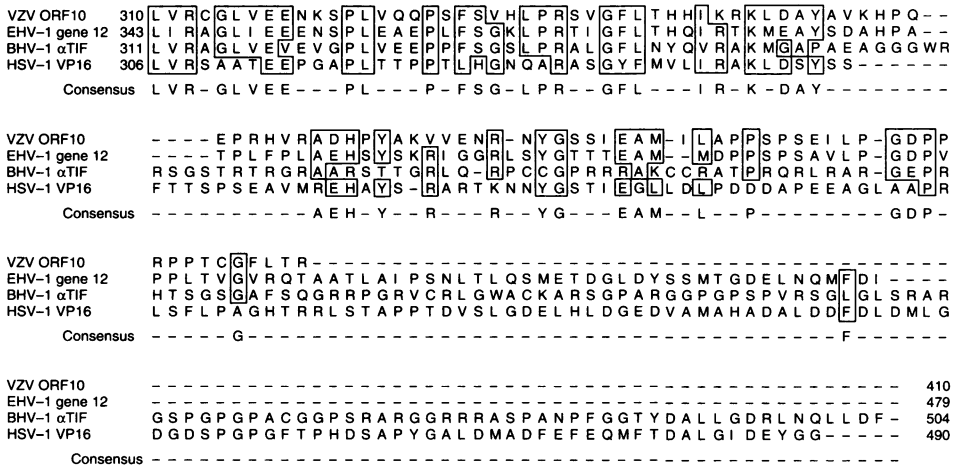


FIG. 6. Comparison of the predicted amino acid sequences of the carboxy portion of VZV ORF10 (4), EHV-1 gene 12 (33), BHV-1 B-αTIF (3), and HSV-1 VP16 (20). Amino acids 310 to 399 of VZV ORF10 are highly conserved among other alphaherpesvirus homologs, while the carboxy terminus (amino acids 400 to 410) is not conserved. Gaps have been introduced into the sequence (dashes) for the best alignment. Boxed amino acids are identical residues or conserved substitutions.

to inadequate expression of the VZV protein. In fact, we found that no or very little (twofold) transactivating ability was detected when VZV ORF10 was expressed from its cognate promoter (24). Because of the concern that ORF10 might be toxic to cells in which it is expressed (like its HSV-1 counterpart VP16 [1]), we chose an alternative promoter that could be induced when desired. Induction of ORF10 expression from the metallothionein promoter resulted in substantial transactivating activity (Fig. 2), although the transactivating ability of ORF10 was weaker than that of HSV-1 VP16 (Fig. 3C).

VZV ORF10, like its HSV-1 homolog VP16 (36), also showed activity in the context of actively replicating HSV-1. Cell lines expressing VZV ORF10 enhanced HSV-1 yield after transfection of the cell lines with HSV-1 virion DNA. Since VZV ORF10 is incorporated into the virion tegument (18) and the protein is thought to be released after infection of cells, transactivation of a major IE VZV gene product (ORF62) by ORF10 would enhance initiation of VZV infection. In addition, since VZV ORF62 transactivates the expression of other putative IE VZV gene promoters (ORF4 [25, 27] and ORF61 [27]) as well as its cognate (ORF62) promoter (28), the transactivation of VZV ORF62 by ORF10 may initiate a cascade of gene activation to support the VZV replicative cycle. It will be important to determine the actual role that ORF10 plays in infection of cells with VZV.

Comparison of the amino acid sequence of VZV ORF10 with those of its alphaherpesvirus homologs indicates that VZV ORF10 lacks the carboxy terminus present in its HSV-1 VP16, BHV-1 α-TIF, and EHV-1 gene 12 homologs. A recent study (29) indicates that EHV-1 possesses a protein that is functionally equivalent to HSV-1 VP16 and can transactivate expression of HSV-1 and EHV-1 IE genes. However, HSV-1 VP16 has an acidic carboxy terminus that activates transcription, while EHV-1 gene 12, BHV-1 α-TIF, and VZV ORF10 lack an acidic carboxy terminus. Thus, the transactivation domain of VZV ORF10 may more closely resemble that of its EHV-1 homolog than the HSV-1 VP16 activation domain.

Our studies show that VZV ORF10 can substitute for the transactivation function of HSV VP16. Cell lines expressing VZV ORF10 were able to complement an HSV-1 VP16 mutant (*in*1814) that lacks the transactivating function of VP16 nearly as effectively as a cell line expressing HSV-1 VP16. In addition to transactivation, the HSV-1 VP16 protein has at least two additional functions. VP16 is an important structural protein in the virion tegument (2, 14), and VP16 forms IE complexes with cellular proteins and an element on HSV-1 IE promoters (19). Substantial amounts of VZV ORF10 have been detected in VZV virions, closely associated with tegument-nucleocapsid structures (18).

At present, it is unknown whether VZV ORF10 transregulatory activity is mediated through binding to cellular proteins in infected cells. HSV-1 VP16 forms IE complexes with the cellular Oct-1 protein, at least one additional cellular factor, and the TAATGARAT element on HSV-1 IE promoters (10, 19, 31, 34). DNA sequences in the VZV ORF62 promoter region possess the element ATGTAAAT GAAAT, representing a combination of the octamer element ATGCAAAT (seven of eight matches) and the HSV TAAT GARAT element (eight of nine matches) (21), as well as several sites that match the octamer element closely (4), while the promoter sequences of ORF4 and ORF61 do not contain any site closely related to TAATGARAT. A previous study of VZV ORF10 was unable to document binding of ORF10 to TAATGARAT elements in the presence of cell extracts (21). Identification of the *cis* elements for VZV ORF10 on IE gene promoters and the cellular factor(s) that may interact with VZV ORF10 will enhance our understanding of the role of ORF10 in regulating VZV IE gene expression.

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