JOURNAL OF VIROLOGY, Dec. 1992, p. 7303–7308
0022-538X/92/127303-06$02.00/0

Vol. 66, No. 12

Varicella-Zoster Virus Open Reading Frame 61 Protein Is Functionally Homologous to Herpes Simplex Virus Type 1 ICP0

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The varicella-zoster virus (VZV) open reading frame 61 (ORF61) protein is thought to be the homolog of herpes simplex virus type 1 (HSV-1) ICP0, based on gene location and limited amino acid homology. However, HSV-1 ICP0 *trans* activates HSV-1 genes, while VZV ORF61 protein *trans* represses the function of VZV *trans* activators on VZV promoters in transient expression assays. To investigate the functional relatedness of HSV-1 ICP0 and VZV ORF61 protein, we established Vero and MeWo cell lines which stably express VZV ORF61 under the control of a metallothionein promoter and performed complementation studies with an HSV-1 ICP0 deletion mutant (7134). Mutant 7134 is impaired for plaque formation and replication at a low multiplicity of infection in cell culture, but these defects were complemented by up to 200-fold in Vero cell lines expressing VZV ORF61. Likewise, the efficiency of plaque formation was improved by up to 100-fold in MeWo cell lines expressing VZV ORF61. A cell line expressing another VZV immediate-early gene product (ORF62) was unable to complement mutant 7134. HSV-1 mutants which are deleted for other HSV-1 immediate-early gene products (ICP4, ICP27) were unable to grow in VZV ORF61-expressing cell lines. These results indicate that, despite marked differences in their sequences and in effects on their cognate promoters in transient expression assays, VZV ORF61 protein is the functional homolog of HSV-1 ICP0.

Varicella-zoster virus (VZV) causes chicken pox with primary infection and shingles on subsequent reactivation from latency. Our knowledge of the mechanism of VZV gene regulation is limited, in part because of the difficulty in obtaining the high titers of cell-free VZV required to synchronously infect the large numbers of cells necessary for studying the kinetics and regulation of gene expression. DNA sequence analysis shows that the overall organization of VZV DNA is similar to that of herpes simplex virus (HSV) and that there are a number of genes whose predicted products are homologous to HSV proteins (8), suggesting functional similarities between them.

The products of two VZV-encoded putative immediate-early (IE) genes, open reading frame 4 (ORF4) and ORF62, *trans* activate expression of viral IE, early, and late genes in transient expression assays (21, 22). VZV ORF4 and ORF62 have amino acid homology to HSV type 1 (HSV-1) ICP27 and ICP4, respectively. ICP27 acts as a *trans* activator or a *trans* repressor, depending on the target gene (30, 35, 36). ICP4 is indispensable to the life cycle of the virus, is involved in negative regulation of IE genes, and is required throughout the viral replicative cycle for the expression of early and late genes (31). We previously showed that ORF62 protein is the functional homolog of HSV-1 ICP4 by demonstrating that ORF62-expressing cell lines can complement ICP4 mutants (15). This was confirmed when an HSV-1 recombinant which contains VZV ORF62 in place of the coding sequences for ICP4 was shown to grow in cell culture (11).

ORF61, another putative VZV IE gene, *trans* represses the function of VZV *trans* activators on putative viral IE, early, and late genes in transient expression assays (27). VZV ORF61 is thought to be the HSV-1 ICP0 (Vmw110) homolog, because both genes are located in similar parts of the genome, and the predicted gene products share a cysteine-rich putative zinc-binding finger in the amino-terminal region and additional limited amino acid homology elsewhere (29). While VZV ORF61 has been shown to be a *trans* repressor in transient expression assays, HSV-1 ICP0 is a strong *trans* activator for homologous (HSV-1 IE, early, and late) as well as several heterologous genes (3, 4, 12, 18, 28). Although a carboxy-terminal deletion mutant of ICP0 shows promiscuous *trans* repressing activity (42), full-length ICP0 does not exhibit *trans* repression. Therefore, it is not clear whether VZV ORF61 protein is a functional homolog of HSV-1 ICP0.

The growth and plaquing efficiency of HSV-1 ICP0 mutants are impaired at a low multiplicity of infection (3, 12, 33, 41). These mutations can be complemented by growth in cell lines expressing HSV-1 ICP0 (2, 33) or by coinfection with VZV or human cytomegalovirus (40). Thus, a VZV gene product(s) must be able to serve as a functional analog of HSV-1 ICP0.

To explore the possible functional similarities between HSV-1 ICP0 and VZV ORF61 protein, we established cell lines which express VZV ORF61 protein and employed them in complementation studies with an HSV-1 ICP0 mutant. Here we show that VZV ORF61 protein can complement an HSV-1 ICP0 deletion mutant in cell culture.

MATERIALS AND METHODS

Cells and viruses. African green monkey kidney cells (Vero; American Type Culture Collection, Rockville, Md.) were propagated as previously described (15). Human ma-

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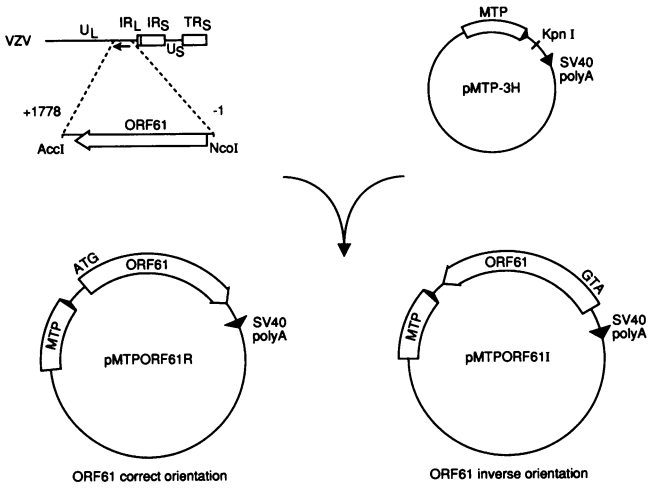

FIG. 1. Construction of VZV ORF61 expression vectors. pMTP-3H contains the metallothionein promoter (MTP) followed by a *Kpn*I restriction endonuclease site and a simian virus 40 (SV40) polyadenylation (polyA) sequence. The 1.8-kb *Acc*I-*Nco*I fragment from the VZV genome contains the protein-coding sequence of ORF61, but no upstream transcriptional regulatory sequences. pMTPORF61R contains the VZV ORF61 gene inserted into the *Kpn*I site of pMTP-3H in the right orientation driven by MTP with a simian virus 40 polyadenylation sequence downstream, while pMTPORF61I contains ORF61 in the inverse orientation.

lignant melanoma (MeWo) cells, obtained from Charles Grose (20), were grown in Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, penicillin G (100 U/ml), and streptomycin (100 μg/ml). FI-14 cells (VZV ORF62-expressing Vero cells [15]) and 3-3 cells (HSV-1 ICP27-expressing Vero cells [24]), provided by Priscilla A. Schaffer, were propagated as previously described. HSV-1 7134 (an ICP0 null mutant [3]), HSV-1 5*dl*1.2 (an ICP27 deletion mutant [32]), and *d*120 (an HSV-1 ICP4 deletion mutant [9]) were provided by Priscilla A. Schaffer. These HSV-1 mutants were all derived from the KOS strain. Stocks of HSV-1 KOS and 7134 used in this study were propagated and titered in Vero cells. Stocks of HSV-1 5*dl*1.2 and *d*120 were propagated in 3-3 cells and E5 cells (HSV-1 ICP4-expressing Vero cells [37]), respectively.

Plasmids. Plasmids pORF61, pGORF4, pGi26 (which expresses ORF62), and p1tkCAT (which contains the promoter for the VZV deoxypyrimidine kinase gene followed by the chloramphenicol acetyltransferase [CAT] gene) have been described previously (21, 22, 27). pSV2neo encodes the G418 resistance gene driven by the simian virus 40 early promoter (39). pMTP-3H, a gift from Dwight Kaufman, contains the human metallothionein promoter followed by a multiple cloning site which includes a *Kpn*I restriction site and a simian virus 40 polyadenylation signal.

ORF61-containing plasmids (Fig. 1) were derived by digesting recombinant VZV DNA with *Acc*I and *Nco*I (which cut at nucleotides 102,708 and 104,486, respectively [8]), filling in the 5′ termini with *Escherichia coli* DNA polymerase I (Klenow fragment), ligating a double-stranded *Kpn*I linker oligonucleotide (5′ GCTGCAGGTACCTGCAGC 3′) to both ends, digesting with *Kpn*I, and inserting into the *Kpn*I site of pMTP-3H. The resulting plasmids, pMTPORF 61R and pMTPORF61I, contain a single copy of the VZV ORF61 gene with the consensus sequence for initiation of translation −1 to −4 bases upstream of the ATG codon and 377 bp of sequence downstream from the stop codon of VZV ORF61 in the right (R) and inverted (I) orientations, respectively, under the control of a human metallothionein promoter.

Establishment of Vero and MeWo cell lines expressing VZV ORF61. Vero and MeWo cells were cotransfected with pSV2neo and pMTPORF61R or pMTPORF61I. G418-resistant cell colonies were selected and subcloned as described previously (15) with the following modifications. About 6×10^5 Vero or 1.2×10^6 MeWo cells were plated the day before transfection in 60-mm-diameter dishes. pSV2neo (0.5 μg, linearized with *Eco*RI) and pMTPORF61R or pMTPORF61I (10 μg each, linearized with *Xmn*I) were transfected into Vero cells by the calcium phosphate procedure (19); this was followed by a 1-min glycerol (15% [vol/vol]) shock 4 h after transfection. For MeWo cell transfections, DNAs were diluted gently in 100 μl of distilled water and then 25 μl of 2.5 M calcium chloride and 375 μl of 1× *N*-2-hydroxylethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-buffered saline. DNA precipitates were added to the MeWo cells in medium. Four hours after addition of precipitates, the medium was removed, cells were shocked with dimethyl sulfoxide (25% [vol/vol]) for 4 min, and the medium was replaced. After 48 h, transfected Vero and MeWo cells were trypsinized and seeded onto 100-mm-diameter dishes at a density of 5×10^3 and 1.5×10^4 cells per cm^2, respectively. The following day, the medium was changed and G418 (geneticin; Life Technologies, Inc./GIBCO, Grand Island, N.Y.) was added at 800 or 400 μg/ml for Vero or MeWo cells, respectively. One week later, the G418 concentration was lowered to 400 or 250 μg/ml for Vero and MeWo cells, respectively. Individual G418-resistant colonies were isolated, amplified, and screened, as below.

Cellular nucleic acid isolation and hybridization. For Southern blot analysis, total cellular DNA was isolated (38), digested with *Kpn*I, separated by agarose gel electrophoresis, and transferred to nylon membranes (Nytran; Schleicher & Schuell, Inc., Keene, N.H.). For Northern (RNA) blot analysis, cadmium chloride was added to the cell culture medium to a final concentration of 10 μM 1 day before the cells were harvested and the cellular RNA was isolated to induce expression from the metallothionein promoter. Total cellular RNA was isolated as described by Chomczynski and Sacchi (7). RNA samples were denatured in 50% formamide–6% formaldehyde–1× MOPS buffer (40 mM 3-morpholinepropanesulfonic acid [pH 7.0], 10 mM sodium acetate, 1 mM EDTA), fractionated on a 6% formaldehyde–1.5% agarose gel, and transferred to a nylon membrane. Southern and Northern blots were prehybridized and then hybridized for 20 h at 42°C in 5× SSPE (1× SSPE is 0.15 M NaCl, 10 mM NaH_2PO_4 [pH 7.7], 1 mM EDTA)–5× Denhardt's solution (0.08% bovine serum albumin, 0.08% Ficoll, 0.08% polyvinylpyrrolidone)–50% formamide–0.1% sodium dodecyl sulfate (SDS)–100 μg of denatured salmon testes DNA per ml. The probe used for both Southern and Northern blots was the VZV ORF61 DNA *Kpn*I fragment from pMTPORF61R that was radiolabelled by random priming with [α-^{32}P]dCTP (Amersham Corp., Arlington Heights, Ill.). Blots were washed twice in 1× SSPE–0.1% SDS at 37°C, and final washes were performed in 0.1× SSPE–0.1% SDS at 65°C for 30 min.

CAT assays. Vero cells were transfected with a total of 15 μg of DNA by the calcium phosphate procedure described above. Immediately after transfection, cadmium chloride was added to cell culture medium to induce expression from the metallothionein promoter. Cells were harvested 40 to 48

h after transfection, and CAT assays were performed as described previously (27). The percentages of acetylated and nonacetylated forms of [^{14}C]chloramphenicol were quantitated by a System 200 imaging scanner (BIOSCAN, Inc., Washington, D.C.).

Viral complementation studies. Virus titers were determined by plaquing on various cell lines in the presence of 0.5% human immune globulin (16) (Gammagard; Baxter Healthcare Corp., Glendale, Calif.). To reduce the level of expression from the metallothionein promoter, cells were maintained in medium containing dialyzed fetal bovine serum before and throughout the experiments. To induce expression from the metallothionein promoter, cadmium chloride was added to a final concentration of 10 μM immediately after infection with virus. Single-step growth studies for HSV-1 KOS and 7134 viruses were done as described previously (15). The yields of KOS and 7134 viruses were determined by titration on Vero cells.

Computer analysis. Predicted amino acid sequences of VZV ORF61, HSV-1 ICP0, bovine herpesvirus 1 (BHV-1) p135, and pseudorabies virus EP0 were simultaneously compared by using the Pileup and Pretty Programs from the Sequence Analysis Software Package (Genetics Computer Group, Inc., Madison, Wis.) (10).

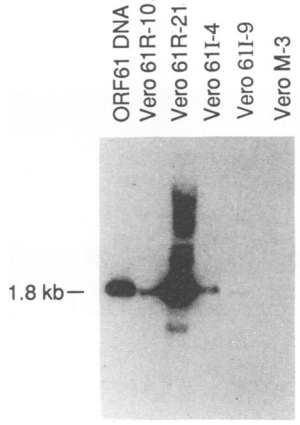

FIG. 2. Southern blot analysis of ORF61-specific sequences in transformed Vero cell lines. Electrophoretically separated *Kpn*I digests (10 μg) of cellular DNAs were transferred to a nylon membrane and probed with the ^{32}P-labelled 1.8-kb *Kpn*I fragment of pMTPORF61R shown in Fig. 1. The leftmost lane contains 50 pg of the same fragment used for the probe. VZV ORF61 sequences were detected in Vero cells transformed with ORF61 (V61R-10, V61R-21, V61I-4, and V61I-9), but not with vector control (VM-3). Marker indicates size in kilobase pairs.

RESULTS

Establishment of VZV ORF61-transformed cell lines. Vero and MeWo cells were cotransfected with pSV2neo (containing the G418 resistance gene as a selection marker) and a 20-fold molar excess of pMTPORF61R or pMTPORF61I (Fig. 1), and this was followed by G418 selection. G418-resistant cell colonies were isolated and subsequently amplified. Thirty colonies were analyzed by Northern blotting, 14 of which expressed VZV ORF61 RNA at various levels. V61R-10 and V61R-21 cell lines (Vero cells expressing VZV ORF61), V61I-4 and V61I-9 cell lines (Vero cells expressing antisense VZV ORF61), and M61R-4 and M61R-37 cell lines (MeWo cells expressing VZV ORF61) were chosen for further characterization, based on high-level expression on Northern blots. VM-3 (Vero) and MM-1 (MeWo) cell lines containing pSV2neo and pMTP-3H but no VZV DNA were used as negative (vector) controls.

Cellular DNAs from the 61R and 61I families of cell lines were digested with *Kpn*I and analyzed for the presence of VZV ORF61 DNA by Southern blotting with a gel-purified *Kpn*I fragment from pMTPORF61R as a probe. The Vero 61R and 61I cell lines chosen for study contained a 1.8-kb band corresponding to the *Kpn*I fragment from pMTPOR F61R. The VM-3 (vector control) cell line did not contain VZV ORF61 DNA (Fig. 2). Hybridization showed the cell line 61R-21 to have multiple copies of VZV ORF61 DNA of various sizes, probably due to rearrangement of transfected DNA accompanied by integration into the cellular genome. DNA from M61R-4 and M61R-37 cells also contained VZV ORF61 DNA of the expected size (26).

To analyze expression of VZV ORF61 in these cell lines, total cellular RNAs were isolated and Northern blots were performed with the ORF61 *Kpn*I fragment as a probe (Fig. 3). RNA from VZV-infected whole human fetal fibroblasts (WHF), which served as a positive control, contained a 1.5-kb transcript (27). RNA from V61R-10 and V61R-21 cells contained transcripts slightly larger than that from VZV-infected WHF, presumably because of differences in the site of initiation or termination of transcription. RNA from V61I-9 cells also contained a transcript larger than that from VZV-infected WHF, while RNA from V61I-4 cells contained a transcript much larger than expected, probably because of differences in both initiation and termination of transcription and rearrangement of VZV DNA in the cell. RNA from M61R-4 and M61R-37 cells contained transcripts of the expected size (26).

***trans* repression of VZV target genes by products of ORF61-expressing cells.** VZV ORF61 acts as a *trans* repressor in transient expression assays (27). To determine whether cells expressing VZV ORF61 made a functionally active product, we transfected cells with p1tkCAT (containing the VZV deoxypyrimidine kinase promoter followed by the CAT gene) alone or in combination with plasmids expressing VZV

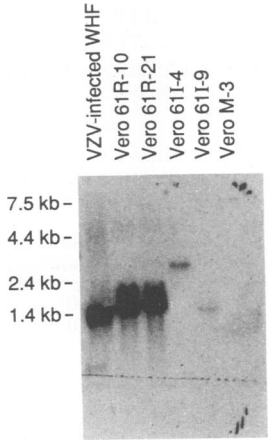

FIG. 3. Northern blot analysis of ORF61-specific transcripts in transformed Vero cell lines. Each lane contains 15 μg of cellular RNA. The blot was hybridized with the same probe as used for Southern blots. The leftmost lane contains RNA from VZV-infected WHF. VZV ORF61 transcripts were detected in V61R-10, V61R-21, V61I-4, and V61I-9 cells, but not in VM-3 cells. Marker indicates size in kilobases.

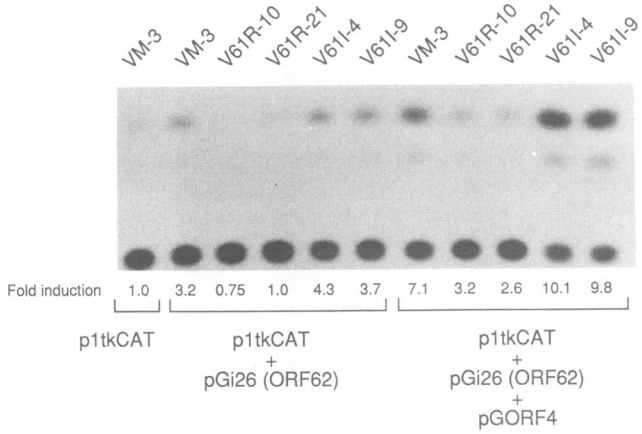

FIG. 4. CAT activity in transformed Vero cell lines. Vero cell lines (top) were cotransfected with 1 μg of p1tkCAT alone or in combination with 5 μg of pGi26 (which expresses ORF62) or pGORF4. Plasmids used for the experiments are indicated at the bottom. Transfected cells were harvested 40 to 48 h after transfection, and cell lysates were assayed for CAT activity. Fold induction of CAT (shown below the autoradiogram) is the CAT activity relative to that obtained for plasmid p1tkCAT alone in VM-3 cells.

ORF62 (pGi26) or ORF4 (pGORF4) (Fig. 4). In VM-3 cells, the expression of CAT was up-regulated over threefold by ORF62 and over sevenfold by ORF4 and ORF62 together. The level of expression of CAT stimulated by ORF62, or ORF62 in combination with ORF4, was down-regulated by 55 to 77% in V61R-10 or V61R-21 cells, but not in V61I-4 and V61I-9 cells relative to VM-3 cells. These results indicate that the ORF61 gene product in the 61R cell lines represses the activation of a VZV early gene promoter by a VZV IE gene, as it does in transient expression assays.

Complementation of ICP0 null mutant of HSV-1 in ORF61-expressing cell lines. (i) Efficiency of plaque formation. ICP0 is not essential for productive infection in cell culture but plays a critical role in viral growth, as indicated by the poor efficiency of plaque formation and the impaired growth of a null mutant (HSV-1 7134 [1]). Transfection of cells with a plasmid expressing ICP0 and HSV-1 7134 viral DNA results in complementation of the 7134 virus (1). To determine whether VZV ORF61 could also complement growth of the HSV-1 ICP0 deletion mutant, we infected cells expressing VZV ORF61 with HSV-1 7134. As shown in Table 1, HSV-1 7134 produced 50- to 60-fold more plaques on Vero cells expressing VZV ORF61 (V61R-10, V61R-21) than on the control cell lines (VM-3, V61I-4). When gene expression was induced with cadmium chloride, HSV-1 7134 produced 100- to 200-fold more plaques on VZV ORF61-expressing cells than on the control cells. Since cadmium chloride increases the level of VZV ORF61 mRNA (26), the degree of complementation of HSV-1 7134 correlates with the level of expression of ORF61. In contrast, cells expressing VZV ORF61 in an antisense orientation (V61I-4) failed to influence the growth of HSV-1 7134, in the presence or absence of cadmium chloride.

HSV-1 7134 infection yielded only slightly more plaques on MeWo cells expressing VZV ORF61 than the control cells in the absence of cadmium; however, induction of VZV ORF61 expression with cadmium chloride resulted in 20- to 100-fold more plaques than on control cells (Table 2).

Complementation of HSV-1 7134 was specific for VZV ORF61 and did not occur with expression of another VZV regulatory gene. The number of plaques obtained with HSV-1 7134 was not increased when the virus was used to infect Vero cells stably expressing VZV ORF62 (Table 3). In addition, ORF61-expressing Vero cells were unable to complement deletion mutants in other HSV-1 IE genes (*5dl*1.2, an ICP27 deletion mutant; *d*120, an ICP4 deletion mutant) (Table 3).

(ii) Efficiency of viral replication. Single-step growth studies of HSV-1 7134 and KOS were performed to determine the yield of virus 24 h after infection. Since prior studies had shown that the growth impairment of HSV-1 7134 is greater at a low multiplicity of infection than at a high multiplicity of infection (3, 12, 33, 41), we performed growth studies with HSV-1 7134 and KOS at both low and high multiplicities of

TABLE 1. Titration of HSV-1 wild type (KOS) and ICP0 deletion mutant (7134) stocks on Vero cell lines expressing VZV ORF61[a]

Cell line	$CdCl_2$	KOS titer (10^8 PFU/ml)	Fold increase[b]	7134 titer (PFU/ml)	Fold increase[b]
VM-3	−	3.3	1.0	1.8×10^5	1.0
	+	2.1	1.0	1.5×10^5	1.0
V61R-10	−	2.4	0.7	8.9×10^6	49
	+	2.5	1.2	1.5×10^7	100
V61R-21	−	3.8	1.2	1.2×10^7	67
	+	3.0	1.4	3.1×10^7	210
V61I-4	−	3.7	1.1	1.3×10^5	0.7
	+	2.9	1.4	9.6×10^4	0.6

[a] Stocks of the indicated viruses were generated in Vero cells; titers were determined on the indicated cell line, as described previously (16). Cells were grown in either the absence (−) or presence (+) of cadmium chloride ($CdCl_2$, 10 μM) immediately after infection with virus.

[b] Ratio of virus titer on the indicated cell line to the titer on the VM-3 cell line, in the absence or presence of cadmium chloride.

TABLE 2. Titration of HSV-1 wild type (KOS) and ICP0 deletion mutant (7134) stocks on MeWo cell lines expressing VZV ORF61

Cell line	$CdCl_2$	KOS titer (10^8 PFU/ml)	Fold increase	7134 titer (PFU/ml)	Fold increase
MM-1	−	3.4	1.0	2.0×10^5	1.0
	+	3.4	1.0	1.0×10^5	1.0
M61R-4	−	2.8	0.82	5.2×10^5	2.6
	+	2.3	0.68	1.0×10^7	100
M61R-37	−	3.0	0.88	3.2×10^5	1.6
	+	2.6	0.76	2.0×10^6	20

TABLE 3. Titrations of HSV-1 ICP0 deletion mutant (7134), ICP4 deletion mutant (*d*120), or ICP27 deletion mutant (*5dl*1.2) on Vero cell lines expressing VZV ORF62 or ORF61 or HSV-1 ICP27[a]

Cell line	$CdCl_2$	Titer (PFU/ml)		
		7134	*d*120	*5dl*1.2
VM-3	−	1.6×10^5	$<10^1$	$<10^1$
	+	1.0×10^5	$<10^1$	$<10^1$
V61R-21	−	1.0×10^7	$<10^1$	$<10^1$
	+	3.1×10^7	$<10^1$	$<10^1$
FI-14	−	6.8×10^3	1.6×10^5	$<10^1$
3-3	−	1.0×10^5	ND	5.5×10^7

[a] Titers were determined on Vero cells expressing VZV ORF61 (V61R-21), ORF62 (FI-14), or HSV-1 ICP27 (3-3). ND, not determined.

TABLE 4. Yield of HSV-1 wild type (KOS) and ICP0 deletion mutant (7134) on Vero cell lines expressing VZV ORF61, 24 h after infection[a]

Cell line	$CdCl_2$	Titer (PFU/dish)			
		KOS		7134	
		Low MOI	High MOI	Low MOI	High MOI
VM-3	−	7.7×10^6	2.7×10^9	2.1×10^4	2.2×10^6
	+	3.2×10^6	5.7×10^8	2.0×10^3	1.5×10^3
V61R-21	−	1.2×10^7	3.3×10^9	7.0×10^5	2.5×10^6
	+	3.3×10^6	2.7×10^8	5.8×10^5	1.8×10^3
V61R-10	+	5.4×10^6	1.2×10^9	7.7×10^5	ND
V61I-4	+	2.2×10^6	3.3×10^8	3.1×10^3	ND

[a] Confluent 60-mm dishes were infected with HSV-1 KOS and 7134 at low (10^{-4} PFU per cell) or high (10^{-1} PFU per cell) multiplicity of infection (MOI) calculated from virus titers on Vero cells in either the absence (−) or presence (+) of cadmium chloride ($CdCl_2$; 10 μM). ND, not determined.

infection (Table 4). At a high multiplicity of infection (0.1 PFU per Vero cell; corresponding to 21 PFU per V61R-21 cell), HSV-1 7134 grew to similar titers on cells expressing VZV ORF61 as on control cell lines. The virus titers were higher on cells grown in cadmium-free medium than on cells grown in cadmium-added medium. In contrast, at a low multiplicity of infection (0.0001 PFU per Vero cell; corresponding to 0.02 PFU per V61R-21 cell), HSV-1 7134 grew to 30- to 300-fold-higher titers on VZV ORF61-expressing cells than on control cell lines in cadmium-free or cadmium-added medium, respectively. The failure to note an increase in titer for a given cell line with addition of cadmium was probably due to two different competing effects of cadmium. While cadmium increases the level of expression of VZV ORF61 in cells expressing this gene (see above), its toxicity reduces the overall growth of both KOS and 7134 viruses in Vero cells.

DISCUSSION

We showed that cell lines expressing VZV ORF61 complement the growth of an HSV-1 ICP0 deletion mutant, and thereby verify that VZV ORF61 is the functional homolog of HSV-1 ICP0. The ability of VZV ORF61-expressing cells to complement an HSV-1 ICP0 deletion mutant is specific for the ORF61 gene product. First, HSV-1 mutants which are deleted for other HSV-1 IE gene products (e.g., ICP4, ICP27) were unable to grow in VZV ORF61-expressing cell lines. Second, a cell line expressing another VZV IE gene product (ORF62) was unable to complement the HSV-1 ICP0 deletion mutant. Third, complementation of VZV ORF61 for HSV-1 ICP0 was dose dependent, corresponding to the amount of VZV ORF61 RNA expressed.

Comparison of the predicted amino acid sequences of VZV ORF61 and HSV-1 ICP0 shows substantial homology in the cysteine-rich motif in the amino-terminal region of VZV ORF61 (Fig. 5). These cysteine-rich motifs are also conserved in homologs of other alphaherpesviruses, including the p135 protein of BHV1 (43) and the EP0 protein of pseudorabies virus (6). Each of these cysteine-rich sequences resembles a zinc-finger motif common to cellular proteins that may interact with DNA (17). The cysteine-rich region of HSV-1 ICP0 is thought to be important for *trans* activation (in the absence of ICP4), for virus reactivation from latency, and for normal growth of HSV-1 in cell culture (1, 5, 12–14). Thus, the homologous cysteine-rich region of ORF61 may play similar critical roles in the biology of VZV.

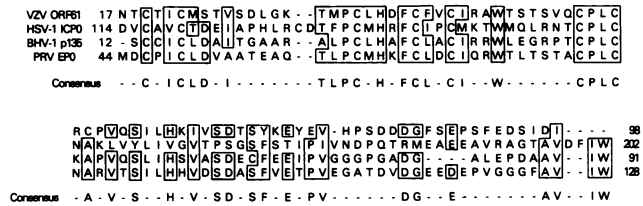

FIG. 5. Comparison of the predicted amino acid sequences of VZV ORF61 (amino acids 17 to 98) with HSV-1 ICP0 (amino acids 114 to 202), BHV-1 p135 (amino acids 12 to 91), and pseudorabies virus (PRV) early protein 0 (EP0) (amino acids 44 to 128). Gaps are introduced into the sequence (in dashes) for the best alignment. Boxed amino acids are identical residues or conserved substitutions.

VZV ORF61 and HSV-1 ICP0 may also share other functional domains. VZV ORF61 has a proline-rich sequence (amino acids 100 to 161) adjacent to the cysteine-rich region; HSV-1 ICP0 contains a proline-rich sequence (amino acids 225 to 551) near its cysteine-rich motif. The proline-rich region lies within the *trans* activation domain of HSV-1 ICP0 (5, 42). Other transcriptional activators, such as CTF/NF-1, AP-2, Jun, and OCT-2 (25), possess proline-rich activation domains. In addition, VZV ORF61 has an acidic domain (amino acids 71 to 105), while HSV-1 ICP0 has two acidic domains (amino acids 1 to 114 and 233 to 243). Acidic domains are important for transcriptional activation in other herpesvirus regulatory proteins such as HSV-1 VP16 (34) and pseudorabies virus IE180 (23). The significance of the acidic domains in VZV ORF61 and HSV-1 ICP0 is uncertain, however, since there is no apparent amino acid homology between the two proteins in this region.

While VZV ORF61 and HSV-1 ICP0 share these very limited areas of amino acid homology, the two proteins exhibit different activities in transient expression assays in vitro. VZV ORF61 *trans* represses the activation of putative viral IE, early, and late gene promoters by VZV ORF4 and ORF62 (27), but it directly *trans* activates an HSV-1 IE and early gene promoter of HSV-1 (26). In contrast, HSV-1 ICP0 *trans* activates both HSV-1 and VZV promoters (3, 4, 12, 18, 26, 28). Although full-length HSV-1 ICP0 does not show *trans* repressing activity, a carboxy-terminal deletion mutant of ICP0 (retaining amino acids 1 to 245) acts as a promiscuous *trans* repressor (42). This mutant is deleted for the proline-rich domain, but retains the cysteine-rich and acidic domains of the molecule. In addition, BHV-1 p135 acts as a *trans* activator or a *trans* repressor, depending on the target promoter (43). Thus, VZV ORF61, HSV-1 ICP0, and BHV-1 p135 may each contain both *trans* activating and *trans* repressing domains, and the net effect observed for each molecule in vitro may depend on the target promoter, on interactions with other viral or cellular proteins, or on undefined properties of transient transfection assays.

In conclusion, we showed that VZV ORF61 is functionally homologous to HSV-1 ICP0. The ability of VZV ORF61 to complement an ICP0 deletion mutant of HSV-1 implies that despite marked differences in amino acid sequences and in activities in transient expression assays, the two proteins have similar functions during virus infection.

ACKNOWLEDGMENTS

We thank D. Kaufman for plasmid pMTP-3H, P. A. Schaffer for the HSV-1 mutants and 3-3 cells, and C. Grose for MeWo cells.

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