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Cell Lines Containing Varicella-Zoster Virus Open Reading Frame 62 and Expressing the "IE"175 Protein Complement ICP4 Mutants of Herpes Simplex Virus Type 1

JAMES M. FELSER,[1] PAUL R. KINCHINGTON,[2] GENEVIEVE INCHAUSPE,[1] STEPHEN E. STRAUS,[1] AND JEFFREY M. OSTROVE[1]*

Medical Virology Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases,[1] and the Uniformed Services University of the Health Sciences,[2] Bethesda, Maryland 20892

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Vero cells were cotransfected with pSV2neo and a recombinant plasmid containing the varicella-zoster virus (VZV) open reading frame 62 (ORF62). Three neomycin-resistant cell lines were isolated and shown to complement two different ICP4 mutants (*ts*B21 and *d*120) of herpes simplex virus (HSV) type 1 (HSV-1). VZV-specific RNA could not be detected in these cell lines, but following infection with *ts*B21, a 4.3-kilobase VZV transcript was detected. This RNA increased in quantity when cells were infected in the presence of cycloheximide. A VZV-specific protein of 175 kilodaltons was detected in extracts of all three cell lines following infection with wild-type HSV-1 but not in uninfected cells. That VZV RNA and protein were detected only in HSV-1-infected cells suggests that a component of the HSV virion activates the expression of VZV ORF62. The increase in RNA expression seen in the presence of cycloheximide indicates that the protein encoded by VZV ORF62, "IE"175, may be autoregulatory. These data provide further evidence that VZV "IE"175 is the functional analog of the HSV ICP4.

Expression of many herpesvirus genes is coordinately regulated and sequentially ordered in a cascade fashion. The most extensive analyses have been done for herpes simplex virus (HSV) type 1 (HSV-1) genes, which are divided into five temporal groupings (α, β_1, β_2, γ_1, and γ_2). Five α or immediate-early genes of HSV are known. These are expressed first in infection and are transcribed in the absence of de novo protein synthesis (20, 21). The HSV β and γ classes of genes are expressed as early and late genes, respectively.

Less extensive analyses of the temporal expression of the genes of other herpesviruses have been conducted. No such studies have been performed with varicella-zoster virus (VZV), in part because of the difficulty in obtaining high titers of cell-free virus required to synchronously infect the large numbers of cells needed to study gene expression. Nevertheless, among the products of the 71 open reading frames (ORFs) predicted by the VZV DNA sequence, there are a number of polypeptide homologs between HSV and VZV (11). From these, Davison and Scott (11) have identified homologs in VZV for three HSV α genes (ICP4, ICP22, and ICP27) (11). The predicted product of VZV ORF62 shares significant amino acid homology with the HSV ICP4. Three regions of the predicted VZV protein, representing approximately 650 amino acids, have over 50% homology to ICP4 (10). The ORF62 product is predicted to be at least 140 kilodaltons (kDa) in size (10, 11) compared with 175 kDa for the HSV-1 ICP4 (36). Recently, Shiraki and Hyman (29) detected four immediate-early proteins of VZV. The one they estimate to be 185 kDa is closest in size to the protein predicted for ORF62. We reported previously that a DNA segment containing VZV ORF62 complements an ICP4 temperature-sensitive (*ts*) mutant of HSV-1, *ts*B21, thereby mapping an ICP4-equivalent activity to ORF62 (16).

In this report, we describe the establishment of transformed cell lines which contain and express the ORF62 gene of VZV. These cell lines efficiently complement two different ICP4 mutants of HSV-1. Under the conditions at which ORF62 is efficiently expressed in these cells, we can detect and characterize its transcript and a 175-kDa protein product.

MATERIALS AND METHODS

Cells and viruses. Human embryonic fibroblasts (Flow 5000), human fetal foreskin fibroblasts (HFFs), and CV-1 and Vero cells were propagated in a 1:1 mixture of minimal essential medium and medium 199 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, penicillin G (100 U/ml), and streptomycin (100 μg/ml). E5 cells, a gift of Neal DeLuca, contain the gene encoding the HSV-1 ICP4 and are able to complement HSV-1 ICP4 deletion mutants (30). The KOS strain of HSV-1 and *ts*B21, an ICP4 temperature-sensitive mutant of HSV-1 (KOS), were provided by Priscilla A. Schaffer (14). HSV strain *d*120, also courtesy of N. DeLuca, is a mutant of KOS which contains a 4.1-kilobase (kb) deletion in the gene encoding ICP4 (13). VZV strains Ellen and Scott were grown in Flow 5000 and HFF cells, respectively, as previously described (35).

Plasmids. pVZVEcoE contains the *Eco*RI E fragment (Fig. 1) of VZV strain Ellen in pBR325. pVZVEcoA contains the *Eco*RI A fragment of VZV strain Ellen in pBR322 (9). pGI26 (Fig. 1) contains the 6.5-kb *Bgl*II-*Hpa*I fragment from pVZVEcoA ligated into the *Sma*I site of pUC9 following blunting of ends with T4 DNA polymerase. Plasmid pSV2neo contains the bacterial gene encoding G418 resistance under the transcriptional control of the simian virus 40 early promoter (32). pSG1, courtesy of Sandra K. Weller, contains the *Eco*RI JK fragment, including the ICP4 gene of HSV-1 cloned into pBR325 (18).

Nucleic acid isolation and hybridization. Cellular DNA was isolated and hybridized as described previously (28, 31). Total cellular RNA was isolated and purified by the guani-

* Corresponding author.

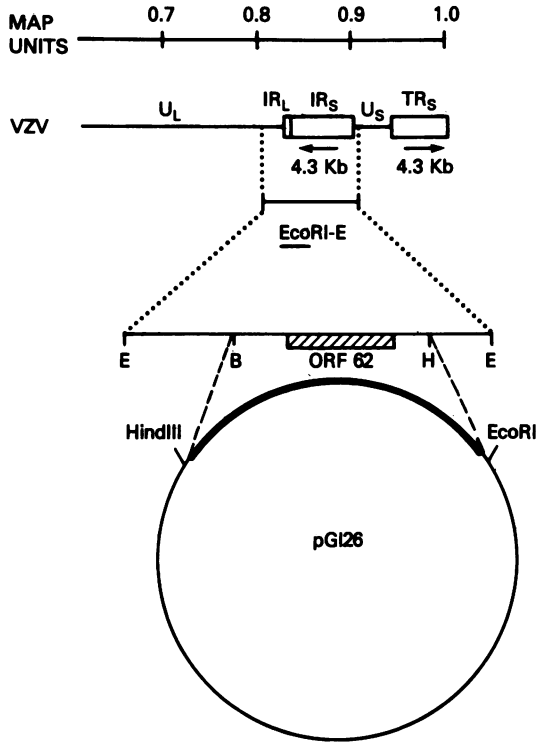

FIG. 1. The map position of ORF62 is shown in the right-hand portion of the VZV genome. Plasmid pGI26 contains the indicated *Bgl*II-*Hpa*I 6.5-kb fragment cloned into pUC9. Transcript mapping data has localized a 4.3-kb transcript to ORF62 as shown previously (26). The locations of restriction sites for enzymes *Eco*RI (E), *Bgl*II (B), and *Hpa*I (H) are based on published sequence data (11). U_L and U_S, Unique long and short segments of the virus; IR_S and TR_S, internal and terminal repeats of the short segment; IR_L, internal repeat of the long segment.

dinium thiocyanate-cesium chloride procedure and hybridized as previously described (7, 17, 26).

Complementation of *ts*B21 by cloned DNA fragments. Complementation of *ts*B21 by protein expressed transiently from transfected DNA fragments was carried out by using a modification of the procedure of Sacks et al. (27). CV-1 cells were seeded into 60-mm-diameter dishes (6.25×10^5 cells per dish). The next day, the cells were transfected in duplicate with 0.5 μg of plasmid DNA and 10 μg of carrier DNA (salmon testes) in 0.5 ml of HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-buffered saline. Four hours later, cells were treated with 15% glycerol in HEPES-buffered saline for 2 min. The cells were infected 24 h later with *ts*B21 at 39°C at a multiplicity of 2 PFU per cell. After incubation for 20 h at 39°C, the cultures were harvested and virus titers were determined by the amount of plaque on Vero cells at 34°C.

Viral complementation studies. Virus titrations were performed on various cell lines as previously described (16). Time course complementation studies were carried out by seeding the indicated cells into 60-mm-diameter dishes in duplicate. The next day, cells were infected with *d*120 at a multiplicity of 2 PFU per cell and were maintained at 37°C. Cultures were harvested at various times after infection. The yield of *d*120 was determined on E5 cells at 37°C.

Biochemical transformation of Vero cells with plasmid DNA. Vero cells were cotransfected with pSV2neo and pGI26, and G418-resistant colonies were selected and grown by using a modification of the technique described by DeLuca et al. (13). Briefly, unlinearized pSV2neo (0.5 μg) and pGI26 (20 μg) were coprecipitated in the presence of 40 μg of salmon testes DNA in a total volume of 1.0 ml by the calcium phosphate procedure of Graham and van der Eb (19). The precipitated DNA was then added to subconfluent monolayers of Vero cells in 60-mm-diameter dishes. After a 4-h incubation at 37°C, the cells were shocked with 15% glycerol and incubated for an additional 2 days at 37°C. The cells were then trypsinized and seeded onto 85-mm-diameter dishes at a density of about 5×10^3 cells per cm^2. After cell attachment, the medium was removed and replaced with medium containing 1 mg of active G418 per ml. After a further 5 to 10 days, the G418 concentration was lowered to 600 μg/ml. At 12 to 18 days after transfection, individual G418-resistant colonies were isolated, amplified, and subsequently maintained in the presence of G418 (600 μg/ml).

Western blot (immunoblot) analysis. Rabbit antisera were prepared against a synthetic peptide corresponding to the C-terminal 12 amino acids predicted from the sequence data for VZV ORF62 (11; P. R. Kinchington, W. Ruyechan, and J. Hay, manuscript in preparation). The peptide was conjugated to keyhole limpet hemocyanin and injected into rabbits, whereupon antisera were derived. A peptide corresponding to the C-terminal 12 amino acids predicted for VZV ORF29 (22a), which has been identified as the VZV major DNA-binding protein by homology to HSV-1 (11), was also synthesized.

A total of 10^6 cells were seeded into 60-mm-diameter dishes in duplicate and infected on the following day with HSV-1 strain KOS at 37°C at a multiplicity of 2 PFU per cell. The infected cultures were harvested 20 h later and the proteins were solubilized in 300 μl of buffer containing 2% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 50 mM Tris hydrochloride, and 5% glycerol (pH 6.7).

The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose in buffer containing 0.25 M Tris, 0.192 M glycine, and 20% methanol. Following transfer, the nitrocellulose was treated with 10% Carnation instant nonfat milk in binding buffer (1 mM EDTA, 10 mM Tris hydrochloride, 0.15 M NaCl, 0.02% Tween 20 [pH 7.6]) for 1 h at 25°C. The blots were then reacted with the anti-peptide sera diluted 1:50 in 1% milk-binding buffer for 2 h at 25°C. Unbound antibodies were removed by five 5-min washes in binding buffer, and the blots were reacted with ^{125}I-labeled staphylococcus protein A (New England Nuclear Corp.) at 10^5 dpm/ml in 1% milk–binding buffer for 2 h at 25°C. Free protein A was removed by extensive washing in binding buffer. Autoradiography was performed on Kodak XAR5 film.

RESULTS

Complementation of *ts*B21 by cloned DNA fragments. Complementation assays were carried out to determine the ability of HSV-1 ICP4 or its putative VZV counterpart to complement *ts*B21 at the nonpermissive temperature of 39°C. Plasmid pSG1, which contains the ICP4 gene of HSV-1, and pVZVEcoE and pGI26, which contain the VZV ORF62, were all found to complement *ts*B21 with complementation indices of 40.4, 11.3, and 13, respectively (Table 1). The only intact VZV ORF contained in pGI26 is ORF62, and this plasmid was therefore used to establish stably transformed cell lines.

Generation of cell lines containing ORF62. pGI26 and pSV2neo were cotransfected into Vero cells. G418-resistant

colonies were isolated and screened for their ability to support the replication of *ts*B21 at 39°C. Three cell lines, termed FI9, FI13, and FI14, efficiently supported the replication of *ts*B21. Twenty hours after infection with *ts*B21 at a multiplicity of 2 PFU per cell, cultures were harvested and their virus yields were compared with those obtained following infection of VN2 cells at 39°C. VN2 cells are a G418-resistant line derived from transfection of Vero cells with pSV2neo alone. Cell lines FI9, FI13, and FI14 yielded, respectively, 26, 39, and 153 times more virus than did infected VN2 cells. The titers of the virus recovered from cell lines FI9, FI13, and FI14 were also determined at 39°C on Vero cells to detect recombinant progeny. Some cell lines cotransformed with the ICP4 gene of HSV-1 have been shown to yield recombinant progeny following infection with *ts*B21 (8). Such cell lines contain an HSV-1 origin of replication. Recombinant screening was performed following our experiments, because a VZV replication origin is contained in pGI26 (34). No increase above background titer was observed, suggesting that the efficient production of recombinant progeny did not occur.

We examined *Eco*RI-digested DNA from the transformed cell lines and Vero cell DNA by Southern blot hybridization analysis by using the gel-purified VZV *Eco*RI E fragment as a probe (Fig. 2). Of four cell lines derived from the initial cotransfection of pSV2neo and pGI26, the three cell lines which complemented *ts*B21 (FI9, FI13, and FI14) were found to contain VZV DNA sequences. The major band noted is 9.2 kb, the size of linearized pGI26. Since intact pGI26 is cut only once with *Eco*RI in the polylinker sequence (Fig. 1), the detection of a single major band suggests integration of the plasmid into the cells as a tandem repeat of pGI26 at one or more sites. The presence of a number of bands smaller and larger than 9.2 kb may reflect integration of VZV sequences into different areas of the cellular genome. The integration pattern is unique for each cell line. One cell line (FI11) which did not complement *ts*B21 was negative for VZV sequences. Two other cell lines (FI6 and FI7) contained VZV sequences but have not been tested for ability to support *ts*B21 replication. VN2 cells were negative for pGI26 sequences, as were Vero cells.

Cell lines containing ORF62 complement ICP4 mutants of HSV-1. Since initial screening demonstrated that three cell lines (FI9, FI13, and FI14) support the replication of *ts*B21, the complementing effect was examined more quantitatively. Plaque formation by *ts*B21 at 39°C was seen on the three cell lines containing ORF62 with titers as shown in Table 2. Plaque formation was also seen on the HSV-1 ICP4-containing E5 cell line. In contrast, *ts*B21 did not form plaques

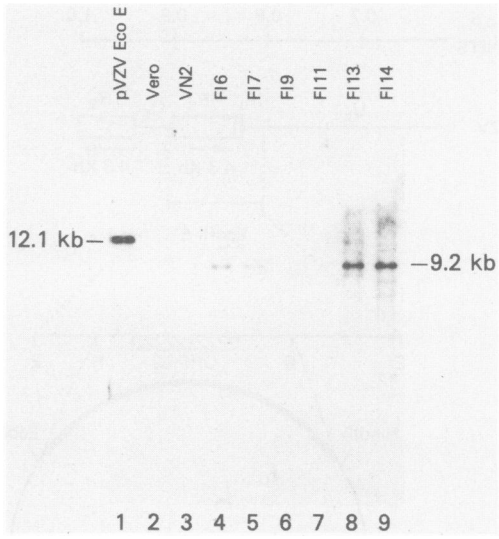

FIG. 2. Southern blot analysis of cellular DNA. Electrophoretically separated *Eco*RI digests (10 μg) of cellular DNAs were transferred to nitrocellulose and probed with a ^{32}P-labeled gel-purified *Eco*RI-E. Lane 1 contains 15 pg of pVZVEcoE plus 10 μg of Vero cell DNA. Lanes 2 (Vero), 3 (VN2), and 7 (FI11) were negative by this analysis. VZV sequences are found in lanes 4 (FI6), 5 (FI7), 8 (FI13), and 9 (FI14). The autoradiograph shown is a 10-day exposure. A single band of >9.2 kb in size was seen with FI9 DNA (lane 6) with a longer exposure.

efficiently on Vero cells under these nonpermissive conditions.

A similar experiment was carried out with *d*120, a mutant of HSV-1 which contains a 4.1-kb deletion in the ICP4 gene (Table 2). At 37°C, *d*120 produced no plaques on Vero or VN2 cells but yielded many plaques on E5, FI14, FI13, and FI9 cells, with E5 cells providing the most efficient support.

Time course studies were conducted to demonstrate the relationship between the replication of the HSV-1 ICP4 mutants and time after infection. Cells were infected with *d*120 at a multiplicity of 2 PFU per cell and maintained at 37°C. Cultures were harvested at the indicated times, and titers were determined on E5 cells at 37°C (Fig. 3). The E5 cell line efficiently complemented this virus, exhibiting fold increases (relative to VN2 cells) of 2.6×10^4 at 24 h and 2.6×10^5 at 48 h, similar to what has been reported earlier (30). Fold increases for FI14 cells were 458 at 24 h and 2.3×10^4 at 48 h. Although the degree of complementation was less for the VZV-derived cell lines than that for E5 cells, significant

TABLE 1. Complementation of *ts*B21 by transfection with cloned DNA fragments[a]

Plasmid	Insert	Virus yield (PFU/ml)	Fold increase
pBR325		4.7×10^2	1.0
pSG1	Contains ICP4 gene of HSV-1	1.9×10^4	40.4
pVZVEcoE	*Eco*RI E fragment of VZV	5.3×10^3	11.3
pGI26	Contains ORF62 of VZV	6.1×10^3	13.0

[a] CV-1 cells were transfected with 0.5 μg of each of the indicated plasmids and 24 h later were infected with *ts*B21 at 39°C at a multiplicity of 2 PFU per cell. After incubation for 20 h at 39°C, cultures were harvested and titers were determined.

TABLE 2. Titer of HSV-1 ICP4 mutants on various cell lines[a]

Cell line	*ts*B21 titer (PFU/ml)	*d*120 titer (PFU/ml)
Vero	2.0×10^2	$<10^1$
E5	1.7×10^8	4.0×10^7
FI14	1.0×10^8	1.2×10^6
FI13	9.8×10^7	4.0×10^5
FI9	3.6×10^7	2.7×10^4
VN2	ND[b]	$<10^1$

[a] Each of the indicated cell lines was seeded in duplicate into 60-mm-diameter dishes and infected with serial dilutions of *ts*B21 or *d*120. Following virus adsorption for 1 h, the cells were overlaid with maintenance medium supplemented with 0.2% human immune serum globulin (6) and incubated for 7 days at 39°C for *ts*B21 or at 37°C for *d*120, after which the cells were fixed and stained with 0.1% crystal violet.

[b] ND, Not determined.

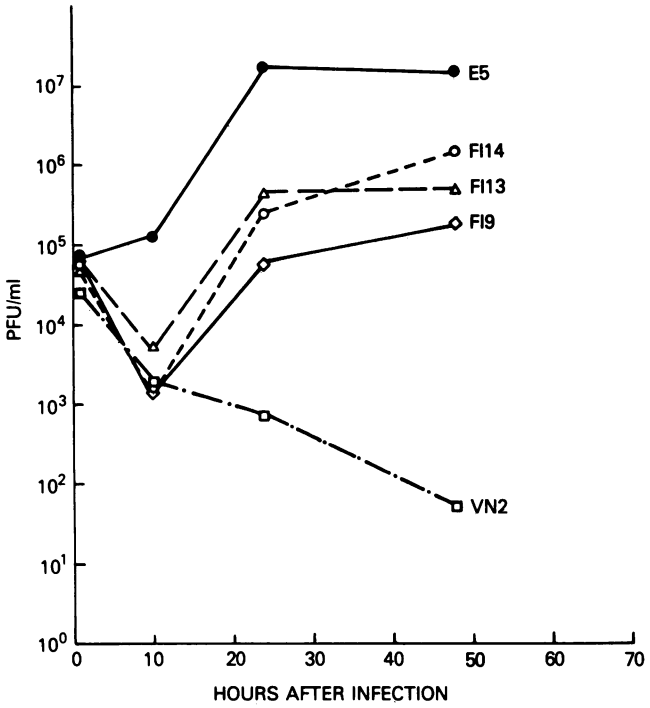

FIG. 3. The growth of *d*120 in cell lines FI9, FI13, and FI14, containing ORF62, and E5 cells compared with its growth in VN2 cells at 37°C over time.

complementation did occur. The VN2 cell line does not contain VZV sequences and served as the nonpermissive line. Complementation of *ts*B21 over time was also studied with lines FI9, FI13, and FI14. FI14 cells produced the greatest degree of complementation, exhibiting fold increases (relative to VN2 cells) of 137 at 24 h, 78 at 48 h, and 292 at 72 h (data not shown). These results indicate that the VZV protein present in these infections is able to complement two different ICP4 mutants of HSV-1.

Progeny viruses recovered from *d*120-infected E5, VN2, and FI14 cells at 24 h were screened for the presence of recombinant progeny. Undiluted culture material was plated on Vero cells at 37°C. No plaques were observed.

Transcription in cells containing ORF62. Transcripts that have been mapped to the short repeat region of VZV include a 4.3-kb RNA corresponding to ORF62 (26). We sought to detect a similar transcript in the transformed cell lines containing ORF62. Total cellular RNA was isolated from FI14, FI13, and FI9 cell lines and Northern (RNA) hybridization analyses were performed by using ^{32}P-labeled pGI26 and pVZVEcoA as probes. VZV transcripts were not detected when 20 μg of RNA were probed under these conditions. However, a 4.3-kb transcript could be demonstrated in FI14 cells following infection with *ts*B21 at 39°C (Fig. 4). The 4.3-kb transcript was present in increased amounts when infection with *ts*B21 was carried out in the presence of 50 μg of cycloheximide per ml. VZV-specific RNA was neither detected in uninfected FI14 cells treated with cycloheximide, nor was it seen in uninfected Vero cells or Vero cells infected with *ts*B21.

Although *ts*B21 does not replicate efficiently in Vero cells at 39°C, immediate-early proteins are synthesized in these cells (12). None of these immediate-early proteins is synthesized in the presence of cycloheximide. Therefore, when *ts*B21 infection occurs in the presence of 50 μg of cycloheximide per ml, the only HSV proteins present in the infected cells are the structural proteins of the infecting virions, suggesting that a virion protein of HSV stimulates the expression of VZV ORF62.

Western blots of cells containing ORF62. Cell lysates from FI14, FI13, and FI9 cells were examined by Western blot analyses and probed with antibody raised in rabbits against a peptide synthesized according to the predicted C-terminal amino acid sequence of ORF62. In the absence of infection, the cell lines contained no detectable VZV polypeptides. However, infection with HSV-1 strain KOS (Fig. 5A) or *ts*B21 (data not shown) led to production of proteins reactive with the ORF62 antibody. The predominant reactive moiety detected in the infected cells was 175 kDa in size. This protein was not seen in KOS-infected or uninfected VN2 cells but was seen in lysates from VZV-infected HFF cells. When ORF62 antibody was preincubated with the ORF62 synthetic peptide (Fig. 5B), the 175-kDa protein was not detected, indicating that the Western blot is specific for the gene product of ORF62. We call this product VZV "IE"175 because of its apparent molecular weight and functional homology to HSV immediate-early gene ICP4. As in the Northern hybridization analyses, we were unable to detect VZV protein in the absence of HSV infection, again suggesting that a component of the HSV virion activates the expression of VZV "IE"175.

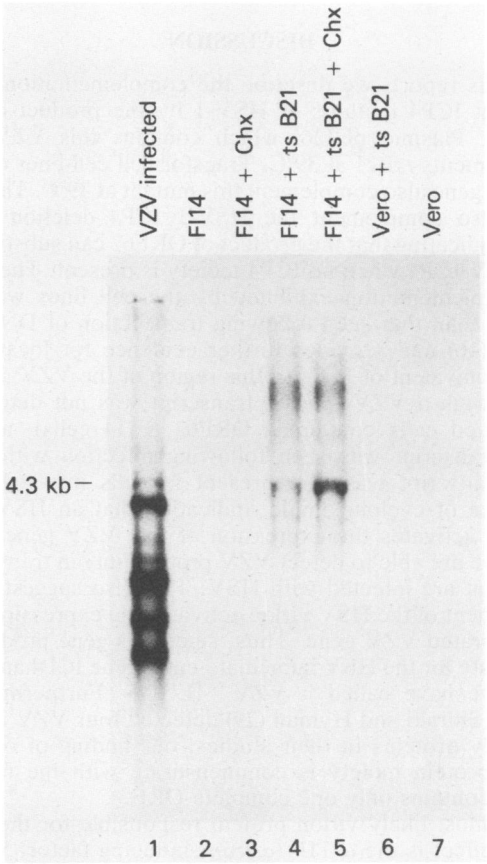

FIG. 4. Northern blot analyses of total cellular RNA. Each lane contains 2 μg of cellular RNA. The blot was hybridized with ^{32}P-labeled pVZVEcoA. Chx indicates that cycloheximide was added to the culture. An 18-h autoradiographic exposure is shown.

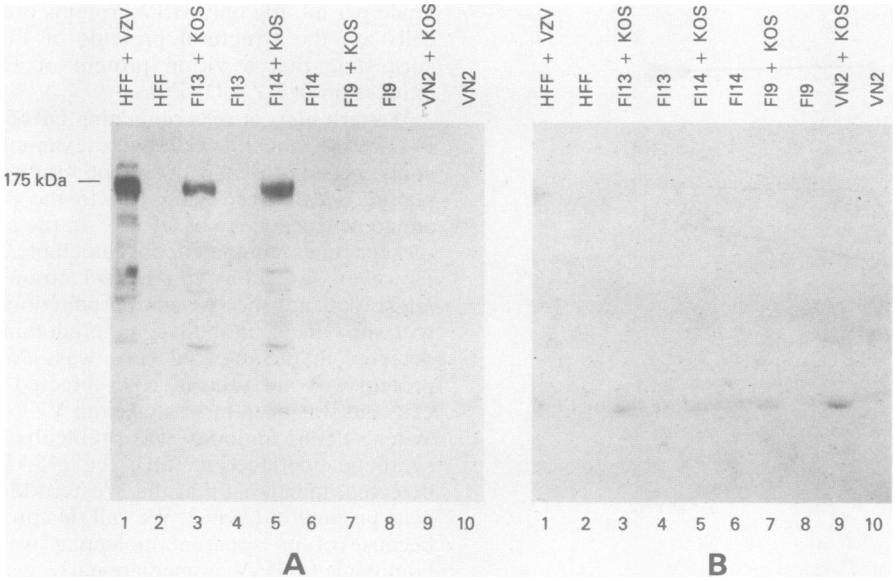

FIG. 5. Western blot analyses of cell lysates. The cell lysates in panels A and B were reacted with antibody to a synthetic ORF62 peptide. The antibody used for panel A was preincubated with 100 μg of synthetic ORF29 peptide per ml, and that used for panel B was preincubated with 100 μg of synthetic ORF62 peptide per ml. In both panels, the lanes contain the following cell lysates: lane 1, VZV-infected HFF cells; lane 2, mock-infected HFFs; lane 3, KOS-infected FI13 cells; lane 4, mock-infected FI13 cells; lanes 5 and 6, KOS-infected and mock-infected FI14 cells, respectively; lanes 7 and 8, KOS-infected and mock-infected FI9 cells, respectively; lanes 9 and 10, KOS-infected and mock-infected VN2 cells, respectively. A 2-day exposure is shown. A 175-kDa moiety is seen in lysates of KOS-infected FI9 cells on a 7-day exposure.

DISCUSSION

In this report, we describe the complementation of two different ICP4 mutants of HSV-1 by the product of VZV ORF62. Plasmid pGI26, which contains this VZV gene, complements *ts*B21 at 39°C. Transformed cell lines containing this gene also complement this mutant at 39°C. These cell lines also complement the HSV-1 ICP4 deletion mutant *d*120, indicating that the product of ORF62 can substitute for the HSV ICP4 when no ICP4 moiety is present. The degree of complementation exhibited by the cell lines was even greater than that seen following transfection of DNA fragments (16) and provides further evidence for locating the VZV equivalent of ICP4 to this region of the VZV genome.

Although a VZV-specific transcript was not detected in uninfected cells containing ORF62 (FI14 cells), a 4.3-kb VZV transcript was seen following infection with *ts*B21. This transcript was also present in cells infected in the presence of cycloheximide, indicating that an HSV virion protein activates the expression of this VZV gene. Moreover, we are able to detect VZV protein only in transformed cells that are infected with HSV. This also suggests that a component of the HSV virion activates the expression of the incorporated VZV gene. Thus, since this gene product can substitute for the HSV immediate-early gene ICP4 and is 175 kDa, we have called it VZV "IE"175. Furthermore, although Shiraki and Hyman (29) detected four VZV immediate-early proteins in their studies, our finding of only one major protein moiety is commensurate with the fact that pGI26 contains only one complete ORF.

The most likely virion protein responsible for the stimulatory effect is the α-TIF (α-*trans*-inducing factor, Vmw65) which has been localized to the HSV tegument and which is known to induce, in *trans*, the expression of HSV α genes, including ICP4 (1, 4). Two upstream DNA elements are thought to be involved in the response of the HSV-1 ICP4 to α-TIF (3). One of these is the TAATGARATTC consensus sequence (R = purine) which, if present, is sufficient for transcriptional activation (3, 23, 37). A second is a GA-rich motif, GCGGAAG, which is thought to activate TAATGARATTC-like motifs with a poor consensus (3).

We analyzed the DNA sequence upstream of VZV ORF62, assigning the AUG of this ORF a value of +1. Scanning 1,000 bases upstream of the AUG, we noted a

TABLE 3. Regulatory elements upstream from the predicted VZV ORF62

Regulatory element	Position[a]	VZV sequence	Orientation[b]
TATA box	−374 to −386	TATATTATATAT	+
CCATT box	−396 to −400	CCAAT	+
	−468 to −472	CAAAT	−
	−521 to −526	GCAATC	−
GA box	−514 to −522	GCGCGAACG	+
TAATGARATTC	−586 to −597	TTATGGGTTAT	+
	−778 to −787	TTATGGTTAT	+
Sp1 binding sites	−568 to −573	GGCGGC	−
	−630 to −635	GGCGGG	+
	−647 to −652	GGCGCG	−
	−657 to −662	GGCGGG	+
	−674 to −679	GGCGCG	−
	−684 to −689	GGCGGG	+
	−701 to −706	GGCGCG	−
	−711 to −716	GGCGGG	+
	−728 to −733	GGCGCG	−
	−738 to −743	GGCGGG	+
	−755 to −760	GGCGCG	−
	−765 to −770	GGCGGG	+

[a] Relative to the ORF62 AUG, which is assigned a position of +1.

[b] The orientation (+ or −) is given with reference to the 5′ to 3′ direction of the associated mRNA. R represents a purine. Sources of consensus elements are given in text.

number of regulatory elements, including sequences sharing homology to both TAATGARATTC and GA-box motifs (Table 3). A candidate TATA box is also shown in Table 3, as are three possible CCAAT boxes (15). Several putative binding sites for Sp1 (22, 25) were also found. Although the 5′ terminus of the transcript associated with ORF62 has not yet been mapped, it is reasonable to place its location within this region. Our analysis therefore indicates that there are regulatory elements upstream of ORF62 that are similar to those found upstream of the HSV ICP4 gene and which could respond to α-TIF-like activities. Two genes adjacent to the HSV-1 α-TIF (ORFA and ORFB) modulate its activity in cotransfection assays (24). Recent data suggest that VZV may encode a related protein, in that its ORF10 shares significant predicted amino acid homology with α-TIF (11, 24). Furthermore, VZV ORF11 and ORF12 adjacent to ORF10 share amino acid homology and identical ordering along the genome with HSV ORFA and ORFB (11, 24).

There have recently been other reports in which cell lines that constitutively contain immediate-early genes have been utilized in studies of regulation. Boom et al. (2) described preparation of a rat cell line containing the major immediate-early gene of human cytomegalovirus. They, too, were unable to find a transcript corresponding to this gene by Northern blotting. However, treatment of their cells with cycloheximide for various amounts of time led to expression of mRNA of the expected size. The interpretation of Boom et al. (2) was that cycloheximide treatment removes the negative autoregulatory effect of the human cytomegalovirus major immediate-early protein by preventing its synthesis. The data of Stenberg and Stinksi (33) also indicate that this protein has an autoregulatory role. In our studies, a transcript was not seen in FI14 cells in the presence of cycloheximide; however, infection of these cells in the presence of cycloheximide led to an increase in the amount of transcript compared with infection in the absence of drug. One explanation for this is that VZV "IE"175 inhibits its own transcription as well.

Smith and Schaffer (30) describe the use of G418-resistant cells containing the ICP4 gene of HSV-2 to complement *d*120. Additionally, E5 cells (containing the ICP4 of HSV-1) were able to complement host-range mutants of HSV type 2, thus showing that ICP4 products encoded by HSV-1 and HSV type 2 are functionally interchangeable during productive infection. In comparison, our cell lines which express VZV "IE"175 are also able to complement *d*120 and *ts*B21. It is of note that our three cell lines complement these mutants to different degrees and that these results parallel the relative amounts of the 175-kDa protein produced on infection with HSV. One explanation for these results is that the three lines differ in the percentage of cells that contain ORF62 or in the percentage of cells competent for infection with HSV. An alternative hypothesis is that all cells contain ORF62 and are infection competent but their ability to make VZV protein differs. The latter theory would imply that VZV "IE"175 is required in stoichiometric, rather than catalytic, amounts for replication to occur.

In summary, we offer evidence that the gene for VZV "IE"175 is responsive to a virion protein of HSV-1, presumably α-TIF. Sequence elements appropriate to such an interaction are present upstream of the gene. VZV sequence data also indicate the presence of homologs of HSV-1 α-TIF (ORF10) and its modulators (ORF11 and ORF12) (11, 24). This is in contrast to the situation for pseudorabies virus, another α-herpesvirus; its major immediate-early gene is α-TIF responsive, and the upstream sequence of this gene contains elements of the TAATGARATTC motif; however, pseudorabies virus itself has been reported to not have an α-TIF homolog (5). We also provide evidence that VZV "IE"175 inhibits its own transcription, an additional property that it shares with its HSV analog ICP4.

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