JOURNAL OF VIROLOGY, Aug. 1993, p. 4474–4483
0022-538X/93/084474-10$02.00/0

Vol. 67, No. 8

A Major Transactivator of Varicella-Zoster Virus, the Immediate-Early Protein IE62, Contains a Potent N-Terminal Activation Domain

L. P. PERERA,[1]* J. D. MOSCA,[2] W. T. RUYECHAN,[3] G. S. HAYWARD,[4] S. E. STRAUS,[1] AND J. HAY[3]

Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892[1]; Retrovirus Research Laboratory, Henry M. Jackson Foundation, Rockville, Maryland 20850[2]; Department of Microbiology, School of Medicine, State University of New York, Buffalo, New York 14214[3]; and Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205[4]

Received 31 March 1993/Accepted 28 April 1993

Accumulating evidence indicates that the product of the putative immediate-early gene ORF62 (IE62) activates varicella-zoster virus (VZV) genes thought to represent all three kinetic classes, namely, immediate-early (α), early (β), and late (γ) classes, of VZV genes as well as a variety heterologous gene promoters. However, the mechanism(s) by which IE62 protein mediates transactivation of these diverse VZV and heterologous gene promoters remains to be elucidated. In this study, by using yeast GAL4 protein chimeras, the coding regions of VZV ORF62 possessing activation domains have been assessed. We demonstrate that the VZV IE62 protein contains a potent activation domain in the N-terminal portion of the molecule, encoded within the first 86 codons of ORF62. The predicted secondary structure profile and the acid-base composition of this IE62 domain resemble those of other transregulatory proteins whose activation is mediated through acidic, hydrophobic elements. In addition, we show that deletion of this activation domain from the 1,310-residue native IE62 protein results in ablation of the transactivator function of IE62. We also present evidence that the mutant IE62 protein lacking the activation domain, though devoid of transactivation ability, was still capable of interfering with the activation of target promoters by the native, full-length IE62.

Varicella-zoster virus (VZV) is a neurotropic alphaherpesvirus and is the etiological agent of chickenpox and zoster in humans. The other members of the alphaherpesvirus group include herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), pseudorabies virus (PRV), and equine herpesvirus 1 (EHV-1). The alphaherpesviruses are enveloped viruses with relatively large double-stranded DNA genomes, and the complete genomic nucleotide sequences of HSV-1, VZV, and EHV-1 are currently available (10, 36, 37, 58). Accumulating evidence indicates that the alphaherpesviruses are essentially colinear with respect to gene distribution and genetic organization. However, much of the current understanding of the regulation of gene expression in alphaherpesviruses has been derived from HSV-1, the most intensively studied prototype member of the alphaherpesvirus group. In HSV-1, gene expression is temporally regulated and the genes are classified as immediate early (IE or α), early (E or β), or late (L or γ), depending on the kinetics of expression and the response to inhibitors of macromolecular synthesis (1, 8, 9, 23, 24, 25, 53). The first set of genes to be expressed are the five IE or α genes, namely, α0, α4, α22, α27, and α47. These five genes are transcribed in the absence of de novo protein synthesis.

The transcription of the HSV α genes is induced by the α *trans*-inducing factor, also referred to as VP16, ICP25, and Vmw65, encoded by the virus (5, 42). This potent 64,000-Da transactivator protein is packaged within the tegument structure of the virion (2). The selective induction of transcription by the α *trans*-inducing factor is dependent upon reiterated elements in the promoter/regulatory domains of α genes (31, 38, 41) which contain a homolog of the octamer element (GYATGnTAATGArATTCyTTGnGGG; the TAATGArAT site). Although at least three of the α gene products are important transactivators that are required for maximal expression of early (β) and late (γ) genes, it now seems clear that ICP4 (IE175), the gene product of the α4 gene, plays an essential central role in the temporal expression of HSV genes during the infectious cycle of the virus (11, 14, 48, 49, 59).

Homologs of the HSV ICP4 gene have been identified in other alphaherpesviruses on the basis of DNA sequence homology and genetic topology; these include the genes that encode the IE62 protein of VZV, the IE1 protein of EHV-1, and the IE180 protein of PRV. IE62 of VZV is a relatively large protein containing 1,310 amino acid residues with a predicted molecular mass of 140 kDa. However, the actual size of the protein appears to be approximately 175 kDa, as determined by polyacrylamide gel electrophoresis (15, 57). It is a potent transactivator capable of transactivating both VZV genes and some heterologous genes (26, 43, 44) and can be found in both the cytoplasm and the nucleus of VZV-infected cells. In addition, the presence of IE62 in the virion tegument in relatively large quantities has been recently demonstrated (30). Although at present there is no definitive proof for an essential role for IE62 in VZV biology, its ability to regulate the expression of VZV genes of all three putative kinetic classes as well as its functional similarity with ICP4 of HSV certainly argues for an important role in the VZV life cycle (13, 16, 26, 43).

The mechanism by which IE62 mediates transactivation of VZV genes and other heterologous genes remains to be

* Corresponding author.

elucidated. However, many regulatory proteins that control transcriptional initiation by RNA polymerase II usually have two domains; one binds to DNA, and the other activates transcription (4, 27, 39). A well-characterized example of this class of activators is the yeast transcriptional activator GAL4, which activates transcription of genes required for catabolism of galactose and melibiose (28). The DNA sequences recognized by GAL4 are 17 bp in length, and each site binds a dimer of the protein. Four such sites are found in the upstream activating sequence of the *GAL* operon (19). The part of GAL4 required for DNA binding is separable from the three discrete regions that activate transcription. This fragment, GAL4(1–147), which bears the N-terminal 147 amino acids, binds to DNA but does not activate transcription (6, 33).

The yeast GAL4 transactivator has been successfully used in domain swap assays to define functional domains of transactivator proteins. Fusion of the GAL4(1–147) fragment with sequences derived from other transactivator proteins (HSV VP16, adenovirus E1A protein, IE2 protein of human cytomegalovirus [HCMV], IE180 protein of PRV, etc. [32, 35, 47, 54]) has allowed the identification of subdomains implicated in transcriptional activation. Using this system, we have identified a potent activation domain in the VZV IE62 protein and have localized this domain to the N terminus of the molecule. The validity of the activation domain identified by the GAL4 chimeras was then confirmed by deleting the putative activation domain from the IE62 protein, which resulted in a mutant polypeptide displaying a dominant-negative phenotype lacking any transactivator capacity.

MATERIALS AND METHODS

Cell lines. A CD4-positive continuous human T-cell line, A3.01 (17), was obtained for the AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases. A3.01 cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) and 2 mM L-glutamine. The CHO-K1 cell line (52) was obtained from the American Type Culture Collection and was grown in Opti-MEM (GIBCO BRL) supplemented with 5% FCS. The human melanoma cell line MeWo was obtained from C. Gross, University of Iowa, and was grown in Opti-MEM supplemented with 5% FCS.

Plasmid constructions. Recombinant plasmids were constructed by standard procedures (56). Plasmids E1bCAT, 1GE1bCAT, 5GE1bCAT, p4CAT, pGR212, and pSV2CAT have been described previously (32, 41, 43). Briefly, E1bCAT contains the minimal promoter of the adenovirus E1b gene fused to the coding region of chloramphenicol acetyltransferase (CAT) gene. In the 1GE1bCAT construct, a 17-bp element which mediates binding of the yeast GAL4 DNA-binding domain, GAL4(1–147), is positioned 5′ to the minimal promoter in plasmid E1bCAT. 5GE1bCAT contains five tandem copies of the GAL 4(1–147)-binding element positioned 5′ to the minimal promoter in plasmid E1bCAT. In p4CAT, the CAT gene is driven from the promoter/upstream elements of the VZV ORF4 gene. Plasmid pGR212 contains the HSV VP16 gene driven from its cognate promoter. Plasmid pGH251 is a modified version of pSG424 (55) bearing a simian virus 40 (SV40) promoter-driven GAL4(1–147) with a unique *Bgl*II site for cloning.

Three different GAL4 fusion vectors in a phagemid backbone were created, each with a specific reading frame overhang when digested with *Bgl*II to facilitate subsequent site-directed mutagenesis, as follows. Plasmid pGH251 was digested with *Xba*I and *Pvu*II. A 750-bp DNA fragment containing the SV40 promoter-driven GAL4(1–147) cassette with a short spacer sequence containing a unique *Bgl*II site was isolated and cloned into the pBS(−) (Stratagene) phagemid vector digested with *Sma*I and *Xba*I. The spacer sequence, 5′(TCG)CCGGAATTCCCGAAGATCT3′, consisted of 19 nucleotides; codon 147 of the GAL4 DNA-binding domain is in parentheses, and the unique *Bgl*II site is underlined. The resultant construct was designated pBS251. Next, pBS251 was cut with *Sal*I (blunted by filling in with Klenow enzyme) and *Xba*I. A 750-bp SV40 poly(A) region was isolated as an *Xba*I-*Sma*I fragment from pJM18 (40) and cloned into *Sal*I-*Xba*I-digested pBS251. The resultant construct, pBSGAL1-147, was then linearized with *Xba*I. A double-stranded oligonucleotide cartridge containing TAA ochre terminators in all three reading frames with *Xba*I overhangs (Table 1) was then cloned into *Xba*I-linearized pBSGAL1-147. The resultant plasmid was designated pBSGAL4-2. From pBSGAL4-2, single-stranded DNA (ssDNA) corresponding to the sense strand of the GAL4 sequence was induced by published procedures (18).

By oligonucleotide-directed site-specific mutagenesis using synthetic oligonucleotides corresponding to the noncoding strand of the GAL4 sequence (Table 1), either one or two A residues were removed from the spacer sequence between codon 147 of GAL4 and the unique *Bgl*II site in the pBSGAL4-2 fusion vector. The resultant constructs were designated pBSGAL4-0 and pBSGAL4-1, respectively.

Plasmid pGi26, which contains the entire coding region of the VZV IE62 gene, has been reported previously (26). This plasmid was digested with *Bam*HI, and a 2.8-kb fragment was isolated by agarose gel electrophoresis and cloned into pBSGAL4-0 that had been digested with *Bgl*II. The resultant construct, p62GAL734-1310, contains the coding region of VZV ORF62 from codon 734 to 1310 cloned in frame behind the GAL4 DNA-binding domain (Table 2). Plasmid p62GAL734-1310R contains the identical fragment in reverse orientation.

To create p62GAL417-866, plasmid pGi26 was digested with *Nru*I (subsequently blunted with mung bean nuclease) and *Eco*RV. A 1.4-kb fragment was isolated by agarose gel electrophoresis and cloned into the pBSGAL4-1 fusion vector that had been digested with *Bgl*II. The resultant construct, p62GAL417-866, contains the coding region of VZV ORF62 from codons 417 to 866 cloned in frame behind the GAL4 DNA-binding domain.

To create p62GAL9-410, plasmid pGi26 was digested with *Sca*I and *Eco*RV, and a 1.3-kb fragment was isolated by agarose gel electrophoresis. This fragment was then cloned into the pBS(−) phagemid vector, from which the polylinker region had been removed by cutting with *Hin*dIII and *Eco*RI followed by mung bean nuclease treatment to blunt the cut ends. This construct was designated pBS1-415; ssDNA corresponding to the sense strand of ORF62 was induced from pBS1-415 to create a *Bgl*II site at codon 8 (the ATG being codon 1) and a *Bam*HI site at codon 411 simultaneously by oligonucleotide-directed site-specific mutagenesis. The sequences of the oligonucleotides used for the mutagenesis are given in Table 1. The modified pBS1-415 containing the novel *Bgl*II and *Bam*HI sites was digested with *Bgl*II and *Bam*HI, and a 1.3-kb fragment was isolated. This fragment was then cloned into the pBSGAL4-1 fusion vector that had been digested with *Bgl*II. The resultant construct, p62GAL9-410, contains the coding region of VZV ORF62 from codons 9 to 410 cloned in frame behind the

TABLE 1. Sequences of the synthetic oligonucleotides used

Oligonucleotide sequence	Corresponding strand	Purpose
5'CTAGTTAAGTTAACTTAA3'		Triple-terminator cartridge with *Xba*I overhangs
5'CCCCGTAGATCTTCGGGTTCCGGCGATACAGTC3'	Minus strand of GAL4	To create pBSGAL4-2
5'CCCCGTAGATCTTCGGGATTCCGGCGATACAGTC3'	Minus strand of GAL4	To create pBSGAL4-1
5'GCGTTGGGGTGTAGATCTCTGCATCGGCGGCG3'	Minus strand of ORF62	To create *Bgl*II site at codon 8 in pBS1-415
5'GATATCCCGGTTGGATCCTTTGTTTCGTCGACCCACC3'	Minus strand of ORF62	To create *Bam*HI site at codon 411 in pBS1-415
5'CCAAATAGTAGATCTTCAGGCTGACTCG3'	Minus strand of ORF62	To create *Bgl*II site at codon 46 in pBS1-415
5'GCCGTTGAGTTTTCTGGAGATCTACTCCGTCCCCCGATGC3'	Minus strand of ORF62	To create *Bgl*II site at codon 233 in pBS1-415
5'CCTGGTGAATTTCCTTACGTCGTTTGTCGGGG3'	Minus strand of ORF62	To create terminator codon 193 in p62GAL9-232
5'CCTCTGCCCGTTTACCCATCGTTCATC3'	Minus strand of ORF62	To create terminator codon 150 in p62GAL9-232
5'CGTAGTCGTCTTATGTTAGCTCTTCGC3'	Minus strand of ORF62	To create terminator codon 121 in p62GAL9-232
5'CGGGTGTGGAAGACCTAGGCCAGCGGGATTAC3'	Minus strand of ORF62	To create terminator codon 108 in p62GAL9-232
5'CGTAGTCGTCTTATGTTAGCTCTTCGC3'	Minus strand of ORF62	To create terminator codon 87 in p62GAL9-232
5'GCATCGGGGGACGGAGTAGATCTCCAGAAAACTCAACGGC3'	Positive strand of ORF62	To create *Bgl*II site at codon 233 in pBS227-733

GAL4 DNA-binding domain. p62GAL9-410R contains the identical fragment in reverse orientation.

To construct 62GAL47-410, a novel *Bgl*II site at codon 46 and a novel *Bam*HI site at codon 411 were introduced into pBS1-415 by oligonucleotide-directed site-specific mutagenesis as described above. The modified pBS1-415 was then cut with *Bgl*II and *Bam*HI to isolate a 1.1-kb fragment. This 1.1-kb fragment was then cloned into the pBSGAL4-0 fusion vector that had been linearized with *Bgl*II. The resultant construct, p62GAL47-410, contains the coding region of VZV ORF62 from codons 47 to 410 cloned in frame behind the GAL4 DNA-binding domain. p62GAL47-410R contains the identical fragment in reverse orientation.

To construct p62GAL9-232, two novel *Bgl*II sites were created at codons 8 and 233 in pBS1-415 simultaneously by oligonucleotide-directed site-specific mutagenesis. The modified pBS1-415 with two *Bgl*II sites was then digested with *Bgl*II, and a 700-bp fragment was isolated by agarose gel electrophoresis. The 700-bp fragment was then cloned into the pBSGAL4-1 fusion vector that had been digested with *Bgl*II. The resultant construct, p62GAL9-232, contains the coding region of ORF62 from codons 9 to 232 cloned in frame behind the GAL4 DNA-binding domain. Plasmid p62GAL9-232R contains the identical fragment in reverse orientation.

To create p62GAL9-192, ssDNA was induced from p62GAL9-232, and an ochre stop codon was introduced at codon 193 by altering the AGA codon to TAA by site-specific mutagenesis. Similarly, plasmid p62GAL9-149 was created by altering codon 150 from GAA to TAA. Plasmids p62GAL9-120, p62GAL9-107, and p62GAL9-86 were created by changing codons 121, 108, and 87, respectively, to either ochre (codons 121 and 87) or amber (codon 108) terminator codons by site-specific mutagenesis.

To create p62GAL 234-734, plasmid pGi26 was digested with *Sph*I and *Bam*HI. A 1.5-kb fragment was then isolated by agarose gel electrophoresis and cloned into pBS(−) that had been digested with *Sph*I and *Bam*HI, resulting in pBS227-733. From pBS227-733, ssDNA corresponding to the noncoding strand of ORF62 was induced to create a novel *Bgl*II site at codon 233 by oligonucleotide-directed site-specific mutagenesis. The modified pBS227-733 was then digested with *Bgl*II and *Bam*HI. A 1.5-kb fragment was isolated by agarose gel electrophoresis and cloned into the pBSGAL4-0 fusion vector digested with *Bgl*II. The resultant construct, p62GAL234-734, contains the coding region of ORF62 from codons 234 to 734 cloned in frame behind the

TABLE 2. GAL4 chimeras evaluated

Chimeric fusion construct	Coding segment of VZV ORF62	Fold induction[a]
p62GAL734-1310	From codons 734 to 1310	NI
p62GAL734-1310R	From codons 734 to 1310 in reverse	NI
p62GAL234-734	From codons 234 to 734	NI
p62GAL234-734R	From codons 234 to 734 in reverse	NI
p62GAL417-866	From codons 417 to 866	NI
p62GAL9-410	From codons 9 to 410	100
p62GAL9-410R	From codons 9 to 410 in reverse	NI
p62GAL9-232	From codons 9 to 232	360
p62GAL9-232R	From codons 9 to 232 in reverse	NI
p62GAL9-192	From codons 9 to 192	440
p62GAL9-149	From codons 9 to 149	510
p62GAL9-120	From codons 9 to 120	765
p62GAL9-107	From codons 9 to 107	1,530
p62GAL9-86	From codons 9 to 86	170
p62GAL47-410	From codons 47 to 410	NI

[a] A3.01 cells were electroporated as described in Materials and Methods. The target (5GE1bCAT) and effector plasmid DNAs were used in 2-μg amounts in cotransfection experiments. Fold induction of CAT activity was calculated relative to the control experiment in which the 5GE1bCAT was transfected with 2 μg of pGEM2 carrier DNA. The value was set equal to 1.0 in the control experiment, and the fold induction data relative to the control experiment are given. NI, no detectable induction above the basal level.

GAL4 DNA-binding domain. Plasmid p62GAL234-734R contains the identical fragment in reverse orientation.

pCMV62, containing the coding region of VZV ORF62 under the control of the strong constitutive HCMV IE1 promoter, has been described previously (43). To generate pCMV62ΔAD, two unique *Bgl*II sites were introduced in the coding region of ORF62 in pCMV62 at codons 8 and 96 by oligonucleotide-directed site-specific mutagenesis. The resultant plasmid, pCMV62-2Bg, was then cut with *Bgl*II. After removal of the 275-bp stuffer fragment by agarose gel electrophoresis, the *Bgl*II-cut ends were blunted by mung bean nuclease treatment prior to religation with T4 DNA polymerase. This plasmid, designated pCMV62ΔAD, lacks the coding region between the codons 7 and 99 of the VZV ORF62 gene. All plasmid constructs generated were sequenced across their manipulated regions to ensure the integrity of the constructions.

DNA sequencing. CsCl density gradient-purified plasmid DNA was sequenced by using the Sequenase sequencing kit (U.S. Biochemical) as published previously (46).

DNA transfections. All DNA transfections in the transient transfection assays were done by electroporation using a Gene-Pulser electroporator (Bio-Rad). For electroporation of A3.01 cells, cells in log-phase growth were resuspended in RPMI 1640 medium with 20% FCS, but without glutamine or antibiotics, at a density of 5×10^7 cells per ml. Aliquots (350 μl) of the cell suspension were mixed with plasmid DNA in sterile 1.5-ml cryovials (Nunc) and incubated on ice for 15 min. The cells were then transferred into an electroporation cuvette with a 0.4-cm electrode gap and were electroporated with a single pulse with the settings at 0.20 V and 960 μF with a capacitance extender. MeWo and CHO-K1 cells were electroporated with the settings at 0.22 V and 960 μF with a capacitance extender. After pulsing, the cells were immediately transferred into either 24-well (for A3.01 cells) or 6-well (for MeWo and CHO-K1 cells) tissue culture plates that had been chilled on ice and incubated for 15 min on ice before addition of the growth medium. The cells were then grown for 48 h before harvesting.

CAT assay. Cells were harvested 48 h after DNA transfection, and CAT assays were performed essentially as described by Gorman et al. (20). Briefly, the cells were washed once with phosphate-buffered saline (PBS), resuspended in 0.25 M Tris-HCl (pH 7.8), and disrupted by three cycles of freeze-thawing. Protein concentration in cell lysates were determined by using the Bio-Rad protein assay kit according to the manufacturer's instructions. CAT activity was assayed by using the same amount of total protein for all samples in an individual experiment. CAT activity was quantitated by using a PhosphorImager scanner with ImageQuant software from Molecular Dynamics Inc. (Sunnyvale, Calif.). All experiments were repeated at least three times with independent DNA transfections.

Immunoblot analysis. Plasmid DNA (25 μg) was transfected into CHO-K1 cells by electroporation as described earlier. After the transfection, cells were plated in 100-mm-diameter tissue culture dishes. The cells were harvested 48 h after transfection and used for immunoblot analysis to detect the proteins expressed from the transfected plasmid DNA as described previously (30), using a 1:50 dilution of the VZV IE62 antipeptide antiserum. The bound antibody was then detected with ^{125}I-labeled protein A. Details of the synthesis and characterization of the VZV IE62 antipeptide antibody have been reported previously (30).

Immunofluorescence. Plasmid DNA (25 μg) was transfected into CHO-K1 cells by electroporation as described above. Following transfection, cells were plated in chamber slides. At 60 h after electroporation, cells were washed with PBS, fixed with 50% methanol–50% acetone (vol/vol) for 2 min at room temperature, and washed again with PBS. Fixed cells were blocked by the addition of 3% (wt/vol) bovine serum albumin (BSA) in PBS for 15 min at room temperature. A 1:100 dilution of the VZV IE62 antipeptide antiserum was then added, and the cells were incubated at 4°C for 1 h in a humidified chamber. The cells were then washed four times for 5 min each time with PBS containing 0.2% (vol/vol) Tween 20. Goat anti-rabbit polyclonal antiserum conjugated to fluorescein isothiocyanate was diluted 1:100 in PBS containing 3% BSA and added to cells for 1 h at 4°C in a humidified chamber, and then the cells were washed as described above and mounted with glycerol containing 50 mM Tris (pH 7.8).

RESULTS

Coarse mapping of the VZV IE62 activation domain. To define the general location of the activation domain of IE62, four overlapping fusion constructs encompassing the entire coding region were made. Each of these four constructs was then cotransfected into A3.01 cells with either of three individual target reporter plasmids: one bearing a single or five promoter-proximal GAL4-binding elements (1GE1b CAT or 5GE1bCAT) or one lacking any GAL4-binding elements in the promoter region (E1bCAT). For each effector plasmid evaluated, a control plasmid in which the identical segment of IE62 in reverse orientation with respect to the GAL4 DNA-binding domain, GAL4(1–147), was included. As shown in Fig. 1, the only effector GAL4 chimeric plasmid capable of activating any of the three target reporter plasmids tested was p62GAL9-410. Although the p62GAL9-410 effector construct was capable of strongly activating 5GE1bCAT, which contains five tandem GAL4-binding elements, it failed to elicit any detectable activation of the 1GE1bCAT target, containing one GAL4-binding element, when 2.5-μg amounts of target and effector DNAs were used in cotransfections in this experiment. However, when p62GAL9-410 was used in increasing amounts in cotransfections with a fixed amount (2.5 μg) of the three targets, a dose-dependent activation of both 1GE1bCAT and 5GE1b CAT was seen (Fig. 2). It should be noted that the CAT assays shown in Fig. 2 were performed with equal amounts of protein in all samples in experiments using three different targets, i.e., 1GE1bCAT, 5GE1bCAT, and E1bCAT. Under such conditions, the level of CAT activity in samples from the experiment in which 5GE1bCAT was used as the target was above the linear range of the assay and necessitated retesting of these samples following dilution for quantitation. It is likely that the enhanced activation seen with 5GE1b CAT, which bears five binding sites for GAL4(1–147), is due to greater accumulation of the activator at the target promoter. The specificity of the system was illustrated by the fact that p62GAL9-410 was unable to activate the E1bCAT target construct lacking any GAL4-binding elements. Thus, the activation domain of VZV IE62 protein is encoded within a segment extending from the codons 9 to 410 of ORF62.

Fine mapping of the activation domain of VZV IE62. To more precisely define the boundaries of the activation domain embedded within the segment extending from codons 9 to 410 of VZV ORF62, a series of deletions from both the 5′ and 3′ ends of this segment was undertaken (Table 2). The construct p62GAL47-410, lacking the first 38 codons from the 5′ end in comparison with p62GAL9-410, was devoid of

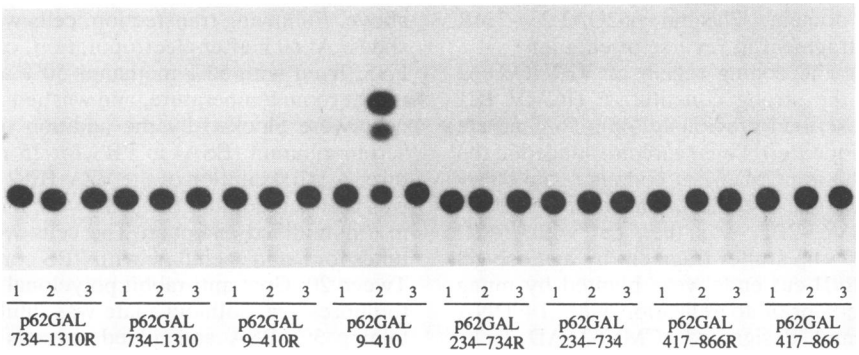

FIG. 1. Coarse mapping of the VZV IE62 activation domain. A panel of GAL4-VZV ORF62 chimeric constructs was cotransfected into A3.01 cells with three different target reporter plasmids, 1GE1bCAT containing one GAL4-binding site (lanes 1), 5GE1bCAT containing five GAL4-binding sites (lanes 2), and E1bCAT lacking any GAL4-binding sites (lanes 3), by electroporation as described in Materials and Methods. The effector chimeric plasmids used are described in Table 2. In cotransfections, equal amounts (2.5 μg) of target and effector DNAs were used. Cells were harvested 48 h after DNA transfection, and the level of CAT activity in the cell lysate was determined by using the same amount of total protein for all samples.

any activity. However, the construct p62GAL9-232, which lacks 178 codons from the 3′ end, was still able to retain potent activator function (Table 2). In addition, when translation terminator codons were introduced at codons 193 (p62GAL9-192), 150 (p62GAL9-149), 121 (p62GAL9-120), 108 (p62GAL9-107), and 87 (p62GAL9-86), there was no loss of activator function (Table 2). On the contrary, there was progressive enhancement of activation potential with successive truncations from the 3′ end. However, the activation potency of p62GAL9-86 was ninefold less than the activation potency of p62GAL9-107. The additional 22 amino acids present in p62GAL9-107 are clearly not critical for the IE62 activation domain. Nonetheless, these additional amino acids could contribute directly to the strength of the IE62 activation domain or, alternatively, could enhance the stability of the chimeric protein. Thus, it appears that the activation domain in IE62 of VZV is encoded by a segment of ORF62 between codons 9 and 86.

To verify the functional validity of the putative IE62 activation domain identified by the GAL4 chimeras, we created a deletion mutant of IE62 devoid of the putative activation domain. The mutant plasmid, pCMV62ΔAD, contains a 94-amino-acid deletion extending from codons 7 through 99 of the coding region of ORF62. As shown in Fig. 3, following transfection of pCMV62 and pCMV62ΔAD DNA into CHO-K1 cells, comparable amounts of mutant and wild-type (full-length IE62) proteins were detected by immunoblot analysis using an antipeptide IE62 antibody that reacts with the C terminus of the IE62 protein, thus demonstrating the expression of IE62 proteins from both plasmids pCMV62 and pCMV62ΔAD. Next, we assessed the ability of the mutant IE62 lacking the putative activation domain to transactivate IE62-responsive promoters, using the VZV ORF4 promoter (p4CAT) as a representative IE62-responsive target in a transient transfection assay. The deletion of putative activation domain resulted in a mutant IE62 lacking any significant transactivator ability within the limits of sensitivity of the assay (Fig. 4). Furthermore, the mutant IE62 lacking the activation domain interfered with the transactivator function of the wild-type IE62 when both wild-type

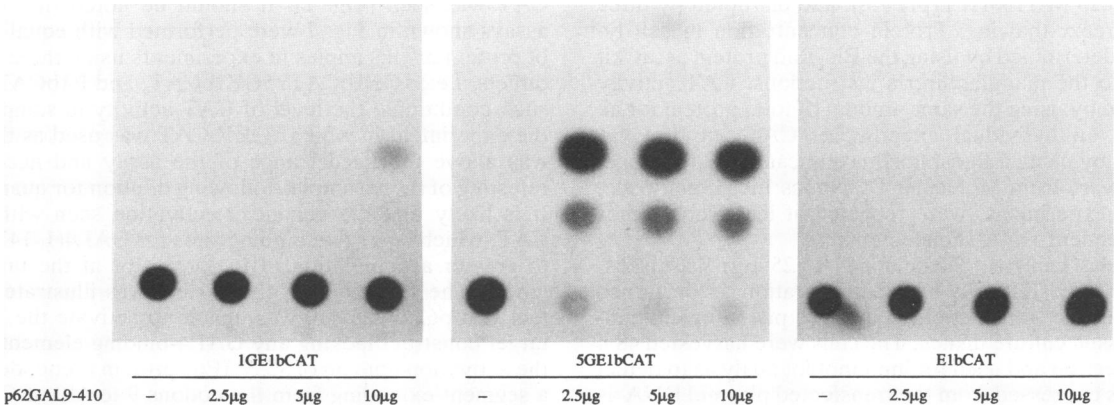

FIG. 2. Dose-dependent activation of the target constructs by the VZV IE62 activation domain. Increasing amounts of p62GAL9-410 DNA were cotransfected with a fixed amount of the target DNA (2.5 μg) into A3.01 cells by electroporation. The cells were harvested 48 h after DNA transfection, and the level of CAT activity was measured. The results represent CAT assays performed with equal amounts of protein in all samples in experiments using three different targets, i.e., 1GE1bCAT, 5GE1bCAT, and E1bCAT. The relative activation levels of 1GE1bCAT target were as follows: no induction (2.5 μg of effector), 2-fold induction (5 μg of effector), and 15-fold induction (10 μg of effector). The relative activation levels of 5GE1bCAT target were as follows: 180-fold induction (2.5 μg of effector), 731-fold induction (5 μg of effector), and 1,400-fold induction (10 μg of effector). No induction of E1bCAT target was seen with 2.5, 5, or 10 μg of effector.

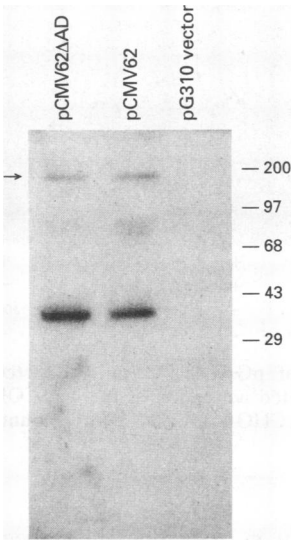

FIG. 3. Expression of wild-type and mutant IE62 from the transfected plasmid DNA. CHO-K1 cells were transfected with 25 μg of plasmid DNA by electroporation. Plasmids pCMV62 and pCMV 62ΔAD express the full-length (wild-type) IE62 and a mutant IE62 devoid of activation domain, respectively, from the strong constitutive HCMV IE1 promoter. The vector plasmid pG310 contains the HCMV IE1 promoter cassette used to generate pCMV62 and pCMV62ΔAD. The cells were harvested 48 h after transfection and solubilized in sample buffer containing 0.1 mM TLCK, 0.1 mM TPCK, and 10 mM phenylmethylsulfonyl fluoride. Equal amounts of crude protein from each sample were used in the immunoblot analysis. The presence of IE62 was detected by using an IE62 antipeptide antibody (30) that reacts with the C terminus of IE62 protein. The bound antibody was then detected with ^{125}I-labeled protein A. The arrow on left indicates the position of undegraded IE62. However, as previously reported (30), a number of smaller species (presumably due to degradation) were also present in both pCMV62- and pCMV62ΔAD-transfected samples. The approximate sizes (in kilodaltons) of marker proteins are shown on the right.

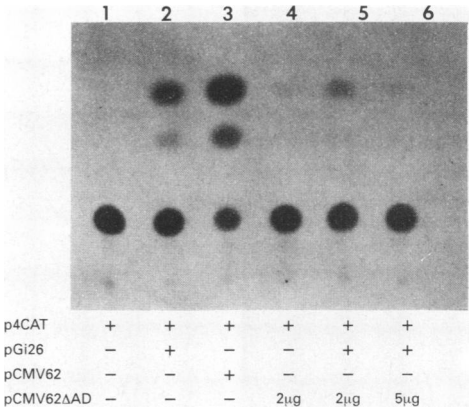

FIG. 4. Ablation of transactivator ability of VZV IE62 upon deletion of the putative activation domain. CHO-K1 cells were transfected with the indicated plasmids or plasmid combinations to assess the effects of deletion of the putative activation domain. Plasmids p4CAT, pGi26, and pCMV62 were used at 2-μg amounts in the cotransfections. The values for percent conversion of [^{14}C]chloramphenicol to acetylated derivatives were 0.4 (lane 1), 20.1 (lane 2), 81.9 (lane 3), 1.2 (lane 4), 4.1 (lane 5), and 1.4 (lane 6).

and mutant IE62 were coexpressed. As shown in Fig. 4, when a fixed amount of pGi26 (cognate promoter-driven ORF62) DNA (2 μg) was cotransfected with increasing amounts of pCMV62ΔAD DNA in transient assays using p4CAT as a target, there was a dose-dependent inhibition of the pGi26-mediated activation of p4CAT by the activation domain-deficient IE62 mutant, thus exhibiting a dominant-negative phenotype. The dominant-negative effect was more pronounced when pGi26 was used than when pCMV62 was used to express IE62 (data not shown), presumably as a result of the presence of mutant IE62 in excess (the mutant IE62 is driven from the HCMV IE-1 promoter) under such conditions since, as shown in Fig. 4, the expression of IE62 from the cognate promoter appears to be less efficient in comparison with the HCMV IE1 promoter. However, the vector plasmid pG310 had no effect on pGi26-mediated activation of p4CAT target (data not shown).

Since it is conceivable that a deletion eliminating the nuclear localization signal of a transactivator protein could render a null phenotype to the mutant protein even though the protein has a fully functional activation domain, we evaluated whether the 94-amino-acid deletion in the mutant IE62 has affected its nuclear localization ability. As shown in Fig. 5, the immunofluorescent profiles for wild-type and mutant IE62 were similar. The abundant localization of both wild-type IE62 and mutant IE62 lacking the activation domain in the nucleus proves that the deletion in pCMV62ΔAD had not eliminated the nuclear localization signal from the mutant IE62 protein.

Since, the activation domains of regulatory proteins are believed to interact with cellular factors in mediating transcriptional activation of genes, we used three different cell types to evaluate whether the functional activity of the IE62 activation domain (p62GAL9-149) was cell type dependent. The A3.01 cell line, representing a human T-lymphocytic background, was chosen for evaluation because of the fact that during the viremic phase of VZV infection, T lymphocytes appear to be important in sustaining the virus growth (reference 43 and references cited therein). Selection of the CHO-K1 cell line was based on the fact that it has been extensively used as a model cell system to evaluate the activity of chimeric GAL4 activators (54). The MeWo cell line was chosen as a line which supports the productive infection of VZV in vitro. Interestingly, the VZV IE62 activation domain demonstrated potent activation of the 5GE1bCAT target in all three cell lines evaluated (data not shown), although activation was 2- to 3-fold higher in the CHO-K1 line than in the other two cell lines (in A3.01, 129-fold; in MeWo, 203-fold; and in CHO-K1, 493-fold). The ability of the IE62 activation domain to function in diverse cell backgrounds thus may reflect the involvement of ubiquitous cellular intermediary factors in transcriptional activation mediated by VZV IE62.

Comparison of VZV IE62 and HSV VP16 activation domains. The predicted acid-base composition of the IE62 activation domain (the region between codons 9 and 86) (Table 3) resembles that of the acidic group of activators. The C-terminal acidic domain of HSV VP16 is one of the most intensively studied potent member of this group of activators currently known (3, 54). Because of the similarity between these two activation domains, we next compared the potency of the VZV IE62 activation domain with that of HSV VP16, using p62GAL9-107 and pGAL4VP16 (54) to express the VZV IE62 and HSV VP16 activation domains, respectively, as GAL4 chimeras. The VZV IE62 activation

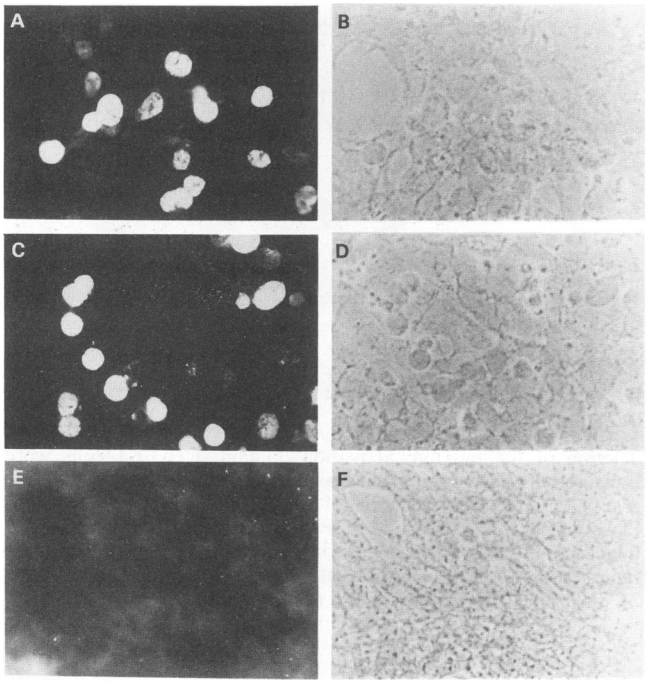

FIG. 5. Fluorescent and bright-field images of the subcellular localization of wild-type and activator domain-deficient IE62 proteins in CHO-K1 cells. Cells were transfected with 25 μg of plasmid DNA as described in Materials and Methods, fixed and permeabilized 60 h after transfection, and subjected to indirect immunofluorescence. The primary antibody was a rabbit IE62 antipeptide antibody that reacts with the C terminus of IE62 protein, and the secondary antibody was fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G. (A and B) Cells transfected with wild-type IE62 (pCMV62); (C and D) cells transfected with activation domain-deficient IE62 (pCMV62ΔAD); (E and F) cells transfected with vector DNA (pG310).

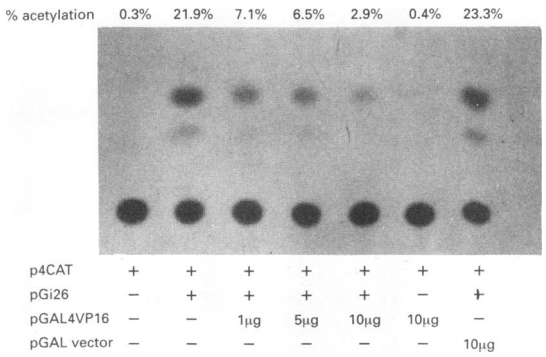

FIG. 6. Effect of pGAL4VP16 on pGi26 (cognate promoter-driven IE62)-mediated activation of the VZV ORF4 promoter. In cotransfections of CHO-K1 cells, 2-μg amounts of p4CAT and pGi26 were used.

domain was sixfold less potent than the VP16 activation domain in activating the target construct 5GE1bCAT (data not shown).

It has been documented that if two activators augment the expression of target genes via a common pathway, for example, through a common adaptor molecule, then the presence of one activator in increasing amounts will exert an inhibitory effect on the activation of a target gene induced by the other activator (3, 29, 33, 50, 51). This phenomenon is commonly referred to as *trans* inhibition or activator interference and is believed to occur by sequestration of an essential common adaptor molecule by the activator present in excess (3, 29). In an attempt to determine whether the VZV IE62 and HSV VP16 activation domains share a common pathway in activating their specific target gene promoters, the following experiments were performed. In one set of experiments, we determined whether coexpression of increasing amounts of GAL4-VP16 chimeric protein had any effect on VZV IE62-mediated activation of a responsive VZV gene promoter, the ORF4 promoter (p4CAT). As expected, plasmid pGi26, containing the cognate promoter-driven IE62, activated the VZV ORF4 gene promoter (Fig. 6). However, the coexpression of increasing amounts of GAL4-VP16 resulted in a dose-dependent inhibition of IE62-mediated activation of the VZV ORF4 promoter. As expected, though, GAL4-VP16 alone had no effect on the basal activity of the ORF4 promoter. Furthermore, the inclusion of GAL4 vector plasmid DNA (pBSGAL4-2) as a control negated the possibility that the inhibition seen with pGAL4VP16 might have been due to titration of a cellular factor by either the SV40 promoter or GAL4(1–147) present in pGAL4VP16 (Fig. 6). However, when pGR212 (native VP16 expression plasmid) was coexpressed with p62GAL9-149, although a similar trend of inhibition of p62GAL9-149-mediated activation of the 5GE1bCAT target was seen, the magnitude of the inhibition was less dramatic (data not shown). In contrast, as shown in Fig. 7, the coexpression of increasing amounts of VZV IE62 activator domain failed to induce any *trans* inhibition of pGR212 (the cognate promoter-driven HSV VP16)-mediated activation of the IE110 (also referred to as ICP0) promoter of HSV-1. In fact, overexpression of the VZV IE62 activation domain (p62GAL9-149) further augmented the HSV VP16-induced activation of the IE110 promoter. It should be noted that p62GAL9-149 alone had no significant effect on the basal activity of the IE110 promoter (Fig. 7). However, the full-length IE62 (expressed from pGi26) was able to activate the IE110 promoter in

TABLE 3. Acidic activation domains of alphaherpesvirus regulatory proteins

Activator	Amino acid sequence[a]	% Acidic residues
VZV IE62	9 STPQRAGSPDTLELMDLLDAAAAAAEHRARVVTSSQPDDLLFGENGVMVGREHEIVSIPSVSGLQPEP RTEDVGEELT 86	21
HSV VP16	400 HTRRLSTAPPTDVSLGDELHLDGEDVAMAHADALDDFDLDMLGDGDSPGPGFTPHDSAPYGALDMADF EFEQMFTDALGIDEYGG 486	24
PRV IE180	1 MADDLFDFIETEGNFSQLLAAAAAAAEEEGIAS 33	24

[a] Acidic amino acids are shown in boldface. Numbers indicate the boundaries of the activation domains in the context of the native proteins.

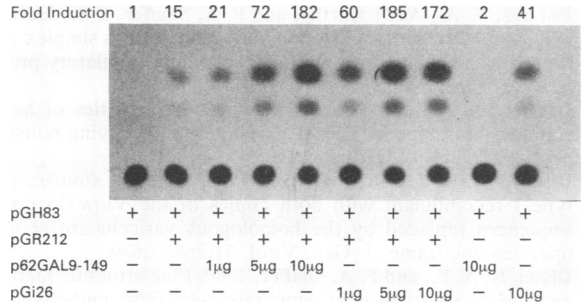

FIG. 7. Effect of p62GAL9-149 on pGR212 (cognate promoter-driven HSV VP16)-mediated activation of the HSV IE110CAT promoter. In cotransfections of CHO-K1 cells, 2-μg amounts of pGH83 (IE110CAT) and pGR212 were used.

addition to being able to enhance the VP16-mediated activation of IE110 promoter. Ideally, a promoter that responds to VP16 but not to IE62 would help to assess the effects of full-length IE62 on the VP16-mediated activation of target promoters. However, the other VP16-responsive HSV IE genes also were induced by IE62 (data not shown), complicating the interpretation of full-length IE62 effects on VP16-mediated activation of target promoters.

DISCUSSION

Many transcriptional activators contain distinct domains dedicated to recognizing specific DNA sequence elements and activation of the transcriptional machinery (4, 27, 39). The DNA-binding domains of activators are classified into four main groups: helix-loop-helix, helix-turn-helix, leucine zipper, and zinc finger (27, 39). Similarly, transcriptional activation domains have been classified according to their structure or composition (27, 39). These include the acidic group (e.g., the HSV VP16 protein and the yeast GCN4 and GAL4 activators), the proline-rich activator group (e.g., AP2 and CTF transcription factors), the glutamine-rich activator group (e.g., Sp1 transcription factor), and the glutamine and proline-rich activator group (e.g., *jun* oncogene).

The exact mechanism by which specific domains mediate transcriptional activation remains to be elucidated; nonetheless, the cumulative evidence suggests two possible models. One such model proposes that the surface of the activation domain contacts a target factor of the basic transcriptional machinery, thereby looping out the intervening DNA. This contact would activate transcription by stabilizing the binding of the target factor to the promoter or by altering the conformation of the bound factor to an active form (4, 50, 51). According to the second model, transactivators function by antagonizing histones which would otherwise constrain the promoter DNA in a repressed chromatin state, thus rendering the promoter region inaccessible to the general cellular transcription factors (22).

The use of a GAL4 protein chimera system offers several advantages in preliminary identification of activator subdomains, especially in large regulatory proteins (for a review, see reference 4) since it allows the analysis of relatively small segments of a complex protein independent of its spatial distribution as determined by the rest of the polypeptide in the native molecule. However, it is crucial to substantiate the functional validity of such subdomains identified by the GAL4 chimeras in the context of the native protein under study.

A major transactivator protein of VZV is the gene product of ORF62. We recently demonstrated that IE62, unlike its HSV counterpart ICP4, not only activates VZV genes of all three putative kinetic classes but also can positively modulate the expression of its own cognate promoter (26, 43, 44). In the present study, we have dissected the coding region of the IE62 gene to define the activation domains of this important VZV regulatory protein and provide evidence to implicate the N-terminal 75 amino acids as a potent activation domain.

It is of interest that p62GAL9-410 was consistently less potent in activating p5GE1bCAT than were constructs bearing smaller versions of the activation domain (Table 2). Similar observations have been made by other investigators who used GAL4 chimeras to define activation domains of regulatory proteins (35, 47). It is conceivable that in the fusion protein encoded by the larger segment of ORF62 in p62GAL9-410, the activation domain is not exposed optimally for interaction with the transcriptional machinery. Another possibility is that the larger fusion proteins were less stable or, alternatively, less efficient in nuclear localization to mediate transcriptional activation.

Although the data from the GAL4-IE62 fusions suggest the presence of a single N-terminal activation domain, one could argue that since the negative GAL4 chimeras were not evaluated for their stability, the other regions of the IE62 molecule may still possess additional activation domains that were not detected in this study because of the instability of such fusion proteins. However, the fact that the mutant IE62 lacking the N-terminal amino acids 7 through 99 was devoid of any activator function strongly suggests that the critical activator elements reside within this segment of the molecule. In addition, the ability of the activation domain-deficient mutant IE62 to exert a *trans* dominant-negative effect when coexpressed with the wild-type IE62 further strengthens the notion that VZV IE62 possesses a critical N-terminal activation domain. However, it should be emphasized that if IE62 contains other activation domains that are dependent on (or augmented by) other VZV-encoded proteins, such domains would not have been detected in the systems used in this study. Although the molecular basis for the dominant-negative phenotype exhibited by the activation domain-deficient IE62 remains to be elucidated, one could speculate that either the formation of functionally crippled, mixed multimers between the wild-type and the mutant IE62 or, alternatively, competition for binding to a critical cellular target or promoter element is responsible for this effect.

Interestingly, the localization of the activation domain of IE62 to a region between amino acids 9 and 86 of the molecule accentuates important differences among the very closely related regulatory proteins in alphaherpesviruses. On the basis of predicted amino acid sequences, the primary structures of VZV IE62, PRV IE180, EHV IE1, and HSV ICP4 have been divided conceptually into five regions that are essentially colinear (7, 10, 21, 37, 58). All four proteins share extensive (approximately 50%) amino acid identity within regions 2 and 4 and have little homology within regions 1, 3, and 5. It has been suggested that the highly conserved region 2 of each of these proteins contains a DNA-binding domain that is essential for regulatory function (60). However, the activation domain of VZV IE62 is contained within a region lacking any significant homology among these proteins. In fact, the N-terminal 147 amino acids of HSV ICP4 have been shown to be functionally nonessential (12). Thus, it appears that these four proteins have evolved functionally similar yet structurally and com-

positionally diverse activation domains for transcriptional regulation.

One curious finding of this study was the ability of the VZV IE62 activation domain (expressed as a GAL4 chimeric protein) to function synergistically with VP16 to upregulate the HSV IE110 promoter bearing three TAATGArAT elements (34). This finding is seemingly in conflict with the observation that the activation of target promoters by VZV IE62 is subjected to *trans* interference when the activation domain of VP16 is coexpressed as a GAL4 chimeric protein. One interpretation consistent with these observations is that both VZV IE62 and HSV VP16 activation domains compete for a common intermediary factor needed for transcriptional activation. Then the question remains as to how one might explain the enhanced VP16-mediated activation of the IE110 promoter by the GAL4 chimeric protein bearing the VZV IE62 activation domain. Since VP16 is the stronger activator, it may have a higher affinity for the intermediary factor(s) essential for transcriptional activation. If that is the case, then overexpression of the VZV IE62 activator domain with an obvious lower affinity for the intermediary factor(s) may not be able to exert any *trans* inhibition. However, if the activation domain of VZV IE62 can also bind other factors, for example, cellular transcriptional repressors, then the coexpression of VZV IE62 may lead to enhanced VP16-mediated activation due to sequestration of the repressor factors. Clearly, more detailed studies are needed to elucidate the functional aspects of the VZV IE62 activation domain that we have identified in this study.

ACKNOWLEDGMENTS

We thank Sumesh Kaushal for assistance with portions of this study.

This work was supported by Public Health Service grants AI18449 (W.T.R. and J.H.; NIAID), AI24489 (J.D.M.; NIAID), and CA28473 (G.S.H.; NCI).

REFERENCES

1. **Alwine, J., W. Stein, and C. Hill.** 1974. Transcription of herpes simplex type I DNA in nuclei isolated from infected HEp-2 and KB cells. Virology **60**:302–307.
2. **Batterson, W., and B. Roizman.** 1983. Characterization of herpes simplex virion-associated factor responsible for the induction of α genes. J. Virol. **46**:371–377.
3. **Berger, S. L., W. D. Cress, A. Cress, S. J. Triezenberg, and L. Guarente.** 1990. Selective inhibition of activated but not basal transcription by the acidic activation domain of VP 16: evidence for transcriptional adaptors. Cell **61**:1199–1208.
4. **Berk, A. J., and M. C. Schmidt.** 1990. How do transcriptional factors work. Gene Dev. **4**:151–155.
5. **Campbell, M. E. E., J. W. Palfreyman, and C. M. Preston.** 1984. Identification of herpes simplex virus DNA sequence which encode a trans-acting polypeptide responsible for stimulation of immediate early transcription. J. Mol. Biol. **180**:1–19.
6. **Carey, M., J. Leatherwood, and M. Ptashne.** 1990. A potent GAL4 derivative activates transcription at a distance in vitro. Science **247**:710–712.
7. **Cheung, A. K.** 1989. DNA nucleotide sequence analysis of the immediate-early gene of pseudorabies virus. Nucleic Acids Res. **17**:4637–4646.
8. **Clements, J. B., R. J. Watson, and N. M. Wilkie.** 1977. Temporal regulation of herpes simplex virus type I transcription: location of transcripts on the viral genome. Cell **12**:275–285.
9. **Constanzo, F., G. Capadelli-Fiume, L. Foa-Tomasi, and E. Cassai.** 1977. Evidence that herpes simplex virus DNA is transcribed by cellular RNA polymerase B. J. Virol. **21**:996–1001.
10. **Davison, A. J., and J. E. Scott.** 1986. The complete DNA sequence of varicella-zoster virus. J. Gen. Virol. **67**:1759–1816.
11. **DeLuca, N. A., A. McCarthy, and P. A. Shaffer.** 1985. Isolation and characterization of deletion mutants of herpes simplex virus type 1 in the gene encoding immediate-early regulatory protein ICP4. J. Virol. **56**:558–570.
12. **DeLuca, N. A., and P. A. Shaffer.** 1987. Activities of herpes simplex virus type 1 (HSV-1) ICP4 genes specifying nonsense peptides. Nucleic Acids Res. **15**:4491–4511.
13. **Disney, G. H., and R. D. Everett.** 1990. A herpes simplex virus type I recombinant with both copies of the Vmw175 coding sequences replaced by the homologous varicella-zoster virus open reading frame. J. Gen. Virol. **71**:2681–2689.
14. **Dixon, R. A. F., and P. A. Shaffer.** 1980. Fine-structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the herpes simplex virus type 1 immediate-early protein VP175. J. Virol. **36**:189–203.
15. **Felser, J. M., P. R. Kinchington, G. I. Inchauspe, S. E. Straus, and J. M. Ostrove.** 1988. 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. J. Virol. **62**:2076–2082.
16. **Felser, J. M., S. E. Straus, and J. M. Ostrove.** 1987. Varicella-zoster virus complements herpes simplex virus type 1 temperature-sensitive mutants. J. Virol. **61**:225–228.
17. **Folks, T., S. Benn, A. Rabson, T. Theodore, M. D. Hoggan, M. Martin, M. Lightfoote, and K. Sell.** 1985. Characterization of a continuous T-cell line susceptible to the cytopathic effects of the acquired immunodeficiency syndrome (AIDS)-associated retrovirus. Proc. Natl. Acad. Sci. USA **82**:4539–4543.
18. **Geisselsoder, J., F. Witney, and P. Yuckenberg.** 1987. Efficient site-directed in vitro mutagenesis. BioTechniques **5**:786–791.
19. **Giniger, E., S. Varnum, and M. Ptashne.** 1985. Specific DNA binding of GAL4, a positive regulatory protein of yeast. Cell **40**:767–774.
20. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
21. **Grundy, F. J., R. P. Bauman, and D. J. O'Callaghan.** 1989. DNA sequence comparative analyses of the equine herpes virus type I immediate early gene. Virology **172**:223–236.
22. **Han, M., and R. Grunstein.** 1989. Nucleosome loss activates yeast downstream promoters in vivo. Cell **55**:1137–1145.
23. **Holland, L. E., K. P. Anderson, J. A. Stringer, and E. K. Wagner.** 1979. Isolation and localization of herpes simplex virus type 1 mRNA abundant before viral DNA synthesis. J. Virol. **31**:447–462.
24. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **14**:8–19.
25. **Honess, R. W., and B. Roizman.** 1975. Regulation of herpesvirus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional viral polypeptides. Proc. Natl. Acad. Sci. USA **72**:1276–1280.
26. **Inchauspe, G., S. Nagpal, and J. M. Ostrove.** 1989. Mapping of two varicella-zoster virus encoded genes that activate the expression of viral early and late genes. Virology **173**:700–709.
27. **Johnson, P., and S. L. McKnight.** 1989. Eukaryotic transcriptional regulatory proteins. Annu. Rev. Biochem. **58**:799–839.
28. **Johnston, M.** 1987. A model fungal gene regulatory mechanism: the *GAL* gene of *Saccharomyces cerevisiae*. Microbiol. Rev. **51**:458–476.
29. **Kelleher, R. J., P. M. Flanagan, and R. D. Kornberg.** 1990. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. Cell **61**:1209–1215.
30. **Kinchington, P. R., J. K. Hougland, A. M. Arvin, W. T. Ruyechan, and J. Hay.** 1992. The varicella-zoster virus immediate-early protein IE62 is a major component of virus particles. J. Virol. **66**:359–366.
31. **Kristie, T. M., and B. Roizman.** 1984. Separation of sequences defining basal expression from those conferring α gene recognition within the regulatory domains of herpes simplex virus 1 α genes. Proc. Natl. Acad. Sci. USA **81**:4065–4069.
32. **Lillie, J. W., and M. R. Green.** 1989. Transcriptional activation by the adenovirus E1A protein. Nature (London) **338**:39–44.

33. **Lin, Y.-S., M. F. Carey, M. Ptashne, and M. R. Green.** 1988. GAL4 derivatives function alone and synergistically with mammalian activators in vitro. Cell **54**:659–664.
34. **Mackem, S., and B. Roizman.** 1982. Structural features of the herpes simplex virus α genes 4, 0, and 27 promoter-regulatory sequences which confer α regulation on chimeric thymidine kinase genes. J. Virol. **44**:939–949.
35. **Martin, K. J., J. W. Lillie, and M. R. Green.** 1991. Transcriptional activation by the pseudorabies virus immediate early protein. Gene Dev. **5**:2376–2382.
36. **McGeoch, D. I., M. A. Dalrymple, A. J. Davison, A. M. Dolan, C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type I. J. Gen. Virol. **69**:1531–1574.
37. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Dolan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the short repeat region in the genome of herpes simplex virus type I. Nucleic Acids Res. **14**:1727–1745.
38. **McKnight, J. L. C., T. M. Kristie, S. Silver, P. E. Pellette, G. Mavromara-Nazos, G. Campadelli-Fiume, M. Arsenakis, and B. Roizman.** 1986. Regulation of herpes simplex virus 1 gene expression: the effect of genomic environments and its implications for model systems. p. 163–173. *In* M. Botchan, T. Grodzicker, and P. Sharp (ed.), Cancer cells, vol. IV. DNA tumor viruses. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
39. **Mitchell, P. J., and R. Tjian.** 1989. Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. Science **245**:371–378.
40. **Mosca, J. D., P. M. Pitha, and G. S. Hayward.** 1992. Herpes simplex virus infection selectively stimulates accumulation of beta interferon reporter gene mRNA by posttranscriptional mechanism. J. Virol. **66**:3811–3822.
41. **O'Hare, P., and G. S. Hayward.** 1985. Three *trans*-acting regulatory proteins of herpes simplex virus modulate immediate-early gene expression in a pathway involving positive and negative feedback regulation. J. Virol. **56**:723–733.
42. **Pellett, P. E., J. L. C. McKnight, F. J. Jenkins, and B. Roizman.** 1985. Nucleotide sequence and predicted amino acid sequence of a protein encoded in a small herpes simplex virus DNA fragment capable of trans-inducing α genes. Proc. Natl. Acad. Sci. USA **82**:5870–5874.
43. **Perera, L. P., J. D. Mosca, W. T. Ruyechan, and J. Hay.** 1992. Regulation of varcella-zoster virus gene expression in human T lymphocytes. J. Virol. **66**:5298–5304.
44. **Perera, L. P., J. D. Mosca, M. Sadeghi-Zadeh, and J. Hay.** 1991. Analysis of cis-acting elements of the human immunodeficiency virus long terminal repeat that mediate transactivation by the immediate early gene products of human alphaherpes viruses, abstr. 10A-27, p. 239. Abstr. XVI Int. Herpesvirus Workshop.
45. **Perera, L. P., J. D. Mosca, M. Sadeghi-Zadeh, W. T. Ruyechan, and J. Hay.** 1992. The varicella-zoster virus immediate early protein, IE62, can positively regulate its cognate promoter. Virology **191**:346–354.
46. **Perera, L. P., J. E. Samuel, R. K. Holmes, and A. D. O'Brien.** 1991. Identification of three amino acid residues in the B subunit of Shiga toxin and Shiga-like toxin II that are essential for holotoxin activity. J. Bacteriol. **173**:1151–1160.
47. **Pizzorno, M. C., M.-A. Mullen, Y.-N. Chang, and G. S. Hayward.** 1991. The functionally active IE2 immediate-early regulatory protein of human cytomegalovirus is an 80-kilodalton polypeptide that contains two distinct activator domains and a duplicated nuclear localization signal. J. Virol. **65**:3839–3852.
48. **Preston, C. M.** 1979. Abnormal properties of an immediate early polypeptide in cells infected with the herpes simplex virus type 1 mutant *ts*K. J. Virol. **32**:357–369.
49. **Preston, C. M.** 1979. Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or the temperature sensitive mutant *ts*K. J. Virol. **29**:275–284.
50. **Ptashne, M.** 1986. Gene regulation by protein acting nearby and at a distance. Nature (London) **322**:697–701.
51. **Ptashne, M.** 1988. How eukaryotic transcriptional activators work. Nature (London) **335**:683–689.
52. **Puck, T. T., S. J. Cieciura, and A. Robinson.** 1958. Genetics of somatic mammalian cells. III. Long-term cultivation of euploid cells from human and animal subjects. J. Exp. Med. **108**:945–955.
53. **Roizman, B., and A. E. Sears.** 1990. Herpes viruses and their replication, p. 1795–1841. *In* B. N. Fields and D. M. Knipe (ed.), Virology—1990. Raven Press, Ltd., New York.
54. **Sadowski, I., J. Ma, S. J. Triezenberg, and M. Ptashne.** 1988. GAL4-VP16 is an unusually potent transcriptional activator. Nature (London) **335**:563–564.
55. **Sadowski, I., and M. Ptashne.** 1989. A vector for expressing GAL4(1-147) fusions in mammalian cells. Nucleic Acids Res. **17**:7539.
56. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
57. **Shiraki, K., and R. W. Hyman.** 1987. The immediate early proteins of varicella-zoster virus. Virology **156**:423–426.
58. **Telford, E. A., M. S. Watson, K. McBride, and A. J. Davison.** 1992. The DNA sequence of equine herpesvirus-I. Virology **189**:304–316.
59. **Watson, R. J., and J. B. Clements.** 1980. A herpes simplex virus type I function continuously required for early and late virus RNA synthesis. Nature (London) **285**:329–330.
60. **Wu, C.-L., and K. W. Wilcox.** 1991. The conserved DNA-binding domain encoded by the herpes simplex virus type 1 ICP4, pseudorabies virus IE180, and varicella-zoster virus ORF62 genes recognize similar sites in the corresponding promoters. J. Virol. **65**:1149–1159.