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# The Extreme Carboxyl Terminus of the Equine Herpesvirus 1 Homolog of Herpes Simplex Virus VP16 Is Essential for Immediate-Early Gene Activation

GILLIAN D. ELLIOTT*

*Molecular Virology Group, Department of Microbiology, University of Leeds, Leeds LS2 9JT, United Kingdom*

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**Gene 12 of equine herpesvirus 1 (EHV-1), the homolog of herpes simplex virus (HSV) VP16 (αTIF, Vmw65), was cloned into a eukaryotic expression vector by PCR and used in transactivation studies of both the EHV-1 and HSV-1 IE1 promoters. Results demonstrated that the product of gene 12 is a potent transactivator of immediate-early gene expression of both viruses, which requires sequences in the upstream HSV-1 promoter for activity. Mutational analysis of the gene 12 open reading frame indicated that removal of the C-terminal 7 amino acids, which contain a short region of homology with the extreme C terminus of VP16, inactivated the protein. Within this region, only a single methionine residue appeared to be essential for activity, implying that gene 12 may have a modular array of organization similar to that of VP16. However, fusion of the gene 12 C terminus to a truncated form of VP16, which contained the complex formation domain, did not restore activity to the HSV-1 protein. These data demonstrate that the EHV-1 immediate-early transactivator may not be functionally colinear with VP16, with transactivation requiring both the C terminus and another region(s) present within the N-terminal portion.**

Equine herpesvirus 1 (EHV-1) is a member of the *Alphaherpesvirinae* subfamily of viruses together with herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV). Like the prototype member, HSV-1, which has a defined pattern of coordinately regulated gene expression (22), EHV-1 transcription is believed to be divided into the three classes of immediate-early (IE), early, and late genes (16). In the case of HSV-1, virus-encoded proteins are known to be involved in both positive and negative regulation of gene expression, producing a finely balanced system of virus replication (reviewed in reference 19). The initial stage of expression, that of the IE genes, is activated by the powerful IE transactivator VP16 (also known as Vmw65 or αTIF). This protein, encoded by the UL48 gene (5, 31, 38), is an essential structural protein of HSV which is present in the tegument of the virus particle (20) and is thus capable of activating transcription from the five IE genes upon infection, in the absence of de novo protein synthesis (5, 38). While the structural function of VP16 is known to be essential for virus assembly (50), the activation function appears to be important only at low multiplicities of infection (2). It is not known, however, if the requirement for the function of VP16 in the virus life cycle is conserved throughout the subfamily *Alphaherpesvirinae*.

VP16 interacts with an element upstream of the IE RNA start site with the consensus TAATGARAT (where R = purine) (12, 26). Unlike classical transcription factors, it does not bind directly to the DNA at this position (33, 48) but forms protein-protein interactions with cellular factors (27, 36, 39, 48), forming a complex which is capable of binding the promoter. In many cases, the TAATGARAT element is overlapped by an octamer binding motif (consensus of ATG CAAAT) which is bound independently by the transcription factor OCT-1, a member of the POU class of cellular proteins (21, 44), and an interaction occurs between OCT-1 and VP16 (13, 25) via the POU homeobox (18, 28) to form a complex on the promoter which contains at least one other cellular protein (13, 25, 52). Consistent with the modularity of other transcription factors, VP16 has been shown to comprise several domains and subdomains (1, 17, 18, 47, 51). The amino-terminal 403 amino acids (aa) are sufficient for complex formation on the promoter (17, 47), and truncated mutants containing only this region have been shown to be negatively transdominant for the full-length protein (11, 47), suggesting that they may compete for the factors involved. The highly acidic C-terminal 60 residues function as an independent activation tail which makes contact with the basal transcription machinery (17, 43, 47). The C terminus has been shown to bind directly to the transcription factors TFIID (23, 45) and TFIIB (30), and the ability to bind the TATA-binding protein-associated factor $TAF_{II}40$ has also recently been reported (14). It has been suggested that this acidic domain may form the structure of an amphipathic helix (6), but mutations designed to break the helix have not indicated any requirement for this structure (7), and nuclear magnetic resonance studies have implied a lack of secondary structure in this region (36). Moreover, detailed mutational analyses of the activation domain have shown that at least 39 residues of the C-terminal acidic tail may be dispensable for activation (17, 47). A phenylalanine (Phe) residue at position 442 appears to be absolutely required for both activity (7) and binding to TFIIB (30) by this truncated activator, and the environment surrounding the Phe-442 residue may also contribute to the ability of VP16 to activate once a complex has been formed (42). More recent studies would seem to indicate that the definition of the exact activation domain of VP16 is still unclear, as two activation subdomains within the long acidic tail, termed H1 and H2, have now been characterized (49).

EHV-1 and VZV both contain gene homologs of the UL48 gene of HSV-1 (9, 46). In the case of VZV, the ORF10 product, which is 410 aa in length and thus lacks the entire

* Corresponding author. Present address: Marie Curie Research Institute, The Chart, Oxted, Surrey RH8 0TL, United Kingdom. Phone: 883 722306. Fax: 883 714375.

C-terminal 80-aa region homologous to the VP16 activation domain, has been shown to be a component of the tegument of the virus particle (24). This protein has also been shown to be capable of transactivating both VZV and HSV IE gene expression, albeit to levels somewhat lower than that produced by VP16 (34), in spite of an apparent inability to form a complex with its homologous TAATGARAT element (32). Likewise for EHV-1, the virions have been demonstrated to possess an IE gene-activating function for both the EHV-1 IE promoter, which contains at least one TAATGARAT motif, and an HSV-1 IE promoter (41). More recently, this activity has been assigned to the gene homolog of VP16, the product of the EHV-1 gene 12 (29, 40). Although the virus product of this gene has yet to be identified, the predicted product has an open reading frame of 479 aa and thus, like the VZV homolog, also lacks the majority of the acidic tail (46). Comparison of the gene 12 and VP16 open reading frames reveals 32% amino acid identity over their N-terminal regions, involved in complex formation in VP16, implying that gene 12 may interact with DNA in a similar manner to VP16. However, the complete divergence over the C-terminal activation domain has led to the suggestion that activation may not occur through this region of gene 12 (29, 40).

To determine the functional homology of gene 12 to VP16, the gene 12 open reading frame was cloned and its product was expressed from a heterologous promoter in mammalian cells. The results indicate that the gene 12 product is a potent transactivator of both EHV-1 and HSV-1 IE promoters and that this activation is dependent on sequences present in the upstream promoter of the HSV-1 IE gene. Mutational analysis of the C terminus of gene 12 showed that removal of the last 7 aa completely inactivates the protein and that within this region a single methionine residue, 3 aa from the end, is absolutely essential for gene 12 activity. However, the lack of activity of a chimeric protein formed by the fusion of the VP16 complex formation domain to the C terminus of gene 12 implies that the mechanism of gene 12 activation may be different from that of VP16 and that the heterologous domains are not interchangeable.

## MATERIALS AND METHODS

**Cells and viruses.** COS-7 and Vero cells were cultured in Dulbecco modified minimal essential medium containing 10% fetal calf serum. RK-13 cells were cultured in Dulbecco modified minimal essential medium containing 10% newborn calf serum.

The Ab1 strain of EHV-1 was propagated in RK-13 cells, and virion DNA was isolated as described previously (11).

**Plasmids.** Plasmid pCAT1.8Hin, generously provided by A. Purewal, contains a 1.8-kb *Hin*dIII fragment of the EHV-1 IE promoter inserted into pCAT (41). Plasmids pPO49 and pAB2, kindly provided by P. O'Hare, contain the HSV-1 IE110K promoter/regulatory region to −785 (plus TAATGA RAT) and −130 (minus TAATGARAT), respectively (35). The EHV-1 gene 12 expression vector was constructed by PCR amplification of gene 12 from Ab1 virion DNA. By using the published sequence of the Ab4 strain (46), primers were designed which hybridized at the exact 5′ and 3′ ends of the gene 12 open reading frame, with *Bam*HI sites being incorporated into the primers to ease manipulation of the amplified fragments (Fig. 1a). Amplification from Ab1 genomic DNA was carried out by using the Perkin-Elmer Cetus Gene-Amp kit as instructed by the manufacturer. Five cycles of 1 min at 97°C, 1 min at 55°C, and 2 min at 72°C, followed by 26 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C, were performed. The amplified product was digested with *Bam*HI and cloned into the *Bgl*II site of the eukaryotic expression vector pKV461 (3), to produce plasmid pGE126 (Fig. 1b). Mutations in gene 12 were also created by PCR, using 3′ primers which contained the necessary mutations and stop codons (Fig. 1a). Cloning of the resulting amplified fragments was carried out as for the full-length gene, and all constructs were sequenced to ensure that the correct mutation had been incorporated. A VP16 expression vector, pGE119, was constructed in the same way as the gene 12 vector, using primers designed according to the published sequence of HSV-1 (31), using the start codon as determined by Pellet and coworkers (38). pGE127 and pGE112 are gene 12 and VP16 expression vectors in the environment of plasmid pGE113 (10), which is identical to pKV461 except for the presence of the simian virus 40 origin of replication at the *Bam*HI site. pGE138, which consists of a truncated version of VP16 lacking the C-terminal 67 residues, was constructed by insertion of the *Sst*I fragment of pGE119 into the *Sst*I site of pGE113. pGE139 was constructed by insertion of the same fragment into *Sst*I-digested pGE127, to produce a fusion of the last seven residues of gene 12 to the truncated VP16. A chimeric molecule consisting of VP16 lacking the C-terminal 79 residues fused to the C-terminal 34 residues of gene 12 was constructed to produce pGE140. This was made by amplification of this region of gene 12, using a 5′ primer containing a *Sal*I site, and insertion of the resulting fragment into *Sal*I-*Bgl*II-digested pGE138. pGE139 and pGE140 were sequenced to ensure that the fusion between the two genes was in the correct reading frame.

**Transfections and CAT assays.** Monolayers of $5 \times 10^5$ cells in 60-mm-diameter tissue culture dishes were transfected by the DEAE-dextran-chloroquine method (8). Transfection mixes containing 100 ng each of reporter plasmid and expression vector were used to transfect the cells, which were harvested after 48 h of incubation at 37°C. Cell extracts were prepared and assayed for chloramphenicol acetyltransferase (CAT) activity as described by Gorman and coworkers (15).

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblotting.** Protein extracts were electrophoresed on 12% acrylamide gels cross-linked with *N,N′*-diallyltartardiamide and then blotted onto nitrocellulose filters. The blots were reacted with the anti-VP16 polyclonal antibody R230, and reactive bands were visualized by using a horseradish peroxidase conjugate.

## RESULTS

**The gene 12 product of EHV-1 activates IE gene expression by interaction with an element(s) in the upstream promoter.** It has been shown previously that the EHV-1 virion possesses an α-transactivating function, capable of activating IE gene expression of both HSV and EHV-1 (41). To confirm that this activation is caused by the product of gene 12, the homolog of HSV VP16 (46), the open reading frame for this gene was amplified by PCR and inserted into the expression vector pKV461 (2), under the control of the human cytomegalovirus IE promoter, to produce pGE126 (Fig. 1b). The results of cotransfections of pGE126 with CAT reporter plasmids driven by the full-length EHV-1 IE promoter (pCAT1.8Hin), full-length HSV-1 IE1 promoter (pPO49), and truncated HSV-1 IE1 promoter lacking TAATGARAT motifs (pAB2) into COS-7 cells indicate that gene 12 is a powerful activator of expression from both the EHV-1 and HSV IE promoters (Fig. 2). This activation is abolished if the TAATGARAT elements

(a)

| PLASMID | 5' PRIMER | 3' PRIMER |
|---|---|---|
| pGE119 | cgcggatccgacatgaacgcggacggc | gcgggatccctacccaccgtactcgtc |
| pGE126 | cgcggatccaccatgtgcctcttacatatttct | gcgggatccttaaatgtcaaacatctggttg |
| pGE130 | " | ggcggatccttactgcagcgtgaggttaga |
| pGE131 | " | ggcggatccttactcatcgcccgtcattga |
| pGE134 | " | ggcggatccttacatctggttgagctcatcg |
| pGE135 | " | cgggatccttaaatgtcaaacatgttgagctcatcgcccgt |
| pGE136 | " | gcgggatccttaaatgtcaaacatctcatcgcccgtcattgat |
| pGE137 | " | gcgggatccttaaatgtcaaactcatcgcccgtcattgat |
| pGE140 | ccggcgtcgacgcttgctattccgtctaac | as for pGE126 |

(b)

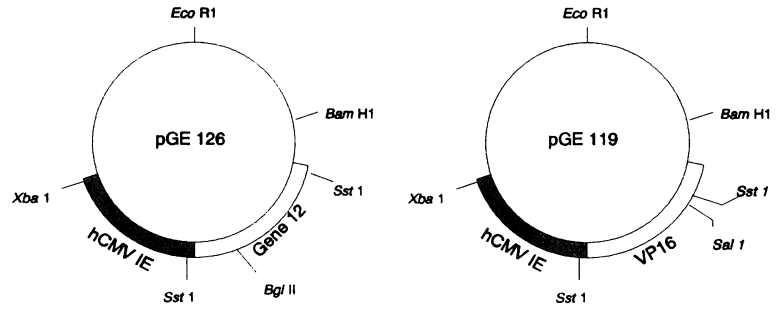


FIG. 1. Construction of EHV-1 gene 12 full-length and mutant expression vectors. (a) Oligonucleotide primers used in PCR amplification. In the case of the gene 12 constructs (pGE126 to pGE137), the open reading frame was amplified by PCR using primers incorporating *Bam*HI sites (underlined) at each end. The primers used to construct a VP16 expression vector (pGE119) are also shown. The pGE140 primers were used to amplify the C-terminal 36 residues of gene 12 incorporating a *Sal*I site (underlined) in the 5' primer. (b) Plasmid maps of the gene 12 and VP16 expression vectors. Following digestion with *Bam*HI, the full-length genes were inserted into the *Bgl*II site of pKV461 to produce the expression vectors pGE126 (gene 12) and pGE119 (VP16). hCMV, human cytomegalovirus.

are removed from the responsive promoter (Fig. 2, HSV-1 IE1 ΔTAAT), implying that gene 12 may activate expression by a molecular mechanism similar to that of VP16.

**Removal of the C-terminal 7 aa inactivates the gene 12 product.** Since the activation domain of VP16 is known to be located in the C-terminal acidic tail of the protein, it was of interest to determine if the gene 12 protein has an analogous region. To assess the importance of this region in transactivation, C-terminal truncations were constructed by PCR to remove the last 23, 7, and 3 residues from the open reading frame (Fig. 3), and each mutant was transfected into COS-7 cells with the EHV-1 and HSV-1 IE-CAT reporter plasmids (Fig. 4). The results indicate that removal of the last 23 aa (pGE130; aa 1 to 456) or of the last 7 aa (pGE131; aa 1 to 472) entirely abolishes the ability of gene 12 to activate expression from the EHV-1 IE promoter. However, removal of the C-terminal 3 aa (pGE134; aa 1 to 476) results in a protein which has an activity approximately 25% of that of the wild type. These three mutants behave identically in conjunction with the HSV-1 IE promoter compared with EHV-1 and produce the same results in Vero cells (data not shown), demonstrating that the region comprising the 4 aa 473 to 476 is involved in the activation function of gene 12.

**A methionine residue close to the carboxyl terminus is absolutely essential for gene 12 activity.** Within the activation domain of VP16, a single Phe residue has been identified as essential for activity (7), and analysis of the C-terminal sequence of gene 12 reveals the presence of a Phe residue 2 aa from the end (Fig. 3b). Other workers have aligned this extreme C terminus of gene 12 with the region of VP16

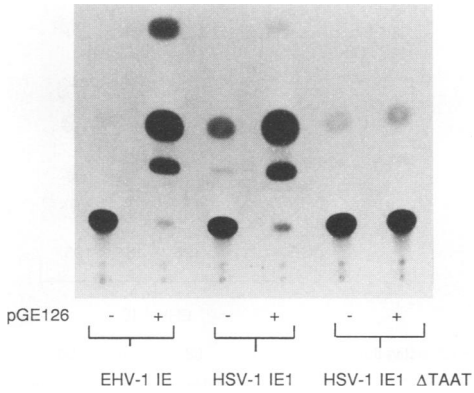


FIG. 2. Activation of herpesvirus IE promoters by the EHV-1 gene 12 product. CAT reporter plasmids driven by the EHV-1 IE (pCAT1.8Hin), the HSV-1 IE1 (pPO49), or the HSV-1 IE1 ΔTAAT GARAT (pAB2) promoter were transfected into COS-7 cells in the absence (−) and or presence (+) of pGE126.

containing the Phe-442 residue (40). However, the mutations described above demonstrate that this Phe can be removed from the protein without abolishing its function (pGE134 in Fig. 4), and therefore it is possible that the activation domain of gene 12 differs structurally from that of VP16. In addition, another short region of homology exists between the C terminus of gene 12 and the extreme C terminus of VP16 (Fig. 5), which includes the four residues already shown above to be essential for gene 12 activity. As this part of VP16 has now also been shown to be involved in activation (42, 49), it is possible that the C-terminal region of gene 12 required for its activity is the homolog of this distal subdomain of VP16.

To investigate this C-terminal region of gene 12 (Leu-Asn-Gln-Met) in more detail, it was mutated further to determine the residue(s) required for gene 12 activity (Fig. 3a). Three internal deletions were constructed to remove the Gln (pGE135), Leu-Asn-Gln (pGE136), and Leu-Asn-Gln-Met (pGE137) residues, which were cotransfected with the EHV-1 IE-CAT reporter into COS-7 cells. Deletion of the glutamine residue had little effect on the activity of the protein (pGE135 in Fig. 6), while a mutant containing an internal deletion of all four residues was entirely inactive (pGE137 in Fig. 6), as would be expected from the results for the truncated mutants. However, deletion of three of these residues such that only the methionine residue was left in the protein resulted in a protein with activity identical to that of wild-type gene 12 (pGE136 in Fig. 6). These mutants exhibit the same activity on both the EHV-1 and HSV-1 IE promoters (data not shown), the conclusion being that this methionine residue, 3 aa from the C terminus, is essential for the function of gene 12. This methionine is also conserved in the short region of homology identified with the extreme C terminus of VP16 (Fig. 5).

**Fusion of the carboxyl 34 residues of gene 12 to the complex formation domain of VP16 does not restore activation to VP16.** To determine if the product of gene 12 has a modular structure similar to that of VP16, with an independent activation domain at the C terminus, chimeric proteins consisting of the N-terminal complex formation domain of VP16 and the C-terminal region of the gene 12 protein were produced (Fig. 3). If an independent activation domain is present in the C terminus of gene 12, then fusion of this region to the complex formation domain of VP16 should result in a protein capable of transactivation. Three plasmids, containing VP16 aa 1 to 423 (pGE138), VP16 aa 1 to 423 fused to the last 7 aa of gene 12 (pGE139), and VP16 aa 1 to 410 fused to the last 34 aa of gene 12 (pGE140), which contained the simian virus 40 origin of replication to enable the detection of the fusion proteins, were constructed (Fig. 3a). Following transfection into COS-7 cells extracts were analyzed by SDS-PAGE and Western blotting. Figure 7a shows that for each construct, except the full-length gene 12 protein, a species of approximately the correct size reacted with an anti-VP16 polyclonal antibody. The proteins

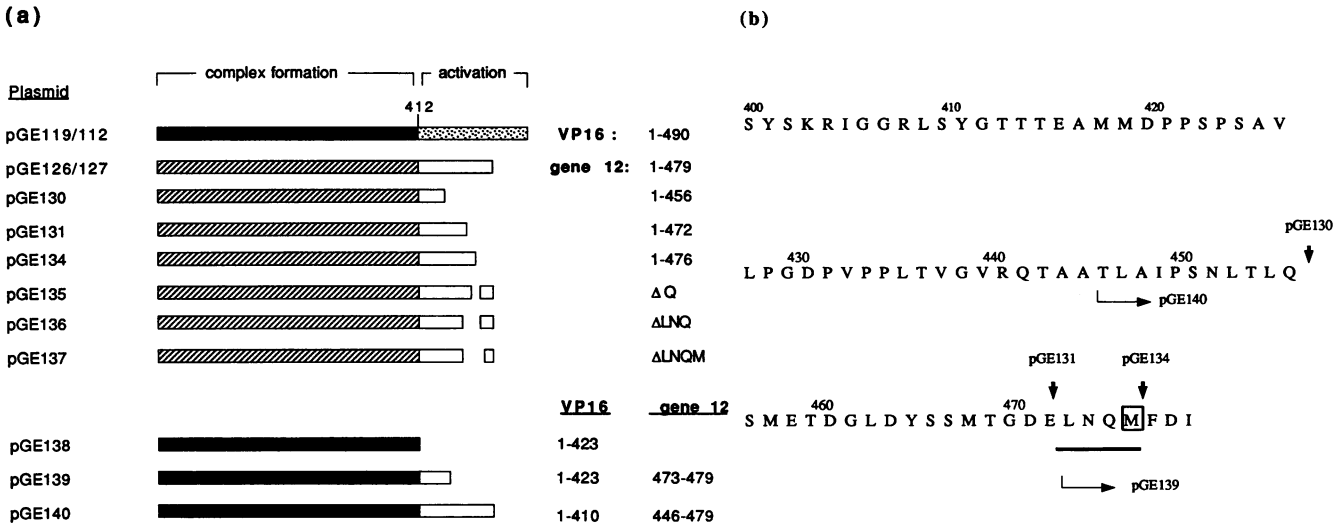


FIG. 3. (a) Line drawings of the constructs used in this study. The VP16 (pGE119) and gene 12 (pGE126) open reading frames have been aligned according to the homology within the N terminus of the proteins. Plasmids pGE130 to pGE137 involve mutations at the C terminus of gene 12, which are described on the right. Plasmids pGE138 to pGE140 contain the VP16 complex formation domain by itself (pGE138) or fused to either the last 7 residues (pGE139) or the last 34 residues (pGE140) of gene 12. (b) Endpoints of the truncations shown in panel a. The essential residues identified by the truncations are underlined. Truncation endpoints are shown above the line; starting points for VP16-gene 12 fusions are shown below.

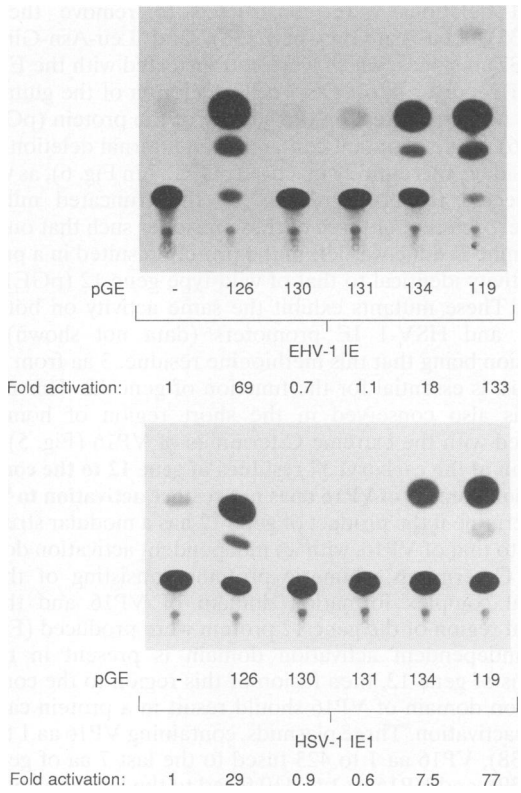


FIG. 4. Activities of the C-terminal truncations of gene 12. Each of the mutants (see Fig. 3) was cotransfected with either the EHV-1 IE (pCAT1.8Hin) or HSV-1 IE (pPO49) CAT reporter into COS-7 cells. The quantification of activation levels was carried out in the linear range of the assay, and the data are averages of three experiments.

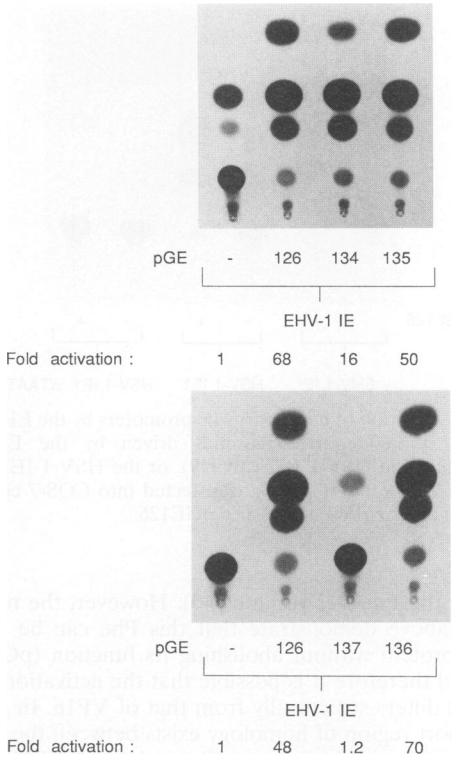


FIG. 6. Activities of the gene 12 internal deletion mutants. Each of the mutants (see Fig. 3) was cotransfected with the EHV-1 IE CAT reporter into COS-7 cells. The quantification of the activation levels was carried out in the linear range of the assay, and the data are averages of two experiments.

were synthesized in equivalent amounts, apart from the truncated VP16 species (pGE138), which was slightly less abundant than the other proteins. However, upon cotransfection with the EHV-1 IE-CAT reporter (Fig. 7b), neither the C-terminal 7 residues (pGE139) or 34 residues (pGE140) of gene 12 were capable of restoring activation to the VP16 N terminus, implying that the C-terminal 34 aa of gene 12 cannot substitute for the C terminus of VP16 or behave as an independent activation domain. Therefore the activation function of gene 12 is not entirely contained within the C terminus.

## DISCUSSION

The HSV-1 transactivator of IE gene expression, VP16, is one of several well-studied transcriptional activators which contains a prototypical acidic activation domain. To investigate the relevance of this protein to the alphaherpesvirus group as a whole, in terms of the life cycle of the viruses and their evolution, the EHV-1 homolog of VP16, the product of gene 12, has been studied. Previous studies have demonstrated the ability of an EHV-1 structural protein to activate the EHV-1 IE promoter (41), an activity more recently assigned to gene 12 (29, 40). In this study, transient expression techniques were used to investigate the ability of the VP16 homolog to transactivate and to define regions of the protein required for this activity. Results show that the gene 12 product is a potent transactivator of IE gene expression, which is capable of activating both the EHV-1 and the HSV-1 IE promoters to levels similar to that of VP16. The gene 12 protein product has not yet been identified, but it is known to be a structural protein (41) like HSV-1 VP16 (20) and VZV ORF10 (24), and identification of this protein will help in the analysis of its role in virus-infected cells and its interaction with other virus proteins and in determination of whether the protein is structurally essential.

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            468
Gene 12     M T G D E L N Q M F D I
            470|     |     | | |
VP 16       M A D F E F E Q M F T D A L G I D E Y G G
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FIG. 5. Homology between the extreme C-terminal regions of EHV-1 gene 12 and HSV-1 VP16. The extreme C terminus of gene 12 has been aligned with part of the second C-terminal subdomain identified by Reiger and coworkers (42) and Walker and coworkers (49).

VP16 is known to interact with the HSV-1 IE promoters through a motif with the consensus TAATGARAT (12, 24). Although several copies of these elements are present in the upstream sequences of these promoters, it has been shown that one is sufficient to allow the full-length VP16 to activate gene expression (49). In the case of the EHV-1 IE promoter, at least one clearly defined TAATGARAT element has been identified (41), providing a possible target for VP16 during activation by the HSV-1 protein. However, little is known about the interaction of the EHV-1 IE transactivator with this class of promoters, and while protein homology over the N termini of the homologs and the inability of gene 12 to activate the truncated HSV-1 IE promoter (lacking TAATGARAT ele-

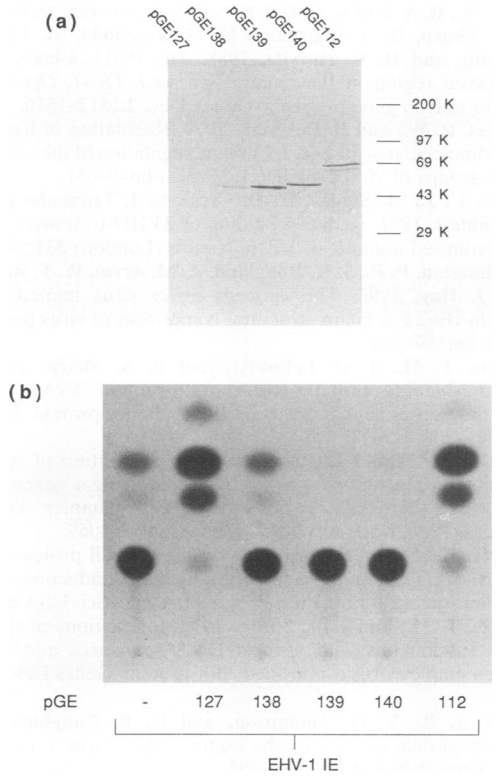


FIG. 7. Analysis of chimeric proteins made between the N terminus of VP16 and the C terminus of gene 12. (a) Western blot analysis of proteins expressed from the chimeric open reading frames. Equal amounts of whole-cell extracts of COS-7 cells transfected with the expression plasmids were electrophoresed on a 12% acrylamide gel and transferred to nitrocellulose. The presence of the simian virus 40 origin of replication in each of these plasmids allowed synthesis of sufficient quantities of the expressed proteins for detection, while the presence of the N terminus of VP16 enabled detection of the proteins by using the anti-VP16 polyclonal antibody R230. The positions of size markers are indicated. (b) The same plasmids as in panel a were cotransfected with the EHV-1 IE CAT reporter plasmid. This set of transfections was carried out in Vero cells to avoid overexpression of the proteins seen in COS-7 cells as a result of the presence of the simian virus 40 origin of replication. Results obtained in COS-7 cells were qualitatively the same (data not shown).

ments) may imply that the EHV-1 protein functions in a manner similar to VP16, the involvement of other promoter elements cannot be discounted. Further studies will be required to determine the precise elements in the EHV-1 IE promoter required for gene 12 transactivation and to determine the role of the other cellular factors known to be involved in VP16 interaction with DNA, namely, OCT-1 and complex-forming factor (CFF) (52). The production of an antibody specific for gene 12 would not only assist the identification of the virion component but also allow studies on gene 12 interaction with DNA and cellular factors in vitro.

Neither the EHV-1 gene 12 protein nor the VZV ORF10 protein, which has also recently been shown to be a transactivator (34), possesses the highly acidic C terminus which contains the activation domain of VP16 (17, 43, 47). However, it has been shown that over half of the VP16 tail is dispensable for activation (17, 47), such that the majority of the negative charge has been removed, and therefore it is possible that the EHV-1 and VZV homologs simply represent a much truncated version of the domain present in VP16. Moreover, the results presented here concerning truncations of the gene 12 C terminus indicate that removal of only the last seven residues is sufficient to inactivate the protein, identifying this region as important for function. This region corresponds linearly to an important 20-aa region identified in VP16, the removal of which also results in an inactive protein (17, 47). However, as shown in this report, fusion of the C-terminal 34 residues of gene 12 to the complex formation domain of VP16 does not restore activity to the truncated protein, indicating that the activation function of gene 12 is not contained entirely within this region of the EHV-1 protein, which therefore cannot substitute for the C terminus of VP16.

By the use of internal deletion mutants at the C terminus of gene 12, it was shown that a single Met residue out of the last 7 aa is essential for activation. This Met-476 residue may be an essential part of the EHV-1 transactivator because it is involved in maintaining the conformation or stability of the protein or because it is involved directly in protein-protein interactions with the cellular transcription machinery. The sequence around Met-476 contains some homology with sequences present at the extreme C terminus of VP16 (Fig. 5), a region which has recently been identified as containing a second transactivation subdomain in VP16 (42), with the methionine itself being conserved in the HSV homolog. This H2 subdomain is capable of restoring activation in a truncated mutant containing a substitution at Phe-442 but does not possess the ability to activate by itself (49). Furthermore, it has recently been shown that when a promoter with only one target site is used, the entire acidic domain of VP16, comprising both the initially identified proximal (H1) and the recently identified distal (H2) subdomains, is essential for activity (49). The H2 region of VP16 has also been reported to be involved in the binding of the TATA-binding protein-associated factor $TAF_{II}40$, which may function as an adaptor between VP16 and the basal transcription machinery (14), for instance with TFIIB, which has been shown to interact with the H1 subdomain (30). Further studies need to be carried out to determine if this region of gene 12 contains a $TAF_{II}40$ binding site and is therefore the homolog of the VP16 H2 domain rather than the homolog of the H1 domain as linear homology might suggest. It would also be of interest to determine if the conserved methionine residue within the H2 subdomain of VP16 is necessary for the activity of this region. Gene 12 and VP16 may therefore not be functionally colinear, with activation by gene 12 requiring some other function of the protein, such as TFIIB binding, which is not present in the C terminus. Thus, it is not yet possible to define the minimum activation domain of gene 12 or determine if it possesses the classical modular array of other transcription factors.

The divergence of the C-terminal domains present in HSV-1, EHV-1, and VZV IE transactivators may reflect the ability of these proteins to interact with selected cellular proteins and thus influence virus growth in different cell types. The EHV-1 gene 12 product provides another useful model for attempting to elucidate the mode of action of this family of transactivators and may help in characterizing the cellular targets involved in complex formation and transcriptional activation. Analysis of the common and divergent domains between HSV, VZV, and EHV-1 may provide a shortcut to identifying these important molecular interactions.

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