Journal of Virology, June 1995, p. 3857–3862 Vol. 69, No. 6
0022-538X/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Nuclear Localization and Transcriptional Activation Activities of Truncated Versions of the Immediate-Early Gene Product of Equine Herpesvirus 1

RICHARD H. SMITH, V. ROGER HOLDEN, and DENNIS J. O'CALLAGHAN*

Department of Microbiology and Immunology, Louisiana State University Medical Center, Shreveport, Louisiana 71130-3932

Received 28 December 1994/Accepted 27 February 1995

The equine herpesvirus 1 (EHV-1) immediate-early (IE) gene product encodes a nuclear regulatory protein capable of negatively autoregulating its own promoter, transactivating representative EHV-1 early promoters, and acting in a concerted fashion with accessory EHV-1 regulatory factors to transactivate EHV-1 late promoters. To identify IE amino acid sequences involved in nuclear localization and to examine the contribution of C-terminal portions of the IE polypeptide to transactivation, vectors that express various carboxy-terminally truncated IE polypeptides were constructed. It is demonstrated that amino acids 963 through 970 of the 1,487-amino-acid IE protein are required for efficient localization of the truncated IE polypeptides to the nuclei of transfected cells. In addition, it is demonstrated that the first 970 amino acids of the IE gene product are sufficient to transactivate the EHV-1 thymidine kinase promoter to significant levels (i.e., approximately 40% of the level of wild-type activation).

Equine herpesvirus 1 (EHV-1), an alphaherpesvirus with worldwide distribution, is a causative agent of equine rhinopneumonitis, as well as epizootic abortion among pregnant mares (1, 20). Within the laboratory, EHV-1 provides a model system with which to study lytic herpesvirus replication, persistent infection, and other important aspects of herpesvirus molecular biology (2, 6, 11, 12, 25, 26). In particular, EHV-1 provides an excellent system for the study of alphaherpesvirus gene regulation (9, 15, 23, 24, 29–31, 35). The immediate-early (IE) gene product of EHV-1 (also referred to as the IR1 gene product) shows significant homology to a number of alphaherpesvirus regulatory proteins, including ICP4 of herpes simplex virus type 1, the ORF62 gene product of varicella-zoster virus, and the IE180 gene product of pseudorabies virus. Two highly conserved regions (domains 2 and 4 [Fig. 1A]) have been identified within each of the IE homologs examined to date (4, 13, 18, 27). As demonstrated for the IE homologs of herpes simplex virus type 1, pseudorabies virus, and varicella-zoster virus, domain 2 contains amino acid sequences associated with site-specific DNA binding (32–34). Recent work in our laboratory has extended this observation to domain 2 of the EHV-1 IE gene product as well (14a).

Characterization of the regulatory functions of the EHV-1 IE gene product has determined that the IE gene encodes a bifunctional regulatory protein capable of both activation and repression of EHV-1 gene expression (29). The IE gene product is capable of down-regulating gene expression directed by its own promoter, activating gene expression directed by model EHV-1 early promoters, and cooperating in a synergistic fashion with accessory regulatory proteins of EHV-1 to induce gene expression directed by EHV-1 late promoters (17, 29, 30). Recently, the transcriptional activation domain of the IE gene product has been mapped to within the first 89 amino acid residues of the IE polypeptide (31). This 89-amino-acid region is negatively charged and therefore is believed to represent a transcriptional activation domain of the acidic class.

In this report, mutant IE polypeptides bearing truncations of the C-terminal portion of the IE molecule were examined for (i) the ability to localize efficiently within the nuclei of transfected cells and (ii) the ability to activate gene expression directed by a model EHV-1 early promoter derived from the viral thymidine kinase (TK) gene. Experimental results identified an 8-amino-acid sequence that is required for nuclear localization of C-terminally truncated IE polypeptides. This 8-amino-acid sequence shows striking similarity to the nuclear localization signal defined for simian virus 40 (SV40) large T antigen (14). In addition, it was determined that IE polypeptides bearing the first 970 residues of the full-length IE protein were capable of transactivating the EHV-1 TK promoter, albeit at levels approximately 40% those of full-length IE polypeptides.

A domain important for nuclear localization occurs between amino acids 951 and 1411 of the IE polypeptide. The EHV-1 IE gene encodes a 1,487-amino-acid phosphoprotein that localizes to the nuclei of transfected cells (3, 13, 31). The EHV-1 IE gene product shows significant homology to a number of corresponding alphaherpesvirus proteins, including ICP4 of herpes simplex virus type 1, IE180 of pseudorabies virus, and the ORF62 gene product of varicella-zoster virus. The amino acid sequence of the respective IE homologs can be divided into five regions on the basis of the degree of homology (Fig. 1A) (4, 13, 18). Regions 2 and 4 represent highly conserved domains, typically showing more than 50% homology in any pairwise comparison among the IE homologs. Regions 1, 3, and 5 generally show less than 50% homology.

To identify regions of the IE polypeptide important for nuclear localization and to determine the contribution of carboxy-terminal portions of the IE molecule to transactivation, plasmids bearing IE nonsense mutants were constructed. Plasmids pSVIE [which expresses IE(1–1487)], pSV12, and pTK-CAT2 have been described previously (29). Plasmids n1411, n951, n627, and n421 were constructed by the method of Pater-

* Corresponding author. Mailing address: Department of Microbiology and Immunology, Louisiana State University Medical Center, 1501 Kings Hwy., P.O. Box 33932, Shreveport, LA 71130-3932. Phone: (318) 675-5750. Fax: (318) 675-5764. Electronic mail address: DCALLA@POP3.LSUMC.EDU.

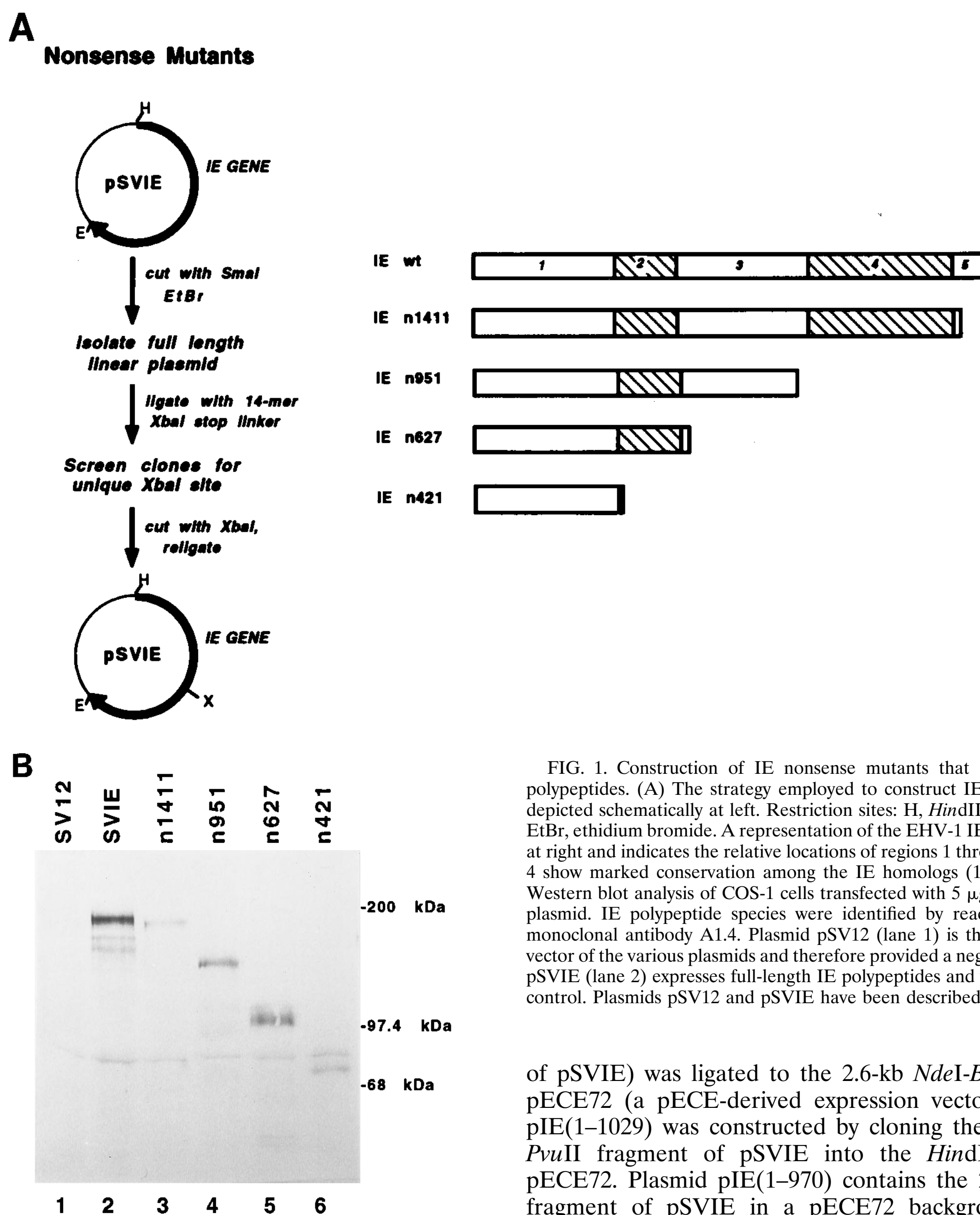

FIG. 1. Construction of IE nonsense mutants that express truncated IE polypeptides. (A) The strategy employed to construct IE nonsense mutants is depicted schematically at left. Restriction sites: H, *Hin*dIII; E, *Eco*RI; X, *Xba*I. EtBr, ethidium bromide. A representation of the EHV-1 IE polypeptide is shown at right and indicates the relative locations of regions 1 through 5. Regions 2 and 4 show marked conservation among the IE homologs (13). wt, wild type. (B) Western blot analysis of COS-1 cells transfected with 5 μg of the indicated test plasmid. IE polypeptide species were identified by reaction with IE-specific monoclonal antibody A1.4. Plasmid pSV12 (lane 1) is the parental expression vector of the various plasmids and therefore provided a negative control. Plasmid pSVIE (lane 2) expresses full-length IE polypeptides and was used as a positive control. Plasmids pSV12 and pSVIE have been described previously (29).

son and Everett (21). Briefly, pSVIE (10 μg) was digested in a 50-μl total volume for 20 min at 30°C with 10 U of *Sma*I in the presence of 50 μg of ethidium bromide per ml. Full-length, linear plasmid DNA was isolated by agarose gel electrophoresis, excised from the gel, and purified by using a GeneClean Kit (Bio101, Inc., La Jolla, Calif.). The linear plasmid DNA was ligated in the presence of a double-stranded *Xba*I linker containing stop codons in all reading frames (1062; New England Biolabs, Inc., Beverly, Mass.). Plasmids were then screened for the acquisition of a unique *Xba*I site. Positive clones were cleaved with *Xba*I, gel purified, and religated to yield a single inserted copy of the linker. The position of the inserts was determined by restriction fragment length polymorphism analysis with *Sma*I as a probe. The plasmid nomenclature indicates the last inclusive IE amino acid. To construct pIE(1–1299), the 4.5-kb *Nde*I-*Bgl*II fragment of n1299 (which contains a *Bgl*II linker inserted within codon 1300 of the IE open reading frame of pSVIE) was ligated to the 2.6-kb *Nde*I-*Bgl*II fragment of pECE72 (a pECE-derived expression vector [10]). Plasmid pIE(1–1029) was constructed by cloning the 3.1-kb *Hin*dIII-*Pvu*II fragment of pSVIE into the *Hin*dIII-*Sma*I site of pECE72. Plasmid pIE(1–970) contains the 2.3-kb *Nde*I-*Asc*I fragment of pSVIE in a pECE72 background (the SV40 promoter region is provided by the *Nde*I-*Asc*I fragment of pSVIE). To construct pIE(1–962), the 1.6-kb *Bam*HI-*Xba*I fragment of pSR122 (an intermediate plasmid bearing the IE open reading frame from positions 1292 to 3865) was ligated to the 4.3-kb *Bam*HI-*Xba*I fragment of pIE(1–1029).

Plasmid pSVIE, an EHV-1 IE gene expression vector (29), was partially digested with *Sma*I restriction enzyme in the presence of ethidium bromide (Fig. 1A). Full-length, linear plasmid DNA was isolated by agarose gel electrophoresis and ligated with a double-stranded oligonucleotide linker bearing stop codons in all reading frames. Plasmids capable of producing IE polypeptides terminating at IE amino acids 1411, 951, 627, and 421 were obtained (Fig. 1A). The ability of these plasmids to express truncated IE polypeptides of the expected size was confirmed by Western blot (immunoblot) analysis of transfected COS-1 cells with an IE-specific monoclonal antibody (Fig. 1B). Equal amounts of protein equivalent to 1.2×10^5 cells were analyzed as described previously (3, 35). A negative control for the Western blot analysis was provided by

transfecting COS-1 cells with pSV12 (Fig. 1B, lane 1) (29), the parental vector of n1411, n951, n627, and n421. Plasmid pSVIE, a plasmid that expresses full-length IE polypeptides (29), was used as a positive control (Fig. 1B, lane 2). Plasmids n1411, n951, n627, and n421 expressed progressively smaller amino-terminal portions of the IE polypeptide as expected (Fig. 1B, lanes 3 through 6, respectively).

To determine the ability of the various IE nonsense polypeptides to accumulate within the nuclei of transfected cells, monolayers of COS-1 cells were transfected with each of the nonsense plasmids and then processed into nuclear and cytoplasmic fractions at approximately 2 days posttransfection. For whole-cell lysates, monolayers of transfected cells were harvested into 1× sample buffer (2% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 10% glycerol, 0.1% bromophenol blue, 62.5 mM Tris-HCl [pH 6.8]), boiled for 5 min, passed several times through a small-gauge needle, and then centrifuged for 10 min in an Eppendorf Microfuge. For subcellular fractions, monolayers of transfected COS-1 cells in 25-cm^2 flasks were rinsed with phosphate-buffered saline (PBS), scraped into PBS, and pelleted by brief centrifugation. The pelleted cells were suspended in a solution consisting of 50 μl of PBS containing 0.5% Nonidet P-40 (Sigma Chemical Co., St. Louis, Mo.) and 0.5 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co.) and incubated for 10 min on ice. The nuclei were pelleted by centrifugation at 800 × *g* for 10 min. The cytoplasmic fraction (supernatant) was removed and stored on ice. The nuclear pellet was rinsed several times with ice-cold PBS and pelleted. The pelleted nuclei and cytoplasmic fractions were brought to equal volumes with 1× sample buffer and processed for Western blot analysis. Polypeptides in equal amounts equivalent to 1.2×10^5 cells were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 7.5% polyacrylamide gels and then transferred electrophoretically to nitrocellulose filters (Schleicher & Schuell, Inc., Keene, N.H.). The filters were rinsed briefly in TBST (10 mM Tris-HCl [pH 8.0], 150 mM NaCl, 0.05% Tween 20) and then blocked with TBST containing 0.4% gelatin. The filters were then incubated for 30 min with a 1:1,000 dilution of monoclonal antibody A1.4, which is specific for the EHV-1 IE gene product (3), rinsed several times in TBST, and then reacted with an alkaline phosphatase-conjugated goat anti-mouse secondary antibody (Promega, Madison, Wis.) for 30 min. Reactive protein bands were identified by incubation in AP buffer (100 mM NaCl, 5 mM $MgCl_2$, 100 mM Tris-HCl [pH 9.5]) containing 0.33 mg of nitroblue tetrazolium and 0.165 mg of 5-bromo-4-chloro-3-indolyl phosphate (both from Bethesda Research Laboratories, Inc., Gaithersburg, Md.) per ml.

The results of SDS-PAGE and Western blot analysis of the subcellular fractions are shown in Fig. 2. Full-length, wild-type IE polypeptides, expressed from plasmid pSVIE, occurred predominantly within the nuclear fraction of transfected cells (compare Fig. 2, lanes 1 and 2). COS-1 cells transfected with pSV12 (a plasmid that does not express IE polypeptides) provided a negative control (Fig. 2, lanes 11 and 12). Similar to full-length IE polypeptides, IE polypeptides expressed from n1411 also occurred predominantly within the nuclear fraction (Fig. 2, lane 3). In contrast, IE polypeptides expressed from n951, n627, and n421 were observed predominantly within the cytoplasmic fraction of transfected cells (Fig. 2, lanes 5 through 10). These results suggest that amino acid sequences contributing to nuclear localization of the IE gene product map within a 460-amino-acid region between residues 951 and 1411.

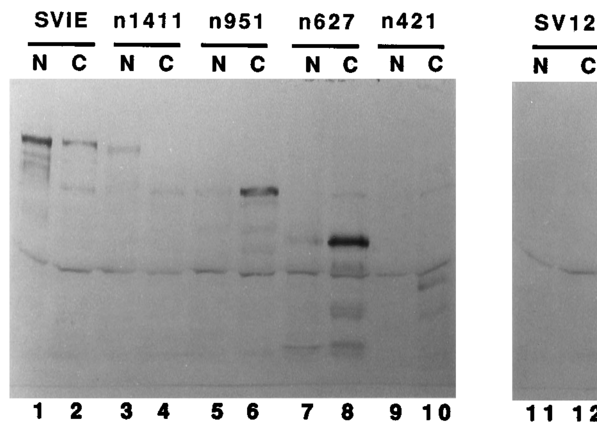

FIG. 2. Immunoblot analysis of nuclear (N) and cytoplasmic (C) fractions of transfected COS-1 cells. COS-1 cells were transfected with 5 μg of the indicated expression vector. At approximately 2 days posttransfection, the transfected cell monolayers were processed into nuclear and cytoplasmic fractions as described in the text. Blotted polypeptides from equivalent numbers of cells (10^5) were probed with the IE-specific monoclonal antibody A1.4.

Amino acids 963 to 970 are necessary for nuclear localization of carboxy-truncated IE polypeptides. To define more clearly the amino acid residues responsible for nuclear localization of IE polypeptides, a series of plasmids that express IE polypeptides terminating within the 460-amino-acid region between residue 951 and residue 1411 was constructed (Fig. 3A). These vectors express IE polypeptides terminating at IE amino acid 1299, 1029, 970, or 962. The ability of these expression constructs to direct the synthesis of the expected IE truncation product was determined by Western blot analysis with the IE-specific monoclonal antibody A1.4 (Fig. 3B). The ability of each of these polypeptide species, as well as those expressed from n1411 and n951, to migrate to the nuclei of transfected cells was determined by indirect immunofluorescence (Fig. 4). IE polypeptides terminating at IE amino acid 1411, 1299, 1029, or 970 demonstrated diffuse nuclear staining characteristic of the full-length IE polypeptide. However, IE polypeptides terminating at residue 962 or residue 951 failed to migrate to the nucleus and, instead, demonstrated a diffuse cytoplasmic staining pattern. Therefore, IE polypeptides possessing amino acids 1 through 970 accumulate efficiently within the nucleus, while those possessing IE amino acids 1 through 951 do not. Examination of the amino acid sequence between the residue 962 and residue 970 endpoints revealed an amino acid sequence (963-PPAPKRRV-970) with striking similarity to the nuclear localization signal of SV40 large T antigen (14) (125-PPK KKRKV-132). The EHV-1 sequence bears a cluster of positively charged amino acids characteristic of eukaryotic nuclear localization signals. As seen in Fig. 4, IE amino acids 963 to 970 are necessary for efficient nuclear localization of truncated IE polypeptides; however, the possibility remains that additional IE amino acid sequences may be necessary and/or sufficient to target the intact IE polypeptide to the nuclei of expressing cells.

IE amino acids 1 to 970 are sufficient to transactivate the EHV-1 TK promoter. We have previously mapped the transcriptional activation domain of the EHV-1 IE gene product to the first 89 amino acids of the IE polypeptide and, in addition, demonstrated that an amino-truncated form of the IE gene product lacking the first 322 amino acids failed to activate gene expression directed by the EHV-1 TK promoter (31). To characterize the contribution of C-terminal portions of the IE polypeptide to transactivation, plasmids expressing various truncated portions of the IE gene product were tested for their ability to transactivate reporter gene expression from a plasmid

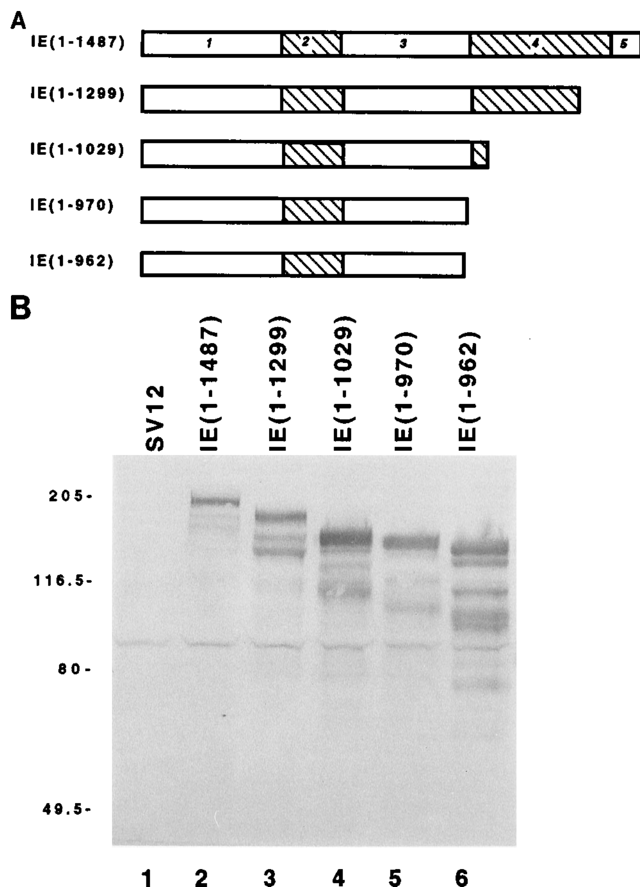

FIG. 3. Western blot analysis of truncated IE polypeptides. (A) The structures of four IE truncation mutants are shown below a schematic representation of the EHV-1 IE polypeptide. (B) COS-7 cells were transfected with the indicated plasmid DNAs and then harvested for Western blot analysis as described in the text. Polypeptides from equivalent numbers of cells were separated by SDS-PAGE, blotted to nitrocellulose filters, and probed with monoclonal antibody A1.4. IE(1–1487) was expressed from plasmid pSVIE.

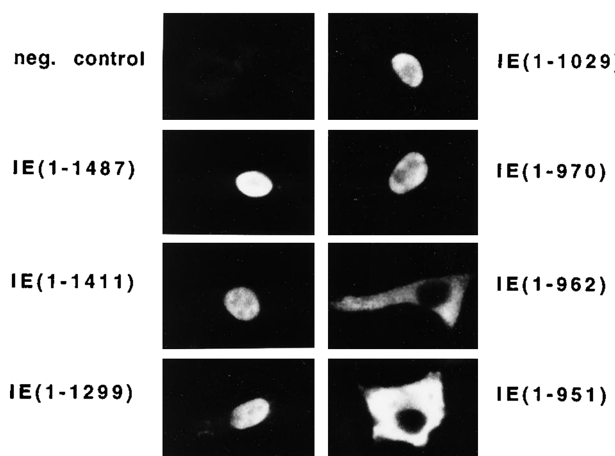

FIG. 4. Nuclear localization of IE truncation mutants. LTK$^-$ cells in 60-mm-diameter dishes were transfected by lipofection with 8 μg of the appropriate plasmid DNA. At approximately 24 h posttransfection, the transfected cells were seeded onto sterile glass slides and incubated an additional 24 h. The cells were fixed in acetone and examined by indirect immunofluorescence with an IE peptide-specific polyclonal rabbit antiserum as described previously (31). The upper left panel represents LTK$^-$ cells transfected with a non-IE-expressing plasmid, pBXG1V, as a negative control. IE(1–1487) was expressed from plasmid pSVIE.

bearing the chloramphenicol acetyltransferase (CAT) gene under the control of the EHV-1 TK promoter (pTK-CAT2). The results are shown in Fig. 5. The wild-type EHV-1 IE gene product induced reporter gene expression by 15-fold compared with basal levels of reporter gene activity observed in the absence of effector plasmid. IE polypeptides terminating at IE amino acid 1411 demonstrated levels of inducing activity approximately 34% that of the full-length IE protein. In contrast, IE polypeptides terminating at IE residue 1299 yielded an 11-fold induction of CAT activity (72% of wild-type levels). The impaired ability of the 1–1411 polypeptide to activate pTK-CAT2 expression, compared with that of the 1–1299 polypeptide, is most likely due to its decreased level of stability in transfected cells (Fig. 1 and 2). Surprisingly, polypeptides terminating at IE amino acid 1029 or 970 demonstrated significant levels of transactivation, yielding levels of activity 30 and 40% that of full-length IE, respectively. In contrast, polypeptides 1–962 and 1–951 gave levels of induction of twofold or less. This observation is consistent with the lack of efficient nuclear localization observed for these polypeptide species. Taken together, the data indicate that although not absolutely required for transactivation, regions 4 and 5 make a significant contribution to the ability of the intact IE polypeptide to transactivate the EHV-1 TK promoter. The contribution of regions 4 and 5 to transactivation may be a structural one since this region of the EHV-1 IE polypeptide, when fused to the DNA-binding domain of GAL4, failed to activate reporter gene expression directed by a GAL4-responsive plasmid (31). Alternatively, amino acid sequences within regions 4 and/or 5 may mediate direct protein-protein interactions that contribute to (but are dispensable for) transcriptional activation.

In this study, IE polypeptides bearing C-terminal truncations were transiently expressed within transfected cells and examined for the ability to (i) localize efficiently within the nuclei of transfected cells and (ii) transactivate a representative EHV-1 early promoter (that of the EHV-1 TK gene). It was determined that amino acids 963 to 970 (PPAPKRRV) of the EHV-1 IE polypeptide were essential for efficient accumulation of the C-terminal truncation mutants within the nucleus. The PPAPKRRV sequence shows marked similarity to the nuclear localization signal defined for SV40 T antigen (PP KKKRKV) (14). Truncated IE polypeptides lacking the se-

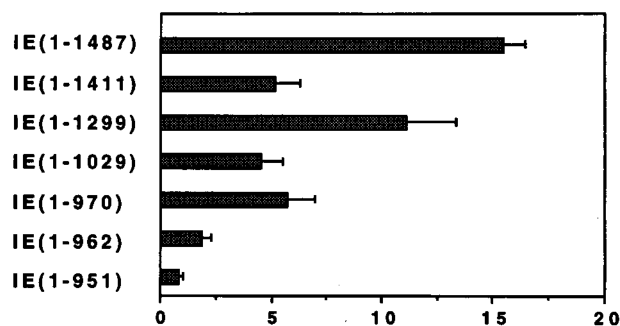

FIG. 5. Fold stimulation of CAT activity directed by pTK-CAT2. L-M cells were transfected in 60-mm-diameter dishes with 1.4 pmol of pTK-CAT2 and 0.3 pmol of a plasmid expressing the indicated IE amino acids. Transfected cells were harvested approximately 2 days posttransfection and assayed for CAT activity as previously described (29, 31, 35). All transfections were performed in duplicate. Data are averages; error bars show standard deviations.

quence from amino acids 963 to 970 failed to activate significantly a TK-CAT reporter plasmid. In contrast, truncated IE polypeptides containing the putative IE nuclear localization signal were capable of inducing reporter gene expression to levels ranging from 30 to 72% that of wild-type genes. Upon examination of the abilities of various truncated and deleted forms of ICP4 of HSV-1 to migrate to the nucleus, DeLuca and Schaffer (8) suggested that ICP4 amino acids 723 to 732 (PR EGRKRKSP) may serve as the ICP4 nuclear localization signal. Paterson and Everett (21) also mapped the ICP4 nuclear localization signal to within the same vicinity (ICP4 amino acids 682 to 774). Recently, Mullen et al. (19) have demonstrated that providing ICP4 amino acids 726 to 732 (GRK RKSP) to nuclear-localization-deficient ICP4 polypeptides can restore efficient nuclear localization. Similar to the EHV-1 IE sequence (963-PPAPKRRV-970), the ICP4 sequence contains a cluster of positively charged amino acids. Although regions 726 to 732 of ICP4 and 963 to 970 of the EHV-1 IE gene product do directly align in a comparison of the intact proteins, both sequences occur within close proximity to the boundary of regions 3 and 4 (13).

Transient-expression results also indicated that although regions 4 and 5 make a significant contribution to the ability of the IE gene product to transactivate the EHV-1 TK promoter, these regions are not essential for transactivation. Similarly, Shepard et al. (28) have demonstrated that a truncated version of ICP4 containing the first 774 amino acid residues (and therefore lacking regions 4 and 5) is sufficient to transactivate the HSV-1 TK gene, as well as other viral genes examined. DeLuca and Schaffer (7) have also reported partial transactivation activity for various forms of ICP4 bearing C-terminal truncations. Examination of various viral regulatory factors has indicated that many of the functional domains are modular and can retain activity when physically separated from the intact polypeptide. In this regard, modular domains have been identified among various members of the ICP4 family of alphaherpesvirus regulatory proteins. For example, use of the GAL4 fusion strategy has identified discrete transcriptional activation domains within the amino termini of the EHV-1 IE gene product (31), the varicella-zoster virus ORF62 gene product (5, 22), and the pseudorabies virus IE180 gene product (16). The transcriptional activation domain of ICP4, however, apparently does not map within the immediate amino terminus of the molecule (7, 8). The DNA-binding domains of ICP4, IE180, and ORF62 have been mapped to conserved region 2 of the respective molecules (33, 34). Region 2 of the EHV-1 IE gene product also specifies a DNA-binding domain (14a). These mapping studies have demonstrated that the DNA-binding and transcriptional activation domains of the various IE homologs can function outside the context of their respective full-length polypeptides and also that, in the case of the ORF62, IE180, and EHV-1 IE gene products, these domains occur within the amino-terminal portions of the protein (i.e., not within region 4 or 5). The location of these domains is consistent with the ability of C-terminally truncated IE polypeptides to transactivate a representative EHV-1 early promoter, since the DNA-binding and transcriptional activation domains are retained by these polypeptides. As noted above for the EHV-1 IE gene product, direct assay of C-terminal portions of the ORF62 and IE180 gene products for transcriptional activation activity in a GAL4 fusion assay has failed to identify significant activity (5, 16). It is possible that regions 4 and 5 make a purely structural contribution to the function of the IE homologs. However, the ability of domains within regions 4 and/or 5 to recruit or interact with factors that, although not essential, facilitate IE homolog-mediated activation of gene expression would also be consistent with current observations. Further experimentation will be required to determine the exact role regions 4 and 5 play in IE gene structure and function.

We thank Suzanne Zavecz for excellent technical assistance.

Support for this investigation was obtained from Public Health Service research grant AI 22001 from the National Institutes of Health and grant 92-37204-8040 from the U.S. Department of Agriculture Animal Molecular Biology Program.

REFERENCES

1. **Allen, G. P., and J. T. Bryans.** 1986. Molecular epizootiology, pathogenesis, and prophylaxis of equine herpesvirus 1 infections, p. 78–144. *In* R. Pandey (ed.), Progress in veterinary microbiology and immunology, vol. 2. S. Karger, Basel.
2. **Baumann, R. P., V. R. R. Yalamanchili, and D. J. O'Callaghan.** 1989. Functional mapping and DNA sequence of an equine herpesvirus 1 origin of replication. J. Virol. **63:**1275–1283.
3. **Caughman, G. B., J. B. Lewis, R. H. Smith, R. N. Harty, and D. J. O'Callaghan.** 1995. Detection and intracellular localization of equine herpesvirus 1 IR1 and IR2 gene products by using monoclonal antibodies. J. Virol. **69:**3024–3032.
4. **Cheung, A. K.** 1989. DNA nucleotide sequence analysis of the immediate-early gene of pseudorabies virus. Nucleic Acids Res. **17:**4637–4646.
5. **Cohen, J. I., D. Heffel, and K. Seidel.** 1993. The transcriptional activation domain of varicella-zoster virus open reading frame 62 protein is not conserved with its herpes simplex virus homolog. J. Virol. **67:**4246–4251.
6. **Dauenhauer, S. A., R. A. Robinson, and D. J. O'Callaghan.** 1982. Chronic production of defective-interfering particles by hamster embryo cultures of herpesvirus persistently infected and oncogenically transformed cells. J. Gen. Virol. **60:**1–14.
7. **DeLuca, N. A., and P. A. Schaffer.** 1987. Activities of herpes simplex virus type 1 (HSV-1) ICP4 genes specifying nonsense peptides. Nucleic Acids Res. **15:**4491–4511.
8. **DeLuca, N. A., and P. A. Schaffer.** 1988. Physical and functional domains of the herpes simplex virus transcriptional regulatory protein ICP4. J. Virol. **62:**732–743.
9. **Elliott, G. D.** 1994. The extreme carboxyl terminus of the equine herpesvirus 1 homolog of herpes simplex virus VP16 is essential for immediate-early gene activation. J. Virol. **68:**4890–4897.
10. **Ellis, L., E. Clauser, D. O. Morgan, M. Edery, R. A. Roth, and W. J. Rutter.** 1986. Replacement of insulin receptor tyrosine residues 1162 and 1163 compromises insulin-stimulated kinase activity and uptake of 2-deoxyglucose. Cell **45:**721–732.
11. **Gray, W. L., R. P. Baumann, A. T. Robertson, G. B. Caughman, D. J. O'Callaghan, and J. Staczek.** 1987. Regulation of equine herpesvirus type 1 gene expression: characterization of immediate early, early, and late transcription. Virology **158:**79–87.
12. **Gray, W. L., R. P. Baumann, A. T. Robertson, D. J. O'Callaghan, and J. Staczek.** 1987. Characterization and mapping of equine herpesvirus type 1 immediate early, early, and late transcripts. Virus Res. **8:**233–244.
13. **Grundy, F. J., R. P. Baumann, and D. J. O'Callaghan.** 1989. DNA sequence and comparative analyses of the equine herpesvirus type 1 immediate early gene. Virology **172:**223–236.
14. **Kalderon, D., W. D. Richardson, S. F. Markham, and A. E. Smith.** 1984. Sequence requirements for nuclear location of simian virus 40 large-T antigen. Nature (London) **311:**33–38.

14a. **Kim, S. K., R. H. Smith, and D. J. O'Callaghan.** Unpublished data.

15. **Lewis, J. B., Y. G. Thompson, and G. B. Caughman.** 1993. Transcriptional control of the equine herpesvirus 1 immediate-early gene. Virology **197:**788–792.
16. **Martin, K. J., J. W. Lillie, and M. R. Green.** 1990. Transcriptional activation by the pseudorabies virus immediate early protein. Genes Dev. **4:**2376–2382.
17. **Matsumura, T., R. H. Smith, and D. J. O'Callaghan.** 1993. DNA sequence and transcriptional analyses of the region of the equine herpesvirus type 1 Kentucky A strain genome encoding glycoprotein C. Virology **193:**910–923.
18. **McGeoch, D. J., A. Dolan, S. Donald, and D. H. K. Brauer.** 1986. Complete DNA sequence of the short repeat region in the genome of herpes simplex virus type 1. Nucleic Acids Res. **14:**1727–1745.
19. **Mullen, M.-A., D. M. Ciufo, and G. S. Hayward.** 1994. Mapping of intracellular localization domains and evidence for colocalization interactions between the IE110 and IE175 nuclear transactivator proteins of herpes simplex virus. J. Virol. **68:**3250–3266.
20. **O'Callaghan, D. J., G. A. Gentry, and C. C. Randall.** 1983. The equine herpesviruses, p. 215–318. *In* B. Roizman (ed.), The herpesviruses, vol. 2. Comprehensive virology, series 2. Plenum Publishing Corp., New York.
21. **Paterson, T., and R. D. Everett.** 1988. Mutational dissection of the HSV-1 immediate-early protein Vmw175 involved in transcriptional transactivation and repression. Virology **166:**186–196.
22. **Perera, L. P., J. D. Mosca, W. T. Ruyechan, G. S. Hayward, S. E. Straus, and**

J. Hay. 1993. A major transactivator of varicella-zoster virus, the immediate-early protein IE62, contains a potent N-terminal activation domain. Virology **67:**4474–4483.
23. **Purewal, A. S., R. Allsopp, M. Riggio, E. A. R. Telford, S. Azam, A. J. Davison, and N. Edington.** 1994. Equid herpesviruses 1 and 4 encode functional homologs of the herpes simplex virus type 1 virion transactivator protein VP16. Virology **198:**385–389.
24. **Purewal, A. S., A. V. Smallwood, A. Kaushal, D. Adegboye, and N. Edington.** 1992. Identification and control of the *cis*-acting elements of the immediate-early gene of equid herpesvirus type 1. J. Gen. Virol. **73:**513–519.
25. **Robinson, R. A., B. E. Henry, R. G. Duff, and D. J. O'Callaghan.** 1980. Oncogenic transformation by equine herpesviruses (EHV). I. Properties of hamster embryo cells transformed by ultraviolet-irradiated EHV-1. Virology **101:**335–362.
26. **Robinson, R. A., R. B. Vance, and D. J. O'Callaghan.** 1980. Oncogenic transformation by equine herpesviruses. II. Coestablishment of persistent infection and oncogenic transformation of hamster embryo cells by equine herpesvirus type 1 preparations enriched for defective interfering particles. J. Virol. **36:**204–219.
27. **Schwyzer, M., C. Vlcek, O. Menekse, C. Fraefel, and V. Paces.** 1993. Promoter, spliced leader, and coding sequence for BICP4, the largest of the immediate-early proteins of bovine herpesvirus 1. Virology **197:**349–357.
28. **Shepard, A. A., A. N. Imbalzano, and N. A. DeLuca.** 1989. Separation of primary structural components conferring autoregulation, transactivation, and DNA-binding properties to the herpes simplex virus transcriptional regulatory protein ICP4. J. Virol. **63:**3714–3728.
29. **Smith, R. H., G. B. Caughman, and D. J. O'Callaghan.** 1992. Characterization of the regulatory functions of the equine herpesvirus 1 immediate-early gene product. J. Virol. **66:**936–945.
30. **Smith, R. H., Y. Zhao, and D. J. O'Callaghan.** 1993. The equine herpesvirus 1 (EHV-1) UL3 gene, an ICP27 homolog, is necessary for full activation of gene expression directed by an EHV-1 late promoter. J. Virol. **67:**1105–1109.
31. **Smith, R. H., Y. Zhao, and D. J. O'Callaghan.** 1994. The equine herpesvirus type 1 immediate-early gene product contains an acidic transcriptional activation domain. Virology **202:**760–770.
32. **Tyler, J. K., K. E. Allen, and R. D. Everett.** 1994. Mutation of a single lysine residue severely impairs the DNA recognition and regulatory functions of the VZV gene 62 transactivator protein. Nucleic Acids Res. **22:**270–278.
33. **Wu, C.-L., and K. W. Wilcox.** 1990. Codons 262 to 490 from the herpes simplex virus ICP4 gene are sufficient to encode a sequence-specific DNA binding protein. Nucleic Acids Res. **18:**531–538.
34. **Wu, C.-L., and K. W. Wilcox.** 1991. The conserved DNA-binding domains 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.
35. **Zhao, Y., V. R. Holden, R. H. Smith, and D. J. O'Callaghan.** 1995. Regulatory function of the equine herpesvirus 1 ICP27 gene product. J. Virol. **69:**2786–2793.