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Protein and DNA Elements Involved in Transactivation of the Promoter of the Bovine Herpesvirus (BHV) 1 IE-1 Transcription Unit by the BHV α Gene *trans*-Inducing Factor

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In herpes simplex virus (HSV)-infected cells, the transcription of immediate-early (α) genes is regulated by a virion component, the α gene *trans*-inducing factor (αTIF). This protein forms a complex with cellular factors and TAATGARAT motifs present in one or more copies in the promoters of all α genes. We have characterized the bovine herpesvirus 1 (BHV-1) homolog of this protein. Like its HSV counterpart, the BHV αTIF was synthesized in the later stages of infection and could be demonstrated to be a component of purified virions. In transient expression assays, BHV αTIF was a strong transactivator and stimulated the activity of IE-1, the major BHV-1 α gene promoter, with an efficiency comparable to that of HSV αTIF. This stimulation was largely dependent on a TAATGAGCT sequence present in a single copy in IE-1, and BHV αTIF, in conjunction with cellular factors, formed a complex with oligonucleotides containing this sequence. Despite these similarities between the two αTIFs, our preliminary observations suggest that the proteins may activate transcription by different mechanisms. Although BHV αTIF strongly transactivated IE-1, it differed from its HSV counterpart in that the carboxyl terminus of BHV αTIF, when fused to the DNA-binding domain of GAL4, was a relatively poor stimulator of a promoter containing GAL4-binding sites. Also unlike HSV αTIF, removal of the carboxyl terminus of BHV αTIF reduced but did not eliminate the ability of the protein to transactivate IE-1. These results are discussed in view of the structural similarities and differences among the αTIFs of alphaherpesviruses.

In cells infected lytically with herpes simplex virus (HSV), the expression of viral genes is coordinately regulated. Viral genes can be categorized as immediate-early (IE or α), early (E or β), delayed early (DE or βγ), or late (L or γ) (45), depending on when they are expressed during the lytic cycle. The transcription of α genes is induced by a component of the infecting virion, which has been variously termed VP16, Vmw65, or α gene *trans*-inducing factor (αTIF) (3, 4, 39). Activation of α genes by the αTIF requires the TAATGARAT sequence (in which R is a purine), which often overlaps the binding motif of the cellular transcription factor Oct-1 and is present in one or more copies in the promoters of α genes (27, 37, 40, 53, 57). Unlike many other transcription activators, αTIF does not itself bind DNA but rather forms complexes with the Oct-1 which directly binds the TAATGARAT motif (2, 13, 23, 28, 30, 38, 41, 50). Another cellular component, CFF or HCF, is required for this association (13, 21, 22, 58, 62).

The αTIF contains two distinct functional domains. The amino-terminal portion of the protein, consisting of 389 amino acids, is sufficient to form a complex with Oct-1, CFF, and the TAATGARAT motif but lacks the elements needed for activating transcription. The carboxyl-terminal 72 amino acids of αTIF are not involved in complex formation with CFF and Oct-1 but contain domains required for transcriptional activation. The carboxyl terminus of αTIF constitutes a powerful activation domain which, when it is linked with a heterologous DNA-binding domain (such as that of the yeast protein GAL4), can activate genes containing GAL4 DNA-binding sites extremely efficiently (9, 47).

Although viral gene expression has been most extensively studied for HSV-infected cells, other members of the *Alphaherpesvirinae* appear to follow a similar transcriptional program in that the genes of these viruses also have the characteristics of α, β, βγ, and γ genes. Proteins structurally homologous to many HSV gene products are specified by the other alphaherpesviruses, and genes coding for homologs of HSV αTIF have been identified in the genomes of varicella-zoster virus (VZV) (12, 29), bovine herpesvirus type 1 (BHV-1) (6), and equine herpesvirus types 1 and 4 (EHV-1 [43] and EHV-4 [42]). Like HSV αTIF, the αTIFs of VZV (38), EHV-1 (24, 48), and EHV-4 (47) can activate the transcription of genes linked to α gene promoters.

The genome of BHV-1 contains an open codon reading frame (ORF), the deduced amino acid sequence of which has considerable homology with HSV αTIF (6). We characterized further the putative BHV αTIF to determine if it could transactivate BHV-1 α promoters with mechanisms similar to that of its HSV type 1 (HSV-1) counterpart. We show that BHV αTIF can indeed transactivate BHV α gene promoters with efficiencies comparable to that of HSV αTIF and that this process requires recognition of a TAATGARAT-like motif by BHV αTIF and cellular factors. However, unlike HSV αTIF, efficient activation of transcription by BHV αTIF requires portions of the protein in addition to its carboxyl terminus

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alone. These results are discussed in light of the structural similarities and differences between the two proteins.

MATERIALS AND METHODS

Tissue culture. Madin-Darby bovine kidney (MDBK), bovine turbinate (BT), COS-7, HeLa, IMR32, and Vero cells were purchased from the American Type Culture Collection. Nonbovine cells were grown in Dulbecco's modified Eagle's medium (Gibco) supplemented with gentamicin or penicillin-streptomycin and 10% newborn calf serum. For growing MDBK and BT cells, the medium was supplemented with 10% horse serum instead of bovine serum. LM-1 bovine endothelial cells were obtained from Lyn Warren (Veterinary Infectious Disease Organization, University of Saskatchewan). These cells were grown in RPMI 1640 containing 20% fetal bovine serum. The cells were labelled metabolically with [^{35}S]methionine as described previously (32). Details of virus purification (32), immunoprecipitation (33), transfection, and chloramphenicol acetyltransferase (CAT) assays (37) have also been published.

Antibodies. The production of antiserum against BHV αTIF is described below, while that of antisera against BHV-gB (35) and VP8 (5) has been published elsewhere. Antiserum against amino acids 1 to 147 of GAL4 was provided by Ivan Sadowski (University of British Columbia).

Plasmids. Plasmids (pM1, pM2, and pM3) for constructing GAL4 fusion proteins, as well as pG5EC, a plasmid containing the coding sequences of the gene for CAT linked to five GAL4-binding sites, were obtained from Ivan Sadowski (46). The plasmids pJuC, containing the *Hin*dIII C fragment of BHV-1 (59), and pIE-2, in which the BHV-1 IE-2 promoter is linked to the coding sequences of CAT, were obtained from Cornel Fraefel and Martin Schwyzer (Universität Zürich). The plasmid pGEXKG for constructing glutathione *S*-transferase (GST) fusion proteins was obtained from Gerry Weinmaster (University of California, Los Angeles).

To construct pcBTIF, an *Eco*RI site was introduced by site-directed mutagenesis 14 nucleotides upstream from the first ATG in the coding sequence of BHV αTIF. An oligonucleotide, GCCGAGTTGAATTCTCTTTGGGATG, was annealed to a deoxyuracil-substituted single-stranded DNA template from an M13 clone with a *Sac*I-*Pst*I insert containing sequences for the amino terminus of BHV αTIF. Details of the procedure for mutagenesis have been published elsewhere (31). An *Eco*RI-*Pst*I fragment generated using the *Eco*RI site thus constructed was cloned along with a *Pst*I-*Hin*dIII fragment and a *Hin*dIII-*Sal*I fragment to reassemble the entire BHV αTIF coding sequence in pcDNA (Invitrogen). In this construct, the coding sequences of BHV αTIF were regulated by the cytomegalovirus (CMV) IE promoter. The plasmid pGEX-BHV-αTIF was constructed by cloning an *Eco*RI-*Xba*I fragment, removed from pcBTIF, into pGEX-KG.

To construct pcBTIFΔ417, containing the coding sequences for BHV αTIF truncated at codon 418, a PCR product derived from pcBTIF by using the oligonucleotides CCAGTGTGCT GGAATTCTCTTTGGGATG and GCTCGCTCTAGAGGC TTAAGGCGCCTGAGC was cloned between the *Eco*RI and *Xba*I sites of pcDNA. PCR was performed using standard reaction conditions (54), except that the reaction mixture contained 20% glycerol, 0.35 mM $MgCl_2$, and 20 ng of template. The reaction mixture was heated at 94°C for 3 min, which was followed by 30 cycles of 1 min and 18 s at 96°C, 1 min and 36 s at 53°C, and 2 min and 30 s at 71°C. The reaction was terminated after 3 min at 71°C. The PCR product was recovered from an agarose gel, digested extensively with *Eco*RI and *Xba*I, recovered again after electrophoresis from an agarose gel, and ligated to pcDNA restricted with *Eco*RI and *Xba*I.

The plasmid pIE-1 was constructed by replacing a *Sph*I-*Xho*I fragment in pBLCAT2 with a 2.3-kb *Sph*I-*Xho*I fragment from pJuC which contained sequences that lie upstream from the TU-1 transcription unit of BHV-1 (see Fig. 3) (60).

The plasmids pMBTIF333-505 and pMBTIF374-505 were constructed by cloning *Hin*dIII-*Xba*I and *Sau*3A-*Xba*I fragments containing DNA coding for amino acids 333 to 505 and 374 to 505 of BHV αTIF into pM1. To construct pMBTIF408-505, the region of BHV αTIF coding for amino acids 408 to 505 was recovered by PCR with oligonucleotides CGGGATC CCTGCCACACATCTGGCTCAGG and GCTCGCTCTAG AGGCCTTACGCCAGAGGG and pcBTIF. The PCR product was purified as described above and cloned between the *Bam*HI and *Xba*I sites of pM1.

pMBTIF1-71 was constructed by cloning the PCR product of primers CCAGTGTGCTGGAATTCTCTTTGGGATG and CAGCTCGGTCTAGATTCACTCCAGCAACGC into the *Eco*RI and *Xba*I sites of pM3.

The plasmid pLSO-TAATGAGCT was constructed by ligating the oligonucleotides CTCCTATTATAATGAGCTTGGC GGCGA and ACGTGAGGATAATATTACTCGAACCGCC GCTCTAG between the *Pst*I-*Bam*HI sites of pSO.puc, which has a portion of the multiple cloning site from pUC and minimal promoter sequences of the HSV-1 thymidine kinase (TK) gene (up to nucleotide −119) cloned upstream from the coding sequence of CAT (37).

Construction of sequential deletions and mutations in IE-1. The IE-1 promoter was excised from pIE-1 with *Sph*I and *Xho*I and cloned at the same sites in pSP73 (Promega). The resulting plasmid was digested with *Kpn*I and *Bam*HI to yield a distal exonuclease III-resistant end and a proximal exonuclease III-sensitive end. Sequential deletions were generated using exonuclease III as described by Sambrook et al. (48), except that the digests were extracted with phenol-chloroform after treatment with S1 nuclease.

Mutagenesis of IE-1. A *Sph*I-*Xho*I fragment containing the IE-1 promoter was cloned into M13 and mutagenized as described earlier (31) with the oligonucleotide GGTTCGC CGCCA (XXXXXXXXX) TAATAGGAGCGG (where X represents any nucleotide) as a mutagenic primer. Mutants were screened by sequencing with a Sequenase 2 kit with 7-deaza-dGTP (U.S. Biochemicals).

Purification of GST BHV αTIF and preparation of antiserum. A liter of L broth containing 50 μg of ampicillin per ml was inoculated from a log-phase culture of TOPP-1 cells (Stratagene) containing pGEX-BTIF. After 1 h at 37°C, isopropyl-β-D-thiogalactopyranoside was added to 0.1 mM, and incubation was continued for an additional 3 h. The cells were then washed in phosphate-buffered saline (PBS) and resuspended in 3 ml of lysis buffer (150 mM NaCl, 50 mM sodium phosphate [pH 7.0], 10 mM mercaptoethanol, 10 mM EDTA, 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride) per g of cells. The cells were then lysed in a French press, and the debris was removed by centrifugation at 8,000 rpm in a Sorvall SS34 rotor.

Glutathione agarose (100 mg; Sigma) was hydrated in 50 ml of water for 2 h, washed with 50 ml of PBS containing 150 mM NaCl (MTPBS), and resuspended in 2 ml of MTPBS. One milliliter of glutathione agarose was added to the cell lysate, which was agitated in the cold for 1 h. The agarose was washed three times with MTPBS, and the GST–BHV αTIF fusion protein was eluted in twice the bed volume (1 ml) of 10 mM glutathione (Sigma) in 50 mM Tris (pH 8.0). The concentra-

tion of protein was measured by using a Bio-Rad protein determination kit and was about 2 mg of protein per ml. Most of the protein in the eluate was in a single polypeptide with a molecular weight of about 100,000.

Two rabbits were immunized using 150 μg of protein in Freund's complete adjuvant. Two weeks following the primary immunization, the rabbits were given booster injections with 150 μg of protein in Freund's incomplete adjuvant and bled for antiserum 2 weeks later.

Extracts of transfected cells and mobility shift assays. COS-7 cells (10^7) in 80-mm-diameter tissue culture dishes were transfected with 20 μg of plasmid DNA (15). Forty-eight hours after transfection, the cells were washed and scraped in 2 ml of PBS. Cell extracts were prepared as described by Wu et al. (61). After pelleting, the cell pellet was resuspended in 200 μl of a buffer containing 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.9), 0.4 M NaCl, 0.1 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetra-acetic acid (EGTA), 0.5 mM dithiothreitol, 5% glycerol, and 0.5 mM phenylmethylsulfonyl fluoride. The cells were lysed by freezing in a dry ice and ethanol bath followed by rapid thawing at 37°C. Cell debris was removed by centrifugation at 11,000 × *g* for 30 min at 4°C, and the supernatant was aliquoted and frozen at −70°C.

Oligonucleotides (20 pmol) representing the BHV-1 TAAT GAGCT and flanking sequences (CTCCTATTATAATGAG CTTGGCGGCGA and GAGGATAATATTACTCGAACC GCCGCT) in 0.1 M Tris (pH 8.0)–0.05 M $MgCl_2$–5 mM dithiothreitol were labelled with polynucleotide kinase and 50 μCi of [γ-^{32}P]ATP for 30 min at 37°C. Incubation was continued for an additional 60 min with 25 mM unlabelled ATP, and the reaction was terminated by heating to 65°C for 10 min. The oligonucleotides were separated from unincorporated material on a Sep-pak column (Waters). NaCl concentration was adjusted to 0.15 M, and the oligonucleotides were allowed to anneal by slowly cooling to room temperature after heating to 65°C for 10 min.

Cell extract (10 μg of protein), 1 μg of bovine serum albumin, and 0.1 μg of poly(dI-dC) were mixed in buffer containing 45 mM KCl, 25 mM HEPES, 1 mM EDTA, 0.5 mM dithiothreitol, and 5% glycerol. After incubation for 5 min at 30°C, 10,000 cpm of labelled oligonucleotides was added, and incubation continued for 30 min. To some samples, antiserum was added after 15 min at 30°C, which was followed by incubation for another 30 min. The reaction mixtures were electrophoresed through a 4% polyacrylamide gel in 0.05 M Tris–0.4 M glycine–2 mM EDTA. The gels were then dried and autoradiographed.

RESULTS

BHV αTIF is a DE (βγ) virion protein. Recently, we reported the nucleotide sequence of a BHV-1 ORF with considerable homology to HSV *UL48* (6). On the basis of this homology, we suggested that the BHV-1 ORF may code for the homolog of the product of HSV *UL48*, the HSV αTIF. However, at that time we had no evidence for the existence of the protein encoded by the ORF.

To detect BHV αTIF, we linked the putative BHV αTIF ORF to the coding sequences for the amino terminus of GST in pGEXKG and raised antibodies against purified GST–BHV αTIF fusion protein. The antibodies precipitated specifically a 68,000-molecular-weight protein from BHV-1-infected cells (Fig. 1).

To examine the time course of BHV αTIF synthesis in infected cells, we immunoprecipitated proteins from lysates of BHV-infected cells, labelled with [^{35}S]methionine (2-h periods) at different times after infection, with antiserum against BHV αTIF (Fig. 1A). To identify whether BHV αTIF could be classified as an E (β) protein made in the absence of viral DNA replication, we also immunoprecipitated proteins from cells radiolabelled 0 to 13 h after infection and treated with phosphonoformic acid (PFA), an inhibitor of BHV-1 DNA replication (33). As a control, untreated infected cells, labelled 0 to 13 h after infection, were used for immunoprecipitation. For comparative purposes, we examined the synthesis of BHV gB, a β-class BHV-1 glycoprotein, from the same samples, using a polyclonal serum against BHV gB (Fig. 1B). Figure 1A shows that the synthesis of BHV αTIF reached a maximum 7 to 9 h after infection, which was followed by a decline in synthesis. In the absence of viral DNA replication (PFA), synthesis of the protein was reduced significantly but was not eliminated completely, suggesting that BHV αTIF is a DE or βγ class protein. In contrast (Fig. 1B), the synthesis of BHV gB, an E or β-class protein (35), and its cleavage products, gBb and gBc, reached a maximum between 4 and 6 h after infection and was not diminished by the interruption of viral DNA replication. (Note that sera against both BHV αTIF and BHV gB nonspecifically precipitated two host cell polypeptides. These polypeptides were not labelled in cells later in the infectious process and were therefore not visible in cells labelled from 7 h after infection.)

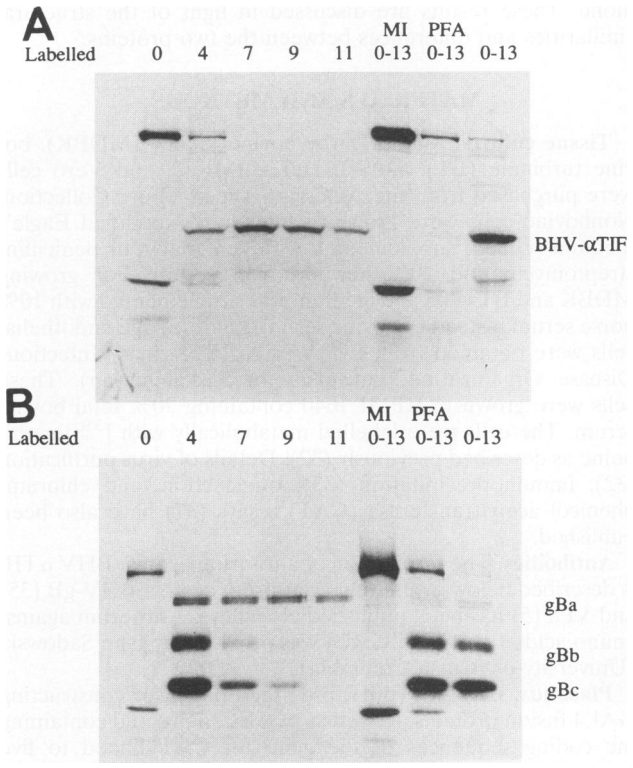

FIG. 1. Comparison of time of synthesis and temporal class (β or γ) of BHV αTIF with those of glycoprotein B. BHV-1-infected or mock-infected (MI) cells were labelled with [^{35}S]methionine for 2-h intervals at 0, 4, 7, 9, and 11 h after infection or were labelled continuously from 0 to 13 h after infection in the presence or absence of PFA. Cell lysates were then immunoprecipitated either with anti-BHV αTIF rabbit serum (A) or with anti-BHV gB rabbit serum (B). The positions of BHV αTIF and gB and its cleavage products gBb and gBc are indicated in the right margin.

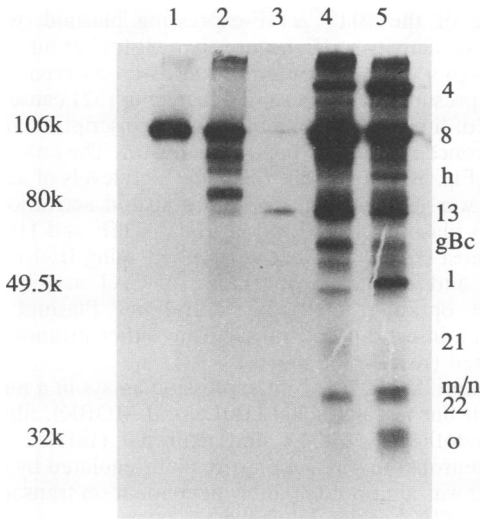

FIG. 2. BHV αTIF is associated with BHV-1 virions. [^{35}S]methionine-labelled infected-cell lysate (lane 5), purified virions (lane 4), BHV αTIF immunoprecipitated from purified virions with anti-BHV αTIF rabbit serum (lane 3), and VP8 immunoprecipitated with anti-VP8 rabbit serum from infected-cell lysate (lane 2) or from purified virions (lane 1) were separated by electrophoresis on SDS-polyacrylamide gels and autoradiographed. The positions of molecular weight standards are listed in the left margin. Designations in the right margin of structural proteins (numbered, except for the small fragment of gB) and nonstructural proteins (lettered) are according to Misra et al. (32). The prefixes VP (virion protein) and ns (nonstructural protein) have been omitted.

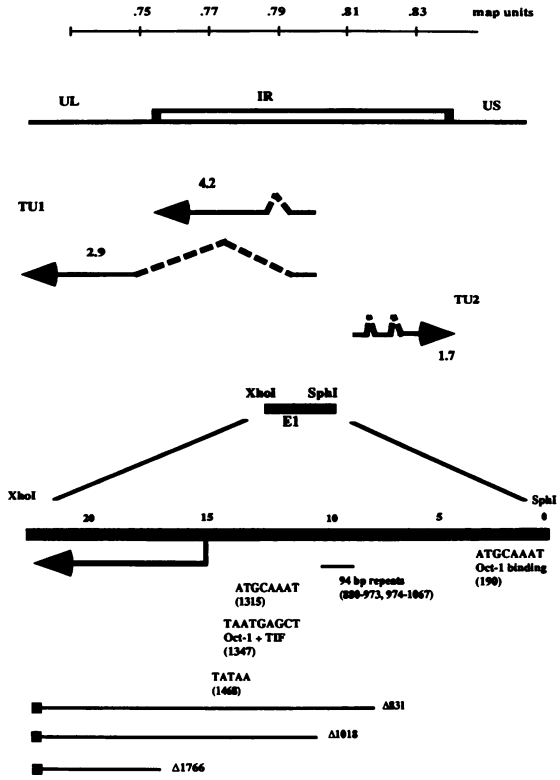

FIG. 3. Major IE (α) transcripts of BHV-1 (information from Wirth et al. [60] and Schwyzer et al. [49]): a portion of the BHV-1 genome from map unit 0.74 to 0.84 including portions of the unique long (UL), unique short (US), and internal repeat (IR). The main transcription units, TU1 and TU2, are shown as arrows with spliced introns designated by interrupted lines. The location of the *Sph*I-*Xho*I fragment used as the IE-1 promoter is shown as a shaded box. The lower portion of the figure shows salient features in the IE-1 promoter and endpoints of deletion mutants used in the study. Numbers above the line indicate lengths in base pairs (×100). Features are shown with the locations in base pairs from the *Sph*I site in brackets. Deletion mutants used in the experiments are represented by lines, with the numbers after the Δ indicating the endpoints of the deletion in base pairs from the *Sph*I site. The position at which transcription begins is indicated by an arrow. The sequence of the fragment (accession no. 14320 [49]) was obtained from GenBank.

To determine if, like HSV αTIF, the BHV-1 protein was associated with virions, we purified ^{35}S-labelled BHV-1 virions by differential centrifugation, which was followed by banding in potassium tartrate gradients (32). Samples of cell lysate, purified virions, and proteins immunoprecipitated from cell lysates and purified virions were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). As a control, we used polyclonal serum raised against the tegument protein VP8, which precipitated VP8 from purified virions (Fig. 2, lane 1) and VP8 as well as some other proteins from infected-cell lysates (lane 2). This serum was raised against VP8 purified by SDS-PAGE from cell lysates (5) and reacted with infected-cell proteins with similar electrophoretic mobilities. The virions were relatively free of nonstructural proteins, since the serum precipitated only VP8 from the purified virion preparation. Lane 3 of Fig. 2 shows that antibodies against GST–BHV αTIF precipitated a polypeptide from purified BHV-1 virions that we had previously identified as the phosphoprotein VP13 (32, 55b). Lysates of BHV-1-infected cells (lane 5) contained BHV-1 structural proteins (numbered) as well as nonstructural proteins (lettered, of which h, l, and o were the most obvious examples). Purified virions were free of detectable levels of nonstructural proteins and were enriched for structural proteins (lane 4) compared with the infected-cell lysate (lane 5). Purified virions contained relatively large amounts of VP13 (lane 4). These results suggest that like its HSV-1 homolog, BHV αTIF is associated with virions.

BHV αTIF transactivates the expression of genes regulated by the BHV-1 IE promoters. BHV-1 contains two main IE or α transcription units (Fig. 3) (60). These units, TU-1 and TU-2, extend in opposite directions in the inverted repeats bracketing the unique short segment of the BHV-1 genome. To examine the effects of BHV αTIF on IE-1, the promoter of TU-1, we linked a 2.3-kb *Sph*I-*Xho*I fragment to the coding sequences for CAT. This fragment contains 1.5 kb of sequences upstream from the start of TU-1 as well as about 850 bp of the transcription unit itself, including the splice donor and acceptor sites (Fig. 3) (49, 60). We designated this construct pIE-1. A construct in which the sequences upstream from TU-2 were cloned in a similar vector was obtained from C. Fraefel and Martin Schwyzer (Universität Zürich) and was termed pIE-2.

To assess the level of activity of the IE-1 and IE-2 promoters without viral transactivators, we measured CAT activities for cells transfected with pIE-1 and pIE-2. Cells transfected with plasmids in which promoters for the HSV-1 α proteins ICP0 (IE110 k) and ICP4 (IE175 k) were linked to CAT were assayed as controls.

When introduced into Vero cells, pIE-1 had extremely low constitutive activity even at DNA concentrations of as high as 500 ng per culture (Fig. 4). In contrast, IE-2 and the promoters for the HSV α genes, ICP4 and ICP0, induced considerable

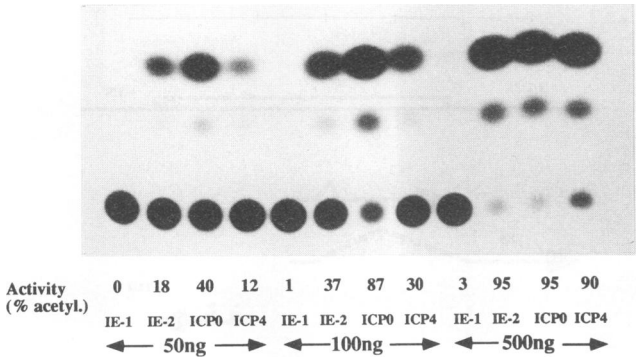

FIG. 4. Constitutive activity of BHV-1 IE-1 and IE-2 and HSV-1 ICP0 and ICP4 promoters in Vero cells. Vero cells were transfected with 50, 100, or 500 ng of plasmids containing the promoters linked to coding sequences for CAT. Cells were harvested and CAT activities were measured 40 h after transfection. Activity is expressed as a percentage of radioactivity in acetylated forms divided by the sum of the radioactivity in acetylated and unacetylate forms of chloramphenicol.

CAT activity, even when as little as 50 ng of DNA was introduced into Vero cells.

To determine if BHV-αTIF could transactivate the BHV-1 IE promoters, we introduced pIE1 or pIE2 mixed with various amounts of vectors expressing BHV αTIF or HSV αTIF into Vero cells. Figure 5A shows that BHV αTIF was a strong *trans* activator of IE-1. Activation of the promoter was dependent on

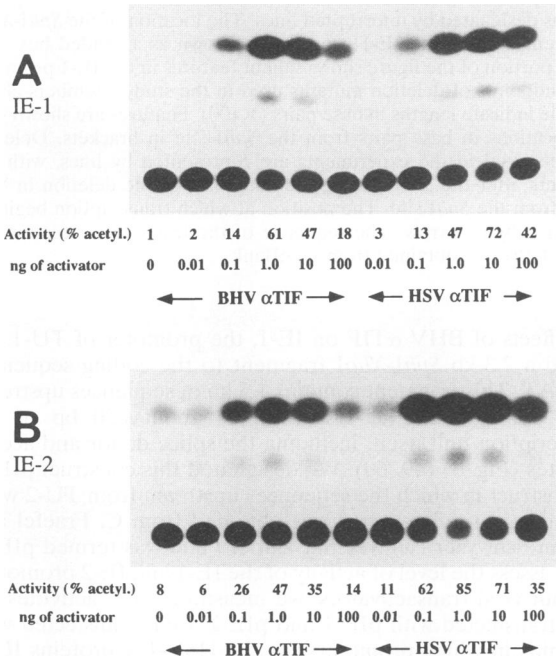

FIG. 5. Activation of BHV-1 IE-1 and IE-2 promoters by BHV αTIF or HSV αTIF. Vero cells were transfected with either 500 ng of pIE-1 (A) or 100 ng of pIE-2 (B) and various amounts of a plasmid containing either BHV αTIF (pcBTIF)- or HSV αTIF (pRG50)-coding sequences linked to the CMV IE promoter. Cells were harvested and CAT activities were measured 40 h after transfection.

the dose of the BHV αTIF-expressing plasmid, which increased the activity of IE-1 more than 60-fold at an input of 1 ng. At higher plasmid concentrations, we observed less CAT activity, presumably because of squelching (52) caused by the removal of limiting components of the transcription apparatus by high concentrations of the transactivator. The enhancement of IE-2 (Fig. 5B), which has relatively high levels of activity on its own, was less pronounced with a sixfold activation at the optimum plasmid concentration. BHV αTIF and HSV αTIF were almost equally efficient at transactivating IE-1 (Fig. 5A), with 61- and 72-fold enhancements of CAT activity, respectively, at optimum DNA concentrations. Plasmid pcDNA without an insert did not transactivate either promoter at any dose tested (results not shown).

We found that in transient expression assays in a number of other cell lines of epithelial (HeLa and MDBK), fibroblastic (BT), endothelial (LM-1), and neuronal (IMR32; primary bovine neurons) origin, gene expression regulated by the IE-1 promoter was almost completely dependent on transactivation by BHV αTIF (results not shown).

Transactivation of IE-1 by BHV αTIF requires a TAAT GAGCT motif. The transactivation of HSV α gene promoters requires TAATGARAT motifs that form binding sites for the HSV αTIF, CFF, and Oct-1 complex. To identify possible *cis*-acting sequences that may be involved in the activation of IE-1 by BHV αTIF, we scanned the nucleotide sequence of IE-1 for the presence of octamer or TAATGARAT-like motifs. Some of these sequences, as well as others that are potentially involved in transcription regulation, are depicted in Fig. 3. IE-1 contains the sequence TAATGAGCT at nucleotide position 1347 (from the *Sph*I site). This sequence differs from the consensus TAATGARAT motif at only one position. There are two potential Oct-1-binding sites, ATGCAAAT, at positions 190 and 1315. The fragment also contains two 94-bp repeats at positions 880 and 974, which are reminiscent of the α gene enhancers of pseudorabies virus (20), and a potential TATAA box at position 1468. To examine the role of these motifs in the transactivation of IE-1 by BHV αTIF, we constructed a series of mutations in which we deleted sequences from the 5′ end of the fragment. Three of these mutations were examined in transient expression assays. IEΔ831 (the numbers indicate the endpoint of the deletion in nucleotides from the *Sph*I site) lacks the potential Oct-1-binding site at position 190; IEΔ1018 also lacks the 94-bp repeats but retains ATGCAAAT at 1315 and TAATGAGCT at 1347. All potential promoter sequences have been removed in IEΔ1766.

Figure 6A shows that both BHV αTIF and HSV αTIF transactivated IEΔ831 and IEΔ1018, although the promoter strength of these constructs was reduced compared with that of intact IE-1 assessed in parallel (compare with the first set of tracks in Fig. 6B). As expected, IEΔ1766 was not transactivated by either αTIF. These data suggested that although sequences upstream from nucleotide 1018 may contribute to the magnitude of the activation, sequences in the fragment extending from position 1018 to the *Xho*I site were sufficient for an efficient response by the αTIFs. This fragment contains a potential Oct-1-binding site and the TAATGAGCT sequence. Since the TAATGAGCT sequence resembles the TAATGA RAT motif, we examined the effects of alterations in this sequence (Fig. 6B). Nucleotides in the TAATGAGCT sequence were mutagenized randomly in the context of the entire *Sph*I-*Xho*I fragment, and two mutants, each with several alterations in the TAATGAGCT sequence, were examined (Fig. 6B). Both mutants appeared to exhibit greatly reduced levels of transactivation by BHV αTIF and HSV αTIF (Fig.

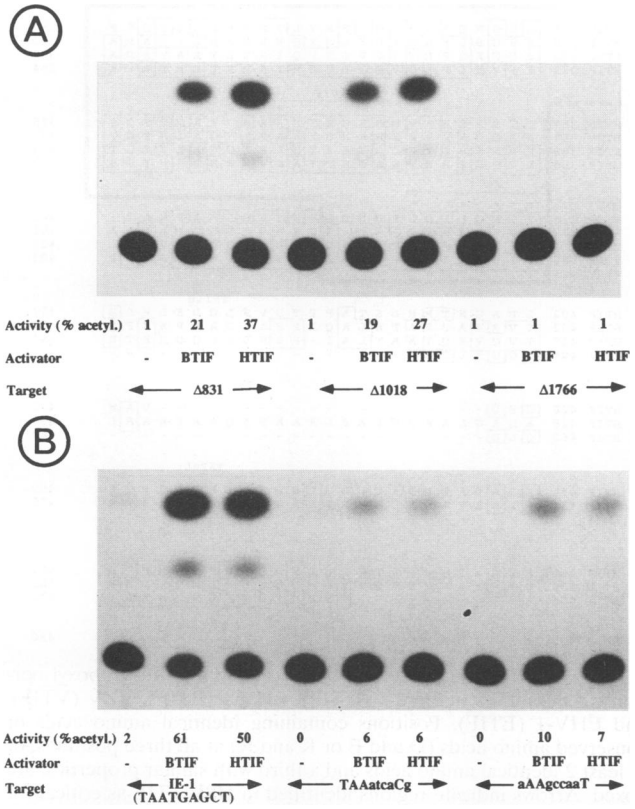

FIG. 6. Effect of deletion of sequences in the IE-1 promoter (A) or mutations in the TAATGAGCT sequence (B) on inducibility by BHV αTIF or HSV αTIF. Vero cells were transfected with 500 ng of plasmids containing fragments of IE-1 from which either 831, 1,018, or 1,766 nucleotides from the *Sph*I site had been deleted (A) or pIE-1 or IE-1 with mutations in the TAATGAGCT sequence (B). Differences from the TAATGAGCT sequence are indicated by lowercase letters. Each transfection also contained 1 ng of either pcDNA, a plasmid with no insert (−), or BHV αTIF (BTIF)- or HSV αTIF (HTIF)-coding sequences linked to the CMV IE promoter. Cells were harvested and CAT activities were measured 40 h later.

6B). However, since the mutants exhibited no detectable basal activity (compared with the very low basal activity of IE-1), it was not possible to unequivocally state that the reduction was due entirely to a reduction in αTIF-mediated transactivation. To clarify the role of the TAATGAGCT sequence, we determined if a single copy of the sequence, when introduced into a basal promoter, could allow BHV αTIF-mediated transactivation.

We inserted oligonucleotides containing the TAATGAGCT sequence as well as nine nucleotides flanking it on either side upstream of the basal HSV-1 TK promoter in pLSO-pUC. This plasmid was designated pLSO-TAATGAGCT. We then compared the abilities of BHV αTIF to stimulate CAT activity in cells transfected with this plasmid, pLSO-pUC, and pIE-1 (Fig. 7). While BHV αTIF had no effect on the minimal TK promoter in pLSO-pUC, the insertion of a single TAAT GAGCT site (pLSO-TAATGAGCT) could confer BHV αTIF responsiveness to this promoter.

When taken together, the data from these experiments suggest that transactivation of the IE-1 promoter by BHV αTIF may involve the TAATGAGCT sequence.

The TAATGAGCT sequence forms a complex with BHV

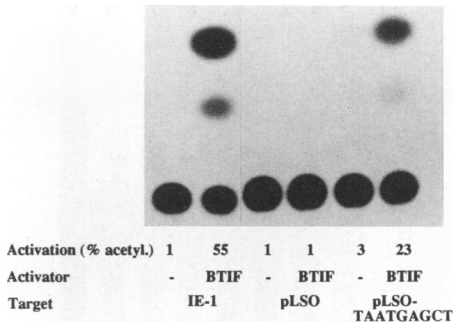

FIG. 7. The TAATGAGCT sequence is sufficient for BHV αTIF-mediated transactivation. Vero cells were transfected with 500 ng of pIE-1, pΔ1018, pLSO (a plasmid containing the minimal HSV-1 TK promoter), or pLSO in which an oligonucleotide containing the TAATGAGCT sequence had been inserted. Each transfection mixture also contained 1 ng of either pcDNA, a plasmid with no insert (−), or BHV-αTIF (BTIF)- or HSV-αTIF (HTIF)-coding sequences linked to the CMV IE promoter.

αTIF and cellular factors. Since our data suggested that the TAATGAGCT sequence may be involved in the ability of BHV αTIF to transactivate IE-1, we determined if this sequence could form complexes with extracts from infected cells. We constructed two homologous 27-bp oligonucleotides that contained sequences from positions 1338 to 1364 of IE-1, including the TAATGAGCT sequence. In band shift assays, these oligonucleotides formed a complex with extracts from uninfected BT cells (solid arrow in Fig. 8). Extracts from BT cells infected with BHV-1 formed this complex, as well as an additional more slowly migrating complex (striped arrow in Fig. 8). Addition of a 100-fold excess of unlabelled oligonucleotide (competitor) abolished the formation of both labelled complexes. Similar results were obtained with MDBK cells infected with BHV-1 (results not shown). These results sug-

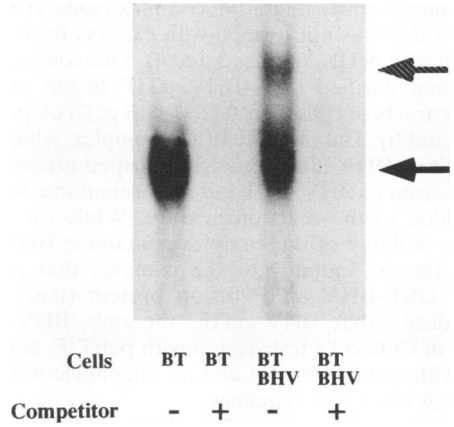

FIG. 8. Binding of the TAATGAGCT sequence by proteins from mock-infected and BHV-1-infected BT cells. ^{32}P-labelled double-stranded oligonucleotide containing the IE-1 TAATGAGCT sequence and nine nucleotides flanking it on either side were incubated with extracts from mock-infected or BHV-1-infected BT cells and were analyzed on a nondenaturing gel. Parallel reaction mixtures (+) were treated with a 100-fold excess of unlabelled oligonucleotide. Solid arrow indicates the position of complex(s) formed with cellular factors, while the striped arrow indicates the position of complex that may contain an infected-cell protein as well.

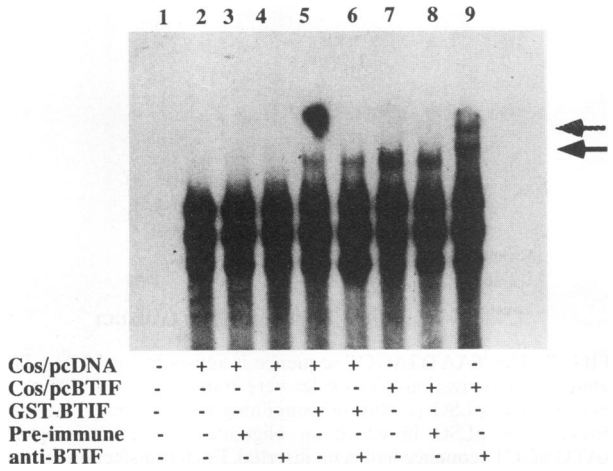

FIG. 9. Complexes formed with BHV αTIF (BTIF), TAAT GAGCT, and cellular factors. ^{32}P-labelled double-stranded oligonucleotide (27 bp) containing the IE-1 TAATGAGCT sequence and nine nucleotides flanking it on either side were incubated with extracts from COS cells transfected with either pcDNA or pcBTIF and were analyzed on nondenaturing polyacrylamide gels. +, reaction mixtures contained, in various combinations, GST-BTIF, serum against BHV αTIF (anti-BTIF), or preimmune serum. Solid arrow indicates the position of complex containing oligonucleotides, cellular factors, and BHV αTIF. Striped arrow indicates the position of this complex shifted by association with antibody.

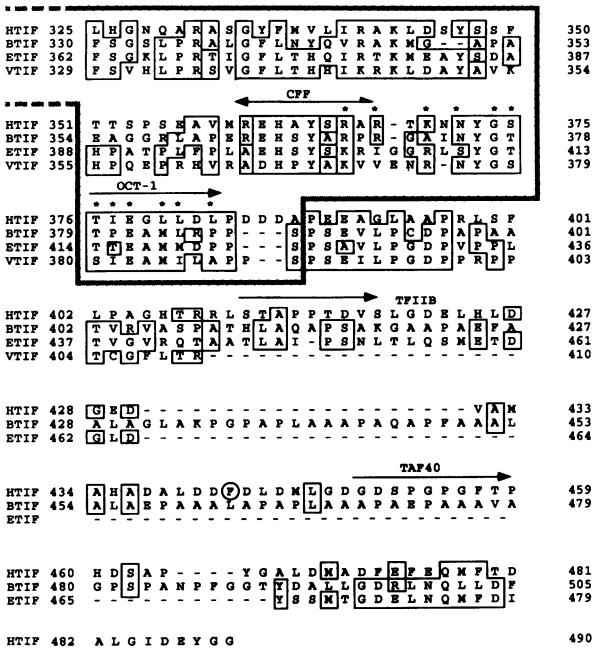

FIG. 10. Alignment of amino acid sequences of the carboxyl portions of the αTIFs of HSV-1 (HTIF), BHV-1 (BTIF), VZV (VTIF), and EHV-1 (ETIF). Positions containing identical amino acids or conserved amino acids (D and E or K and R) at all three positions, or at least 2 identical amino acids and a third with similar properties, are boxed. Arrows indicate regions identified in HSV αTIF as critical for binding to CFF (16), Oct-1 (51), TFIIB (8, 14), and TAF40 (14). Shaded box indicates the portion of the displayed portion of HSV αTIF required for complex formation with TAATGARAT and cellular factors (16). The dashes in the box in the top left corner indicate that sequences upstream from the displayed sequence are also required. Asterisks above the HSV αTIF sequence identify positions where changes affect complex formation (16). F442 in HSV αTIF, which is critical for transactivation (11), is circled. Alignment of the published BHV αTIF sequence (6) with the sequences of the HSV, VZV, and EHV homologs led us to think that the sequence at the carboxyl terminus of the ORF may contain some errors. Therefore, we resequenced the ORF using 40% formamide-containing gels (following manufacturer's instructions with the Sequenase 2 kit) and identified three frameshift mutations not observed on sequencing gels containing only urea. The corrected sequence of the carboxyl terminus is displayed in this figure. No errors were found in the amino portion of the sequence. The appropriate corrections have also been made in the sequence in the data base (EMBL accession number Z11610).

gested that BHV-1-infected cells contained proteins that allowed the formation of a unique complex with the BHV-1 TAATGAGCT sequence.

To determine if BHV-αTIF could form a complex with the oligonucleotides, we analyzed extracts of COS cells transfected with either pcBTIF or pcDNA, the parental plasmid lacking BHV αTIF. Figure 9 shows that all cell extracts contained prominent bands representing complexes formed with cellular factors. In addition, COS cells transfected with pcBTIF contained a unique slowly migrating complex (solid arrow in lane 7 of Fig. 9) that was not formed with extracts from COS cells transfected with pcDNA (lanes 3 and 4). This complex was also formed when purified GST–BHV αTIF fusion protein was added to extracts of cells transfected with pcDNA (solid arrow in lanes 5 and 6). The mobility of the complex, which presumably contained BHV αTIF, changed (striped arrow in lane 9) when antiserum to BHV αTIF but not preimmune serum (lane 8) was added to the reaction mixture. While the antiserum shifted the mobility of the complex containing BHV αTIF, it did not alter the mobility of the complex that presumably contained GST–BHV αTIF fusion protein (lane 6). These results indicate that BHV αTIF, the only BHV-1 protein expressed in COS cells transfected with pcBTIF, could form a complex with cellular factors and an oligonucleotide containing the TAATGAGCT sequence.

Analysis of requirements within BHV αTIF for transcriptional activation. The HSV αTIF contains two distinct modules. The amino-terminal 389 amino acids contain elements required for interaction with Oct-1 and CFF and for assembly of the complex on the α gene promoter-specific TAATGA RAT motif (for a review, see reference 36). While the carboxyl-terminal 90 amino acids are dispensable for these interactions, they are critical for the subsequent activation of transcription. The ability of the carboxyl terminus to activate transcription is not dependent on the amino-terminal 389 amino acids, and it can transactivate genes extremely efficiently when it is linked to an appropriate DNA-binding domain, such as that of GAL4 (9, 47).

There is considerable homology between the αTIFs of HSV and BHV at their amino termini, and several features identified as important for the interaction of HSV αTIF, CFF, and Oct-1 are conserved in BHV αTIF (arrows within the stippled box in Fig. 10) (see Discussion). However, considering the efficiency with which BHV αTIF activates IE-1, there appears to be surprisingly little resemblance between the highly acidic carboxyl terminus of HSV αTIF and the largely hydrophobic carboxyl terminus of BHV αTIF. Few of the features identified as being important for the ability of the carboxyl terminus of HSV αTIF to activate transcription (see Discussion) are retained in the BHV homolog.

To determine if the carboxyl terminus of BHV αTIF could

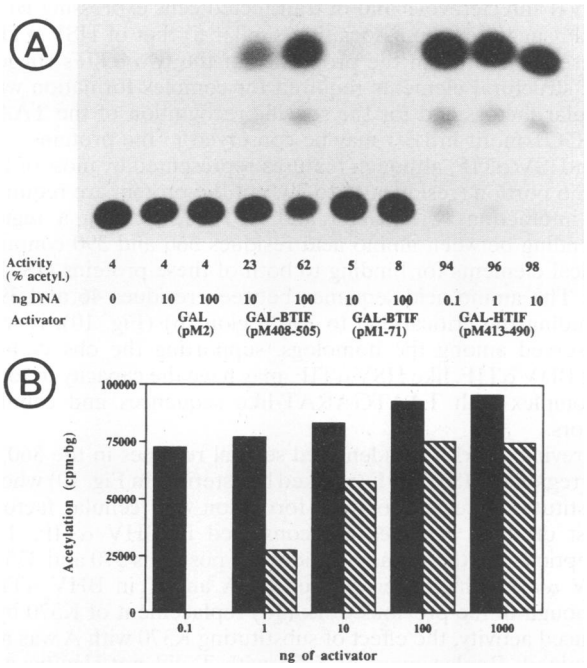

FIG. 11. Activation of a GAL4-binding site containing promoter by GAL4-αTIF fusion proteins. (A) COS cells were transfected with 100 ng of pG5EC (46), a plasmid containing the coding sequences of CAT linked to a promoter containing five UAS sites. Transfection mixtures also contained various amounts (0 to 100 ng) of either pM2, a plasmid containing the DNA (UAS)-binding domain of GAL4, or plasmids in which different segments of BHV αTIF or HSV αTIF were linked to the DNA-binding domain of GAL4. Numbers in brackets indicate the residues of BHV-αTIF (BTIF) or HSV αTIF (HTIF) in the construct. (B) COS cells transfected with 500 ng of pG5EC and various amounts of pMBTIF408-505 (striped bars) and pMHTIF413-490 (black bars). The *y* axis shows counts per minute in acetylated chloramphenicol from which background activation (counts per minute in a sample with pG5EC and 1,000 ng of pM2) has been subtracted.

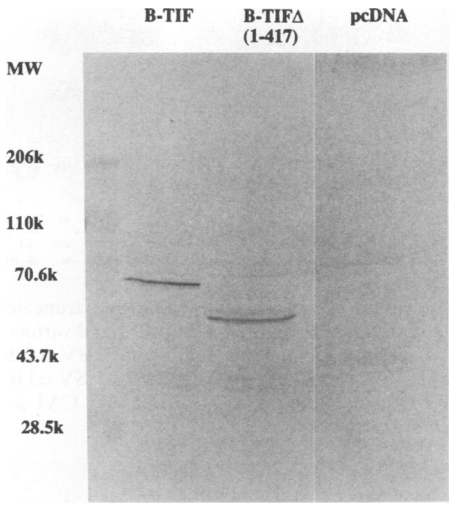

FIG. 12. Expression of full-length and truncated BHV αTIF in COS cells. Cell lysates from COS cells transfected with pcDNA, pcBTIF expressing full-length BHV-αTIF, or pcBTIFΔ, in which a stop codon was introduced at codon 418, were analyzed by SDS-PAGE, blotted to a nitrocellulose membrane, and incubated with anti-BHV αTIF rabbit serum. Numbers in the left margin indicate the position of molecular weight (MW) markers.

function as an activation domain when linked to a DNA-binding protein, we constructed plasmids in which amino acids 333 to 505, 374 to 505, and 408 to 505 of BHV αTIF were linked to the amino-terminal 147 amino acids of GAL4 (pM). Figure 11A and B show the results of two experiments comparing the abilities of pMBTIF408-505 and a construct in which the carboxyl-terminal 77 amino acids of HSV αTIF are linked to GAL4 (pM2VP16 [46], renamed pMHTIF413-490) to activate a promoter containing five GAL4-binding sites. As expected, pMHTIF413-490 (GAL-HTIF in Fig. 11A; solid bars in Fig. 11B) transactivated the promoter very efficiently. Even at 0.1 ng, the lowest concentration of plasmid used, considerable CAT activity was detected. In contrast, a 1,000-fold higher concentration of pMBTIF408-505 (striped bar in Fig. 11B) was required for comparable levels of CAT activity. Similar concentrations of plasmid expressing just the DNA-binding domain of GAL4 (pM2 in Fig. 11A; background in Fig. 11B) or the DNA-binding domain fused to the first 71 amino acids of BHV αTIF (pM1-71 in Fig. 11A) did not activate the promoter. pMBTIF1-71 was also unable to act synergistically with pMBTIF408-505 to activate the promoter (results not shown; see Discussion). The levels of activation achieved by pMBTIF333-505 and pMBTIF374-505 did not exceed that of pMBTIF408-505 (results not shown). To rule out the possibility that the relative inability of pM408-505 to transactivate was due to insufficient protein synthesized in COS cells, extracts from cells transfected with equivalent amounts of pM2, pMBTIF408-505, or pMHTIF413-490 were analyzed in Western blots (immunoblots) using antiserum against amino acids 1 to 147 of GAL4. The serum specifically identified the GAL fusion proteins with molecular weights of approximately 28,000 in the cell lysates. The band corresponding to GAL-BTIF was about twice as intense as that for GAL-HTIF (data not shown), indicating that the relative inability of GAL-BTIF to transactivate could not be attributed to lack of its synthesis in COS cells.

These data suggested either that the carboxyl terminus of BHV αTIF is not its activation domain or that, unlike that of HSV αTIF, it cannot function well as an autonomous module. To explore these possibilities further, we introduced a stop codon at position 418 in the coding sequence of BHV αTIF. We then compared the ability of the truncated BHV αTIF to activate IE-1 with those of full-length BHV αTIF (pcBTIF), full-length HSV αTIF (pRG50), and HSV αTIF truncated at position 410 (pRG14). To establish that the wild-type and mutant BHV αTIF proteins were synthesized in equivalent amounts in transfected cells, we analyzed equal amounts of extracts from cells transfected with plasmids expressing either protein by Western blotting. Figure 12 shows that the cell extracts contained comparable amounts of the two proteins, and, as expected, that the truncated mutant had a higher electrophoretic mobility than BHV αTIF.

The ability of HSV αTIF to activate transcription lies entirely in its carboxyl terminus (15), and, as expected, the removal the carboxyl-terminal 70 amino acids of HSV αTIF completely eliminated the ability of this protein to transactivate IE-1 (Fig. 13). By comparison, the removal of the corresponding portion of BHV αTIF reduced the ability of the protein to transactivate IE-1 by about 70% but did not eliminate it completely (Fig. 13). These results indicated that the carboxyl terminus is required for the optimal level of activation but that the amino terminus may make a significant

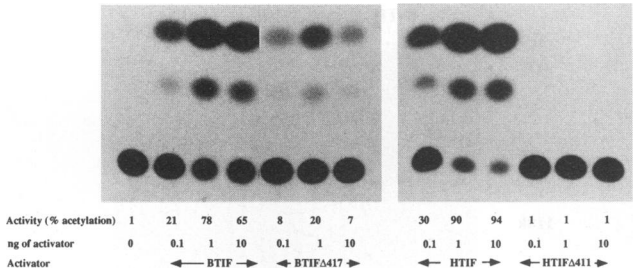

FIG. 13. Activation of IE-1 by full-length and truncated αTIFs. Vero cells were transfected with 500 ng of pIE-1 and various amounts (0 to 10 ng) of plasmids expressing full-length BHV αTIF (BTIF), BHV αTIF truncated at codon 418 (BTIFΔ417), HSV αTIF (HTIF), or HSV-αTIF truncated at codon 412 (HTIFΔ411). CAT activity was measured 40 h after transfection.

contribution. These data are also consistent with the observation that the carboxyl terminus of BHV αTIF, when linked to the DNA-binding domain of GAL4, functioned only as a weak activator. Since the intact BHV αTIF is an efficient transactivator of IE-1, while its carboxyl- and amino-terminal portions separately are weak activators, we are led to the conclusion that unlike its HSV homolog, both portions of BHV αTIF must contribute to the efficient transcriptional activation of the major BHV-1 α gene promoter.

Our results demonstrate a fundamental difference between the HSV αTIF and BHV αTIF. While the carboxyl terminus of the HSV protein contains all attributes needed for transactivation and can function as an autonomous module that is separate from the 420 amino acids of the amino portion of HSV αTIF, the BHV-1 protein may require features contributed by the entire protein, including the carboxyl terminus.

DISCUSSION

Viral gene expression during a lytic infection by several alphaherpesviruses is initiated by the transcription of the IE or α genes (7, 20, 32, 45). In HSV, α gene expression is regulated by HSV αTIF, a component of the infecting virion, which redirects the ubiquitous cellular factor Oct-1 to the activation of viral α promoters. HSV αTIF has well-characterized and functionally separable domains for interaction with cellular factors responsible for promoter recognition and for transcriptional activation. Homologs for HSV αTIF have been described for five other herpesviruses (6, 10, 12, 17, 42). Although all of these homologs (with the exception of that of HSV-2) have little primary structural homology with the transcriptional activation domain of HSV αTIF, it is becoming evident that they may participate in the regulation of α gene expression as well (24, 34, 42). To HSV αTIF homologs with this role we now add BHV αTIF.

Our results show that BHV αTIF is similar functionally to HSV αTIF. It is synthesized in the later stages of infection, and it is associated with virions. At least in transient expression assays, it transactivates an α gene promoter via mechanisms involving similar, TAATGARAT-like sequences and cellular factors. Although we have not yet identified Oct-1 and CFF as components of the complex involving BHV αTIF, cellular factors, and the BHV-1 TAATGAGCT sequence, the major complex formed between cellular factors and the TAAT GAGCT sequence has an electrophoretic mobility similar to that of the HSV TAATGARAT sequence bound to Oct-1 (data not shown). The mobility of this complex is increased in BHV-1-infected cells and in transfected cells expressing BHV αTIF, suggesting an association similar to that of HSV αTIF. These similarities in the properties of the two αTIFs suggest that structural elements required for complex formation with cellular factors and for the specific recognition of the TAAT GAGCT motif in IE-1 may be conserved in the proteins.

In HSV αTIF, although features represented by most of the amino portion (residues 46 to 397) of the protein are required for interactions with Oct-1 and CFF (1, 16, 56), a region extending between amino acid residues 360 and 390 contains critical elements for binding to both of these proteins (18, 19, 51). The amino acid sequence between residues 46 and 397, including the critical 360 to 390 region (6) (Fig. 10), is well conserved among the homologs, supporting the observation that BHV αTIF, like HSV αTIF, may have the capacity to form a complex with TAATGARAT-like sequences and cellular factors.

Previous work (16) identified several residues in the 360 to 390 region of HSV αTIF (marked by asterisks in Fig. 10) whose substitution affected complex formation with cellular factors. Most of these residues are conserved in BHV αTIF. The exceptions are the K and S residues at positions 370 and 375 in HSV αTIF, which are replaced by A and T in BHV αTIF. Although in the previous work (16) replacement of K370 by I reduced activity, the effect of substituting K370 with A was not examined. Replacement of S375 with T did not significantly reduce activation or the ability to form a complex. The replacement of S375 at the corresponding position in BHV αTIF with T would, therefore, not be expected to affect adversely its ability to form a complex.

While most amino acids required by HSV αTIF for complex formation are conserved in BHV αTIF, a critical segment consisting of three consecutive D residues (385 to 387) is not. Deletion of these residues dramatically reduces the ability of HSV αTIF to form a complex with cellular factors and TAATGARAT (16). The D residues are not conserved in either BHV αTIF, VZV αTIF, or EHV αTIF. Although McKee et al. (29) were unable to demonstrate VZV αTIF in a complex with TAATGARAT, we have shown that BHV αTIF can form a complex with cellular factors and the TAAT GAGCT-containing oligonucleotides. Our results are consistent with the findings of Elliott (12a) that EHV αTIF (which also lacks the three D residues) can form a complex as well and suggest that the lack of D385-D387 in the BHV-1 and EHV-1 proteins does not interfere sufficiently with complex formation to prevent transactivation of α promoters.

Since all αTIFs examined to date can transactivate homologous α gene promoters, the dramatic differences in their carboxyl termini are puzzling. The carboxyl-terminal 79 amino acids of HSV αTIF constitute its activation domain. It contains elements required for binding to TFIIB (8, 26, 55), TAF40 (14), and other possible components (25, 55) of the transcriptional complex. The region is highly acidic in character, and, although the net negative charge is not sufficient for transactivation, it contributes to it (11). The region also contains several aromatic and hydrophobic residues, including the critical F at position 442 (circled in Fig. 10), that are important for activity (11, 44).

Unlike the activation domains of HSV αTIF, the carboxyl terminus of BHV αTIF is largely hydrophobic in character. Seventy of the 98 residues from amino acids 408 to 505 have hydrophobic side chains. There are few acidic amino acids. The drastic differences between the activation domains of the HSV αTIF and BHV αTIF carboxyl termini suggest that the proteins may activate transcription by distinct mechanisms. Consistent with this, we found that unlike HSV αTIF, the activa-

tion capacity of BHV αTIF does not lie entirely in its carboxyl terminus. While deletion of the carboxyl terminus of HSV αTIF (residues 412 to 490) completely eliminates its ability to transactivate, removal of a similar segment from BHV αTIF reduced the activity by 60 to 70% but did not eliminate it. This suggested that in contrast to HSV αTIF, the amino terminus of BHV αTIF retained some elements required for transactivation. Similarly, while the intact proteins have comparable capacities for transactivating IE-1, the carboxyl terminus of BHV αTIF, fused to the DNA-binding domain of GAL4, could transactivate a promoter containing five UAS sites but at efficiencies more than 1,000-fold lower than those of the GAL4–HSV αTIF fusion protein. Unlike GAL–HSV αTIF (55), the GAL–BHV αTIF fusion protein was incapable of activating transcription from promoters containing a single UAS site (data not shown). These data suggest that the carboxyl terminus of BHV αTIF has some capacity to transactivate; however, efficient transactivation requires structural or functional contributions from the remainder of the protein. Consistent with differences in the primary sequences and functional capacities of the carboxyl termini of BHV αTIF and HSV αTIF, preliminary experiments in our laboratory (55a) also point to a different mechanism of transactivation for BHV αTIF. In pull-down experiments using GST-linked TFIIB and TAF40, we were unable to demonstrate an association with either full-length or truncated BHV αTIF. In contrast, HSV αTIF bound efficiently both TFIIB and TAF40.

HSV αTIF may activate transcription by contacting two or more components of the transcriptional complex (14, 55). The entire carboxyl terminus (412 to 490) is required for contact with TFIIB, while the distal segment of the terminus (452 to 490) contacts TAF40 (14). Other contacts have also been postulated (14, 55). It is possible that BHV αTIF also provides more than one signal to the transcriptional apparatus. Some of these signals may be provided by the carboxyl terminus; the remainder may be provided by other portions of the protein. These signals may act synergistically for complete activity. Despite the overall differences within the carboxyl termini of the αTIFs, there is a significant similarity among the HSV-1, BHV-1, and EHV proteins at their extreme carboxyl termini (Fig. 10). It is possible that this region contributes to a signal provided by the H2 activation domain of HSV αTIF (55), which may be common to the three αTIFs.

At present, we have not identified the portion of BHV αTIF that complements signals provided by its carboxyl terminus. This signal is probably not provided exclusively by the amino-terminal (1 to 71) amino acids of BHV αTIF, a region of divergence in the αTIFs. This region is the only part of the amino terminus of BHV αTIF that has no homology to HSV αTIF (6) and could have accounted for the functional differences in the amino termini of the two proteins. However, a construct containing the amino-terminal 71 amino acids of BHV αTIF linked to GAL4 had the ability neither to transactivate nor to enhance the ability of the GAL4 fusion protein containing the carboxyl terminus of BHV αTIF.

Because of the relevance of HSV to human disease, the relative ease with which it can be cultivated in vitro, its broad cell tropism, and the availability of convenient experimental hosts, it is the most extensively studied herpesvirus. HSV is regarded as a prototype for other herpesviruses, and often understanding of molecular mechanisms of host cell interactions, gained from studies with HSV, is extended to explain the replicative cycles of other herpesviruses. There is some justification for this extrapolation. Most HSV proteins are structurally and functionally conserved in closely related herpesviruses, and it is intuitive to assume that these viruses may have evolved similar biochemical strategies to deal with the same biological problems. However, while there are many similarities among the alphaherpesviruses, it is becoming increasingly evident that there are interesting differences as well.

HSV-1 has long served us as a convenient prototype for understanding other herpesviruses and as a model for cellular processes. This extrapolation may not always be justified. Our results indicate that other herpesviruses may have evolved distinct mechanisms for dealing with the same physiological problems, and a study of these unique mechanisms may provide insights into cellular physiology and the mechanism of viral pathogenesis that may not be possible from a study of HSV alone.

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REFERENCES

1. **Ace, C. I., M. A. Dalrylmple, F. H. Ramsay, V. G. Preston, and C. M. Preston.** 1988. Mutational analysis of the herpes simplex virus type 1 trans-inducing factor VMW65. J. Gen. Virol. **69:**2595–2605.
2. **apRhys, C. M., D. M. Ciufo, E. A. O'Neill, T. J. Kelly, and G. S. Hayward.** 1989. Overlapping octamer and TAATGARAT motifs in the VF65-response elements in herpes simplex virus immediate-early promoters represent independent binding sites for cellular nuclear factor III. J. Virol. **63:**2798–2812.
3. **Batterson, W., and B. Roizman.** 1983. Characterization of the herpes simplex virion-associated factor responsible for the induction of α genes. J. Virol. **46:**371–377.
4. **Campbell, M. E. M., J. W. Palfreyman, and C. M. Preston.** 1984. Identification of herpes simplex virus DNA sequences which encode a trans-acting polypeptide responsible for stimulation of immediate early transcription. J. Mol. Biol. **180:**1–19.
5. **Carpenter, D. E., and V. Misra.** 1991. The most abundant protein in bovine herpes 1 virions is a homologue of herpes simplex virus type 1 UL47. J. Gen. Virol. **72:**3077–3084.
6. **Carpenter, D. E., and V. Misra.** 1992. Sequences of the bovine herpesvirus 1 homologue of herpes simplex virus α-trans-inducing factor (UL48). Gene **119:**259–263.
7. **Caughman, G. B., J. Staczek, and D. J. O'Callaghan.** 1985. Equine herpesvirus type 1 infected cell polypeptides: evidence for immediate early/early/late regulation of viral gene expression. Virology **145:**49–61.
8. **Choy, B., and M. R. Green.** 1993. Eukaryotic activators function during multiple steps of preinitiation complex assembly. Nature (London) **366:**531–536.
9. **Cousens, D. J., R. Greaves, C. R. Goding, and P. O'Hare.** 1989. The C-terminal 79 amino acids of the herpes simplex virus regulatory protein, Vmw65, efficiently activate transcription in yeast and mammalian cells in chimeric DNA binding proteins. EMBO J. **8:**2337–2342.
10. **Cress, A., and S. J. Triezenberg.** 1991. Nucleotide and deduced amino acid sequences of the gene coding virion protein 16 of herpes simplex virus type 2. Gene **103:**235–238.
11. **Cress, W. D., and S. J. Triezenberg.** 1991. Critical structural elements of the VP16 transcriptional activation domain. Science **251:**87–90.
12. **Davison, A. J., and J. E. Scott.** 1986. The complete DNA sequence of varicella-zoster virus. J. Gen. Virol. **67:**1759–1816.

12a. **Elliott, G.** 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.
13. **Gerster, T., and R. G. Roeder.** 1988. A herpesvirus trans-activating protein interacts with transcription factor OTF-1 and other cellular proteins. Proc. Natl. Acad. Sci. USA **85:**6347–6351.
14. **Goodrich, J. A., T. Hoey, C. J. Thut, A. Admon, and R. Tjian.** 1993. Drosophila TAFii40 interacts with both a VP16 activation domain and the basal transcription factor TFIIB. Cell **75:**519–530.
15. **Greaves, R. F., and P. O'Hare.** 1989. Separation of requirements for protein-DNA complex assembly from those for functional activity in the herpes simplex virus regulatory protein Vmw65. J. Virol. **63:**1641–1650.
16. **Greaves, R. F., and P. O'Hare.** 1990. Structural requirements in the herpes simplex virus type 1 transactivator Vmw 65 for interaction with the cellular octamer-binding protein and target TA ATGARAT sequences. J. Virol. **64:**2716–2724.
17. **Greaves, R. F., and P. O'Hare.** 1991. Sequence, function, and regulation of the Vmw65 gene of herpes simplex virus type 2. J. Virol. **65:**6705–6713.
18. **Haigh, A., R. Greaves, and P. O'Hare.** 1990. Interference with the assembly of a virus-host transcriptional complex by peptide competition. Nature (London) **344:**257–259.
19. **Hayes, S., and P. O'Hare.** 1993. Mapping of a major surface-exposed site in herpes simplex virus protein Vmw65 to a region of direct interaction in a transcription complex assembly. J. Virol. **67:**852–862.
20. **Ihara, S., L. Feldman, S. Watanabe, and T. Ben-Porat.** 1983. Characterization of the immediate early functions of pseudorabies virus. Virology **131:**437–454.
21. **Katan, M., A. Haigh, C. P. Verrijzer, P. C. van der Vliet, and P. O'Hare.** 1990. Characterization of a cellular factor which interacts functionally with Oct-I in the assembly of a multiprotein transcription complex. Nucleic Acids Res. **18:**6871–6880.
22. **Kristie, T. M., J. H. LeBowitz, and P. A. Sharp.** 1989. The octamer binding proteins form multi-protein-DNA complexes with the HSV alpha TIF regulatory protein. EMBO J. **8:**4229–4238.
23. **Kristie, T. M., and B. Roizman.** 1987. Host cell proteins bind to the cis-acting site required for virion-mediated induction of herpes simplex virus α genes. Proc. Natl. Acad. Sci. USA **84:**71–75.
24. **Lewis, J. B., Y. G. Thompson, and G. Caughman.** 1993. Transcriptional control of the equine herpesvirus 1 immediate early gene. Virology **197:**788–792.
25. **Liao, S. M., I. C. Taylor, R. E. Kingston, and R. A. Young.** 1991. RNA polymerase II carboxy-terminal domain contributes to the response to multiple acidic activators in vitro. Genes Dev. **5:**2431–2440.
26. **Lin, Y. S., and M. R. Green.** 1991. Mechanism of action of an acidic transcriptional activator in vitro. Cell **64:**971–981.
27. **Mackem, S., and B. Roizman.** 1982. Differentiation between alpha promoter and regulatory regions of herpes simplex virus 1: the functional domains and sequence of a movable alpha regulator. Proc. Natl. Acad. Sci. USA **79:**4917–4921.
28. **Marsden, H. S., M. E. M. Campbell, L. Haarr, M. C. Frame, D. S. Parris, M. Murphy, R. G. Hope, M. T. Muller, and C. M. Preston.** 1987. The 65,000-M_r DNA-binding and virion *trans*-inducing proteins of herpes simplex virus type 1. J. Virol. **61:**2428–2437.
29. **McKee, T. A., G. H. Disney, R. D. Everett, and C. M. Preston.** 1990. Control of expression of the varicella-zoster virus major immediate early gene. J. Gen. Virol. **71:**897–906.
30. **McKnight, J. L., T. M. Kristie, and B. Roizman.** 1987. Binding of the virion protein mediating alpha gene induction in herpes simplex virus 1 infected cells to its cis site requires cellular proteins. Proc. Natl. Acad. Sci. USA **84:**7061–7065.
31. **Misra, V., and E. L. Blewett.** 1991. Construction of herpes simplex virus that are pseudodiploid for the glycoprotein B gene: a strategy for studying the function of an essential herpesvirus gene. J. Gen. Virol. **72:**385–392.
32. **Misra, V., R. M. Blumenthal, and L. A. Babiuk.** 1981. Proteins specified by bovine herpesvirus 1 (infectious bovine rhinotracheitis virus). J. Virol. **40:**367–378.
33. **Misra, V., J. E. Gilchrist, G. Weinmaster, L. Qualtiere, S. van den Hurk, and L. A. Babiuk.** 1982. Herpesvirus induced "early" glycoproteins: characterization and possible role in immune cytolysis. J. Virol. **43:**1046–1054.
34. **Moriuchi, H., H. Moriuchi, S. E. Straus, and J. I. Cohen.** 1993. Varicella-zoster virus open reading frame 10 protein, the herpes simplex virus VP16 homolog, transactivates herpesvirus immediate-early gene promoters. J. Virol. **67:**2739–2746.
35. **Nelson, R., A.-M. Adachi, H. Chisholm, and V. Misra.** 1989. Temperature sensitive mutants of bovine herpesvirus type 1: mutants that make unaltered levels of 'early' glycoproteins but fail to synthesize a 'late' glycoprotein. J. Gen. Virol. **70:**125–132.
36. **O'Hare, P.** 1993. The virion transactivator of herpes simplex virus. Sem. Virol. **4:**145–155.
37. **O'Hare, P., and C. R. Goding.** 1988. Herpes simplex virus regulatory elements and the immunoglobulin octamer domain bind a common factor and are both targets for virion transactivation. Cell **52:**435–445.
38. **O'Hare, P., C. R. Goding, and A. Haigh.** 1988. Direct combinatorial interaction between a herpes simplex virus regulatory protein and a cellular octamer-binding factor mediates specific induction of virus immediate-early gene expression. EMBO J. **7:**4231–4238.
39. **Post, L. E., S. Mackem, and B. Roizman.** 1981. Regulation of α genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with α gene promoters. Cell **24:**555–565.
40. **Preston, C. M., M. G. Cordingley, and N. D. Stow.** 1984. Analysis of DNA sequences which regulate the transcription of a herpes simplex virus immediate early gene. J. Virol. **50:**708–716.
41. **Preston, C. M., M. C. Frame, and M. E. Campbell.** 1988. A complex formed between cell components and a HSV structural polypeptide binds to a viral immediate early gene regulatory DNA sequence. Cell **52:**425–434.
42. **Purewal, A. S., R. Allsopp, M. Riggio, E. A. R. Telford, S. Azam, A. J. Davison, and N. Edington.** 1994. Equid herpesvirus 1 and 4 encode functional homologues of the herpes simplex virus type 1 virion transactivator protein, VP16. Virology **198:**385–389.
43. **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.
44. **Regier, J. L., F. Shen, and S. J. Triezenberg.** 1993. Pattern of aromatic and hydrophobic amino acids critical for one of two subdomains of the VP16 transcriptional activator. Proc. Natl. Acad. Sci. USA **90:**883–7.
45. **Roizman, B.** 1991. Herpesviridae: a brief introduction, p. 841–849. *In* B. N. Fields and D. M. Kea (ed.), Virology, 2nd ed. Raven Press, Ltd., New York.
46. **Sadowski, I., B. Bell, P. Broad, and M. Hollis.** 1992. GAL4 fusion vectors for expression in yeast or mammalian cells. Gene **118:**137–141.
47. **Sadowski, I., J. Ma, S. Triezenberg, and M. Ptashne.** 1988. GAL4-VP16 is an unusually potent transcriptional activator. Nature (London) **335:**563–564.
48. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
49. **Schwyzer, M., V. Cestmir, O. Menekse, C. Fraefel, and V. Paces.** 1993. Promoter, spliced leader and coding sequences of BICP4, the largest of the immediate-early proteins of bovine herpesvirus 1. Virology **197:**349–357.
50. **Stern, S., M. Tanaka, and W. Herr.** 1989. The Oct-1 homeodomain directs formation of a multiprotein-DNA complex with the HSV transactivator VP16. Nature (London) **341:**624–630.
51. **Stern, S., and W. Herr.** 1991. The herpes simplex virus transactivator VP16 recognizes the Oct-1 homeo domain: evidence for a homeo domain recognition subdomain. Genes Dev. **5:**2555–2566.
52. **Tasset, D., L. Tora, C. Fromental, E. Scheer, and P. Chambon.** 1990. Distinct classes of transcriptional activating domains function by different mechanisms. Cell **62:**1177–1187.
53. **Triezenberg, S. J., K. L. LaMarco, and S. L. McKnight.** 1988. Evidence of DNA: protein interactions that mediate HSV-1 immediate early gene activation by VP16. Genes Dev. **2:**730–742.
54. **Wagner, W. N., J. Bogdan, D. Haines, H. G. G. Townsend, and V. Misra.** 1992. Detection of equine herpesvirus and differentiation of equine herpesvirus type 1 from type 4 by the polymerase chain

reaction. Can. J. Microbiol. **38:**1193–1196.
55. **Walker, S., R. Greaves, and P. O'Hare.** 1993. Transcriptional activation by the acidic domain of Vmw65 requires the integrity of the domain and involves additional determinants distinct from those necessary for TFIIB binding. Mol. Cell. Biol. **13:**5233–5244.
55a. **Walker, S., and S. Hayes.** Personal communication.
55b. **Weinmaster, G.** 1982. M. S. thesis. University of Saskatchewan, Saskatoon, Saskatchewan, Canada.
56. **Werstuck, G., and J. P. Capone.** 1989. Mutational analysis of the herpes simplex virus trans-inducing factor Vmw65. Gene **75:**213–224.
57. **Whitton, J. L., and J. B. Clements.** 1984. Replication origins and a sequence involved in coordinate induction of the immediate early gene family are conserved in an intergenic region of herpes simplex virus. Nucleic Acids Res. **12:**2061–2079.
58. **Wilson, A. C., K. LaMarco, M. G. Peterson, and W. Herr.** 1993. The VP16 accessory protein HCF is a family of polypeptides processed from a large precursor protein. Cell **74:**115–125.
59. **Wirth, U. V., C. Fraefel, B. Vogt, C. Vicek, V. Paces, and M. Schwyzer.** 1992. Immediate-early RNA 2.9 and early RNA 2.6 of bovine herpesvirus 1 are 3′ coterminal and encode a putative zinc finger transactivator protein. J. Virol. **66:**2763–2772.
60. **Wirth, U. V., B. Vogt, and M. Schwyzer.** 1991. The three major immediate-early transcripts of bovine herpesvirus 1 arise from two divergent and spliced transcription units. J. Virol. **65:**195–205.
61. **Wu, C.** 1984. Two protein-binding sites in chromatin implicated in the activation of heat shock genes. Nature (London) **309:**229–334.
62. **Xiao, P., and J. P. Capone.** 1990. A cellular factor binds to the herpes simplex virus transactivator Vmw65 and is required for Vmw65-dependent protein-DNA complex assembly with Oct-1. Mol. Cell. Biol. **10:**4974–4977.