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Identification and Characterization of a Human Herpesvirus 6 Gene Segment Capable of Transactivating the Human Immunodeficiency Virus Type 1 Long Terminal Repeat in an Sp1 Binding Site-Dependent Manner

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The human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) is transactivated by various extracellular signals and viral cofactors that include human herpesviruses. These transactivators are capable of transactivating the HIV-1 LTR through the transactivation response element, NF-κB, or other regulatory binding elements. Human herpesvirus 6 (HHV-6) is a potential cofactor of HIV-1. Here, we report that an HHV-6 gene segment, ZVH14, which can neoplastically transform NIH 3T3 and human keratinocytes, is capable of transactivating HIV-1 LTR chloramphenicol acetyltransferase constructs in an Sp1 binding site-dependent manner. Transactivation increased synergistically in the presence of multiple Sp1 sites and was dramatically reduced by cotransfection with oligomers designed to form triplex structures with HIV-1 LTR Sp1 binding sites. HIV-1 LTR NF-κB sites were not essential for ZVH14-mediated transactivation. A putative open reading frame in ZVH14, B115, which may encode a highly basic peptide consisting of 115 amino acid residues, showed transactivation capacity similar to that of ZVH14. This open reading frame also transactivated the HIV-1 LTR in an Sp1 site-dependent fashion in African green monkey kidney cells and human T cells. These data suggest that HHV-6 may stimulate HIV-1 replication via transactivation of Sp1 binding sites present in the HIV-1 promoter.

Human immunodeficiency virus type 1 (HIV-1) is the widely accepted etiologic agent for AIDS. Gradual depletion of immune regulatory T cells and apoptosis (programmed cell death) may occur by direct effects of cytopathic HIV variants that may contribute to the attenuation and collapse of the immune system. The mechanism of cell depletion may include gp120 binding to CD4 (29) and the upregulated release of cytokines such as tumor necrosis factor alpha or transforming growth factor beta (39). Virus expression can be enhanced by activation with cofactors such as CD3 antibodies (29) or by activation of HIV long terminal repeat (LTR)-directed viral gene expression by exogenous viral infection, such as by herpes simplex virus (13, 30, 31, 34), human cytomegalovirus (3, 5, 8), human hepatitis B virus (41), and human herpesvirus 6 (HHV-6) (9). Among them, HHV-6 may play a prominant role because HHV-6 and HIV-1 are similar in $CD4^+$ cell tropism.

HHV-6 was first isolated from the peripheral blood mononuclear cells of six adult patients from the United States and Jamaica who had lymphoproliferative diseases and AIDS (40, 43). The virus has also been isolated from infants with exanthem subitum (1, 42) and from patients with various other diseases. The virus is identified as the etiologic agent of exanthem subitum (42). HHV-6 infects many of the same cell types as HIV-1 does (26), and coinfection of T lymphocytes with HIV-1 and HHV-6 leads to accelerated cytopathic effects (26). Infection of $CD8^+$ cells by HHV-6 induces surface expression of CD4, rendering them susceptible to infection with HIV-1 (27). The HIV-1 LTR is activated in HHV-6-infected primary human T lymphocytes (9) and T-cell lines (18) and leads to increased HIV-1 gene expression and virus replication. This activation is via nuclear factors binding to the HIV-1 LTR enhancer (9). These observations suggest that HHV-6 may be an important cofactor in HIV-1 infection and may contribute to the progression of AIDS. However, other reports show that HHV-6 can suppress HIV-1 infection in vitro (6, 23).

Previous studies conducted by Razzaque et al. (37, 38) have demonstrated that a genomic fragment, ZVH14, of HHV-6 strain GS can neoplastically transform NIH 3T3 and human keratinocyte cells. Horvat et al. (19) have shown that the same fragment can transactivate the HIV-1 LTR in primary lymphocytes. In this study, we provide evidence that the HHV-6 ZVH14 gene segment transactivates the HIV-1 LTR predominantly through Sp1 binding sites. Within ZVH14, a fragment containing a small open reading frame (ORF) retains the capacity to transactivate the HIV LTR.

MATERIALS AND METHODS

Cells. The African green monkey kidney cell line CV1 (ATCC CCL 70) was routinely maintained in Dulbecco's modified Eagle's medium (DMEM). The human T-cell line H9 (ATCC HTB 176) was maintained in RPMI 1640 medium. Both media were supplemented with 10% fetal calf serum (GIBCO BRL), 100 U of penicillin G per ml, 100 μg of streptomycin per ml, and 2 mM L-glutamine. Cells were fed twice weekly and split at ratios of 1:5 to 1:10 as needed.

Plasmids. Plasmid pZVH14 (8.7-kb *Hin*dIII fragment in pBS vector) of HHV-6 strain GS was generated as described

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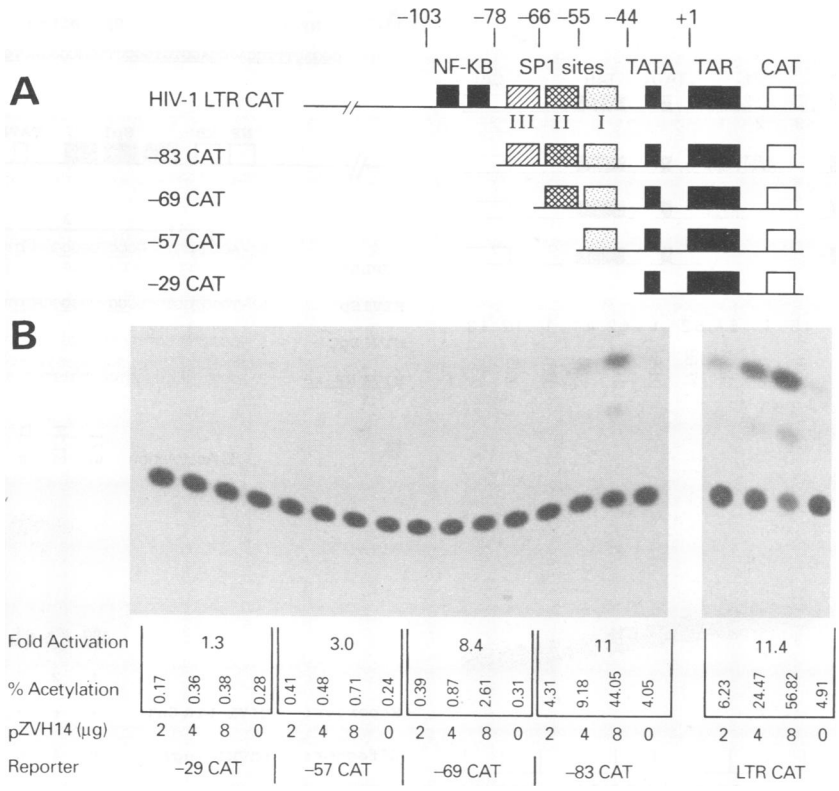


FIG. 1. Determination of the elements necessary for transactivation by pZVH14. (A) CAT reporter constructs used in cotransfection assays. (B) Relative CAT activities of constructs used in cotransfection of CV1 cells with pZVH14. CV1 cells were transfected with 1 μg of different deletion constructs of the HIV-1 LTR CAT expression vector with or without pZVH14 by the calcium phosphate method. Conversion of chloramphenicol to acetylated forms was calculated by scintillation counting after thin-layer chromatography as described previously (2). The data are representative of four independent experiments.

previously (22). Subclones pZVH14-1, pZVH14-2, pZVH14-3, pZVH14-5, pZVH14-6, pZVH14-7, pZVH14-8, and pZVH14-A were constructed by digestion of pZVH14 with *Sma*I, *Pst*I, *Cla*I, *Hin*cII, *Sma*I-*Eco*RV, *Mlu*I, *Nsi*I, and *Acc*I, respectively, and self-ligated by T4 DNA ligase. Plasmid pZVH14-4 was constructed by ligation of pZVH14 partially digested with *Xba*I. Plasmid pZVH14-12 was constructed by inserting the 0.8-kb *Pst*I-*Hin*dIII segment of pZVH14 into *Pst*I- and *Hin*dIII-digested pBS vector. pZVH14-10 and pZVH14-11 were constructed by digesting pZVH14-1 first with *Hin*dIII then with *Eco*RV and *Mlu*I respectively, filling in the ends with DNA polymerase fragment, and then ligating the ends with T4 DNA ligase. All clones have been confirmed by restriction enzyme analysis. The intact HIV LTR chloramphenicol acetyltransferase (CAT) −57CAT, and −29CAT constructs were gifts from Charles Wood. −83CAT and −69CAT were constructed in the laboratory. −117Sp3CAT, −117Sp3/2CAT, −117Sp3/1CAT, and −117CAT were described previously (46). Plasmid DNAs used in transfections were purified by the alkaline lysis method followed by double CsCl-ethidium bromide density gradients. After dialysis, the plasmid DNAs were precipitated with ethanol and redissolved in sterile water. Concentrations were calculated from A_{260} determinations and checked on agarose gels.

Synthesis of oligonucleotides. Oligonucleotides were synthesized on a model 8700 nucleic acid synthesizer (Millipore) by the H phosphoramidite method. After synthesis, oligonucleotides were purified by C18 high-pressure liquid chromatography (Waters). For cellular studies, the oligonucleotides were further purified on an 8% denaturing polyacrylamide gel, electroeluted into dialysis tubes, desalted by using NAP-25 chromatography (Pharmacia LKB), ethanol precipitated, and resuspended in distilled sterile water.

Transfection. Transfections of CV1 cells were performed by the calcium phosphate-DNA coprecipitation technique (15). Precipitates were added to cells that had been plated 48 h before at a density of 3×10^5 to 5×10^5 cells per 100-mm-diameter dish. For most transfections, 1 μg of CAT plasmid was coprecipitated with effector plasmids and calf thymus DNA (to adjust the total DNA to 30 μg). Precipitates were left on the cells overnight (16 h), and then the monolayers were subjected to a 2-min 15% glycerol–DMEM shock. After being washed in phosphate-buffered saline, the cells were fed with 10 ml of DMEM containing 10% fetal bovine serum. Cells were harvested 48 h later for CAT enzyme activity analysis (2). Each transfection was performed a minimum of two times in duplicate, with the contents of the two plates pooled at the time of cell harvest. Transfections of H9 cells were carried out by the DEAE-dextran method (25).

CAT assay. CAT assays were carried out as described by Gorman et al. (14), and the percentage of acetylation and fold induction were determined as described previously (2).

Expression vectors. Two putative ORFs, RF2 and RF5, of pZVH14 were amplified by PCR with the following temperature profile: 96°C, 20 s; 55°C, 20 s; and 72°C, 30 s (total of 20 cycles). The PCR products were purified by miniprep spun column chromatography (Pharmacia LKB) and codigested with pSV-SPORT 1 expression vector (GIBCO BRL), which is

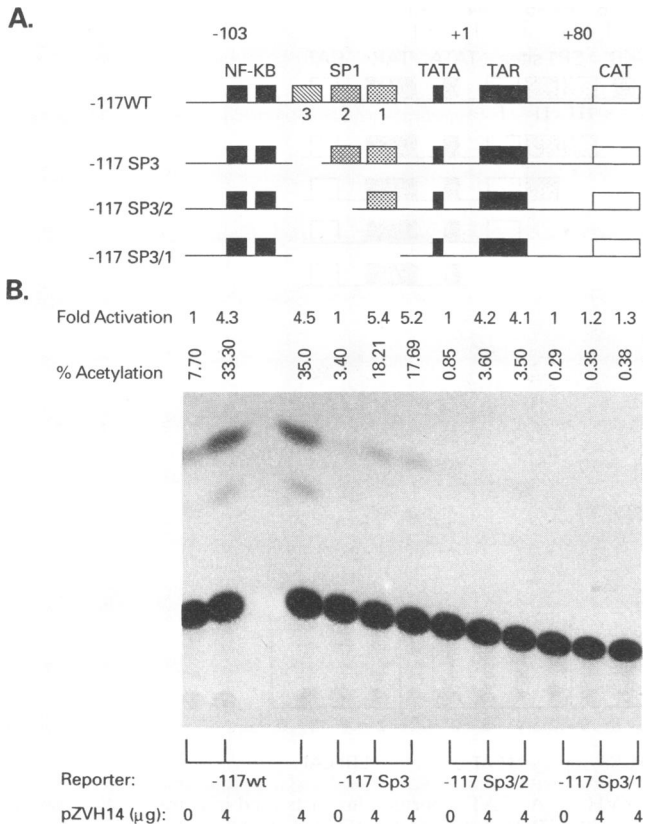


FIG. 2. Determination of the critical role of Sp1 sites in transactivation of the HIV LTR by pZVH14 in the presence of NF-κB sites. (A) CAT reporter constructs used in cotransfection assays. (B) Relative CAT activities of constructs used in cotransfection of CV1 cells with pZVH14. The data are representative of at least three independent experiments.

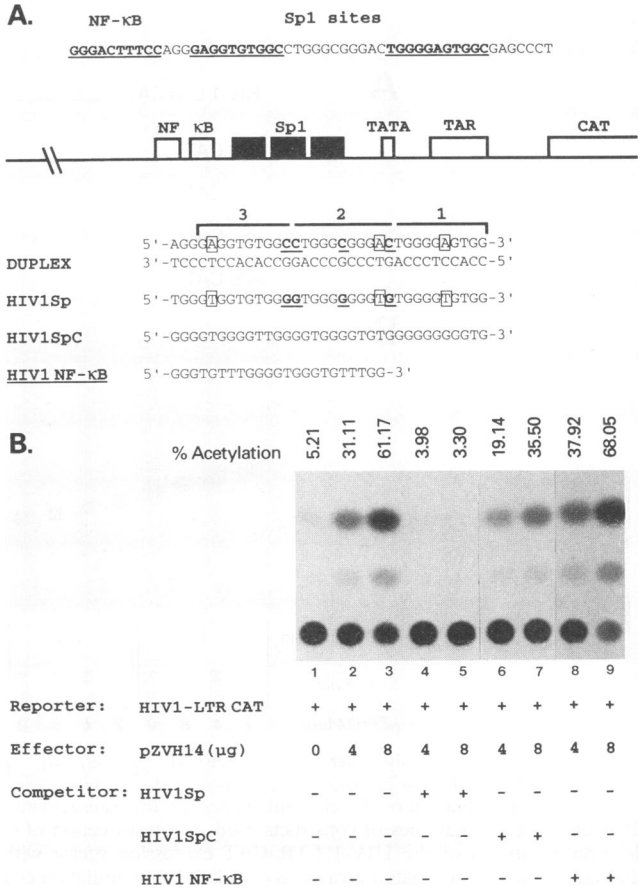


FIG. 3. Inhibition of pZVH14-induced transactivation of the HIV-1 LTR by cotransfection with oligomers designed to form triplex DNA with three Sp1 binding sites. (A) Sequence of the HIV-1 LTR. The sequences of oligomers are shown under the duplex target sequence. (B) Representative thin-layer chromatography of [^{14}C] chloramphenicol and its acetylated products showing the effects of different oligomers in ZVH14-induced transactivation. One microgram of HIV-1 LTR CAT and 4 μg (lanes 2, 4, 6, and 8) or 8 μg (lanes 3, 5, 7, and 9) of pZVH14 were cotransfected with 1 μg of each oligomer into CV1 cells. The data were reproduced in three independent experiments.

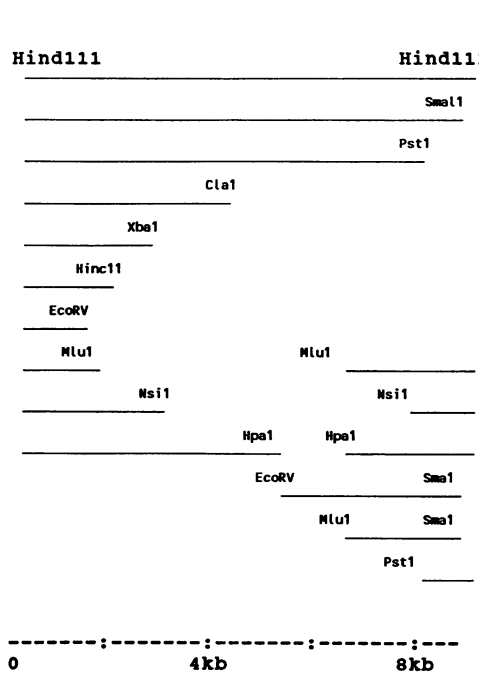
driven by the simian virus 40 early promoter, and ligated by T4 DNA ligase. Clones were screened by the colony hybridization method described by Hanahan and Meselson (17) and confirmed by restriction analysis and sequencing.

DNA sequencing and sequence analysis. DNA sequencing was conducted by using a Sequenase version 2.0 kit (United States Biochemical) as specified by the manufacturer. Sequences were analyzed by the method of Pearson and Lipman (35) on an NIH Convex system.

RESULTS

Transactivation of the HIV LTR by the HHV-6 genomic fragment in pZVH14 is not just T-cell specific. The HIV LTR can be transactivated by HHV-6 infection in human T-cell lines and in primary human T cells (18, 19). The 8.7-kb ZVH14 genomic segment of HHV-6 (GS) able to transactivate the HIV LTR when cotransfected into human T cells (19). To determine whether HIV-1 LTR transactivation by pZVH14 is cell type specific, CV1 cells were used in transfection. CV1 cells have been used to study transactivation of the HIV LTR with various cofactors. Cotransfection of the HIV LTR CAT construct with pZVH14 resulted in a more than 10-fold increase in CAT enzymatic activity (Fig. 1). Consistent with previous report by Horvat et al. (19), the pZVB70 fragment had stronger activity than pZVH14 (data not shown). Our data show that HHV-6 (GS) genomic fragment ZVH14 can also transactivate the HIV LTR in CV1 cells.

Role of NF-κB and Sp1 binding sites in pZVH14-induced transactivation of the HIV LTR. It has been reported that HHV-6 increases HIV-1 expression in coinfected T cells via nuclear factors binding to the HIV-1 enhancer (9) and that NF-κB sites are required for another HHV-6 22-kb genomic subclone, pZVB70, to transactivate the HIV LTR (11, 19). To map the elements required for transactivation by pZVH14, a series of HIV LTR deletion constructs containing from one to three Sp1 sites but without NF-κB sites was tested for response to pZVH14. Deletion of NF-κB enhancer elements did not cause a large reduction of basal-level promoter activity (Fig. 1). These elements were not required for transactivation, as shown by cotransfection of pZVH14 with the −83CAT construct (containing three Sp1 sites, a TATA box, and a transactivation response element [TAR]), in which case CAT activity was elevated 11-fold. This result is consistent with a previous report showing that pZVH14 can transactivate the HIV-1 LTR even with NF-κB sites mutated (19).



	Acetylation (%)	Fold
pZVH14	18.36	15.3
pZVH14-1	20.28	16.9
pZVH14-2	21.72	18.1
pZVH14-3	39.24	32.7
pZVH14-4	26.40	22.0
pZVH14-5	2.52	2.1
pZVH14-6	1.56	1.3
pZVH14-7	3.72	3.1
pZVH14-8	12.84	10.7
pZVH14-9	19.44	16.2
pZVH14-10	5.28	4.4
pZVH14-11	4.68	3.9
pZVH14-12	4.80	4.0
pBS	1.21	1.0

FIG. 4. Schematic outline of deletion and truncation clones of pZVH14 and their CAT activities. The entire insert of pZVH14 is schematically depicted with the restriction sites used in cloning. CV1 cells were cotransfected with 1 μg of the HIV-1 LTR CAT expression vector and 8 μg of a pBS or pZVH14 subclone; 50 μl of a 200-μl extract (from two duplicate dishes) was used for measuring CAT activity after incubation for 30 min. Values represent averages of two assays.

Using constructs varying in number of Sp1 sites, we found that pZVH14 activated transcription at a moderate level (threefold) from −57CAT containing Sp1 site I, as determined by liquid scintillation counting (Fig. 1). This activation was more than eightfold with −69CAT, which contains two Sp1 sites (I and II). The presence of the third Sp1 site (site III) in −83CAT further enhanced the basal-level transcription, and transactivation was elevated up to 11-fold. No elevated CAT activity was observed in the −29CAT construct, which contains the TATA and TAR elements with all three Sp1 binding sites being deleted. The data presented here indicate that for efficient transactivation, more than one Sp1 site is required, which is consistent with the reports of Jones et al. (20).

To confirm this finding, constructs containing the two NF-κB sites and the TATA and TAR sites with variable numbers of Sp1 sites were used (Fig. 2). Internal deletion of all three Sp1 sites with the presence of NF-κB, TATA, and TAR sites (−117SP3/1) caused the complete loss of transactivation by pZVH14, while this construct can still be transactivated by *tax* and *tat* (46). This finding indicates that even at low basal activity of an Sp1-less construct, transactivation is possible, and pZVH14 failed to transactivate it because of the absence of Sp1 sites. With the presence of NF-κB, Sp1 site I, TATA, and TAR (−117SP3/2), subtle levels of transactivation were observed. With the presence of NF-κB, Sp1 sites I and II, TATA, and TAR (−117SP3), enhanced activation was observed. These studies indicated that Sp1 binding sites are essential and that maximal stimulation of the HIV LTR by pZVH14 requires Sp1 sites I and II.

Inhibition of pZVH14-elevated transcription of the HIV-1 LTR by oligomers designed to form a triplex with Sp1 binding sites. Several studies have demonstrated that oligomers can inhibit promoter activities through formation of triplex with certain elements of promoters. The formation of a triplex DNA molecule (forming a T:AT triplet or C^+:GC triplet) occurs through the binding of polypurine oligonucleotides to a polypurine/polypyrimidine site in a sequence-specific manner (32). It has also been demonstrated that the presence of strictly polypurine/polypyrimidine DNA sequences is not absolutely necessary for the formation of triplex DNA (4, 45). A non strictly polypyrimidine:polypurine/polypyrimidine site can form a triplex which can also functionally inhibit transcription in vitro and in vivo (4, 7, 10, 33, 44, 45). Although the sequence of the Sp1 binding site of the HIV LTR is not purely polypurine/polypyrimidine, it has been demonstrated that it may form triplex DNA under certain conditions and suppress viral replication by inhibiting transcription and transactivation (28). In this experiment, three oligomers, HIVSp, HIVSpC, and HIV NF-κB, were designed. HIVSp is the parallel form of HIV LTR −46 to −79 with substitutions of A to T and C to G. HIV NF-κB is the parallel form of HIV LTR −82 to −103 with substitutions of A to T and C to G. HIVSpC has the same base composition as HIVSp but contains designated point mutations with respect to HIVSp. To test whether these oligomers can inhibit pZVH14-induced transactivation of the HIV LTR, they were cotransfected with HIV LTR CAT and pZVH14 into CV1 cells, and CAT activity was measured. As illustrated in Fig. 3, the activity of the HIV LTR was significantly (≈90% by liquid scintillation counting) inhibited when cotransfected with HIVSp, as expected. HIVSp also substantially inhibited the basal LTR transcription. Although suppression was observed with HIVSpC, the level of suppression was much lower than that of HIVSp. No inhibition was observed with the treatment of HIV NF-κB oligomers. We also found that the triplex oligomers (HIVSp and HIVSpC) failed to inhibit transcription from the Rous sarcoma virus LTR and the

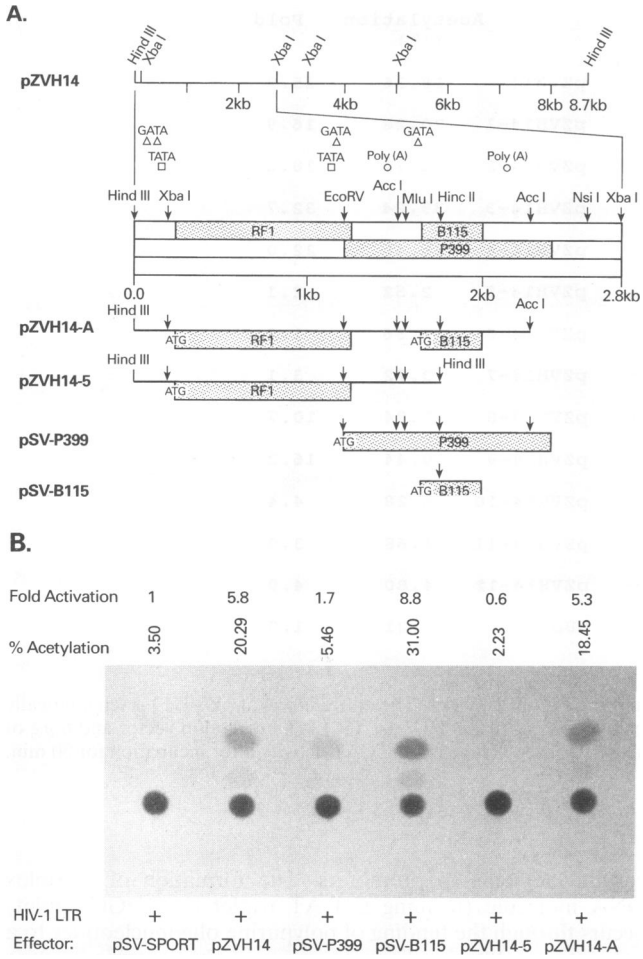


FIG. 5. Identification of ORFs involved in transactivation of the HIV-1 LTR. (A) Schematic outline of the three left-hand subclones of pZVH14 and two expression vectors, pSVB115 and pSVP399, with the putative ORFs. ORFs of >300 bp are displayed, and all three ORFs are on the rightward strand. Arrows show the locations of restriction sites in the amplified *Hin*dIII-*Xba*I fragment. RF1, P399, and B115 are the ORFs described in the text. ATG, initiation codon; △, GATA box; □, TATA box; ○, poly(A); RF5 (B115). (B) CAT activity in CV1 cells. The cotransfections were performed with 1 μg of the HIV LTR CAT construct and 4 μg of effectors. The data are representative of at least four independent experiments.

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5'- GCATACGATCTACTCCCCGGAGACAAACCTTCTCGTAAGCATTCTACTAAACGCGTATAC
    CAACTACCTCCAAAAATACGTAAACCCTTGGATCAAACAAACCATAACGGCAAACTTCTC
    CCTGATACAGACGTACATAACTTTCACGAAACAATGCGCCAGCATCTTGGCGACCAAATG
                                    M  R  Q  H  L  G  D  Q  M
    CAACATAAACTTAGACAATCTATTTATCTACATAACTATCGGAACAGACAAAATTGTCAC
    Q  H  K  L  R  Q  S  I  Y  L  H  N  Y  R  N  R  Q  N  C  H
    CACTTCGTTTTGCTCCTTCATCGCTACATGCAGAAATTTAGTACGTCAACACGAAGAATT
    H  F  V  L  L  L  H  R  Y  M  Q  K  F  S  T  S  T  R  R  I
    CGAAAAATCACTACAAACGATCCAAATTTCCGAAACCACACTCACGGGGATGCTACGAAA
    R  K  I  T  T  N  D  P  N  F  R  N  H  T  H  G  D  A  T  K
    CATAATCACGAGCGTCTCCTCCTCCAAAGAGCTGCTAACCAACGAAGCACTACAAAAATT
    H  N  H  E  R  L  L  L  Q  R  A  A  N  Q  R  S  T  T  K  I
    CATCGACACTGTCCAACGCATCTCCCAACACGTAAACGAAACATACCAGTCTATTTCCAT
    H  R  H  C  P  T  H  L  P  T  R  K  R  N  I  P  V  Y  F  H
    GAACCTCGAAAAATGTAAAACCTCAAACGACATCCTAATCGAATCCCTGAAGAAGATCAT
    E  P  R  K  M  *
    CTACATAGTTGATGTACTCAGCTCCAATGCAATCCTAAACACGTCGTTAGCTTCCAGATG
    TCTGGAAGCCGCCAACCTCGCGGTTTCGAACAATTCTTTCACAATACTCGAAATTAAAAA
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FIG. 6. Predicted amino acid sequence of ORF B115.

simian virus 40 early promoter (data not shown). These results demonstrate that an oligonucleotide which is capable of forming a triplex with Sp1 binding sites may inhibit the ability of pZVH14 to transactivate the HIV LTR.

More than one subfragment of ZVH14 possesses transactivational function. To further identify and characterize the specific gene(s) involved in transactivation of the HIV-1 LTR, several deletions and truncation clones of the 8.7-kb ZVH14 insert were constructed and tested for the ability to transactivate the HIV LTR. The transactivation level of each clone is shown in Fig. 4. All clones (pZVH14-1, pZVH14-2, pZVH14-3, pZVH14-4, pZVH14-8, and pZVH14-9) which contain the 1-kb *Hin*cII-*Xba*I fragment were capable of transactivating the HIV LTR. Clones which contained the right-hand 0.8-kb *Pst*I-*Hin*dIII fragment were also able to consistently transactivate the HIV LTR (fourfold). These studies localized the major transactivating domain of pZVH14 to a 1-kb *Hin*cII-*Xba*I fragment.

B115 is as strong as ZVH14 in transactivation of the HIV-1 LTR. Three putative ORFs, RF1, P399, and B115, are present in the 2.8-kb *Hin*dIII-*Xba*I fragment (Fig. 5). B115, encoding 115 amino acid residues, was amplified by PCR and cloned into pSV-SPORT 1 (GIBCO BRL) under the control of simian virus 40 early promoter. The resulting plasmid was designated pSV-B115. Similarly, P399, encoding 399 amino acid residues, was cloned and designated pSV-P399. A subclone, pZVH14-A, which maintains the entire RF1 and B115 with disrupted P399, was also constructed as described in Materials and Methods. pSV-P399, pSV-B115, pZVH14-A, or pZVH14-5 (in which only RF1 is complete) was cotransfected along with HIV-1 LTR CAT into CV1 cells. Both pSV-B115 and pZVH14-A containing B115 showed transactivating potential similar to that of pZVH14 (Fig. 5), but pSV-P399 and pZVH14-5 showed little or no effect. The sequence of B115 was obtained from GenBank (accession number S57540) as part of pZVH14 (21) and confirmed by us after PCR amplification and cloning into pSV-SPORT 1. The DNA sequence of B115 together with the putative 5′ leading sequence, TATA site, 3′-end poly(A) site, and protein sequence is shown (Fig. 6). The putative ORF B115 contains 42% basic amino acid residues. No classic leucine zipper and zinc finger motifs were detected in B115.

Transactivation of the HIV-1 LTR by pSV-B115 is Sp1 site dependent. To determine whether pSV-B115 required Sp1 sites to transactivate the HIV LTR, CV1 cells were cotransfected with pSV-B115 and either −83CAT or −29CAT into CV1 cells. B115 appears to function like pZVH14 in CV1 cells, since it transactivated −83CAT but not −29CAT (Fig. 7A). Similar experiments were performed with H9 cells (human T cells). Both pSV-B115 and pZVH14 transactivated −83CAT but not −29CAT (Fig. 7B). This finding demonstrated that pSV-B115 is capable of transactivating the HIV-1 LTR in an Sp1-dependent manner in CV1 and human T cells.

DISCUSSION

Studies regarding the role of HHV-6 or its gene segments in regulating the expression of HIV-1 may be important to understanding the pathogenesis of HIV-1, since HHV-6 is considered an important cofactor of HIV-1. Activation of HIV expression by human herpesviruses or viral genes involves different regulatory elements of the HIV-1 promoter. Using

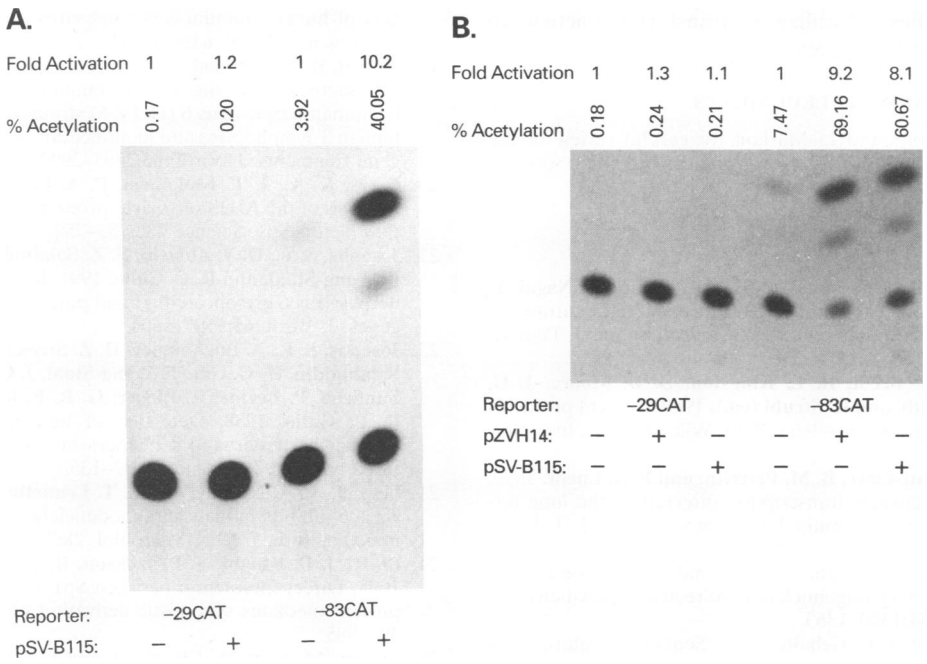

FIG. 7. Transactivation of the HIV-1 LTR by pSV-B115 in CV1 (A) and H9 (B) cells. The amount of pZVH14 or pSV-B115 used in cotransfections was 8 μg. The data are representative of five independent experiments.

cotransfection experiments of various HIV-1 LTR CAT constructs with HHV-6 (GS) gene segment pZVH14, we have examined the function of individual known regulatory elements of the HIV-1 LTR in pZVH14-induced transactivation. Our data indicated that pZVH14 transactivated the HIV-1 LTR through the Sp1 binding sites and that NF-κB sites are not essential in transactivation. Another study has demonstrated that NF-κB binding sites are necessary for transactivation of the HIV-1 LTR by another HHV-6 (GS) gene segment, pZBV70 (11). Together, the data suggest that activation of the HIV-1 LTR by HHV-6 involves at least two HHV-6 genes and multiple response elements within the HIV promoter. A similar phenomenon has been observed whereby both Sp1 and NF-κB sites in the HIV LTR are required for transactivation by HSV-1 infection (30).

From this study, it is clear that efficient transactivation of the HIV LTR by pZVH14 requires functional Sp1 binding sites. Since an HIV LTR construct containing Sp1 binding sites I and II (−117SP3) was efficiently transactivated by pZVH14. In contrast, the HIV LTR construct containing only Sp1 binding site I showed poor activation, suggesting that pZVH14 requires at least two tandem Sp1 binding sites to exert its optimal function. Also, the triplex formation data in Fig. 3 suggest that activation is Sp1 site specific, since NF-κB did not show significant blocking function. The same triplex oligomer, HIVSp, could also specifically block transcription of the HIV LTR but did not form a triplex with regulatory regions of c-*myc* and interleukin 2 receptor promoters as shown by McShan et al. (28). The major *trans*-acting domain of pZVH14 is localized to a small fragment which contains a 115-amino-acid ORF (B115). It is likely that this 115-amino-acid ORF is responsible for Sp1-dependent transactivation.

There are several possible mechanisms by which Sp1-dependent transactivation may occur. First, the putative protein may directly bind to Sp1 sites in a similar fashion as the cellular transcription factor Sp1. Second, it may act as a coactivator by directly or indirectly interacting with Sp1 or its family members (12, 24, 36), such as bovine papillomavirus E2 (24). Third, it may interfere with the posttranslational modification of Sp1, such as phosphorylation. Finally, it may activate expression of cellular genes which may be involved in transactivation, such as tumor necrosis factor-induced Sp1 in micromuscular cells (16), although expression of Sp1 family members, unlike that of NF-κB or AP-1 family members, is relatively stable and usually constitutive.

Sequence analyses indicate that there are one GATA box 30 bp and one TATA box 98 bp upstream of the first ATG codon and a polyadenylation signal sequence (ATTAAA) at position 154 bp downstream of the putative ORF B115 (Fig. 6). The HHV-6 B115 is, therefore, likely to be expressed. The putative protein consists of 115 amino acids, is highly basic, and is rich in histidine. Sequence comparisons show that B115 does not have sequence similarity with the cytomegalovirus AD169 genome. No significant sequence homology has been detected between B115 and human transcription factor Sp1. Preliminary experiments with B115, expressed in vitro in a wheat germ transcription-translation system, found the protein to have a molecular mass of 18 kDa as expected. Using a 139-bp *Ava*I-*Pvu*II fragment of the HIV-1 LTR (containing NF-κB, Sp1, and TATA sites) as a probe, no binding of the in vitro-translated B115 was detected in gel shift assays, while purified Sp1 protein showed a band shift (data not shown). Furthermore, no additional band shift was detected when B115 and Sp1 proteins were added together. These data suggest that B115 does not bind to Sp1 sites or to Sp1 protein. Whether B115 acts through another cellular factor to interact with Sp1 motifs remains to be elucidated.

Current studies are in progress to further identify and characterize the protein encoded by B115. Since pZVH14 also has oncogenic potential and has been detected in human

lymphoma (22), studies to localize the transforming activity in this segment are also in progress.

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