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Hepatitis B virus X gene can transactivate heterologous viral sequences

(human immunodeficiency virus long terminal repeat/gene regulation/transactivation/RNase mapping)

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ABSTRACT **The smallest open reading frame of hepatitis B virus (HBV) has been designated the X gene and its biological function during HBV infection and replication is not known. Experiments described here demonstrate that expression of the HBV X gene in HepG2 cells containing a plasmid with the chloramphenicol acetyltransferase (CAT) gene under control of the human immunodeficiency virus (HIV-1) long terminal repeat (LTR) sequence leads to a marked increase in CAT gene transcription as well as expression of the gene product (CAT). The HIV-1 *tatIII* gene and the HBV X gene together increased HIV-1 LTR-regulated CAT expression above that observed with either gene alone, suggesting a synergistic effect of the X gene and *tat*. HBV X gene also stimulated expression of the CAT gene under control of the simian virus 40 enhancer and early promoter but not the visna virus LTR or the human T-cell lymphotropic virus type I (HTLV-I) LTR, indicating that the HBV X gene can transactivate some but not other heterologous viral sequences. Transactivation of the HIV-1 LTR by the HBV X gene varied in different cell lines, suggesting that it may be mediated by a cellular factor(s).**

Hepatitis B virus (HBV) contains a small circular DNA genome that has four open reading frames, and three of these appear to encode virion structural proteins (i.e., proteins with HBV surface antigen and core antigen/e antigen specificity, and probably the virion DNA polymerase) (reviewed in refs. 1 and 2). The biological function of the smallest open reading frame, designated the X gene, which could encode a polypeptide of 154 amino acids, is uncertain. A protein with antigenic cross-reactivity with a synthetic peptide, the sequence of which was derived from the nucleotide sequence of the X gene (3), and with an X protein–β-galactosidase fusion protein made in bacteria (4) has been detected in some HBV-infected livers, suggesting that the X gene may be expressed in liver during HBV infection. Recently, introduction of a DNA fragment containing the HBV X gene under control of a HBV promoter and enhancer into Vero cells containing a plasmid with the bacterial chloramphenicol acetyltransferase (CAT) gene under control of the human β-interferon promoter and enhancer led to increased expression of CAT activity (5). Although this result suggests that the HBV X gene can act in trans to stimulate expression of a gene under control of β-interferon control sequences, the role of HBV X gene during natural infection is still unclear. Efforts in this lab to demonstrate a trans effect of the X gene on known HBV control sequences have so far failed to demonstrate such an effect (J.-S.T., A. Siddiqui, and W.S.R., unpublished data).

Here we demonstrate that the expression of the HBV X gene in HepG2 cells containing a plasmid with the CAT gene under control of the human immunodeficiency virus 1 (HIV-1) long terminal repeat (LTR) led to a marked increase in CAT expression. Similar transactivation of simian virus 40 (SV40) control sequences but not visna virus or human T-cell lymphotropic virus type I (HTLV-I) was observed. The HIV-1 *tatIII* gene and the HBV X gene together increased HIV-1-regulated CAT expression above that observed with either gene alone, suggesting a synergistic effect of X gene and *tat*. The transactivation effect of the HIV-1 LTR varied in different cell lines, suggesting that certain cellular factor(s) may be involved in the HBV X gene effect.

MATERIALS AND METHODS

Plasmids. Recombinant plasmids were constructed to place the HBV X gene under control of different heterologous promoters. A plasmid with the HBV X gene controlled by the mouse mammary tumor virus (MMTV) LTR (pMMTV.X; Fig. 1) was constructed by inserting the 612-base-pair (bp) *Nco* I/*Sal* I HBV DNA fragment [map position 1372–1984 of HBV genome subtype *adw* (6)] containing the HBV X open reading frame between the *Sma* I and *Sal* I restriction sites of plasmid pMSG (7) provided by A. Roach (Indiana University). A plasmid with the HBV X gene controlled by the human cytomegalovirus (CMV) immediate early promoter (phCMV.X; Fig. 1) was constructed by blunt ending the *Nco* I end of the *Nco* I/*Hin*dIII DNA fragment of pTWU14 (ref. 5 and Fig. 1) containing the HBV X open reading frame and inserting this DNA fragment between *Hin*cII and *Hin*dIII restriction sites of pUC19 to form a pUC19-X intermediate. A 1.2-kilobase *Xba* I to *Bam*HI DNA fragment of plasmid pON249 (8) obtained from E. Mocarski (Stanford University) and containing the desired CMV promoter sequence was then inserted in front of the HBV X open reading frame between *Xba* I and *Hin*dIII sites of the pUC19-X intermediate to form phCMV.X. A plasmid with the HBV X gene controlled by the metallothionein promoter (pMT.X; Fig. 1) was constructed by blunt ending the *Nco* I end of the 503-bp *Nco* I/*Alu* I DNA fragment of pTWU14 (ref. 5 and Fig. 1), which contained only the HBV X open reading frame, and inserting this DNA fragment into the *Hin*cII restriction site of pUC19. The *Xba* I/*Hin*dIII DNA fragment containing the HBV X open reading frame of this intermediate construct was then inserted between the *Xba* I and *Hin*dIII sites of plasmid pMT.NeoI (provided by A. Roach) to form pMT.X with the HBV X gene downstream of the metallothionein promoter. Other plasmids used in this study included pHIVCAT0 (9) with the HIV-1 LTR containing DNA fragment between map positions −470 to +80 (+1 being the RNA start site) in front of the CAT gene, a gift of E. Holland (Stanford University); pVisLTRCAT

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Abbreviations: HBV, hepatitis B virus; CAT, chloramphenicol acetyltransferase; HIV, human immunodeficiency virus; LTR, long terminal repeat; SV40, simian virus 40; MMTV, murine mammary tumor virus; CMV, cytomegalovirus; HTLV, human T-cell lymphotropic virus.

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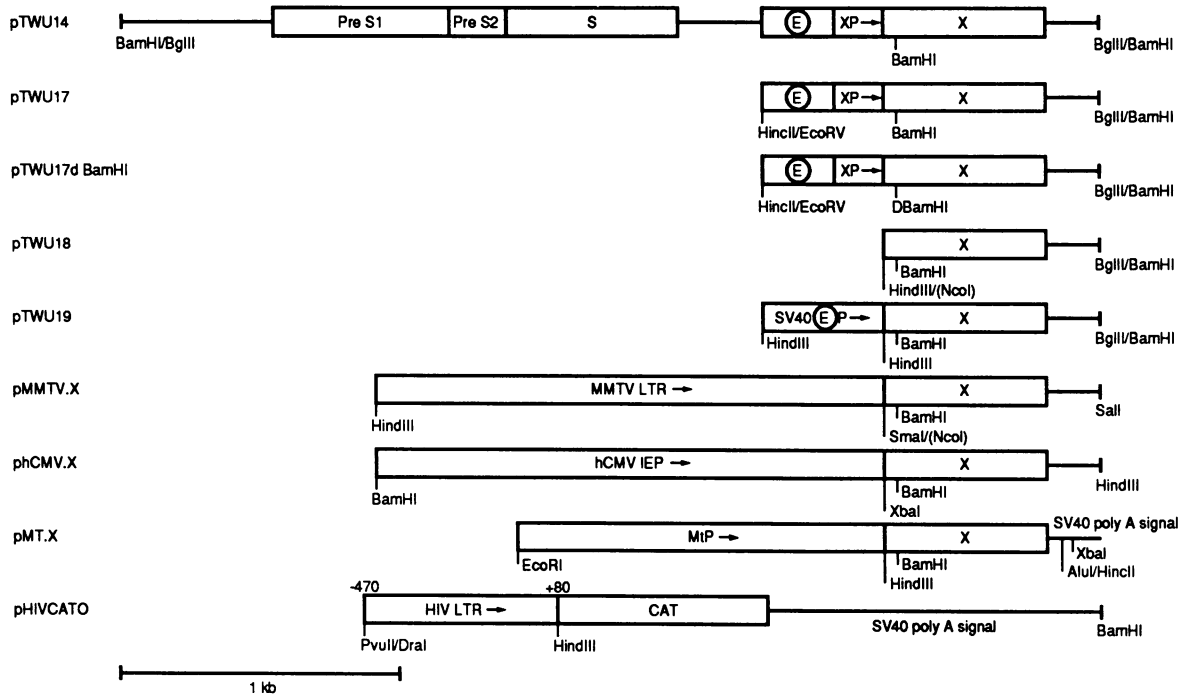

FIG. 1. Schematic representation of plasmids used in this study. Pre S1, HBV pre-S1 region; Pre S2, HBV pre-S2 region; S, HBV S gene coding region; E, HBV enhancer region; XP, HBV X gene promoter region; X, HBV X gene coding region; SV40EP, SV40 early promoter and enhancer region; hCMV IEP, human CMV immediate early promoter; MtP, metallothionein promoter; kb, kilobase.

(10), obtained from J. Clements (Johns Hopkins University), with the CAT gene under control of the visna virus LTR; pU3R-I (11), with the CAT gene under control of the HTLV-I LTR; and pSV2CAT (12), provided by G. Khoury (National Institutes of Health), with the CAT gene under control of the SV40 early promoter and enhancer elements. The plasmid pcDEBtat, kindly provided by E. Holland, contained the HIV-1 tatIII cDNA regulated by the SV40 early promoter and SV40 RNA processing signal (9). The cloning vectors pUC9 and pUC19 were purchased from New England Biolabs. The construction of the plasmids pTWU14, pTWU17, pTWU18, pTWU19, and pTWU17dBamHI (Fig. 1) has been described (5). Plasmid DNAs were constructed by standard recombinant techniques (13) and purified twice by centrifugation in CsCl density gradients prior to use for transfections.

Cells and Transfections. Vero cells were cultured in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS). HepG2 cells, HepG2T14 cells (14), HuH7 cells, and HeLa cells were cultured in a 1:1 ratio of Dulbecco's modified essential medium (DMEM) and RPMI 1640 medium supplemented with 10% FCS. MT2 cells were cultured in RPMI 1640 medium supplemented with 10% FCS. NIH 3T3 cells were cultured in DMEM supplemented with 10% calf serum. Cells were plated at a density of 10^6 cells per 100-mm plate for transfection. After 24 hr, the medium was replaced and 2–4 hr later, DNA transfections were performed by the calcium phosphate method according to the procedure of Wigler *et al.* (15). After incubation with DNA–calcium phosphate precipitate for 4 hr, the cell monolayer was rinsed with serum-free medium, treated with 15% (vol/vol) glycerol for 1.5 min at room temperature, and then received 10 ml of fresh medium. Unless specified, 10 μg of plasmids containing HBV DNA and 1 μg of plasmids containing the CAT gene were used in all transfections.

CAT Assays. Cells were harvested and CAT assays were performed essentially as described by Gorman *et al.* (12). Cells were washed with phosphate-buffered saline and removed by scraping. Cells were pelleted and resuspended in 0.25 M Tris·HCl (pH 7.8) and disrupted by two cycles of freeze–thawing. Protein concentration of cell lysates was determined according to the method of Bradford (16) and CAT activity was assayed by using the same amount of total protein for all samples in an individual experiment. The activity of CAT was determined quantitatively by excising spots corresponding to nonacetylated and acetylated forms from TLC plates and counting in a liquid scintillation spectrometer. All experiments were performed on multiple occasions and with independent DNA transfections.

RNA Analysis. Total cellular RNA was isolated from transfected cells by lysing cells in guanidium isothiocyanate followed by centrifugation through a CsCl gradient (13). SP6 RNA polymerase and pSPtkCAT (Fig. 2) as template were used to synthesize [^{32}P]UTP-labeled CAT RNA probe complementary to mRNA transcribed from transfected pHIVCAT0 (17). Fifty micrograms of total cellular RNA was used in hybridization reactions with 2.5×10^6 cpm of CAT RNA probe. After 16 hr of hybridization in 40% formamide/40 mM Pipes, pH 6.7/0.4 M NaCl/1 mM EDTA at 45°C, the reaction mixture was treated with RNase A type III-A (4 μg/ml) at 37°C for 1 hr and analyzed by electrophoresis on 6% polyacrylamide/8 M urea sequencing gel (17). The gel was dried and exposed to Kodak XAR-5 x-ray film. The intensity of protected bands was measured with a scanning densitometer from Helena Laboratories.

RESULTS

To determine whether the HBV X gene can transactivate expression of a gene under control of the HIV-1 LTR, plasmids with an intact X gene, pTWU17 (containing the HBV X gene under control of the X promoter and HBV enhancer) and pTWU14 (containing the entire S and X open reading frames) were separately used to cotransfect HepG2 cells with pHIVCAT0 (a plasmid containing a HIV-1 DNA fragment −470 to +80 with the viral LTR inserted in front of the CAT gene). HepG2 cells were used because the HBV enhancer and promoters exhibit specificity for liver cells of human origin (18). It is clear that the presence of pTWU14 or pTWU17 in HepG2 cells with pHIVCAT0 led to a marked increase in the HIV-1 LTR-directed CAT expression com-

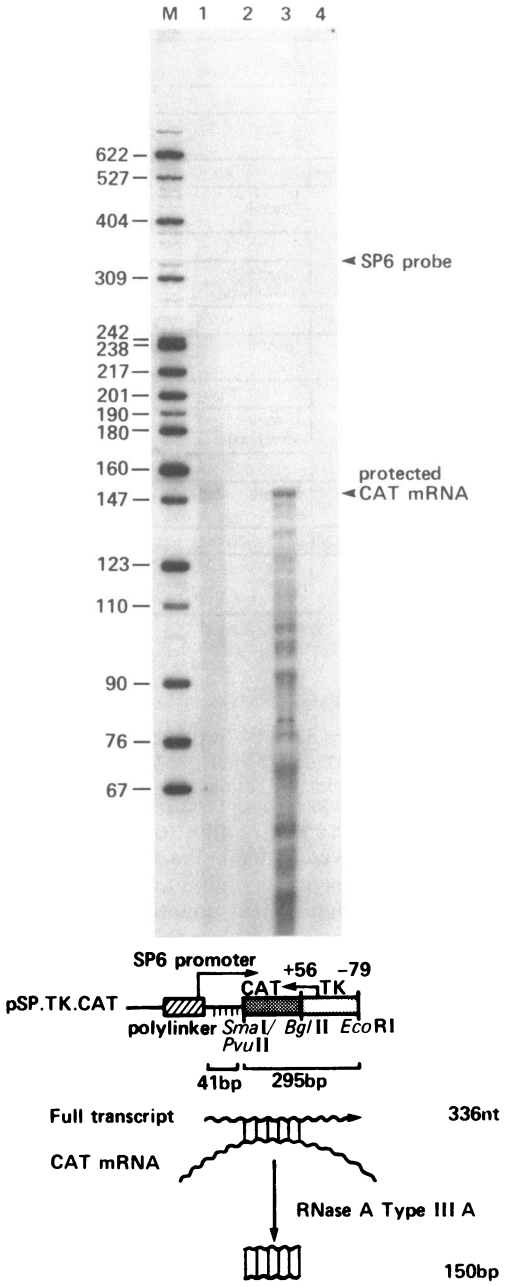

FIG. 2. Transactivation of HIV-1 LTR-directed transcription by the HBV X gene. HepG2 cells were cotransfected with 1 μg of pHIVCAT0 and 10 μg of pUC9 (lane 2) or pTWU17 (lane 3) for 48 hr and then harvested for RNA preparation. Fifty micrograms of total cellular RNA (lanes 2 and 3) or yeast tRNA as negative control (lane 4) was analyzed for CAT mRNA by RNase mapping (17). Markers (lane M) were end-labeled *Msp* I fragments of pBR322 DNA. TK, thymidine kinase; nt, nucleotides.

pared with cells without the HBV X gene (Table 1). As controls, plasmid pTWU17dBamHI, with an insertion mutation in the *Bam*HI site at map position 1400 within the HBV X gene (ref. 5; Fig. 1), and plasmid pTWU18 (ref. 5; Fig. 1), containing only the HBV X gene coding region without its regulatory sequences, were each separately used with pHIVCAT0 for cotransfection of HepG2 cells. The levels of CAT activity observed were no higher than the basal levels detected in cells cotransfected with pHIVCAT0 and pUC9. The failure to demonstrate transactivation of the HIV-1 LTR in cells with the plasmid containing the mutated X gene or the X gene coding region without enhancer and promoter elements and the strong transactivation with constructs containing the intact X gene with its promoter and the HBV

Table 1. Relative CAT activity of pHIVCAT0 used to transfect HepG2 cells in the presence or absence of different HBV X gene constructs

	X DNA/HIV-1 LTR*		X DNA/HIV-1 LTR (10:10)	
Test plasmid	10:1	10:10	Uninduced†	Induced†
pUC9	2.2	41.4	37.8	48.3
pTWU14	104.2	ND		
pTWU17	91.1	238.1		
pTWU17dBamHI	2.1	ND		
pTWU18	1.9	ND		
pTWU19	84.6	595.1		
pMMTV.X	80.3	807.3	739.1	1802.8
phCMV.X	80.9	1718.1		
pMT.X	69.4	1278.2		

The values shown have been normalized and represent the relative CAT activity of each DNA compared to the activity obtained following cotransfection with 10 μg of pUC9 and 1 μg (10:1 ratio) or 10 μg (10:10 ratio) of pSV2CAT in the same cell line. The results represent the average of three independent transfections with results differing by no more than 20%. ND, not done.

*HepG2 cells were cotransfected with test plasmid and pHIVCAT0 at a ratio of 10:1 or 10:10 and CAT assays were done with cell extracts prepared 48 hr after transfections.

†HepG2 cells were cotransfected with 10 μg of pUC9 or pMMTV.X and 10 μg of pHIVCAT0 and incubated in medium in the absence (uninduced) or presence (induced) of 1 μM dexamethasone for 48 hr.

enhancer suggest that X gene expression is necessary for this HIV-1 LTR transactivation.

Similar results were obtained with plasmids containing the HBV X gene under control of a heterologous promoter such as the SV40 early promoter and enhancer elements (pTWU-19), the MMTV LTR (pMMTV.X), the human CMV immediate early promoter (phCMV.X), or the metallothionein promoter (pMT.X). Cotransfection of HepG2 cells with each of the above plasmids containing the X gene and pHIVCAT0 resulted in a 30- to 40-fold increase in CAT expression above the basal level (pUC9, pTWU17dBamHI, and pTWU 18) (Table 1). When the ratio of the test plasmid to pHIVCAT0 was changed from 10:1 to 10:10, significantly higher levels of CAT activity above the basal level were observed for the constructs containing the strongest promoters such as the human CMV immediate early promoter and the metallothionein promoter even though the basal level of CAT expression was increased. Dexamethasone stimulated the expression of CAT activity in HepG2 cells cotransfected with pHIVCAT0 and pMMTV.X (Table 1), a result consistent with stimulation of HBV X gene expression (and consequently increased CAT expression) by dexamethasone through its effect on the glucocorticoid-responsive MMTV LTR (7). These results suggest that the HBV enhancer and X gene promoter are not necessary for HIV-1 LTR transactivation by the HBV X gene because similar transactivation occurs when X gene expression is controlled by heterologous regulatory elements.

To determine whether transactivation of CAT expression in these experiments was associated with activation of transcription, total cellular RNA prepared from HepG2 cells cotransfected with 1 μg of pHIVCAT0 and 10 μg of pUC9 (Fig. 2, lane 2) or pTWU17 (lane 3) was analyzed for CAT transcripts by quantitative RNase mapping (17) with single-stranded uniformly labeled RNA probe synthesized *in vitro* with SP6 RNA polymerase from pSPtkCAT linearized with *Eco*RI to yield a 336-ribonucleotide RNA (lane 1). Correctly initiated transcripts protected a 150-base fragment of this probe (lane 3). Densitometry of the autoradiograms revealed that the X protein transactivated the HIV-1 LTR-initiated CAT transcripts by ≈125-fold (lane 3 versus lane 2). This

result indicates that the HBV X gene transactivates transcription controlled by the HIV-1 LTR.

To determine whether the HBV X gene can transactivate other heterologous viral control sequences, plasmids containing CAT gene under control of the HIV-1 LTR (pHIVCAT0), the visna virus (the prototype of the lentivirus family) LTR (pVisLTRCAT), the HTLV-I LTR (pU3R-I), and the SV40 early promoter and enhancer elements (pSV2CAT) were each used to cotransfect HepG2 cells with the plasmid pTWU17. Fig. 3 shows that the HBV X gene transactivated the HIV-1 LTR (lanes 1 and 2) and the SV40 early promoter and enhancer elements (lanes 7 and 8), and no transactivation was apparent for the visna virus LTR (lanes 3 and 4) or the HTLV-I LTR (lanes 5 and 6), which has been shown to respond to their own transactivator proteins (10, 11). Thus, the HBV X gene appears to transactivate control sequences of some heterologous viruses (e.g., HIV-1 and SV40) but not others (e.g., HTLV-I and visna virus).

To investigate the cell specificity of the transactivating function of the HBV X gene upon the HIV-1 LTR, HepG2 cells, HuH7 cells, MT2 cells, HeLa cells, Vero cells, and NIH 3T3 cells were each cotransfected with plasmids pTWU17 and pHIVCAT0. Table 2 shows that the highest levels of HIV-1 LTR-controlled CAT gene expression in the presence of pTWU17 (containing the X gene) occurred in HepG2 cells, MT2 cells, and Vero cells. A much lower level of transactivation was observed in HeLa cells. There was no apparent effect of pTWU17 on CAT expression in HuH7 cells containing pHIVCAT0, although CAT under control of the HIV-1 LTR was expressed in these cells at a basal level in the absence of pTWU17. NIH 3T3 cells containing pHIVCAT0 had no detectable CAT activity and no transactivation by pTWU17 was observed. The ability of the HBV X gene to transactivate HIV-1 LTR is not the same in all cells, suggesting that cellular factors could be essential for this effect.

Recently, HBV has been shown to replicate to a limited extent in HepG2 cells in culture transfected with closed circular or head-to-tail dimer HBV DNA (14, 20) and infectious HBV has been detected in some cases (20). To investigate whether such cells provide an environment for expression of a gene under control of the HIV-1 LTR, as might be expected if the HBV X gene was expressed under these conditions, pHIVCAT0 was introduced into HepG2T14 cells containing detectable replicating forms of HBV (14) and uninfected HepG2 cells, and CAT gene expression was studied. Table 1 shows that >200-fold higher CAT activity was detected in HepG2T14 cells than in uninfected HepG2 cells, suggesting that the HBV X gene may be expressed at levels sufficient to transactivate HIV-1 LTR in cells replicating HBV at a low level.

Table 2. Effect of HBV X gene on HIV-1 LTR-controlled CAT expression in different cell types

Cell line	Origin	CAT activity, % Ac	
		pUC9	pTWU17
HepG2*	Human hepatoma HBV negative	0.2	13.7
HuH7*	Human hepatoma HBV negative	20.3	21.9
MT2*	Human T-cell leukemia	0.1	1.9
HeLa*	Human cervix carcinoma	0.2	0.7
Vero*	African green monkey kidney	1.1	35.8
NIH 3T3*	NIH Swiss mouse	<0.1	<0.1
HepG2†	Human hepatoma HBV negative	0.2	
HepG2T14†	HepG2 cell transfected with closed-circular HBV DNA, HBV positive	47.0	

*Cells (1×10^6) were cotransfected with 1 μg of pHIVCAT0 and 10 μg of pUC9 or pTWU17. Forty-eight hours later, cells were harvested and same amount of protein of each cell lysate was assayed for CAT activity (expressed as percentage acetylation, % Ac). MT2 cells (1×10^7) were cotransfected with 1 μg of pHIVCAT0 and 10 μg of pUC9 or pTWU17 by the DEAE-dextran method of Sompayrac and Danna (19). Cells were harvested 44 hr posttransfection and cell lysate was assayed for CAT activity.

†HepG2 or HepG2T14 cells (1×10^6) were cotransfected with 1 μg of pHIVCAT0 and 10 μg of pUC9. Forty-eight hours later, cells were harvested and assayed for CAT activity.

Since HBV X protein and HIV-1 tatIII protein each transactivate the HIV-1 LTR, an experiment was done to determine whether a synergistic effect on the HIV-1 LTR would occur when both were introduced into cells containing pHIVCAT0. Fig. 4 shows that when HepG2 cells were cotransfected with 1 μg of pHIVCAT0 plus either 10 μg of pTWU17 (lane 2) or 1 μg of pdCEBtat (lane 3), CAT expression was increased over that observed in cells with pHIVCAT0 alone (lane 1). When plasmids pTWU17 (10 μg) and pdCEBtat (1 μg) were used together with pHIVCAT0 (1 μg) for transfection of HepG2 cells (lane 4), CAT expression was significantly greater than in pHIVCAT0-containing cells with either plasmid alone, suggesting a cooperative effect of

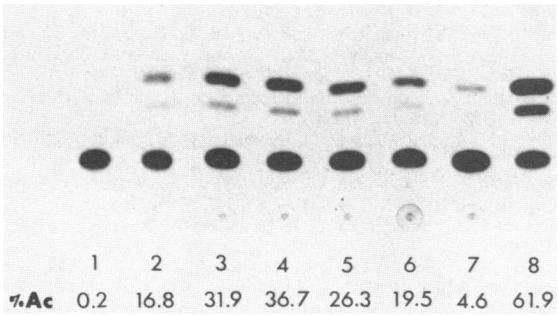

FIG. 3. Effect of the HBV X gene on expression of the CAT gene under control of HIV-1, visna virus, HTLV-I, and SV40 regulatory elements. Cells (1×10^6) were transfected with 1 μg of pHIVCAT0 (lanes 1 and 2), pVisLTRCAT (lanes 3 and 4), pU3R-I (lanes 5 and 6), or pSV2CAT (lanes 7 and 8) in the presence (even-numbered lanes) or absence (odd-numbered lanes) of 10 μg of pTWU17. Forty-eight hours later, cells were harvested and an equal amount of protein of each cell extract was assayed for CAT activity (expressed as percent acetylation, % Ac).

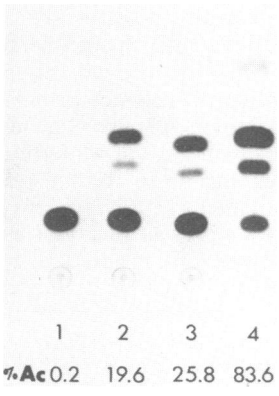

FIG. 4. Cooperative effect of the HBV X gene and the HIV-1 *tatIII* gene on the HIV-1 LTR-directed CAT gene expression in HepG2 cells. HepG2 cells (1×10^6) were cotransfected with 1 μg of pHIVCAT0 plus 10 μg of pUC9 (lane 1), 10 μg of pTWU17 (lane 2), 1 μg of pcDEBtat (lane 3), or 10 μg of pTWU17 and 1 μg of pcDEBtat (lane 4). The difference in the total amount of plasmid DNA used in each transfection was made by the addition of pUC9. Cells were harvested 48 hr later and equal amounts of protein of each cell extract were assayed for CAT activity (expressed as percent acetylation, % Ac).

the HBV X gene and the HIV-1 *tatIII* gene in the HIV-1 LTR transactivation.

DISCUSSION

We have demonstrated that the HBV X gene can transactivate the HIV-1 LTR and SV40 sequences containing the SV40 enhancer and early promoter as it apparently can a human β-interferon gene regulatory region (5). The expression of the HBV X gene, either controlled by HBV regulatory sequences or by heterologous control sequences, appears to be required for this transactivation because a frame-shift mutation in the X gene or removal of the X gene promoter and enhancer abolished the effect on the HIV-1 LTR. Transactivation of HIV-1 LTR-directed transcription by the HBV X gene was shown by an RNase mapping experiment. The HBV X gene appears not to transactivate all heterologous viral control sequences since the visna virus LTR and the HTLV-1 LTR were not responsive to the HBV X gene in these experiments. The apparent synergism of HBV X protein and HIV-1 *tatIII* gene product in stimulating CAT gene expression under control of the HIV-1 LTR in cells containing specific plasmid constructs suggests that the HIV-1 *tat* gene and the HBV X gene products may act by different mechanisms and raises the question of whether HBV could exert a similar effect on HIV-1 during coinfection of cells with the two viruses under natural conditions. Both HIV-1 and HBV have been shown to infect T cells *in vivo* (21), and we show here that the HIV-1 LTR is activated in HepG2 cells replicating HBV (Table 2) and that the HIV-1 LTR can be transactivated by the HBV X gene in a T4-positive cell line MT2 (Table 2).

Several cis-acting regulatory sequences have been identified in the HIV-1 LTR and the function of these cis-acting elements is regulated in part by interaction with protein factors including at least one HIV-1-encoded trans-acting factor as well as cellular regulatory protein factors, some of which have been identified. The trans-acting responsive (TAR) sequence mapped in the HIV-1 LTR between −17 to +80 nucleotides (+1 being the messenger RNA start site) is regulated in trans by the HIV-1 *tatIII* gene (for transactivator gene of HTLV-III) (22, 23). Additional control sequences identified in the HIV-1 LTR include three tandem regulatory factor SP1 binding sequences (between −77 and −45) shown to be functionally responsive to SP1 and only two of which are essential for promoter function (24); two core transcriptional enhancer elements (−104 to −95 and −90 to −81, respectively) (25), both of which are essential for responsiveness to extracts of cells induced with phorbol esters (26, 27) and these are identical in sequence to enhancer core regions of SV40 early and immunoglobulin K genes; a region homologous to 5′ flanking sequences of γ-interferon (−125 to −109) (28); a region homologous to the interleukin 2 distal and proximal control elements (−302 to −246) (28); a TATA box (−28 to −24); and a repressor region or negative regulatory element (−340 to −185) (26). In addition, there is an apparent binding sequence (CCCCAGCG) for the transcriptional regulatory protein AP-2 that is very similar to the AP-2 binding sequence in SV40 (CCCCAGGC) (29). Which (if any) of these regulatory elements of the HIV-1 LTR are required for the transactivating effect of the HBV X gene is not yet known. However, it is noteworthy that the HIV-1 LTR (28), the SV40 enhancer region (30), and the human β-interferon regulatory region (31) share a common core enhancer sequence that is not found in the visna virus LTR (10) or the HTLV-I LTR (32), raising the possibility that this core enhancer sequence may be required for the HBV X gene regulatory effect.

The HBV X gene could exert its effect on the HIV-1 LTR and SV40 control sequences by acting directly on a viral sequence or it could affect HIV-1 LTR function indirectly by altering the activity of one or more cellular regulatory protein factors. The variation in the effect of the HBV X gene on transactivating the HIV-1 LTR in different cell types suggests that tissue-specific cellular factor(s) may be involved in this HBV X gene regulatory effect.

The HBV X gene appears to transactivate regulatory sequences of certain cellular genes and viruses, and this suggests that the X gene could serve a regulatory function during natural HBV infection although this has not yet been shown. Regulatory effects on both HBV and cellular genes must be considered. Our demonstration that cells in culture replicating HBV activate HIV-1 LTR-controlled CAT expression suggests the HBV X gene is expressed during HBV replication.

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