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Activation of the HTLV-I Long Terminal Repeat by the Hepatitis B Virus X Protein

SUSAN J. MARRIOTT,[1] TEH-HSIU LEE, BETTY L. SLAGLE, and JANET S. BUTEL

Division of Molecular Virology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030

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The human T-cell leukemia virus type I (HTLV-I) Tax protein and the hepatitis B virus (HBV) X protein have each been shown to activate transcription of their respective viral promoters as well as a subset of cellular gene promoters. Here we show that the HTLV-I long terminal repeat (LTR) is responsive to HBV X transactivation. Maximum levels of X-mediated transactivation of the LTR were 8-fold. An X-responsive-region (XRR) of the LTR is located between nucleotides −355 and −276 and contains an AP-2 binding site, a previously recognized X-responsive element. We demonstrated that Tax and X synergize to activate transcription from the HTLV-I LTR, although the AP-2 binding site was not required for this synergy. These results raise the possibility that the HBV X protein may affect the level of HTLV-I gene expression in co-infected individuals.

INTRODUCTION

Human T-cell leukemia virus type I (HTLV-I) and hepatitis B virus (HBV) both cause human diseases characterized by long latency periods. HTLV-I is the etiologic agent of adult T-cell leukemia (ATL) and tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) (Gessain *et al.,* 1985; Hinuma *et al.,* 1982; Poiesz *et al.,* 1980; Posner *et al.,* 1981; Yoshida *et al.,* 1982), whereas chronic infection with HBV has been associated with an increased risk of developing hepatocellular carcinoma (Beasley *et al.,* 1981; Buendia, 1992; Slagle *et al.,* 1992; Szmuness, 1978). Both HTLV-I and HBV can infect T lymphocytes (Davison *et al.,* 1987; Pasquielli *et al.,* 1986) and it is known that certain patients are co-infected with both viruses (Asakura *et al.,* 1991; Yamaguchi *et al.,* 1993). It is possible that co-infected individuals might display a more aggressive disease progression.

HTLV-I encodes a nonstructural protein, Tax, which is a powerful transcriptional transactivator and is the transforming protein of this virus (reviewed in Sodroski, 1992). Tax activates transcription of the HTLV-I long terminal repeat (LTR), as well as a variety of cellular gene promoters. At least three different Tax-responsive elements have been identified in these promoters, including cAMP response element (CRE)/activating transcription factor (ATF), serum response element (SRE), and nuclear factor κB (NF-κB) sites.

Regulation of HTLV-I transcription is complex and involves numerous cellular transcription factors (reviewed in Sodroski *et al.,* 1992). Two different Tax-responsive elements have been described in the HTLV-I LTR, including three copies of a 21-basepair repeat known as Tax responsive element 1 (TRE-1). Two or more copies of TRE-1 are necessary to confer Tax responsiveness to a promoter. Multimerized copies of TRE-1 are also responsive to the mitogens TPA and cAMP, and TRE-1 contains a core element which resembles a CRE. TRE-1 has been shown to bind cellular transcription factors which are members of the CRE-binding (CREB) and ATF protein families. Further, Tax has been shown to form a physical complex with CREB and ATF (Suzuki *et al.,* 1993; Wagner and Green, 1993; Zhao and Giam, 1991, 1992). A single copy of the second Tax-responsive element, TRE-2, is located between −117 and −163 in the LTR. Multimerized copies of TRE-2 are not Tax responsive, but a single copy of TRE-2 in combination with a single copy of TRE-1 confers Tax responsiveness to a promoter. TRE-2 contains binding sites for Sp1, Myb, Ets1, NF-κB, and a 36-kDa protein (Bosselut *et al.,* 1990, 1992; Gitlin *et al.,* 1991; Marriott *et al.,* 1990; Numata *et al.,* 1991; Nyborg *et al.,* 1990). Tax has been shown to form a protein–protein complex with the 36-kDa protein (Marriott *et al.,* 1990) and can cooperate with Ets1 in transactivation of the LTR (Gitlin *et al.,* 1993).

HBV encodes a 17-kDa nonstructural protein, X, which can transactivate transcription from many viral and cellular promoters (Avantaggiati *et al.,* 1992; Levrero *et al.,* 1990; Menzo *et al.,* 1993; Rossner, 1992; Seto *et al.,* 1988; Siddiqui *et al.,* 1989; Spandau and Lee, 1988; Twu and Schloemer, 1987; Twu and Robinson, 1989; Twu *et al.,* 1989, 1993; Zahm *et al.,* 1988; Zhou *et al.,* 1994). Although the role of X in HBV pathogenesis is not clearly defined, almost all infected individuals have antibodies to X (Feitelson, 1992; Kay *et al.,* 1985; Moriarty *et al.,* 1985), suggesting that it is expressed during the replication cycle. The viral enhancer(I) serves as the X-responsive

[1] To whom correspondence and reprint requests should be addressed. Fax: (713) 798-3590. E-mail: susanm@bcm.tmc.edu.

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element in the HBV promoter (Spandau and Lee, 1988; Twu and Schloemer, 1987). The X protein is frequently termed a "promiscuous" transactivator as numerous transcriptional regulatory sequences from heterologous viruses and cellular genes have been reported as targets for transactivation. X has been reported to transactivate adenovirus, herpes simplex virus, human immunodeficiency virus, HTLV-I, HTLV-II, mouse mammary tumor virus, Rous sarcoma virus, SV40, and Visna virus, as well as a variety of cellular genes (reviewed in Rossner, 1992). The X-responsive elements in these promoters include AP-1, AP-2, AP-3, and NF-κB sites, as well as HBV enhancer elements. Like Tax, the HBV X protein does not bind DNA specifically and recent studies suggest that the X protein acts indirectly to activate transcription through cellular signaling pathways involving the serine/threonine kinases, protein kinase C, and Raf-1 (Benn and Schneider, 1994; Cross *et al.,* 1993; Kekule *et al.,* 1993). In addition, the X protein is able to interact with a cellular DNA repair protein (Lee *et al.,* 1995), providing another means by which X may modify cellular replication processes.

The results that follow demonstrate that the HBV X protein can activate transcription of the HTLV-I LTR. An X-responsive region (XRR) of the LTR was localized to nucleotides −355 to −276 and was shown to contain an AP-2 binding site. Further, Tax and X synergistically activated transcription of the LTR; however, the AP-2 binding site was not required for this synergy. These results suggest the possibility that the HBV X protein may affect the level of HTLV-I gene expression in co-infected cells.

MATERIALS AND METHODS

Plasmids

Expression constructs containing the HTLV-I Tax and Gal-Tax genes under the control of the SV40 promoter have been described previously (Connor *et al.,* 1993). Construction of the HTLV-I deletion constructs 10-1, 6-3, and 11-2 (Brady *et al.,* 1987) and promoters containing gal4 binding sites, 11-2-35ASgalS and 6-2galSS (Conner *et al.,* 1993), have been described previously.

5′LTR-CAT was constructed by PCR amplification of the 5′ region of the HTLV-I LTR from a pU3RCAT (Sodroski *et al.,* 1984) template using the 5′LTR-S and 5′LTR-AS primers. The primers were designed to convert the ends of the resulting fragment to *Bgl*II sites with an internal *Pvu*II site at the 3′ end of the double-stranded PCR product: 5′LTR-S, 5′-GACTCTAGATCTCATAGTTTACATCTCC-3′; 5′LTR-AS, 5′-GGGCTGAGATCTCAGCTGACTTCTGTTTCTCGG-3′. The resulting PCR product was digested with *Bgl*II and cloned into the *Bgl*II site of pCAT-Promoter (Promega), which contains the enhancer-less SV40 promoter upstream of the CAT gene. Gal-X was constructed by cloning the X-coding sequence (Lee *et al.,* 1995) into the vector pSG424 (Sadowski and Ptashne, 1989), which contains the coding sequence for amino acids 1–147 of Gal4 followed by a polylinker. The X gene was cloned in-frame into the *Sma*I site of the polylinker. LTR△AP-2 was constructed by cloning oligonucleotides containing a mutant AP-2 binding site into the *Sma*I site of pU3RCAT. This cloning disrupted the native AP-2 site in the LTR and replaced it with a mutant element. The sense strand of the mutant AP-2 oligonucleotide is shown with the mutant bases underlined: HTLV mut AP-2S 5′-CCCAAATATCCCTTGGGGGCTTAG-3′.

Cells and transfections

HeLa cells were maintained in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal calf serum (FCS). For transfections, 2×10^5 cells were seeded in 35-mm dishes. After 24 hr the medium was replaced and approximately 5 hr later the cells were transfected by the calcium phosphate method as described (Connor *et al.,* 1993). The specific amounts of plasmids used in each transfection are provided in the figure legends. Approximately 18 hr following transfection the medium was removed and replaced with fresh medium. Cells were harvested for CAT assays 48 hr following transfection.

CAT assays

Cells were harvested and CAT assays were performed as described (Connor *et al.,* 1993). Briefly, cells were washed in phosphate-buffered saline and collected by scraping in TEN buffer (40 m*M* Tris, pH 7.8, 1 m*M* EDTA, 150 m*M* NaCl). Cells were pelleted, resuspended in 0.25 *M* Tris, pH 7.8, and disrupted by three freeze/thaw cycles. The amount of extract used in each CAT assay was selected so that the resulting conversion to acetylated chloramphenicol would be less than 70%. Each experiment was repeated a minimum of two times. TLC plates were quantitated using a Betagen blot analyzer. The percentage conversion of CAT to the acetylated form was calculated by dividing the cpm found in ^{14}C-labeled acetylated chloramphenicol by the total of acetylated and nonacetylated chloramphenicol. All data are reported as fold activation, which was calculated by dividing the percentage CAT conversion of a particular sample by the percentage CAT conversion achieved with the corresponding vector alone.

Gel shift assays

Oligonucleotides encompassing nucleotides −309 to −326 in the HTLV-I LTR were synthesized with overhanging *Xba*I compatible ends using a Beckman Oligo1000 DNA synthesizer. The sequence of the sense strand oligonucleotide is HTLV AP-2S, 5′-CTAGCAAATATCCCCCGGGGGT-3′. Following synthesis, the oligonucleotides were annealed and labeled with [^{32}P]dCTP using the

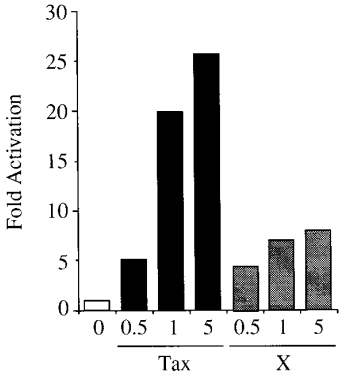


FIG. 1. The HBV X protein activates the HTLV-I LTR. HeLa cells were transfected with 5 μg of pU3RCAT reporter plasmid alone (□) or with increasing concentrations (0.5, 1, 5 μg) of Tax (■) or X (▨) expression plasmids as indicated. Fold activation was calculated by dividing the CAT activity of activated constructs by the basal activity of pU3RCAT.

Klenow fragment of DNA polymerase. The labeled, double-stranded oligonucleotide (50,000 cpm/0.71 ng) was incubated with 2 μl of HeLa nuclear extract (6.2 μg/μl), 1 μg of poly(dI–dC), and 6 μl of 5× reaction buffer (50 m*M* HEPES, pH 7.9, 20% Ficoll, 250 m*M* KCl, 1 m*M* EDTA, and 1 m*M* DTT). Buffer D (20 m*M* HEPES, pH 7.9, 100 m*M* KCl, 12.5 m*M* $MgCl_2$, 17% glycerol, 1 m*M* EDTA, and 1 m*M* DTT) was added to each reaction to bring the total volume of buffer D plus nuclear extract to 18 μl. A 50-fold molar excess of unlabeled competitor oligonucleotides was added to certain reactions as indicated. The reactions were incubated for 20 min at room temperature. The antibody supershift experiment was performed by adding the specified rabbit polyclonal antibody following the initial 20-min incubation, then incubating for an additional 30 min at room temperature. The samples were separated on a 5% nondenaturing polyacrylamide gel in 0.5× TBE (45 m*M* Tris-borate, 1 m*M* EDTA) at 150 V for 2.5 hr. The gel was dried and exposed to Kodak XAR5 X-ray film for 9 hr at −70° with a screen. The AP-2 consensus and mutant competitors as well as the anti-AP-2, anti-AP-1, and anti-Sp1 antibodies were purchased from Santa Cruz Biotechnology Inc. The AP-1, AP-3, and Sp1 competitors were purchased from Stratagene.

RESULTS

HBV X protein transactivates the HTLV-I LTR

To investigate the possibility that the HBV X protein may activate the HTLV-I LTR, HeLa cells were cotransfected with a plasmid encoding the HTLV-I LTR driving chloramphenicol acetyl transferase (CAT) expression (pU3RCAT) and increasing concentrations of expression vectors encoding either the HTLV-I Tax or the HBV X proteins. Both Tax and X genes were under the control of the SV40 early promoter. Transfection of 0.5, 1, and 5 μg of Tax resulted in dose dependent activation of the LTR with a maximum of 25-fold activation (Fig. 1). Transfection of 0.5, 1, and 5 μg of X also resulted in dose-dependent activation of the LTR with a maximum of 8-fold activation. The 8-fold activation of the HTLV-I LTR by X is typical of other X responsive promoters. While this experiment demonstrates that HBV X can activate the HTLV-I LTR in HeLa cells, X appears to be a weaker activator of the HTLV-I LTR than Tax.

An X-responsive element resides in the 5′ end of the HTLV-I LTR

X-responsive elements have been mapped to NF-κB, AP-1, AP-2, and AP-3 sites. The HTLV-I LTR does not contain any previously recognized AP-1, or AP-3 sites, although the location of putative AP-2 and NF-κB sites has been suggested (Numata *et al.,* 1991; Nyborg *et al.,* 1990). To determine which portion of the HTLV-I LTR was involved in X activation, a series of LTR deletion mutants was tested (Fig. 2A). As shown in Fig. 2B, Tax activated expression of the full-length LTR (pU3RCAT) as well as two deletion mutants (10-1 and 6-3). The increased Tax activation of the deletion mutants, as compared to the full-length LTR, has been previously reported (Brady *et al.,* 1987; Marriott *et al.,* 1990) and could result from deletion of a negative regulatory element combined with reduced basal activities of the deletion mutants. As seen previously, HBV X activated the full-length HTLV-I LTR. In contrast, X activation of the deletion mutants was diminished in mutant 10-1 and could not be distinguished from basal activity in mutant 6-3. The 10-1 reporter has deleted nucleotides −355 to −306 in the viral LTR relative to the transcription start site, while in the 6-3 construct, additional nucleotides were deleted to −242 (Brady *et al.,* 1987). Thus, this result suggested that an X-responsive element resides in the 5′ region of the LTR between nucleotides −355 and −242. To test this possibility, X transactivation of nucleotide sequences −355 to −276 from the LTR (5′LTR-CAT) was examined. Nucleotides −242 to −276 were not included in this reporter construct because they contain a portion of TRE-1. The 5′LTR-CAT reporter was not responsive to Tax but was activated fourfold by HBV-X, demonstrating that an X responsive element resides between nucleotides −355 and −276 in the HTLV-I LTR. These nucleotides are thus referred to as the X-responsive region of the HTLV-I LTR. The nucleotide sequence of the XRR is shown in Fig. 2C. Activation of the XRR by X protein is approximately 75% of that observed with the full LTR, suggesting that sequences outside of the XRR may contribute to optimal X transactivation.

Visual inspection of the XRR for elements which had previously been recognized as X responsive, revealed a sequence at position −328 to −320 which resembles an AP-2 binding site (Fig. 3A). To determine whether this element functions as a specific AP-2 binding site, gel shift analysis was performed using a series of oligonu-

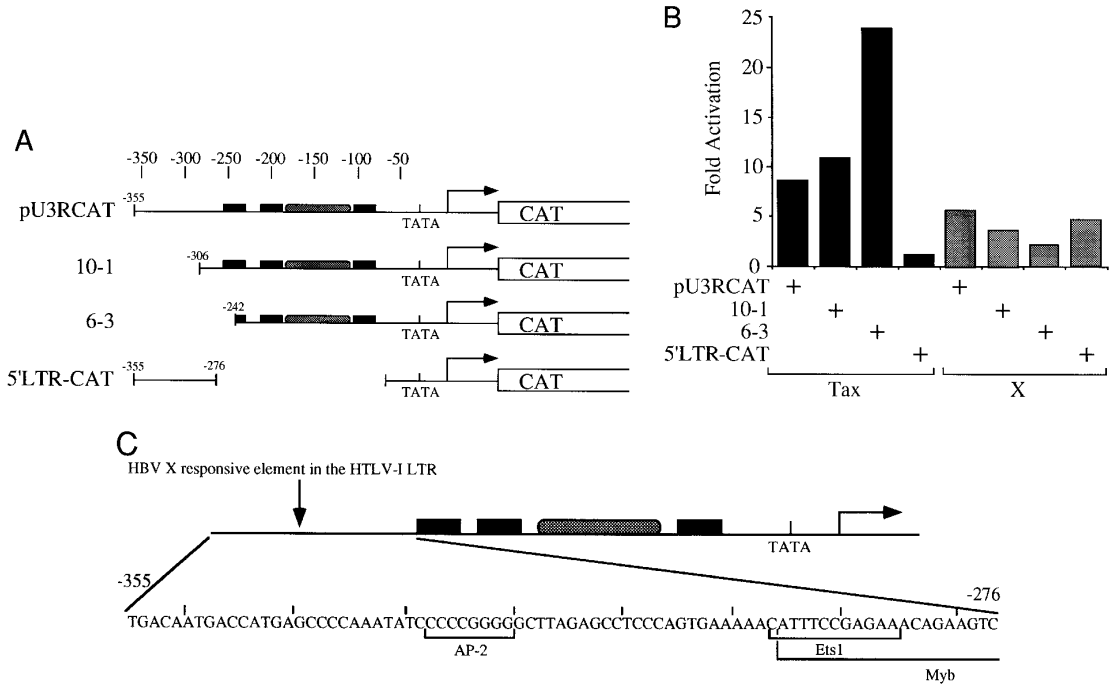
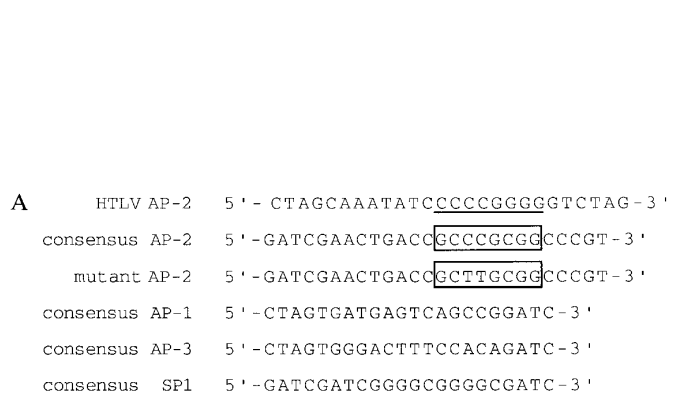


FIG. 2. The X-responsive element resides in the 5′ end of the LTR. (A) Diagram of reporter constructs used in this experiment. TRE-1 and TRE-2 elements are shown as black and shaded rectangles, respectively. The position of the TATA box (⊥), transcription start site (→), and CAT reporter gene (▭) is shown. Nucleotide numbering of the LTR relative to the transcription start site is shown above. (B) HeLa cells were transfected with 5 μg of the indicated reporter plasmid (pU3RCAT, 10-1, 6-3, 5′LTR-CAT) and either 1 μg of Tax (■) or 3 μg of X (▩) expression plasmids. The basal activity of each reporter was normalized to one and fold activation was calculated by dividing the CAT activity of activated constructs by their basal activity. (C) Detailed diagram of the X responsive element in the HTLV-I LTR. LTR elements are depicted as described in A. The nucleotide sequence of the X-responsive region of the LTR is shown at the bottom. The position of previously reported Ets1 and Myb binding sites is shown. An LTR sequence containing 75% identity with a consensus AP-2 site is underlined.

cleotide competitors. When the ^{32}P-labeled oligonucleotide probe representing the putative AP-2 binding site in the HTLV-I LTR was mixed with HeLa nuclear extract a gel shift complex was formed. Formation of this complex was reduced when the reaction contained a 50-fold excess of the unlabeled probe or an oligonucleotide representing the consensus AP-2 binding site. A mutation in the consensus AP-2 binding site which has previously



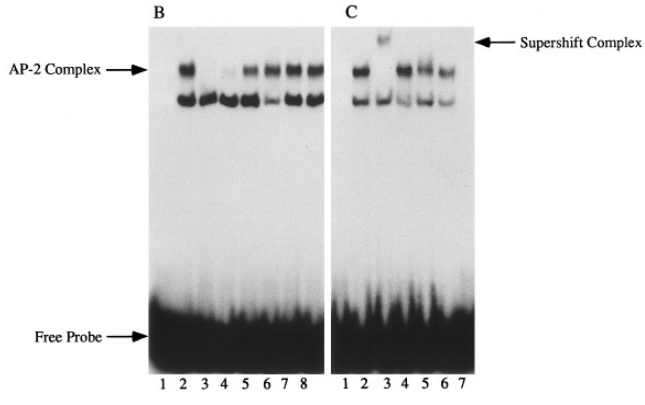


FIG. 3. Gel shift analysis of the X-responsive element in the HTLV-I LTR. (A) The nucleotide sequence of the HTLV-I LTR used as a probe is shown (HTLV AP-2) as well as the sequences of competitor oligonucleotides: consensus AP-2, mutant AP-2, consensus AP-1, consensus AP-3, and consensus SPl. The core element necessary for protein binding is boxed in the AP-2 and mutant AP-2 sequences. The HTLV AP-2 element showing sequence conservation with the consensus AP-2 core element is underlined. (B) The ^{32}P-labeled HTLV AP-2 probe was incubated alone (lane 1) or with HeLa nuclear extract (lanes 2–8). A 50-fold molar excess of unlabeled oligonucleotide competitor was added to lanes 3–8 (lane 3, HTLV AP-2; lane 4, consensus AP-2; lane 5, mutant AP-2; lane 6, consensus AP-1; lane 7, consensus AP-3; lane 8, consensus SPl). The position of the specific protein complex is indicated by the arrow. The location of the free probe is also indicated. (C) The ^{32}P-labeled HTLV AP-2 probe was incubated alone (lane 1) or with HeLa nuclear extract (lanes 2–6). Antibodies against specific transcription factors were added to lanes 3–7 (lanes 3 and 7, anti-AP-2; lane 4, anti-SPl; lane 5, anti-CREB; lane 6, anti ATF-3). The positions of the specific protein complex and the supershifted complex are indicated. The location of the free probe is also indicated.

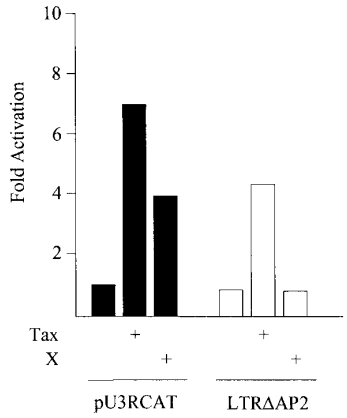


FIG. 4. The AP-2 binding site is required for X transactivation of the HTLV-I LTR. HeLa cells were transfected with 5 μg of the reporters pU3RCAT or LTRΔAP-2 alone, or in combination with 1 μg of Tax or 3 μg of X expression plasmids. Fold activation was calculated as described in the legend to Fig. 2.

been shown to abolish AP-2 binding did not compete for complex formation. Three other transcription factor binding site oligonucleotides (AP-1, AP-3, and Sp1) were similarly unable to compete for complex formation. These results demonstrate that a specific protein complex can form on the HTLV-I probe and that this complex is likely to be AP-2. To confirm this result, a supershift assay was performed (Fig. 3B). Addition of anti-AP-2 antibody to the gel shift reactions caused the complex to shift to a slower mobility while antibodies directed toward three other transcription factors (Sp1, CREB, and ATF-3) were unable to supershift the complex (Fig. 3C). In the absence of nuclear extract, the AP-2 antibody did not affect the mobility of the labeled probe. These results verify the identity of the protein involved in the complex as AP-2.

The AP-2 element is required for X transactivation of the HTLV-I LTR

To directly determine whether the AP-2 element is involved in X transactivation of the HTLV-I LTR the element was mutated within the context of the complete LTR (LTRΔAP-2). This mutation introduced a 2 base pair C to T change that was identical to the mutation which abolished AP2 binding in the previous experiment. LTRΔAP-2 was completely unresponsive to HBV-X but retained responsiveness to Tax (Fig. 4). Thus, the binding of AP-2 correlates with X transactivation of the HTLV-I LTR and specifically, the AP-2 binding site located at -320 was required for X transactivation.

Tax and X synergistically activate the HTLV-I LTR

Tax and X share many similar characteristics including transforming properties, transcriptional activity, and an inability to bind DNA specifically. Therefore, it was of interest to determine whether these two transactivators could cooperate in activation of the HTLV-I LTR. The full-length LTR was activated 25-fold by Tax and 4-fold by X (Fig. 5). When transfected together, Tax and X activated transcription of the LTR 45-fold. To control for the possible effects of increased vector and promoter concentrations in the transfection containing both Tax and X, a plasmid expressing β-galactosidase under control of the SV40 promoter was used to replace the appropriate transactivator. No synergistic activity was observed with either Tax and β-gal or X and β-gal. In addition, β-gal activities were similar when transfected alone or when transfected with Tax or X, demonstrating that neither protein affected levels of expression from the SV-40 promoter under the conditions tested (data not shown). These results demonstrate that HTLV-I Tax and HBV X proteins can specifically synergize to activate the HTLV-I LTR.

Synergistic activation of the HTLV-I LTR by Tax and X does not require the XRR

Since Tax and X utilize distinct elements for independent activation of LTR transcription, we examined the possibility that the synergistic effect of Tax and X required both response elements. Reporter constructs containing either functional Tax response elements and mutated X response elements (10-1, 6-3, 11-2, and LTRΔAP-2) or a functional X response element in the absence of Tax response elements (5′LTR-CAT) were examined for synergistic activation. Each of the reporters containing a functional Tax response element in the absence of an X response element were activated by Tax, failed to be activated by X, and displayed synergistic activation in the presence of both proteins (Fig. 5). The reporter 5′LTR-CAT, containing a functional X response element in the absence of Tax response elements, was activated by X, failed to be activated by Tax, and did not display synergistic activation in the presence of both proteins. These results demonstrate that synergistic activation of the HTLV-I LTR by Tax and X requires a Tax response element but does not require an X response element. Thus, the ability of X to activate transcription of the HTLV-I LTR and its ability to work in combination with Tax to synergistically activate transcription may be separable activities.

DISCUSSION

The results described here demonstrate that the HBV X protein can transactivate the HTLV-I LTR through an element located in the 5′ end of the LTR. This region of the LTR contains an AP-2 binding site which is required for X activation. In combination, Tax and X proteins synergistically activate the LTR, although the synergy does not require the AP-2 binding site. Based on these results, we propose that co-infection with HTLV-I and HBV may influence the progression of HTLV-I-associated disease. A direct analysis of the effect of HBV on HTLV-I replica-

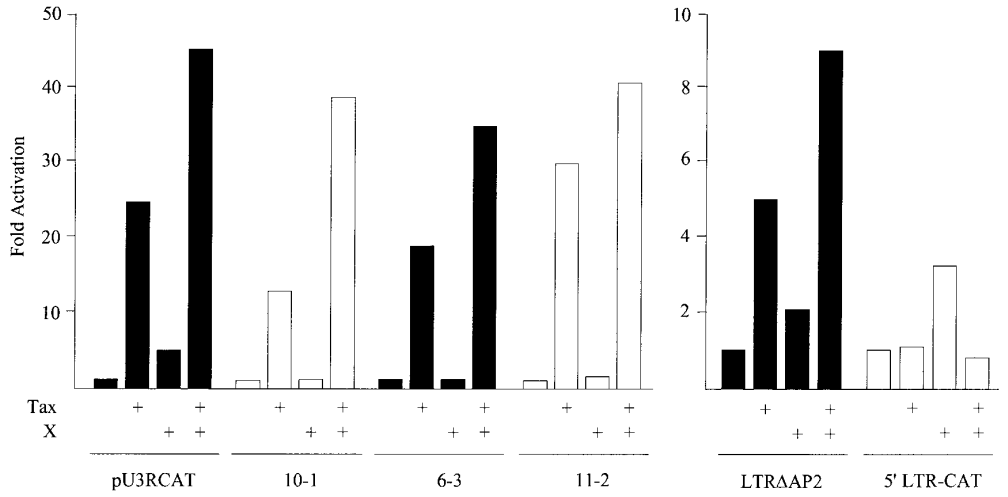


FIG. 5. Tax and X synergistically transactivate the HTLV-I LTR. HeLa cells were transfected with 5 μg of the indicated reporters alone, or in combination with 0.5 μg of Tax or 3 μg of X expression plasmids as indicated. Fold activation was calculated as described in the legend to Fig. 2.

tion awaits the optimization of infectious HTLV-I and HBV clones.

There are several possible mechanisms by which the HBV X protein could activate the HTLV-I LTR. The X protein may act directly on a sequence within the HTLV-I LTR, although X does not appear to bind DNA specifically (Faktor and Shaul, 1990; Rossner, 1992). It has previously been reported that X activation may be mediated through NF-κB, CREB/ATF, AP-1, AP-2, or AP-3 elements (Lucito and Schnieder, 1992; Maguire *et al.,* 1991; Seto *et al.,* 1990; Twu *et al.,* 1989). A potential NF-κB site has been identified within the TRE-2 region of the HTLV-I LTR, but it is clear from studies presented here that this site is not sufficient for X activation (Fig. 2B). The TRE-1 elements within the HTLV-I LTR share identity with CREB and ATF binding sites and these proteins can bind the TRE-1 elements. Although X has been shown to bind CREB/ATF-2 proteins (Maguire *et al.,* 1991), our data demonstrate that these elements are not sufficient for X transactivation of the HTLV-I LTR. No AP-1 or AP-3 elements have been identified within the HTLV-I LTR, although two AP-2 sites have been recognized at approximately −180 and −230 (Nyborg *et al.,* 1990). These AP-2 sites are not present in the 5′ LTR construct which was activated by X and the construct 6-3, which contains these elements, was not efficiently activated by X, suggesting that they are also not sufficient for maximal activation by X. However, an additional site containing a 6 of 8 nucleotide match with the AP-2 consensus sequence located within the X-responsive 5′ region of the LTR was identified in this study. We demonstrated that this sequence does serve as a functional AP-2 binding site which is both necessary and sufficient for X transactivation of the HTLV-I LTR. It is possible that X may bind AP-2 directly, forming a physical complex with enhanced transcriptional activity, as has been shown with X and CREB/ATF family members (Maguire *et al.,* 1991). Alternative mechanisms of X transactivation which do not require that X become associated with a promoter have been suggested. For example, X has been shown to affect the affinity of AP-1 for DNA, possibly by modulating its phosphorylation state (Natoli *et al.,* 1994). Supporting this model, X has been shown to affect the activity of cellular kinases (Benn and Schneider, 1994; Cross and Rutter, 1993; Luber *et al.,* 1993). Thus, it appears likely that activation of the HTLV-I LTR by X protein occurs through an indirect mechanism and may involve multiple elements within the LTR.

The ability of Tax and X to synergistically activate transcription may involve cooperative interactions between their two activation pathways. It has recently been demonstrated that X can function as a coactivator in combination with a variety of potent activation domains (Haviv *et al.,* 1995), although Tax was not included in that study. It is possible that X may provide a coactivator function for the Tax activation domain, resulting in the synergistic activation of LTR transcription which is observed in the presence of both proteins. As synergistic activation of the HTLV-I LTR by Tax and X does not require the presence of the X response element, it is possible that the ability of X to modulate cellular serine/threonine kinases may increase the pool of phosphorylated transcription factors available for Tax-mediated transactivation.

Whereas these studies are of general interest due to the insights they provide into basic transcription mechanisms, the finding that X and Tax synergistically activate LTR transcription more specifically suggests that co-infection by HTLV-I and HBV might lead to a more aggressive HTLV-I infection and possibly affect the development of HTLV-I-associated disease. In a survey of 13 villages in Papua, New Guinea, 3 of 13 (23%) serum samples which tested positive for HTLV-I were also positive for HBV, demonstrating that co-infection with these two viruses is relatively prevalent in this population (Yama-

guchi *et al.,* 1993). In a study of HTLV-I and HBV infection among Japanese blood donors, approximately 3% of those positive for HTLV-I were also positive for HBV (Asakura *et al.,* 1991). Both HTLV-I and HBV have been shown to infect T cells *in vivo* (Colucci *et al.,* 1988; Davison *et al.,* 1987; Laure *et al.,* 1985; Zeldis *et al.,* 1986), although it has not been determined whether the same cells are infected with both viruses in co-infected individuals. This study highlights the importance of future efforts to determine the role of HTLV-I and HBV co-infection in the pathogenesis of associated diseases.

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