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Activation of Enhancer Sequences in Type II Human T-Cell Leukemia Virus and Bovine Leukemia Virus Long Terminal Repeats by Virus-Associated *trans*-Acting Regulatory Factors

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The ability of the sequences present in the long terminal repeats (LTRs) of human T-cell leukemia viruses type I and II (HTLV-I and HTLV-II) and of bovine leukemia virus to function as enhancer elements was investigated. Recombinant plasmids that contained the HTLV-I, HTLV-II, and bovine leukemia virus LTRs at a distance from a simian virus 40 promoter element located 5′ to the bacterial gene encoding chloramphenicol acetyltransferase (EC 2.3.1.28) were constructed. We report that all three LTR sequences contain enhancer elements capable of increasing the level of gene expression directed from a distal heterologous promoter. The enhancer present in the HTLV-I LTR was active in uninfected cells of lymphoid and nonlymphoid origin. In contrast, the enhancer activity of the HTLV-II and bovine leukemia virus LTR sequences was evident only in virus-infected cells. This activity is likely due to virus-associated *trans*-acting transcriptional factors previously shown to be present in HTLV- and bovine leukemia virus-infected cells. The implication of these observations for virus replication and transforming activity are discussed.

Human T-cell leukemia virus type I (HTLV-I) is the etiologic agent of adult T-cell leukemia-lymphoma (22, 30, 31, 42, 43). HTLV-II is an infrequent isolate derived from a patient with a clinically benign T-cell variant of hairy cell leukemia (7, 21). Bovine leukemia virus (BLV) is the etiologic agent of enzootic bovine leukosis (4, 24). These three retroviruses share common structural and biological features that distinguish them from other members of the retroviral family. One unique feature is the presence of a long open reading frame (*x-lor*) region located between the 3′ end of the *env* gene and the 5′ end of the long terminal repeat (LTR) (17, 32, 35). Another common feature of these viruses is the phenomenon of transcriptional *trans*-activation of the LTR in infected cells (*trans*-acting transcriptional regulation) (9, 13, 33, 38). The LTRs of HTLV-II and BLV function poorly, if at all, as promoters in many uninfected cells, but function very well in cells infected with the corresponding virus (13, 33, 38). Although the HTLV-I LTR sequences promote gene expression in uninfected cells, the rate of transcription is greatly augmented in infected cells. Recent studies indicate that the protein product encoded by the HTLV *x-lor* region mediates the *trans*-acting transcriptional regulation phenomenon (9, 37).

Our previous investigation focused on the ability of the viral LTR sequences to function as promoters when located immediately 5′ to the CAT gene. Here we investigate the ability of the HTLV-I, HTLV-II, and BLV LTR sequences to function as enhancer elements. Enhancers are sequences that are capable of increasing the rate of transcription of a distal promoter irrespective of their orientation and distance (1, 2, 10, 15, 26). In particular, we wished to determine whether *trans*-acting factors present in virus-infected cells regulate the enhancer activity of the viral LTR. For this purpose, the ability of the viral LTR sequence to augment expression of a distal promoter was examined in infected and in uninfected cells. The experiments presented here demonstrate that the LTR sequences of HTLV-II and BLV contain enhancer elements, the activities of which are dependent on the presence of virus associated *trans*-acting factors. The LTR of HTLV-I contains an enhancer sequence that is active in both uninfected and infected cells.

MATERIALS AND METHODS

Cells and media. The uninfected and HTLV-II in vitro-infected feline epithelial $CCCS^+L^-$ cells were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum. BLV-infected and uninfected fetal lamb kidney (FLK) cells and HeLa cells were grown in Dulbecco modified Eagle medium plus 10% fetal calf serum. The HTLV-II-infected $CCCS^+L^-$ cells and the BLV-infected FLK cells are productively infected and produce *trans*-acting factors that augment expression of the respective LTRs (33; our unpublished observations). Human lymphocyte lines Raji (Epstein-Barr virus-immortalized B lymphocyte) and C81-66/45 (HTLV-I-immortalized T lymphocyte) were maintained in RPMI medium plus 20% fetal calf serum.

Recombinant DNA constructions. Figure 1a shows the vector plasmid pSVIXCAT (5). Plasmid pSVIXCAT contains the bacterial chloramphenicol acetyltransferase (CAT) gene under control of the simian virus 40 (SV40) early region promoter sequences lacking the 72-base-pair (bp) repetitive enhancer sequences.

The plasmids that contain the HTLV-I LTR sequence 5′ to the SV40 promoter-CAT gene were constructed as follows. Plasmid pU3R-I (38), which contains the HTLV-I LTR, was first cleaved with *Xho*I. The protruding ends were filled with T4 DNA polymerase, and the DNA was ligated to synthetic *Bam*HI linkers. After cleavage with *Bam*HI-*Bgl*II the 700-bp fragment that contained the HTLV-I LTR was isolated from a low-melting-point agarose gel and ligated to *Bam*HI-cleaved pSVIXCAT. Two resultant plasmids chosen, pU3RI-IXS and pU3RI-IXA, contain the HTLV-I LTR

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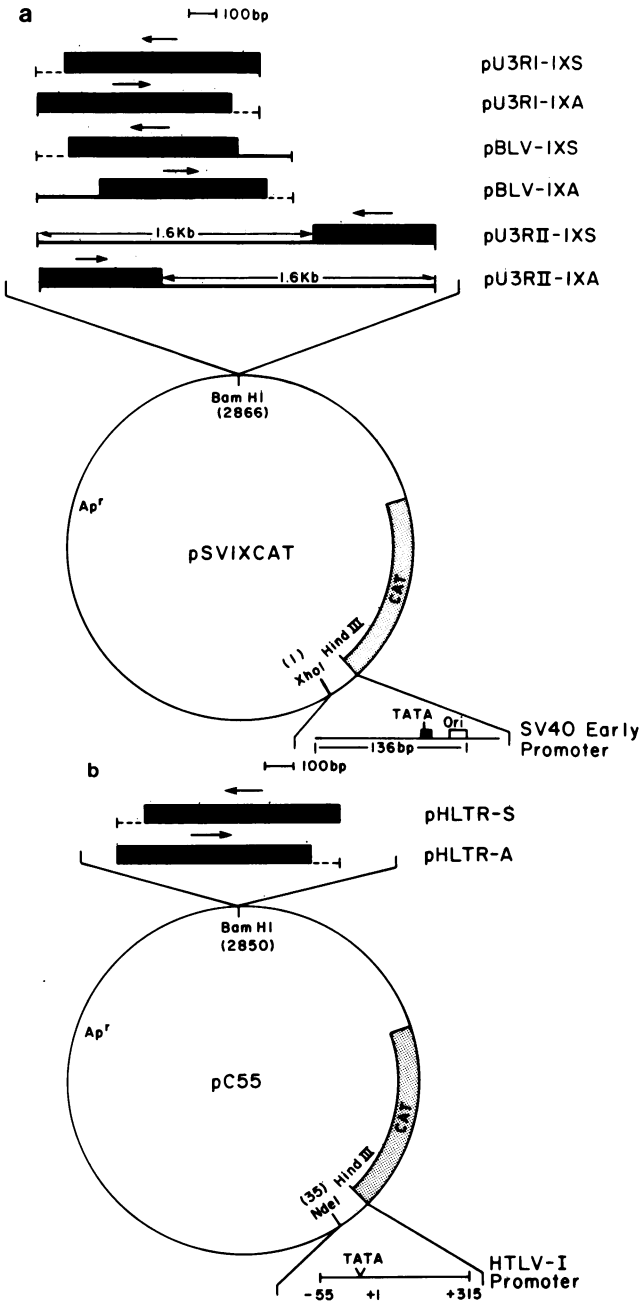

FIG. 1. Construction of the recombinant plasmids. (a) The construction of the vector plasmid pSVIXCAT that contains the SV40 early region promoter sequences has been described previously (5). The HTLV-I and HTLV-II LTR sequences were derived from plasmids pU3R-I (38) and pU3R-II (38), respectively. The BLV LTR sequences were derived from plasmid pBLVCAT (33). Construction of the individual plasmids shown above is described in Materials and Methods. Plasmids pU3RII-SD and pU3RII-AD (not shown) are identical to pU3RII-IXS and pU3RII-IXA, except the region between the *Nde*I-*Hin*dIII sites that encompasses the SV40 promoter sequence has been deleted. Solid bars depict LTR sequences. Solid and dotted lines represent cellular and viral flanking sequences, respectively. Transcriptional orientation of the LTR sequence with respect to the SV40 promoter is shown by arrows. (b) The plasmids shown are identical to the plasmids that contain the HTLV-I LTR shown in a, except the SV40 promoter sequence has been replaced by the HTLV-I promoter. The HTLV-I promoter sequence was derived from plasmid pC55, the construction of which has been described elsewhere (32a).

sequences in the sense and antisense orientation 5′ to the SV40 promoter. Plasmids pHLTR-S and pHLTR-A (Fig. 1b) are identical to those described above, except the SV40 promoter sequences have been replaced by the HTLV-I promoter sequence present on plasmid pC55 (32a). Plasmids pU3RII-IXS and pU3RII-IXA that contain the HTLV-II LTR sequences were constructed in a similar manner. An HTLV-II LTR sequence was obtained by cleaving plasmid pU3R-II (38) with *Bam*HI. The 2-kilobase (kb) fragment was isolated from a low-melting-point agarose gel and ligated to *Bam*HI-cleaved pSVIXCAT.

To construct plasmids pBLV-1XS and pBLV-1XA, which contain the BLV LTR sequences 5′ to the SV40 promoter, plasmid pBLVCAT (33) was first cleaved with *Eco*RI. The DNA ends were filled with T4 DNA polymerase, and the blunt-ended DNA was ligated to *Bam*HI linkers. After *Bam*HI digestion, the 900-bp fragment that contained the BLV LTR sequences was ligated to *Bam*HI-cleaved pSVIXCAT.

Final constructions were confirmed by extensive restriction enzyme analysis. All plasmid DNA was purified by banding in CsCl. Recombinant DNA techniques were done by standard established procedures (27). Enzyme reactions were according to the manufacturers' specifications.

Eucaryotic cell transfections and CAT assays. Adherent cell lines were transfected by the calcium phosphate precipitation method of Wigler et al. (41). Lymphoid cell lines were transfected by the DEAE-dextran method of Queen and Baltimore (29). Between 2 and 5 μg of plasmid DNA was used for each transfection.

Between 40 and 48 h posttransfection cells were harvested, washed once with phosphate-buffered saline, and suspended in 200 μl of 250 mM Tris hydrochloride (pH 8.0). Cell extracts were prepared by three freeze (−70°C)-thaw (37°C) cycles. Cellular debris was removed by a brief centrifugation, and protein determinations were made on the cell extracts. Samples of 200 to 500 μg of total protein were used in each assay. CAT assays were performed as previously described (15, 38). After analysis by thin-layer chromotography the percent conversion of chloramphenicol to its acetylated forms was determined by liquid scintillation counting.

Analysis of DNA. The BLV-infected FLK cells and the HTLV-II-infected $CCCS^{+}L^{-}$ cells were transfected with 5 μg of pBLVCAT and pU3R-II, respectively. Hirt supernatants were prepared at 6, 24, and 48 h posttransfection (19). The DNA was dot blotted onto nitrocellulose and hybridized to a [^{32}P]CTP-labeled CAT gene probe (3).

RESULTS

The ability of the HTLV-I, HTLV-II, and BLV LTR sequences to function as enhancers was examined. For this purpose plasmids that contain the various LTRs distal (2.8 kb) to the SV40 early region promoter (which is immediately 5′ to the CAT gene) were constructed (Fig. 1). The SV40 sequences used in these experiments lack the 72-bp repetitive elements that contain the SV40 early region enhancer activity (5). The HTLV and BLV LTR sequences were inserted in both transcriptional orientations relative to the 3′ promoter (Fig. 1). Recombinant plasmids were introduced into the target cells by using either calcium phosphate or DEAE dextran coprecipitation. Cells were harvested 48 h posttransfection, and the level of CAT enzyme activity was measured. Previous studies have demonstrated that for constructions such as those used here, in which the sequences surrounding the RNA start site are identical, the

TABLE 1. Analysis of HTLV-II LTR function[a]

Plasmid	Upstream control element	Orientation	Downstream		CAT activity in the following cell lines:			
			Enhancer	Promoter	$CCCS^+L^-$	$CCCS^+L^-$ (plus HTLV-II)	HeLa	Raji
pSVIXCAT				SV40	1.0	1.0	1.0	1.0
pU3RII-IXS	HTLV-II	Sense		SV40	0.8	10.5	0.6	0.3
pU3RII-IXA	HTLV-II	Antisense		SV40	0.9	3.5	0.8	0.5
pU3RII-SD	HTLV-II	Sense			0.0	0.0	ND	0.0
pU3RII-AD	HTLV-II	Antisense			0.0	0.0	ND	0.0
pU3R-II			HTLV-II	HTLV-II	2.6	100.0	0.05	12.0
pSV2CAT			SV40	SV40	71.0	80.0	ND	38.0

[a] The CAT activity directed by each plasmid is expressed relative to the activity obtained from transfection with plasmid pSVIXCAT in the same cell line. Plasmid pU3R-II contains the CAT gene under control of the HTLV-II transcriptional regulatory sequences (38). Plasmid pSV2CAT contains the SV40 enhancer and promoter sequences upstream of the CAT gene. The results represent the average of at least three independent transfections. CAT activity differed by no more than 25%. ND, Not determined.

level of indicator gene activity (including CAT) corresponds directly to the transcription rate (18, 23, 28, 39, 40). Furthermore, since the constructions described here leave the promoter region intact, the CAT assay is well suited for the study of enhancer function, since any bias due to translational efficiency or differential RNA processing should be eliminated because the CAT messages will be identical for each construction.

HTLV-II LTR function. To test the ability of the HTLV-II sequences to augment gene expression from a distance, plasmids that contain the entire HTLV-II LTR inserted 2.8 kb upstream to the SV40 early promoter (pU3RII-IXS and pU3RII-IXA) were introduced into both uninfected and HTLV-infected $CCCS^+L^-$ feline epithelial cells. Table 1 and Fig. 2 show that, relative to the pSVIXCAT plasmid, no increase in CAT gene expression was observed in uninfected cells transfected with these plasmids. However, the HTLV-II LTR sequences increased the level of gene expression directed by the SV40 promoter in the HTLV-II-infected $CCCS^+L^-$ cells by 4- to 10-fold, dependent upon the orientation of the LTR. The increased level of CAT gene expression does not reflect differences in transfection efficiencies between the infected and uninfected cells, because the level of CAT activity observed upon transfection with plasmid pSV2CAT was similar in both cell lines (Table 1). In addition, DNA dot-blot analysis indicates that a similar amount of plasmid DNA was present in both the uninfected and infected cells (data not shown).

To determine whether CAT gene expression was dependent upon the SV40 promoter, plasmids were constructed (pU3RII-SD and pU3RII-AD) that lacked the 3′ SV40 promoter sequence. No CAT activity was detected upon transfection of all cells with these plasmids (Table 1). We conclude that the increased level of CAT gene expression in the infected cells is dependent upon both the HTLV-II LTR and the SV40 promoter.

A recent report demonstrated that enhancer elements of polyomavirus can activate replication of polyomavirus DNA (11). This raised the possibility that the increased level of CAT gene expression directed by the HTLV-II LTR in the infected cells might reflect plasmid replication. To test this possibility, the plasmid DNA present in the transfected cells was measured in Hirt supernatants prepared at 6, 24, and 48 h posttransfection. Plasmid DNA was present at a similar level throughout the transfection period (Fig. 3). This result is in marked contrast to the prolific replication detected upon transfection of plasmid pSV2CAT into COS cells. COS cells contain SV40 T antigen that drives replication of plasmids that contain the SV40 origin of replication (14). These results indicate that the presence of HTLV-II LTR sequences does not elicit an increase in plasmid copy number in the infected cells.

We conclude that the HTLV-II LTR contains an enhancer that is capable of increasing the level of gene expression

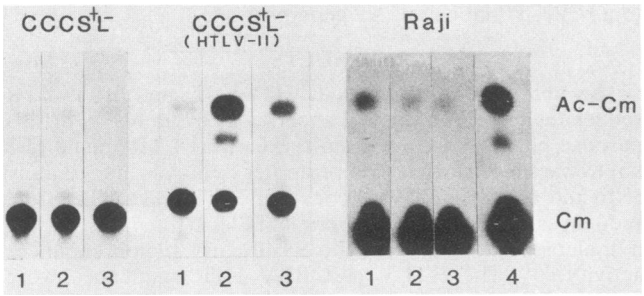

FIG. 2. CAT activity from transfected CCS^+L^- cells and Raji cells. Shown are autoradiograms of CAT assays performed with cell extracts prepared from cells transfected with the following plasmids: pSVIXCAT (lane 1), pU3RII-IXS (lane 2), pU3RII-IXA (lane 3), and pU3R-II (lane 4). The CAT assays shown were taken from a time point within the linear range of the assay. The unreacted chloramphenicol (Cm) and the acetylated reaction products (Ac-Cm) are as indicated.

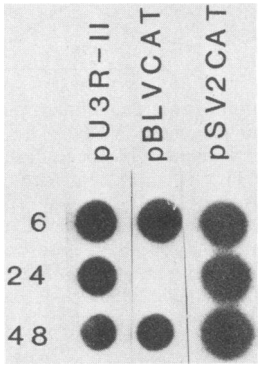

FIG. 3. Dot-blot analysis of transfected DNA. BLV-infected FLK cells and HTLV-II-infected CCS^+L^- cells were transfected with the plasmids pBLVCAT and pU3R-II, respectively. Plasmid pSV2CAT, which contains the SV40 enhancer and promoter sequences, was transfected into COS cells (14). Hirt supernatants were prepared at the indicated times, and DNA was hybridized to a CAT gene probe.

TABLE 2. Analysis of BLV LTR function[a]

Plasmid	Upstream control element	Orientation	Downstream		CAT activity in the following cell lines:		
			Enhancer	Promoter	FLK	FLK (plus BLV)	HeLa
pSVIXCAT				SV40	1.0	1.0	1.0
pBLV-IXS	BLV	Sense		SV40	0.60	8.0	0.60
pBLV-IXA	BLV	Antisense		SV40	0.30	6.0	0.40
pBLVCAT			BLV	BLV	0.05	26.0	0.05
pSV2CAT			SV40	SV40	15.0	16.0	ND

[a] Plasmids were transfected into the indicated cells as described in Materials and Methods. The CAT activity directed by each plasmid is expressed relative to the activity obtained from transfection with pSVIXCAT in the same cell line. Plasmid pBLVCAT contains the CAT gene under control of the BLV LTR transcriptional regulatory sequences (33). The results represent the average of at least three independent transfections. CAT activity differed by no more than 20%. ND, Not determined.

directed by a heterologous promoter irrespective of distance and orientation. Furthermore, the HTLV-II enhancer activity is only evident in HTLV-II-infected cells.

Promoter activity of the HTLV-II LTR in certain cell lines. Although the HTLV-II transcriptional regulatory sequences are unable to promote transcription in many uninfected cell lines (38), a low level of CAT activity was detected upon transfection of certain cells. Table 1 and Fig. 2 demonstrate that when the HTLV-II LTR sequences were present proximal to the CAT gene (plasmid pU3R-II) they directed a measurable level of CAT gene expression in the human B-cell lymphoma line (Raji) and in the $CCCS^+L^-$ feline epithelial cell line. Although low, the level of CAT activity directed by the HTLV-II LTR in these cells was somewhat higher than the level directed by the SV40 early region promoter. To determine whether the HTLV-II enhancer was active in these cells, the level of CAT activity directed by plasmids pU3RII-1XS and pU3RII-IXA was measured. Table 1 and Fig. 2 show that CAT gene expression was not increased relative to that directed by the SV40 promoter alone.

We suggest that the ability of the HTLV-II LTR sequences to direct a low level of transcription in certain uninfected cell lines may be attributed to promoter activity of the sequences and not to an enhancer activity, since no increase in gene expression was observed when the HTLV-II LTR sequences were located distal to the promoter. We can conclude that functional activity of the HTLV-II promoter and enhancer is not lymphoid specific, since these elements are active in cell lines of lymphoid and nonlymphoid origin.

BLV LTR function. As is the case for the HTLV-II LTR, the transcriptional activity of the BLV LTR is restricted to BLV-infected cells (9, 33). To determine whether BLV-associated *trans*-acting factors activate an enhancer element within the BLV LTR, the activity of plasmids that contain the entire BLV LTR distal to the SV40 promoter was examined in BLV-infected and uninfected FLK cells and in uninfected HeLa cells. CAT gene expression directed by these plasmids in uninfected cells was somewhat lower than that directed by the vector plasmid pSVIXCAT (Table 2). In contrast, the level of CAT activity was substantially elevated in cells infected with BLV. The elevation of CAT gene expression did not reflect replication of the BLV plasmid in the infected cells (Fig. 3). We therefore conclude that activation of the BLV transcriptional sequences, like those of HTLV-II, is mediated at least in part by an enhancer function that is dependent upon the presence of BLV-associated *trans*-acting regulatory factors.

HTLV-I LTR function. In contrast to our findings with HTLV-II and BLV, the HTLV-I LTR functions as a promoter in a wide variety of uninfected cell types (38). The level of CAT gene expression directed by plasmids that contain the HTLV-I LTR proximal to the CAT gene is similar to that directed by the SV40 early region enhancer-promoter sequence (38). However, the level of transcription directed by the HTLV-I LTR is greatly increased in HTLV-I-infected cells (13, 38).

We recently demonstrated that the HTLV-I LTR contains enhancer elements (32a). To determine whether the HTLV-I LTR enhancer effect was greater in infected cells than in uninfected cells, plasmids pU3RI-IXS and pU3RI-IXA, which contain the HTLV-I LTR distal to the SV40 promoter, were introduced into HTLV-infected and uninfected cells. In uninfected cells, the level of CAT gene expression was increased three- to fourfold relative to the plasmid that lacked the HTLV-I LTR sequences (Table 3). The level of CAT activity was augmented, but only to a minor extent, in HTLV-II-infected $CCCS^+L^-$ cell lines. The latter result is surprising, since the level of CAT gene expression directed by the HTLV-I LTR when located proximal to the CAT gene (pU3R-I) was greatly elevated in the same cell line (Table 3). The enhancer activity of the HTLV-I LTR sequences was also examined in the HLTV-I-immortalized T-lymphocyte line C81-66/45. For these experiments we constructed an additional set of plasmids (pHLTR-S and pHLTR-A) that contain HTLV-I LTR sequences 5′ to the HTLV-I promoter element (Fig. 1b). The LTR sequences increased the level of HTLV-I promoter directed gene expression by eightfold in the sense orientation and threefold in the antisense orientation (Table 3). A similar stimulation was evident upon transfection of the uninfected B-lymphocyte cell line Raji and HeLa cells. We conclude that the HTLV-I LTR contains an enhancer sequence that is active in infected as well as uninfected cells. Moreover, the enhancer activity is not augmented by viral associated *trans*-acting regulatory factors.

DISCUSSION

The studies presented here demonstrate that the LTR sequences of HTLV-I, HTLV-II, and BLV contain elements that conform to the formal definition of enhancer sequences. By definition, enhancers are DNA sequences that increase the rate of gene expression irrespective of orientation and distance to a promoter sequence. The enhancer elements of the HTLV-I LTR are active in uninfected cells of lymphoid and nonlymphoid origin. In contrast, the enhancer activity of the HTLV-II and BLV LTR sequences is only evident in cells infected with the corresponding virus. The dependence of the HTLV-II and BLV LTR enhancer activity on factors present in infected cells may explain the very low transcriptional activity of the entire LTR previously observed in

TABLE 3. Analysis of HTLV-I LTR function[a]

Expt	Plasmid	Upstream control element	Orientation	Downstream		CAT activity in the following cell lines:			
				Enhancer	Promoter	$CCCS^+L^-$	$CCCS^+L^-$ (plus HTLV-II)	Raji	C81-66/45
1	pSV1XCAT				SV40	1.0	1.0	ND	ND
	pU3R1-1XS	HTLV-I	Sense		SV40	4.0	6.5	ND	ND
	pU3R1-1XA	HTLV-I	Antisense		SV40	2.2	7.2	ND	ND
	pU3R-1			HTLV-I	HTLV-I	75.0	160.0	ND	ND
	pSV2CAT			SV40	SV40	85.0	85.0	ND	ND
2	pC55				HTLV-I	1.0	ND	1.0	1.0
	pHLTR-S	HTLV-I	Sense		HTLV-I	4.0	ND	11.0	8.5
	pHLTR-A	HTLV-I	Antisense		HTLV-I	3.0	ND	3.5	3.0
	pU3R-1			HTLV-I	HTLV-I	40.0	ND	16.0	190.0

[a] In experiment 1, the CAT activity directed by each plasmid is expressed relative to the activity obtained from transfection with plasmid pSVIXCAT in the same cell line. In experiment 2, the CAT activity is expressed relative to the CAT activity obtained from transfection with plasmid pC55 in the same cell line. Plasmid pU3R-I contains the CAT gene under control of the HTLV-I enhancer and promoter sequences (38), whereas in plasmid pC55 only the HTLV-I promoter sequences are present (32a). The results represent the average of at least three independent transfections. CAT activity differed by no more than 20%. ND, Not determined.

uninfected cells (9, 33, 38). The ability of the HTLV-I enhancer to function in uninfected cells possibly explains the efficient function of the LTR in a wide variety of cells.

Whether *trans*-activation of the HTLV-II and BLV enhancers fully accounts for the very high level of transcription directed by the respective LTRs in the infected cells remains to be determined. For example, when the LTRs are proximal to the CAT gene, stimulation of transcriptional activity appears to be greater than that which can be attributed to the enhancer activity alone. Alternatively, since the measurement of enhancer activity requires placement of the enhancer at a considerable distance from the promoter, the actual effect of *trans*-activation may be greatly diminished.

The HTLV-II and BLV enhancer elements are formally analogous to the mouse mammary tumor virus enhancer. In the case of mouse mammary tumor virus, binding of the dexamethasone receptor complex to the LTR is required for enhancer activity (6). We speculate that binding of viral or virus-induced transcription factors to the HTLV-II and BLV enhancer or other sequences activates the enhancer function. In this view, such factors might serve a dual function in HTLV and BLV infection; that is, they might activate the viral enhancer and stimulate the promoter activity. We suggest that for HTLV-I the virus-associated *trans*-acting factors may only interact with promoter region sequences, since the enhancer exhibits a constitutive activity in the absence of these factors. In this regard, we note that the organization of the HTLV-I repetitive sequences, believed to contain the enhancer element (20, 32a), differs from that of the HTLV-II LTR. The repetitive elements of the HTLV-I LTR are located some distance from the site of transcription initiation (20, 36). For HTLV-II and BLV the repetitive elements are much closer to the TATA box (8, 34). Conceivably, the BLV and HTLV-II LTR recognition sites for viral *trans*-acting factors overlap the regions that specify enhancer and promoter function, whereas these regions are further separated in the HTLV-I LTR. Thus, such factors may stimulate both enhancer and promoter sequences in HTLV-II and BLV but only promoter sequences in HTLV-I.

The dependence of the HTLV-II and BLV enhancer activity on virus-associated *trans*-acting factors raises the intriguing question as to how viral replication is initiated. It is possible that the weak promoter activity evident in certain uninfected cells is sufficient to initiate a transcription cycle. Once initiated, viral *trans*-acting factors could maintain transcription at a high level. Alternatively, factors that stimulate virus-specific transcription may be packaged in the virion or be transmitted from cell to cell via spontaneous cell fusion. The epidemiology of BLV transmission is consistent with a mechanism of cell-mediated virus infection (4).

The mechanisms that govern HTLV and BLV leukemogenesis are unknown. By classical definition both groups of viruses lack oncogenes, since they do not contain sequences that are similar to those of conserved mammalian genes. Furthermore, since proviral integration is random in BLV and HTLV tumors (12, 25, 43), it is unlikely that leukemogenesis results via downstream activation of a nearby cellular oncogene. The presence of virus-associated transcriptional regulatory factors in infected cells raises the possibility of their involvement in pathogenesis. The experiments described here suggest that these factors may exert different types of regulatory functions; stimulation of enhancer activity or interaction with sequences that influence promoter function or both. We suggest that recognition sites for the viral *trans*-acting factors, similar to those present in the LTR, are also present within the regulatory regions of genes that govern lymphocyte growth. Consequently, activation of these genes via the mechanisms described above might be responsible for the novel transforming effects associated with this group of viruses.

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