Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 6502–6506, October 1985
Biochemistry

Location of *cis*-acting regulatory sequences in the human T-cell leukemia virus type I long terminal repeat

(immortalization/*tat* protein/*trans*-activation)

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Communicated by Paul Zamecnik, June 12, 1985

ABSTRACT **The location of *cis*-acting regulatory regions within the long terminal repeat (LTR) of the human T-cell leukemia virus type I (HTLV-1) was determined. The sequences present between nucleotides −350 and −55 (cap site +1) contain an enhancer element that is active in lymphoid and nonlymphoid cell lines. The sequences located near the "TATA" and RNA initiation sites contain a promoter, the activity of which can be augmented by homologous and heterologous enhancer elements. A region responsive to *trans*-acting transcription factors present in HTLV-I- and HTLV type II-infected cells is located between nucleotides −159 and +315. HTLV-I LTR deletion mutants respond in a similar manner both to the *trans*-acting factors present in infected cells and to the tat protein encoded by the x-*lor* region of the genome, thus providing further evidence that the tat protein mediates transcriptional *trans*-activation of the LTR in HTLV-infected cells.**

The human T-cell leukemia virus type I (HTLV-I) is the etiologic agent of adult T-cell leukemia lymphoma, a disease endemic to certain geographic regions (1–4). HTLV-I, HTLV type II (HTLV-II), and bovine leukemia virus share common structural and biological features that distinguish them from the other nonacute retroviruses. These include: unusually large long terminal repeat (LTR) sequences (5–7), the presence of an x-*lor* or *tat* gene between the *env* gene and the 3′ LTR (8–11), and the ability of viral-associated *trans*-acting transcription factors to increase the rate of LTR-directed gene expression (12–15). In addition, both HTLV-I and HTLV-II are able to immortalize primary lymphocytes *in vitro* (16, 17). Recent studies have shown that the HTLV-I and HTLV-II *tat* genes encode 42- and 38-kDa nuclear proteins, respectively (18–20) and that the tat products, in the absence of other viral proteins, activate in *trans* gene expression directed by the HTLV-I and -II LTRs (21).

In the present study we used a transient gene expression assay to identify the functional domains within the HTLV-I LTR. The results demonstrate that the LTR contains functional enhancer and promoter sequences. The enhancer activity is evident in cells of lymphoid and nonlymphoid origin. Furthermore, neither the enhancer nor the promoter sequences contain in their entirety those sequences responsive to the viral *trans*-acting factors.

MATERIALS AND METHODS

Cell Lines. HeLa cells, and *in vitro* HTLV-infected and uninfected feline kidney epithelial cells, $CCCS^+L^-$ (Moloney murine sarcoma virus-transformed, leukemia virus negative) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The human Epstein–Barr virus-immortalized B-lymphocyte cell line Raji, human T lymphocytes HUT 78 (22), and C81-66/45 (23) were grown in RPMI 1640 medium containing 20% fetal bovine serum. The C81-66/45 cell line is a nonproducer line that expresses the 42-kDa x-*lor* product (18).

Plasmid Constructions. Plasmid pU3R-I (12) contains the HTLV-I LTR sequences upstream of the bacterial chloramphenicol acetyltransferase gene (*CAT*) (24). Deletion mutants of the LTR were constructed as follows. Plasmid pU3R-1 was first cleaved at the unique *Sma* I site at nucleotide −332 within the LTR, and deletion mutants were generated with the exonuclease BAL-31 (25). Synthetic *Xho* I linkers were ligated to the DNA, and Maxam and Gilbert DNA sequence analysis (26) was used to determine the end point of each deletion. The name of each plasmid is indicative of the number of nucleotides present relative to the transcription start site (5). Construction of the additional plasmids used to assess promoter and enhancer activity was performed by using standard recombinant DNA methodology (27). LTR regions used for each construction are described in the text and figure legends.

Eukaryotic Cell Transfections and CAT Assays. The HeLa and $CCCS^+L^-$ cells were transfected with 2 μg of plasmid DNA by a modification of the calcium phosphate coprecipitation technique (28). Lymphoid cells were transfected by the DEAE-dextran method as described by Queen and Baltimore (29). CAT assays were performed as described (12, 24).

RESULTS

Deletion Analysis. To identify *cis*-acting regulatory sequences 5′ to the "TATA" sequence, a series of 5′ deletion mutants were constructed in plasmid pU3R-I (Fig. 1). Previous studies have shown that for constructions in which the sequences surrounding the RNA start site are identical and which differ only in the sequences located remotely from the promoter, the level of indicator gene activity, including *CAT*, correlates closely with the indicator gene mRNA levels (31–36).

The location of the deletions with respect to the repetitive elements in the HTLV-I LTR and the effect on activity in lymphoid and upon nonlymphoid cells is shown in Table 1.

Transfection with plasmid pC294 in which 70 base pairs (bp) from the 5′ end of the LTR has been deleted resulted in a decrease in CAT activity, an unexpected result as none of the repetitive elements remote from the cap site are affected by this deletion. The level of *CAT* gene expression in uninfected cells was further diminished by a deletion (pC240) that extends into the first 21-bp imperfect repeat. *CAT* gene expression following transfection with plasmid pC230 in

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Abbreviations: HTLV-I and HTLV-II, human T-cell leukemia virus types I and II; LTR, long terminal repeat; *CAT*, gene for chloramphenicol acetyltransferase; bp, base pair(s); SV40, simian virus 40; RSV, Rous sarcoma virus.

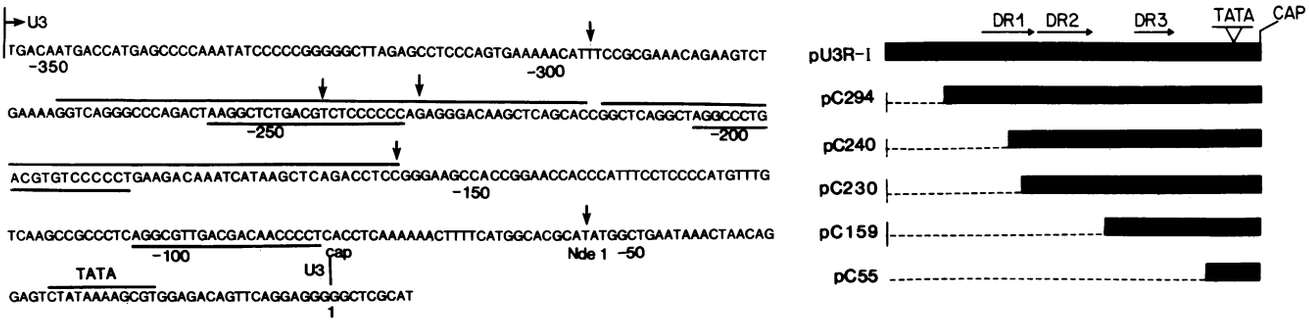

FIG. 1. Deletion analysis of the HTLV-I LTR. (*Left*) The sequence present within the HTLV-I LTR U3 region of strain HTLV-CR is shown (30). Two 51-base-pair imperfect repeats are overlined and three 21-base-pair imperfect repeats are underlined. Arrows indicate the position of each deletion. (*Right*) Deletion analysis of plasmid pU3R-I was performed as described.

which the entire 21-bp repeat has been deleted was almost completely abolished. Evidently, neither the two remaining 21-bp repeats nor the 51-bp imperfect direct repeat is sufficient to direct high levels of gene expression in uninfected cells.

We note that the extent of diminution of the activity experienced with each deletion differs depending upon the cell line used. This finding suggests that transcription factors unique to each cell type may interact with different LTR sequences.

HTLV-I Enhancer Activity. The LTR sequences of many retroviruses have been shown to harbor enhancer elements (31, 37–40). Since the deletion analysis demonstrated a loss in promotional activity following removal of LTR U3 sequences, we sought to determine whether sequences within this region might serve as enhancer elements.

For this purpose, the portion of the LTR extending from the 5′ terminus to a position 35 nucleotides 5′ to the TATA sequence was inserted in both the sense and antisense orientation 5′ to the simian virus 40 (SV40) early region promoter (Fig. 2 *Left*). Transfection with plasmid pSV1XCAT (37) that contains the SV40 early region promoter minus the 72-bp SV40 enhancer demonstrated little CAT activity in HeLa and feline epithelial $CCCS^+L^-$ cells (Fig. 3*A*).

However, the level of CAT activity increased significantly after addition of the HTLV U3 sequences in either the sense (pHE1CAT) or antisense (pHE2CAT) orientation. To determine if the activity of the distal HTLV-I sequences was dependent upon the SV40 promoter, we deleted the SV40 promoter sequence. The remaining sequences present on these plasmids (pHE2CAT-D and pHLTR-D) did not direct a detectable level of CAT enzyme activity. Hence, the increase in CAT activity after addition of the HTLV-I U3 sequences is dependent upon the SV40 promoter. To examine whether promoter function could be stimulated over a longer distance, plasmids pHLTRS and pHLTRA that contain the entire HTLV-LTR U3 region 2.8 kilobases away from the HTLV-I promoter region (nucleotides −55 to +315) were tested. Transfection of HeLa, $CCCS^+L^-$, and Raji cells with these plasmids gave significantly higher levels of CAT activity as compared with that directed by plasmid pC55 alone (Fig. 3*b*).

Table 1. Relative activity of LTR deletion mutants

Plasmid	Cell line				*trans*-induction*
	HeLa	$CCCS^+L^-$	Raji	HUT 78	
pU3R-I	1.00	1.00	1.00	1.00	95
pC294	0.66	0.78	0.74	0.37	132
pC240	0.09	0.59	0.14	0.51	65
pC230	0.05	0.33	0.14	0.07	100
pC159	0.06	0.09	0.31	0.05	72
pC55	0.05	0.05	0.40	0.05	1

Cells were transfected with the deletion mutants shown in Fig. 1. Forty-eight hours after transfection, CAT assays were performed. The values shown have been normalized to the activity obtained after transfection with plasmid pU3R-I in the same cell line. The results shown represent the average of at least two independent transfections, with results differing by no more than 25%.

**trans*-induction: to arrive at this value, the CAT activity directed by each deletion mutant in the C81-66/45 cell line was divided by the activity obtained after transfection with plasmid pSV2CAT. This calculation was repeated in the uninfected T-lymphocyte line, and the value obtained in the C81-66/45 cells was divided by the value obtained in the uninfected cells to arrive at the *trans*-induction number.

From these experiments we conclude that U3 region sequences located between nucleotides −350 and −55 function as enhancer elements.

HTLV-I Promoter Sequences. The promoters of most viral LTRs are located near the site of initiation and often include the sequence TATA. To determine whether nucleotides near the TATA site could serve as an initiation sequence for a heterologous enhancer, the HTLV-I LTR sequence extending from −55 to +315 was tested for its ability to respond to enhancers. For this purpose sequences excised from the RSV LTR (31) that possess enhancer activity were placed 5′ to the deleted HTLV-I LTR (Fig. 2 *Left*). The HTLV enhancer sequences (located between positions −350 and −55) were also placed in reverse orientation 5′ to the test HTLV-I sequence. Hybrid plasmids containing the SV40 early promoter region and the U3 enhancer regions of HTLV and RSV were also constructed to permit comparison of promoter function (Fig. 2 *Left*).

Addition of either the RSV or HTLV-I enhancer elements 5′ to the HTLV-I promoter sequence significantly increased the level of *CAT* gene expression in human T and B lymphocytes and $CCCS^+L^-$ cells (Table 2 and Fig. 3). Similar levels of CAT activity were observed upon transfection of cells with plasmid (pHE1CAT) that contains the SV40 promoter element rather than the corresponding HTLV-I sequence. To determine whether RNA initiated from within the HTLV-I sequence in these hybrid recombinant plasmids, plasmids identical to pREH1 and pU3R-I were constructed, except that the *CAT* coding region was replaced by the cDNA of rabbit β-globin (pREHI-β and pU3R-I-β) (43). S1 nuclease analysis (44) shows that the length of the protected fragments was similar for the transcripts derived from the intact and hybrid LTRs (Fig. 4).

From these experiments, we conclude that the HTLV-I nucleotides near the TATA sequence serve a promoter function equivalent to the SV40 promoter sequence in this assay.

The Region Responsive to Viral *trans*-Acting Factors. The U3–R region of the HTLV-I LTR is responsive to *trans*-

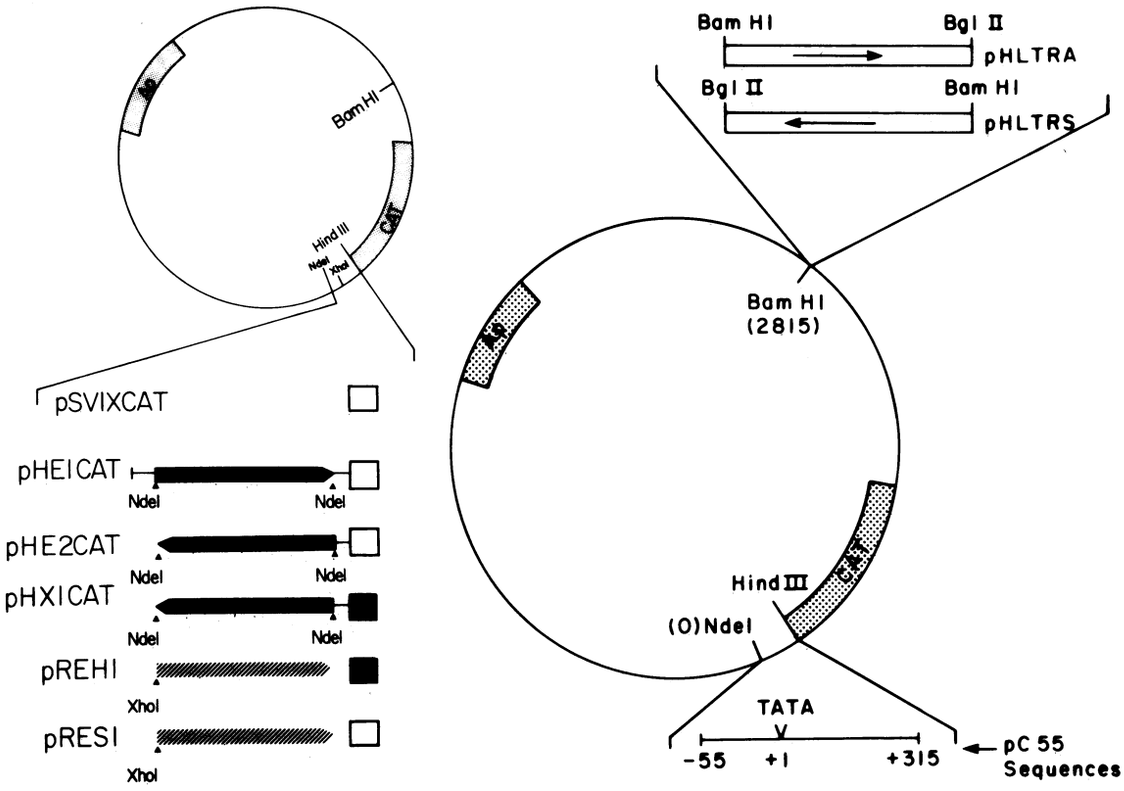

FIG. 2. Construction of recombinant plasmids. The upper circle denotes the vector plasmid pSV1XCAT (37). Plasmids pHE1CAT and pHE2CAT contain the LTR U3 sequence (−350 to −55) located 5′ to the SV40 early promoter. Plasmid pHX1CAT contains the same HTLV U3 sequence but in the reverse orientation on the HTLV promoter. Plasmids pREH1 and pRES1 contain the LTR enhancer sequence of Rous sarcoma virus (RSV) (41) (*Acc* I–*Sph* I fragment) located 5′ to the HTLV and SV40 promoter sequence, respectively. Plasmids pHLTRS and pHLTRA contain the HTLV LTR sequences in the sense and antisense orientation 2.8 kb upstream to the 5′ terminus of the HTLV promoter sequence present on plasmid pC55. Plasmid pHLTRS-D (not shown) is identical to plasmid pHLTRS except that it lacks the HTLV promoter sequence (*Nde* I–*Hin*dIII fragment) 5′ to the *CAT* gene. Blackened and crosshatched rectangular bars represent HTLV and RSV sequences, respectively. Arrow directions indicate the orientation of sequences with respect to their orientation in the LTR. Blackened and open squares represent HTLV and SV40 promoter sequences, respectively. Sequences derived from plasmid pBR322 are shown as solid lines.

acting factors present in HTLV-I- and HTLV-II-infected cells (12).

To determine if the responsive sequences were located within the enhancer sequence (−350 to −55), the level of CAT activity directed by plasmids pHE2CAT and pHX1CAT was examined in infected and in uninfected cells (Table 2). The level of CAT activity directed by these plasmids was found to be the same in both cell types. Thus, sequences responsive to the *trans*-acting factors are not present in their entirety within the region that contains the HTLV-I enhancer.

The response of the HTLV-I promoter sequence to viral *trans*-acting factors was also determined. For this purpose the level of CAT activity directed by plasmid pC55 and the hybrid enhancer/promoter plasmids was examined. Fig. 5 and Table 2 show that *CAT* gene expression directed by these plasmids is comparable in infected and in uninfected cells. We conclude that the sequences responsive to *trans*-acting factors are not present, in their entirety, in the promoter region.

The 5′ boundary of the region responsive to the *trans*-acting factors was determined by comparison of the activity of the U3 deletions in several cell lines including C81-66/45, a cell line that contains the 42-kDa tat protein (18).

In a separate experiment, the activity of several deleted LTR plasmids and hybrid recombinants was compared in HUT 78 cells that were cotransfected with a plasmid that expressed an active 42-kDa *tat* gene alone ($pCATLOR_{II}$) to the activity in cells cotransfected with a plasmid that contained the *tat* gene inactivated by a frameshift mutation ($pCATLOR_{II}fs$) (21). The results of these experiments are shown in Table 1 and Fig. 5.

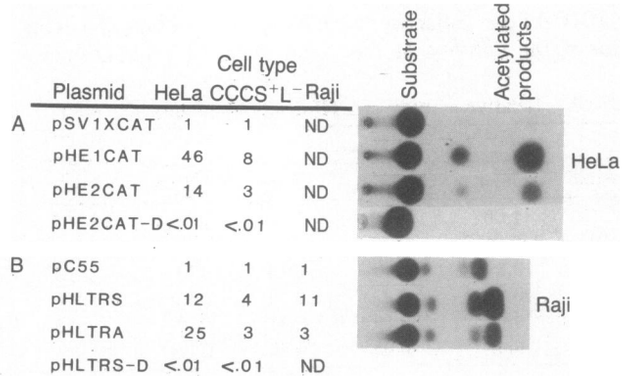

FIG. 3. Enhancer activity of the LTR sequences. Cells were transfected as described. Forty-eight hours after transfection, cells were harvested and CAT assays were performed. (*A*) The CAT activity is normalized relative to the absolute activity directed by the pSV1XCAT plasmid in each cell line. (*B*) The CAT activity is normalized relative to the absolute activity directed by plasmid pC55 in each cell line. All values represent an average of at least two independent transfections. Autoradiograms shown are of CAT assays performed with extracts prepared from cells transfected with the plasmid cited to the left. Spots on the right correspond to the acetylated forms of [^{14}C]chloramphenicol monoacetate, while spots on the left are unreacted substrate.

Table 2. Relative activity of hybrid enhancer/promoter constructions

Enhancer origin	Promoter origin	Plasmid	Cell line			
			CCCS$^+$L$^-$	CCCS$^+$L$^-$ (HTLV-II)	C81-66/45	Raji
	SV40	pSV1XCAT	1.0	1.0	1.0	1.0
HTLV	SV40	pHE2CAT	3.0	5	2.0	1.4
RSV	SV40	pRES1	12	7.3	2.0	4
	HTLV	pC55	4.7	3.5	2.25	1.0
HTLV	HTLV	pHX1CAT	10	15	5.5	ND
RSV	HTLV	pREH1	5.8	6	10	33
HTLV	HTLV	pU3R-I	38	271	165	56
HTLV	deleted	pHE2CAT-D	0	0	ND	ND
HTLV	deleted	pHLTRS-D	ND	ND	ND	0

The CAT activity of each plasmid is normalized to the activity directed by pSV1XCAT in each cell line. The CCCS$^+$L$^-$ (HTLV-II) feline epithelial cell line is infected with HTLV-II as demonstrated by reverse transcriptase and Southern blot analysis (unpublished observations).

Deletions extending to within 159 nucleotides of the initiation site do not affect the ability of the LTR sequences to respond to *trans*-acting factors, although the upstream sequences do contribute to the overall activity of the LTR even in the presence of these factors. In addition, as demonstrated in the infected cells (Table 2), neither the HTLV-I enhancer nor the HTLV-I promoter sequence contains in their entirety those sequences responsive to the tat protein (Fig. 5). The shortest fragment that was found to respond to the *trans*-acting factors in these experiments extends from position −159 to +315.

DISCUSSION

In the present study, the location of the *cis*-acting regulatory sequences within the HTLV-I LTR was determined. The sequences from −350 to −55 conform to the formal definition of enhancer elements as they increase the rate of gene expression directed by a heterologous promoter from a distance in an orientation-independent fashion. We note that the HTLV-I enhancer does not contain core enhancer consensus sequences that are present in several other viral and cellular enhancer elements (46). Since the level of enhancer activity is similar in uninfected and HTLV-infected cells, we conclude that the HTLV-I enhancer element is not responsive to the viral-associated *trans*-acting regulatory factors. In this respect, the enhancer element of HTLV-I differs from those of HTLV-II and bovine leukemia virus that are active only in cells that contain the viral-associated *trans*-acting regulatory factors (our unpublished results).

The function of the HTLV-I enhancer is not restricted to cells of lymphoid origin as enhancer activity is evident in feline epithelial cells and HeLa cells. These observations suggest that the T-cell tropism associated with HTLV-I infection *in vivo* is not determined by the enhancer element alone. In this respect, the HTLV-I enhancer differs from the T-cell tropic murine leukemia virus enhancers (37, 42, 47).

The location of the region that responds to the *trans*-acting factors extends from −159 to +315. The integrity of sequences near −55 must be important for responsiveness as an inversion of the LTR at this position, as in the plasmid pHX1CAT, eliminates the *tat* response but does not significantly diminish activity of the altered LTR in uninfected cells. The 3′ border of the responsive sequences has not been mapped in these experiments. In experiments not presented here, the response to *trans*-acting factors is retained after removal of sequences 3′ to nucleotide +3.

The HTLV-I LTR sequences that are responsive to viral *trans*-acting factors are not contained in their entirety in the

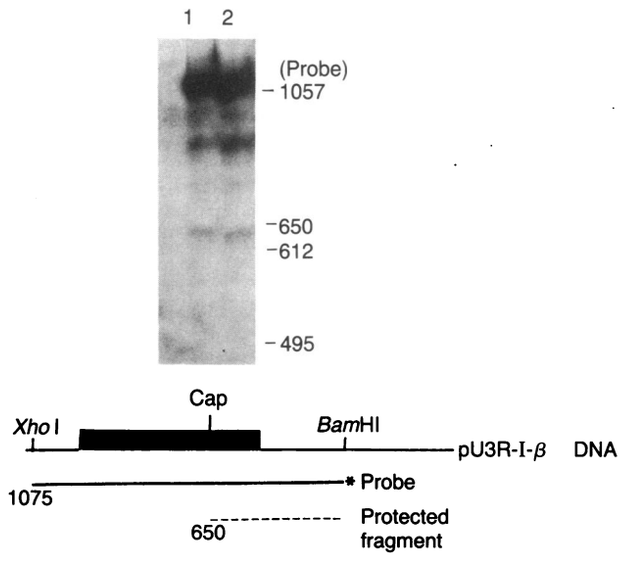

FIG. 4. S1 nuclease analysis of RNA from transfected CCCS$^+$L$^-$ cells. Cells were transfected with plasmids pU3R-I-β (lane 1) and pREH1-β (lane 2), total RNA was extracted (45) 48 hr after transfection, and the 5′ ends of the transcripts were mapped with S1 nuclease (44). The structure of the DNA probe and of the expected DNA fragment protected by the 5′ terminal nucleotides of the β-globin RNA are shown at the bottom. Sizes are shown in bp.

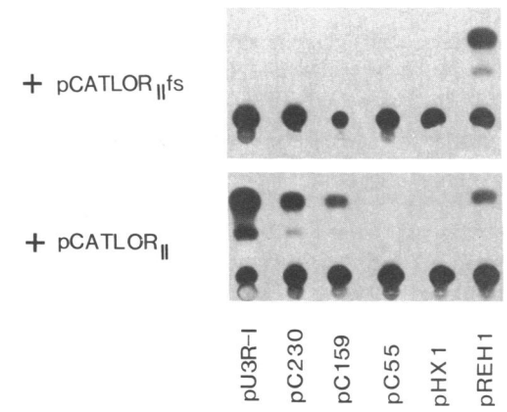

FIG. 5. CAT assays after cotransfection of HUT 78 human T lymphocytes with x-*lor* expressor plasmids (21). Cells were transfected with 0.5 μg of the indicated plasmid together with 3 μg of plasmid pCATLOR$_{II}$ (expressor) or pCATLOR$_{II}$fs (frame-shift mutation). CAT assays were performed 48 hr after transfection.

enhancer or promoter sequence. We propose that the sequence responsive to the viral *trans*-acting factors be termed the *TAR* element, for *trans*-acting response (or target) sequence. The location of the *TAR* sequence, proximal to the promoter and separate from the enhancer, suggests that *trans*-acting factors serve to increase the efficiency of transcriptional initiation. This speculation is in accord with the observation that the level of mRNA directed by the HTLV-I LTR is greatly increased in infected cells as compared to uninfected cells (12). In this regard, the *tat* gene should play an important role in the life cycle of the virus, inducing an autostimulatory replication cycle, whereby a product of HTLV-I transcription positively activates the HTLV-I LTR. We have proposed that the specificity for transformation of HTLV I and II for T lymphocytes is conferred by the specificity of the tat protein (12, 21). Although the tat protein can stimulate HTLV LTR-directed transcription in many cells, we speculate that the ability to regulate cellular genes that control cellular proliferation may be limited to lymphoid cells, or alternatively, that genes regulated by the tat protein only induce proliferation in lymphoid cells.

Further studies regarding mechanisms that regulate control of viral gene expression may provide insight into those mechanisms by which viral proteins regulate cellular genes.

We thank Drs. R. C. Gallo, F. Wong Staal, Z. Salahuddin, M. Essex, and R. Weiss for gifts of cell lines; C. Gorman, B. Howard, and D. Celander for gifts of plasmids; K. Campbell, N. Shiomi, D. Perkins, and D. Briggs for technical assistance; and D. Artz and L. DeFurio for help in preparing the manuscript. This work was supported by National Institutes of Health Grant CA36974 and by an American Cancer Society Directors Grant to W.A.H.; C.A.R. and J.G.S. were supported by CA07580 and CA07094, respectively.

1. Uchiyama, T., Yodoi, J., Sagawa, K., Takatsuki, K. & Uchino, H. (1977) *Blood* **50,** 481–491.
2. Poiesz, B. J., Ruscetti, R. W., Gazdar, A. F., Bunn, P. A., Minna, J. D. & Gallo, R. C. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7415–7419.
3. Kalyanaraman, V. S., Sarngadharan, M., Bunn, P., Minna, J. & Gallo, R. C. (1981) *Nature (London)* **294,** 271–273.
4. Yoshida, M., Miyoshi, I. & Hinuma, Y. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2031–2035.
5. Seiki, M., Hattori, S. & Yoshida, M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6899–6902.
6. Josephs, S., Manzari, V., Wong-Staal, F., Trus, M., Sodroski, J., Gallo, R. C. & Haseltine, W. A. (1984) *Virology* **139,** 340–345.
7. Couez, D., Deschamps, J., Kettman, R., Stephens, R. M., Gilden, R. V. & Burny, A. (1984) *J. Virol.* **49,** 615–620.
8. Seiki, M., Hattori, S., Hirayama, Y. & Yoshida, M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3618–3622.
9. Haseltine, W. A., Sodroski, J. G., Patarca, R., Briggs, D., Perkins, D. & Wong-Staal, F. (1984) *Science* **225,** 419–421.
10. Rice, N. R., Stephens, R. M., Covez, D., Deschamps, J., Kettman, R., Burny, A. & Gilden, R. V. (1984) *Virology* **138,** 82–90.
11. Shimotohno, K., Wachsman, W., Takahaski, Y., Golde, D. W., Miwa, M. M., Sugimura, T. & Chen, I. S. Y. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6657–6661.
12. Sodroski, J. G., Rosen, C. A. & Haseltine, W. A. (1984) *Science* **225,** 381–384.
13. Rosen, C. A., Sodroski, J. G., Kettman, R., Burny, A. & Haseltine, W. A. (1985) *Science* **227,** 321–323.
14. Derse, D., Caradonna, S. J. & Casey, J. W. (1985) *Science* **227,** 318–320.
15. Fujisawa, J., Seiki, M., Kiyokawa, T. & Yoshida, M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2277–2281.
16. Popovic, M., Lange-Wantzin, G., Sarin, P., Mann, D. & Gallo, R. C. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5402–5406.
17. Chen, I. S. Y., Quan, S. G. & Golde, D. W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7006–7009.
18. Lee, T. H., Coligan, J. E., Sodroski, J. G., Haseltine, W. A., Wong-Staal, F., Gallo, R. C. & Essex, M. (1984) *Science* **226,** 57–61.
19. Slamon, D., Shimotohno, K., Cline, M. J., Golde, D. W. & Chen, I. S. Y. (1984) *Science* **226,** 61–65.
20. Goh, W. C., Sodroski, J. G., Rosen, C. A., Essex, M. & Haseltine, W. A. (1985) *Science* **27,** 1227–1229.
21. Sodroski, J. G., Rosen, C. A., Goh, W. C. & Haseltine, W. A. (1985) *Science* **227,** 171–174.
22. Manzari, V., Gallo, R. C., Franchini, G., Westin, E., Cecherini-Nelli, L., Popovic, M. & Wong-Staal, F. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 11–15.
23. Salahuddin, S. Z., Markham, P. D., Wong-Staal, F., Franchini, G., Kalyanaraman, V. S. & Gallo, R. C. (1983) *Virology* **129,** 51–64.
24. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
25. Legerski, R. J., Hodnett, J. F. & Gray, H. B. (1978) *Nucleic Acids Res.* **5,** 1445–1455.
26. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
27. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
28. Graham, F. & van der Eb, A. (1973) *Virology* **52,** 456–467.
29. Queen, C. & Baltimore, D. (1983) *Cell* **33,** 741–748.
30. Josephs, S., Manzari, V., Wong-Staal, F., Trus, M., Sodroski, J., Gallo, R. C. & Haseltine, W. A. (1984) *Virology* **139,** 340–345.
31. Gorman, C. M., Merlino, G. T., Willingham, M. C., Pastan, I. & Howard, B. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6667–6781.
32. Walker, M. D., Edlund, T., Boulet, A. M. & Rutter, W. J. (1983) *Nature (London)* **306,** 557–561.
33. Keller, J. M. & Alwine, J. C. (1984) *Cell* **36,** 381–389.
34. Herrera-Estrella, L., Van den Broeck, G., Maenhaut, R., Van Montagu, M., Schell, J., Timko, M. & Cashmore, A. (1984) *Nature (London)* **310,** 115–120.
35. McKnight, S. L., Gavis, E. R. & Kinsbury, R. (1981) *Cell* **25,** 385–398.
36. Spandidos, D. A. & Wilkie, N. M. (1983) *EMBO J.* **2,** 1193–1199.
37. Celander, D. & Haseltine, W. A. (1984) *Nature (London)* **312,** 159–162.
38. Laimins, L. A., Khoury, G., Gorman, C., Howard, B. & Gruss, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6453–6457.
39. Laimins, L. A., Tsichlis, P. & Khoury, G. (1984) *Nucleic Acids Res.* **12,** 6427–6442.
40. Gluzman, Y. & Shenk, T. (1982) *Enhancers and Eukaryotic Gene Expression* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
41. Luciw, P. A., Bishop, J. M., Varmus, H. E. & Capecchi, M. R. (1983) *Cell* **33,** 705–716.
42. Chatis, P. A., Holland, C. A., Hartley, J. W., Rowe, W. P. & Hopkins, N. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4408–4411.
43. Mulligan, R. C., Howard, B. H. & Berg, P. (1979) *Nature (London)* **277,** 108–114.
44. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12,** 721–729.
45. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1977) *Biochemistry* **18,** 5294–5299.
46. Weiher, H., Konig, M. & Gruss, P. (1983) *Science* **219,** 626–631.
47. Rosen, C. A., Haseltine, W. A., Lenz, J., Ruprecht, R. & Cloyd, M. (1985) *J. Virol.* **55,** 862–866.