Trans-Acting Transcriptional Activation of the Long Terminal Repeat of Human T Lymphotropic Viruses in Infected Cells

Joseph G. Sodroski, Craig A. Rosen, William A. Haseltine

Abstract. *The transcription initiation signals for retroviruses lie within the long terminal repeat (LTR) sequences that flank the integrated provirus. Two subtypes of human T lymphotropic virus (HTLV) are associated with different disease phenotypes. In this article it is shown that marked differences exist in the ability of LTR sequences of these subtypes to function as transcriptional elements in differentiated cell types. It is also shown that* trans*-acting regulatory factors present in HTLV-infected cells stimulate gene expression directed by these LTR sequences in a type-specific manner. These results have implications for understanding the diverse biological effects of HTLV infection.*

The human T lymphotropic viruses (HTLV), a family of exogenous human retroviruses (*1*), are distinguished by the different diseases with which they are associated and by their structural features (*2*). HTLV-I is the etiologic agent of clinically aggressive adult T-cell leukemia-lymphoma (*1, 3*). HTLV-II is an infrequent isolate originally derived from a patient with a clinically benign T-cell variant of hairy cell leukemia (*2*). Recently, a new group of HTLV, HTLV-III, was isolated from patients with the acquired immune deficiency syndrome (AIDS) (*4*).

We wished to characterize the differences between the genomes of HTLV-I and HTLV-II that might account for their different disease associations. Experience with murine leukemia viruses indicated that transcriptional differences mediated by the viral long terminal repeats (LTR's) are major determinants of virulence (*5*). The LTR regions of HTLV-I and -II diverge markedly in sequence (*2*). To determine whether these structural differences have functional significance, we investigated the ability of the LTR sequences of HTLV-I and -II to act as transcriptional elements.

Construction of HTLV-CAT recombinant plasmids. The transcriptional control elements of animal retroviral LTR's are contained within the U3 region (*6*). The LTR U3-R boundary, defined by the RNA transcriptional start site, has been determined for HTLV-I and -II (*2*). For our studies, we inserted the entire U3 and R regions of HTLV-I and -II at a site 5′ to the chloramphenicol acetyltransferase (CAT) gene (pU3R-I and pU3R-II) (Fig. 1) (*7*). A third plasmid that contains only the U3 and a small portion of the R region of HTLV-II was also constructed, pU3-II (Fig. 1). Other plasmids used for comparative purposes contain either the entire SV40 enhancer-promoter region (pSV2CAT), the promoter of SV40 without the 72-base repeat regions that comprise the SV40 enhancer (pSVIXCAT), or the entire LTR of Rous sarcoma virus located 5′ to the CAT gene (pRSVCAT) (*7*).

To test the transcriptional activity of the inserted HTLV sequences, we introduced the plasmid DNA into cells via transfection using either the calcium phosphate or DEAE-dextran methods (*8*). For each experiment the CAT activity directed by the plasmids that contained HTLV sequences was normalized to that of plasmids that contain the SV40 enhancer-promoter elements, known to function in a wide variety of cell types (*7, 9*). Levels of CAT enzymatic activity at 48 hours after transfection correlate closely with CAT-related messenger RNA (mRNA) levels, even when such messages differ in their 5′ start sites, thereby providing a measure of the ability of the sequences 5′ to the CAT gene to promote transcription (*7, 9*).

Expression of the CAT gene in fibroblast and epithelial cell lines. We tested the ability of the LTR sequences of HTLV-I and -II to act as transcriptional elements in fibroblasts and epithelial cells of murine, simian, and human origin. The data are summarized in Table 1 and Fig. 2.

In murine fibroblasts (NIH 3T3) (*10*), the activity of plasmids that contained the LTR sequences of HTLV-I was comparable to that of pSV2CAT and much higher than that produced by the plasmid that contained the SV40 promoter sequence alone. The HTLV-I sequences also yielded significant CAT activity upon transfection of simian (CV-1) cells (*11*), although this was lower than that observed for the pSV2CAT plasmid. The LTR sequences of HTLV-II directed no appreciable levels of CAT activity in either of these cell lines. These experiments suggest that the LTR sequences of HTLV-I function as efficient transcriptional elements in cells of different species, whereas the HTLV-II LTR sequences do not.

Upon transfection of pU3R-I into human epithelial and fibroblast cells [HeLa (*12*) and M1 (*13*)] the level of CAT activity was greater than that observed upon transfection of the same cells with pSV2CAT. Negligible CAT activity was observed upon transfection of these cells with pU3R-II or pU3-II. Thus, HTLV-I LTR sequences appear to function as transcriptional control elements in human cells that are not the natural targets for HTLV infection, whereas the HTLV-II LTR sequences do not.

In retrovirus-infected cells, LTR-mediated gene expression occurs from a provirus stably integrated into the host cell genome, whereas in the CAT assay, the transfected DNA directing transcription occurs primarily in an extrachromosomal state (*14*). To determine if the results obtained from the CAT assays are relevant to an understanding of HTLV LTR function in the integrated provirus, the HTLV LTR sequences used to direct CAT expression were positioned upstream of the neomycin phosphotransferase (*neo*) gene (*15*). These plasmids were transfected into murine and human cell lines and stably transfected colonies were selected in the presence of the neomycin analog G-418. The numbers of G-418–resistant colonies in NIH 3T3 cells transfected with pU3R-I-neo and pSV2neo were equal. In HeLa

The authors are members of the Dana Farber Cancer Institute, Department of Pathology, Harvard Medical School, and W. A. Haseltine is also in the Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115.

cells, pU3R-I-neo yielded about twice as many colonies as did pSV2neo. In both these cell lines, the neo plasmid directed by HTLV-II LTR failed to yield any G-418–resistant colonies. We conclude that the levels of CAT activity correlate well with the function of the LTR in the integrated state.

Expression in human lymphoid cells. In naturally acquired HTLV infections, most of the infected cells are of the T-cell lineage (*16*). However, some HTLV-producing cells expressing B-cell markers have been isolated (*17*). To determine the function of the HTLV LTR sequences within lymphocytes, we transfected cell lines of lymphoid origin with the plasmids described above. The cell lines used were: HUT 78, an $OKT4^+$ (helper-inducer) human T-cell line derived from an HTLV-negative patient with Sézary syndrome (this cell line lacks HTLV-I or HTLV-II proviral sequences) (*18*); and NC37, a B-cell line established from a normal donor and immortalized with Epstein-Barr virus (*18*).

The LTR sequences of HTLV-I directed the synthesis of significant amounts of the CAT gene product in the human T and B cells (Fig. 2 and Table 1). The ratio of pU3R-I activity to pSV2CAT activity was approximately twice as high as this ratio in fibroblasts and epithelial cell lines. These findings demonstrate that factors specific for the cell type modulate the activity of the HTLV-I LTR.

To our surprise, transfection of the HUT 78 or NC37 cells with plasmids containing HTLV-II LTR sequences resulted in no appreciable CAT activity. Thus, there are substantial differences in the cellular requirements for the function of HTLV-I and HTLV-II LTR sequences. Some human lymphoid cells apparently lack factors required for the efficient function of the HTLV-II LTR.

Expression in HTLV-infected cells. The inactivity of the HTLV-II LTR in the lymphoid cell lines suggested that factors specific to the HTLV target cell might be required for efficient expression of the CAT gene under control of the HTLV LTR's. For this reason we transfected HTLV-producing cell lines derived from infected individuals, or cell lines established by cocultivation of primary human lymphocytes with HTLV producer cell lines. The cell lines used include: HUT 102, an HTLV-I–producing $OKT4^+$ T-lymphocyte line established from a patient with an HTLV-associated adult T-cell leukemia-lymphoma (mycosis fungoides) (*18*); MT2, an HTLV-I–producing cell line established by immortalization of primary T lymphocytes after cocultivation with an HTLV producer cell line (*19*); and C81-66-45, a subclone of $C63/CR_{II}$-4, derived by fusion of primary umbilical cord blood cells with an HTLV-I–producing cell line established from an adult T-cell leukemia-lymphoma patient (this cell line does not produce virus but expresses a limited number of viral proteins) (*20*).

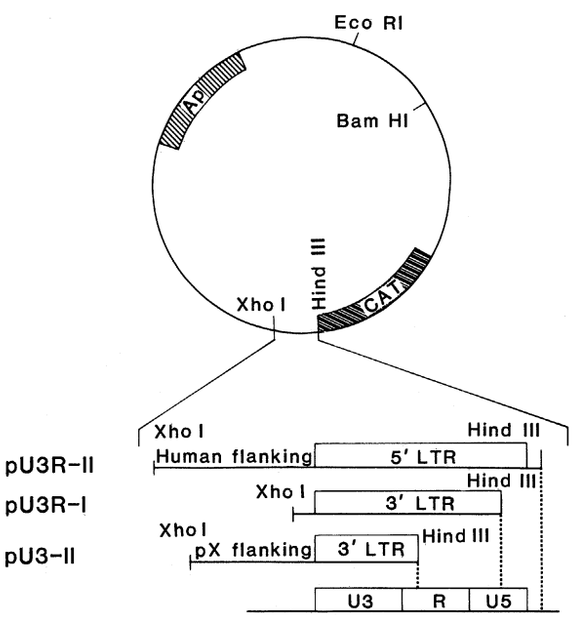

Fig. 1. Construction of recombinant plasmids. The diagram depicts the region of the HTLV LTR's placed 5′ to the CAT gene (Ap, ampicillin resistance gene). pSV2CAT contains the SV40 early promoter region 5′ to CAT (*7*). The recipient vector contains a Hind III–Xho I fragment of pSVIXCAT, a variant of pSV2CAT lacking the SV40 72-base pair enhancer region. Digestion with Hind III–Xho I removes the SV40 promoter region, allowing HTLV sequences to be placed 5′ to CAT. HTLV LTR sequences were inserted into this vector by using natural restriction enzyme sites or by converting convenient sites to Hind III and Xho I sites with synthetic linkers. The sites used for cloning were as follows: (i) pU3R-I: pCR1, a defective proviral clone containing the HTLV-I 3′ LTR (*35*), was digested with Rsa I and Mbo I, resulting in a fragment containing the U3, R, and 105 bp of the U5 region. (ii) pU3R-II: The complete 5′ LTR from MO15A, a complete HTLV-II proviral clone (*2*), was cleaved with Bgl II (in the 5′ cellular flanking sequence) and Eco RI, located 25 bp 3′ to the terminus of the LTR. (iii) pU3-II: MO1A, a 3′ HTLV-II LTR clone (*2*), was cleaved with Xho I (in the envelope gene) and Bam HI (47 bp downstream from the cap site). All recombinant DNA techniques were performed according to enzyme manufacturer's specifications. Plasmid DNA's were purified by centrifugation in $CsCl_2$ gradients prior to transfection.

Table 1. Relative CAT activity in transfected cells. The percent conversion of chloramphenicol to its acetylated forms in HTLV-CAT recombinant transfected cells was normalized against the percent conversion in similar cells transfected with pSV2CAT. To arrive at the values shown, we divided the slope of the time course from the CAT assays of the HTLV-CAT transfected cells by the slope of the pSV2CAT time course. Thus the values represent the percent acetylation per hour relative to that directed by pSV2CAT. The numbers represent the average of a minimum of three independent experiments with a variation no greater than ±20 percent between experiments. N.D., not done.

Cell line	Description	pSV2CAT	pU3R-I	pU3-II	pU3R-II	pRSVCAT
CV-1	Simian fibroblast cell line	1.0	0.2	<0.01	<0.01	N.D.
NIH 3T3	Murine fibroblast cell line	1.0	0.9	<0.01	<0.01	N.D.
M1	SV40-transformed human fibroblasts	1.0	2.2	<0.01	<0.01	N.D.
HeLa	Human cervical carcinoma line	1.0	2.2	<0.01	<0.01	1.6
NC37	Human B-lymphocyte line immortalized by Epstein-Barr virus	1.0	4.5	<0.01	<0.01	N.D.
HUT 78	HTLV-negative human T-lymphocyte line	1.0	4.5	<0.01	<0.01	2.1
C81-66-45	HTLV-I–immortalized nonproducer	1.0	74	<0.01	<0.01	N.D.
HUT 102	HTLV-I producer T-lymphocyte line	1.0	28	1.4	1.7	N.D.
MT2	HTLV-1 producer T-lymphocyte line	1.0	180	N.D.	5.5	2.4
C3-44	HTLV-II producer T-lymphocyte line	1.0	140	40	95	N.D.
HOS	Human osteosarcoma cell line	1.0	0.7	<0.01	<0.01	N.D.
HOS/PL	HTLV-I–infected HOS cell line	1.0	75	<0.01	<0.01	1.0

The results obtained upon transfection of these cell lines with pU3R-I were remarkable and unexpected. The level of CAT activity was 25- to 180-fold higher than that obtained with pSV2CAT.

Normalization of CAT activities for DNA uptake indicates that the increased ratio of CAT activity directed by the pU3R-I plasmid in infected cells represents a real increase and not only a relative increase compared to the activity in uninfected cells. Since the transfected DNA that expresses the CAT activity is mostly extrachromosomal, these results suggest that *trans*-acting factors in HTLV-infected cells stimulate the expression of genes directed by the HTLV-I LTR. Although low, the CAT activity directed by pU3R-II and pU3-II was substantially higher in most of the cells that contained HTLV-I proviruses than it was in uninfected cells.

Lymphocytes infected with HTLV exhibit changes characteristic of T cells activated by exposure to mitogenic or antigenic stimuli (*21*). To examine whether T-cell activation alone might permit increased expression of genes under the control of the HTLV LTR sequences, we transfected the HTLV-CAT and control plasmids into an HTLV-negative immature human T-cell line, Jurkat, both in the presence and absence of the T-cell mitogen phytohemagglutinin (PHA) (*18*). The Jurkat cell line displays many of the responses typical of activated T cells when treated with PHA (*18*). No effect of PHA stimulation on the relative levels of CAT activity directed by any of the plasmids was observed (data not shown). We conclude that mitogenic activation of T cells is probably insufficient to account for the stimulation of CAT activity in infected cells transfected with the HTLV-CAT plasmids.

Activity of the HTLV-II LTR. To determine if the HTLV-II LTR sequences could function in a cell line producing type II virus, the plasmids were transfected into the C3-44 cell line. C3-44 is an HTLV-II–producing cell line established by immortalization of primary lymphocytes following cocultivation with cells derived from an HTLV-II–infected individual (*18*). In this cell line, the level of CAT activity produced by both pU3R-II and pU3-II was approximately 40 times that of the same cells transfected with pSV2CAT DNA. Evidently, in the proper cellular environment, the HTLV-II LTR can function as an efficient transcriptional element.

The CAT activity of the plasmid containing the HTLV-I LTR sequence was also high, approximately 135 times that of pSV2CAT in C3-44 cells. The ratio of CAT activity directed by the plasmids containing the HTLV-I and -II sequences was 25:1 in HTLV-I–infected cells, and only 3:1 in HTLV-II–infected cells. This suggests the presence of *trans*-acting factors, that is, factors that can regulate the transcriptional activity of DNA molecules other than those that encode them. The *trans*-acting factors in HTLV-I–infected cells appear to differ from those of HTLV-II–infected cells in the ability to act on HTLV LTR sequences of different types. The high CAT activity in HTLV-infected cells might thus be a consequence of viral infection rather than of the specific type of cell infected.

A test for viral-associated trans*-acting factors.* To test the possibility that the high CAT production in cells containing HTLV proviruses could be attributed to the virus infection, we examined the ability of the plasmids containing the HTLV LTR sequences to function in uninfected (HOS) and HTLV-I–infected (HOS/PL) human osteogenic sarcoma cells (*22*). The HOS/PL cells were derived in vitro by cocultivation of HOS cells with HTLV-I–producing lymphocytes and do not express T-cell markers (*22*). The data of Table 1 and Fig. 2 show that the CAT activity induced by pU3R-I in the HOS/PL cells was more than 90 times that in uninfected cells. Neither

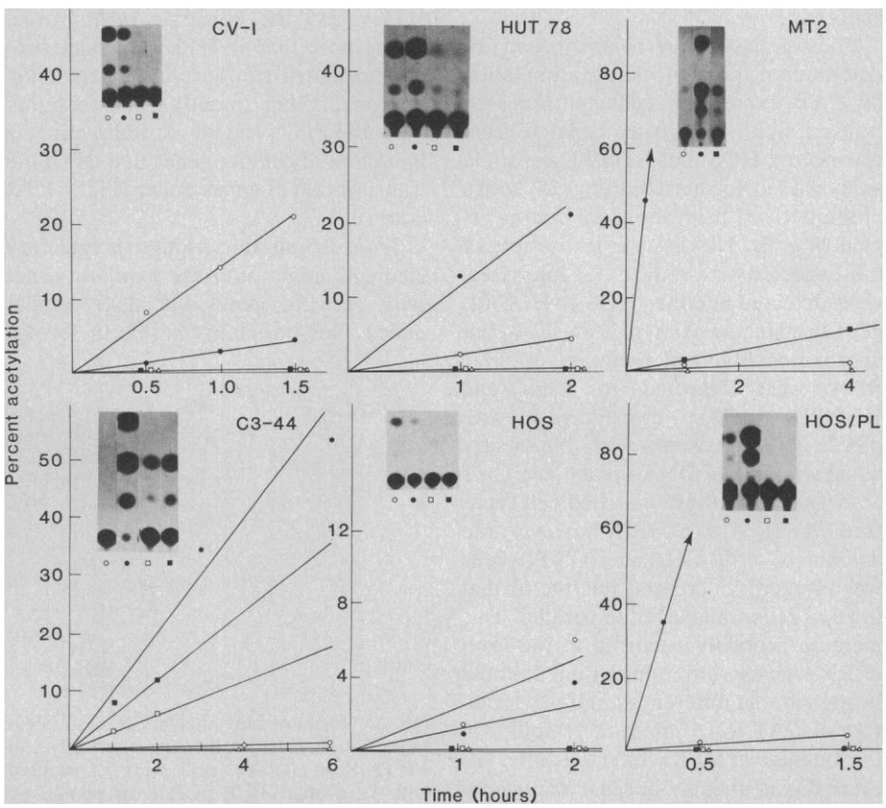

Fig. 2. Transient expression of the CAT gene directed by the HTLV LTR transcriptional elements. NIH 3T3 and CV-1 cells were transfected by a modification of the calcium phosphate coprecipitation technique (*8*). Approximately 1×10^6 cells were seeded on 100-mm dishes 24 hours prior to addition of the $CaPO_4$-DNA precipitate. One milliliter of the precipitate containing 5 to 10 μg of DNA was added to the medium, and the cells were incubated 4 hours at 37°C and then exposed to glycerol for 3 minutes. CV-1 cells were exposed to 10 percent dimethyl sulfoxide 24 hours after transfection. All other cell lines were transfected by a modification of the DEAE-dextran technique (*8*). Twenty-four hours before transfection adherent cells were seeded at a density of 1×10^6 cells per 10-cm^2 dish. Immediately prior to transfection cells were trypsinized, washed, and transfected in suspension with 5 to 8 μg of DNA, an amount shown to be less than saturating. Lymphocyte lines were transfected at a density of 1×10^6 to 5×10^6 cells per milliliter with 5 to 10 μg of DNA. Cells were harvested 48 hours after transfection, and cellular extracts were prepared by freeze-thawing three times. After a brief centrifugation to remove cell debris, extracts were analyzed for CAT activity as described (*7*) except that acetyl coenzyme A was present at 24 m*M*. Percent conversion of chloramphenicol to the acetylated forms was determined by ascending thin-layer chromatography and liquid scintillation counting of the spots cut from the plate. The graphs depict typical CAT assays over the time course indicated. Arrowheads indicate other data points that could not be accommodated in the graph. All experiments were performed a minimum of three times, and the slopes of the curves differ by no more than 20 percent from experiment to experiment. Symbols represent CAT activity directed by the plasmids: ●, pU3R-I; ○, pSV2CAT; □, pU3-II; and ■, pU3R-II; and △, pSVIXCAT. Insets show actual autoradiograms of a CAT assay and represent conversions obtained from one time period within the linear range of the assay.

HOS nor HOS/PL cells transfected with the plasmids containing HTLV-II LTR sequences expressed significant CAT activity.

We therefore conclude that *trans*-acting factors in HTLV-infected cells, either encoded directly by the virus or induced by viral infection, augment gene expression directed by the HTLV LTR sequences. This effect is not observed upon transfection of these plasmids into murine T lymphocytes infected with nonacute murine leukemia viruses (*23*). The expression of the *neo* gene directed by the HTLV-II LTR as reported by Chen *et al.* (*24*) is probably due to *trans*-acting factors present in the HTLV-II–infected recipient cells and not only due to tissue type-specific elements as they suggest.

Trans-*activation of transcription*. To determine if the *trans*-acting stimulation of CAT expression reflects increased transcription, RNA was isolated from transfected HOS and HOS/PL cells and analyzed by Northern blotting (*25*) with a probe derived from the CAT coding region (Fig. 3). No discrete transcripts of the expected size (1.6 to 1.7 kilobases) were detected in either HOS or HOS/PL cells transfected with pU3-II. Low but approximately equal amounts of CAT RNA were detected in HOS and HOS/PL cells transfected with pSV2CAT, consistent with the nearly equal amounts of DNA uptake and CAT activity seen in these matched cell types (see Fig. 2). With pU3R-I, however, the amount of CAT RNA in HOS/PL cells was markedly increased relative to that in HOS cells transfected in parallel. This increase probably occurred at the level of RNA transcription initiation. It could be argued that differences in the 5′ structure of CAT RNA affect the stability of the message in HTLV-infected cells. We think this is unlikely because *trans*-activation in HTLV-II–infected cells occurs with plasmids pU3R-I, pU3R-II, and pU3-II, which differ substantially in R region sequence and, therefore, in the leader sequence of the CAT transcripts. Moreover, we find that a plasmid containing a sequence rearrangement 5′ of the pU3R-I promoter (TATA box), but that leaves the known RNA transcriptional start site (*2*) unaltered, directs the same level of CAT activity in both uninfected and HTLV-infected cells (*26*). Thus, the *trans*-acting stimulation of HTLV LTR-directed gene expression must affect sequences distant from those present in the CAT transcripts, showing that the *trans*-activation is mediated at the level of initiation of RNA transcription.

Discussion

The phenomenon of *trans*-activation distinguishes HTLV from other nonacute transforming retroviruses. The ability of HTLV-I and -II to immortalize primary lymphocytes in vitro, the apparent absence of preferential integration sites in HTLV-induced tumors, and the absence of chronic viremia also distinguish these viruses from most others (*19–21, 27–29*). The genome of HTLV also differs from the genomes of nonacute retroviruses by the presence of a long, 1600-base sequence located between the 3′ end of the envelope gene and the LTR (*30*).

To explain the differences in the structure and biological activity between HTLV and the nonacute retroviruses, we propose that an HTLV-encoded protein mediates transcriptional regulation in *trans*, either directly via interaction with the HTLV LTR or indirectly via induction of cellular genes that effect the transcription of genes under HTLV LTR control.

Trans-acting transcriptional regulatory elements might alter the expression not only of viral genes but also cellular genes. One possibility is that in T cells,

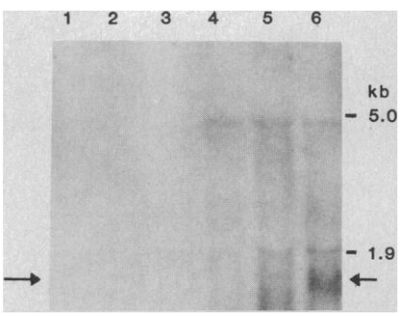

Fig. 3. Northern blot analysis of CAT messages in transfected cells. Approximately 2×10^6 HOS or HOS/PL cells were transfected with 5 μg of pU3R-I, pU3-II, or pSV2CAT DNA by using calcium phosphate–DNA coprecipitation. Forty-eight hours after transfection, RNA was isolated. Fifty micrograms of total cellular RNA was sized on a denaturing gel, transferred to nitrocellulose, and hybridized to a nick-translated probe made from the Hind III–Bam HI CAT fragment as described (*25*). Nonspecific hybridization serves to identify the positions of the 28*S* and 18*S* ribosomal bands. Lanes 1 to 3 represent RNA from HOS cells, and lanes 4 to 6 from HOS/PL cells. Cells were transfected with pU3-II DNA (lanes 1 and 4), pSV2CAT DNA (lanes 2 and 5), or pU3R-I DNA (lanes 3 and 6). Arrows indicate the expected position of CAT messages with appropriate start and termination sites. Since the message sizes differ only minimally between the plasmids, comparison of hybridization signals between adjacent bands is appropriate. After Northern transfer, the nitrocellulose filter was stained with methylene blue to ensure equal transfer between lanes.

HTLV-I and -II regulate cellular genes that control proliferation, whereas HTLV-III regulates genes that terminate cell growth. In this regard, we note that the receptor for T-cell growth factor (interleukin-2), the HT3 gene product, and the JD-15 product are regularly expressed at high levels in lymphocytes infected with HTLV-I or -II (*18*). The specificity of HTLV transformation for the $OKT4^+$ (helper-inducer) subset of T lymphocytes may result from a restricted ability of the *trans*-acting transcriptional element to regulate growth control in specific differentiated T-cell subpopulations. Expression of a viral protein that mediates the biological effects of HTLV infection provides a natural explanation for the ability of HTLV-I and -II to transform cells in vitro and for the absence of preferential integration sites in tumor cells. We call this hypothesis the *trans*-acting transcriptional regulation hypothesis. The transforming proteins of some DNA tumor viruses, notably adenovirus and SV40, also activate expression of viral and cellular genes in *trans* (*31, 32*).

What might the identity of the virus-induced *trans*-acting product be? We note that the level of CAT activity directed by the HTLV-I LTR in the C81-66-45 line is comparable to the high activity in HTLV producer cells. Since the C81-66-45 line produces no virus, yet contains a 42-kilodalton viral protein precipitable by human antiserum to HTLV (*33*) the production of this protein may be sufficient to maintain the *trans*-activation phenomenon. In a separate study, we have shown that the region located between the envelope gene and the 3′ LTR of both HTLV-I and HTLV-II can encode a polypeptide of about this size (*34*). This coding region includes sequences that are highly conserved (approximately 75 percent identical) in the two virus types, but the immediate flanking sequences are almost entirely divergent. The predicted amino acid sequences of these proteins are 77 percent identical. A subgenomic spliced 2.2-kilobase mRNA species that contains the 3′ sequences, but not the *gag, pol*, or *env* genes, has been detected in HTLV-producing cells and in some fresh HTLV-associated tumors (*35, 36*). It is possible that this region encodes the 42-kilodalton protein seen in the C81-66-45 line, and that this protein mediates the *trans*-acting transcriptional effects of HTLV infection.

The T-cell malignancies associated with HTLV-I and HTLV-II infection differ in their clinical aggressiveness (*1, 2*). Here we have demonstrated marked dif-

ferences in the ability of the HTLV-I and HTLV-II LTR sequences to direct CAT expression in a range of differentiated cell types. The number and type of cells infected by these different groups of HTLV may vary as a consequence of differences in LTR promoter strength, host cell range, or response to *trans*-acting transcriptional factors. The level of a viral protein effecting lymphocyte transformation may vary in a critical target cell as a result of differences in LTR function. Further work to identify the HTLV-associated *trans*-acting factor, as well as its target sequences in the viral and host cell genome, should provide insight into the mechanism of transformation by this family of retroviruses.

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ASSOCIATION AFFAIRS

Gerard Piel: President-Elect of the AAAS

Dennis Flanagan

Gerard Piel, president-elect of the American Association for the Advancement of Science, has devoted his entire career to advancing public understanding of science, technology, and medicine. He has been president and publisher of *Scientific American* since 1947, a span of 37 years. Before that time he had been science editor of *Life* for 6 years.

Piel was born in 1915 at Woodmere, a suburb of New York City on Long Island, the fourth of six children of Loretto Scott Piel and William F. J. Piel. William Piel was president of Piel Brothers, a family-owned brewery. Gerard Piel was educated at Phillips Academy in Andover, Massachusetts, and at Harvard College, from which he graduated magna cum laude in 1937.

Since Piel is the publisher of a magazine mostly devoted to the natural sciences, it is sometimes taken for granted that he was educated in those sciences. Such is not the case. At Andover he flunked physics, and at Harvard he kept a respectful distance between himself and such subjects. (One explanation of this apparent contradiction in the background of someone in Piel's occupation is that, if someone studies science and does well at it, the probability is high that he will become a scientist. Therefore the main population from which someone in Piel's occupation can be recruited is one consisting of nonscientists.)

Piel's education did not, however, lack for influences that played a strong role in his career of advancing public understanding of science. At Harvard he first took up sociology, and under the Harvard tutorial system his first academic mentor was a graduate student named Robert K. Merton. (Merton, now professor of sociology at Columbia University, went on to found the sociology of science.) Later Piel concentrated in history, with an emphasis on political economy; his senior thesis dealt with the history of the French Socialist Party up to World War I. The interaction of economics and politics in social history struck a deep chord that has persisted throughout Piel's career. (At Harvard, Piel was also a leading member of the varsity wrestling team, which may signify a willingness to grapple with such problems.)

Piel left Harvard in the middle of the Great Depression, when by definition jobs were scarce. He found work in the

Dennis Flanagan is editor emeritus of *Scientific American*.