The half-life of the $p40^{xI}$ protein is relatively short (120 minutes) (Fig. 2), which is what might be expected for a protein with regulatory function. Proteins encoded by two other transforming viruses, the *myc* protein of the MC-29 virus and the E1A protein of adenovirus are involved in regulation of gene transcription (*20*). Both of these proteins also have short half-lives (*21, 22*), and both are found in the nuclei of infected cells (*12, 23, 24*).

If the $p40^{xI}$ protein is responsible for activation of the viral LTR and for viral gene transcription as postulated (*17–19*), a remaining question is how viral transcription is initially carried out in newly infected cells before the *x* gene is transcribed. At least two possibilities exist: (i) like reverse transcriptase, the $p40^{xI}$ protein may be packaged in the virion, thus facilitating viral gene transcription upon infection, or (ii) low levels of gene transcription occur in the absence of the $p40^{xI}$ protein and subsequently increase as the amount of $p40^{xI}$ increases. We found no detectable levels of $p40^{xI}$ protein in disrupted viral particles (Fig. 5), which argues against the first possibility. Moreover, we have found that low levels of viral transcription do occur in the absence of the x^{II} gene in HTLV-II–infected cells (*18*). A similar mechanism is likely for HTLV-I–infected cells.

It has been postulated that the product of the *x* gene interacts with viral LTR sequences to facilitate viral gene transcription. This function may also activate a cellular gene or genes involved in T-cell proliferation, thus inducing malignancy.

Note added in proof: After submission of this manuscript, Goh *et al.* published results in agreement with Fig. 3b of this study, that is, nuclear localization of the *x* protein (*36*).

DENNIS J. SLAMON
Division of Hematology-Oncology
Department of Medicine,
UCLA School of Medicine,
Los Angeles, California 90024
MICHAEL F. PRESS
Department of Pathology,
University of Chicago School of
Medicine, Chicago, Illinois 60637
LAWRENCE M. SOUZA
DOUGLAS C. MURDOCK
Amgen
Thousand Oaks, California 91324
MARTIN J. CLINE
DAVID W. GOLDE
JUDITH C. GASSON
IRVIN S. Y. CHEN
Division of Hematology-Oncology
Department of Medicine
UCLA School of Medicine

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A Transcriptional Activator Protein Encoded by the x-*lor* Region of the Human T-Cell Leukemia Virus

Abstract. *Human T-cell leukemia viruses type I and II (HTLV-I and -II) exhibit several features characteristic of this retroviral family: the presence of an x-*lor *gene encoding a nuclear protein, transformation properties suggesting the involvement of a virus-associated* trans*-acting factor, and transcriptional* trans*-activation of the long terminal repeat (LTR) in infected cells. In the study described here the HTLV x-*lor *products, in the absence of other viral proteins, were able to activate gene expression in* trans *directed by the HTLV LTR. The regulation of the expression of particular genes in* trans *by HTLV x-*lor *products suggests that they play a role in viral replication and possibly in transformation of T lymphocytes.*

Human T-cell leukemia viruses (HTLV) comprise a retroviral family associated with lymphoid disorders. HTLV types have been identified on the basis of immunocompetition analysis of *gag* proteins (*1*). HTLV-I is associated with adult T-cell leukemia-lymphoma (ATLL) that is endemic in certain geographic regions (*2*). HTLV-II is a rare isolate associated with a benign form of hairy T-cell leukemia (*1*). HTLV-III has been identified as a probable cause of the acquired immune deficiency syndrome (AIDS) (*3*). Bovine leukemia virus (BLV), the etiological agent of enzootic leukosis in domestic cattle, demonstrates antigenic relatedness to the HTLV viruses in some of the virion proteins (*4*).

HTLV-I, -II, and BLV have been categorized as a separate family (HTLV-BLV) of transforming retroviruses (*5*) because of their characteristic structural and biological properties. The HTLV-BLV viruses can be distinguished from chronic murine leukemia viruses in that the former exhibit *trans*-acting transcriptional activation of the viral long terminal repeat (LTR), which governs viral gene expression, in infected cells (*6, 7*). Cells infected by these viruses contain factors that greatly augment steady-state levels of RNA produced by the viral LTR, without stimulating the replication of the template DNA (*7, 8*). The *trans*-acting factors act most efficiently on the LTR of the infecting virus (*7*), suggesting that a virus-specific rather than host cell-

specific factor mediates *trans*-activation.

In addition to the *gag*, *pol*, and *env* genes typical of chronic leukemia viruses, the HTLV-BLV viruses possess a sequence (called the X region) located between the envelope gene and the 3′ LTR (*5*, *9*). This region of HTLV-I and HTLV-II contains a gene called x-*lor* that encodes a 42- and 38-kilodalton (kD) protein in HTLV-I and -II, respectively (*10*). These proteins are greater than 90 percent homologous at the amino acid level (*5*, *9*) and are located predominately in the nuclei of infected cells (*11*). Studies correlating the presence of the x-*lor* protein in various nonvirus-producing HTLV transformed cell lines with LTR *trans*-activation provide indirect evidence implicating an x-*lor* product in transcriptional regulation (*12*). Here we examine directly the effects of x-*lor* products on gene expression under the control of the viral LTR's.

The x-*lor* genes of HTLV-I and -II lack initiator methionine codons but have splice acceptor consensus sequences at their 5′ ends (*5*, *9*, *13*). The x-*lor* proteins are made from spliced 2-kilobase (kb) messages that often contain, in addition to regions homologous to LTR and x-*lor*, sequences surrounding the *pol-env* junction (*14*). To gain insight into the composition of the natural x-*lor* protein, we mapped selected splice donors and acceptors in the HTLV-I–immortalized C81-66-45 cell line. Our experience, and that of other investigators (*10*, *12*), is that this cell line has only HTLV-related messenger RNA (mRNA) that is 2 kb in size and expresses only the 42-kD x-*lor* product detectable by antiserum from patients with adult T-cell leukemia-lymphoma. A 3′ end-labeled probe spanning from 86 base pairs (bp) 5′ of the start codon of the *env* gene to the middle of the *env* gene (Fig. 1) was annealed to C81-66-45 RNA and treated with S1 nuclease. The size of the protected fragment was determined by comparison with Maxam-Gilbert sequencing reactions performed on the probe (*15*). The terminus of the protected fragment maps at a splice donor consensus sequence located immediately 3′ of the *env* gene start codon. In a similar manner, we mapped the C81-66-45 splice acceptor to a consensus sequence located 172 bp 5′ of the Cla I site in the x-*lor* gene, in a position identical to that determined for the HTLV-II x-*lor* message (*13*). Since C81-66-45 RNA does not hybridize to probes derived from the region bounded by these splice donor and acceptor sites, we conclude that these sites are juxtaposed in the C81-66-45 cell line to produce the 2-kb x-*lor* message. A probable scheme for production of the natural x-*lor* message and protein product is presented in Fig. 1. By means of a dual splicing event, the HTLV *env* gene contributes its initiation codon plus a single guanosine residue to the x-*lor* gene, resulting in an in-frame coding sequence.

Knowledge of the splicing pattern for the x-*lor* message suggested that the natural x-*lor* products could be expressed in eukaryotic cells by placing the HTLV-I LTR 5′ to the *env*-X regions of the HTLV-I and HTLV-II genomes (plasmids $envX_I$ and $envX_{II}$ in Fig. 2). These plasmids could potentially express envelope proteins, x-*lor* proteins, and other products from the 3′ half of the HTLV genome. Another plasmid ($pCATLOR_{II}$) was designed to express only a fusion protein consisting of nine NH_2-terminal residues encoded by the bacterial chloramphenicol acetyltransferase (CAT) gene (*16*) and the product of the entire HTLV-II x-*lor* gene extending from the splice acceptor to the termination codon (*5*, *9*). This hybrid gene was also placed under the transcriptional control of the HTLV-I LTR. As negative controls, three additional plasmids containing the same sequences as $envX_I$ or $pCATLOR_{II}$ in a configuration not suitable for protein expression were constructed: $IenvX_I$, containing the entire env-X region of HTLV 3′ to the HTLV-I LTR, but in an orientation opposite to that of $envX_I$; $pTACLOR_{II}$, containing the HTLV-I LTR and bacterial CAT gene sequences in the opposite orientation to that of $pCATLOR_{II}$; $pCATLOR_{II}fs$, identical to $pCATLOR_{II}$ except for a 2-bp deletion in the CAT gene sequences of $pCATLOR_{II}$, resulting in a +1 frameshift mutation in the x-*lor* reading frame.

The ability of each of these plasmids to affect the rate of gene expression directed by the HTLV-I and -II LTR sequences was examined. Cells were cotransfected with test plasmids and indicator plasmids in which the CAT gene was placed under the control of various eukaryotic promoter elements. The

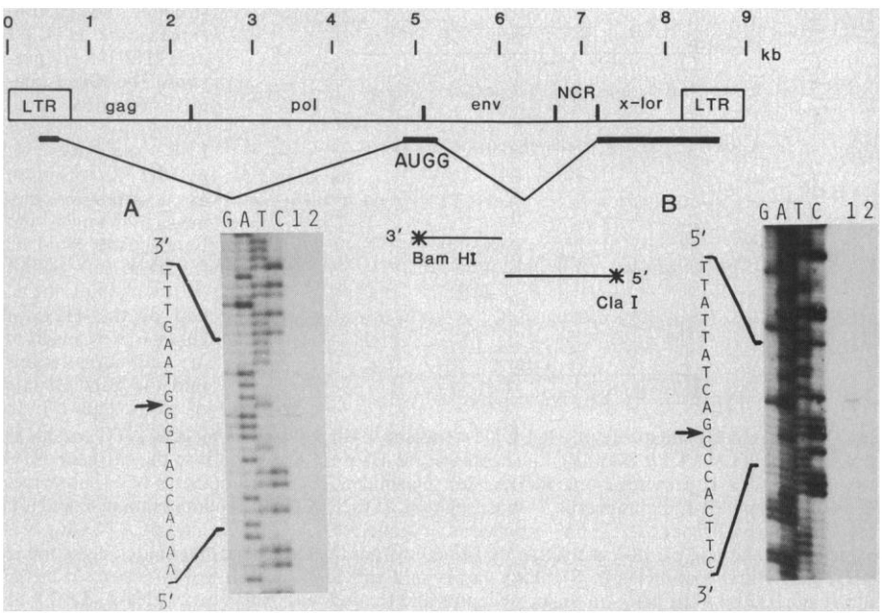

Fig. 1. Mapping of splice sites of the HTLV-related message in the C81-66-45 cell line. NCR represents the region that is not conserved between HTLV-I and HTLV-II (*5*). A model for generation of the 2-kb x-*lor* message from the HTLV-I genome is shown beneath the genome. Solid horizontal bars represent exons, and thin diagonal lines encompass the regions spliced from the mature message. The C81-66-45 mRNA was characterized for splice donor and acceptor sites relevant to the generation of the x-*lor* open reading frame. A 0.6-kb Bam HI-Sal I fragment [nucleotides 5094 to 5672 of the HTLV-I sequence of Seiki *et al.* (*9*)], end-labeled at the 3′ position of the Bam HI site, was used as a probe for the splice donor (A). A 1.8-kb Sal I-Cla I fragment (nucleotides 5672 to 7474) end-labeled at the 5′ position of the Cla I site was used to define the splice acceptor (B). Denatured probes were annealed to total RNA of C81-66-45 (lane 1) or HUT 78 (an HTLV-negative T-lymphocyte line) (lane 2) and treated with S1 nuclease (*24*). The protected fragments were then analyzed on denaturing acrylamide gels alongside Maxam-Gilbert sequencing reactions (*15*) performed on the probe fragments. The sequencing gels are oriented so that the sequence of the sense strand can be read directly (G, guanine reaction; A, adenine plus guanine reaction; T, thymine plus cytosine reaction; C, cytosine reaction). Arrows denote the position of the protected fragments. The 5′-most splice donor of the HTLV-I genome was not mapped; thus, the 5′-most splice donor depicted in the figure is based only upon the presence of consensus sequences located in the R region of the LTR. The splice acceptor sequence in the *pol* gene has been mapped approximately to positions 4990 to 5100 of the sequence of Seiki *et al.* (*9*).

CAT-containing plasmids include those previously shown to respond to HTLV-I or -II *trans*-acting factors, pU3R-I (containing HTLV-I LTR sequences) and pU3-II (containing HTLV-II LTR sequences (*7*). To assess the specificity of any observed effect, CAT-containing plasmids that do not respond to HTLV-I or -II *trans*-acting factors (*7*) were used. The latter include pSV2CAT (with the SV40 early promoter), pBLVCAT (with the bovine leukemia virus LTR), pU3R-III (with the HTLV-III LTR), and pC55 (with the HTLV-I LTR deleted so as to render it unresponsive to viral *trans*-acting factors) (*7, 8, 17, 18*). Cell lines used as recipients include uninfected human OKT4+ T lymphocytes (HUT 78), a simian kidney cell line containing a functional SV40 T antigen (COS-1), a feline kidney cell line (CCCS+L−), and a murine fibroblast line (NIH 3T3). Forty-eight hours after transfection, CAT enzymatic activity was determined, a measurement previously shown to correlate with CAT mRNA levels (*17, 19*).

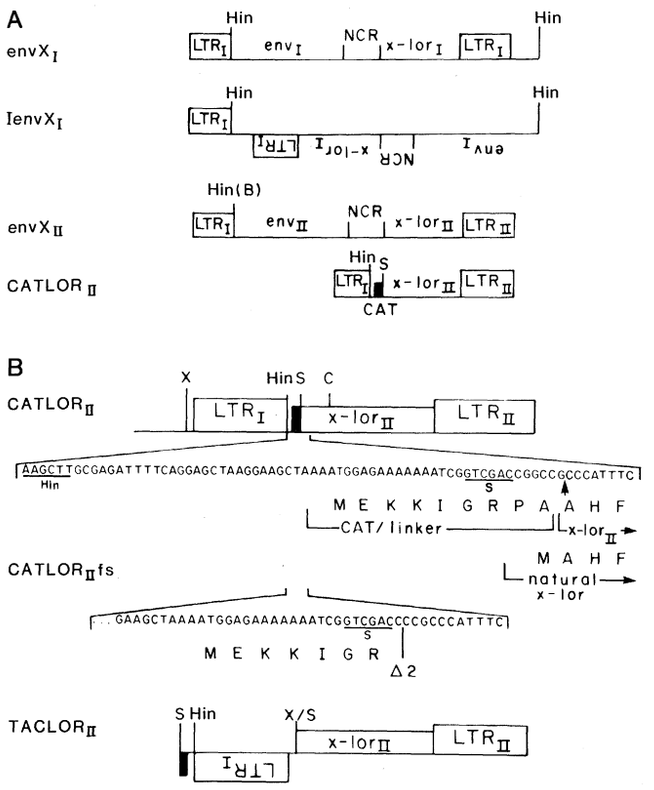

Fig. 2. HTLV x-*lor* expressor and control plasmids. The darkened boxes show sequences derived from the CAT gene. Hin, Hind III site; Hin(B), Hind III adjacent to a Bgl II site via a polylinker; S, Sal I; C, Cla I; X/S, Xho I-Sal I ligation; X, Xho I; NCR, nonconserved region between HTLV-I and HTLV-II. (A) Constructions designed to express the HTLV-I or HTLV-II x-*lor* proteins. The Xho I-Hind III fragment containing the HTLV-I LTR sequences of pU3R-I (*7*) was used as a promoter element. For envX$_I$ and IenvX$_I$, the Hind III fragment of pCR-1 (*25*) containing the 3′ end of the HTLV-I genome was inserted in both orientations into the Hind III site of pU3R-Igpt (*7*). In envX$_I$, the Hind III site of the pU3R-I LTR is located 190-bp 5′ to the *env* gene ATG codon. In envX$_{II}$, the HTLV-I LTR of pU3R-I is located at the Bam HI site 88 bp 5′ to the *env* gene ATG codon of HTLV-II proviral clone MO1A (*26*). Plasmid pCATLOR$_{II}$ consists of 5′ sequences derived from pU3R-I, including the 5′-most segment of the CAT-gene coding sequence, and a 3′ sequence derived from the HTLV-II provirus in clone MO1A (*26*). Plasmid pCATLOR$_{II}$ was made by digestion of pU3R-I at a single Pvu II site within the CAT gene and limited digestion of DNA with Bal31 exonuclease. Similarly, a plasmid subclone of MO1A was linearized at the Bgl II site 227 bp 5′ to the x-*lor* splice acceptor and treated with Bal31 exonuclease. Sal I 8-bp linkers (New England Biolabs) were ligated to the exonuclease-treated fragments, which were subsequently digested with Sal I, recircularized, and cloned after transfection of *Escherichia coli*. Fragments from selected clones were then religated to yield constructions with the CAT coding sequence fused in-frame (pCATLOR$_{II}$) and out-of-frame (pCATLOR$_{II}$fs) with the x-*lor* gene. The structure of these plasmids near the Sal I junction was confirmed by DNA sequencing. In addition to the sequences illustrated, all plasmids contain pBR322 sequences (origin of replication and ampicillinase gene) and the SV40 early-region promoter. Plasmid envX$_{II}$ contains enhancer sequences derived from the Moloney murine leukemia virus LTR (*27*) (from the 5′ limit of the LTR to the Sac I site located upstream of the TATA box) located immediately 5′ to and in the same orientation as the HTLV-I LTR. (B) Details of pCATLOR$_{II}$ and variants. The nucleotide sequences surrounding the junction of the CAT gene and the HTLV-II x-*lor* gene in pCATLOR$_{II}$ are shown. In addition to the methionine of the natural x-*lor* product, the potential pCATLOR$_{II}$-derived product has eight NH_2-terminal residues derived from the CAT gene. The vertical arrow designates the position of the known x-*lor*$_{II}$ splice acceptor (*13*). The plasmid pCATLOR$_{II}$fs is identical to pCATLOR$_{II}$ except for a 2-bp deletion (Δ2) just 5′ to the junction between CAT and x-*lor* resulting in a +1 frameshift in the potential protein product. The plasmid pTACLOR$_{II}$ has the HTLV-I LTR and ATG codon of the CAT gene in an orientation opposite to that of pCATLOR$_{II}$. All plasmids were purified by centrifugation in cesium chloride prior to transfection. Amino acid designations: A, alanine; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; M, methionine; P, proline; R, arginine.

The results of these experiments are summarized in Table 1 and Fig. 3. In all cell lines examined, cotransfection with the plasmids that contained the x-*lor* gene of either HTLV-I or -II in a configuration suitable for expression led to a marked increase in the level of HTLV-I LTR-directed CAT gene expression as compared to experiments done in parallel with the control plasmids. For example, the level of CAT activity directed by pU3R-I in HUT 78, COS-1, and NIH 3T3 cells transfected with pCATLOR$_{II}$ is 66 times, 30 times, and 7.7 times that observed upon cotransfection of the same cell lines with pU3R-I and pCATLOR-$_{II}$fs. No stimulation of CAT activity was detected upon cotransfection of the test plasmids with CAT plasmids containing the SV40 early promoter or the LTR's of BLV or HTLV-III. Similarly, none of the x-*lor* expressor plasmids stimulated the CAT activity directed by pC55. The plasmid pC55 is identical to pU3R-I except that sequences 5′ to the promoter (TATA box), which are necessary for response to viral *trans*-acting factors, have been deleted (*8*). This suggests that HTLV-I LTR sequences necessary for *trans*-activation in infected cells are also required for the response to the cotransfected x-*lor* expressor plasmids. Furthermore, the stimulation of pU3R-I CAT activity by the envX$_{II}$ and pCATLOR$_{II}$ constructions is consistent with the ability of the *trans*-acting factors in HTLV-II–infected cells to activate HTLV-I LTR transcription (*7*). We conclude that products of the HTLV x-*lor* region are able to activate specifically gene expression directed by the HTLV-I LTR.

The *trans*-acting factors in all HTLV-I–infected cells examined to date preferentially activate HTLV-I LTR-mediated gene expression, with detectable, but relatively weak, activation of the HTLV-II LTR (*7*). To examine the effect of the x-*lor* products on HTLV-II LTR promoter activity, the control plasmids and the x-*lor* expressor plasmids were cotransfected into eukaryotic cells along with the HTLV-II LTR-CAT plasmid, pU3-II. In CCCS+L− feline kidney cells, COS-1 monkey kidney cells, or NIH 3T3 murine fibroblasts, both the x-*lor*$_I$ expressor plasmid (envX$_I$) and the x-*lor*$_{II}$ expressor plasmids (envX$_{II}$ and pCATLOR$_{II}$) stimulated CAT activity directed by the HTLV-II LTR (Fig. 3, Table 1). By contrast, in HUT 78 human T lymphocytes, the x-*lor*$_I$ and x-*lor*$_{II}$ expressor plasmids had only slight stimulatory effects on the HTLV-II LTR, despite the marked stimulation of the HTLV-I LTR

by these plasmids in the same cell line. That both x-lor_I and x-lor_{II} expressor plasmids efficiently activate the HTLV-II LTR in some cell lines only, suggests that the apparent LTR type-selectivity observed in HTLV-I–infected cells (*7*) is probably not due to structural differences between the HTLV-I and HTLV-II x-*lor* products. A more tenable explanation is that the HTLV-II LTR is more dependent both upon viral and cellular transcriptional factors than is the HTLV-I LTR. Thus, even in the presence of a functional x-*lor* product, the lack of appropriate cellular factors can prevent efficient HTLV-II LTR-directed gene expression. The ability of the HTLV-II LTR to be *trans*-activated in COS-1, NIH 3T3, and CCCS+L− cells and the poor *trans*-activation in HUT 78 cells suggests that such cellular factors are not necessarily cell type (lymphocyte)-specific.

Our experiments provide direct evidence that products of the HTLV x-*lor* gene are able to act in *trans* to stimulate gene expression directed by the HTLV LTR. The magnitude of LTR *trans*-activation after cotransfection with x-*lor* expressor plasmids approximates that observed in HTLV-infected cell lines (*7*). In the $pCATLOR_{II}$ construction, only the HTLV-II x-*lor* region is available for expression, suggesting that products of this region are sufficient for *trans*-activation. Disruption of the open reading frame known to encode the 38-kD HTLV-II protein results in a complete loss of *trans*-activating ability. This observation suggests that the known products of the HTLV x-*lor* genes are necessary for *trans*-activation. They are probably sufficient as the x-lor_I and x-lor_{II} expressor plasmids activate both the HTLV-I and -II LTR in the appropriate cell type, despite the observation that no other potential protein products of the HTLV-I and -II x-*lor* region are structurally related (*5, 9*).

Consistent with their involvement in the stimulation of gene expression by the LTR, we will refer to the 42- and 38-kD x-*lor* products as the tat_I and tat_{II} proteins, for *trans*-activating transcriptional proteins. We also propose that the HTLV gene encoding this protein be called the *tat* gene, in keeping with the tradition that retroviral genes be named according to function. Viral proteins are likely to mediate the *trans*-acting stimulation of LTR-directed gene expression in cells infected with BLV and HTLV-III (*7, 18*) as well. Since retroviral replication is highly dependent on proviral transcription, these products serve critical functions in virion production.

In addition to their role in the virus life cycle, the *tat* products may mediate the pathogenic effects of these retroviral agents. Tumors associated with HTLV-I are monoclonal, yet lack preferred proviral integration sites (*20*). In addition, HTLV-I and HTLV-II can immortalize primary lymphocytes in vitro despite their lack of a host cell-derived oncogene (*9, 21*). The HTLV *tat* product is maintained in all in vitro–immortalized cells examined to date, in some cases to the exclusion of all other detectable viral proteins (*10, 12*). These observations suggest that HTLV might transform lymphocytes by production of this viral

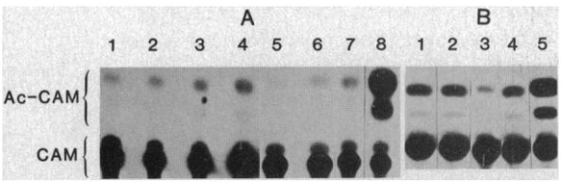

Fig. 3. CAT assays after cotransfection of HUT 78 lymphocytes and CCCS+L− cells with x-*lor* expressor plasmids. Transfection was performed as described in the legend to Table 1. Forty-eight hours after transfection, equivalent amounts of protein from cell lysates were incubated with ^{14}C-labeled chloramphenicol in the presence of acetyl coenzyme A as described (*7, 17*). Unacetylated chloramphenicol (CAM) and acetylated forms (Ac-CAM) were determined by thin layer chromatography after a 1-hour reaction. (A) HUT 78 and (B) CCCS+L− cells are the transfected cells. The CAT plasmids used for transfection are: (lanes A1 and A2) pC55 [an HTLV-I LTR-deletion mutant that is unresponsive to viral *trans*-acting factors (*8*)]; (lanes A3, A4, B1, and B2) pSV2CAT (containing SV40 sequences); (lanes A5 and A6 and B3 to B5) pU3-II (containing HTLV-II LTR sequences); and (lanes A7 and A8) pU3R-I (containing HTLV-I LTR sequences). Cotransfected plasmids used were $pCATLOR_{II}$fs (lanes A1, A3, A5, and A7), $pCATLOR_{II}$ (lanes A2, A4, A6, and A8), $IenvX_I$ (lanes B1 and B3), $envX_I$ (lane B4), and $envX_{II}$ (lanes B2 and B5).

Table 1. CAT activity after cotransfection. Transfections were carried out as described (*7*), with the DEAE-dextran technique for lymphocytes and $CaPO_4$-DNA coprecipitation for adherent cell lines. For lymphocytes, 8 μg of CAT plasmid DNA and 4 μg of cotransfected test plasmid were used for approximately 10^7 cells. For adherent cell lines, 2 μg CAT plasmid DNA and 2 μg test plasmid DNA were transfected onto 10^6 cells. Forty-eight hours after transfection, equivalent amounts of protein (representing roughly one half of the total cell lysate) were analyzed for CAT activity (*7*). Percentage conversion of chloramphenicol to acetylated forms per 1-hour reaction time is shown. The pSV2CAT plasmid replicates in COS-1 cells, whereas the other CAT-containing plasmids do not. For this cell line, shorter reaction times and lower amounts of cell lysate were used to remain within the linear scale of the reaction; values obtained were subsequently adjusted to correspond to those obtained with the other cell lines over a 1-hour reaction. All experiments were performed at least twice with a variation of less than 30 percent in CAT activities observed between experiments. ND, not done.

Cotransfected test plasmid	Acetylation of chloramphenicol (%)					
	pU3R-I	pU3-II	pSV2CAT	pC55	pBLVCAT	pU3R-III
			HUT 78 (human T lymphocytes)			
$pCATLOR_{II}$	79	0.5	1.0	0.5	0.2	1.0
$pCATLOR_{II}$fs	1.2	0.3	0.9	0.5	ND	ND
$pTACLOR_{II}$	1.1	0.3	0.9	0.5	0.2	0.7
$envX_I$	4.5	1.0	1.0	0.5	0.2	0.7
$envX_{II}$	4.9	0.9	1.0	ND	ND	ND
$IenvX_I$	0.8	0.8	1.0	0.5	0.2	0.9
			COS-1 (simian kidney cells)			
$pCATLOR_{II}$	11900	2970	2990			
$pCATLOR_{II}$fs	391	11.4	2980			
$pTACLOR_{II}$	390	14.5	3000			
$envX_I$	900	305	3000			
$envX_{II}$	1402	290	2990			
$IenvX_I$	418	14.9	2990			
			NIH 3T3 (murine fibroblasts)			
$pCATLOR_{II}$	18.5	6.6	2.4			
$pCATLOR_{II}$fs	2.4	ND	ND			
$pTACLOR_{II}$	2.8	2.1	3.5			
$envX_I$	52.5	19.6	3.5			
$envX_{II}$	81.6	31.5	2.9			
$IenvX_I$	2.5	1.8	3.6			
			CCCS+L− (feline kidney cells)			
$pCATLOR_{II}$		19.9	7.2			
$pCATLOR_{II}$fs		1.1	4.9			
$pTACLOR_{II}$		ND	ND			
$envX_I$		4.3	7.0			
$envX_{II}$		34.1	7.4			
$IenvX_I$		1.9	6.6			

product (*10, 12*). This report directly links the HTLV *tat* product to transcriptional *trans*-activation, whereby host cellular genes involved in T-cell proliferation might be modulated in the absence of adjacent *cis*-acting proviral integrations (*7*). The HTLV *tat* product may share functional similarities to the immortalizing proteins of adenovirus and SV40, as well as to the cellular *myc* oncogene product. Like the HTLV *tat* product, these proteins are located in the nucleus (*22*) and can exert regulatory effects on the transcription of viral or cellular genes (*23*). The expression of a biologically active HTLV *tat* protein in eukaryotic cells should allow a direct examination of the ability of this protein to regulate the transcription of host cell genes and to immortalize primary lymphocytes.

JOSEPH SODROSKI
CRAIG ROSEN
WEI CHUN GOH
WILLIAM HASELTINE*

Dana-Farber Cancer Institute,
Department of Pathology,
Harvard Medical School,
Boston, Massachusetts 02115

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* W.A.H. is also associated with the Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115.

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Oxidation of Persistent Environmental Pollutants by a White Rot Fungus

Abstract. *The white rot fungus* Phanerochaete chrysosporium *degraded DDT [1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane], 3,4,3',4'-tetrachlorobiphenyl, 2,4,5,2',-4',5'-hexachlorobiphenyl, 2,3,7,8-tetrachlorodibenzo*-p-*dioxin, lindane (1,2,3,4,5,6-hexachlorocyclohexane), and benzo*[a]*pyrene to carbon dioxide. Model studies, based on the use of DDT, suggest that the ability of* Phanerochaete chrysosporium *to metabolize these compounds is dependent on the extracellular lignin-degrading enzyme system of this fungus.*

Many toxic or carcinogenic organohalides persist in the environment and tend to accumulate in the body fat of animals occupying higher trophic levels (*1*). One reason for the environmental persistence of these compounds is that microorganisms are either unable to degrade them or do so very slowly (*2*).

Lignin is a naturally occurring, highly complex, nonrepeating heteropolymer that provides structural support in woody plants (*3*). Like many synthetic organohalides, lignin is resistant to attack by most microorganisms. Its biodegradation is thought to be the rate-limiting step in the carbon cycle (*3–5*). Microorganisms that are able to metabolize lignin include some species of fungi and a relatively small number of species of bacteria (*5, 6*). Studies of the lignin-degrading system of *Phanerochaete chrysosporium*, a common white rot fungus (*3, 5–8*), have shown that in nitrogen-, carbohydrate-, or sulfur-deficient cultures this fungus secretes a unique H_2O_2-dependent extracellular lignin-degrading enzyme system (*8*). Because of its ability to generate carbon-centered free radicals (*9*), this enzyme system is able to catalyze numerous, nonspecific cleavage reactions on the lignin "lattice." The resultant heterogeneous mixture of low molecular weight aromatic compounds may then undergo further modification or ring cleavage and metabolism to CO_2 by more conventional enzyme systems. Furthermore, chlorinated lignin-derived by-products of the Kraft pulping process, as well as chlorinated lignin, are also readily degraded by *P. chrysosporium* (*10, 11*). The ability of this fungus to degrade lignin and to metabolize halogenated aromatics suggested to us that

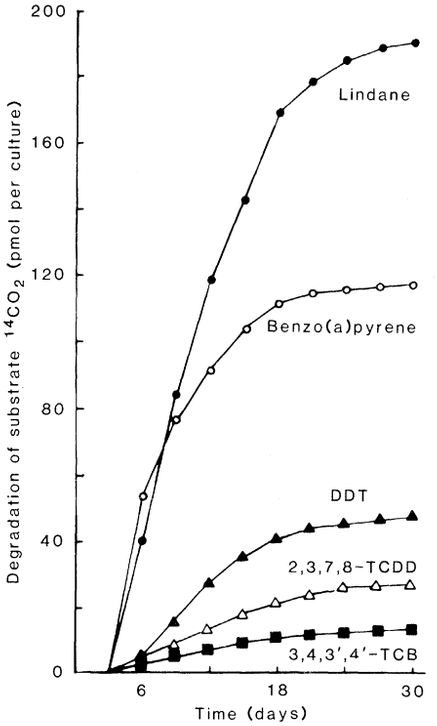

Fig. 1. Rate of oxidation of five environmentally persistent organopollutants to CO_2 by *P. chrysosporium* (*13, 17*).