medial does not [J. E. Krettek and J. L. Price, *J. Comp. Neurol.* **172**, 723 (1977)].
16. It is suggested that in the normal animal lower frequency volleys, perhaps of the order of 100 Hz or less (the reported firing rate of granule cells), arriving asynchronously on a number of perforant path fibers may constitute the composite 400-Hz input produced here by asynchronous 200-Hz volleys. (In the normal animal the asynchrony could be paced by internal circuitry within the entorhinal cortex or could result from the asynchrony inherent in diverse neocortical input to the entorhinal cortex.) The 400-Hz composite volley could then activate the 400-Hz–sensitive mechanism demonstrated here to produce LTP in selected synapses.
17. As reported previously following tetanic stimuli [B. L. McNaughton, in *Neurobiology of the Hippocampus*, W. Seifert, Ed. (Academic Press, London, 1983), p. 233].
18. Supported by NIMH Research Scientist Development Award 5-K02-MH00232, NSF grant BNS 8209875, and a grant from the Harry F. Guggenheim Foundation to J.W.

9 May 1986; accepted 19 September 1986

Expression and Characterization of the *Trans*-Activator of HTLV-III/LAV Virus

CONNIE M. WRIGHT, BARBARA K. FELBER, HARRY PASKALIS, GEORGE N. PAVLAKIS

The human T-lymphotropic retrovirus HTLV-III/LAV encodes a *trans*-activator that increases viral gene expression. We expressed this *trans*-activator in animal cells and studied its structural and functional characteristics. The putative *trans*-activator protein was immunoprecipitated from overproducing stable cell lines and shown to migrate as a 14-kilodalton polypeptide on sodium dodecyl sulfate–polyacrylamide gels. S1 nuclease mapping experiments showed that the *trans*-activator increases the levels of steady-state messenger RNA transcribed from the viral long terminal repeat promoter. Sequences within the R region of the HTLV-III/LAV long terminal repeat are essential for *trans*-activation. Quantitations of messenger RNA and protein showed that the protein increase was greater than the messenger RNA increase in CV1 and HeLa cells, indicating that more than one mechanism was responsible for the *trans*-activation and that cell type–specific factors may determine the final level of *trans*-activation.

THE HUMAN RETROVIRUS HTLV-III/LAV [or human immunodeficiency virus (HIV) (*1*)] is the etiologic agent of acquired immune deficiency syndrome (AIDS) (*2–4*). Elucidation of the molecular structure of the virus (*5*) revealed that it is related to lentiviruses, such as visna virus (*6*). In addition to the three genomic retroviral regions *gag, pol*, and *env*, all the AIDS virus isolates contain additional reading frames that encode proteins of mostly unknown function. One genomic region necessary for the activation of the HTLV-III/LAV long terminal repeat (LTR) has been identified. Complementary DNA (cDNA) clones and deletion mutants of the virus were used in cotransfection experiments to identify the genomic region that encodes the *trans*-activator (*7, 8*). The genomic region was called the *tat*-III gene and was proposed to encode a transcriptional *trans*-activator of the HTLV-III/LAV virus (*8*). Within this genomic region, an open reading frame (ORF) is conserved in all of the viral isolates that have been sequenced to date. Cotransfections of cell lines with plasmids containing this intact ORF for the putative *trans*-activator protein increased the amounts of chloramphenicol acetyltransferase (CAT) produced from the LTR promoter in transient expression assays (*7, 8*). Substantial evidence indicates that the protein product of this conserved reading frame is the *trans*-activator of HTLV-III/LAV. The cDNA clones and deletion mutants that destroy the reading frame are negative, whereas all the constructs that contain the conserved ORF are positive in *trans*-activation assays (*7, 8*). We have identified the protein product of the *tat*-III ORF. Since the mode of action of this protein is not yet clear, we refer to this putative *trans*-activator as TA-III. Here we demonstrate that the *trans*-activator increases the steady-state level of messenger RNA (mRNA) transcribed from the viral LTR promoter as well as the level of the produced protein. Quantitative comparisons indicate that the level of protein increases more than can be accounted for by the mRNA levels.

Three types of vectors were used for the expression of the *trans*-activator of HTLV-III/LAV in animal cells (Fig. 1). A bovine papilloma virus (BPV) vector was constructed containing a Sal I–Xho I fragment from either of two HTLV-III/LAV proviral clones [pHXB2C or pBH10R (*9, 10*)] next to the mouse metallothionein-I (mMT-I) promoter. This construct (pB2MX3) was introduced into mouse C127 cells by the calcium coprecipitation technique (*11*). Two days later the cells were placed in medium containing 10 μM $CdCl_2$ to select for cells resistant to cadmium because of overproduction of human metallothionein (hMT) encoded by the vector. Cadmium-resistant colonies were cloned and maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum and 20 μM $CdCl_2$. Independent cadmium-resistant cell lines were assayed for the presence of the HTLV-III/LAV *trans*-activator. A convenient functional assay (Fig. 2A) was used to screen 22 cell lines for the presence of functional *trans*-activator expressed from the mMT-I promoter. Cells were transfected with plasmid pL3CAT (Fig. 1B), which contains the LTR promoter of HTLV-III/LAV that transcribes the bacterial CAT gene (*12*). CAT assays on 2 of the 22 cell lines (CB2MX3-23 and CB2MX3-24) are shown in Fig. 2A. All 22 cell lines selected on the basis of cadmium resistance stimulated the production of CAT at levels 40 to 300 times those of cell lines that contained only the BPV vector. Clones CB2MX3-2, CB2MX3-23, and CB2MX3-24 were selected for further analysis. The induction of CAT production in these cell lines compared with control CBMG7-4 cells were 300-fold, 100-fold, and 300-fold, respectively.

To identify the expressed protein, we labeled CB2MX3-2 cells with [^{35}S]cysteine for 1 hour, lysed them in RIPA buffer (Fig. 3), and the cellular extracts were immunoprecipitated with sera from AIDS patients or a rabbit antiserum to the *tat*-III ORF expressed in bacteria (*13*) (Fig. 3). The putative *trans*-activator protein was immunoprecipitated as a 14-kilodalton (kD) protein by the rabbit antiserum. The discrepancy between the predicted molecular weight of 10 kD (*7*) and the observed migration on SDS gels could be caused either by post-translational modifications or by anomalous migration due to the basic nature of this protein. Sera from three AIDS patients used in the same assay were not able to immunoprecipitate the 14-kD protein under the same conditions. However, other studies have shown that sera from some AIDS patients recognize this 14-kD protein (*13, 14*). A higher molecular weight band of 26 kD was also present in some immunoprecipitations.

The BPV construct as well as expression vectors based on simian virus 40 (SV40) (Fig. 1) were also used for the transient

C. M. Wright, Bionetics Research, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701, and Department of Biology, University of Maryland, Baltimore County, Catonsville, MD 21228. B. K. Felber, H. Paskalis, G. N. Pavlakis, Bionetics Research, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701.

expression of the *trans*-activator protein after transfection of mouse NIH 3T3 and C127 and monkey CV1 cell lines (Fig. 2). In these cell lines, we observed a 20- to 50-fold increase in levels of CAT in the presence of *trans*-activator–producing plasmids. Since the Sal I–Xho I fragment of HTLV-III/LAV (Fig. 1) has the potential to express other ORF's (such as *env* products) in addition to the *tat*-III ORF, we constructed plasmids that contained smaller viral fragments (Fig. 1). Plasmid pMX3Bneo contains a Sal I–Bam HI fragment, and pMX3Nneo contains a Sal I–Nde I fragment. Alternatively, a retroviral vector, pGV1 (*15*), was used to express the *trans*-activator (Fig. 1). All of these vectors encoded functional *trans*-activators as determined by the activation of pL3CAT after cotransfection in CV1 cells. Since all the HTLV-III/LAV fragments in the various vectors gave similar results in the *trans*-activation assay, we conclude that the Sal I–Nde I fragment, which contains only the first coding exon of the *trans*-activator protein, is able to produce fully functional *trans*-activator. There are no other known viral proteins encoded in the Sal I–Nde I fragment of the AIDS virus except for the first coding exon of the putative antirepressor of translation (*art*) gene product, which has been mapped by deletion mutagenesis (*16*). Therefore, these results suggest that the *trans*-activator can activate the LTR in the absence of a functional *art* product and possibly in the absence of any other viral protein.

Table 1. Induction of expression in LTR constructs.

LTR construct	Activator*		Induction	
	–	+	CAT	mRNA
pL3CAT	1†	38	38× (11)‡	11× (5)
pL31CAT (Δ3′ + 38)	2	3	1× (6)	2× (1)
pL32CAT (Δ3′ + 25)	3	4	1× (8)	
pΔ5′−91L3CAT	0.6	28	46× (5)	10× (2)
pΔ5′−33L3CAT	0.4	0.4	1× (4)	1× (2)
Stable cell line HL3T1‖			680× (4)	19× (2)

*Activator was expressed in the cells by cotransfections of different producing plasmids (Fig. 1A). No difference was detected among these various constructs. †For comparisons, the level of expression of pL3CAT in the absence of activator was assigned the value of 1. The results represent the average of 4 to 11 transfections in CV1 cells with different DNA preparations. CAT assays were quantitated by liquid scintillation counting. The mRNA levels represent the average of one to five experiments. Two methods were used to quantitate the S1 nuclease mapping results: densitometry scanning and comparisons of different amounts of RNA under conditions in which the S1 nuclease mapping assay gave linear results. ‡Numbers in parentheses represent the numbers of experiments used for the calculation of relative expression. ‖The stable HeLa cell line HL3T1 contains integrated copies of pL3CAT. The cells were transfected with either a *trans*-activator–producing plasmid or a control plasmid, and 24 hours later the cells were analyzed by S1 mapping and CAT assays.

To further characterize the interaction between the LTR and the *trans*-activator of HTLV-III/LAV, we constructed deletion mutants of the HTLV-III/LAV LTR. Some of the mutants characterized by sequencing are shown in Fig. 1B. A 5′ deletion to nucleotide −91 (+1 is the transcriptional start site) is activated to a similar level as pL3CAT in the presence of the *trans*-activator (Fig. 2 and Table 1). Deletion to nucleotide −33 eliminated inducibility and severely affected the basal level of the expression.

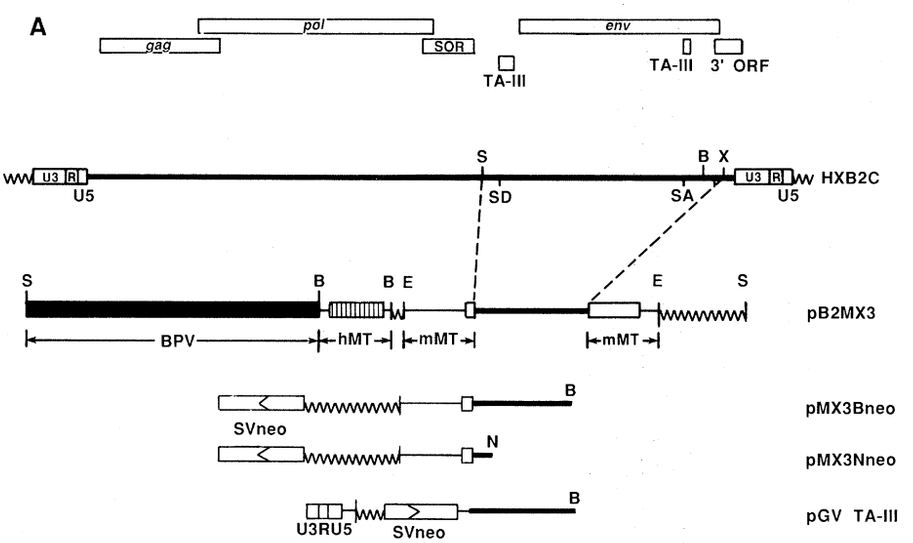
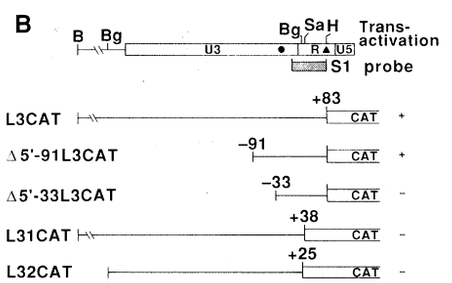


Fig. 1. (A) Construction of plasmids for the expression of HTLV-III/LAV *trans*-activator. The ORF's encoding known proteins of the AIDS virus, including the two coding exons of the *trans*-activator protein, are indicated at the top (*7*). The plasmid pHXB2C is a complete HTLV-III/LAV proviral clone that produces infectious virions when transfected into normal human T cells (*9*). A Sal I–Xho I fragment (nucleotides 5365 to 8473) inserted into the BPV vector pBMT2X under the control of the mMT-I promoter resulted in plasmid pB2MX3. Vector pBMT2X contains the hMT-IA gene and the mMT-I gene with a unique Xho I site within the 5′ untranslated region of the mMT-I gene. After transfections, cells that replicate the BPV vector are transformed and resistant to toxic concentrations (20 to 80 μ*M*) of cadmium. Plasmids pMX3Bneo and pMX3Nneo contain a Sal I–Bam HI fragment (nucleotides 5365 to 8051) and a Sal I–Nde I fragment (nucleotides 5365 to 5979), respectively, next to the mMT-I promoter. In addition, these plasmids contain a fragment of plasmid SV2neo, which consists of the SV40 early promoter-enhancer region and the bacterial gene encoding neomycin (*neo*) resistance (*24*). Plasmid pGV TA-III, a derivative of pGV1 (*15*), contains the murine sarcoma virus (MSV–HT-I) LTR and viral packaging signal, the pBR322 origin of replication, the SV40 origin of replication and early promoter, *neo*, and a Sal I–Bam HI fragment from HTLV-III/LAV (nucleotides 5365 to 8051). The wavy lines represent pBR322 sequences. All these constructs directed the synthesis of functional *trans*-activator as determined by CAT assays upon transfection into animal cells. (B) Deletion mutants of HTLV-III/LAV LTR. The HTLV-III/LAV LTR is represented by a box. A dot represents the TATA box, and a triangle the polyadenylation site. CAT construct pL3CAT contains a Bam HI–Hind III fragment of HTLV-III/LAV (nucleotides −1068 to +83) ligated to the CAT gene. pΔ5′-91 L3CAT contains the region −91 to +83. pΔ5′-33 L3CAT contains the region −33 to +83. pL31CAT contains the region −1068 to +38. pL32CAT contains the region −492 to +25. At the right side of the figure, + and − indicate the ability of the LTR constructs to be activated in the presence of the *trans*-activator as assessed by CAT assays and S1 nuclease mapping experiments. A hatched box represents the uniformly labeled probe used in the S1 nuclease experiments (nucleotides −18 to +83). Abbreviations: SOR, short open reading frame; TA-III, *trans*-activator coding sequences; 3′ ORF, 3′ open reading frame; SD, splice donor; SA, splice acceptor; hMT, human metallothionein IA gene; mMT, mouse metallothionein I gene; SVneo, SV40neo transcription unit; B, Bam HI; Bg, Bgl II; E, Eco RI; H, Hind III; N, Nde I; S, Sal I; Sa, Sac I; X, Xho I.



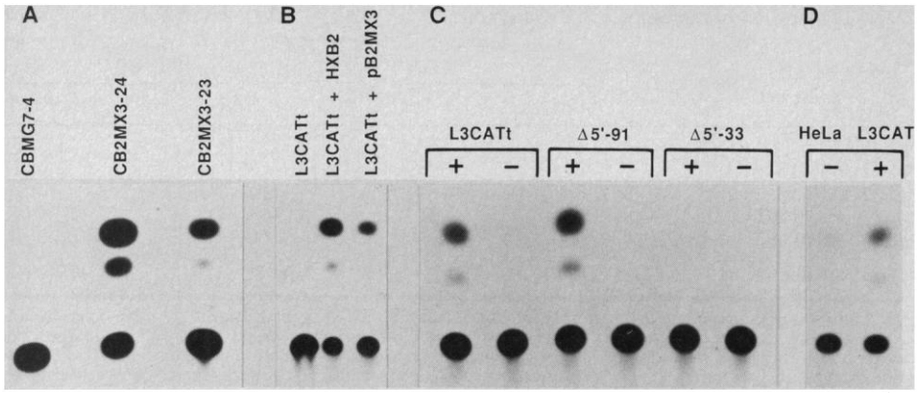


Fig. 2. CAT assays after transfections of the LTR-CAT constructs in animal cells. CAT-producing plasmid (10 μg) was mixed with 10 μg of *trans*-activator–producing plasmid when appropriate. In all cases, the total amount of transfected DNA was brought to 35 μg/ml with plasmid DNA or salmon sperm DNA. (A) Transfections of pL3CAT into stable cell lines. Two mouse lines producing *trans*-activator, CB2MX3-24 and CB2MX3-23, and one control cell line CBMG7-4 (*25*) that contains a BPV vector without any HTLV-III/LAV sequences were transfected with 10 μg of pL3CAT per milliliter. (B) CAT assays after cotransfections of 10 μg of pL3CAT per milliliter with 10 μg of *trans*-activator–producing plasmids pHXB2C and pB2MX3 per milliliter. (C) Analysis of 5′ deletion mutants (10 μg/ml) in the presence (+) or absence (−) of 10 μg of *trans*-activator–producing plasmid per milliliter. (D) Transfection of one pool of colonies of HeLa cells that contain integrated copies of pL3CAT after selection with G418. −, Cells transfected with salmon sperm DNA; +, cells transfected with 10 μg of the *trans*-activator–producing plasmid pMX3Nneo per milliliter. Two similar pL3CAT constructs, pL3CATt and pL3CATc, used throughout these experiments gave similar results.

The 3′ deletions of R region sequences to nucleotide +38 or to nucleotide +25 eliminated activation in the presence of the *trans*-activator protein (Table 1). The smallest fragment of the LTR identified by deletion mutagenesis to be activated by the *trans*-activator was between nucleotide −91 and +83. We conclude that the HTLV-III/LAV *trans*-activator requires sequences within the R region for appropriate function. Similar results have been obtained after transfections of a series of deletion mutants in H9 cells infected with HTLV-III/LAV (*17*).

To examine the effect of the *trans*-activator on the mRNA transcribed from the LTR of HTLV-III/LAV, LTR-CAT constructs were transfected into CV1 cells in the presence or absence of *trans*-activator–producing plasmids. One or two days later, total RNA was isolated and analyzed by S1 nuclease mapping (*18*) through the use of a probe spanning the transcriptional initiation site of the AIDS virus mRNA (Fig. 1B). In the presence of the *trans*-activator protein, CV1 cells contained approximately ten times as much mRNA initiated at the correct cap site of the virus as did cells without *trans*-activator (Fig. 4 and Table 1). Comparison of the levels obtained for mRNA and CAT in parallel experiments (Table 1) indicate that the increase in mRNA can account only partially for the increased levels of CAT.

To verify that the results of the S1 mapping experiments were not influenced by the CAT gene sequences ligated to the LTR promoter, two additional constructs of the LTR promoter were transfected in CV1 cells in the presence or absence of the *trans*-activator, and the extracted mRNA was quantitated by S1 mapping. The first construct, pL3TPA, contained sequences of the human tissue plasminogen activator gene. The second construct, pCCMV, is a deletion mutant of the HXB2C provirus clone that is missing nucleotides 2198 to 8473. These constructs gave results similar to those obtained with the pL3CAT plasmid, demonstrating that mRNA levels are not influenced by the nature of sequences ligated to the viral LTR. In addition, the results with pCCMV show that the presence of the complete R and U5 region of the viral LTR and of the *gag* region do not affect the activation.

To verify that the amount of mRNA measured by S1 mapping reflects the levels of intact polyadenylated mRNA, we electrophoresed total or oligodT-selected RNA from transfected CV1 cells on 1% agarose gels under denaturing conditions. The RNA was transferred onto nitrocellulose and hybridized to a nick-translated CAT probe (Fig. 5). A band of approximately 1.6 kilobases (kb) hybridized specifically to the probe as expected for the correctly spliced and polyadenylated CAT mRNA. This band was more prominent after oligodT enrichment, indicating that it represented polyadenylated RNA. Furthermore, this RNA band was greatly increased in the presence of the HTLV-III/LAV *trans*-activator. These experiments show that the full-length, polyadenylated CAT mRNA is increased in the presence of the *trans*-activator.

To determine whether the HTLV-III/LAV LTR behaves similarly when integrated into the cellular DNA, we constructed HeLa cell lines containing integrated copies of the pL3CAT plasmid. For this, HeLa cells were cotransfected with plasmids pL3CAT and pSV2neo, G418-resistant colonies were obtained, and pools of approximately 200 colonies were transfected either with salmon sperm DNA as a control or with a *trans*-activator–producing plasmid. Pooled colonies transfected with the *trans*-activator–producing plasmid expressed 11 to 48 times as much CAT as pools transfected with control DNA (Fig. 2D). Thus, the HTLV-III/LAV LTR-CAT constructs behaved similarly in both the episomal and integrated states. Individual colonies of G418-resistant HeLa cells were cloned and examined for the presence and inducibility of the pL3CAT construct. Results obtained with a representative cell line, HL3T1, are shown in Table 1. The mRNA measurements after S1 mapping and parallel mea-

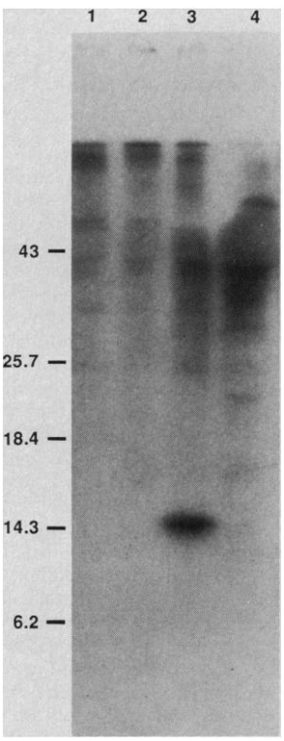


Fig. 3. Immunoprecipitation of the *trans*-activator of HTLV-III/LAV from an overproducing cell line. Cell line CB2MX3-2 was labeled for 1 hour with 200 μCi of [^{35}S]cysteine per milliliter of cysteine-free Dulbecco's modified Eagle's medium. Cells were lysed in RIPA buffer [50 m*M* tris-HCl (*p*H 7.4), 150 m*M* NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS], and the cell lysates were immunoprecipitated with either normal human (lane 1), normal rabbit (lane 2), rabbit immune (lane 3), or human AIDS patient RJ-7 (lane 4) serum. The immunoprecipitated proteins were subjected to electrophoresis on 10% SDS–polyacrylamide gel, and the gel was soaked in a 1*M* solution of sodium salicylate (*26*), dried, and subjected to fluorography at −70°C.

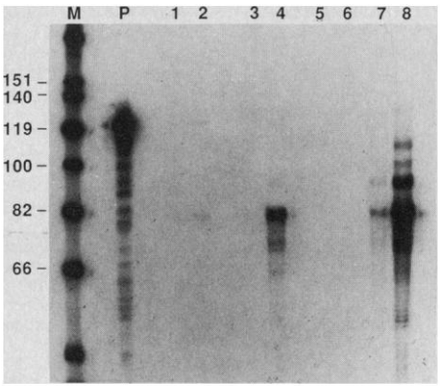


Fig. 4. S1 nuclease protection analysis. Monkey CV1 cells were transfected with plasmid DNA. The total amount of DNA was kept constant at 35 μg/ml in all transfections. After 24 hours, total RNA was isolated (*27*). In lanes 1 to 8, different amounts of RNA were hybridized with a uniformly labeled single-stranded DNA probe spanning the transcriptional start site of the HTLV-III/LAV (nucleotides −18 to +83) (Fig. 1B). After treatment with S1 nuclease (*18*), the protected fragments were resolved on a 6% acrylamide–8*M* urea gel. Lanes are M, molecular weight markers (ϕX 174 DNA digested with Hinf I); the size of the markers in nucleotides is indicated to the left; P, undigested, uniformly labeled probe. 1, 3 μg of RNA from cells transfected with pL3CAT (10 μg/ml); 2, 30 μg of RNA from cells as in lane 1; 3, 3 μg of RNA from cells cotransfected with pL3CAT (10 μg/ml) and pB2MX3 (10 μg/ml), a plasmid producing functional *trans*-activator; 4, 30 μg of RNA from cells as in lane 3; 5, 3 μg of RNA isolated from H9 cells (*4*); 6, 30 μg of RNA from cells as in lane 5; 7, 3 μg of RNA from H9 cells infected with HTLV-III/LAV virus; and 8, 30 μg of RNA from cells as in lane 7.

surements of CAT showed that these cell lines were inducible by a factor of 10 to 20 at the level of mRNA and a factor of 300 to 800 at the level of protein (Table 1). Therefore, in these HeLa clones the levels of induction of mRNA and of protein are more discrepant than they are in CV1 cells. More than one mechanism must be involved in the *trans*-activation of HTLV-III/LAV virus, and cell type–specific factors may determine the final level of *trans*-activation.

To understand the regulatory mechanism of the AIDS virus, it will be necessary to characterize in detail the mechanism of both basal and induced expression in different cell types. The LTR promoter of the AIDS virus seems to function in a number of cell lines in the absence of *trans*-activator, as measured by CAT assays. Substantial amounts of correctly initiated mRNA are detected by S1 nuclease mapping after transfections in CV1 and HeLa cells. This result contrasts with those achieved with the LTR promoter of HTLV-I virus, where, in the absence of the specific transcriptional activator TA-I, no correctly initiated mRNA could be detected by S1 nuclease mapping (*19*). One element that may contribute to the basal level of expression in vivo is the three Sp1 binding sites between positions −46 and −77. These sites are important for the basal level expression of the promoter in vitro (*20*). In the presence of the AIDS virus *trans*-activator, a 10- to 20-fold increase at the level of correctly initiated mRNA can be detected by S1 nuclease mapping in CV1 and HeLa cells. This increase is not detected with mutants pΔ5′−33L3CAT, pL31CAT, and pL32CAT. These characteristics may be the result of transcriptional activation of the LTR promoter, stabilization of the mRNA, or both. Direct measurements of RNA polymerase density by nuclear runoff experiments and of mRNA half-life are necessary to distinguish between these possibilities. Reports regarding the level at which regulation of *trans*-activation is exerted are in conflict (*17, 21*). Rosen *et al.* (*21*) reported similar amounts of mRNA in the presence and absence of *trans*-activator, which differs from our results (Fig. 4). Increased mRNA levels in the presence of the *trans*-activator have been observed by others (*22*).

We have found that sequences located both upstream and downstream of the TATA box are important for the regulated expression of the AIDS virus promoter. The organization of the AIDS virus promoter resembles that of the heat-shock promoter and of the adenovirus major late promoter. For these promoters, sequences downstream of the cap site are involved in activation (*23*). A sequence necessary for *trans*-activation exists within the R region of the HTLV-III/LAV virus (nucleotides +38 to +83). This region is necessary for induction at both the mRNA and protein levels. Since this region is transcribed, the transactivator could act post-transcriptionally to increase the stability or translatability (or both) of the mRNA. In CV1 cells, we observed a 10- to 20-fold increase of specific mRNA and a 20- to 50-fold increase in CAT. This small but reproducible discrepancy between induction levels may reflect a difference in the translatability of mRNA in the presence of *trans*-activator. In the HeLaL3CAT clones (HL3T1) the discrepancy between the induction of mRNA (a 10- to 20-fold increase) and of protein (a 300- to 800-fold increase) is even greater. The data suggest that more than one mechanism determines the level of expression from the LTR promoter and that cell type specificity contributes to the overall effect of these different mechanisms. Therefore, in HeLa cells, in addition to the mRNA increase, a great increase in mRNA translatability results in high levels of activation at the protein level. In CV1 cells the major mechanism of *trans*-activation is an increase in mRNA. The simplest explanation of these results is that the *trans*-activator binds to the R region of the mRNA and increases mRNA's stability and translational efficiency. Alternatively, the *trans*-activator may increase both the transcription from the LTR promoter and the translational efficiency of the mRNA. The first model predicts that all the mRNA molecules that initiate at the R region of HTLV-III/LAV virus should be equally inducible by the *trans*-activator, irrespective of the promoter. That this is not the case (*17*) suggests alternative models for *trans*-activation. Further experiments are needed to test the possibility that in other cell types, such as human lymphoid cell lines, the contribu-

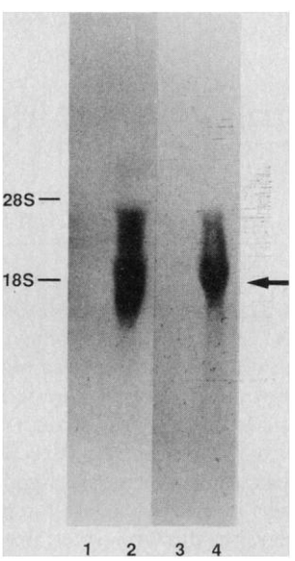


Fig. 5. Northern blots of total (lanes 1 and 2) and oligodT-selected RNA (lanes 3 and 4) from CV1 cells transfected with pL3CAT in the presence or absence of *trans*-activator. Total mRNA was isolated (*27*) and run on a 1% agarose–formaldehyde gel (*28*). The RNA was blotted on nitrocellulose, and the filter was incubated in 5× standard saline citrate, 50% formamide, 1× Denhardt's solution, 1% glycine, 50 m*M* sodium phosphate at *p*H 6.6, 250 μg of transfer RNA per milliliter, and 150 μg of salmon sperm DNA per milliliter. The filter was subsequently hybridized at 42°C in the above buffer containing 2% glycine, 10% dextran sulfate, and the nick-translated L3CAT DNA probe. Lanes 1 and 2 were exposed for 18 hours, and lanes 3 and 4 were exposed for 1 hour. The positions of 18S and 28S ribosomal RNA molecules are indicated. (Lane 1) 20 μg of total RNA from cells transfected with pL3CAT; (lane 2) 20 μg of total RNA from cells transfected with pL3CAT and the *trans*-activator–producing plasmid pB2MX3; (lane 3) 5 μg of RNA from cells transfected with pL3CAT selected by binding to an oligodT column; and (lane 4) 5 μg of RNA as in lane 3 from cells transfected with pL3CAT and pB2MX3.

tion of mechanisms increasing message translatability is higher (*21*). The difference between CV1 and HeLa cells suggests that an additional intracellular control mechanism for HTLV-III/LAV replication could be the extent of *trans*-activation in different cellular environments.

REFERENCES AND NOTES

1. J. M. Coffin *et al.*, *Science* **232**, 697 (1986).
2. F. Barré-Sinoussi *et al.*, *ibid.* **220**, 868 (1983).
3. S. M. Broder and R. C. Gallo, *N. Engl. J. Med.* **311**, 1292 (1984).
4. M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *Science*, **224**, 497 (1984); R. C. Gallo *et al.*, *ibid.*, p. 500, M. G. Sarngadharan *et al.*, *ibid.*, p. 506.
5. L. Ratner *et al.*, *Nature (London)* **313**, 277 (1985); S. Wain-Hobson *et al.*, *Cell* **40**, 9 (1985); R. Sanchez-Pescador *et al.*, *Science* **227**, 484 (1985); M. A. Muesing *et al.*, *Nature (London)* **313**, 450 (1985).
6. M. A. Gonda *et al.*, *Science* **227**, 173 (1985); P. Sonigo *et al.*, *Cell* **42**, 369 (1985).
7. S. K. Arya *et al.*, *Science* **229**, 69 (1985); L. Siegel *et al.*, *Virology* **148**, 228 (1986).
8. J. Sodroski *et al.*, *Science* **227**, 171 (1985); J. Sodroski, R. Patarca, C. Rosen, F. Wong-Staal, W. Haseltine, *ibid.* **229**, 74 (1985).
9. A. G. Fisher, E. Collalti, L. Ratner, R. C. Gallo, F. Wong-Staal, *Nature (London)* **316**, 262 (1985).
10. B. H. Hahn *et al.*, *ibid.* **312**, 166 (1984).
11. F. J. Graham and A. J. Van der Eb, *Virology* **52**, 456 (1973).
12. C. M. Gorman, S. L. Moffat, B. H. Howard, *Mol. Cell. Biol.* **2**, 1044 (1982); C. M. Gorman, G. T. Merlino, M. C. Willingham, I. Pastan, B. H. Howard, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6777 (1982).
13. A. Aldovini *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6672 (1986).
14. W. C. Goh, C. Rosen, J. Sodroski, D. D. Ho, W. A. Haseltine, *J. Virol.* **59**, 181 (1986).
15. T. S. Robins *et al.*, in preparation.
16. J. Sodroski *et al.*, *Nature (London)* **321**, 412 (1986).
17. C. A. Rosen, J. G. Sodroski, W. A. Haseltine, *Cell* **41**, 813 (1985).
18. A. J. Berk and P. A. Sharp, *ibid.* **12**, 721 (1977); R. F. Weaver and C. Weissman, *Nucleic Acids Res.* **7**, 1175 (1979).
19. B. K. Felber, H. Paskalis, C. Kleinman-Ewing, F. Wong-Staal, G. N. Pavlakis, *Science* **229**, 675 (1985).
20. K. A. Jones, J. T. Kadonaga, P. A. Luciw, R. Tjian, *ibid.* **232**, 755 (1986).
21. C. A. Rosen *et al.*, *Nature (London)* **319**, 555 (1986).
22. B. Cullen, *Cell* **46**, 973 (1986).
23. D. Hultmark, R. Klemenz, W. J. Gehring, *ibid.* **44**, 429 (1986); E. D. Lewis and J. L. Manley, *Mol. Cell. Biol.* **5**, 2433 (1985); S. L. Mansour, T. Grodzicker, R. Tjian, *ibid.* **6**, 2684 (1986).
24. P. J. Southern and P. Berg, *J. Mol. Appl. Genet.* **1**, 327 (1982).
25. G. N. Pavlakis and D. H. Hamer, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 397 (1983).
26. J. P. Chamberlain, *Anal. Biochem.* **98**, 132 (1979).
27. V. Glisin, R. Crkvenjakov, C. Byus, *Biochemistry* **13**, 2633 (1974).
28. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning—A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).

29. B.K.F. was supported by a Swiss National Science Foundation grant. H.P. is a fellow of the Leukemia Society of America. We thank A. Aldovini and C. Debouk for the rabbit antibody to *trans*-activator and F. Wong-Staal and R. Gallo for the HTLV-III/LAV clones and for sharing unpublished data. We also thank G. Kaplin and T. Tse for expert technical assistance, H. Marusiodis for typing and editorial assistance, and K. Bivens for editorial assistance. Supported by the National Cancer Institute, under contract NO1-CO-23909 with Bionetics Research, Inc.

3 July 1986; accepted 17 September 1986

Distribution of Airborne Radon-222 Concentrations in U.S. Homes

A. V. NERO, M. B. SCHWEHR,* W. W. NAZAROFF,† K. L. REVZAN

Apparently large exposures of the general public to the radioactive decay products of radon-222 present in indoor air have led to systematical appraisal of monitoring data from U.S. single-family homes; several ways of aggregating data were used that take into account differences in sample selection and season of measurements. The resulting distribution of annual-average radon-222 concentrations can be characterized by an arithmetic mean of 1.5 picocurie per liter (55 becquerels per cubic meter) and a long tail with 1 to 3% of homes exceeding 8 picocuries per liter, or by a geometric mean of 0.9 picocurie per liter and a geometric standard deviation of about 2.8. The standard deviation in the means is 15%, estimated from the number and variability of the available data sets, but the total uncertainty is larger because these data may not be representative. Available dose-response data suggest that an average of 1.5 picocuries per liter contributes about 0.3% lifetime risk of lung cancer and that, in the million homes with the highest concentrations, where annual exposures approximate or exceed those received by underground uranium miners, long-term occupants suffer an added lifetime risk of at least 2%, reaching extraordinary values at the highest concentrations observed.

RADON-222 AND ITS DECAY PRODUCTS are universally present in the air we breathe, with typical levels indoors estimated—on the basis of epidemiology among uranium miners—to cause thousands of cases of lung cancer annually in the United States. A special concern has been the frequent appearance of homes with concentrations that imply individual lifetime risks of lung cancer exceeding 1%, and the occasional occurrence of levels with estimated risks an order of magnitude or more higher. These risks, large compared with ordinarily considered environmental risks, have led to diverse studies characterizing indoor concentrations, the factors affecting them, and health implications (*1, 2*).

However, there has not been a quantitative characterization of the distribution of radon concentrations in U.S. residences. Monitoring has been limited to modest local efforts varying markedly in scientific objectives, selection of homes, and measurement techniques, providing no direct estimate either of average exposures or of the number of homes above proposed action levels, such as the criterion recently recommended by the U.S. National Council on Radiation Protection and Measurements (NCRP): 2 working-level months (WLM) per year (*3, 4*). Nonetheless, taken together, data from U.S. studies are substantial. We present results of a systematic appraisal of these data, designed to aggregate them in a consistent way, explicitly accounting for their differences, and thereby to estimate the frequency distribution of concentrations in U.S. homes. We demonstrate the utility of lognormal parameters for representing this distribution and extract quantitative values for average concentrations and for the incidence of high levels; both lognormal and nonparametric analyses give similar results.

From the literature and from direct communication with researchers, we have accumulated data from 38 U.S. areas, typically urban centers or states (Table 1). Approximately 99% of these data were taken in single-family houses, typically selected by asking for volunteers (for example, from among employees of a given institution) or by choosing from participants in energy conservation programs. Thus, while few, if any, of the homes monitored were selected by statistically based sampling procedures, virtually all selection processes contained a strong random element. The studies varied substantially in size: eight have 50 or more homes and an equal number have fewer than 10. Each data set still gives a useful indication of concentrations in the corresponding area and, taken together with the other data sets, of concentrations in U.S. single-family houses.

Indoor Environment Program, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

*Present address: California Air Resources Board, Sacramento, CA 95816

†Present address: Department of Engineering Sciences, California Institute of Technology, Pasadena, CA 91125.