stunted in animals treated with TGF-α at doses higher than 0.3 μg/g per day. Again, the inhibition of growth rate correlated well with the dose administered. At 4 μg/g per day, the growth of animals treated with either rat TGF-α or mEGF was 25 percent slower than that of the control animals.

Although the biochemical events leading to these effects of TGF-α on the newborn mouse remain unresolved, the results clearly support those obtained from studies (*1–3*) in vitro and provide direct evidence in vivo that TGF-α is a member of the EGF family. Preliminary data from other physiological studies of TGF-α on whole animals or tissues also support the conclusion that TGF-α is physiologically similar to EGF. For example, TGF-α stimulates ornithine decarboxylase activity and protein synthesis (*13*) but significantly inhibits histamine-induced gastric acid secretion (*14*). These and other results suggest that TGF-α, although originally found in malignancy, might be physiologically as important as its normal counterpart, EGF. One possibility is that EGF is required to maintain the normal state of the animal, whereas TGF-α, which is found in states of rapid growth such as pregnancy and malignancy, perhaps serves as the additional growth factor required to meet the needs for such rapid growth.

The present results also provide greater insight to the structural relation between TGF-α and EGF. The similar behavior of TGF-α and EGF is remarkable in that only 16 of the 50 amino acids of TGF-α have sequence homology to mouse EGF. Ten of the 16 amino acid residues are related to conformational and structural requirements for the proper refolding of the molecule—such as disulfide formation and β-bends (six cysteines, three glycines, and one proline)—and only six amino acid residues may have functional roles. It is likely that the common secondary structure of TGF-α and EGF has an important role in determining the biological properties of these growth factors. The use of synthetic TGF-α analogs will be useful to test this hypothesis.

References and Notes

1. H. Marquardt, M. W. Hunkapiller, L. E. Hood, G. J. Todaro, *Science* **223**, 1079 (1984).
2. J. Massague, *J. Biol. Chem.* **258**, 13606 (1983).
3. J. E. Delarco and G. J. Todaro, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4001 (1978).
4. C. R. Savage, T. Inagami, S. Cohn, *J. Biol. Chem.* **247**, 7612 (1972).
5. H. Gregory, *Nature (London)* **257**, 325 (1975).
6. J. Scott *et al.*, *Science* **221**, 236 (1983).
7. A. Gray, T. J. Dull, A. Ullrich, *Nature (London)* **303**, 722 (1983).
8. R. Derynck, A. B. Roberts, M. E. Winkler, E. Y. Chen, D. V. Goeddel, *Cell* **38**, 287 (1984).
9. J. P. Tam *et al.*, *Nature (London)* **309**, 376 (1983).
10. R. B. Merrifield, *J. Am. Chem. Soc.* **85**, 2149 (1963).
11. S. Cohen, *J. Biol. Chem.* **237**, 1555 (1962).
12. G. P. M. Moore, B. A. Panaretto, D. Robertson, *J. Endocrinol.* **88**, 293 (1981).
13. A. M. Nakhla and J. P. Tam, paper presented at 69th annual meeting of the Federation of American Societies for Experimental Biology, Anaheim, California, April 1985.
14. R. A. Murphy *et al.*, paper presented at the 69th annual meeting of the Federation of American Societies for Experimental Biology, Anaheim, California, April 1985.

15. This investigation was supported by PHS 36544 awarded by the National Cancer Institute, Department of Health and Human Services. I thank L. Tsai for her technical assistance during the course of this work.

25 January 1985; accepted 26 June 1985

The pX Protein of HTLV-I Is a Transcriptional Activator of Its Long Terminal Repeats

Abstract. *Expression of the pX protein of human T-cell leukemia virus type I (HTLV-I) in animal cells demonstrates that this protein is a specific transcriptional activator of the long terminal repeats (LTR) of HTLV-I. Several other promoters are not affected by pX. No lymphocyte-specific factors are required for this activation. pX can be detected in the nucleus of transfected monkey kidney cells (line CV1) by indirect immunofluorescence. These results indicate that the pX protein is essential for the replication cycle of the virus and that it may be directly involved in the immortalization of human lymphocytes by HTLV-I.*

Barbara K. Felber
Harry Paskalis
Carol Kleinman-Ewing
LBI-Basic Research Program, NCI–Frederick Cancer Research Facility, Frederick, Maryland 21701
Flossie Wong-Staal
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205
George N. Pavlakis
LBI-Basic Research Program, NCI–Frederick Cancer Research Facility

The exogenous retrovirus human T-cell leukemia virus type I (HTLV-I) appears to be the etiologic agent of adult T-cell leukemia-lymphoma (*1, 2*). HTLV-I is a member of a family of human retroviruses that display some structural and functional similarities, including the tropism for a restricted cell type, the OKT4 helper T lymphocyte. HTLV-I resembles a chronic leukemia virus in that it is replication competent, causes a monoclonal malignancy after a long latency period, and does not contain any recognizable cell-derived oncogene. However, HTLV-I can efficiently immortalize normal human lymphocytes in vitro, a property previously associated with acute transforming viruses containing oncogenes (*3*). In spite of the monoclonality of HTLV-I associated malignancies, no specific chromosomal sites have been detected for the integration of the provirus (*4*). There is evidence that the HTLV viruses, HTLV-I, HTLV-II, and HTLV-III, together with bovine leukemia virus (BLV), may constitute a group of retroviruses that can be characterized by the way in which they interact with the infected cell. There is a great increase in the level of steady-state messenger RNA (mRNA) directed from the promoter in the long terminal repeats (LTR's) in cell lines infected with HTLV-I compared to uninfected parent cells (*5–7*). It has been postulated that this is a transcriptional activation caused directly or indirectly by a viral product (*5, 8*). In addition to the typical retroviral genes *gag*, *pol*, and *env*, the HTLV-I genome contains four overlapping open reading frames at the 3′ end of the genome (*9*). One of these, the extended X_{IV} (*9*) or LOR (*8*) reading frame (here referred to as the X-LOR) is highly conserved between HTLV-I and HTLV-II (*10*). A splice acceptor at the beginning of this reading frame is also conserved among HTLV-I, HTLV-II (*11*), and probably BLV (*12*). Furthermore, a protein of the expected molecular weight was identified in cells infected with HTLV-I or HTLV-II by immunoprecipitation and partial radiosequencing (*13*). It was proposed that this viral product (here referred to as pX) may be responsible for the observed *trans*-activation. Alternatively, virus-permissive cell lines may constitutively produce specific factors that, upon infection with virus, allow the increased transcription from the LTR (*6, 7*). To distinguish between these explanations, and to understand the role of pX in the life cycle of the virus and its relation to leukemogenesis, we have expressed the pX protein in animal cells and studied its structural and functional properties.

The strategy we used is depicted in Fig. 1A. The promoter and the first structural exon of the mouse metallothionein I (MT) gene were ligated to HTLV-I upstream of the mapped splice

acceptor within the X-LOR reading frame (*11*). If the MT splice donor can splice to the HTLV-I splice acceptor, a protein should be produced that would contain seven amino acids from the NH_2 terminal end of MT and the entire pX reading frame from the splice acceptor to the terminator within the LTR. A frameshift mutation within the X-LOR reading frame was constructed by inserting a dinucleotide at the Cla I site at position 7472 of HTLV-I. These two constructs were inserted into vector pJYM that contains a part of the plasmid pBR322 and the entire SV40 genome linearized at the Bam HI site (*14*). This resulted in plasmids pMXL and pMXfL, containing the X-LOR and the frameshift mutant, respectively.

Plasmids pMXL and pMXfL were introduced into CV1 cells, a monkey kidney cell line, by using the calcium phosphate coprecipitation technique (*15*). Total RNA was isolated and analyzed by S1 mapping (*16*) with the use of probes a and b (Fig. 1A). S1 mapping with probe a revealed that most of the stable mRNA was correctly spliced (Fig. 2A). Two bands of 174 and 192 nucleotides were specifically protected in the transfected

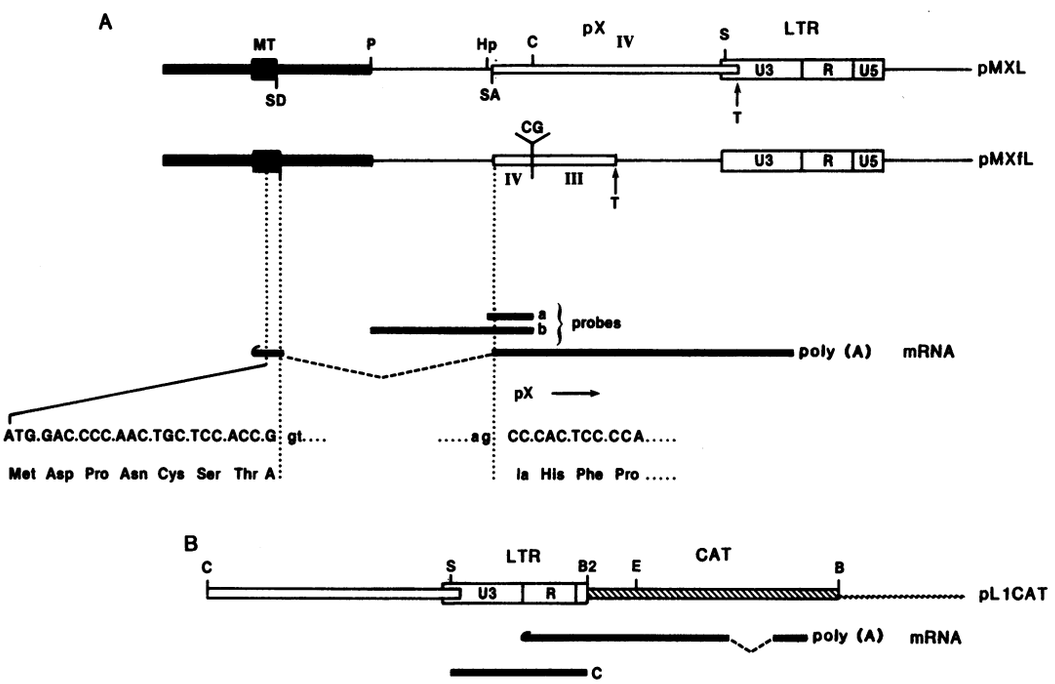

Fig. 1 (top right). (A) Structure of the recombinant plasmids for the expression of pX of HTLV-I. A 2.3-kb piece of the mouse MT gene (solid rectangles) containing the promoter, the first exon, and part of the first intervening sequence was ligated upstream from the splice acceptor (SA) of HTLV-I. This construct was inserted into vector pJYM that contains the complete SV40 linearized at the Bam HI site (plasmid pMXL). The SV40 genome is adjacent to the LTR. The direction of transcription of the MT promoter is the same as that of the late region of SV40. The expected protein contains seven amino acids from the NH_2 terminus of metallothionein and the complete X-LOR reading frame from the splice acceptor [position 7301; the sequence is numbered as in (*9*)]. The HTLV-I clone used (*31*) was partially sequenced. Only three differences were found between this clone and that of Seiki *et al.* (*9*) within the sequenced region. These were G to A at position 5195, T to C at position 5234, and C to T at position 7271, which destroys a Pst I site. A frameshift was constructed in the X-LOR reading frame by inserting a CG dinucleotide in the Cla I site by filling in with the Klenow fragment of DNA polymerase I and converting it into a Nru I site (plasmid pMXfL). The expected protein contains seven amino acids of metallothionein, 58 amino acids of the X-LOR reading frame, and 111 amino acids of the X_{111} reading frame from position 7464 to 7809. The structures of the expected mRNA and protein are indicated. The bars labeled a and b are the S1 mapping probes used in Fig. 2: a, Hpa I–Cla I probe; b, Pst I–Cla I probe. For the S1 mapping experiments, uniformly ^{32}P-labeled single-stranded probes were generated by primer extension from appropriate restriction fragments subcloned into M13 vectors. (B) Structure of plasmid pL1CAT. A Cla I–Sau 3AI fragment containing the U3, R, and part of the U5 region of the LTR of HTLV-I was ligated upstream of the CAT coding sequences in plasmid CAT3M (*32*). The bar labeled c represents the Sma I–Sau 3AI S1 mapping probe used in Fig. 4F. Symbols: MT, metallothionein; SD, splice donor; SA, splice acceptor; T, terminator; LTR, long terminal repeat; P, Pst I; Hp, Hpa I; C, Cla I; S, Sma I; B2, Bgl II; E, Eco RI; B, Bam HI.

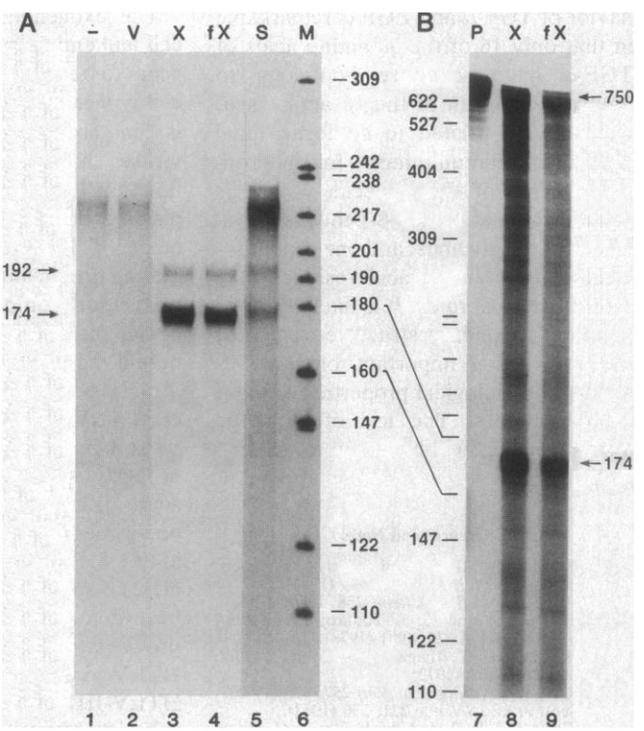

Fig. 2 (bottom right). (A) S1 mapping analysis of mRNA with the use of probe a. At 48 hours after transfection, total RNA was prepared by the guanidinium isothiocyanate–cesium chloride method. Ten micrograms of RNA were hybridized at 40°C overnight and treated with S1 nuclease (*16*). We expected a band of 192 nucleotides from the unspliced mRNA and a band of 174 nt from the spliced mRNA. CV1 cells were transfected with lane 1, salmon sperm DNA; lane 2, pJYMMT(L) vector DNA; lane 3, pMXL DNA; lane 4, pMXfL DNA. Lane 5, S1 mapping of total RNA isolated from the stable producer cell line BXL40 with probe a. Lane 6, end-labeled Hpa II digest of pBR322 DNA as molecular weight markers. (B) S1 mapping of the same RNA preparations using probe b. Lane 7, probe b. Lanes 8 and 9, CV1 cells transfected with pMXL DNA and pMXfL DNA, respectively. The same 174-nucleotide band is protected by the spliced mRNA.

cells. The lower band is derived from hybridization to correctly spliced mRNA and the upper band from hybridization to unspliced mRNA. No bands were protected after S1 mapping of RNA from cells transfected with salmon sperm DNA or with vector pJYMMT(L) (*17*), a JYM plasmid (*14*) with a 4-kilobase (kb) MT gene insert. S1 mapping experiments with probe b, which spans the entire HTLV-I part of the intervening sequence, gave a band at 174 nucleotides corresponding to spliced mRNA, and a substantial amount of full-length probe, indicating the presence of unspliced mRNA (Fig. 2B). There appeared to be no other major splice donors or acceptors within this region. Therefore, most (>90 percent) of the mRNA is correctly spliced, and both plasmids pMXL and pMXfL direct the synthesis of similar amounts of mRNA after transfection.

Cells transfected with plasmid pMXL were also examined by indirect immunofluorescense with the use of patient sera containing high titers of antibody to HTLV-I (*18*). Approximately 10 percent of the CV1 cells were positive in this assay. All positive cells contained strongly fluorescing nuclei, indicating that most of the HTLV-I protein encoded by plasmid pMXL was localized in the nucleus (Fig. 3A). Identical results were obtained after transfection with plasmid pMXfL. The numbers of cells positive in transfections with pMXL and with pMXfL were about equal. Untransfected cells or cells transfected with vector pJYMMT(L) DNA were not stained by the antibody (Fig. 3B). Recently Goh *et al.* (*19*) reported that pX is present in both the nuclear and the cytoplasmic fractions of HTLV-I–infected transformed lymphocytes.

We have also established permanent cell lines that overexpress pX protein by inserting the constructs shown in Fig. 1A into bovine papilloma virus vectors as described (*20*). These cell lines, which will be described elsewhere, contain correctly spliced pX mRNA (Fig. 2A, lane 5). They also express the pX protein as determined by indirect immunofluorescence as well as by immunoprecipitations and immunoblots of cell lysates.

The effect of the pX protein on the transcription of the LTR of HTLV-I was next tested by cotransfection experiments. First, plasmid pL1CAT (Fig. 1B) was constructed by ligating the LTR of HTLV-I 5′ to the body of the gene for chloramphenicol acetyltransferase (CAT). Transcription from the promoter in the LTR is expected to produce CAT, which was monitored by the acetylation

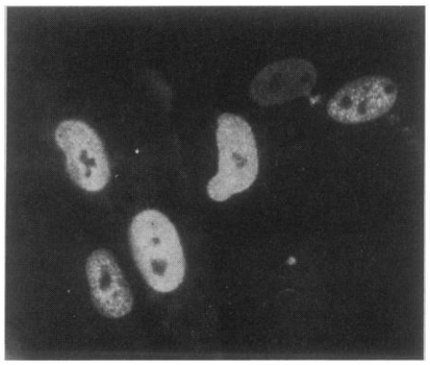

Fig. 3. Localization of pX by indirect immunofluorescence in CV1 cells transfected with plasmid pMXL. At 48 hours after transfection, the cells were fixed in 3.7 percent formaldehyde, made permeable with 0.1 percent NP-40 in phosphate-buffered saline (PBS), treated with serum from a human patient and then with FITC-conjugated goat anti–human immunoglobulin G (Cappel) to identify the pX protein.

of ^{14}C-labeled chloramphenicol as described (*21*). Plasmid pL1CAT was introduced into CV1 cells in the presence or absence of plasmid pMXL or plasmid pMXfL. Cell lysates were assayed for CAT activity, or total RNA was isolated and used for quantitative S1 mapping (Fig. 4A). pL1CAT alone directed the synthesis of low levels of CAT in CV1 cells [Fig. 4A, lane (−)]. When a coprecipitate of pMXL and pL1CAT was applied on the cells, a 50-fold increase of the CAT activity was observed (lane X). Cotransfection of pL1CAT with pMXfL (lane fX) resulted in a small increase in CAT activity, approximately fourfold greater than that seen with pL1CAT alone. Mixing plasmid pMXL and pMXfL increased the CAT activity to the same extent as pMXL alone (lane X + fX). These results indicate that the activation is caused by the gene product of the X-LOR reading frame acting in *trans*, and not by competition of the incoming plasmid DNA for cellular factors. To verify this conclusion, we cotransfected increasing amounts of pMXL into CV1 cells with 10 μg of pL1CAT (Fig. 4B). The CAT activity was significantly stimulated by 0.1 μg of pMXL (1:200 molar ratio, pMXL:pL1CAT). As a control, the same experiment was repeated with a number of other promoters linked to the CAT gene, such as pSV2CAT and pRSVCAT (*21*). The presence of various amounts of pMXL did not affect the promoter activity of the SV40 early promoter (Fig. 4C), the Rous sarcoma virus LTR promoter, or the mouse metallothionein promoter.

The extent of HTLV-I LTR activation in a lymphoid cell line was also tested. The human T-cell line H9 (*22*) was transfected by the DEAE-dextran technique (*23*) (Fig. 4D). The results obtained by cotransfection of pL1CAT with pMXL or pMXfL were similar to the results in the CV1 cell line. No factors that are unique to lymphoid cells appeared to be required for the activation of the LTR by pX. Similar results were obtained after transfection of plasmid pL1CAT into the stable pX-producing cell lines BXL14 and BXL40. The CAT activity was 20-fold higher in BXL40 cells (Fig. 4E) than in control 7-4 cells containing a similar BPV vector without any HTLV-I sequences.

The increased CAT levels observed in these assays could, in principle, be due to a transcriptional activation of the LTR promoter, an increase in CAT mRNA accumulation, a change in translational efficiency of the hybrid mRNA or in protein stability, or some combination of the above. To distinguish among these possibilities and to examine whether the same promoter is used in the presence of pX, we transfected CV1 cells with pL1CAT in the presence or absence of pX. Total RNA was isolated and mapped by S1 nuclease for the presence of correctly initiated LTR mRNA with the use of probe c (Fig. 1B). Because plasmid pMXL contains a copy of the HTLV-I LTR, a different plasmid, pMX ΔL, was used in these experiments. pMX ΔL has the entire LTR deleted 3′ to the Sma I site (Fig. 1A); therefore, it produces a pX protein that lacks 16 amino acids from the carboxy terminus. This protein was found to be functional in the pL1CAT transactivation assay.

Correctly initiated mRNA was detected in the cells cotransfected with pL1CAT and pMX ΔL (Fig. 4F, lanes 2 and 3). No mRNA starting at the correct initiation site was detected in cells transfected only with pL1CAT, even after the addition of 50 times more RNA (Fig. 4F, lane 1). Therefore, the induction ratio of the correctly initiated mRNA from the LTR promoter in the presence of pX is approximately 500-fold. This is almost one order of magnitude greater than the estimate based on the CAT assay. There appears to be a high basal level in the CAT assay due to transcripts initiated in other places within the transfected plasmid. Such possible aberrant initiation sites can explain the protected bands in Fig. 4F, lane 1. When we included a plasmid containing a human α-globin gene in the transfection coprecipitates, no difference was detected in the amounts of α-globin mRNA, indicating that the transfection efficiencies of the

different coprecipitates were similar. Nuclear runoff experiments, deletion mutagenesis of the pL1CAT plasmid (*24*), and the data presented above indicated that the activation of LTR by pX is primarily, if not exclusively, a transcriptional event.

Since the product of pMXL in animal cells is a mutant pX protein containing seven additional amino acids at the NH_2-end, the question arises whether the properties of this mutant are similar to those of pX. The authentic pX protein is generated by a double-spliced mRNA that brings the envelope AUG next to the X-LOR reading frame. Using a similar approach, we have expressed the authentic pX protein in animal cells by splicing of the viral RNA and have shown that the two proteins give qualitatively similar results in the *trans*-activation assays (*24*). The observed fourfold induction of CAT with the frameshift mutant pfX indicates that pfX protein is partially active. We did not determine whether the 111 amino acids of the X_{111} reading frame contribute to this activity, or whether a major functional domain of pX exists within the 58 NH_2-terminal amino acids that are identical in pX and pfX. The low induction by pMXfL could also be due to a frameshift suppression that generates a small amount of pX from pMXfL. Our *trans*-activation experiments were done in the presence of SV40 T antigen, a known modulator of gene expression (*25*). We obtained similar results with another set of pX-producing vectors that do not contain SV40 sequences (*24*). Therefore, *trans*-activation of the LTR by pX is not dependent on the presence of T antigen.

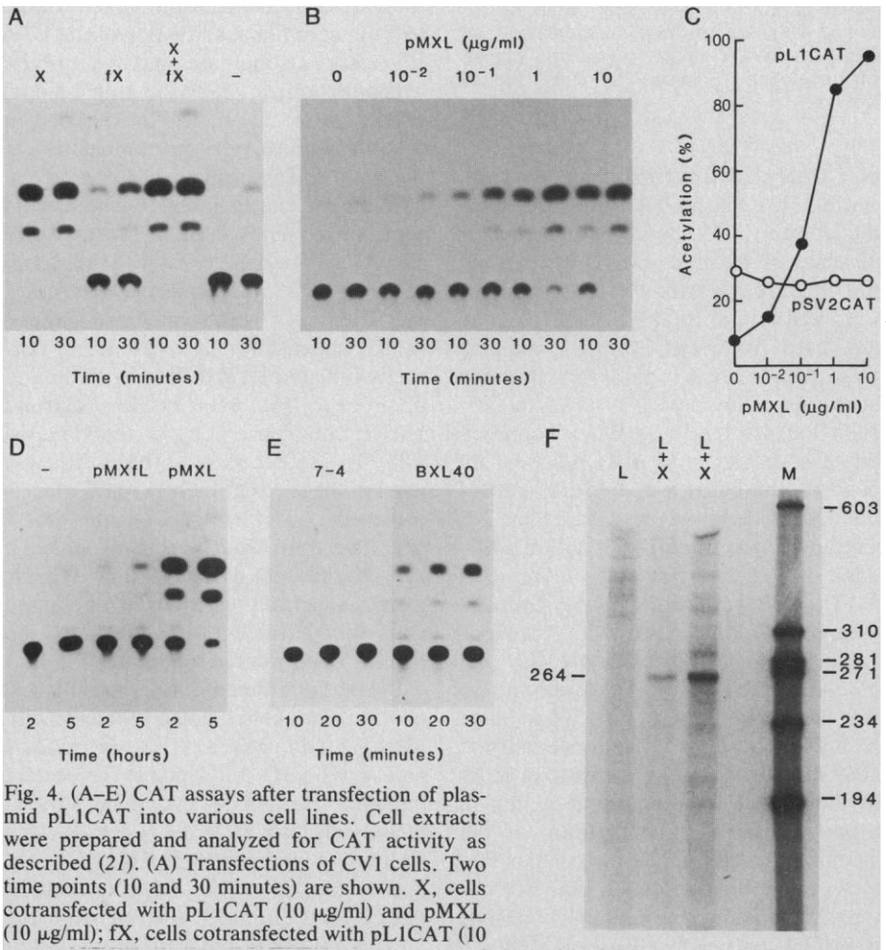

Fig. 4. (A–E) CAT assays after transfection of plasmid pL1CAT into various cell lines. Cell extracts were prepared and analyzed for CAT activity as described (*21*). (A) Transfections of CV1 cells. Two time points (10 and 30 minutes) are shown. X, cells cotransfected with pL1CAT (10 μg/ml) and pMXL (10 μg/ml); fX, cells cotransfected with pL1CAT (10 μg/ml) and pMXfL (10 μg/ml); X+fX, cells cotransfected with pL1CAT (10 μg/ml), pMXL (10 μg/ml) and pMXfL (10 μg/ml); (–), cells transfected only with pL1CAT (10 μg/ml). (B) Cotransfection of pL1CAT (10 μg/ml) in CV1 cells with different amounts of pMXL (0 to 10 μg) as indicated. Two time points for each concentration are shown (10 and 30 minutes). In all cases, the final DNA concentration in the precipitate was adjusted to 35 μg/ml by the addition of JYMMT(L) vector DNA. (C) Plot of data in (B). The 10-minute time points were quantitated by liquid scintillation counting. Under the same conditions, the expression of pSV2CAT is not affected by pMXL. (D) Cotransfections of plasmids pL1CAT with pMXL or pMXfL in H9 cells, a lymphoid cell line permissive for HTLV-I. Two time points (2 and 5 hours) of the CAT assay are shown. –, Cells transfected with pL1CAT (1 μg/ml); pMXfL, cells transfected with pL1CAT (1 μg/ml) and pMXfL (1 μg/ml); pMXL, cells transfected with pL1CAT (1 μg/ml) and pMXL (1 μg/ml). (E) Transfections of pL1CAT (10 μg/ml) into a stable cell line producing pX. Three time points of the CAT assay are shown (10, 20, and 30 minutes). 7-4 is a control cell line transformed with a BPV vector not containing HTLV-I sequences. BXL40 produces high quantities of pX. (F) S1 mapping of the LTR promoter of HTLV-I in the presence and absence of pX, using probe c. Lane 1, 50 μg of total RNA from cells transfected with pL1CAT. Lane 2, 1 μg of total RNA from cells cotransfected with pL1CAT and pMX ΔL. Lane 3, 5 μg of total RNA as in lane 2. Lane 4, molecular weight markers (end-labeled ϕX Hae III digest).

Two general models of pX action may now be proposed. In the first, pX would bind directly to a specific site in the LTR and influence the binding of other cellular factors or RNA polymerase with the DNA. In the second model, pX would modify the expression or the activity of other cellular factors that interact with the LTR. Neither model is sufficient to explain the behavior of HTLV-I or BLV in vivo without further assumptions. BLV RNA cannot be detected in fresh lymphocytes from infected animals (*26*), but virus is produced shortly after culturing the cells (*27*). Fresh leukemic cells from four of five ATL patients do not express detectable quantities of HTLV-I mRNA (*28*). Therefore, at least the majority of peripheral blood cells containing the provirus appear to counteract the activation of the LTR promoter by pX. This could be caused either by an inhibition of pX production or by an inhibition of transcription by cellular repressors. The existence of additional regulatory circuits is supported by the observation that after cultivation of leukemic cells, the provirus is actively transcribed and transmissible virus can be detected. The production of pX may be linked to cell cycle. Therefore, nondividing peripheral blood lymphocytes of leukemic patients would not contain substantial quantities of pX mRNA.

These data demonstrate that HTLV-I carries its own transcriptional activator that functions well in other cell types as well as in lymphoid cells. HTLV-I, HTLV-II, and BLV appear to have properties that justify their classification in the same class of retroviruses. To our knowledge this is the first identification of a group of retroviruses (*29*) that can code for a protein that has some properties characteristic of the transforming proteins of DNA tumor viruses, such as nuclear localization, ability to activate a homologous viral promoter (or promoters), and ability to modulate the expression of certain cellular promoters (*24*). It will therefore be interesting to examine whether pX is a transforming protein of HTLV-I, perhaps analogous

to adenovirus E1A products or SV40 and polyoma T antigens (*25, 30*). The ability of pX to modulate the activity of other promoters may be important for triggering the sequence of events that lead to leukemogenesis. Since little or no pX protein occurs in fresh leukemic cells from patients, it could be involved in the initiation but not the maintenance of transformation. Alternatively, low levels of pX acting during inappropriate stages in the cell cycle may be enough to maintain the transformed phenotype in the leukemic cell. Availability of vectors producing authentic and mutant pX proteins in animal cells may help answer these questions. Since the name pX implies an unknown function of the gene product, it is no longer adequate. We propose to call this the Ta I protein, for transcriptional activator of the LTR.

References and Notes

1. B. J. Poiesz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7415 (1980); V. S. Kalyanaraman *et al.*, *Nature (London)* **294**, 271 (1981); M. Robert-Guroff *et al.*, *J. Exp. Med.* **154**, 1957 (1981); M. Yoshida *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2031 (1982).
2. For recent reviews, see R. C. Gallo, M. Essex, L. Gross, Eds., *Human T-Cell Leukemia Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1984); F. Wong-Staal and R. C. Gallo, *Blood* **65**, 253 (1985).
3. I. Miyoshi *et al.*, *Nature (London)* **294**, 770 (1981); M. Popovic *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5402 (1983); S. Merl *et al.*, *Blood* **64**, 967 (1984).
4. M. Seiki *et al.*, *Nature (London)* **309**, 640 (1984).
5. J. Sodroski *et al.*, *Science* **225**, 381 (1984); J. Sodroski *et al.*, *ibid.* **227**, 171 (1985).
6. I. S. Y. Chen *et al.*, *Nature (London)* **309**, 276 (1984).
7. D. Derse *et al.*, *Science* **227**, 317 (1984).
8. W. A. Haseltine *et al.*, *ibid.* **225**, 419 (1984).
9. M. Seiki *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3618 (1983).
10. K. Shimotono *et al.*, *ibid.* **81**, 6657 (1984).
11. W. R. Wachsman *et al.*, *Science* **226**, 177 (1984); T. Okamoto *et al.*, *Virology*, in press.
12. N. R. Rice *et al.*, *Virology* **138**, 82 (1984); N. R. Rice, *ibid.* **142**, 357 (1985); N. Sagata *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 677 (1985).
13. T. H. Lee *et al.*, *Science* **226**, 57 (1984); D. J. Slamon *et al.*, *ibid.*, p. 61; T. Kiyokawa *et al.*, *Gann* **75**, 747 (1984).
14. M. Lusky and M. Botchan, *Nature (London)* **293**, 79 (1981).
15. F. J. Graham and A. J. Van der Eb, *J. Virol.* **52**, 456 (1973).
16. A. J. Berk and P. A. Sharp, *Cell* **12**, 721 (1977); R. F. Weaver and C. Weissmann, *Nucleic Acids Res.* **7**, 1175 (1979).
17. D. H. Hamer and M. J. Walling, *J. Mol. Appl. Genet.* **1**, 273 (1982).
18. J. Schüpbach *et al.*, *Cancer Res.* **43**, 886 (1983).
19. W. C. Goh, J. Sodroski, C. Rosen, M. Essex, W. A. Haseltine, *Science* **227**, 1227 (1985).
20. N. Sarver *et al.*, *Mol. Cell. Biol.* **1**, 486 (1981); G. N. Pavlakis and D. H. Hamer, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 397 (1983); *Recent Prog. Horm. Res.* **39**, 353 (1983); unpublished results.
21. C. M. Gorman *et al.*, *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).
22. M. Popovic *et al.*, *Science* **224**, 497 (1984).
23. J. J. McCutchan and J. S. Pagano, *J. Natl. Cancer Inst.* **41**, 351 (1968).
24. G. N. Pavlakis and B. K. Felber, in preparation.
25. J. Tooze, Ed., *DNA Tumor Viruses: Molecular Biology of Tumor Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1981); S. I. Reed *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3083 (1976); D. Rio *et al.*, *ibid.* **77**, 5706 (1980); R. Tjian, *Cell* **13**, 165 (1981); J. Brady, F. Wong-Staal, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2040 (1984).
26. R. Kettmann *et al.*, *Leuk. Res.* **4**, 509 (1980).
27. V. Baliga and J. F. Ferrer, *Proc. Soc. Exp. Biol. Med.* **156**, 388 (1977).
28. G. Franchini *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6207 (1984).
29. A potential addition is Rous sarcoma virus; see S. Broome in *Eucaryotic Viral Vectors*, Y. Gluzman, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982); S. Broome and W. Gilbert, *Cell* **40**, 538 (1985).
30. N. Jones and T. Shenk, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3665 (1979); J. R. Nevins, *Cell* **26**, 213 (1981); C. T. Feldman, M. J. Imperiale, J. R. Nevins, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4952 (1982); A. Berk *et al.*, *Cell* **17**, 935 (1979).
31. V. Manzari *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1574 (1983).
32. L. A. Laimins *et al.*, *J. Virol.* **49**, 183 (1984).

33. We thank M. Satake for help with the immunofluorescence, M. G. Sarngadharan and R. C. Gallo for human patient sera and for discussions, B. Howard and L. Laimonis for CAT vectors, H.-U. Affolter for advice, S. Hughes, D. Hamer, and Y. Ito for discussions and comments, and H. Marusiodis and V. Koogle for editorial assistance. Research sponsored by NCI, DHHS, under contract No. N01-C0-23909 with Litton Bionetics, Inc.

4 April 1985; accepted 1 July 1985

Origin of Human Small Cell Lung Cancer

Ruff and Pert (*1*) describe the presence of antigens (including OKM1) previously believed specific for macrophages on two small cell carcinoma (SCLC) cell lines (originally isolated by us) and four SCLC autopsy specimens. They suggest that SCLC arises from macrophages of bone marrow origin. Although their findings are of interest, we believe that they do not support the conclusions they have reached.

Our own work with 27 SCLC lines indicates that certain "lymphoreticular" antigens detected by monoclonal antibodies can be expressed on SCLC cell lines and tumor tissue samples (*2*). These include the Leu-7 (HNK-1) antigen [present on natural killer (NK) cells] which is found in more than 90 percent of SCLC; Leu M1 (expressed on macrophages and granulocytes); and Leu M2 (present on macrophages). However, certain other NK and macrophage-associated antigens, including Leu 11 and Leu M3, are seldom expressed on SCLC. Specifically, we found the OKM1 antigen on only 8 of 27 SCLC. Of interest, OKM1 was also found on two of five non-SCLC tumors. Thus, our observations based on a much larger sample of tumors only partially confirm their data and actually point to the Leu-7 antigen as a much more frequently expressed lymphoreticular antigen in SCLC. The widespread development of monoclonal antibodies has demonstrated many hitherto unsuspected antigenic relationships between cell groups of diverse function and embryologic origin. For example, cerebellar Purkinje cells express the T-lymphocyte antigen Leu-4 (*3*). The NK cell antigen Leu-7 is also expressed on normal and neuroendocrine cells and tumors, including SCLC (*2, 4*). In addition, SCLC expresses an antigen, lacto-*N*-fucopentaose III, present on other cell types including proximal renal tubules (*5*). Thus, shared antigenicity does not necessarily indicate common lineage or embryologic origin. In addition, Ruff and Pert presented no biochemical evidence that the molecules bearing the shared antigenic determinants on SCLC cells and macrophages are identical.

SCLC cells express many neuroendocrine features (*6*). This phenotype is very similar to that of normal pulmonary endocrine cells, the putative precursor cells of SCLC and bronchial carcinoids (*6*). In contrast, we know of no evidence that macrophages, monocytes, or NK cells express neuronal features. Pearse (*7*) postulated that all neuroendocrine cells have a neuroectodermal origin. However, embryologic studies strongly suggest that the bronchial mucosa is composed entirely of cells of endodermal origin (*6*). Pathologic studies support the notion that SCLC arises in the bronchial mucosa and it expresses morphological and biochemical features of epithelial cells such as desmosomes and the intermediate filament keratin (*6*). Recently, several groups have shown that bronchial mucosal and SCLC cells may demonstrate simultaneous differentiation along endocrine and nonendocrine pathways (*6, 8*). While SCLC selectively expresses certain lymphoreticular antigens, there is much data in support of a unitarian origin for all of the bronchial mucosa and the tumors arising from it, including SCLC (*6*).

ADI F. GAZDAR
PAUL A. BUNN, JR.
JOHN D. MINNA
NCI–Navy Medical Oncology Branch, National Cancer Institute, and *Naval Hospital, Bethesda, Maryland 20814*
STEPHEN B. BAYLIN
Oncology Center, Johns Hopkins Hospital, Baltimore, Maryland 21205

References

1. M. R. Ruff and C. B. Pert, *Science* **225**, 1034 (1984).
2. P. A. Bunn, Jr., I. Linnoila, J. D. Minna, D. Carney, A. F. Gazdar, *Blood* **65**, 764 (1985).
3. J. A. Garson, P. C. L. Beverly, H. B. Coakham, E. I. Harper, *Nature (London)* **298**, 375 (1982).