*Acta Med. Nagasaki* 33 : 38－47

# Transcriptional modulation of a flanking gene by HTLV-1 integration, and demonstration of a cellular transcript homologous to HTLV-1 LTR-gag region

Ryozo MORIUCHI, Hisanaga IGARASHI, Daisuke NAKAYAMA
Tsutomu MIYAMOTO and Shigeo HINO*

*Department of Bacteriology, Nagasaki University School of Medicine, 12-4 Sakamoto, Nagasaki 852*

*Received for publication, February 12, 1988*

**SUMMARY :** We have cloned a human T-lymphotropic virus type-1 (HTLV-1 ) proviral genome, λ 255 M, of a cell line, TL-Su, which has been established from peripheral blood lymphocytes of a healthy HTLV-1 carrier. The λ 255M had a very similar restriction map to that of a previous isolate, λ ATK-1, but a different junctional repeat, TGAAAG, consistent with the random integration sites of HTLV-1. We found two species of interesting cellular gene transcripts. The first gene homologous to LTR-gag region of HTLV-1 was expressed as 4.5 kb RNA only in the cells of lymphoid cell lineage as far as tested. The RNA was present even in uninfected cells including a T-cell line, Jurkat, and a B-cell line, FLEB-12-3-4, but not in another T-cell line, CEM. Because of strong viral RNA signals, we could not demonstrate the RNA in virus producing cell lines. The results suggested the presence of human cellular gene related with LTR-gag region of HTLV-1. The other gene homologous to 5' flanking gene of the λ 255 M was expressed as 1.8 kb mRNA in all human cells tested except for the TL-Su cell. In the TL-Su cell, HTLV-1 integration modulated this gene transcription into 3.8, 3.2. 2.0 and 1.6kb mRNAs qualitatively, and at least 10 times more than other human cells quantitatively. The data suggests that HTLV-1 gene integration may cause the transcriptional modulation of cellular genes by insertional mutagenesis. (key words, HTLV-1; HTLV-1 related human gene; transcriptional modulation; flanking sequence; insertional mutagenesis )

## INTRODUCTION

Human T-lymphotropic virus type-1(HTLV-1) is closely associated with adult T-cell leukemia (ATL ) (POIESZ *et al.* 1980[18] ), HINUMA *et al.* 1981[39] ), YOSHIDA *et al.* 1982[60] ), WONG-STAAL *et al.* 1983[59] ), YOSHIDA *et al.* 1984[61] ). ATL usually develops after age of 40 in HTLV-1 carriers (UCHIYAMA *et al.* 1977[55]), whose infection is established early in life mainly via milk of carrier mothers (HINO *et al.* 1985[17]), KINOSHITA *et al.* 1985[55] ). Chronic development of ATL by HTLV-1 is consistent with the absence of typical oncogene of cellular origin (SEIKI *et al.* 1983[44] ). Retroviruses without oncogene may cause genetic changes of infected cells leading to cellular oncogenesis by 1 ) promoter /enhancer insertional activation (HAYWARD *et al.* 1981[16], PAYNE *et al.* 1982[38], NUSSE and VARMUS 1982[35], NUSSE *et al.* 1984[36], NILSEN *et al.*

* To whom correspondence should be addressed

1985[34]), 2)trans-acting transcriptional activation (INOUE *et al.* 1986[20], CROSS *et al.* 1987[7]), 3) transduction and subsequent alteration (BISHOP *et al.* 1983[1], BISHOP, 1984[2], BISHOP and VARMUS, 1984[3], BISHOP, 1985[4]), and 4) insertional inactivation or modulation (VARMUS *et al.* 1981[56], HAWLEY*et al.* 1982[15], SCHNIEKE *et al.* 1983[42], HARBERS *et al.*1984[14], WOLF and ROTTER 1984[58], FRANKEL*et al.* 1985[9], KING *et al.* 1985[24]). The HTLV-1 seems to have no preferential sites for the integration (SEIKI *et al.* 1984[45]), suggesting that the leukemogenesis of ATL is not mediated by promoter/enhancer insertional activation of an adjacent cellular oncogene as demonstrated in avian lymphoma and erythroblastosis induced by avian leucosis virus (ALV) (HAYWARD *et al.* 1981[16]).

HTLV-1 has a unique *pX* gene, whose counterpart is not present in type C leukemia viruses (SEIKI *et al.* 1983[44] SEIKI *et al.* 1985a[46]. HTLV-1 does not seem to induce ATL by a hit-and-run mechanism, since every monoclonal tumor cell of ATL contains the HTLV-1 proviral genome, at least *pX* and LTR (YOSHIDA *et al.* 1984[61]). Thus, the *pX* gene has been extensively studied as a candidate for responsible gene of leukemogenesis. Recent studies have shown that p40$^{tax}$ encoded by ORFIV (KIYOKAWA *et al.* 1984[26], LEE *et al.* 1984[30], MIWA *et al.* 1984[31], SALMON *et al.* 1984[40]) of the *pX* gene is a trans-acting transcriptional activator (SODROSKI *et al.* 1984[50], CANN *et al.* 1985[5], FELBER *et al.* 1985[8], FUJISAWA *et al.* 1986[12], PASKALIS *et al.* 1986[37], SEIKI *et al.* 1986[48], SHIMOTOHNO *et al.* 1986[49]) which promotes transcription from LTR responding to the enhancer. The p40$^{tax}$ also activates cellular genes, such as interleukin 2 and its receptor genes (INOUE *et al.* 1986[20], CROSS *et al.* 1987[7]). The activation of these genes may be essential to the initial step of transformation in polyclonal HTLV-1 infected cells. Another *pX* protein, p27$^{rex}$ encoded by ORFIII (KIYOKAWA *et al.* 1985[27]), has been indicated to switch splicing of RNAs, from ones directed to early proteins to ones for late proteins, as a post-transcriptional modulator of viral RNA (INOUE *et al.* 1987[21]). However, the conjecture may not explain the very low rate and long incubation period of oncogenic transformation among HTLV-1 carrier cells.

The transduction and insertional modulation models are both related to flanking regions of viral genome. To focus on the matter, we analyzed the flanking region of an HLTV-1 proviral genome in TL-Su cells (SUGAMURA *et al.* 1984[54]); the cell line was established from PBL of an HTLV-1 healthy carrier. In this study, we found two interesting species of RNAs. One expressed even in some uninfected cells was homologous to HTLV-1 LTR-gag region, and the other expressed in every human cells tested was homologous to 5' flanking region and specifically modulated in TL-Su cells by HTLV-1 integration.

## MATERIALS AND METHODS

*Cells:* Table 1 shows the list of cells used in this study. Cell lines infected with HTLV-1 include MT-2 and MT-4 (MIYOSHI *et al.* 1982[32], HARADA *et al.* 1985[13]), TL-OmI (SUGAMURA *et al.* 1984[54]) and TL-Su. The first cell line was established by coculture of peripheral blood lymphocytes (PBL) of ATL patients with uninfected cord blood cells, while the others by culture of PBL of an ATL patient or a carrier, respectively. Fresh PBL obtained from a patient with ATL (ATLPBL) was prepared by Ficoll-Conray density gradient centrifugation. At least 70% of ATLPBL were leukemic ATL cells by the hematological standard, but they did not express viral transcripts. The list also includes uninfected cell lines originated from T-cell, B-cell, and various tumor and non-tumor cells of ectodermal, mesodermal or entodermal origin. We added a non-human cell line of bovine origin (ENDO).

We cultured lymphoid cells in RPMI 1640 (GIBCO) medium supplemented with 10% fetal bovine serum and 2.0 mM L-glutamine, and other cells in Dulbecco's modified Eagle's MEN (GIBCO) supplemented with 5% fetal bovine serum.

*Molecular cloning of HTLV-1 proviral genomes:* After complete digestion with EcoRI, the high molecular weight DNA of TL-Su cells was sized with a sucrose density gradient centrifugation. The DNA in franctions containing 9-20 kbp

length was ligated to the arms of EMBL-4 phage DNA (FRISCHAUF *et al.* 1983[10]), and subjected to *in vitro* packaging (STERNBERG *et al.* 1977[53]). Approximately 5 × $10^5$ plaques were screened with $^{32}$P-labelled pATK-33 probe, 2.3 kbp *pol* gene fragment of HTLV-1 genome ( λ ATK-1) (SEIKI *et al.* 1983[44]). We used a plasmid pUC118 for subcloning, and pMT-2/64/17 containing whole HTLV-1 genome as another probe.

*Hybridization analysis:* DNA probes were prepared by multi-prime method using $^{32}$P-dCTP. DNA-DNA hybridization was performed by the method of Southern (1975)[52] on nylon membranes (Zeta probe, Biorad) at 42℃ for 18 hr in a mixture of 50% formamide, 3 × SSPE (1 × SSPE: 0.1 M NaCl, 0.01 M sodium phosphate, pH 7.0), 10% dextran sulfate, 1% SDS, 0.5% skim milk and 0.2 mg/ml yeast RNA.

To analyze cellular RNA, total cellular RNAs were extracted by the procedures of CHIRGWIN *et al.* (1979)[6], and poly(A) contaning RNAs were isolated by oligo (dT)-cellulose column chromatography. The RNAs were fractionated by agarose gel electrophoresis in the presence of 2.2 M formaldehyde, transferred onto nylon membranes, and hybridized at the same condition as the southern analysis. The filter was finally washed at 42 ℃ for 30 min with a buffer of 0.1 x SSC and 0.1% SDS.

*Nucleotide sequence determination:* We employed the method of SANGER *et al.* (1977)[41] for the nucleotide sequence analysis.

**Table I.** Cells used for Nothern hybridization analysis

| | origin | HTLV-1 genome | HTLV-1 antigens | supplied from |
|---|---|---|---|---|
| TL-Su | HTLV-1 carrier | + | + | K. Sugamura |
| MT-2 | ATL[b] | + | + | I. Miyoshi |
| MT-4 | ATL[b] | + | + | I. Miyoshi |
| TL-Oml | ATL | + | − | K. Sugamura |
| ATLPBL | fresh ATL cells | + | − | K. Kinoshita |
| Jurkat | ALL | − | − | H. Shiku |
| ELEB 12-3-4 | EBV[c]transformed preB cell | − | − | S. Katamine |
| ENDO | endothelial cells | − | − | S.A. Aaronson |
| T 98 G | glioblastoma | − | − | do. |
| G 401 | Wilm's tumor | − | − | do. |
| T 24 | bladder carcinoma | − | − | do. |
| A 388 | epidermoid carcinoma | − | − | do. |
| HA 117 | GI carcinoma | − | − | do. |
| U 937 | histiocytic lymphoma | − | − | JCRB-Cell |

a) All cells are originated from human exept ENDO from bovine.
b) Established by co-cutivation with cord blood lymphocytes.
c) Epstein-Barr virus.

## RESULTS

*Isolation of λ 255M, an HTLV-1 proviral genome:* We isolated six independent clones contaning HTLV-1 *pol* gene from a genomic library of TL-Su cells, with pATK-33 as a probe. We selected λ255M for further analysis, since it contained the large enough insert to cover whole HTLV-1 genome. Fig 1A shows a simplified restriction cleavage map of λ255M. We estimated the location of LTRs in comparison to the genetic map of λ ATK-1. Thirteen restriction sites of enzymes in λ255M (Fig. 1A) were identical to those of λ ATK-1, if we estimate the presence of approximately 1 kbp deletion in *gag-pol* region. However, there was some difference between these two clones, such as the absence of NdeI site in the λ255M LTR (data not shown). The result was consistent with the previous data that HTLV-1s isolated in the world are very closely related each other, approximately 2% difference in LTR base sequences (WATANABE *et al.* 1984[57], JOSEPHS *et al.* 1984[22]).

We first subcloned fragments of λ255M, p255M-01 through -12, into pUC118 (Fig. 1A),

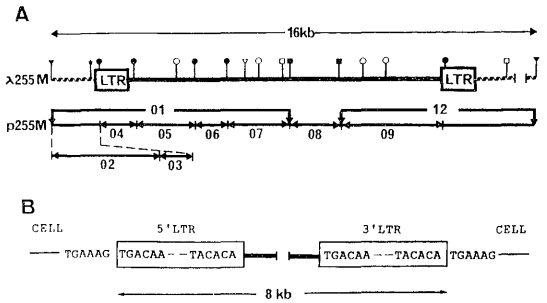


Fig. 1. (A) Restriction map of λ255M integrated proviral genome of HTLV-1. Thick line denotes the location of proviral DNA with a LT R (box) at each end. Bottom half shows the map of p255M, p255M-01 through -09, and -12 plasmid subclones. Restriction site symbols: ▼, EcoRI; ●, SmaI; ■, BamHI; ○, PstI; □, HindIII; ▽, XbaI; ★, HpaI.
(B) Nucleotide sequences of the virus-cellular junction in the clone λ255M.

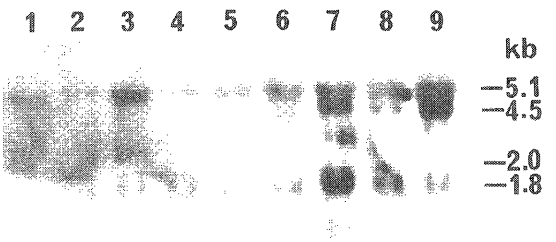


Fig. 2. Northern hybridization analysis of total cellular RNAs by p255M-01 as probe. Total cellular RNAs, 10 μg were appplied per lane. Transcript sizes are shown in kilobases. Lanes: 1, ENDO; 2, T98G; 3, G401; 4, T24; 5, A388; 6, HA117; 7, Jurkat; 8, FLEB12-3-4; 9, ATLPBL.

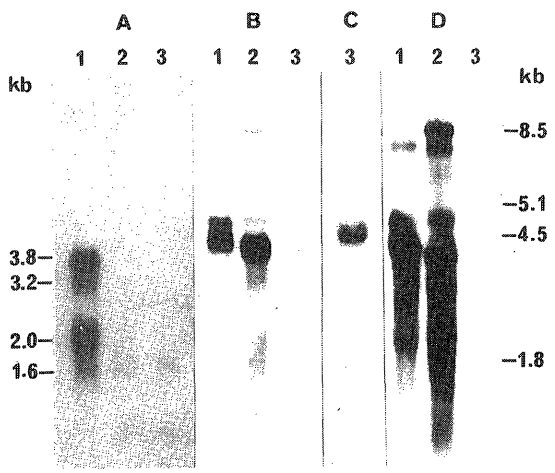


Fig. 3. Northern hybridization analysis of total cellular RNAs of TL-Su (lane 1), MT-4 (lane 2), and Jurkat(lane 3) using p255M -02(A), -03(B), -04(C), and -09(D) as probes, respectively. Profiles of RNAs of virus producers, TL-Su and MT-4 are not shown in (C) because of strong signals of viral RNAs.

and sequenced the junctions of the LTR and flanking DNA, which revealed the precise location of LTRs and a pair of 6-bp cellular direct repeat, TGAAAG, on each external end of the LTRs (Fig. 1B). The direct repeat was different from the other reported couterparts of HTLV-1 proviral clones, for example, TAGTTC in case of λATK-1. The result is consistent with the previous observations that HTLV-1 integrates in a random fashion. From the exact location of the junctions, we found that λ255M has one and seven kbp cellular flanking DNA in the 5' and 3' ends of the viral genome, respectively.

*Expression of 5' cellular flanking sequences:* To look for possible expression of flanking sequences in human cells, we screened total cellular RNAs by p255M-01 consisting of the whole 5' flanking sequences and approximately 4 kbp 5' end of viral genome as a probe. A northern hybridization analysis showed the presence of 1.8 kb RNA in every human cell tested (Fig. 2) except for TL-Su cells (Fig. 3, lane A1) from which λ255M was isolated. More interestingly, we also found a 4.5 kb RNA in some of the lymphoid cells tested (Fig. 2).

*The 4.5 kb RNA homologous to p255M-01:* The 4.5 kb RNA was present only in lymphoid cells as far as tested, and in uninfected as well as infected cells(Fig. 2-4). Of cells positive for the 4.5 kb RNA, Jurkat and FLEB12-3-4 (KATAMINE *et al.* 1984[23]) are unrelated to HLTV-1 in origin. We confirmed that these cells are negative for HTLV-1 by southern and northern hybridization analyses using *pol* (pATK-33) or *pX*

(p255M-09) as probes. None of them was positive. Fig. 3, Lane D3 shows an example of data indicating the absence of any RNA hybridizable to *pX* probe in Jurkat cells. Of HTLV-1 infected but not producing cells, TL-OmI (data not shown) and ATLPBL (Fig. 2, lane 9) were positive for the 4.5 kb RNA. However, in cells replicating HTLV-1, such as MT-2, MT-4 and TL-Su, we could not identify the presence of the 4.5 kb RNA because of the strong viral signals.

To localize the sequences with homology, we used 3 subclones of p255M-01, EcoRI-HpaI (p255M-02), HpaI-SmaI (p255M-03) and SmaI-SmaI (p255M-04) as probes which correspond 700 bp flanking, 169 bp flanking and 31 bp LTR U3, and most LTR through 50 bp 5' end of *gag* gene, respectively. Surprisingly, the 4.5 kb RNA did not hybridize with either of the p255M-02 or p255M-03 probe, i.e. the RNA was not homologous to the 5' flanking DNA of λ255M. Instead, the 4.5 kb RNA hybridized with the p255M-04 (Fig. 3C). The RNA hybridized very weakly to p255M-05 probe, but did not to p255M-06 through -09 (data not shown). Homology of the 4.5 kb RNA and HTLV-1 was confirmed by a northern hybridization analysis using pMT-2/64/17 as a probe (Fig. 4A). The specificity of hybridization was high, since more stringent wash at 67℃ did not remove the signals (Fig. 4B). Thus, it was clear that 4.5kb RNA expressed in certain lymphoid cells is transcribed from a cellular gene and related to LTR-gag region of HTLV-1.

*The 1.8 kb RNA homologous to p255M-01:* To localize the sequences homologous to the 1.8 kb RNA present in all human cells tested except for the TL-Su, we surveyed the 1.8 kb RNA with the p255M-02, -03, and -04 as probes. The RNA hybridized only with the p255M-02. Poly (A) selected RNA showed the same profile suggesting the RNA is mRNA and derived from a gene commonly expressed in wide spectrum of cell differentiation (Fig. 5) The region of 3' flanking sequence homologous to the 1.8 kb mRNA was surveyed with seven subclones covering p255M-12 in Fig. 1A, but it was unsuccessful because of very strong *Alu* signals (data not shown).

*Nucleotide sequence of 5' cellular flanking DNA:* To analyze the primary structure of the entire

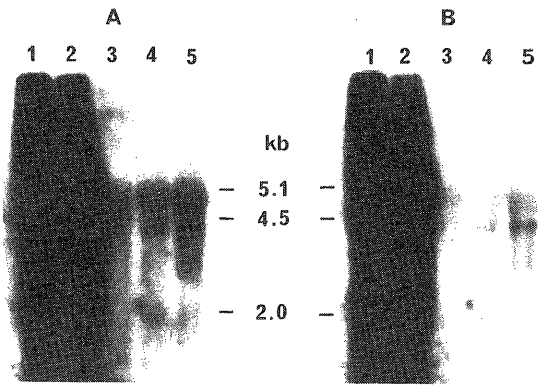


Fig. 4. Detection of 4.5 kb RNAs using pMT-2/64/17 as a probe. RNA sample, 10 μg of each, was applied per lane. A: The filter was washed with 0.1 × SSC, 0.1% SDS at 42℃, B: washed at 67℃. Lanes: 1, TL-Su; 2, MT-4; 3, Jurkat; 4, FLEB12-3-4; 5, ATLPBL.

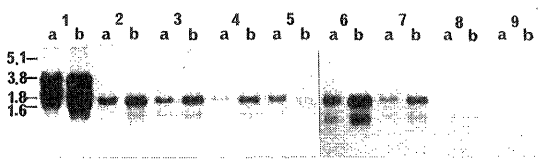


Fig. 5. Northern hybridization analysis of various human cellular RNAs using p255M-02 as a probe. Total cellular RNA (10 μg each, lane a) or polyA-selected RNA (1 μg each, lane b) was applied. Lanes: 1, TL-Su; 2, MT-4; 3, Jurkat; 4, FLEB12-3-4; 5, T24; 6, TL-OmI; 7, U937; 8, Rabbit spleen; 9, Mouse spleen.

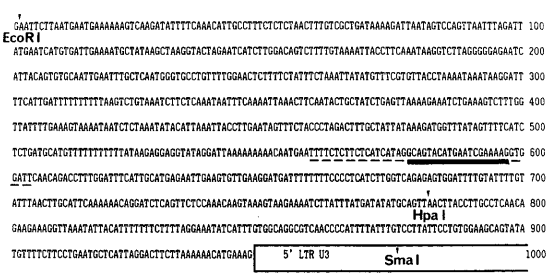


Fig. 6. Nucleotide sequence of the 5' cellular flanking DNA in λ255M. Numbering is from the EcoRI site. EcoRI, HpaI, and SmaI sites are shown by vertical arrows. Underline shows putative exon.

5' cellular flanking region, we determined the sequences using fragments of p255M-02 and 03 by dideoxy chain termination method on both strands (Fig. 6). The flanking DNA is extraordinary AT rich (GC content: 30%). The sequence did not include a typical initation codon that conforms reasonably well to Kozak's consensus sequence for 3' splice acceptor site was found at nucleotides 559 through 577 ($(Py)_{>11}$ NPyAGG) and 5' splice donor site at nucleotides 595 through 603 (AAGGTGAGT) (Mount 1982[33]). The data implies that the region of putative exon, the sequence between the splicing acceptor and donor sites, hybridized to the 1.8 kb mRNA in various human cells. Homologous sequence to this putative exon was not found in a data bank, LASL-GDB, release 48.0. *Modulation of cellular gene expression by insertion of HTLV-1:* The TL-Su cell from which λ255M originated was the only human cell not expressing the 1.8 kb mRNA, so far as tested. However, the cells expressed several species of mRNAs hybridizable to the p255M-02, 3.8, 3.2, 2.0 and 1.6 kb (Fig. 5, Lane 3). Using subfragments of junctional region (p255M-03) and viral genes (p255M-04 to -09) as probes, we tried to see if these mRNAs are read through or spliced into the viral gene. The excessive expression of viral RNA made the analysis difficult. However, in short term exposure of these northern blottings, none of these mRNAs hybridizable to p255M-02 corresponded with the major viral bands (for example, Fig. 3, lanes A1, B1 and D1). The results did not exclude the possibility of the presence of lower amount of RNA read through or spliced into the viral gene. On the other hand, as shown in Fig. 5, these mRNAs expressed in TL-Su cells are at least 10 times more abundant than 1.8 kb mRNA in other cells. These results indicated that the flanking cellular gene adjacent to proviral HTLV-1 genome may be qualitatively or quantitatively modulated, or both.

## DISCUSSION

Since HTLV-1 has no oncogene or no specific integration site (Seiki *et al.* 1983[44], 1984[45]), the function of its unique *pX* gene has been studied extensively. However, it seems difficult to explain leukemogenesis by the functions of *pX* gene alone. On the other hand, it has been well known that carcinogens or radiation hit DNA in a random fashion, and induce tumors. Therefore, we are interested in some possible role of flanking gene expression modified by integration of HTLV-1.

We cloned HTLV-1 provirus genome from TL-Su cell line established from PBL of a healthy carrier, and analyzed expression of its flanking DNA in various human cells. In λ255M, the proviral genome integrated into a cellular gene different from any of previously reported HTLV-1 proviral genomes, since λ255M had a 6-bp direct repeat (TGAAAG) on the external end of 5' and 3' LTRs, in comparison to λATM-1 (CATTCC) (Seiki *et al.* 1982[43]), λATK-1 (TAGTTC), and pMT-2-42 (GGATAT) (Ino *et al.* 1986[19]).

Northern hybridization analysis with the p255M-01 showed two species of RNA with the length of 4.5 and 1.8 kb. The 4.5 kb RNA is expressed in certain lymphoid cells, such as HTLV-1 related TL-Oml and ATLPBL and HTLV-1 uninfected Jurkat and FLEB12-3-4 cells. However, not all lymphoid cells were positive for the 4.5 kb RNA. For example, neither of an ATL lymphoma nor a CD4-positive cell line, CEM, was positive (data not shown). None of tested non-lymphoid cells was positive for the 4.5 kb RNA. We failed to define the cell types which express the 4.5 kb RNA. In HTLV-1 producers, we could not analyze the 4.5 kb RNA because of strong signals of viral RNA.

The 4.5 kb RNA hybridized to p255M-04 consisted of most LTR through 50 bp of *gag* gene, but to p255M-02 consisted of only the flanking region of λ255M. The result strongly suggested the presence of a human cellular gene homologous to HTLV-1 LTR, which may be a target of trans-acting transcriptional activation by $p40^{tax}$ in HTLV-1 infected cells. Detailed study is in progress.

The other 1.8 kb RNA was present in all human cells tested except for TL-Su cells from which λ255M originated. Since the rabbit and mouse spleen cells (Fig. 5) and the bovine cells (Fig. 2, lane 1) did not show any RNA hybridizable to the probe, we considered the finding to be specific. We fouud 3.8, 3.2, 2.0 and 1.6 kb mRNAs in TL-Su cells. Since the 5' flanking

region of 255M contained a splicing donor consensus sequence, these mRNAs transcribed from this cellular gene may use unusual splicing acceptors within the proviral genome. We tried to detect these mRNAs species using viral genome as probe, such as p255M-04 through -09. However it was unsuccessful because the extraordinary large amount of viral transcripts made it difficult to detect the unusually spliced mRNAs.

The results suggested that the provirus of λ255M integrated within a cellular gene whose expression is essential for most cells, although we have no information about the biological function of the 1.8 kb mRNA. However, the integration of HTLV-1 qualitatively modulated transcription of the gene in TL-Su cells as shown by unusual size of 1.8 kb mRNA species In addition, the same integration quantitatively modulated transcription of the gene as shown by at least 10-fold increase of the 1.8 kb mRNA species. The qualitative and/or quantitative modulation of cellular gene expression by HTLV-1 integration may affect normal cellular functions and lead to transformation.

On the other hand, oncogenesis is associated with recessive mutation rather than activation or alteration of genes, such as oncogenes. For example, specific chromosomal changes which result in homozygosity or hemizygosity for the 'mutant' or inactive allele are claimed as a key mechanism leading to tumor formation, in case of retinoblastoma or Wilms' tumor (KNUDSON 1985[28]). HTLV-1 integration into one of these genes may increase the possibility of malignant transformation of human T-cells. In this report, we have shown the first evidence of the insertional mutagenesis by HTLV-1 gene integration.

## ACKNOWLEDGMENTS

The authors are grateful to Drs. M. YOSHIDA, F. WANG-STAAL, K. SUGAMURA and I. MIYOSHI, for providing pATK-33, pMT-2/64/17, TL-OmI and TL-Su and MT-2 and MT-4, respectively. Part of this study was supported by Grant-in-Aid for Cancer Res. from the Japanese Ministry of Education, Science and Clure. They also thank to Japanese Cancer Res. Resorces Bank (JCRB)-Cell for U937.

## REFERFNCES

1) BISHOP, J.M. (1983) Cellular oncogenes and retroviruses. *Annu. Rev. Biochem.* **52**, 301-354.
2) BISHOP, J.M. Transforming genes. (1984)In: *RNA tumor viruses*, 2nd ed. vol. 1, edited by R. Weiss, N. Teich, H. Varmus & J Coffin, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., pp. 999-1108.
3) BISHOP, J.M. & VARMUS, H.E. (1984)Transforming genes. In: *RNA tumor viruses*, 2nd ed. vol. 2, edited by R. Weiss, N. Teich, H. Varmus & J. Coffin, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., pp. 249-356.
4) BISHOP, J.M. (1985) Viral oncogenes. *Cell.* **42**, 23-38.
5) CANN, A.J., ROSENBLATT, J.D., Wachsman, W., Shah, N.P. & Chen, I.S.Y. (1985)Identification of the gene responsible for human T-cell leukemia virus transcriptional regulation. *Nature,* **318**, 571-574.
6) CHIRGWIN, J.M, PRZYBYLA, A. E., MACDONALD, R.J. & RUTTER, W.J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochmistry.* **18**, 5294-5299.
7) CROSS, S.L., FEINBERG, M.B., WOLF, J.B., HOLBROOK, N.J., WONG-STAAL, F. & LEONARD, W.J. (1987 ) Regulation of the human interleukin-2 receptor α chain promotor: activation of a nonfunctional promotor by the transactivator gene of HTLV-I. *Cell,* **49**, 47-56.
8) FELBER, B.K., PASKALIS, H., KLEINMAN-EWING, C., WONG-STAAL, F. & PAVLAKIS, G.N. (1985) The *pX* protein of HTLV-I is a transcriptional activator of its long terminal repeats. *Science*, **229**, 675-679.
9) FRANKEL, W., POTTER, T.A., ROSENBERG, N., LENZ. J. & RAJAN, T.V. (1985) Retroviral insertional mutagenesis of target allele in a heterozygous murine cell line. *Proc. nat. Acad. Sci. USA,* **85**, 6600-6604.
10) FRISCHAUF, A.M., LEHRACH, H., POUSTKA, A. & MURRAY, N. (1983 ) Lambda replacement vectors carrying polylinker sequences. *J. mol. Biol.,* **170**, 827-842.
11) FUJISAWA, J., SEIKI, M., KIYOKAWA, T.&YOSHIDA, M. (1985) Functional activation of the long terminal repeat of human T-cell leukemia virus type I by a trans-acting factor. *Proc.*

nat. Acad Sci. USA, **82**, 2277-2281.

12) Fujisawa, J., Seiki, M., Sato, M. & Yoshida, M. (1986) A transcriptional enhancer sequence of HTLV-1 is responsible for transactivation mediated by $p40^{X}$ of HTLV-1. *EMBO J.*, **5**, 713-718.

13) Harada, S., Koyanagi, Y. & Yamamoto, N. (1985) Infection of HTLV-III /LAV in HTLV-I carrying cells MT-2 and MT-4 and application in a plaque assay. *Science*, **229**, 563-566.

14) Harbers, K., Kuehn, M., Delius, H.& Jaenisch, R. (1984) Insertion of retrovirus into the first intron of $\alpha 1$(I) collagen gene leads to embryonic lethal mutation in mice. *Proc. nat. Acad. Sci. USA*, **81**, 1504-1508.

15) Hawlyey, R.G., Shulman, M.J., Murialdo, H., Gibson, D.M & Hozumi, N. (1982) Mutant immunoglobulin genes have repetitive DNA elements inserted into their intervening sequences. *Proc. nat. Acad. Sci. USA*, **79**, 7425-7429.

16) Hayward, W.S., Neel, .G. & Astrin, S.M. (1981) Activation of a cellular *onc* gene by promoter insertion in ALV-induced lymphoid leukosis. *Nature*, **290**, 475-480.

17) Hino, S., Yamaguchi, K., Katamine, S., Sugiyama, H., Amagasaki ,T., Kinoshita, K., Yoshida, Y., Doi, H., Tsuji, Y. & Miyamoto, T. (1985) Mother-to-child transmission of human T-cell leukemia virus type-I. *Gann*, **76**, 474-480.

18) Hinuma, Y., Nagata, K., Hanaoka, M., Nakai, M., Matsumoto, T., Kinoshita, K., Shirakawa, S. & Miyoshi, I. (1981) Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc. nat. Acad. Sci. USA*, **78**, 6476-6480.

19) Iino, T., Takeuchi, K., Nam, S.H., Siomi, H., Sabe, H., Kobayashi, N. & Hatanaka, M. (1986) Structural analysis of p28 adult T-cell leukemia-associated antigen. *J. gen. Virol.* **67**, 1373-1379.

20) Inoue, J., Seiki, M., Taniguchi, T., Tsuru, S. & Yoshida, M. (1986) Induction of interleukin 2 receptor gene expression by $p40^{X}$ encoded by human T-cell leukemia virus type 1. *EMBO. J.*, **5**, 2883-2888.

21) Inoue, J., Yoshida, M. & Seiki, M. (1987) Transcriptional ($p40^{X}$) and post transcriptional ($p27^{X\text{-}III}$) regulators are required for the expression and replication of human T-cell leukemia virus type I genes. *Proc. nat. Acad. Sci. USA*, **84**, 3653-3657.

22) Josephs, S.F., Wong-Staal, F., Manzari, V., Gallo, R.C., Sodroski, J.G., Trus, M.D., Perkins, D., Patarca, R. & Haseltine, W.A. (1984) Long terminal repeat structure of an American isolate of type I human T-cell leukemia virus. *Virology*, **139**, 340-345.

23) Katamine, S., Otsu, M., Tada, K., Tsuchiya, S., Sato, T., Ishida, N., Honjo, T. & Ono, Y. (1984) Epstein-Barr virus transforms precursor B cells even before immunoglobulin gene rearrangements. *Nature*, **309**, 369-372.

24) King, W., Patel, M.D., Lobel, L.I., Goff S.P. & Nguyen-Huu, M.C. (1985) Insertion mutagenesis of embryonal carcinoma cells by retroviruses. *Science*, **228**, 554-558.

25) Kinoshita, K., Yamanouchi, K., Ikeda, S., Momita, S., Amagasaki, T., Soda, H., Ichimaru, M., Moriuchi, R., Katamine, S., Miyamoto, T. & Hino, S. (1985) Oral infection of a common marmoset with human T-cell leukemia virus type-I (HTLV-I) by inculating fresh human milk of HTLV-I carrier mothers. *Jpn. J. Cancer Res. (Gann)*, **76**, 1147-1153.

26) Kiyokawa, T., Seiki, M., Imagawa, K., Simizu, F. & Yoshida, M. (1984) Identification of a protein ($p40^{X}$) encoded by a unique sequence *pX* of human T-cell leukemia virus type I. *Gann*, **75**, 747-751.

27) Kiyokawa, T., Seiki, M., Iwashita, S., Imagawa, K., Shimizu, F. & Yoshida, M. (1985) $p27^{X\text{-}III}$ and $p21^{X\text{-}III}$, proteins encoded by the *pX* sequence of human T-cell leukemia virus type I. *Proc. nat. Acad. Sci. USA*, **82**, 8359-8363.

28) Knudson, A.G.,Jr. (1985) Hereditary cancer, oncogenes, and antioncogenes. *Cancer Res.*, **45**, 1437-1443.

29) Kozak, M. (1984) Compilation and analysis of sequence upstream from the translational start site in eukaryotic mRNAs. *Nucleic Acids Res.*, **12**, 857-872.

30) Lee, T.H., Coligan, J.E., Sodroski, J.G., Haseltine, W.A., Salahuddin, S.Z., Wong-Staal, F., Gallo, R.C. & Essex, M. (1984) Antigens encoded by the 3'-terminal region of human T-cell leukemia virus: evidence for a functional gene. *Science*, **226**, 57-61.

31) Miwa, M., Shimotohno, K., Hoshino, H., Fujino, M. & Sugimura, T. (1984) Detection of *pX* proteins in human T-cell leukemia virus (HTLV) infected cells by using antibody against peptide deduced from sequences of *X*-IV DNA of HTLV-I and *Xc* DNA of HTLV-II proviruses. *Gann*, **75**, 752-755.

32) Miyoshi, I., Taguchi, H., Kubonishi, I.,

YOSHIMOTO, S., OHTSUKI, Y., SHIRAISHI, Y. & AKAGI, T. (1982) Type C-virus producing cell lines derived from adult T-cell leukemia. *Gann Monogr. Cancer Res.*, **28**, 219-228.

33) MOUNT, S.M. (1982) A catalogue of splice junction sequences. *Nucleic Acids Res.*, **10**, 459-472.

34) NILSEN, T.W., MARONEY,P.A., GOODWIN, R.G., ROTTMAN, F.M., CRITTENDEN, L.B., RAINES, M.A. & KUNG, H.J. (1985) c-*erb* B Activation in ALV-induced erythroblastosis: novel RNA processing and promoter insertion result in expression of an amino-truncated EGF receptor. *Cell*, **41**, 719-726.

35) NUSSE, R. & VARMUS, H. (1982) Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell*, **31**, 99-109.

36) NUSSE, R., OOYEN, A., COX, D., FUNG, Y.K.T. & VARMUS, H. (1984) Mode of proviral activation of a putative mammary oncogene (*int*-1) on mouse chromosome 15. *Nature*, **307** 131-136.

37) PASKALIS, H., FELBER, B.K. & PAVLAKIS, G.N. (1986) Cis-acting sequences responsible for the transcriptional activation of human T-cell leukemia virus type-I constitute a conditional enhancer. *Proc. nat Acad. Sci. USA*, **83**, 6558-6562.

38) PAYNE, G.S., BISHOP, J.M. & VARMUS, H.E. (1982) Multiple arrangements of viral DNA and an activated host oncogene in bursal lymphomas. *Nature*, **295**, 209-214.

39) POIESZ, B.J., RUSCETTI, F.W., GAZDAR, A.F., BUNN, P.A., MINNA, J.D. & GALLO, R.C. (1980) Detection and isolation of type-C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. nat. Acad. Sci. USA*, **77**, 7415-7419.

40) SALMON, D.J., SHIMOTOHNO, K., CLINE, M.J., GOLDE, D.W. & CHEN, I.S.Y. (1984) Identification of the putative transforming protein of the human T-cell leukemia viruses HTLV-I and HTLV-II. *Science*, **226**, 61-65.

41) SANGER, F., NICKLEN, S. & COULSON, A.R. (1977) DNA sequencing with chain terminating inhibitors. *Proc. nat. Acad. Sci. USA*, **74**, 5463-5467.

42) SCHNIEKE, A., HARBERS, K. & JAENISCH, R. (1983) Embryonic lethal mutation in mice induced by retrovirus insertion into the $\alpha$1(I) collagen gene. *Nature*, **304**, 315-320.

43) SEIKI, M., HATTORI, S. & YOSHIDA, M. (1982)Human adult T-cell leukemia virus: molecular cloning of the provirus DNA and the unique terminal structure. *Proc. nat. Acad. Sci. USA*. **79**, 6899-6902.

44) SEIKI, M., HATTORI, S., HIRAYAMA, Y.& YOSHIDA, M. (1983) Human adult T-cell leukemia virus: complete nucleotide sequeuce of the provirus genome integrated in leukemia cell DNA. *Proc. nat. Acad. Sci. USA*, **80**, 3618-3622.

45) SEIKI, M., EDDY, R., SHOWS, T.B. & YOSHIDA, M. (1984) Nonspecific integration of the HTLV provirus genome into adult T-cell leukemia c ells. *Nature*, **309**, 640-642.

46) SEIKI, M., HIKIKOSHI, A., TANIGUCHI T. & YOSHIDA, M. (1985a) Expression of the *pX* gene of HTLV-I: general splicing mechanism in the HTLV family. *Science*, **228**, 1532-1534.

47) SEIKI, M., INOUE, J., TAKEDA, T., HIKIKOSHI, A., SATO, M. & YOSHIDA, M. (1985b) The $p40^X$ of human T-cell leukemia virus type-I is a *trans*-acting activator of viral gene transcription. *Jpn. J. Cancer Res. (Gann)*, **76**, 1127-1131.

48) SEIKI, M., INOUE, J., TAKEDA, T. & YOSHIDA, M. (1986) Direct evidence that $p40^X$ of human T-cell leukemia virus type-I is a trans-acting transcriptional activator. *EMBO J.*, **5**, 561-565.

49) SHIMOTOHNO, K., TAKANO, M., TERUUCHI, T. & MIWA, M. (1986) Requirement of multiple copies of a 21-nucleotide sequence in the U3 regions of human T-cell leukemia virus type-I and type-II long terminal repeats for trans-acting activation of transcription. *Proc. nat. Acad. Sci. USA*, **83**, 8112-8116.

50) SODROSKI, J.G., ROSEN, C.A.& HASELTINE, W.A. (1984) Trans-acting transcriptional activation of the long terminal repeat of human T-lymphotropic viruses in infected cells. *Science*, **225**, 381-385.

51) SODROSKI, J.G., ROSEN, C., GOH, W.C. & HASELTINE, W. (1985) A transcriptional activator protein encoded by the x-*lor* region of the human T-cell leukemia virus. *Science*, **228**, 1430-1434.

52) SOUTHERN, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. mol. Biol.* **98**, 503-517.

53) STERNBERG, N., TIEMEIER, D. & ENQUIST, L. (1977) In vitro packaging of a lambda dam vector containing EcoRI DNA fragments of *Escherichia coli* and phage P1. *Gene*, **1**, 255-280.

54) SUGAMURA, K., FUJII, M., KANNAGI, M., SAKITANI, M., TAKEUCHI, M. & HINUMA, Y. (1984) Cell surface phenotypes and expression of viral antigens of various human cell lines carrying human T-cell leukemia virus. *Int. J. Cancer*, **34**, 221-228.

55) UCHIYAMA, T., YODOI, J., SAGAWA, K., TAKATSUKI, K, & UCHINO, H. (1977) Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* **50,** 481-492.
56) VARMUS, H.E., QUINTRELL, N. & ORTIZ, S. (1981) Retroviruses as mutagens: insertion and excision of a non-transforming provirus alter expression of a resident transforming provirus. *Cell,* **25,** 23-36.
57) WATANABE, T., SEIKI, M. & YOSHIDA, M. (1984) HTLV type-I (U.S. isolate) and ATLV (Japanese isolate) are the same species of human retrovirus. *Virology,* **133,** 238-241.
58) WOLF, D. & ROTTER, V. (1984) Inactivation of p53 gene expression by an insertion of Moloney murine leukemia virus-like DNA sequence. *Mol. cell. Biol.,* **4,** 1402-1410.
59) WONG-STAAL, F., HAHN, B., MANZARI, V., COLONBINI, S., FRANCHINI, G., GELMANN, E.P. & GALLO, R.C. (1983) A survey of human leukaemias for sequence of a human retrovirus. *Nature,* **302,** 626-628.
60) YOSHIDA, M., MIYOSHI, I. & HINUMA Y. (1982) Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc. nat. Acad. Sci. USA,* **79,** 2031-2035.
61) YOSHIDA, M., SEIKI, M., YAMAGUCHI, K & TAKATSUKI, K. (1984) Monoclonal integration of human T-cell leukemia provirus in all primary tumors of adult T-cell leukemia suggests causative role of human T-cell leukemia virus in the disease. *Proc. nat. Acad. Sci. USA,* **81,** 2534-2537.