

Subcellular Localization of the Product of the Long Open Reading Frame of Human T-Cell Leukemia Virus Type I

Abstract. Human T-cell leukemia virus type I (HTLV-I) is a retrovirus associated with adult T-cell leukemia and lymphoma. In addition to containing the gag, pol, and env genes of the chronic leukemia viruses, the genome of HTLV-I contains a long open reading frame (LOR) located between the 3' end of the envelope gene and the 3' long terminal repeat sequence (LTR). It has been suggested that a protein of 42 kilodaltons that is encoded by the LOR region may participate in both trans-acting transcriptional regulation of the viral LTR as well as in the transforming properties of HTLV-I. It is reported here that a significant fraction of the 42-kilodalton HTLV LOR product is located in the nucleus of HTLV-I-infected transformed lymphocytes, a finding that is consistent with its proposed functions.

Human T-cell leukemia virus type I (HTLV-I) is a retrovirus that is thought to be the etiologic agent of adult T-cell leukemia-lymphoma (ATLL) (1, 2). Infection with HTLV-I results in a low incidence of ATLL after a long latent period. The mechanism of disease induction by HTLV-I differs from that of the chronic leukemia viruses (3), however, in that no specific chromosomal sites of proviral integration have been detected in HTLV-I-induced tumors (4). Moreover, HTLV-I transforms primary cells in culture (5), heretofore a property associated only with the acute retroviruses that contain oncogenes derived from the host cell. HTLV-I does not appear to contain an oncogene, because its genome lacks sequences that are similar to conserved cellular genes (6). The genomes of HTLV-I and of the related virus HTLV-II contain long open reading frames (LOR) located between the envelope gene and the 3' long terminal repeat (LTR). The LOR region encodes proteins of 42 kilodaltons (kD) (HTLV-I) and 38 kD (HTLV-II) (6, 7, 8). This raises the possibility that the product of the LOR region mediates both the transforming activity of these viruses and trans-acting transcriptional activation of the viral LTR evident in HTLV-I-infected cells (9). To obtain insight into the possible mechanism whereby the LOR product might exert these effects, we characterized the intracellular location of the 42-kD LOR product of HTLV-I.

The lymphocyte cell lines used for this work were HUT 102, a virus-producing line established from a patient with adult T-cell leukemia (1, 10), and C81-66-45, a T-cell line derived by fusion of primary umbilical cord blood cells with HUT 102 (11). The virus-encoded proteins present in HUT 102 cells include the gag gene products (phosphoprotein 19, p24, p15, and p55), and env gene products (glycoproteins gp61, gp45, and gp21) (12, 13) as well as a 42-kD protein recently demonstrated to be encoded by the LOR region of this genome (Fig. 1) (7). Previous

studies have shown that the 42-kD protein is neither a phospho- nor a glycoprotein and cannot be iodinated by treatment of the intact cells with lactoperoxidase (7, 13). The 42-kD LOR protein is recognized by serum from some ATLL patients and by serum from some infected but asymptomatic HTLV-I carriers

(7, 13). The C81-66-45 cell line does not produce virus, and the only viral protein detected by serum from ATLL patients in this cell line is the 42-kD LOR product (7).

To identify the subcellular location of the HTLV-I LOR region product, we labeled cells of both lines with [³⁵S]cysteine and then separated them into nuclear and membrane-cytoplasmic fractions by treatment with a nonionic detergent in the presence of an iso-osmotic solution under conditions that stabilize the nuclear membrane (14). The nuclei were then separated from the other cellular components by differential centrifugation. The membrane-cytoplasmic fraction was further cleared of insoluble material by a high-speed centrifugation.

The nuclear fraction appeared to be free of intact cells under microscopic inspection. Assays for the lysosomal enzyme β -N-acetylglucosaminidase (15),

Fig. 1. Open reading frame (LOR) in the 3' region of the HTLV genome. The positions of the nonconserved region between HTLV-I and HTLV-II and the single open reading frame encoding the p42 LOR product are shown. The splice acceptor site used in production of the LOR message is indicated (8).

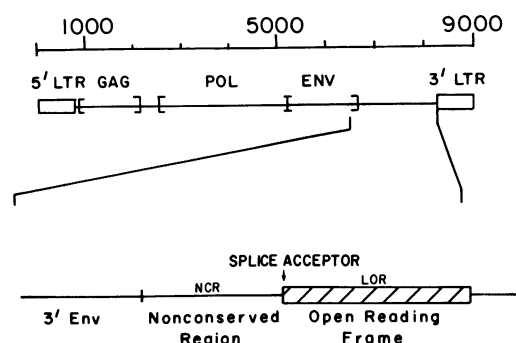
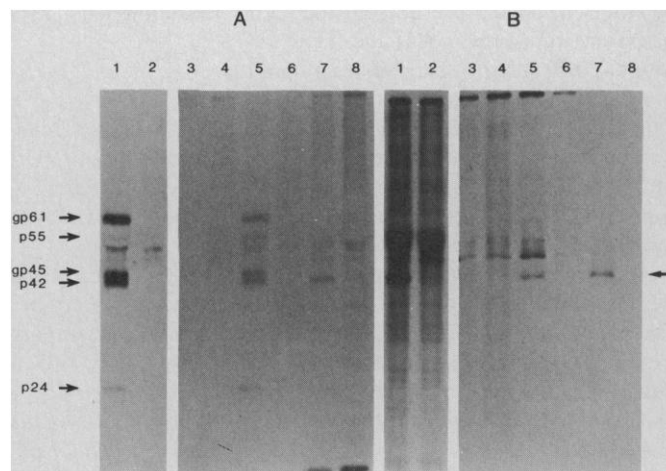


Fig. 2. Subcellular localization of the p42 LOR product. HUT 102 and C81-66-45 cells were labeled with [³⁵S]cysteine for 12 hours in RPMI 1640 medium containing 20 percent fetal calf serum. Cells were washed twice with cold phosphate-buffered saline and resuspended (5×10^7 cells per milliliter) in buffer A [10 mM tris-Cl, pH 8.5, 0.25M sucrose, 3.7 mM CaCl₂, 12 mM MgCl₂, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1 percent NP40]. The cells were incubated for 20 minutes on ice and pipetted up and down gently. A portion was removed for microscopic analysis. Nuclei were centrifuged at 600g for 5 minutes at 4°C and washed once with buffer A. The supernatant, termed the S-100 fraction, was further centrifuged at 100,000g. Pelleted debris was called the P-100 fraction. The nuclear and P-100 fractions were resuspended in RIPA lysis buffer (0.15M NaCl, 0.05M tris-HCl, pH 7.2, 1 percent Triton X-100, 1 percent sodium deoxycholate, and 0.1 percent sodium dodecyl sulfate) while the S-100 fraction was adjusted to 1 percent Triton X-100. (A and B) Immunoprecipitations of HUT 102 and C81-66-45 cells, respectively. The amount of the cell fraction used for the immunoprecipitation corresponds to the amount derived from equivalent cell numbers in each experiment. Lanes 1 and 2, total unfractionated cell lysates; lanes 3 and 4, the P-100 fractions; lanes 5 and 6, the S-100 fractions; lanes 7 and 8, the nuclear fractions. Serum samples from ATLL patients (lanes 1, 3, 5, and 7) and normal subjects (lanes 2, 4, 6, and 8) that were described previously (7, 13) were used.



which provides an independent biochemical marker for cytoplasmic contamination of the nuclear fraction, indicated that only about 7 percent of the total cellular activity of the enzyme was in the nuclear fraction. Moreover, the nuclei remained intact, as most of the DNA is retained in the nuclear fraction as demonstrated by the fractionation of cells labeled with [³H]thymidine (Table 1).

The location of the 42-kD protein in the cell fractions was determined by immunoprecipitation of the labeled proteins with serum from an ATLL patient. This serum had previously been shown to react strongly with the 42-kD protein as well as with HTLV-I *gag* and *env* gene products (7, 13). All three fractions, nuclear, membrane-cytoplasmic (S-100), and the resuspended pellet of the S-100 fraction (P-100) were tested.

The reactivity of the ATLL patient serum for the unfractionated HUT 102 and C81-66-45 cells is compared to that of normal human serum in Fig. 2. The 42-kD LOR protein was evident in both immunoprecipitates. When the different fractions were tested separately, a significant amount of the 42-kD LOR protein was found in the nuclear fraction of both HUT 102 and C81-66-45 cells. By contrast, the p24 and p55 *gag* products as well as the gp61 and gp45 *env* gene products were totally absent from the nuclear preparations (Fig. 2), providing further evidence of the lack of cytoplasmic or outer membrane contamination in the nuclear fraction. These viral proteins are known to be present in the cell membrane (gp61 and gp45) and the cytosol (p55, p24) (12, 13). Negligible amounts of viral proteins were detected in the P-100 fractions. Densitometric tracing of the autoradiograms of the immunoprecipitated protein in the subcellular fractions showed that between 50 and 60 percent of the 42-kD LOR protein was present in the nuclear fraction of both the HUT 102 and C81-66-45 cell lines. The actual fraction of the 42-kD protein present in the nucleus is likely to be higher because some of it may have been lost from the nuclei during the isolation procedure.

From these data we conclude that a significant fraction of the 42 kD protein is located within the nucleus of HTLV-I-transformed lymphocytes. The results also suggest that a fraction of the 42-kD protein is located outside the nucleus. Such nuclear localization of a retrovirus-coded protein, other than those proteins encoded by cell-derived oncogenes including the *myc*, *fos* and *myb* (16), has not, to our knowledge, been reported previously. The product of the HTLV-I

Table 1. Assays for β -N-acetylglucosaminidase in subcellular fractions and incorporation of [³H]thymidine.

Fraction	Activity of β -N-acetylglucosaminidase* (%)	[³ H]Thymidine incorporation† (%)
S-100	93	4
Nuclear	7	96

*Cells were fractionated with buffer A as described in Fig. 2, sonicated and centrifuged at 10,000 rev/min for 10 minutes to remove debris. Lysates from the S-100 and nuclear fractions were incubated in 0.1M sodium citrate buffer (pH 4.5), 0.16 percent Triton X-100, and 1 mM *p*-nitrophenol- β -N-acetylglucosaminide for 60 minutes at 37°C. Reactions were stopped by the addition of sodium carbonate buffer and the optical densities at 420 nm were determined. †Cells were labeled with [³H]thymidine at 1 μ Ci/ml for 16 hours, washed with phosphate-buffered saline thrice, and fractionated as described in Fig. 2. Portions of the cell fractions were precipitated with cold trichloroacetic acid and tested for incorporation of radioactivity by scintillation counting.

LOR region has been implicated in the transcriptional regulation of the LTR, as well as in mediation of cell transformation by this virus, for example, in the immortalization of primary lymphocytes in vitro (6, 9, 17). It has been suggested that the HTLV LOR region product might exert these effects by way of sequence-specific transcriptional regulation of both viral LTR and host cellular genes (9). The nuclear location of the 42-kD HTLV-I LOR product is consistent with these roles. We note that the transforming proteins of some DNA tumor viruses, which also exert transcriptional regulatory effects, are also located within the nucleus (18).

WEI CHUN GOH

JOSEPH SODROSKI

CRAIG ROSEN

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

MAX ESSEX

Department of Cancer Biology,
Harvard School of Public Health,
Boston, Massachusetts 02115

WILLIAM A. HASELTINE

Dana-Farber Cancer Institute and
Department of Cancer Biology,
Harvard School of Public Health

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