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lck Suppresses Gene Expression from Various Promoters Including Human T-Cell Leukemia Virus Type I Promoter

Masataka Ohta, Toshirou Morita and Kunitada Shimotohno

Virology Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104

The T-lymphocyte-specific tyrosine kinase gene, *lck*, is expressed in T-lymphocyte cell lines, except for several human T-cell leukemia virus type I(HTLV-I)-transformed T-lymphocyte cell lines, which produce HTLV-I. By introducing an *lck*-expression vector, we found that *lck* product suppresses gene expression from HTLV-I promoter in a transient assay. Moreover, various other promoters of cellular genes or viruses were found to have their transcriptional activity repressed by *lck*.

Key words: *lck* — HTLV-I — Promoter

The *lck* gene is a member of the *src*-related gene family that encodes a class of closely related tyrosine-specific protein kinases. The product of *lck* gene was originally identified as a 56 kilodalton (kDa) protein ($p56^{lck}$) and was shown to be a membrane-associated phosphoprotein abundantly present in murine T-cell lines (LSTRA) derived from a thymoma induced by the Moloney murine leukemia virus (MoMuLV).[1] In LSTRA cell lines, the MoMuLV genome is interposed between two *lck* promoters that normally generate *lck* transcripts differing in the 5′ untranslated regions.[2,3] The interposition of MoMuLV permits the generation of an *lck* mRNA with a novel 5′ untranslated region that may be more efficiently translated, resulting in the overexpression of *lck*.[4–6] Human *lck* cDNA was isolated from T-cell leukemia cell line JURKAT by two different groups.[7,8] Human *lck* gene is located at chromosome 1 at 1p32-35 near a site of frequent structural abnormalities in human lymphoma and neuroblastoma.[9] These studies raise the possibility that alteration of *lck* gene through chromosomal rearrangement may contribute to cellular transformation in malignancy.

The expression of murine and human *lck* gene is restricted to cells of T-lymphoid lineage, although human *lck* gene is expressed ectopically in several cell lines derived from colon carcinoma and small cell lung cancer.[1,10–12] These findings suggested that $p56^{lck}$ plays some specific role in T-lymphocytes. It has been shown that $p56^{lck}$ is functionally and physically associated with CD4/CD8 T-cell antigen.[13–18] Furthermore $p56^{lck}$ is activated by CD45, the phosphotyrosine phosphatase which binds to the cell-surface membrane of leukocytes.[19,20] These findings suggest that $p56^{lck}$ is an important regulatory molecule that mediates signal transduction in T-cells.

Recently, it has been reported that some T-lymphocyte cell lines, established *in vitro* by infection with HTLV-I, are negative for *lck* expression.[21] One of the features of these cell lines is that they express viral mRNA. This finding raises the possibility that the infection of HTLV-I somehow down-regulates the expression of *lck*, or that the gene expression from HTLV-I provirus is inhibited by the *lck* product. The latter possibility implies that viral gene expression is suppressed in *lck*-positive lymphocytes, and thus, immortalization, which is induced by the viral putative transforming proteins, *tax* and/or *rex*,[22] would be blocked in these lymphocytes. At the same time, the *lck*-negative T-cells, which could express viral transforming genes, would grow in culture, resulting in the generation of an immortalized cell line. To explore whether the HTLV-I-gene expression is inhibited by the *lck* product, we studied the effect of the *lck* expression on transcriptional activities of HTLV-I promoter, and we found that *lck* suppresses gene expression from HTLV-I and various other gene promoters in a transient assay.

YT16 is a plasmid containing full-length human *lck* cDNA derived from JURKAT cell line.[12] The *Nco*I/*Hin*dIII fragment of YT16, containing the entire open reading frame of *lck*, was subcloned into *Hin*dIII/*Eco*RI fragment of pCMVCAT with *Hin*dIII/*Nco*I linker and SV40 DNA fragment containing a polyadenylylation signal[23,24] (Fig. 1). In the resultant construct, pCLS, *lck*-gene expression was directed by cytomegalovirus promoter. When introduced into COS-1 cells, pCLS expressed a 2.7 kb mRNA, the predicted size, as evidenced by northern (RNA) blot analysis (data not shown). As a negative control, another construct, pCLA, was created which has a similar structure to pCLS except that *lck* cDNA was arranged in the opposite orientation.

Abbreviations: HTLV-I, human T-cell leukemia virus; ATL; adult T-cell leukemia; LTR, long terminal repeat; CAT, chloramphenicolacetyltransferase.

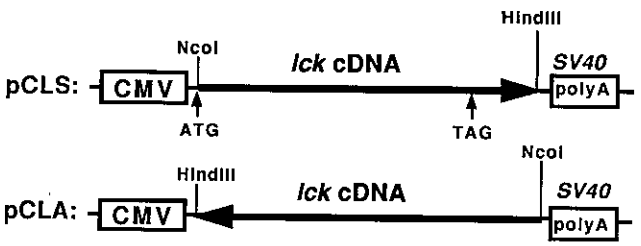

Fig. 1. Schematic representation of the *lck*-expressing vector pCLS or non-expressing plasmid pCLA constructed as described in the text.

pILTRCAT is a construct which carries a chloramphenicol acetyltransferase (CAT) gene which is directed by HTLV-I promoter as described elsewhere.[25] pILTRCAT was co-transfected into COS-1 cells with pCLS or PCLA by the calcium phosphate precipitation method as described previously.[26] Co-transfection of pILTRCAT with PCLA or other vector plasmids, which do not carry the structure to express gene products in eukaryotic cells, had no significant effect on CAT gene expression from pILTRCAT (data not shown). After incubation for 48 h at 37°C and 5% CO_2, the cell extract was prepared and CAT enzyme activities were assayed as previously described.[24] The indicated transfections were performed in parallel at least three times independently. The amounts of cell extract and the reaction time were determined arbitrarily in each set of experiments to compare the CAT activities within the linear range of reaction.

Co-transfection of pILTRCAT with pCLS makedly reduced the CAT activity expressed from pILTRCAT (Fig. 2, A; lanes a and b). Since pCLS carries the same promoter element as the control plasmid pCLA, it is suggested that the suppression by pCLS is not due to removal of limiting factor(s) by the cytomegalovirus promoter. The extent of suppression was dependent on the dose of pCLS DNA that was co-transfected (Fig. 2, B). This suppression was confirmed by northern (RNA) blot analysis using CAT gene DNA as a probe (Fig. 2, C). However, co-transfection of a v-*src*-expressing vector resulted in enhancement of CAT activity expressed from pILTRCAT (data not shown), as has already been reported.[27,28]

In order to examine the effect of *lck* product on gene expression from other promoters, the same sets of experiments were performed using other promoter-CAT constructs. The results show that expression of *lck* suppresses gene expression from promoters derived from various genes or viruses (Fig. 3). These include human immunodeficiency virus (Fig. 3, lane a; pH3LTRCAT),[29] Raus sarcoma virus (Fig. 3, lane b; pRSVCAT),[30]

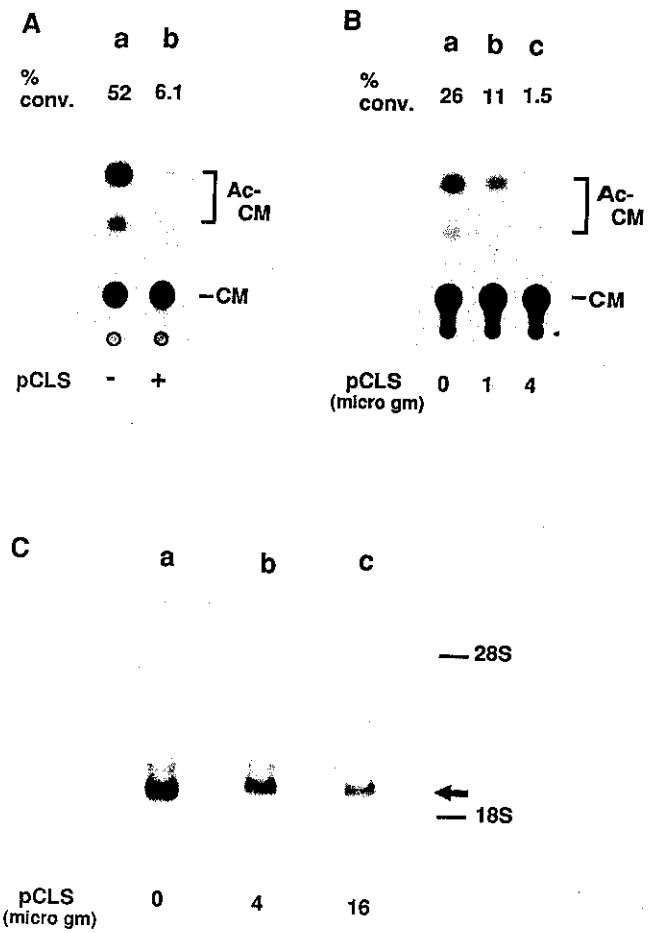

Fig. 2. *lck* Product suppresses gene expression from HTLV-I promoter in a transient CAT assay (A, B) and northern blot analysis (C). A: One μg of pILTRCAT DNA was co-transfected into COS-1 cells with 4 μg of pCLA (a), or pCLS (b) in tissue culture dishes 3.5 cm in diameter. CAT assays were performed as described in the text. The figure at the top of each lane represents percent conversion rate of acetylated chloramphenicol versus total chloramphenicol, which is measured as described previously.[24] This experiment was repeated independently more than 3 times and representative data are shown. Abbreviations; Ac-CM, acetylated chloramphenicol; CM, chloramphenicol. B: One μg of pILTRCAT was co-transfected with 4 μg of pCLA (a), 1 μg of pCLS plus 3 μg of PCLA (b), or 4 μg of pCLS (c). CAT assays were performed as described in A. C: 4 μg of pILTRCAT DNA was co-transfected into COS-1 cells with 16 μg of pCLA (a), 4 μg of pCLS plus 12 μg of pCLA (b), or 16 μg of pCLS (c) in tissue culture dishes 10 cm in diameter. Total cellular RNA was extracted after incubation for 48 h, and 2 μg of the poly(A)$^+$ RNA was analyzed by northern blot analysis as described elsewhere[34] using the radiolabeled *Hin*dIII/*Bam*HI CAT DNA fragment from pCMVCAT as a probe. The arrow indicates the 2.3-kilobase mRNA from pILTRCAT.[25] The origin of a minor band above the main band is not known, but may represent a read-through product from pILTRCAT.

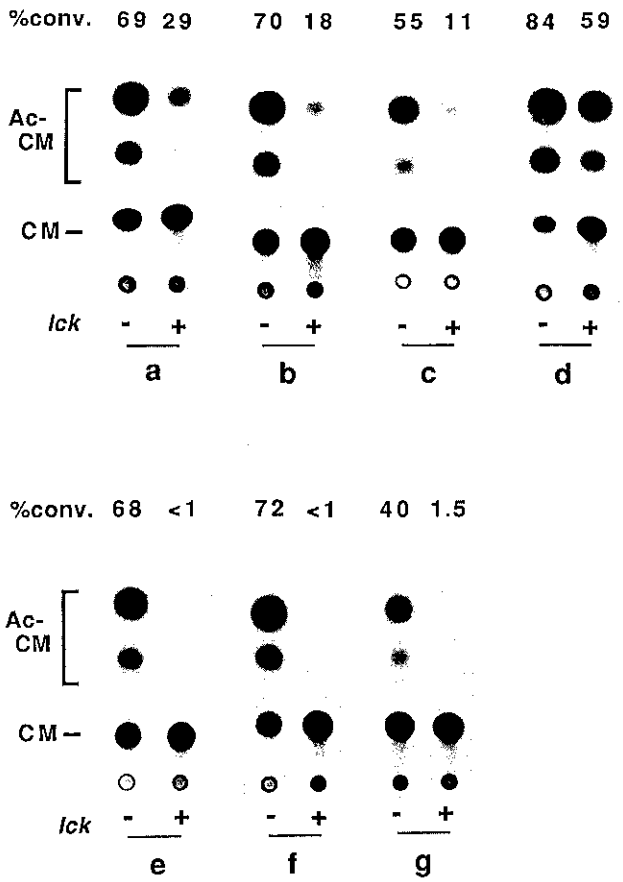

Fig. 3. *lck* Product suppresses gene expression from various gene promoters. One μg of various promoter-CAT constructs (a, pH3LTRCAT; b, pRSVCAT; c, pCMVCAT; d, pSV2CAT; e, pXATCAT; f, pSI319CAT; g, pSRPXCAT) was co-transfected with 4 μg of pCLA (left lane in each pair of experiments) or 4 μg of pCLS (right lane in each pair of experiments). CAT assays were performed and data are represented as described in the legend to Fig. 2, A.

cytomegalovirus (Fig. 3, lane c; pCMVCAT),[23] c-*myc* (Fig. 3, lane e; pXATCAT; the promoter contains rat c-*myc* gene fragment from the *SalI* site in Exon I and the adjacent *SalI* site in the 5′ flanking region), interleukin 2 (Fig. 3, lane f; pSI319CAT)[31] and interleukin 2 receptor α (Fig. 3, lane g; pSRPXCAT).[31] The extent of suppression was variable among these promoters. However, the expression of *lck* had a negligible effect on gene expression from SV40 early promoter (Fig. 3, lane d; pSV-2CAT).[30]

These data suggest that the product of *lck* suppresses the expression of various genes. Although the mechanism of suppression by *lck* gene is not known at present, the suppressive effect on various gene promoters suggests some common step(s) in the general gene transcription process. In this context, the observation that *lck* hardly suppresses SV40 early promoter may help to identify this common step. Since *lck* protein is a membrane-bound tyrosine kinase, suppression might be mediated by the specific phosphorylation of tyrosine residues of cellular phosphoproteins. This possibility is interesting in the light of the recent observation that v-*src*, an activated form of the same *src*-family gene, activates transcription from various promoters including HTLV-I promoter.[27] Possibly, the difference in substrate specificity between the two tyrosine kinases may reflect the mode of suppression or activation of gene transcription. In other words, some proteins, which are phosphorylated on tyrosine residues, may mediate the functions of these two closely related, *src*-family genes through reciprocal modes of gene transcription.

Suppression of gene transcription from HTLV-I promoter suggests some biological significance of *lck* in viral expression *in vivo*. *lck* is not expressed in some HTLV-I-producing T-cell lines.[22] However, fresh peripheral blood lymphocytes from adult T-cell leukemia (ATL) patients express *lck* mRNA, but do not express HTLV-I mRNA at a level detectable by northern blot analysis (unpublished observation). In view of these observations, our result suggests that *lck* product may be a suppressor of HTLV-I-gene expression in ATL tumor cells. To test this possibility, it is important to examine whether the levels of HTLV-I expression depends on the extent of *lck* expression.

lck also supppresses gene expression from various cellular gene promoters. Since *lck* is expressed in normal resting T-cells, and these cells are in the G0 phase of the cell cycle where most cellular gene expression is kept at low levels, $p56^{lck}$ may, presumably, exist as a suppressor of many cellular genes in quiescent T-lymphocytes, and activation of lymphocytes may, somehow, be followed by turning off of this suppression mechanism, resulting in the activation of various gene promoters. This is consistent with the observation that the expression of $p56^{lck}$ is down-regulated during T-cell activation.[32] However, this possibility is disfavored by the finding that the tyrosine-specific kinase activity of *lck* is increased by CD4-cross-linking mediated by antibodies against the extracellular domain of CD4.[13-17] Moreover, recent studies suggest that CD45-phosphotyrosine phosphatase, whose presence in T-cells is essential for T-cell activation, activates $p56^{lck}$ via the dephosphorylation of the tyrosine at the 505th amino-acid residue (tyrosine-505), a putative negative regulatory site.[19, 20, 33] These studies suggest that the activation of lymphocytes is accompanied with the activation of *lck*-tyrosine kinase through dephosphorylation at tyrosine-505, and that $p56^{lck}$, which is present in resting T-cells, is an inactive form of tyrosine kinase.

These results may argue against our hypothesis that the tyrosine-kinase activity of *lck* suppresses gene transcription in resting lymphocytes. However, not all of the $p56^{lck}$ molecules in a normal T-cell are suggested to be phosphorylated on tyrosine-505. The molecules which are not phosphorylated at tyrosine-505 in resting T-cells may have significant basal enzymatic activity,[20] and be responsible for the suppression of gene expression. Alternatively, *lck* protein, which is phosphorylated on tyrosine-505, may have a suppressive effect on gene transcription. Another possibility is that the crosslinking of CD4 may result in transient alterations of the cellular localizations of both CD4 and $p56^{lck}$, allowing tyrosine kinase to interact with different substrates, as suggested by Veillette *et al.*[13] Such alterations in the substrate-specificity of $p56^{lck}$ may lead to changes in the suppressive function of *lck*. To investigate these possibilities, we are examining whether the increase in the tyrosine-kinase activity of *lck* has a positive correlation to the suppressive effect of *lck* on gene expression.

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