MOLECULAR AND CELLULAR BIOLOGY, Sept. 1992, p. 3784–3795
0270-7306/92/093784-12$02.00/0

Vol. 12, No. 9

Characterization of the Promoter Region of the *src* Family Gene *lyn* and Its *trans* Activation by Human T-Cell Leukemia Virus Type I-Encoded $p40^{tax}$

FUMIAKI UCHIUMI,[1] KENTARO SEMBA,[1] YUJI YAMANASHI,[1] JUN-ICHI FUJISAWA,[2] MITSUAKI YOSHIDA,[2] KAZUSHI INOUE,[3] KUMAO TOYOSHIMA,[3] AND TADASHI YAMAMOTO[1]*

Department of Oncology[1] and Department of Cellular and Molecular Biology,[2] Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108, and Department of Oncogene Research, Research Institute for Microbial Diseases, Osaka University, 3-1, Yamadaoka, Suita, Osaka 565,[3] Japan

Received 30 January 1992/Returned for modification 28 April 1992/Accepted 9 June 1992

The *src* family gene *lyn* is expressed preferentially in B lymphocytes but very little in normal T lymphocytes. Transcription of the *lyn* gene in T lymphocytes was shown to be induced by the $p40^{tax}$ protein encoded by human T-cell lymphotropic virus type I. For determination of the mechanism of $p40^{tax}$-mediated *trans* activation, the transcriptional promoter region of the *lyn* gene was characterized. By endonuclease S1 mapping, the transcriptional initiation sites were identified within the 770-bp *Eco*RI-*Sac*I fragment of the 5′-terminal portion of the human *lyn* gene. This fragment showed promoter activity when placed upstream of the bacterial chloramphenicol acetyltransferase gene and transfected into various cell lines. Nucleotide sequence analysis revealed that the *lyn* promoter region contained four GC box-like sequences but not a TATA or CCAAT box. In addition, it contained sequences characteristic of a cyclic AMP-responsive element, octamer-binding motif, PEA3-like motifs, and NFκB-binding motif-like sequence. Mutational analysis suggested that the octamer-binding motif sequence is of primary importance for the *lyn* promoter activity but that the other elements are not. Cotransfection of various chloramphenicol acetyltransferase constructs containing different length of the *lyn* promoter together with $p40^{tax}$ expression plasmids into Jurkat T cells showed that the sequence responsible for $p40^{tax}$-induced transcription is present around the transcription initiation sites.

The *lyn* gene is a member of the *src* gene family and encodes the tyrosine kinase Lyn proteins $p56^{lyn}$ and $p53^{lyn}$ (52, 66, 68). Products of the *src* family are generally associated with the internal portion of the plasma membrane. Several lines of evidence suggest that some members of this family participate in signal transduction in association with surface receptors that lack an intracellular catalytic domain (19, 42, 58). Furthermore, we recently showed that the Lyn protein is associated with membrane-bound surface immunoglobulins M and D, major B-cell antigen receptors (64, 65). Stimulation of B-cell antigen receptors results in activation of the Lyn kinase (64), suggesting that Lyn is important in antigen receptor-mediated signaling.

The human *lyn* gene was originally characterized as a close homolog of the *yes* oncogene of avian sarcoma virus Y73 and the *lck* gene (63). The *lck* gene has been shown to be overexpressed in T-cell lymphoma LSTRA cells by retroviral insertion (31). Interestingly, the expression pattern of the *lyn* gene contrasts with that of *lck*. The *lck* gene is highly expressed in mature T lymphocytes (22, 57), whereas the *lyn* gene is expressed in B cells, macrophages/monocytes, and platelets but only at low levels in T lymphocytes (67). However, the *lyn* gene is expressed in T-cell lines infected with and producing human T-cell leukemia virus type I (HTLV-I) (67). Transcription of *lck* is down-regulated in interleukin-2 (IL-2)-independent, HTLV-I-infected T-cell lines, suggesting that the transcriptions of *lyn* and *lck* are both regulated by a factor(s) present in HTLV-I-producing T cells.

HTLV-I is an etiological agent of adult T-cell leukemia (ATL) (20, 62, 70). This human retrovirus contains genes coding for Gag, Pol, and Env structural proteins, but unlike many other acutely transforming retroviruses, it does not carry a typical oncogene. Instead, the HTLV-I genome encodes a 40-kDa protein designated $p40^{tax}$. This virus-encoded protein is able to *trans* activate its own promoter in the long terminal repeat (LTR) (4, 8, 12, 13, 45, 51). In addition, $p40^{tax}$ *trans* activates certain cellular genes, including those encoding IL-2 (32, 48), IL-2 receptor α (IL-2Rα) (7, 21, 40), c-Fos (11, 37), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (34). Induction of IL-2 and IL-2Rα by $p40^{tax}$ is thought to contribute to proliferation of HTLV-I infected cells in an early stage of ATL when T cells are proliferating in an IL-2-dependent manner (49, 56, 69). Here we report the identification and characterization of the human *lyn* promoter region and show that $p40^{tax}$ can *trans* activate expression of the *lyn* gene. We also report identification of the promoter region responsible for $p40^{tax}$-mediated *trans* activation.

MATERIALS AND METHODS

Cells and cell culture. Raji is a human Burkitt lymphoma cell line. Jurkat is a human acute lymphocytic leukemia T-cell line (67). J24 cells are derivatives of Jurkat cells into which a plasmid construct containing the $p40^{tax}$ gene under the control of the metallothionein promoter is stably introduced (21a). Expression of $p40^{tax}$ in J24 cells was induced by the addition of 1.25 μM $CdSO_4$ to the cultured medium. HBC21.7.31, a class II-restricted mouse helper T-cell hybridoma (hereafter referred to as HBC), was prepared by fusion between immunized T cells of a B10 mouse ($I\text{-}A^b$) and the AKR-derived thymoma BW5147. These cells were cul-

* Corresponding author.

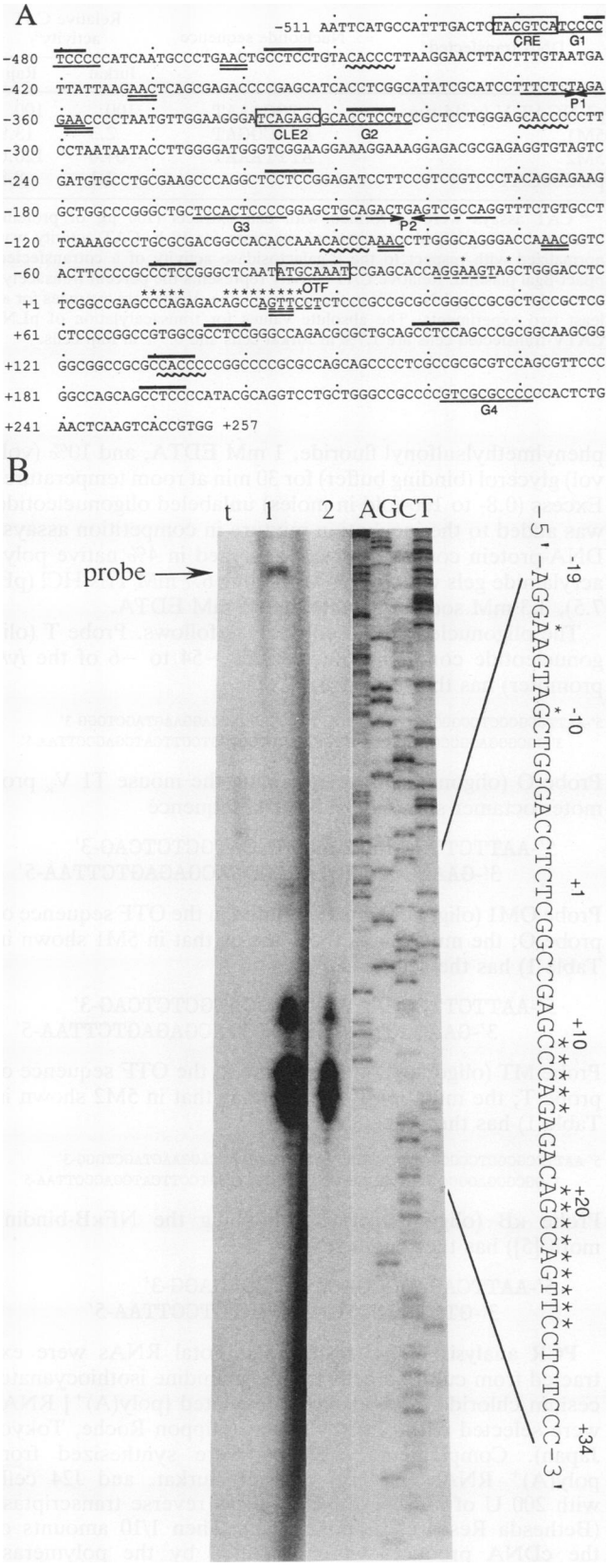


FIG. 1. (A) Nucleotide sequence of the 5′-flanking region of the human *lyn* gene. The nucleotide sequence of a 768-bp cloned fragment is shown. The 5′ end of the cDNA is designated nucleotide +1 of the gene. Transcription start sites, determined by S1 nuclease analysis (see Fig. 2), are indicated by asterisks. The size of each asterisk indicates the relative start site usage in Raji cells. The doubly underlined AAC and GTT indicate the cores of the consensus sequences for c-Myb protein recognition, 5′-(C/Pu)(Py/A)Py AACPyPu-3′ (38), allowing 2-bp mismatches. Four GC box-like sequences (G1 to G4) are underlined. These GC box-like sequences are allowed to carry a 1-bp mismatch from the consensus sequence for Sp1 recognition, 5′-(G/T)(G/A)GG(C/A)G(G/T)(G/A)(G/A)(C/T)-3′ (3). The bold lines above the sequences indicate the p40^{tax}-responsive sequence 5′-CC(A/T)CC-3′ that is present in the LTR of HTLV-I (30). The dotted lines indicate PEA3-like motifs (60). CACCC box elements (47) are indicated by wavy lines. (B) S1 nuclease mapping of human *lyn* transcripts in Raji cells. Poly(A)$^+$ RNA (20 μg) from Raji cells (lanes 1 and 2) and 20 μg of *E. coli* tRNA (lane t) were hybridized to a 288-nucleotide-long (nucleotides −172 to +116) single-stranded DNA probe at 60°C for 3 h (lanes t, 1, and 2). The hybridization mixture was treated with S1 nuclease (200 U for lane 1; 400 U for lanes t and 2) at 37°C for 30 min. S1 nuclease-resistant products were analyzed in a 7 M urea–6% polyacrylamide gel. Sizes of protected bands were determined by comparison with the adjacent genomic sequence, which was generated by primer extension with the same oligonucleotide primer as used for producing the S1 probe (see Materials and Methods). X-ray film was exposed to the gel for 7 days (lanes t, 1, and 2) or 3 h (lanes A, G, C, and T).

tured in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, and antibiotics. The monkey kidney fibroblast cell line CV-1 and human epidermoid carcinoma cell line A431 were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, and antibiotics.

DNA cloning and sequencing. A human genomic library in the λgt10 vector was screened with the ^{32}P-labeled 0.9-kbp *Hin*dIII fragment of pLY2.2 that contained the 5′-proximal sequence of the human *lyn* cDNA (63). A 0.77-kbp *Eco*RI-*Sac*I fragment of one of the positive clones was inserted into the vector plasmid pUC119, and the resulting plasmid was termed pLYN-EcoSac. The nucleotide sequence of this 0.77-kbp fragment was determined by the dideoxy-chain termination method but with deoxy-7-deazaguanosine triphosphate instead of dGTP (35).

S1 nuclease mapping. A single-stranded DNA extending from nucleotide positions −173 to +116 (Fig. 1) was used as a probe for S1 mapping and was prepared as follows. A 17-mer nucleotide corresponding to an antisense sequence from positions +100 to +116 (5′-TTGCCGCGGGCTG GAGG-3′; Fig. 1) was annealed to single-stranded DNA of the template plasmid containing the 0.77-kbp *Eco*RI-*Sac*I fragment. The annealing mixture was incubated with the Klenow enzyme, and the products were digested with *Sma*I. The probe DNA generated was purified by electrophoresis in a 7 M urea–6% polyacrylamide gel.

Nuclease S1 mapping was performed by the modified procedure described elsewhere (29). Briefly, the probe DNA was annealed with 20 μg of poly(A)$^+$ RNA prepared from Raji cells in a solution (20 μl) of 80% formamide, 50 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES; pH 6.4), 1 mM EDTA, and 0.4 M NaCl at 85°C for 5 min and then at 60°C for 3 h. The annealed products were digested with S1 nuclease (200 or 400 U) in a solution (200 μl) of 290 mM NaCl, 4.5 mM $ZnCl_2$, and 30 mM sodium acetate (pH 4.5) at 37°C for 30 min. The reaction was terminated by the addition

of 2.5 volumes of ice-cold ethanol, and the ethanol precipitate was analyzed in a 7 M urea–6% acrylamide sequencing gel.

Construction of chloramphenicol acetyltransferase (CAT) expression plasmids. The 0.77-kbp *Eco*RI-*Sac*I fragment containing the *lyn* promoter was excised from pLYN-EcoSac. This fragment was blunt ended with T4 DNA polymerase and inserted in the correct orientation into pSV0CAT, which differed from pSV2CAT in having no simian virus 40 promoter sequence (17). The plasmid generated was designated pLNCAT03. The blunt-ended 0.77-kbp *Eco*RI-*Sac*I fragment was also inserted into the blunt-ended *Sph*I site of the pUC00CAT vector. The plasmids with the insert in the correct and reverse orientations were designated pLN CAT11 and pLNCAT12, respectively. The inserted DNA of pLNCAT11 was then deleted from the 5′ end. One of the deletion constructs generated from pLNCAT11 was digested by *Eco*RI and *Hin*dIII. The 0.31-kbp *Eco*RI-*Hin*dIII fragment was blunt ended with T4 DNA polymerase and then inserted into pUC00CAT at the *Sma*I sites. The resultant plasmid was used to generate deletions from the 3′ end of the *lyn* promoter. Sequences with unidirectional deletions in both the 5′-to-3′ and 3′-to-5′ directions were obtained with a Kilo Sequence Deletion kit (Takara, Kyoto, Japan).

Site-directed mutagenesis. Oligonucleotide primers (28-mers) corresponding to mutated sequences of the octamer-binding motif (OTF) were synthesized in a DNA synthesizer (Applied Biosystems model 381A). Plasmids carrying mutations were obtained according to a modification of the site-directed mutagenesis method described by Kunkel (24). Introduction of the mutated bases was confirmed by sequence analysis as described above.

Transfection assay. Plasmid DNAs were transfected into CV-1 or A431 cells by the lipofection method (9), using Lipofectin (Bethesda Research Laboratories, Gaithersburg, Md.). DNAs were transfected into Raji, Jurkat, and J24 cells by the DEAE-dextran method (16). Cells (2×10^6) were suspended in 0.4 ml of TBS (25 mM Tris [pH 7.4], 137 mM NaCl, 5 mM KCl, 0.6 mM Na_2HPO_4, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$) containing 2.5 μg of the reporter plasmid and 0.3 μg of $p40^{tax}$ expression plasmid or vector plasmid, 0.5 μg of pβact-βgal plasmid in which the β-actin promoter was placed upstream of the β-galactosidase gene, and 500 μg of DEAE-dextran per ml for 30 min at room temperature. The cells were then washed with TBS to remove unadsorbed DNA and cultivated for another 48 h in RPMI medium. CAT assays and thin-layer chromatography were performed as described elsewhere (33). Briefly, cell extracts containing 100 μg of protein were incubated with [^{14}C]chloramphenicol and acetyl coenzyme A for 3 to 12 h. CAT activities of the reaction products were examined by thin-layer chromatography and quantitation with a Fuji BAS 2000 image analyzer system (Fuji Film, Tokyo, Japan). The CAT activities indicated in Fig. 4 and Table 1 were normalized with respect to the β-galactosidase activity.

Electrophoretic mobility shift assay. DNA-binding reactions and electrophoretic mobility shift assays were performed by a modification of a procedure described elsewhere (59). Briefly, oligonucleotides (see below) were synthesized in a DNA synthesizer and labeled with [α-^{32}P]dATP with Klenow enzyme. Samples of about 0.1 ng of ^{32}P-labeled oligonucleotides (10,000 cpm) were incubated with 2 μg of nuclear extracts from Raji and Jurkat cells and 2 μg of poly(dI-dC) (Pharmacia) in a solution (20 μl) of 25 mM *N*-2-hydroxyethylpiperazine-*N*′-ethanesulfonic acid (HEPES)-KCl (pH 7.9), 50 mM KCl, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, and 10% (vol/vol) glycerol (binding buffer) for 30 min at room temperature. Excess (0.8- to 100-fold in moles) unlabeled oligonucleotide was added to the incubation mixture in competition assays. DNA-protein complexes were analyzed in 4% native polyacrylamide gels with buffer containing 6.7 mM Tris-HCl (pH 7.5), 3.3 mM sodium acetate, and 1 mM EDTA.

TABLE 1. Effects of mutations on OTF of pLNCATV

DNA transfected	Nucleotide sequence from −36 to −29	Relative CAT activity[a]	
		Jurkat	Raji
pLNCAT V (wild type)	ATGCAAAT	100	100
5M1	ATGCGGAT	2.3	13.9
5M2	ATTTAAAT	64.0	120.6
pUC00CAT		2.1	8.8

[a] CAT assays were performed with cell extracts (100 μg of protein) incubated with [^{14}C]chloramphenicol substrate for 10 h. CAT activity was normalized with respect to the β-galactosidase activity of a cotransfected pβact-βgal plasmid. Relative CAT activity represents the percent transacetylation of that with pLNCATV in the same cell line. Values are averages for at least two experiments. The absolute values for transacetylation of pLNCATV-transfected cells are 1.7% in Jurkat cells and 0.9% in Raji cells.

The oligonucleotides used were as follows. Probe T (oligonucleotide containing nucleotides −54 to −6 of the *lyn* promoter) has the sequence

5′-AATTCCGCCCTCCGGGCTCAATATGCAAATCCGAGCACCAGGAAGTAGCTGGG-3′
3′-GGCGGGAGGCCCGAGTTATACGTTTAGGCTCGTGGTCCTTCATCGACCCTTAA-5′

Probe O (oligonucleotide containing the mouse T1 V_κ promoter octamer site [6, 43]) has the sequence

5′-AATTCTTCCCAATGATTTGCATGCTCTCAG-3′
3′-GAAGGGTTACTAAACGTACGAGAGTCTTAA-5′

Probe OM1 (oligonucleotide mutated in the OTF sequence of probe O; the mutation is the same as that in 5M1 shown in Table 1) has the sequence

5′-AATTCTTCCCAATGATCCGCATGCTCTCAG-3′
3′-GAAGGGTTACTAGGCGTACGAGAGTCTTAA-5′

Probe MT (oligonucleotide mutated in the OTF sequence of probe T; the mutation is the same as that in 5M2 shown in Table 1) has the sequence

5′-AATTCCGCCCTCCGGGCTCAATATTTAAATCCGAGCACCAGGAAGTAGCTGGG-3′
3′-GGCGGGAGGCCCGAGTTATAAATTTAGGCTCGTGGTCCTTCATCGACCCTTAA-5′

Probe κB (oligonucleotide containing the NFκB-binding motif [5]) has the sequence

5′-AATTCAGAGGGGACTTTCCCAGAGG-3′
3′-GTCTCCCCTGAAAGGGTCTCCTTAA-5′

PCR analysis of *lyn* transcripts. Total RNAs were extracted from cultured cells by the guanidine isothiocyanate-cesium chloride method. Polyadenylated [poly(A)$^+$] RNAs were selected with oligo(dT)-latex (Nippon Roche, Tokyo, Japan). Complementary DNAs were synthesized from poly(A)$^+$ RNAs (2.5 μg) of Raji, Jurkat, and J24 cells with 200 U of murine leukemia virus reverse transcriptase (Bethesda Research Laboratories). Then 1/10 amounts of the cDNA products were amplified by the polymerase chain reaction (PCR) as described by Yamanashi et al. (66). Namely, synthetic 28-mers pr85 (5′-AAGAAAG

GAGAGAAGATGAAAGTCCTGG-3') and αpr204 (5'-GA TACAGGGAAAAGTGATTCGTGGAGAG-3') were used as sense and antisense primers, respectively, to amplify the sequence corresponding to the SH3/SH2 domain of the Lyn protein. PCR conditions were as follows: denaturing at 94°C for 1 min, annealing at 37°C for 2 min, and elongation at 72°C for 3 min for 30 cycles. PCR products were separated on 4% agarose gels (FMC BioProducts, Philadelphia, Pa.). After electrophoresis, DNAs on the gels were subjected to Southern hybridization as described previously (63). The 360-bp PCR product prepared from Raji mRNA was used as a Southern probe and was labeled with [α-^{32}P]dCTP with a nick translation kit (Amersham). After completion of this procedure, filters were exposed to Kodak X-Omat film with an intensifying screen at −70°C. Signal intensities were quantitated with a Fuji BAS 2000 image analyzer system.

Northern (RNA) blotting. Poly(A)$^+$ RNA (2.5 μg) was fractionated in formaldehyde-containing 1.0% agarose gel (Nippon Gene, Toyama, Japan) and transferred to a nitrocellulose filter. Hybridization of this filter with a ^{32}P-labeled 1,074-bp fragment covering whole coding sequence of p40^{tax} was carried out as described before (63).

RESULTS

Characterization of the 5'-flanking region of the human *lyn* gene. A clone containing a 0.75-kbp *Eco*RI-*Sac*I fragment that hybridized to the 5' end of the cDNA was obtained by screening a human genomic library. Nucleotide sequence analysis of this *Eco*RI-*Sac*I fragment revealed the presence of an exon containing the sequence at the 5' end of the cDNA (63), which was designated nucleotide position +1 (Fig. 1A). No sequence similar to a TATA or CCAAT box was found in this region. Instead, four GC box-like sequences were identified at positions −485, −331, −165, and +223, based on a 10-bp consensus and allowing a 1-bp mismatch. Computer-assisted analysis of the 768-bp 5'-flanking sequence of the *lyn* gene demonstrated characteristic sequences of a cyclic AMP (cAMP)-responsive element (−492), lymphokine element 2 (−338), OTF (−36), and two PEA3-like motifs (−19 and +23). In addition, possible c-Myb-binding sequences (AAC and GTT) were found, and footprint analysis showed that bacterially expressed c-Myb protein bound to the sequence at positions −67 and +24 (data not shown).

To identify the transcription initiation site of the *lyn* mRNA, we carried out S1 nuclease protection analysis by using a single-stranded DNA probe extending from −172 to +116 (Fig. 1B). The probe was 5' end labeled with ^{32}P on the noncoding strand and hybridized to poly(A)$^+$ RNA isolated from Raji cells. As shown in Fig. 1B, several S1-resistant fragments were detected. The main protected fragments were 107 to 103 and 98 to 90 bases long, indicating transcription initiation sites at nucleotide positions +10 to +14 and +19 to +27. Although the *lyn* cDNA does not correspond to a major start site, minor S1-resistant fragments indicated transcription initiations at positions −16 and −11. Perhaps the cDNA was synthesized from the mRNA which had been transcribed at a minor start site. In our experimental conditions, no S1 nuclease-resistant fragment was detected when an equal amount of *Escherichia coli* tRNA was used instead of poly(A)$^+$ RNA from Raji cells.

Identification of promoter activity of the *lyn* gene. For examination of promoter activity, the 768-bp 5'-flanking sequence of the *lyn* gene was inserted upstream of the bacterial CAT gene of pUC00CAT in both orientations. The resulting plasmids, pLNCAT11 and pLNCAT12, in correct and reverse orientations, respectively, were transfected into CV-1, A431, Raji, Jurkat, and HBC cells, and CAT activity was examined. When transfected with pLNCAT11, all of these cells except HBC cells expressed CAT activity (Fig. 2, lanes 2, 7, 12, 17, and 22). The CAT activity of pLNCAT11-transfected cells was about 5 to 30% of that of pSV2CAT. On the other hand, pLNCAT12 showed a barely detectable level of CAT activity in all but CV-1 cells (lanes 3, 8, 13, 18, and 23). The CAT activity directed by pLNCAT12 in CV-1 cells suggests the presence of a gene transcribed preferentially in CV-1 cells in the opposite direction to the *lyn* gene. However, it is also possible that the relatively high CAT activity of pLNCAT12 in CV-1 cells is due to the presence of a cryptic promoter that is fortuitously active in CV-1 cells. Scarcely any *lyn* expression was detected in T cells by Northern blot analysis (67), but CAT expression with pLN CAT11 was observed in Jurkat T cells (lane 17). Possibly the amount of a suppressing factor(s) for *lyn* transcription is limited in Jurkat cells, and so it could not suppress the exogenous promoter activity. As CAT expression was not observed when pLNCAT11 was transfected into HBC (mouse T-cell hybridoma) and Molt4 (human T-cell line) cells, (lane 22 and data not shown), the CAT activity of pLNCAT11 in Jurkat cells seems to be peculiar to this cell line. The data strongly suggested that the 768-bp *Eco*RI-*Sac*I fragment contains a functional promoter of the *lyn* gene.

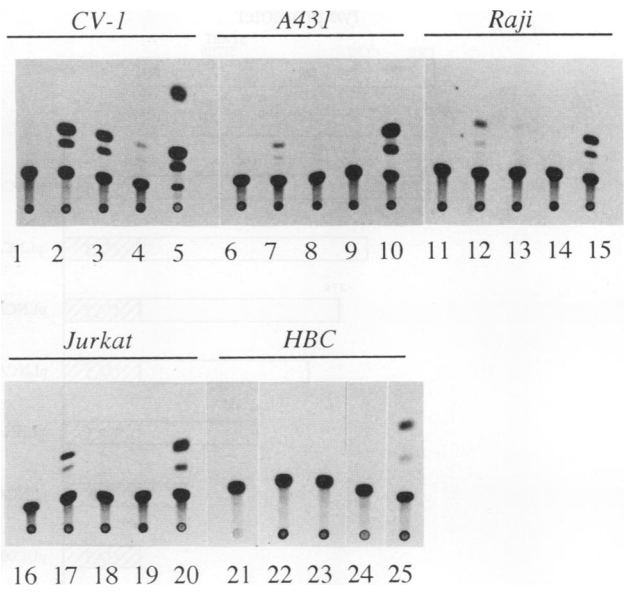


FIG. 2. Transient expression of the CAT gene directed by the 5'-flanking sequence of the human *lyn* gene. Each reporter plasmid (2.5 μg) was transfected into CV-1, A431, Raji, Jurkat, and HBC cells. After 48 h, the cells were harvested and CAT activity was measured as described in Materials and Methods. The reporter plasmids used for transfection were as follows: pLNCAT11 (lanes 2, 7, 12, 17, and 22), pLNCAT12 (lanes 3, 8, 13, 18, and 23), pSV00CAT (lanes 4, 9, 14, 19, and 24), and pSV2CAT (lanes 5, 10, 15, 20, and 25). As controls for CAT activity, extracts from untransfected cells were analyzed (lanes 1, 6, 11, 16, and 21).

Deletion analysis of the promoter region of the human *lyn* gene. To identify DNA sequences that are primarily responsible for promoter activity, we made progressive 5' deletions in the *lyn* promoter sequence of the pLNCAT11 and introduced the resulting constructs into various cell lines. Figure

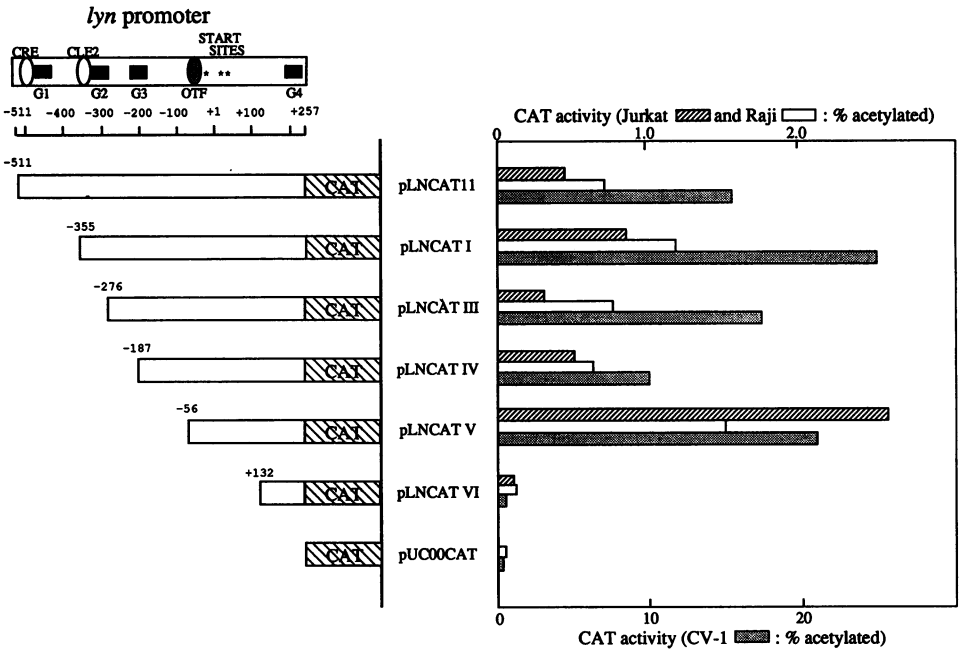


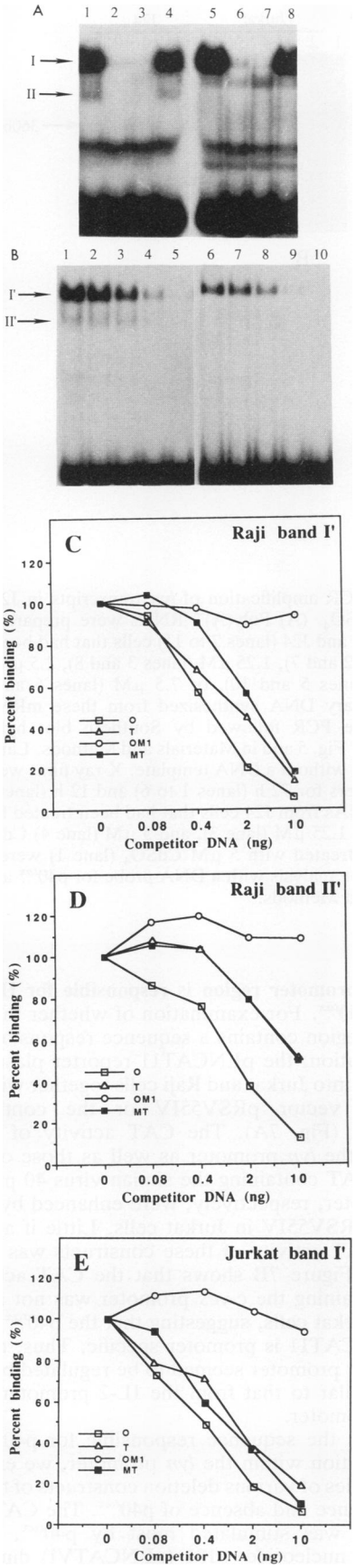
FIG. 3. Deletion analysis of the human *lyn* promoter. Reporter CAT plasmids containing progressive 5′ deletions of the human *lyn* promoter and regulatory region of pLNCAT11 are illustrated schematically on the left. A diagram of the 5′ upstream region of the human *lyn* gene promoter is shown at the top. Several DNA elements indicated in Fig. 1 are included in this diagram. These reporter plasmids were transfected into CV-1, Raji, and Jurkat cells, and cell extracts (50 μg of protein from CV-1; 100 μg from Raji and Jurkat) were subjected to a CAT assay. The histogram shows the CAT activities of the constructs transfected into CV-1, Raji, and Jurkat cells. CRE, cAMP-responsive element; CLE2, conserved lymphokine element 2.

3 shows the CAT activities of these deletion constructs in CV-1, Raji, and Jurkat cells. In all cell lines, the promoter activity was prominent with a 5′-flanking sequence up to position −56. The sequence between −187 and −57 seemed to contain a negative *cis*-acting element(s), because removal of this sequence stimulated the promoter activity in all cell lines, especially in Jurkat cells. A preliminary study indicated that the negative element was located between −110 and −79 (data not shown). We also found that deletion to +132 drastically decreased transcription initiation to 2% of that observed with pLNCATV in Jurkat cells. A similar level of suppression of transcription in Jurkat cells was observed with the *lyn* promoter construct deleted to −4 (see Fig. 8B). Thus, the sequence between −56 and −4, which includes OTF motif, is important for *lyn* promoter activity.

Role of the OTF motif in *lyn* promoter activity. As indicated in Fig. 1, the OTF motif is located at positions −36 to −29 of the *lyn* promoter region. To investigate the role of the OTF sequence in the *lyn* promoter, we introduced point mutations (Table 1) into the OTF sequence of plasmid pLNCATV, which contained the nucleotide sequence from −56 to +257 of the *lyn* promoter and showed the strongest CAT activity among the deletion constructs (Fig. 3). Table 1 shows the relative CAT activities of the mutant constructs transfected into Raji and Jurkat cells. The 5M1 mutant construct greatly down-regulated the promoter activity significantly in all cell lines, whereas mutant construct 5M2 had less effect. Therefore, we tentatively concluded that transcription of the *lyn* gene is positively regulated by a factor(s) that interacts with the OTF sequence and is expressed in all of these cell lines. In addition, the observation that the mutations in 5M2 had effects on the *lyn* promoter activity in Jurkat cells but not in Raji cells suggests the presence of an additional specific regulatory factor that interacts with the OTF motif in T cells.

Binding of nuclear factors to the OTF element of the *lyn* gene. We examined whether any nuclear factor(s) binds to the sequence between −56 and −4 which includes OTF motif. Nuclear extracts prepared from Jurkat and Raji cells were incubated with a 57-bp synthetic double-stranded DNA probe (DNA fragment T, containing nucleotides −54 to −6 of the *lyn* promoter) that was end labeled with ^{32}P. Electrophoresis of the reaction products on a polyacrylamide gel revealed a shifted band (band I), indicating that fragment T binds to a nuclear protein of these cell lines (Fig. 4A). Formation of the DNA-protein complex was abolished by the addition of 100-fold molar excess of unlabeled DNA fragment T. The DNA fragment O, which contains the OTF-binding sequence of the murine κ light-chain promoter (6, 43), also competed with complex formation, suggesting that the 57-bp DNA fragment T interacts with the OTF-1. Another shifted band (band II) was formed only with the nuclear extract from Raji cells (Fig. 4A, lane 1). The DNA-protein interaction detected as band II was inhibited by the presence of excess fragment T or fragment O. Since band II was scarcely observed with extracts from other non-B cells (data not shown), it may represent the interaction of DNA fragment T with OTF-2, whose expression is restricted in B cells (43, 53). We also observed two shifted bands (band I′ and II′) upon incubation of radiolabeled DNA fragment O with the nuclear extract from Raji cells (Fig. 4B, lane 1), although only band I′ was produced with nuclear extract from Jurkat cells (lane 6).

Since the 5M1 reporter plasmid that had mutations in the OTF sequence directed much less CAT activity than did the parental pLNCATV plasmid in all cell lines (Table 1), the mutations seemed to reduce the interaction between the OTF-1 and the *lyn* promoter. This possibility was examined by a gel shift competition assay with radiolabeled DNA



fragment O (Fig. 4C to E). With the nuclear extract from Raji (Fig. 4C and D) or Jurkat (Fig. 4E) cells, fragment OM1, which had the same mutations as those in the 5M1 construct (see Materials and Methods), did not compete with the formation of DNA-protein complexes in bands I′ and II′, whereas DNA fragments O, T, and MT (see Materials and Methods) were competitive. From these results, we conclude that the interaction of the OTF-binding protein with the *lyn* promoter region plays an important role in transcription of the *lyn* gene.

p40^{tax} *trans* activates transcription of the *lyn* gene. We have reported that the *lyn* gene is expressed very little in T cells but is expressed in HTLV-I-producing T-cell lines (67). From these findings, we suspected that the *lyn* gene promoter may be *trans* activated by p40^{tax} encoded by HTLV-I. To confirm this possibility, we used a sensitive method, namely, PCR amplification in conjunction with Southern blot analysis, to detect the *lyn* transcripts. The primers used for the PCR directed the synthesis of a 360-bp DNA fragment that corresponded to the SH3/SH2 domain of the Lyn protein (66). The results of the control experiment (Fig. 5A) show that the amount of the 360-bp PCR product is proportional to the amount of poly(A)$^+$ RNA added.

RNAs were prepared from Jurkat cells transfected for 24 h with different concentrations of the p40^{tax} expression plasmid pRSV55IV (14). The level of the 360-bp PCR product synthesized with mRNAs from untransfected Jurkat cells was about 1/1,000 of that synthesized from mRNAs of Raji cells (Fig. 5B; compare the density of bands in lanes 2 and 8), as estimated with a Fuji image analyzer system. The amount of the PCR product from mRNA of pRSV55IV-transfected Jurkat cells were elevated (lanes 3 to 6) to about 1/100 of that from an equal amount of Raji mRNA. Assuming that the transfection efficiency was about 10^{-1} to 10^{-2}, the level of the *lyn* mRNA in pRSV55IV-transfected cells should be over 10^2-fold. Therefore, we concluded that endogenous *lyn* transcripts were *trans* activated by p40^{tax}. We also examined the level of *lyn* transcripts in Jurkat cells at different times after transfection of pRSV55IV. The levels of p40^{tax}-induced *lyn*

FIG. 4. Binding of nuclear factors to probes containing the OTF sequence. (A) Oligonucleotide probe T (see Materials and Methods) was labeled with [α-^{32}P]dATP with Klenow enzyme, and the labeled probe (approximately 0.1 ng) was incubated in binding buffer with 2 μg of poly(dI-dC) and 2 μg of nuclear extract from Raji (lanes 1 to 4) or Jurkat (lanes 5 to 8) cells for 30 min at room temperature. The reaction mixture was electrophoresed in a 4% nondenaturing polyacrylamide gel. The unlabeled competitors used for the binding reaction were as follows: 10 ng of probe T (lanes 2 and 6), 10 ng of probe O containing the mouse octamer motif (lanes 3 and 7), or 10 ng of probe κB (lanes 4 and 8). Lanes 1 and 5 show reaction mixtures without competitor. (B) An electrophoretic mobility shift assay was carried out by using ^{32}P-labeled oligonucleotide probe O with nuclear extract from Raji (lanes 1 to 5) or Jurkat (lanes 6 to 10) cells. Unlabeled probe O was used for the competition assay. The amounts of competitor DNA were as follows: 0.08 ng (lanes 2 and 7), 0.4 ng (lanes 3 and 8), 2 ng (lanes 4 and 9), and 10 ng (lanes 5 and 10). Lanes 1 and 6 represent a binding assay without competitor. (C to E) Binding of nuclear factors to the mutated OTF sequence. Binding of the nuclear extracts to the ^{32}P-labeled probe O was analyzed as described above, using unlabeled probe O, OM1, T, or MT as the competitor. The radioactivities of band I′ (C) and band II′ (D), which were formed by incubating the labeled probe O with a nuclear extract from Raji cells, were quantified with a Fuji BAS 2000 image analyzer system. In panel E, the radioactivity of band I′ produced by incubating probe O with a nuclear extract from Jurkat cells was quantified. The positions of bands I′ and II′ were as shown in panel B.

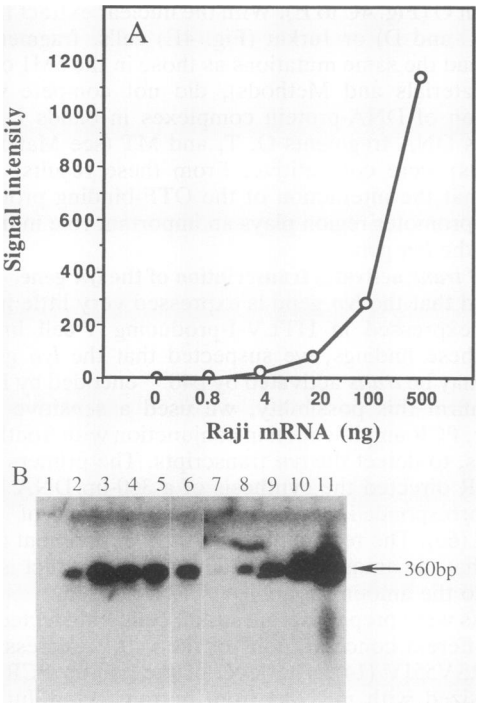


FIG. 5. PCR analysis of endogenous *lyn* transcripts. (A) Poly(A)$^+$ RNAs were prepared from Raji cells. Before the cDNA synthesis by reverse transcriptase, poly(A)$^+$ RNA solutions, (2.5 μg/20 μl) were diluted with *E. coli* tRNA solution (2.5 μg/20 μl). PCR products of cDNA synthesized from each samples were electrophoresed in a 4% low-melting-point agarose gel and detected by Southern blot analysis, and then the signal at 360 bp was quantified as described in Materials and Methods. (B) PCR amplification of *lyn* transcripts in Jurkat cells transfected with the p40^{tax} expression plasmid. PCR products of cDNA synthesized from mRNA (2.5 μg) of untransfected Jurkat cells (lane 2) and Jurkat cells transfected with the p40^{tax} expression plasmid (0.375, 0.75, 1.5, and 6 μg/10^7 cells [lanes 3, 4, 5, and 6, respectively]) were electrophoresed and detected by Southern blot analysis. For quantification of the PCR products from each sample, approximately 50 ng of PCR product from Raji cDNA was used (lane 11) and was diluted 1/10, 1/100, 1/1,000, and 1/10,000 (lanes 10, 9, 8, and 7, respectively). Lane 1 represents the PCR product without a template for the PCR reaction.

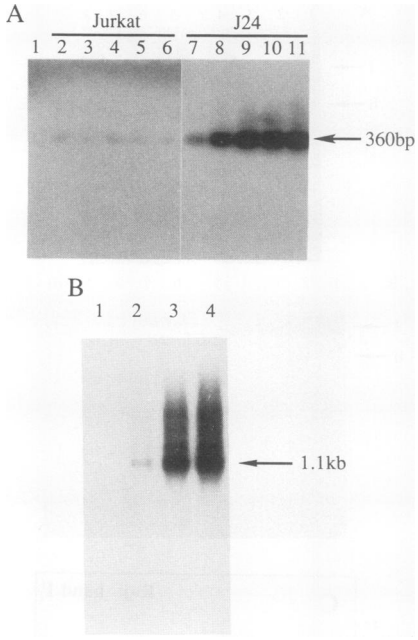


FIG. 6. PCR amplification of *lyn* transcripts in J24 cells stimulated by $CdSO_4$. (A) Poly(A)$^+$ RNAs were prepared from Jurkat (lanes 2 to 6) and J24 (lanes 7 to 11) cells that had been treated with 0 μM (lanes 2 and 7), 1.25 μM (lanes 3 and 8), 2.5 μM (lanes 4 and 9), 5 μM (lanes 5 and 10), or 7.5 μM (lanes 6 and 11) $CdSO_4$. Complementary DNA synthesized from these mRNAs was subjected to the PCR followed by Southern blot hybridization as described for Fig. 5 and in Materials and Methods. Lane 1 shows the PCR product without a DNA template. X-ray films were exposed to Southern filters for 72 h (lanes 1 to 6) and 12 h (lanes 7 to 11). (B) Poly(A)$^+$ RNAs from J24 cells that had been treated for 24 h with 0 μM (lane 2), 1.25 μM (lane 3), and 5 μM (lane 4) $CdSO_4$ and from Jurkat cells treated with 5 μM $CdSO_4$ (lane 1) were subjected to Northern blot analysis with a DNA probe for p40^{tax} as described in Materials and Methods.

transcripts 36 and 48 h after transfection were similar to those 24 h after transfection (data not shown).

To confirm the conclusion presented above, we measured the level of *lyn* transcripts in J24 cells upon Cd^{2+} induction of the exogenously introduced p40^{tax} gene. J24 cells are permanent transformants of the p40^{tax} gene which was placed under the control of the transcriptional promoter of the metallothionein gene. The level of *lyn* transcripts was increased 15- to 30-fold, depending on the level of p40^{tax} induction (Fig. 6A, lanes 7 to 11; Fig. 6B, lanes 2 to 4). The parental cell line Jurkat did not show any changes in the amount of *lyn* transcripts upon treatment with $CdSO_4$ (Fig. 6A, lanes 2 to 6). The amount of the *lyn* PCR product obtained from nonstimulated J24 mRNA was about 10-fold higher than that obtained from Jurkat mRNA, suggesting slight expression of p40^{tax} in unstimulated J24 cells (Fig. 6B, lane 2). Although several explanations are possible for the increased *lyn* transcripts in p40^{tax}-expressing Jurkat cells, we reasoned that endogenous *lyn* transcripts might be *trans* activated by p40^{tax}.

The *lyn* promoter region is responsible for HTLV-I *trans* activator p40^{tax}. For examination of whether the 768-bp *lyn* promoter region contains a sequence responsible for p40^{tax} *trans* activation, the pLNCAT11 reporter plasmid was cotransfected into Jurkat and Raji cells together with the p40^{tax} expression vector pRSV55IV or the control plasmid pRSV55TK (Fig. 7A). The CAT activity of pLNCAT11 containing the *lyn* promoter as well as those of pSV2CAT and pIL2CAT containing the simian virus 40 promoter and IL-2 promoter, respectively, were enhanced by cotransfection with pRSV55IV in Jurkat cells. Little if any enhancement of CAT activity of these constructs was observed in Raji cells. Figure 7B shows that the CAT activity of pY SCAT containing the c-*yes* promoter was not activated by p40^{tax} in Jurkat cells, suggesting that the p40^{tax} responsibility of pLNCAT11 is promoter specific. Thus, transcription from the *lyn* promoter seemed to be regulated by p40^{tax} in a fashion similar to that from the IL-2 promoter and simian virus 40 promoter.

To locate the sequence responsible for p40^{tax}-mediated *trans* activation within the *lyn* promoter, we examined the CAT activities of various deletion constructs of the promoter in the presence and absence of p40^{tax}. The CAT activity of pLNCATV was stimulated most by p40^{tax}, and further deletion to nucleotide +131 (pLNCATVI) diminished the

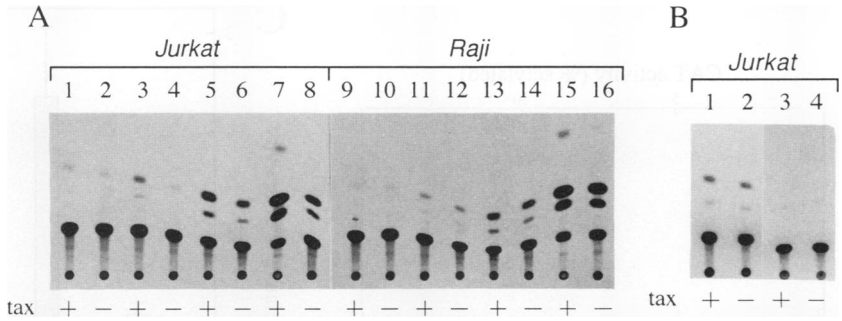


FIG. 7. Effects of p40^{tax} on the CAT activity of the *lyn* promoter-CAT construct. (A) The CAT activities of Jurkat cells (lanes 1 to 8) and Raji cells (lanes 9 to 16) that had been transfected with 2.5 μg of pSV00CAT (lanes 1, 2, 9, and 10), pIL2CAT (lanes 3, 4, 11, and 12), pLNCAT03 (lanes 5, 6, 13, and 14), or pSV2CAT (lanes 7, 8, 15, and 16) with (+) or without (−) the p40^{tax} expression plasmid, examined as described in Materials and Methods. (B) Similar experiment done by using pYSCAT (lanes 1 and 2) and pUC00CAT (lanes 3 and 4) for cotransfection with (+) or without (−) the p40^{tax} expression plasmid into Jurkat cells.

stimulation (Fig. 8A). Thus, the p40^{tax}-responsive element was located within the sequence between −56 and +131. For confirmation of the responsive sequence, another series of deletion constructs made on pLNCATIV was transfected into Jurkat cells together with the p40^{tax} expression plasmid. *trans* activation by p40^{tax} was apparent with the pLNCA TIV-4 constructs which had a deletion to −54 and contained the OTF motif (Fig. 8B). Although removal of the OTF motif resulted in a drastic decrease of the promoter activity of the *lyn* gene, the promoter activity of pLNCATIV-5 with a deletion to position −4 was significantly activated by p40^{tax}. Moreover, CAT expression of the 5M1 construct was activated by p40^{tax} significantly (data not shown), suggesting that the OTF sequence does not generate p40^{tax} *trans* activation. It should be noted that the CAT activity of the constructs with further deletion still responded weakly to p40^{tax}. These data suggest that the region between −4 and +257 is responsible for p40^{tax} activation. To demonstrate the effect of *trans* activation by p40^{tax} on the region from −4 to +257, the promoter region of the *lyn* gene in pLNCATIV-5 was inserted in both orientations upstream of the c-*yes* promoter in pYSCAT. As shown in Fig. 8C, CAT activities of both constructs were activated by p40^{tax}, although the degree of activation by p40^{tax} was somewhat diminished. Our preliminary data obtained from the 3′ deletion experiment on the *lyn* promoter region suggested that the region between −56 and +12 was important for p40^{tax} *trans* activation (data not shown). These results together suggest that the sequence between −4 and +12 is important for p40^{tax}-mediated *trans* activation, though this possibility should be verified by further experiments. Our present data do not exclude the possibility that another p40^{tax}-responsive element is located outside the 770-bp promoter region.

DISCUSSION

In this study, we identified the *lyn* gene promoter in a genomic clone from a human placenta library. The 768-bp *Eco*RI-*Sac*I fragment showed the promoter activity in all cells tested. We also showed that p40^{tax} encoded by HTLV-I could *trans* activate transcription of the *lyn* gene in Jurkat T cells through the sequence within the 768-bp promoter region.

The sequence of the *lyn* promoter region resembles that of other members of the *src* family such as *hck* (28), c-*yes* (33), c-*fgr* (39), and *lck* (55) in that it carries no apparent TATA or CCAAT box. Transcription initiation of these genes from multiple sites could be due to the lack of a TATA sequence. Among these genes, the c-*yes* gene is expressed rather ubiquitously, and its promoter does not carry any typical enhancer motif (33). In contrast, the expressions of c-*fgr* and *lck* are rather cell type specific; the c-*fgr* gene is expressed in mature cells of the monocyte/macrophage lineage (27). Within the c-*fgr* promoter region, there are five pairs of sequences homologous to the AP-2-binding site, which is suggested to be responsible for cell-specific expression of the c-*fgr* gene (39). The *lck* gene is expressed specifically in T cells of lymphoid lineage (22). The *lck* promoter region (type I and type II) does not contain any consensus sequences for known eukaryotic *cis*-acting elements such as a GC box or TATA box. Except in the type I *lck* promoter, there is an oligonucleotide sequence identical to the consensus sequence for the immunoglobulin heavy-chain enhancer (CCAGGTGG) (55), which may play a part in T-cell-specific expression of the *lck* gene. In contrast to the *lck* promoter, the 768-bp *lyn* promoter contains sequences similar to those of well-characterized DNA elements, including a cAMP-responsive element, conserved lymphokine element 2, PEA3 elements, an NFκB-binding motif like sequence, GC box-like sequences, and OTF. In addition, putative c-Myb-binding sites and two palindromic sequences were found in this promoter region. The biological importances of these elements for *lyn* gene expression remain to be established. However, deletion and mutational analyses of the *lyn* promoter region suggested that the OTF motif, with its downstream sequence containing multiple transcription initiation sites, is of primary importance for *lyn* promoter activity.

Two enhancer proteins, OTF-1 and OTF-2, in the B-cell nuclear extract bind to the OTF motif within the promoter of the immunoglobulin gene and regulate cell-type-specific expression of this gene (53, 61). Since OTF-1 and OTF-2 from B-cell nuclear extracts bound to the *lyn* promoter sequence (Fig. 4A and 4B), B-cell-specific expression of the *lyn* gene may be regulated by these factors. As expected, in studies with a T-cell nuclear extract, the *lyn* promoter interacted only with OTF-1, which is expressed ubiquitously and known to be important for the expression of various genes (10, 36, 54). OTF-1 binding to the *lyn* promoter should be important for its basal level of expression. This notion is supported by our observation that mutations in the OTF sequence of the *lyn* promoter, which diminished OTF-1 binding (Fig. 4C to E), drastically decreased its promoter activity (Table 1). In addition, our data showed that the

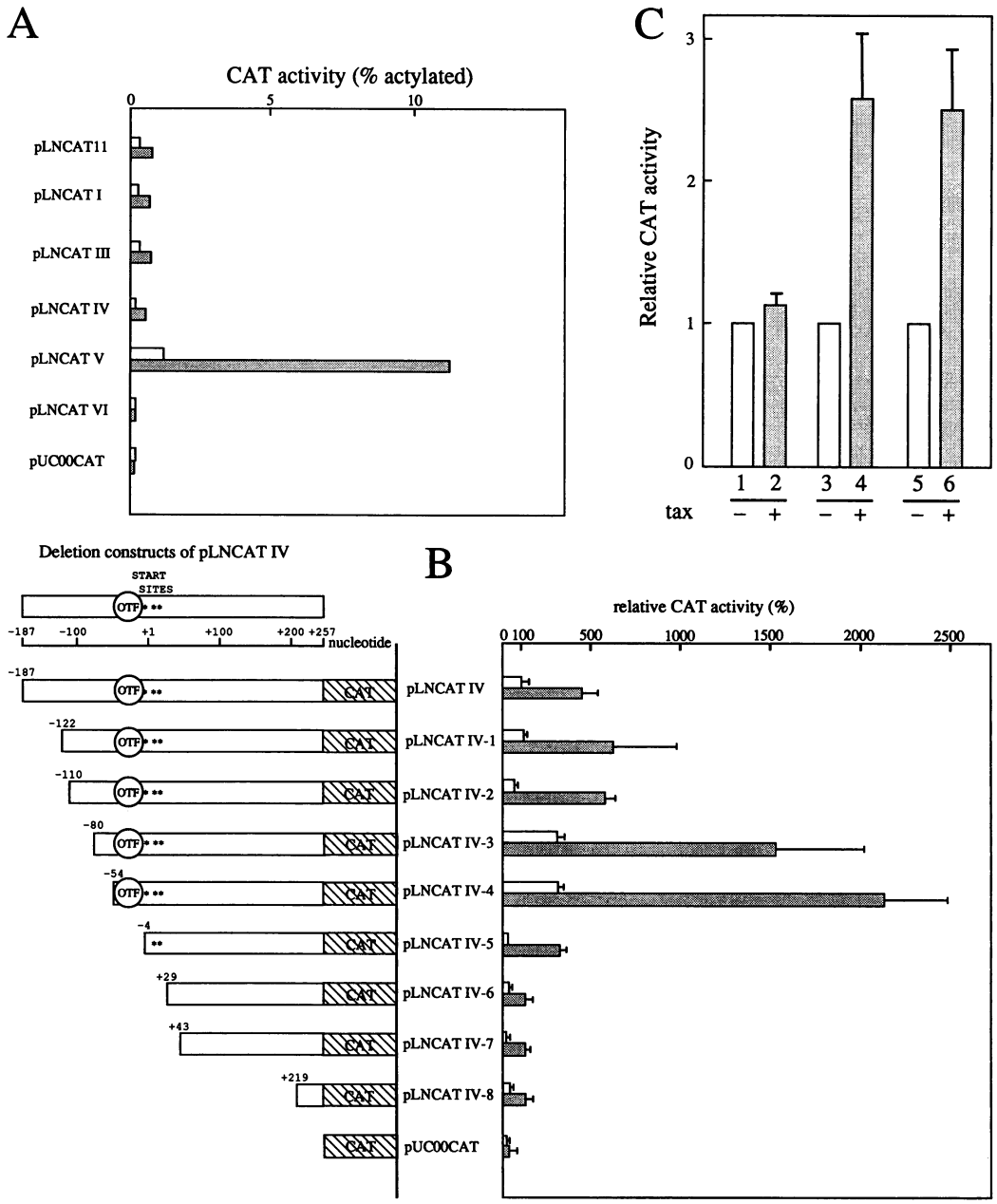


FIG. 8. Location of the p40^{tax}-responsive sequence on the *lyn* promoter. (A and B) Reporter constructs (2.5 μg of each) containing 5'-deleted forms of the human *lyn* promoter were transfected into Jurkat cells with (dotted bars) or without (open bars) the p40^{tax} expression plasmid (0.3 μg). The CAT activity of each construct was measured as described in Materials and Methods. Histograms show the average of the absolute percent transacetylation in two independent experiments (A) and the relative CAT activity compared with the percent transacetylation in cells transfected with pLNCATIV without the p40^{tax} expression vector (B). At least three experiments were done, and results are shown as means and standards errors. (B). (C) *trans* activation by p40^{tax} on *lyn-yes* hybrid promoter constructs. The *lyn* promoter region from pLNCATIV-5 (nucleotides −4 to +257) were inserted upstream of the human c-*yes* promoter of plasmid pYSCAT in head-to-tail orientation to generate pLYCAT-5 (columns 3 and 4). In pLYCAT-5R (columns 5 and 6), the *lyn* promoter region was in the reverse orientation upstream of the c-*yes* promoter. These constructs and pYSCAT (columns 1 and 2) were transfected in Jurkat cells with (columns 1, 3, and 5) or without (columns 2, 4, and 6) the p40^{tax} expression vector. CAT assays were performed as in panels A and B. The level of transacetylation of [^{14}C]chloramphenicol obtained with each construct in the presence of p40^{tax} was normalized to that obtained in the absence of p40^{tax}. Results are shown as means and standard errors of four independent experiments. Average values of acetylation in columns 1, 3, and 5 were 0.85, 0.35, and 0.33%, respectively.

sequences from −110 to −79 and from +75 to +126 acted negatively on the promoter activity of *lyn* in Jurkat T cells (Fig. 8). These *cis*-regulatory elements may be partly responsible for suppression of *lyn* gene expression in T cells (67).

Although normal T lymphocytes express the *lyn* gene at very low levels, *lyn* mRNA and the Lyn protein are easily detectable in T cells producing HTLV-I (67). This finding suggests that transcription of the *lyn* gene is stimulated by a

protein(s) encoded by HTLV-I. The present data clearly show that $p40^{tax}$ up-regulates the *lyn* promoter activity (Fig. 7 and 8). A 21-bp element within the LTR of HTLV-I is known to be required for *trans* activation by $p40^{tax}$ (13, 46). No sequence homologous to this element was identified in the *lyn* promoter region. However, it should be noted that the magnitude of *trans* activation of gene expression through the 21-bp element is much greater than that observed upon *lyn trans* activation (13). This finding suggests multiple mechanisms of $p40^{tax}$-mediated *trans* activation. In fact, the NF-κB-binding motif in the promoter regions of the IL-2R gene (1, 5, 26) and GM-CSF gene (34, 44) are also responsible for $p40^{tax}$-mediated *trans* activation. The sequence at positions −8 to +2 (5′-GGGACCTCTC-3′) of the *lyn* promoter is similar to the NF-κB-binding motif, but this sequence did not compete with factor binding to the NF-κB-binding motif (5′-GGGACTTTCC-3′ [5]) of the murine κ light-chain enhancer (data not shown). Therefore, NF-κB may not mediate $p40^{tax}$-induced *lyn* transcription through its interaction with the decanucleotide present at position −8. The CC(A/T)CC sequence recently demonstrated to act as the $p40^{tax}$-responsive element has been found in the HTLV-I LTR (2, 30). This pentanucleotide sequence appears nine times in the *lyn* promoter region. It should be noted that the sequence between +29 and +257 showed weak $p40^{tax}$ responsiveness and contains four CC(A/T)CC motifs. Recent evidence suggested direct interaction between a general transcription factor and a *trans*-acting factor(s) encoded by DNA tumor virus (15, 25). Possibly $p40^{tax}$ stimulates *lyn* gene expression by directly or indirectly interacting with a general factor such as an initiator for transcription.

Several cellular genes, including those encoding IL-2, IL-2R, c-*fos*, and GM-CSF, have been reported to be *trans* activated by $p40^{tax}$. IL-2 is known to play a major role in proliferation of T cells by interacting with the IL-2R complex (18, 50). The $p40^{tax}$-induced expression of IL-2R and IL-2 could lead to polyclonal growth of HTLV-I-infected T cells in an early stage of ATL. However, these effects may not be sufficient for cellular transformation or malignancy in ATL, because T cells at a later stage of ATL proliferate in a growth factor-independent manner. The *lyn* gene product may also play a part in HTLV-I-induced leukemogenesis. Recent studies revealed that Lyn is physically and functionally associated with membrane-bound immunoglobulin M in B cells (64–66). This finding suggests that the Lyn protein is important in antigen-mediated signal transduction of B cells. However, the function of Lyn in normal T cells has not been examined because Lyn is expressed only at very low levels in peripheral T cells (67). The present data clearly show that expression of *lyn* is induced by $p40^{tax}$. On the other hand, expression of the *lck* gene, another member of the *src* family, has not been detected in IL-2-independent, HTLV-I-infected T-cell lines (23). The *lck* gene product Lck is physically associated with CD4 and CD8 cell surface molecules and becomes activated by their cross-linking to these molecules (41, 58). Moreover, Lck has been found to interact with IL-2R and appears to be activated in IL-2-stimulated T cells (19). The amino acid sequence in the kinase domain of the Lck protein is responsible for its association with IL-2R. Because of the similarities of the primary structures of the kinase domains of the Lyn and Lck proteins, IL-2R may also interact with Lyn kinase in HTLV-I-infected T cells. Further investigations are needed to prove this hypothesis and elucidate the role of the Lyn protein in HTLV-I-producing T cells.

ACKNOWLEDGMENTS

We thank S. Ishii for advice and M. Sudol for critical reading of the manuscript.

This work was supported in part by grants-in-aid for Special Project Research, Cancer-Bio Science, from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1. **Ballard, D. W., E. Bohnlein, J. W. Lowenthal, Y. Wano, B. R. Franza, and W. C. Greene.** 1988. HTLV-I Tax induces cellular proteins that activate the κB element in the IL-2 receptor α gene. Science **241**:1652–1655.
2. **Brady, J., K.-T. Jeang, J. Duvall, and G. Khoury.** 1987. Identification of p40x-responsive regulatory sequences within the human T-cell leukemia virus type I long terminal repeat. J. Virol. **61**:2175–2181.
3. **Briggs, M. R., J. T. Kadonaga, S. P. Bell, and R. Tjian.** 1986. Purification and biochemical characterization of the promoter specific transcription factor, Sp1. Science **234**:47–52.
4. **Chen, I. S. Y., D. J. Slamon, J. D. Rosenblatt, N. P. Shan, S. G. Quan, and W. Wachsman.** 1985. The *x* gene is essential for HTLV replication. Science **229**:54–58.
5. **Cross, S. L., N. F. Halden, M. J. Lenardo, and W. J. Leonard.** 1989. Functionally distinct NF-κB binding sites in the immunoglobulin κ and IL-2 receptor α chain genes. Science **244**:466–469.
6. **Currie, R. A., and R. G. Roeder.** 1989. Identification of an octamer-binding site in the mouse kappa light-chain immunoglobulin enhancer. Mol. Cell. Biol. **9**:4239–4247.
7. **Doi, T., M. Hatakeyama, S. Itoh, and T. Taniguchi.** 1989. Transient induction of IL-2 receptor in cultured T cell lines by HTLV-1 LTR-linked *tax*-1 gene. EMBO J. **8**:1953–1958.
8. **Felber, B. K., H. Paskalis, C. Kleinman-Ewing, F. Wong-Staal, and G. N. Pavlakis.** 1985. The pX protein of HTLV-I is a transcriptional activator of its long terminal repeats. Science **229**:675–679.
9. **Felgner, P. L., T. R. Gadek, M. Holm, R. Roman, H. W. Chan, M. Wenz, J. P. Northrop, G. M. Ringold, and M. Danielsen.** 1987. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proc. Natl. Acad. Sci. USA **84**:7413–7417.
10. **Fletcher, C., N. Heintz, and R. G. Roeder.** 1987. Purification and characterization of OTF-1, a transcription factor regulating cell cycle expression of a human histone H2b gene. Cell **51**:773–781.
11. **Fujii, M., P. Sassone-Corsi, and I. M. Verma.** 1988. c-*fos* promoter trans-activation by the tax_1 protein of human T-cell leukemia virus type I. Proc. Natl. Acad. Sci. USA **85**:8526–8530.
12. **Fujisawa, J., M. Seiki, T. Kiyokawa, and M. Yoshida.** 1985. Functional activation of the long terminal repeat of human T-cell leukemia virus type I by a *trans*-acting factor. Proc. Natl. Acad. Sci. USA **82**:2277–2281.
13. **Fujisawa, J., M. Seiki, M. Sato, and M. Yosida.** 1986. A transcriptional enhancer sequence of HTLV-I is responsible for *trans*-activation mediated by $p40^{x}$ of HTLV-I. EMBO J. **5**:713–718.
14. **Fujisawa, J., M. Toita, and M. Yoshida.** 1989. A unique enhancer element for the *trans* activator ($p40^{tax}$) of human T-cell leukemia virus type I that is distinct from cyclic AMP- and 12-*O*-tetradecanoylphorbol-13-acetate-responsive elements. J. Virol. **63**:3234–3239.
15. **Fujisawa, J., M. Toita, T. Yoshimura, and M. Yoshida.** 1991. The indirect association of human T-cell leukemia virus *tax* protein with DNA results in transcriptional activation. J. Virol. **65**:4525–4528.
16. **Fujita, T., H. Shibuya, T. Ohashi, K. Yamanishi, and T. Taniguchi.** 1986. Regulation of human interleukin-2 gene: functional DNA sequences in the 5′ flanking region for the gene expression in activated T lymphocytes. Cell **46**:401–407.
17. **Gorman, C.** 1985. High efficiency gene transfer into mammalian cells, p. 143–165. *In* D. M. Glover (ed.), DNA cloning, vol. 2. IRL Press, Oxford.
18. **Greene, W. C., and W. J. Leonard.** 1986. The human interleukin-2 receptor. Annu. Rev. Immunol. **4**:69–95.

19. **Hatakeyama, M., T. Kono, N. Kobayashi, A. Kawahara, S. D. Levin, R. M. Perlmutter, and T. Taniguchi.** 1991. Interaction of the IL-2 receptor with the *src*-family kinase $p56^{lck}$: identification of novel intermolecular association. Science **252:**1523–1528.
20. **Hinuma, Y., K. Nagata, M. Hanaoka, M. Nakai, T. Matsumoto, K. Kinoshita, S. Shirakawa, and I. Miyoshi.** 1981. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proc. Natl. Acad. Sci. USA **78:**6476–6480.
21. **Inoue, J., M. Seiki, T. Taniguchi, S. Tsuru, and M. Yoshida.** 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.

21a. **Inoue, J., and M. Yoshida.** Unpublished data.

22. **Koga, Y., N. Kimura, J. Minowada, and T. W. Mak.** 1988. Expression of the human T-cell-specific tyrosine kinase YT16 (*lck*) message in leukemic T-cell lines. Cancer Res. **48:**856–859.
23. **Koga, Y., N. Oh-hori, H. Sato, N. Yamamoto, G. Kimura, and K. Nomoto.** 1989. Absence of transcription of *lck* (lymphocyte specific protein tyrosine kinase) message in IL-2-independent, HTLV-I-transformed T cell lines. J. Immunol. **142:**4493–4499.
24. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82:**488–492.
25. **Lee, W. S., C. C. Kao, G. O. Bryant, X. Liu, and A. J. Berk.** 1991. Adenovirus E1A activation domain binds the basic repeat in the TATA box transcription factor. Cell **67:**365–376.
26. **Leung, K., and G. J. Nabel.** 1988. HTLV-1 transactivator induces interleukin-2 receptor expression through an NF-κB-like factor. Nature (London) **333:**776–778.
27. **Ley, T. J., N. L. Connolly, S. Katamine, M. S. C. Cheah, R. M. Senior, and K. C. Robbins.** 1989. Tissue-specific expression and developmental regulation of the human *fgr* proto-oncogene. Mol. Cell. Biol. **9:**92–99.
28. **Lichtenberg, U., N. Quintrell, and J. M. Bishop.** 1992. Human protein-tyrosine kinase gene HCK: expression and structural analysis of the promoter region. Oncogene **7:**849–858.
29. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 207–209. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
30. **Marriott, S. J., I. Boros, J. F. Duvall, and J. N. Brady.** 1989. Indirect binding of human T-cell leukemia virus type I Tax_1 to a responsive element in the viral long terminal repeat. Mol. Cell. Biol. **9:**4152–4160.
31. **Marth, J. D., R. Peet, E. G. Krebs, and R. M. Perlmutter.** 1985. A lymphocyte-specific protein-tyrosine kinase gene is rearranged and overexpressed in the murine T cell lymphoma LSTRA. Cell **43:**393–404.
32. **Maruyama, M., H. Shibuya, H. Harada, M. Hatakeyama, M. Seiki, T. Fujita, J. Inoue, M. Yoshida, and T. Taniguchi.** 1987. Evidence for aberrant activation of the interleukin-2 autocrine loop by HTLV-I-encoded $p40^{x}$ and T3/Ti complex triggering. Cell **48:**343–350.
33. **Matsuzawa, Y., K. Semba, J. Kawamura-Tsuzuku, T. Sudo, S. Ishii, K. Toyoshima, and T. Yamamoto.** 1991. Characterization of the promoter region of the c-*yes* proto-oncogene: the importance of the GC boxes on its promoter activity. Oncogene **6:**1561–1567.
34. **Miyatake, S., M. Seiki, M. Yoshida, and K. Arai.** 1988. T-cell activation signals and human T-cell leukemia virus type I-encoded $p40^{x}$ protein activate the mouse granulocyte-macrophage colony-stimulating factor gene through a common DNA element. Mol. Cell. Biol. **8:**5581–5587.
35. **Mizusawa, S., S. Nishimura, and F. Seela.** 1986. Improvement of the dideoxy chain termination method of DNA sequencing by use of deoxy-7-deazaguanosine triphosphate in place of dGTP. Nucleic Acids Res. **14:**1319–1324.
36. **Murphy, S., A. Pierani, C. Scheidereit, M. Melli, and R. G. Roeder.** 1989. Purified octamer binding transcription factors stimulate RNA polymerase III-mediated transcription of the 7SK RNA gene. Cell **59:**1071–1080.
37. **Nagata, K., K. Ohtani, M. Nakamura, and K. Sugimura.** 1989. Activation of endogenous c-*fos* proto-oncogene expression by human T-cell leukemia virus type I-encoded $p40^{tax}$ protein in the human T-cell line, Jurkat. J. Virol. **63:**3220–3226.
38. **Nakagoshi, H., T. Nagase, C. Kanei-Ishi, Y. Ueno, and S. Ishii.** 1990. Binding of the c-myb proto-oncogene product to the simian virus 40 enhancer stimulates transcription. J. Biol. Chem. **265:**3479–3483.
39. **Patel, M., S. J. Leevers, and P. M. Brickell.** 1990. Structure of the complete human c-*fgr* proto-oncogene and identification of multiple transcriptional start sites. Oncogene **5:**201–206.
40. **Ruben, S., H. Poteat, T. Tan, K. Kawakami, R. Roeder, W. Haseltine, and C. A. Rosen.** 1988. Cellular transcription factors and regulation of IL-2 receptor gene expression by HTLV-I *tax* gene product. Science **241:**89–92.
41. **Rudd, C. E., J. M. Trevillyan, J. D. Dasgupta, L. L. Wong, and S. F. Schlossman.** 1988. The CD4 receptor is complexed in detergent lysates to a protein-tyrosine kinase (pp58) from human T lymphocytes. Proc. Natl. Acad. Sci. USA **85:**5190–5194.
42. **Samelson, L. E., A. F. Phillips, E. T. Luong, and R. D. Klausner.** 1990. Association of the fyn protein-tyrosine kinase with the T-cell antigen receptor. Proc. Natl. Acad. Sci. USA **87:**4358–4362.
43. **Scheidereit, C., A. Heguy, and R. G. Roeder.** 1987. Identification and purification of a human lymphoid-specific octamer-binding protein (OTF-2) that activates transcription of an immunoglobulin promoter in vitro. Cell **51:**783–793.
44. **Schreck, R., and P. A. Baeuerle.** 1990. NF-κB as inducible transcriptional activator of the granulocyte-macrophage colony-stimulating factor gene. Mol. Cell. Biol. **10:**1281–1286.
45. **Seiki, M., J. Inoue, T. Takeda, and M. Yoshida.** 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.
46. **Shimotohno, K., M. Takano, T. Teruuchi, and M. Miwa.** 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. Natl. Acad. Sci. USA **83:**8112–8116.
47. **Shule, R., M. Muller, H. O. Murakami, and R. Reukawitz.** 1988. Cooperativity of the glucocorticoid receptor and the CACCC-box binding factor. Nature (London) **332:**87–90.
48. **Siekevitz, M., M. B. Feinberg, N. Holbrook, F. Wong-Staal, and W. C. Greene.** 1987. Activation of interleukin 2 and interleukin 2 receptor (Tac) promoter expression by the trans-activator (tat) gene product of human T-cell leukemia virus, type I. Proc. Natl. Acad. Sci. USA **84:**5389–5393.
49. **Smith, K. A.** 1980. T-cell growth factor. Immunol. Rev. **51:**337–357.
50. **Smith, K. A.** 1984. Interleukin 2. Annu. Rev. Immunol. **2:**319–333.
51. **Sodroski, J., C. Rosen, W. C. Goh, and W. Haseltine.** 1985. A transcriptional activator protein encoded by the x-*lor* region of the human T-cell leukemia virus. Science **228:**1430–1434.
52. **Stanley, E., S. Ralph, S. McEwen, I. Boulet, D. A. Holtzman, P. Lock, and A. R. Dunn.** 1991. Alternatively spliced murine *lyn* mRNAs encode distinct proteins. Mol. Cell. Biol. **11:**3399–3406.
53. **Staudt, L. M., H. Singh, R. Sen, T. Wirth, P. A. Sharp, and D. Baltimore.** 1986. A lymphoid-specific protein binding to the octamer motif of immunoglobulin genes. Nature (London) **323:**640–643.
54. **Sturm, R. A., G. Das, and W. Herr.** 1988. The ubiquitous octamer-binding protein Oct-1 contains a POU domain with a homeo box subdomain. Genes Dev. **2:**1582–1599.
55. **Takedera, T., S. Leung, A. Gernone, Y. Koga, Y. Takihara, N. G. Miyamoto, and T. W. Mak.** 1989. Structure of the two promoters of the human *lck* gene: differential accumulation of two classes of *lck* transcripts in T cells. Mol. Cell. Biol. **9:**2173–2180.
56. **Taniguchi, T., H. Matsui, T. Fujita, M. Hatakeyama, N. Kashima, A. Fuse, J. Hamuro, C. Nishi-Takaoka, and G. Yamada.** 1986. Molecular analysis of the interleukin-2 system. Immunol. Rev. **92:**121–133.
57. **Trevillyan, J. M., C. Canna, D. Maley, T. J. Linna, and C. A. Phillips.** 1986. Identification of the human T-lymphocyte protein-tyrosine kinase by peptide-specific antibodies. Biochem.

Biophys. Res. Commun. **140:**392–398.
58. **Veillette, A., M. A. Bookman, E. M. Horak, and J. B. Bolen.** 1988. The CD4 and CD8 cell surface antigens are associated with the internal membrane tyrosine-protein kinase $p56^{lck}$. Cell **55:**301–308.
59. **Wang, J., K. Nishiyama, K. Araki, D. Kitamura, and T. Watanabe.** 1987. Purification of an octamer sequence (ATGCAAAT)-binding protein from human B cells. Nucleic Acids Res. **15:** 10105–10116.
60. **Wasylyk, C., P. Flores, A. Gutman, and B. Wasylyk.** 1989. PEA3 is a nuclear target for transcription activation by non-nuclear oncogenes. EMBO J. **8:**3371–3378.
61. **Wirth, T., L. Staudt, and D. Baltimore.** 1987. An octamer oligonucleotide upstream of a TATA motif is sufficient for lymphoid-specific promoter activity. Nature (London) **329:**174–178.
62. **Wong-Staal, F., and R. C. Gallo.** 1985. Human T-lymphotropic retroviruses. Nature (London) **317:**395–403.
63. **Yamanashi, Y., S. Fukushige, K. Semba, J. Sukegawa, N. Miyajima, K. Matsubara, T. Yamamoto, and K. Toyoshima.** 1987. The *yes*-related cellular gene *lyn* encodes a possible tyrosine kinase similar to $p56^{lck}$. Mol. Cell. Biol. **7:**237–243.
64. **Yamanashi, Y., Y. Fukui, B. Wongsasant, Y. Kinoshita, Y. Ichimori, K. Toyoshima, and T. Yamamoto.** 1992. Activation of Src-like protein-tyrosine kinase Lyn and its association with phosphatidylinositol-3 kinase upon B cell antigen receptor-mediated signaling. Proc. Natl. Acad. Sci. USA **89:**1118–1122.
65. **Yamanashi, Y., T. Kakiuchi, J. Mizuguchi, T. Yamamoto, and K. Toyoshima.** 1991. Association of B cell antigen receptor with protein tyrosine kinase Lyn. Science **251:**192–194.
66. **Yamanashi, Y., M. Miyasaka, M. Takeuchi, D. Ilic, J. Mizuguchi, and T. Yamamoto.** 1991. Differential responses of $p56^{lyn}$ and $p53^{lyn}$, products of alternatively spliced lyn mRNA, on stimulation of B cell antigen receptor. Cell Regul. **2:**979–987.
67. **Yamanashi, Y., S. Mori, M. Yoshida, T. Kishimoto, K. Inoue, T. Yamamoto, and K. Toyoshima.** 1989. Selective expression of a protein-tyrosine kinase, $p56^{lyn}$, in hematopoietic cells and association with production of human T-cell lymphotropic virus type I. Proc. Natl. Acad. Sci. USA **86:**6538–6542.
68. **Yi, T., J. B. Bolen, and J. N. Ihle.** 1991. Hematopoietic cells express two forms of *lyn* kinase differing by 21 amino acids in the amino terminus. Mol. Cell. Biol. **11:**2391–2398.
69. **Yoshida, M.** 1987. Expression of the HTLV-1 genome and its association with a unique T-cell malignancy. Biochim. Biophys. Acta **907:**145–161.
70. **Yoshida, M., I. Miyoshi, and Y. Hinuma.** 1982. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. Proc. Natl. Acad. Sci. USA **79:**2031–2035.