MOLECULAR AND CELLULAR BIOLOGY, Sep. 1994, p. 5812–5819
0270-7306/94/$04.00+0

Vol. 14, No. 9

Functional Dissection of p56^{lck}, a Protein Tyrosine Kinase Which Mediates Interleukin-2-Induced Activation of the c-*fos* Gene

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Received 7 March 1994/Returned for modification 1 April 1994/Accepted 3 June 1994

Members of the newly identified receptor family for cytokines characteristically lack the intrinsic protein tyrosine kinase domain that is a hallmark of other growth factor receptors. Instead, accumulating evidence suggests that these receptors utilize nonreceptor-type protein tyrosine kinases for downstream signal transduction by cytokines. We have shown previously that the interleukin-2 receptor β-chain interacts both physically and functionally with a Src family member, p56^{lck}, and that p56^{lck} activation leads to induction of the c-*fos* gene. However, the mechanism linking p56^{lck} activation with c-*fos* induction remains unelucidated. In the present study, we systematically examined the extent of c-*fos* promoter activation by expression of a series of p56^{lck} mutants, using a transient cotransfection assay. The results define a set of the essential amino acid residues that regulate p56^{lck} induction of the c-*fos* promoter. We also provide evidence that the serum-responsive element and *sis*-inducible element are both targets through which p56^{lck} controls c-*fos* gene activation.

p56^{lck}, a lymphocyte-specific member of the Src family of protein tyrosine kinases (PTKs) (14, 30), is expressed almost exclusively in T lymphocytes, especially thymocytes (5, 20). Expression of catalytically active p56^{lck} is required for normal T-cell development, in part because p56^{lck} drives the maturation of CD4$^+$8$^+$ cells from immature CD4$^-$8$^-$ progenitors (reviewed in reference 3). In addition, p56^{lck} associates with the cytoplasmic portions of the CD4 and CD8 coreceptors and participates in delivering signals from these cell surface glycoproteins. We have previously demonstrated that the catalytic domain of p56^{lck} associates intracellularly with the cytoplasmic "acidic region" of the interleukin-2 receptor (IL-2R) β-chain (10). Moreover, p56^{lck} kinase activity was shown to be significantly augmented by interleukin-2 (IL-2) treatment (16).

Our studies on IL-2-mediated signal transduction through the IL-2R β-chain have revealed the presence of two distinct signaling pathways: a Src family tyrosine kinase-dependent pathway and a Src family tyrosine kinase-independent pathway (24). The former is linked to the induction of c-*fos* and c-*jun* gene expression, whereas the latter is linked to c-*myc* gene induction. Furthermore, we had reported that IL-2 activates the serum-responsive element (SRE) of the c-*fos* gene, but not the *sis*-inducible element (SIE), to induce c-*fos* transcription in a mouse pro-B-cell line, BAF-B03 (9).

The p56^{lck} kinase can be divided into several distinct functional domains (reviewed in reference 21). The catalytic domain is located in the carboxy-terminal half of the protein, where a regulatory site of tyrosine phosphorylation, Tyr-505, is positioned. Mutation of Tyr-505 results in augmented kinase activity and the acquisition of transforming potential (1, 2, 13). Sequences necessary for myristylation and membrane association are contained in the amino-terminal region of p56^{lck}, which also contains unique sequence motifs, centered around cysteine residues 20 and 23, that permit interaction with the coreceptor molecules CD4 and CD8 (reviewed in references 21 and 28).

The N-terminal half of p56^{lck} also contains two noncatalytic domains, known as Src homology domains (SH2 and SH3), common to Src family PTKs and to a large member of unrelated proteins. The SH2 domains have been identified in phospholipase C-γ, the Ras GTPase-activating protein, the p85 subunit of phosphatidylinositol 3′ kinase, and the oncoproteins Nck, Vav, and Crk, among others. The Src family PTKs, and most of these other proteins as well, also contain a distinct motif termed the SH3 domain. The presence of these domains in a wide variety of proteins suggests that they are important for the formation of protein complexes to mediate signaling through common effector pathways (reviewed in references 15 and 19).

Several reports have demonstrated that the SH2 and SH3 domains may be important in modulating p56^{lck} functions. For example, mutation of the SH3 domain of p56^{lck} affects its kinase activity and its ability to induce morphological changes associated with cellular transformation (27). Mutations within the SH2 domain partially abrogate the ability of p56^{lck} to induce cellular transformation, suggesting that the SH2 domain may direct the interaction of p56^{lck} with cellular proteins required for maintenance of transformation (27). It has been shown that SH2 domains can mediate protein-protein interactions by binding to phosphotyrosine residues of target proteins. In addition, the SH2 domain of p56^{lck} interacts intramolecularly with the distal phosphorylated Tyr-505 residue, thereby repressing p56^{lck} kinase activity (reviewed in reference 21). In this model, activation of p56^{lck} by dephosphorylation of Tyr-

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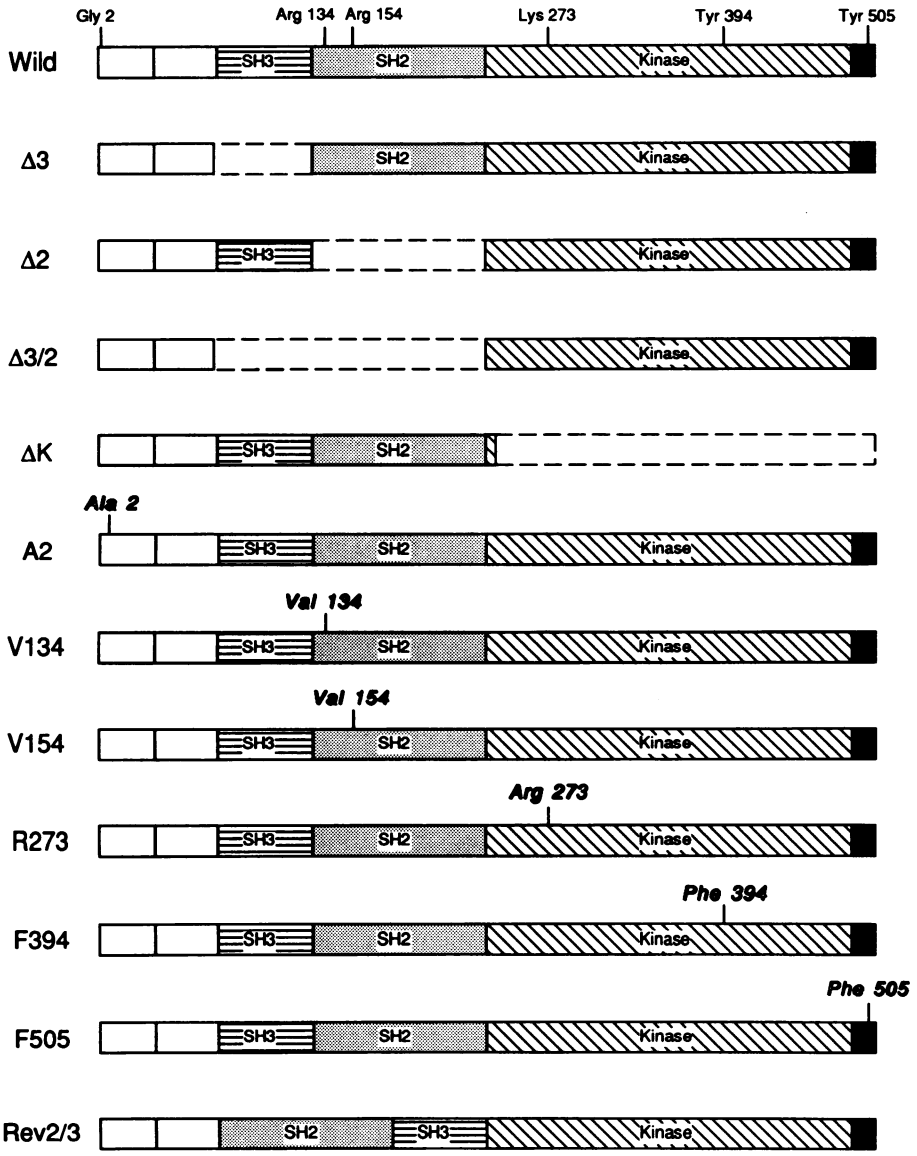


FIG. 1. Construction of deletion and point mutants of p56^{lck}. Construction of the cDNAs encoding the mutant proteins is described in Materials and Methods.

505 would release the SH2 domain to interact with other appropriate target proteins.

To further delineate the role of p56^{lck} in growth signal transduction, we have constructed a series of *lck* mutants with deletion or point mutations. Transient transfection assays were then employed to identify regions required for Lck-induced c-*fos* gene expression. Here, we provide evidence that the tyrosine kinase activity of p56^{lck} is absolutely required for induction of c-*fos* gene expression. Interestingly, the SRE and SIE of the c-*fos* gene both appear to mediate c-*fos* gene activation by p56^{lck}.

MATERIALS AND METHODS

Plasmid construction. The *lck* expression vectors, pKWild (pdKCR-*lck*), pKΔ2 (LM-3), pKΔK (LM-4), and pKF505 (pdKCR-*lck* F505) were constructed as described previously (10, 16). To generate the other mutant *lck* cDNAs, the pKWild plasmid was modified. To construct pKΔ3 and pKΔ3/2, pK-Wild was digested with *Bgl*II and *Eco*T14I or with *Bgl*II and *Nco*I, respectively. A synthesized oligonucleotide linker (G A TCTCGCTGCCCATCCGGAATGGCTCTGAAGTGCGG GACC), which contains *Bgl*II and *Nco*I (*Eco*T14I) sites, was annealed to an antisense strand and then ligated. All single mutants were generated by the recombinant PCR method with the following oligonucleotides: for the Gly-2-to-Ala-2 mutant, CTCCGGATCCCTCTCTACATTCCTTCAGGGATCAT GGCGGTGT (sense) and GAAGAACAAGGTTCAGGCT CCAGGCTGTTTGCTTTCGCCACGA (antisense); for the Tyr-394-to-Phe-394 mutant, CAGAAACCATGGTGGGAG GACGAATGGGAA (sense) and CCCCTCCCGGGCCGT GAACTCATTGCCTC (antisense). To construct pKRev2/3, pKWild was digested with *Stu*I and *Xba*I, and pKΔ3 was digested with *Stu*I and *Nco*I. The 150-bp *Stu*I-*Nco*I fragment from pKΔ3 was isolated, and the 330-bp *Nco*I-*Xba*I fragment

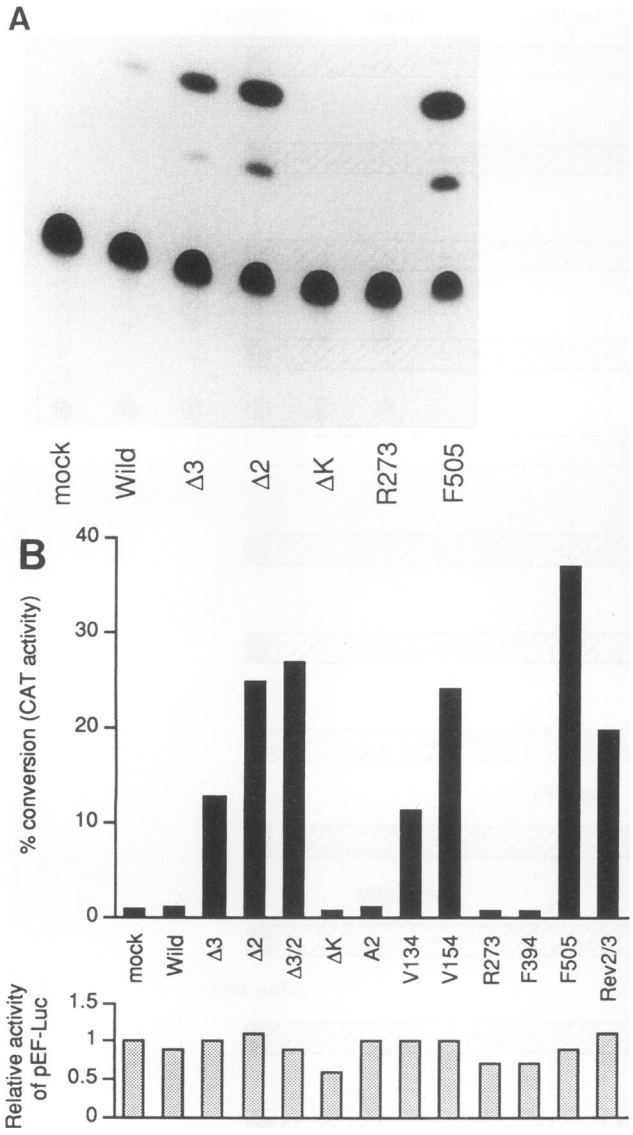


FIG. 2. Activation of c-*fos* promoter by *lck* mutants. (A) *lck* mutant plasmids were cotransfected with FC3. The transfected cells were deprived of WEHI conditioned medium for 18 h, and CAT activity was assayed as described in Materials and Methods. (B) The various *lck* mutants were cotransfected with FC3 and elongation factor promoter-luciferase gene expression plasmid (pEF-Luc). Luciferase activity was determined as described in Materials and Methods. The transfection experiments were repeated at least three times, and the results were reproducible.

which contains both the SH3 and the catalytic domains was generated by PCR from a pKΔ2 template by using the oligonucleotide AAGTGCGGGACCCATGGGTCACCTATGAG GGATCTCTCCC (sense) and GGTTCAGGCTCCAGTCTA GATGCTTTCGCCACGAAGTTGA (antisense). These fragments were ligated into the pKWild vector. For generation of double mutants, chimeric cDNAs were constructed by replacing the *Stu*I-*Nco*I or *Nco*I-*Sma*I fragment of the F505 *lck* cDNA with that of either the A2 or the F394 *lck* cDNA, respectively. Mutant *lck* sequences were determined by using oligonucleotide primers based on the previously determined DNA sequences. In each case the sequence information was generated by the dideoxynucleotide chain termination method.

pEF-Luc was generated by cleaving pEF-MSS1 (23) with *Eco*RI and *Bam*HI and inserting an *Eco*RI-*Bam*HI fragment from BHlucOL1 (29) containing the luciferase coding sequences and poly(A) addition signal sequences.

The point mutants of the c-*fos* promoter, mSRE and mSIE, were constructed as described previously (9). The plasmid mSIE has AAATTA instead of CCCGTC within the c-*fos* SIE sequences.

Cells and cell culture. F7 cells are BAF-B03-derived stable transformants expressing the human IL-2R β-chain (11). F7 cells were maintained in RPMI 1640 medium supplemented with 10% (vol/vol) fetal calf serum and 10% (vol/vol) conditioned medium from the WEHI-3B cell line (10% WEHI conditioned medium) as a source of IL-3.

DNA transfection, CAT assay, and luciferase assay. The *lck* mutant genes as described above and the reporter gene (FC3) were cotransfected with pEF-Luc as the internal reference gene for chloramphenicol acetyltransferase (CAT) assay into F7 cells by using the DEAE-dextran method as described previously (25). After the transfection, the cells were deprived of IL-3 in RPMI 1640–10% fetal bovine serum without WEHI conditioned medium for 18 h. The cells were washed with phosphate-buffered saline and then divided into two portions for CAT assays and luciferase assays. Cell extracts for CAT assays were prepared, and CAT assays were performed, as described previously (25). Data were analyzed quantitatively with the use of the BAS2000 image analyzer (FUJI). For the luciferase assay, cells were lysed in 500 μl of buffer (25 mM Tricine [pH 7.8], 0.5 mM EDTA, 0.54 mM sodium tripolyphosphate, 16.3 mM $MgSO_4$, 0.3% Triton X-100, 0.1% dithiothreitol, 1.2 mM ATP, 270 μM coenzyme A). The lysates (400 μl) were reacted with 1 mM luciferin (20 μl), and the luminescence was immediately quantitated with a luminometer (Berthold).

In vitro kinase assay. Transiently transfected F7 cells were solubilized with lysis buffer (50 mM Tris-HCl [pH 8.0], 0.5% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, 50 mM NaF, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, 10 μg of leupeptin per ml, 10 μg of aprotinin per ml) for 30 min at 4°C. The lysates were centrifuged to remove the insoluble materials, and the resultant supernatants were precleared for 1 h at 4°C with protein A-Sepharose (Pharmacia). Immunoprecipitates were then formed with anti-Lck antiserum plus protein A-Sepharose for 2 h at 4°C. The immunoprecipitates were washed seven times with lysis buffer prior to the in vitro kinase assay and then washed once with kinase buffer (25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid] [pH 7.2], 0.1% [vol/vol] Nonidet P-40, 10 mM $MgCl_2$, 30 μM Na_3VO_4) and resuspended in 30 μl of the kinase buffer containing 2 μg of rabbit muscle enolase as an exogenous substrate. Reactions were initiated by the addition of 3.75 μM [γ-^{32}P]ATP per sample (3,000 Ci/mmol; Amersham), and reaction mixtures were incubated for 5 min at 25°C. The reaction was terminated by the addition of Laemmli buffer. Subsequently, the samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10 to 20% gradient gels) under reducing conditions. Phosphorylated proteins, including enolase, were detected by exposure of the dried gels to X-ray film.

RESULTS

Construction of *lck* mutants. To dissect the functional domains of $p56^{lck}$, a series of mutants was generated as follows: by deletion of the SH3 region (pKΔ3; amino acids 54 to 119), deletion of the SH2 region (pKΔ2; amino acids 126 to 232),

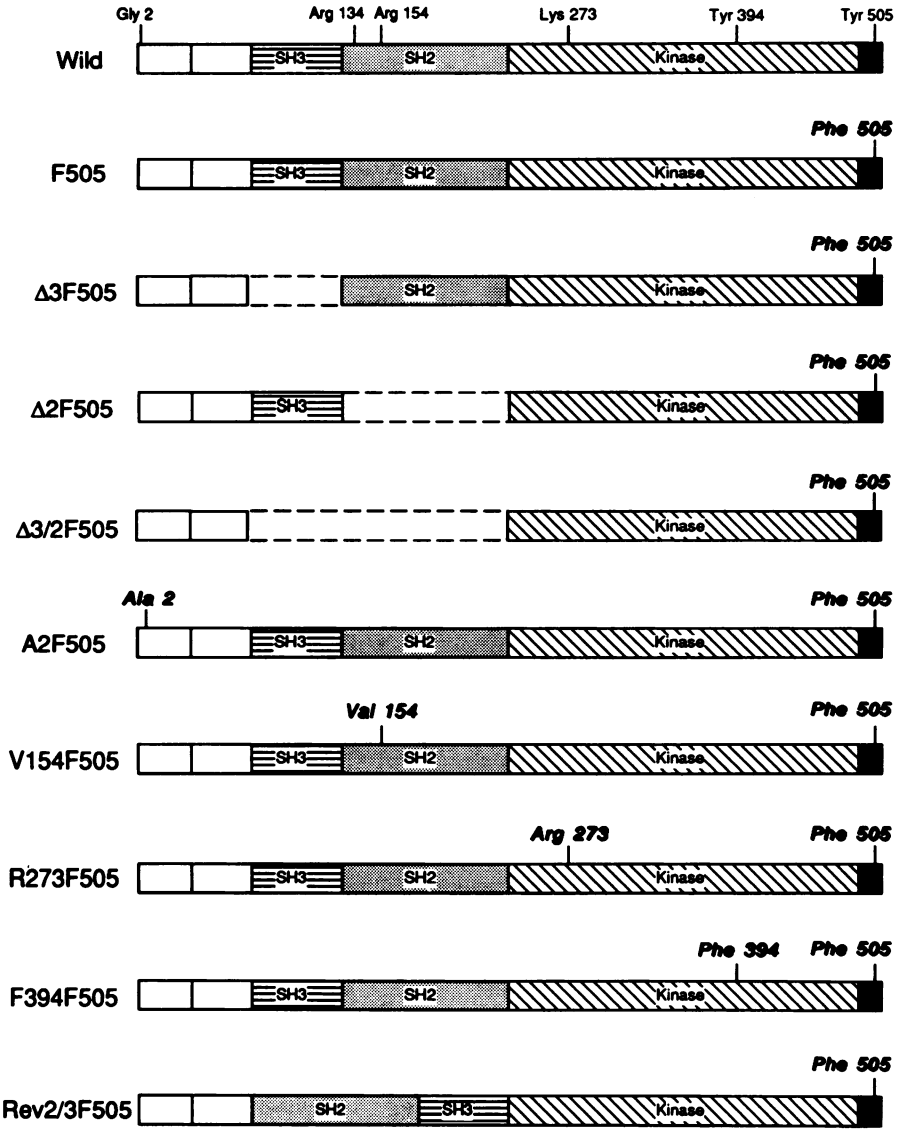


FIG. 3. Additional mutations for F505 *lck* mutant.

deletion of both the SH3 and SH2 regions (pKΔ3/2; amino acids 54 to 232), deletion of the kinase region (pKΔK; amino acids 233 to 509), mutation of the myristylation site (pKA2), mutation of the phosphotyrosine association sites within the SH2 region (pKV134 and pKV154), mutation of the ATP interaction site within the kinase region (pKR273), mutation of the autophosphorylation tyrosine residue within the kinase region (pKF394), and mutation of the regulatory phosphotyrosine residue in the carboxy-terminal region (pKF505) (Fig. 1). The mutant (pKRev2/3) with the positions of the SH2 and SH3 regions reversed was also constructed. In addition, double mutants carrying the F505 mutation with a series of deletions or point mutations were created (see Fig. 3). All mutant cDNAs were sequenced and found to contain no additional alterations. Each mutant expression plasmid was transiently transfected into F7 cells (11) and subjected to Western blotting (immunoblotting) analysis using anti-Lck antibodies which recognize the amino-terminal region or the carboxy-terminal region of p56^{lck}. The products of all the *lck* constructs were efficiently detected (data not shown).

Activation of the c-*fos* promoter by the *lck* mutants. To examine the effect of the *lck* mutants on c-*fos* promoter activity, we cotransfected F7 cells, which are BAF-B03-derived stable transformants expressing the human IL-2R β-chain (11), with the *lck* mutant expression plasmids and with constructs containing the CAT gene under the control of the promoter from the c-*fos* gene (FC3) (4). The BAF-B03 cell is a hematopoietic cell line useful for the analysis of Lck function because it lacks endogenous *lck* transcripts (16). A luciferase reporter plasmid under the control of the elongation factor promoter was included as a reference to permit normalization with respect to transfection efficiency. As expected, we noted that expression of the activated F505 *lck* mutant, but not the wild-type kinase, resulted in a marked increase in CAT activity (Fig. 2). In order to address the importance of the topographic sequence of SH2 and SH3 in the Lck molecule and the

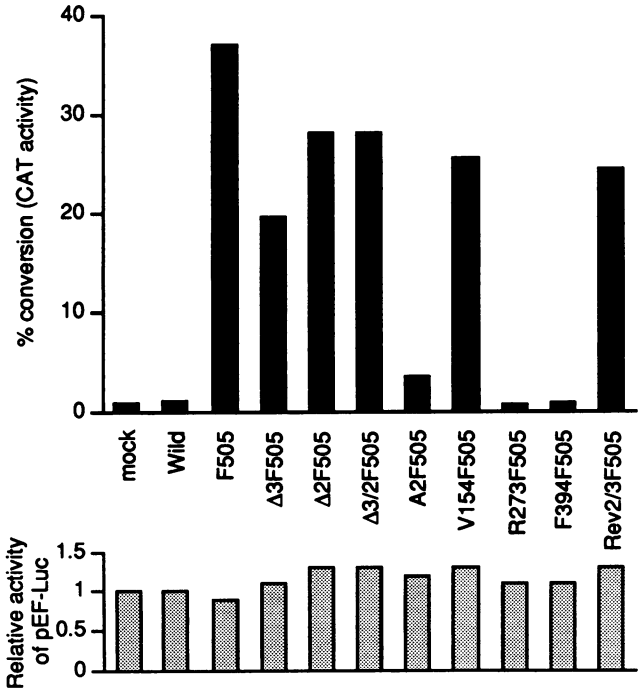


FIG. 4. Effect of additional mutations on activated *lck* mutant F505 for the expression of the c-*fos* gene. The c-*fos*–CAT fusion gene (FC3) was cotransfected with a series of double mutant expression plasmids. CAT activity and luciferase activity were determined 18 h after the transfection. Essentially identical results were obtained in at least three independent experiments.

potential role of SH3 in the IL-2-mediated c-*fos* signal, the ΔSH2, ΔSH3, ΔSH3/2, and Rev2/3 *lck* mutants were generated and tested. Moreover, the role of the conserved Arg-134 and Arg-154 residues in the function of the SH2 domain with respect to c-*fos* gene activation was explored. Expression of ΔSH2, ΔSH3, or ΔSH3/2 *lck* constructs also stimulated c-*fos* promoter activity (Fig. 2B). Interestingly, the Rev2/3 *lck* mutant, in which the positions of the SH3 and SH2 regions were reversed, provoked activation of the c-*fos* promoter. In addition, constructs bearing mutations at Arg-134 and Arg-154 in the SH2 region were also activated in this assay. Furthermore, to explore the importance of the cell membrane association of Lck and the integrity of the kinase domain, we generated the A2, R273, and F394 *lck* mutants. As shown in Fig. 2B, no effect was observed when the A2, R273, or F394 *lck* mutant was expressed. These findings emphasize the critical importance of the SH2 and SH3 regions, and of the distal Tyr residue of the carboxy-terminal region, in the signaling pathway for c-*fos* gene expression.

Identification of the critical domains of p56^{lck} required for c-*fos* gene expression. By means of a number of point mutations and deletions along the entire structure of p56^{lck}, we investigated the importance of the structural features of F505 *lck* in the context of a constitutively active kinase domain for the activation of the c-*fos* promoter. The various mutants are shown in Fig. 3, and the assay system is the same as described above. As shown in Fig. 4, the c-*fos*-inducing activities of R273F505 *lck*, which contains an Lys-to-Arg mutation in the catalytic domain at amino acid residue 273, and of F394F505 *lck*, which contains a Tyr-to-Phe mutation in the catalytic domain at amino acid residue 394, were completely abolished. On the other hand, the F505 *lck* mutants lacking the SH2 domain or the SH3 domain or both retained the ability to stimulate the c-*fos* promoter, indicating that the kinase domain per se can act independently of the SH2 and SH3 domains in c-*fos* promoter activation. Taken together, the data presented above indicate that only the tyrosine kinase activity of Lck is required for c-*fos* promoter activation.

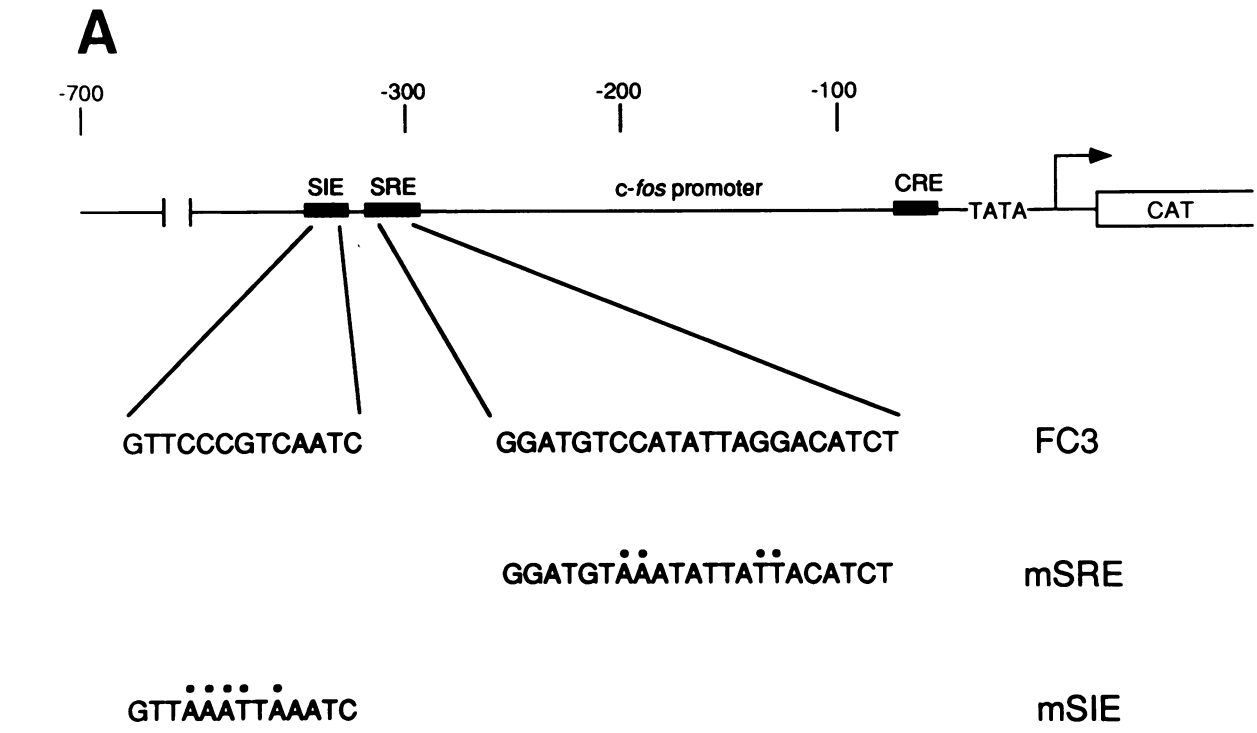
Previous studies have shown that p56^{lck} contains covalently bound myristic acid at its amino terminus, which assists association with the plasma membrane (reviewed in references 21 and 28). Mutating Gly-2 to an Ala residue abolishes both myristylation and cell membrane association (1). However, the critical role of Lck membrane association in PTK activity and downstream signaling is not sufficiently clear. Membrane association of p56^{lck} is not necessary for the kinase activity (see "Activation of the c-*fos* promoter by the *lck* mutants" above for details). On the other hand, a dramatic reduction in the Lck-induced c-*fos* promoter activity was observed with F505 *lck* carrying the Gly-to-Ala substitution at position 2 (A2F505; Fig. 4). This result indicates the importance of cell membrane association of activated p56^{lck} for downstream signaling to activate the c-*fos* promoter.

Both SRE and SIE are required for c-*fos* gene activation by p56^{lck}. In a previous study, we had identified the *cis*-acting response element(s) of the c-*fos* promoter required for an IL-2-mediated signal (9). Moreover, IL-2R β-chain association with p56^{lck} was also demonstrated (10, 16). However, the details of the effects of p56^{lck} on the c-*fos* promoter have not been exhaustively explored. In fact, recent evidence on signaling mediated by epidermal growth factor and platelet-derived growth factor points to the critical role of the SIE (22, 31). Moreover, new data indicate the presence of independent pathways for the SRE and SIE in c-*fos* gene expression (reviewed in reference 17). The point mutants of the FC3 reporter plasmid (Fig. 5A) were cotransfected into F7 cells with the active *lck* mutants, and the Lck-induced CAT expression levels were monitored. Figure 5B shows that mutation of either the SRE or SIE of the c-*fos* promoter abrogates p56^{lck}-mediated transcriptional regulation, although the inducing level of the wild-type c-*fos* promoter (FC3) was found to be different from that caused by each *lck* mutant. Hence, both the SRE and SIE sequences are required for the induction of expression of the c-*fos* gene by Lck.

Analysis of the PTK activities of the p56^{lck} mutants. To evaluate the basis for the lack of activating potential of several *lck* mutants, the tyrosine kinase activities of a series of *lck* mutants were compared by in vitro kinase assays. The Lck-associated tyrosine kinase activity was measured by in vitro kinase reactions with rabbit muscle enolase as an exogenous substrate (Fig. 6). The experiments revealed that the specific activities of the tyrosine kinase-deficient *lck* mutants (R273F505 and F394F505) were approximately 5- to 10-fold less than that of F505 *lck*. Interestingly, the specific activity of the A2F505 *lck* mutant was nearly identical to that of the F505 *lck* mutant. Hence, the catalytic activity of p56^{lck} is not by itself sufficient to provoke c-*fos* gene induction; activated p56^{lck} must also be localized correctly in the cell.

DISCUSSION

In the present study, we have examined the effect of the p56^{lck} on c-*fos* gene expression. Using a cotransfection system, we found that p56^{lck} activates c-*fos* transcriptionally in the hematopoietic cell line BAF-B03 (F7). We also demonstrated that a functional tyrosine kinase domain is essential for the activation of c-*fos* gene expression.



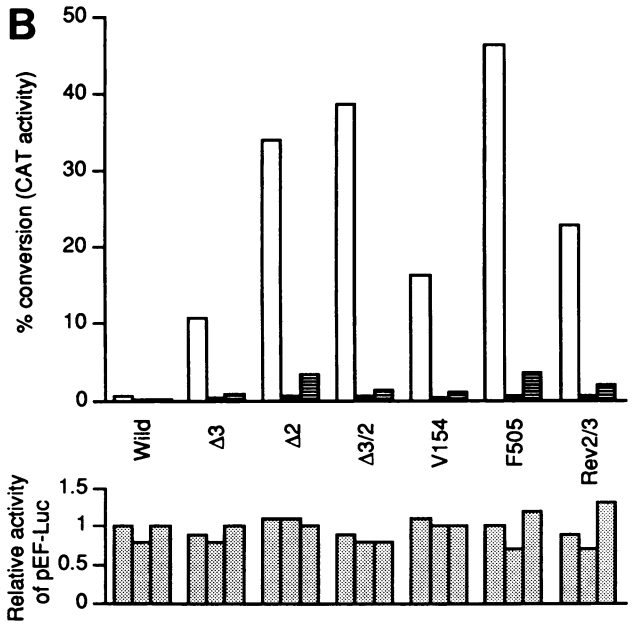


FIG. 5. Role of SRE and SIE in p56^{lck}-induced c-*fos* activation. (A) Structure of the upstream sequences of the c-*fos* gene and mutant c-*fos* promoter sequences. Site-directed mutations were introduced into the SRE and SIE sequences in the FC3 plasmid to make mSRE and mSIE, as described in Materials and Methods. (B) FC3 (□), mSRE (■), or mSIE (▤) plasmid was cotransfected with the indicated *lck* mutant expression plasmids. CAT activity and luciferase activity were determined 18 h after the transfection.

The SH2 and SH3 domains of p56^{lck} are believed to be critical for modulating its PTK activity and for directing protein-protein interactions required for p56^{lck}-mediated changes in cell behavior. The sequence of the SH2 and SH3 domains and the functional role of either or both motifs in Lck function were systematically explored by using deletion mutants or by reversing the order of these domains. Deletion of the SH2 domain, the SH3 domain, or both or reversal of the positions of the SH2 and SH3 domains resulted in a striking elevation in the kinase activity of p56^{lck}. This was further indicated by the ability of these mutants to activate c-*fos* gene expression in F7 cells. Thus, the enhancement of catalytic activity correlated well with the enhancement of c-*fos* gene expression. Moreover, neither the SH2 nor the SH3 domain contributed to the activation of c-*fos* gene expression by the catalytically activated *lck* mutant (F505) (Fig. 4). In fact, expression of the F505 mutants lacking either SH2 or SH3 or both still induced the c-*fos* promoter, albeit with a slightly lower efficiency. Thus, the PTK activity of p56^{lck} is by itself required to invoke downstream signaling and the activation of the c-*fos* promoter. It remains possible that the SH2 and SH3 regions may also be necessary for full-scale activation of the c-*fos* gene (Fig. 4). Further work will be required to elucidate the mechanism of interplay between the SH2 and SH3 domains in the overall regulation of c-*fos* gene expression by p56^{lck}, particularly with respect to other SH2- or SH3-interacting cellular proteins necessary for the amplification or the specification of the signal.

Like those of prior studies (reviewed in reference 21), our results implicate the SH2 and SH3 domains in negatively regulating the kinase activity of p56^{lck}. It has been proposed that there may be an intramolecular interaction between the amino-terminal half of p56^{lck} and the catalytic domain of the molecule to regulate kinase activity. Several lines of evidence support the model that the SH2 domain interacts with the regulatory phosphotyrosine at residue 505 in p56^{lck} to restrict its enzymatic activity (reviewed in references 18 and 21). Indeed, high-resolution crystallographic analysis of the SH2 domain of p56^{lck} has revealed a conserved fold in this domain

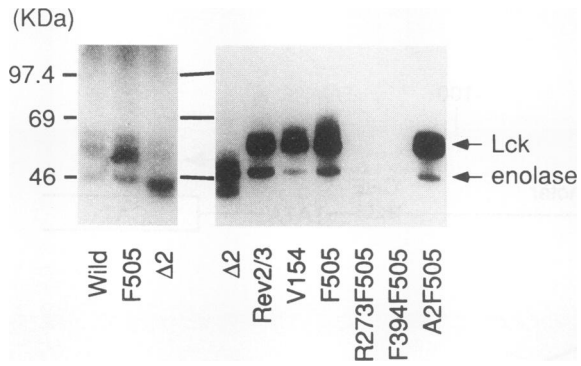


FIG. 6. Activation of PTK for *lck* mutants transfected in F7 cells. After transfection of each *lck* mutant expression plasmid, cell lysates were prepared as described in Materials and Methods. $p56^{lck}$ was immunoprecipitated with anti-Lck antiserum and subjected to an in vitro kinase assay with enolase as an exogenous substrate to evaluate its tyrosine kinase activity. The positions of tyrosine-phosphorylated Lck and enolase are indicated by the arrows.

and properties of a phosphotyrosine-binding pocket (7). In addition, the SH3 domain is also likely to play a significant role in regulating kinase activity, possibly by interacting directly with the catalytic domain. One model proposes that kinase activity may be inhibited by binding of the catalytic domain to substrate-like sequences found in the SH3 domain. Indeed, a recent report shows that the C-terminal tail of $p56^{lck}$ binds at the intermolecular SH3-SH2 contact site (6). The data presented here are consistent with these models for the regulation of $p56^{lck}$ activity, since deletion of either the SH2 or SH3 domain augments the Lck catalytic activity (Fig. 2B).

Upon activation by mutation of Tyr-505, $p56^{lck}$ becomes extensively phosphorylated in vitro at Tyr-394, the site of in vitro autophosphorylation (2, 13). To evaluate whether Tyr-394 phosphorylation may participate in regulation of the tyrosine-specific protein kinase activity of $p56^{lck}$, this residue within F505 *lck* was replaced by a Phe, and the resultant mutant, F394F505, was expressed in F7 cells. Transient expression analyses revealed that this mutation completely abolishes the ability of the F505 *lck* mutant to stimulate c-*fos* gene expression (Fig. 4). In agreement with these findings, the tyrosine-specific protein kinase activity of the F394F505 *lck* mutant (measured in in vitro kinase assays) was determined to be fivefold lower than that of the F505 *lck* protein (Fig. 6).

Myristylation of $p56^{lck}$ at its amino-terminal glycine residue is required for stable association of $p56^{lck}$ with the cellular membrane (reviewed in references 21 and 28). Mutation of Gly-2 of $p56^{lck}$ to Ala prevents myristylation and membrane association of this polypeptide (1). We have shown that Gly-2 is also required for the activation of c-*fos* gene expression. On the other hand, the PTK activity of the A2F505 *lck* mutant was approximately the same as that of the F505 *lck* mutant. These results indicate that the membrane association of $p56^{lck}$ is not required for PTK activation but is required for its action on downstream signal transducers.

Previously, the importance of the SRE in the promoter region of c-*fos* in the IL-2R β-chain-mediated signal was identified (9). However, the details of $p56^{lck}$ effects on the c-*fos* promoter were not exhaustively explored. In the present study, we have provided evidence that $p56^{lck}$ activates both the SRE and SIE sequences to induce c-*fos* proto-oncogene expression. The SRE has been shown to be the primary target of growth factors such as platelet-derived growth factor and epidermal growth factor that also induce c-*fos* gene expression (8, 26). However, recent findings point to the importance of the SIE in addition to the SRE motif in c-*fos* induction. This alternative pathway is activated by c-*sis* and functions via another element located ~60 bp upstream of the SRE in the c-*fos* gene (12, 31). Moreover, recent studies have shown that there are independent pathways for the SRE and SIE in c-*fos* gene expression and that different transcription factors bind to these elements (22; reviewed in reference 17). The present study further points to the general importance of the SIE as a target of Src family tyrosine kinase-mediated signal transduction. Our constructs harbor additional sequences from the c-*fos* gene, such as the cyclic AMP responsive element; therefore, we cannot exclude the possibility that some elements distinct from the SRE and SIE may also be involved in the $p56^{lck}$ response in F7 cells.

Previously, we demonstrated that $p56^{lck}$ interacts with the cytoplasmic acidic region of the IL-2R β-chain, that $p56^{lck}$ kinase activity is at least partly regulated by the IL-2 signal (16), and that IL-2 activation of the c-*fos* gene in F7 cells is mediated through its SRE (9). The present findings indicate that both the SRE and SIE are required for $p56^{lck}$-mediated c-*fos* gene activation. However, we also observed that induction of c-*fos* gene expression requires both the acidic and the serine-rich regions of the IL-2R β-chain (9, 16, 24). Therefore, another molecule(s) functionally coupled with the serine-rich (or the acidic) region of the IL-2R β-chain may be critical for c-*fos* gene activation mediated through the SRE. Our findings clearly demonstrate the complexity of the events leading to cell growth following cytokine signaling. Further studies will be required to elucidate the mechanism underlying the fine tuning of IL-2-induced c-*fos* gene expression through intermediary effectors, such as $p56^{lck}$.

ACKNOWLEDGMENTS

We thank Y. Minami, T. Kono, and A. Kawahara for valuable comments.

This work was supported by a Grant-in-Aid for Special Project Research, Cancer Bioscience, from the Ministry of Education, Science and Culture of Japan and by a research grant from Nippon Boehringer Ingelheim Co., Ltd., Kawanishi, Japan.

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