Creation and Characterization of Temperature-Sensitive Mutants of the *lck* Tyrosine Protein Kinase

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Temperature-sensitive mutants of the *lck* tyrosine protein kinase were created by the introduction of mutations known to cause temperature sensitivity of the v-*src* tyrosine protein kinase of Rous sarcoma virus. p56^{lck} activated by mutation of the regulatory site of tyrosine phosphorylation, Tyr-505, to Phe transforms fibroblasts in culture. Mutations identical to those responsible for the temperature-sensitive phenotypes of the tsNY68 and tsNY72-4 v-*src* mutants rendered this activated *lck* gene temperature sensitive for both morphological transformation and induction of growth in soft agar. The mutant proteins were incapable of cellular transformation at the nonpermissive temperature in part because of failure of the *lck* protein to accumulate to normal levels. Morphological transformation of fibroblasts was detectable within 24 h of a shift of cells to the permissive temperature and was essentially complete in 48 to 72 h. These mutants should prove useful for the study of the function of the *lck* kinase in hematopoietic cells.

p56^{lck}, a member of the *src* family of tyrosine protein kinases, is expressed in T cells, some B cells, and natural killer cells (13, 17, 29, 30). In T cells, p56^{lck} is found bound to the cytoplasmic domains of either CD4 or CD8 (23, 24, 27, 28). p56^{lck} may participate in antigen-induced T-cell activation. Mutants of CD4 (7, 19) or CD8 (31) that lack the ability to bind p56^{lck} do not augment antigen-induced interleukin-2 (IL-2) production of CD4-dependent or CD8-dependent T cells. p56^{lck} has also been reported to associate with the β chain of the IL-2 receptor (9), Thy-1, Ly-6, CD59, CD55, and CD48 (26) in T cells and surface immunoglobulin in B cells (4). The significance of these associations is not yet clear.

The activity of p56^{lck} in vivo is regulated by phosphorylation of Tyr-505 (3, 16). Mutation of Tyr-505 to Phe activates p56^{lck} (3, 16), and expression of this activated mutant in fibroblasts causes morphological transformation. One approach to understanding the function of p56^{lck} in hematopoietic cells is to introduce the activated form and look for effects on the function of these cells. Indeed, Abraham et al. (1) have shown that activated p56^{lck} renders a normally nonresponsive T-cell hybridoma cell line responsive to antigen, and we have found that activated p56^{lck} induces antigen-independent production of IL-2 in at least four T-cell hybridomas (15). It would obviously be of interest to examine the effect of activated p56^{lck} on other types of cells in which p56^{lck} is expressed. For example, it is of interest to know whether it affects the requirement of IL-2-dependent cells for IL-2 and the response of B cells to antigen.

These experiments can be problematical. Recombinant retroviruses expressing p56^{F-505lck} and Neo^r appear to be somewhat toxic in hematopoietic cells because cells selected in G418 often do not express p56^{F-505lck} (our unpublished results). In contrast, equivalent viruses expressing wild-type p56^{lck} readily yield cells expressing the exogenous *lck* protein. We therefore sought a means of circumventing this

apparent toxicity. One approach is to use a temperature-sensitive mutant of p56^{F-505lck}. Cells infected with a retrovirus encoding such a mutant protein and a selectable marker could be selected at the nonpermissive temperature, at which the inactive p56^{F-505lck} should not be toxic, and the effect of p56^{lck} could be examined following a shift to the permissive temperature.

We initially attempted to create a temperature-sensitive p56^{F-505lck} by random mutagenesis but had no success. We therefore turned to site-directed mutagenesis. We introduced into F-505lck eight sets of point mutations known to render p60^{v-src} temperature sensitive in its transforming activity. Although most of these mutations in *lck* were not useful, three rendered the protein temperature sensitive. The properties of two of these mutants are described here.

MATERIALS AND METHODS

Construction of the temperature-sensitive *lck*. Mimicking mutations known to render p60^{v-src} temperature sensitive (6, 18), eight mutants of murine p56^{lck} were constructed by oligonucleotide-directed mutagenesis (14). All mutations were introduced into *lck* that contained the activating mutation of Tyr-505 to Phe. First, Gly-456 was mutated to Asp alone (D-456F-505lck) or in combination with mutation of Tyr-470 to His (D-456H-470F-505lck). The H-470 mutation was also combined with mutation of Arg-458 to His (H-458H-470F-505lck). Further, Leu-303 was mutated to Pro alone (P-303F-505lck), or in combination with mutation of Ile-439 to Met (P-303M-439F-505lck). The M-439 mutation was also made alone (M-439F-505lck), in combination with deletion of Thr-330, Pro-331, and Ser-332 (Δ 330-332M-439F-505lck), or in combination with mutation of Pro-481 to Ser (M-439S-481F-505lck). For mutagenesis, template DNA was obtained by infection of *Escherichia coli* CJ236 (*dut ung F'*) with an M13mp18 construct containing the EcoRI fragment of murine *lck* activated by a Tyr-to-Phe mutation at position 505 (3). Only Δ 330-332M-439F-505lck (Δ MFlck) M-439S-481F-505lck (MSFlck), and M-439F-505lck (MFlck) were found to be temperature sensitive, so the mutagenesis involved in construction of only these mutants is described in

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detail. Site-directed mutagenesis of Ile-439 to Met and Pro-481 to Ser was performed by using oligonucleotides 5'-GTAA GGCATTGACCGTG-3' and 5'-GTCCTCTGAGCGCTCC TT-3', respectively. Deletion of amino acids Thr-330, Pro-331, and Ser-332 was performed by using the oligonucleotide 5'-ATTCAACTTGATGCCCTTGAGAAAATCTAC-3'. The presence of all mutations was confirmed by DNA sequencing. Both mutant *lck* DNAs were digested with *Bam*HI, and the fragment encoding amino acid residues 150 to 509 was used to replace the equivalent fragment in the retroviral vector LXS_N (20) (generously provided by A. D. Miller) containing the *Eco*RI cDNA fragment of wild-type *lck* (22). In this construct, expression of the *lck* gene is under the control of the Moloney murine leukemia virus long terminal repeat, and expression of the Neo^r gene is under the control of the simian virus 40 promoter.

Generation of infectious virus. The LXS_N *lck* constructs and a viral helper plasmid, SV-ψ⁻-E-MLV (generously provided by N. R. Landau [21]), were introduced into the monkey kidney cell line COS-7 by CaPO₄-mediated transfection (8) as previously described (22). Viral stocks were collected after 48 h.

Infection of cells. We seeded 1.75×10^5 rat 208F fibroblasts on 5-cm dishes containing Dulbecco-Vogt modified Eagle's medium plus 10% bovine calf serum and 6 µg of Polybrene per ml. After attachment, the medium was aspirated, and the cells were infected with 500 µl of viral supernatant for 30 min at 37°C. Medium containing Polybrene was then added, and the cells were incubated for 48 h at 39°C. The cells were then grown at 39°C in medium containing 600 µg of the drug G418 (GIBCO/BRL) per ml. Pools and clones of cells expressing Δ*MFck* and MS*Fck* were identified by temperature-dependent morphological transformation and by Western immunoblotting with anti-*lck* antibodies and anti-phosphotyrosine antibodies after growth at 32°C. Representative clones of both were chosen for further study. A clone of cells expressing F-505*lck* which exhibited a highly transformed morphology was used as a positive control. Cells expressing wild-type *lck* were not cloned. Rather, a pool of G418-resistant cells was used.

Growth in soft agar. A total of 10^4 208F rat fibroblasts expressing wild-type *lck*, F-505*lck*, Δ*MFck*, or MS*Fck* were seeded at a concentration of 2.5×10^3 cells per ml in Dulbecco-Vogt modified Eagle's medium plus 5% calf serum and 0.3% agarose on base layers of 0.6% agarose in the same medium. One set of plates was incubated at 39°C, and the other set was incubated at 32°C. Colonies that formed at 39°C were photographed after 4 weeks, and those that formed at 32°C were photographed after 6 weeks.

Analysis of the temperature dependence of morphological transformation of cells expressing MS*Fck* and Δ*MFck*. Cells were grown at 39°C for 7 to 14 days and then seeded on 5-cm dishes and shifted to 32°C. At designated times, the cells were photographed, washed with Tris-buffered saline, and lysed in 350 µl of hot sodium dodecyl sulfate (SDS)-sample buffer. Lysates were boiled for 5 min, stored at -70°C, and used for Western blotting (see below).

Western blotting. Lysates were adjusted to contain equal amounts of total protein. Proteins were fractionated on 15% low bis polyacrylamide gels and transferred electrophoretically to Immobilon-P (Millipore). Western blotting was performed with anti-phosphotyrosine antibodies or anti-p56^{lck} antibodies as previously described (11).

Thermostability assay. Cells expressing F-505*lck*, MS*Fck*, and Δ*MFck* were grown at 32°C and then lysed in 3% Nonidet P-40-150 mM NaCl-20 mM Tris-HCl (pH 8.4 at

4°C)-2 mM EDTA-200 mM Na₃VO₄-50 mM NaF. *lck* protein was immunoprecipitated with anti-p56^{lck} antibodies or normal rabbit serum as previously described (11, 12). After two washes in TN (50 mM Tris-HCl [pH 7.2], 150 mM NaCl), immunoprecipitates were split into four equal fractions, resuspended in 10 µl of kinase buffer [40 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES; pH 7.1), 10 mM MnCl₂], incubated at 30°C for designated times, and stored on ice until all the samples were ready. In vitro kinase assays were then performed with [γ -³²P]ATP and the exogenous substrate, acid-denatured enolase, as previously described (11). The kinase assays were fractionated on a polyacrylamide gel, and incorporation of label into enolase was determined by scintillation counting of the enolase band.

RESULTS

We constructed mutants of *lck* that mimicked five temperature-sensitive mutants of *v-src*: *tsLA24*, *tsLA31* (6), *tsNY68*, *tsNY72-4*, and PA104 (18). All of the mutations were in the catalytic domain of the *lck* protein and were introduced into *lck* that contained the activating mutation of Tyr-505 to Phe; they are summarized in Fig. 1. When expressed in fibroblasts, p56^{F-505lck} induces morphological transformation (3, 16). Gly-456 was replaced with Asp alone or in combination with mutation of Tyr-470 to His, mimicking *tsLA31*. Cells expressing p56^{D-456F-505lck} were not transformed at any temperature (data not shown). This was probably due, at least in part, to the fact that p56^{D-456F-505lck} had an extremely short half-life and never reached the same steady-state levels as p56^{F-505lck} did (data not shown). p56^{D-456H-470F-505lck} also did not induce the transformation of cells at any temperature, although it was expressed at levels comparable to p56^{F-505lck} (data not shown). Mutation of Arg-458 to His in combination with the H470 mutation (mimicking *tsLA24*) also produced a protein that had no detectable transforming activity (data not shown). Mutation of Leu-303 to Pro alone or in combination with replacement of Ile-439 with Met (mimicking PA104) was also performed. Neither p56^{P-303F-505lck} nor p56^{P-303M-439F-505lck} induced transformation of cells at either temperature (data not shown). In contrast, mutations mimicking those responsible for the temperature sensitivity of the *v-src* proteins encoded by *tsNY68* and *tsNY72-4* (18)—replacement of Ile-439 with Met alone or in combination with either deletion of Thr-330, Pro-331, and Ser-332 or mutation of Pro-481 to Ser—produced proteins that exhibited temperature-sensitive behavior. p56^{M-439S-481F-505lck} (p56^{MSFck}) and p56^{Δ330-332M-439F-505lck} (p56^{ΔMFck}) are described herein. p56^{M-439F-505lck} has not yet been characterized fully.

Effect of growth temperature on the properties of fibroblasts expressing mutant *lck* proteins. To examine the temperature sensitivity of the mutant *lck* proteins, we infected rat 208F fibroblasts with retroviruses encoding the various *lck* proteins, selected G418-resistant clones, and examined the morphology of the cells grown at 39 and 32°C. Uninfected rat 208F fibroblasts have a flat, nontransformed morphology and display contact-inhibited growth (Fig. 2). Cells expressing wild-type *lck* were indistinguishable from uninfected cells at both 39 and 32°C (Fig. 2). Cells expressing *lck* with the activating mutation Phe-505 displayed a rounded, highly transformed morphology at both temperatures (Fig. 2). At the nonpermissive temperature of 39°C, cells expressing MS*Fck* and Δ*MFck* had a flat, nontransformed morphology, indistinguishable from that of uninfected cells (Fig. 2).

FIG. 1. Comparison of the mutations which induce temperature sensitivity in $p56^{lck}$ and $p60^{src}$. Known temperature-sensitive mutants of src are shown below the amino acid sequence for src . Only the amino acid substitutions required for the temperature-sensitive phenotype of $p60^{src}$ are shown. All of the lck mutations were introduced into lck that contained the activating mutation of Tyr-505 to Phe. Mutants of lck that did not display a temperature-sensitive phenotype are $p56^{D-456F-505lck}$ (DF), $p56^{D-456H-470F-505lck}$ (DHF), $p56^{H-458H-470F-505lck}$ (HHF), $p56^{P-303F-505lck}$ (PF), and $p56^{P-303M-439F-505lck}$ (PMF) (see Results). Mutants which displayed temperature sensitivity are $p56^{M-439F-505lck}$ (MF), $p56^{A330-332M-439F-505lck}$ (Δ MF), and $p56^{M-439S-481F-505lck}$ (MSF).

At 32°C the same cells were much more refractile and rounded but not as altered in morphology as cells transformed with F-505/*lck* (Fig. 2).

We next investigated whether cells expressing these proteins could grow in agarose suspension. Uninfected 208F rat fibroblasts can form rudimentary colonies in soft agar containing 5% calf serum (21a) (Fig. 3). Expression of wild-type *lck* did not affect the size of these colonies (Fig. 3). Expression of F-505*lck* induced the growth of extremely large

colonies at both 39 and 32°C (Fig. 3). The slightly smaller size of the colonies grown at 32°C is due to the slower growth of the cells at the lower temperature. At 39°C, cells expressing *MSFlick* or Δ *MFlick* did not form colonies that could be distinguished from those of uninfected cells (Fig. 3). Cells expressing either *MSFlick* or Δ *MFlick* formed large colonies when grown at 32°C (Fig. 3). The colonies formed by cells expressing Δ *MFlick* grown at 32°C were somewhat larger than those formed by cells expressing *F-505lick* grown at the

FIG. 2. Mutant *lck* proteins induce temperature-sensitive transformation of fibroblasts. Uninfected (UN) rat 208F cells or cells expressing wild-type *lck* (WT), F-505*lck* (F505), MSF*lck* (MSF), or Δ MF*lck* (Δ MF) were incubated at 39 or 32°C for at least 7 days and then photographed.

same temperature (Fig. 3). The colonies formed by cells expressing MSF*lck* at 32°C were somewhat smaller than those formed by cells expressing F-505*lck* (Fig. 3).

Levels of phosphotyrosine in cellular proteins increase at the permissive temperature. We then examined whether the level of phosphotyrosine in protein in cells expressing the mutant

FIG. 3. Mutant *lck* proteins induce temperature-sensitive growth in agarose suspension. A total of 10^4 uninfected (UN) rat 208F cells or cells expressing wild-type *lck* (WT), F-505*lck* (F505), MSF*lck* (MSF), or Δ MF*lck* (Δ MF) were seeded in Dulbecco-Vogt modified Eagle's medium plus 5% calf serum and 0.3% agarose and grown at 39 and 32°C. Colonies that formed at 39°C were photographed after 4 weeks, and colonies that formed at 32°C were photographed after 6 weeks.

FIG. 4. Levels of phosphotyrosine in cellular proteins increase at the permissive temperature. Cells were grown for 5 days at 32 and 39°C and then lysed directly in SDS-sample buffer. The lysates were normalized for levels of total cellular protein, fractionated on polyacrylamide gels, and subjected to Western blotting with anti-phosphotyrosine antibodies and 125 I-protein A. Lanes: 1, wild-type *lck* at 32°C; 2, wild-type *lck* at 39°C; 3, F-505*lck* at 32°C; 4, F-505*lck* at 39°C; 5, MSF*lck* at 32°C; 6, MSF*lck* at 39°C; 7, Δ MF*lck* at 32°C; 8, Δ MF*lck* at 39°C.

lck proteins exhibited temperature sensitivity. Cells expressing the various forms of *lck* were incubated at 39 or 32°C for 5 days and then lysed directly in SDS-sample buffer and analyzed by Western blotting with anti-phosphotyrosine antibodies. As previously described (3), cells expressing wild-type *lck* showed no increase in phosphotyrosine in protein, whereas those expressing F-505*lck* had dramatically increased levels of phosphotyrosine in protein (Fig. 4). Growth temperature had little effect on the level of tyrosine phosphorylation in cellular protein in cells expressing wild-type *lck* and F-505*lck*. Cells expressing MSF*lck* contained very little phosphotyrosine in protein when incubated at 39°C but contained high levels when incubated at 32°C (Fig. 4). At the nonpermissive temperature, cells expressing Δ MF*lck* had slightly elevated levels of phosphotyrosine in protein compared with cells expressing wild-type *lck*. The level of phosphotyrosine was much higher when the cells expressing Δ MF*lck* were incubated at the permissive temperature. The patterns of phosphotyrosine-containing proteins in cells expressing MSF*lck* and in those expressing Δ MF*lck* incubated at the permissive temperature both appeared very similar to the pattern seen for cells expressing F505*lck* at either temperature.

The temperature-sensitive *lck* proteins are more thermolabile in vitro. We investigated whether p56^{MSF*lck*} and p56 ^{Δ MF*lck*} exhibited increased thermolability in vitro. To do this, we immunoprecipitated the *lck* proteins from cells expressing F-505*lck*, MSF*lck*, and Δ MF*lck* grown at 32°C. The immunoprecipitates were preincubated for 0 to 12 min at 30°C and then subjected to an in vitro kinase assay. Incorporation of label into the exogenous substrate enolase was quantified by scintillation counting. After an 8-min incubation at 30°C, p56^{F-505*lck*} lost 68% of its kinase activity, whereas p56 ^{Δ MF*lck*} and p56^{MSF*lck*} lost 77 and 94%, respectively (Fig. 5). The calculated half-life of kinase activity at 30°C was 5.9 min for p56^{F-505*lck*}, 3.8 min for p56 ^{Δ MF*lck*}, and 2.3 min for p56^{MSF*lck*}.

FIG. 5. Thermostability of mutant *lck* proteins in vitro. *lck* proteins were immunoprecipitated from cells expressing F-505*lck*, MSF*lck*, or Δ MF*lck* at 32°C. The immunoprecipitates were incubated for 0 to 12 min at 30°C and then subjected to an in vitro kinase assay in the presence of [γ - 32 P]ATP and the exogenous substrate enolase. Incorporation of label into enolase was quantified by scintillation counting. The fraction of the kinase activity remaining was determined by dividing the cpm incorporated into enolase after preincubation at 30°C by the cpm incorporated into enolase without a 30°C preincubation.

Steady-state levels of the temperature-sensitive *lck* proteins increase at the permissive temperature. To determine whether the temperature-sensitive phenotype of the mutants was due, in addition, to differences in the steady-state levels of the mutant *lck* proteins at the permissive and nonpermissive temperatures, we analyzed cell lysates by Western blotting with anti-p56^{*lck*} antibodies. Whereas steady-state levels of wild-type p56^{*lck*} and p56^{F-505*lck*} differed less than 1.5-fold between 39 and 32°C, the steady-state level of p56^{MSF*lck*} decreased by 9-fold at the nonpermissive temper-

FIG. 6. The level of p56^{*lck*} increases at the permissive temperature. The lysates prepared for Fig. 4 were subjected to Western blotting with anti-p56^{*lck*} antibodies and 125 I-protein A. Lanes: 1, wild-type *lck* at 32°C; 2, wild-type *lck* at 39°C; 3, F-505*lck* at 32°C; 4, F-505*lck* at 39°C; 5, MSF*lck* at 32°C; 6, MSF*lck* at 39°C; 7, Δ MF*lck* at 32°C; 8, Δ MF*lck* at 39°C.

FIG. 7. Kinetics of change of phosphotyrosine in cellular protein and cell morphology after a temperature shift. Duplicate plates of cells expressing *MSFlck* were grown at 39°C for at least 48 h and then shifted to 32°C for 3, 6, 12, 27, 48, and 72 h. Duplicate plates of uninfected 208F cells or cells expressing *F-505lck* were grown for 72 h at 39 and 32°C. (a) The cells were photographed and then lysed directly in SDS-sample buffer. (b) The proteins were analyzed by Western blotting with anti-phosphotyrosine antibodies as described in Materials and Methods.

plates of cells expressing the various *lck* proteins were grown at 39°C and then shifted to 32°C for 3, 6, 12, 27, 48, and 72 h. The cells were photographed (Fig. 7a) and then lysed in SDS-sample buffer. The proteins were analyzed by Western blotting with anti-phosphotyrosine antibodies (Fig. 7b). A slight rounding of the cells expressing *MSFlck* was first seen 27 h after the shift to the permissive temperature (Fig. 7a). The morphological transformation became more noticeable after 48 h and quite dramatic after 72 h (Fig. 7a). Similar morphological changes were seen in cells expressing Δ *MSFlck* over the same period, although a small percentage of cells were transformed at the nonpermissive temperature (data not shown).

The effect of the temperature shift on the levels of phosphotyrosine in cellular protein, as assayed by Western blotting with anti-phosphotyrosine antibodies, essentially paralleled the effect on morphology. As expected, there was essentially no change in the amount of phosphotyrosine in uninfected 208F cells or cells expressing either wild-type *lck* or *F505lck* during the temperature shift (Fig. 7b). With cells expressing *MSFlck*, the amount of phosphotyrosine in protein increased very gradually, reaching maximal levels 48 to 72 h after the initial shift of the cells to the permissive temperature (Fig. 7b). The amount of phosphotyrosine in cells expressing *MSFlck*, however, did not reach the high levels seen in cells expressing *F-505lck*. Cells expressing Δ *MSFlck* showed increases in phosphotyrosine over the same period that were very similar to those seen for *MSFlck* (data not shown). The level of phosphotyrosine in the cells ex-

ature (Fig. 6). The level of $p56^{\Delta$ *MSFlck* was reduced by half at the nonpermissive temperature.

Kinetics of change of cell morphology and phosphotyrosine in cellular proteins following a temperature shift. Duplicate

pressing *MSFlck* reflected the level of *lck* protein. $p56^{MSFlck}$ expression increased approximately twofold after 27 h, reaching a fourfold increase 48 to 72 h after the initial shift of the cells to the permissive temperature (data not shown).

DISCUSSION

We have created three temperature-sensitive mutants of activated (F-505) *lck* by site-directed mutagenesis and have characterized two of these mutants in detail. Two regions of *lck* were targeted for mutagenesis on the basis of the location of mutations known to make the closely related tyrosine protein kinase $p60^{v-src}$ temperature sensitive. Mutations in *lck* that were identical to the mutations in *v-src* responsible for the temperature sensitivity of *tsLA24* and *tsLA31* (6) eliminated the transforming activity of the activated *lck* protein. These results are somewhat surprising since mutations in *v-abl* (5) and *Drosophila* sevenless (25) similar to those in *tsLA24* and *tsLA31* produce temperature-sensitive mutants of those proteins. Additionally, introduction of the mutation(s) responsible for the temperature-sensitive character of PA104 (18) into $p56^{F505lck}$ eliminated the transforming activity even at 32°C. These mutations are apparently more deleterious in *lck* than they are in *v-src*, *v-abl*, and sevenless. These results point out the unpredictability of this approach and serve as a reminder that although the tyrosine protein kinase catalytic domains are highly related, they are not identical.

In contrast, introduction of the mutations shown to produce the temperature-sensitive phenotype in *tsNY68* and *tsNY72-4* (18) produced a temperature-sensitive F-505*lck* protein. The properties of $p56^{MSFlck}$ and $p56^{\Delta MFck}$ were similar to those of *tsNY68* in that they induced morphological transformation and growth in agar suspension at the permissive temperature, 32°C, but had no effect at the nonpermissive temperature, 39°C. Unlike *tsNY68*, however, the temperature sensitivity of $p56^{MSFlck}$ was due in large part to a more rapid turnover of the mutant *lck* polypeptide at the nonpermissive temperature. As a consequence, the transformation of fibroblasts following a shift to the permissive temperature was slow with $p56^{MSFlck}$, because transformation required synthesis of active protein. In *tsNY68*, the inactive *src* protein is renatured and reactivated quickly after a shift to the permissive temperature (2, 10). The temperature sensitivity of $p56^{\Delta MFck}$ was also due in part to decreased steady-state levels of the mutant *lck* protein at the nonpermissive temperature. The decrease in *lck* protein levels at the nonpermissive temperature was, however, less dramatic with $p56^{\Delta MFck}$ than with $p56^{MSFlck}$. $\Delta MFck$ is a more leaky mutant than *MSFlck*. Cells expressing $\Delta MFck$ had a slightly elevated level of phosphotyrosine in protein at the nonpermissive temperature. This is probably because $p56^{\Delta MFck}$ accumulated to a higher level than did $p56^{MSFlck}$ at the nonpermissive temperature and was less rapidly inactivated.

We developed these mutant *lck* proteins to facilitate experiments with hematopoietic cells in which $p56^{F505lck}$ shows some apparent toxicity (our unpublished results). Cells infected with retroviruses encoding these genes and a selectable marker can be selected at the nonpermissive temperature, at which both $p56^{MSFlck}$ and $p56^{\Delta MFck}$ are relatively inactive and therefore presumably less toxic than $p56^{F505lck}$. The effect of $p56^{F505lck}$ activity on lymphokine production, lymphokine dependence, or cytolytic activity can then be assessed following a shift to the permissive temperature. In fact, $p56^{MSFlck}$ induces IL-2 production by

the DO11.10 T-cell hybridoma cell line in as little as 6 h after the cells are shifted to the nonpermissive temperature (14a, 15). The rapidity of this activation may be due in part to greater stability of $p56^{MSFlck}$ at the nonpermissive temperature in lymphoid cells (14a). We anticipate that these mutants will prove useful in the dissection of the role of $p56^{lck}$ in other types of hematopoietic cells.

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